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SSAT QUICK SHOT PRESENTATION

Perioperative Mortality After Non-hepatic General Surgery in Patients with Liver Cirrhosis: an Analysis of 138 Operations in the 2000s Using Child and MELD Scores

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Abstract
Introduction Despite of advances in modern surgical and intensive care treatment, perioperative mortality remains high in patients with liver cirrhosis undergoing nonhepatic general surgery. In the few existing articles, mortality was reported to be as high as 70% in patients with poor liver function (high Child or model for end-stage liver disease (MELD) score). Since data are limited, we analyzed our recent experience with cirrhotic patients undergoing emergent or elective nonhepatic general surgery at a German university hospital.
Methods Since 2000, 138 nonhepatic general surgical procedures (99 intra-abdominal, 39 abdominal wall) were performed in patients with liver cirrhosis. Liver cirrhosis was preoperatively classified according to the Child (41 Child A, 59 B, 38 C) and the MELD score (MELD median 13). Sixty-eight (49%) of the patients underwent emergent operations. Most abdominal wall operations were for hernias. Intra-abdominal operations consisted of GI tract procedures (n=53), cholecystectomies (n=15), and various others (n=31). Perioperative data were gained by retrospective analysis.
Results Overall perioperative mortality in all 138 cases was 28% (9% in elective surgery, 47% in emergent surgery; $p<0.001$). Perioperative mortality was higher after intra-abdominal than after abdominal wall operations (35% vs. 8%; $p=0.001$) or in patients requiring transfusions (43% vs. 5% without transfusions; $p<0.001$). Perioperative mortality increased with the Child score (10% Child A, 17% Child B, 63% Child C; $p<0.01$) and the MELD score (9% MELD <10, 19% MELD 10–15, 54% MELD >15; $p<0.001$). Univariately, further factors like American Society of Anesthesiologists (ASA) score and various preoperative laboratory values were also associated with perioperative mortality. By multivariate analysis of all 138 operations, the Child and ASA classifications, intraoperative transfusions, and a preoperative sodium <130 mmol/l, but not the MELD score, were independent prognostic factors.
Analysis of elective operations revealed only a preoperatively increased creatinine as risk factor for perioperative mortality. In emergent operations again, Child class, blood transfusions, and low sodium level, but not the MELD score, predicted postoperative mortality.
Conclusions Our results demonstrate that perioperative mortality remains high in patients with liver cirrhosis undergoing general surgery, especially in emergent situations. Patients with poor liver function and/or need for blood transfusions even had a very high mortality. In our experience, the Child score (together with other variables) independently correlates with perioperative mortality in emergent operations whereas the MELD score was inferior in predicting the outcome.

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Introduction

While liver resection for hepatocellular carcinoma and liver transplantation itself are the kind of surgical procedures most often associated with in liver cirrhosis, other surgical conditions are also quite frequent in those patients. In general, perioperative morbidity and mortality have been proven to be much higher in cirrhotic patients than in controls.[1–4] Prognostic scoring systems for the underlying surgical disease in combination with the state of liver disease have therefore been investigated. Both of the clinically most common scoring systems for chronic liver diseases (Child–Turcotte–Pugh classification, CTP; model for end-stage liver disease, MELD) have been correlated with outcome.[1,5,6] Among the diseases requiring surgical procedures, cirrhotic patients are especially prone to the following: abdominal wall hernias of all kinds due to ascites, gastrointestinal bleeding disorders due to portal hypertension, and also perforations of the intestine. While surgical resection and transplantation for hepatocellular carcinoma have been looked at intensively in cirrhotic patients,[7–11] general and abdominal surgery procedures (e.g., cholecystectomy, abdominal wall hernias, procedures of the GI tract) have not been scrutinized in the same manner. For reference, studies including larger numbers of patients (>40) are either few or limited[1,6,12–14] or date from the past decade.[2,15] Some do provide a large number of patients but lack sufficient detail with regard to the exact extent of liver cirrhosis (i.e., CTP or MELD score).[4] Other studies have looked at various surgical procedures including cardiac surgery and orthopedic surgery.[1,3,16]

The present results unanimously reflect an extremely high rate of morbidity but more importantly a high mortality in cirrhotic patients having to undergo surgery.[1,13,14]

In addition, the situation becomes aggravated if the surgical procedure in those patients has to be done in an emergency (e.g., bleeding, perforation, incarceration).

The aim of this study was to evaluate the perioperative outcome after general and digestive surgical procedures done in cirrhotic patients at a single European university hospital since the year 2000 and to analyze potential risk factors for perioperative mortality.

Patients and Methods

Patients with liver cirrhosis were identified using our computerized hospital information system for procedure codes in combination with international codes of disease (ICD-10). Included in this retrospective analysis were all patients with cirrhosis having undergone any surgical treatment at our department, with the exception of hepatic resection, from 2000 until 2007. The hospital charts were assessed in all 138 cases (123 patients) for the following specific parameters: indication for surgery, etiology of cirrhosis (if known), laboratory values (complete blood count, prothrombin time, partial thromboplastin time, electrolytes, liver function tests), and further data in order to calculate CTP and MELD scores, type of operation, timing of operation (elective/emergent), length of surgery, blood loss, substitution of blood or other blood products, general complications, surgical complications, and in-hospital mortality.

The definition of cirrhosis was mainly based on clinical and laboratory parameters in conjunction with imaging, mostly ultrasonography, if underlying liver disease supported the diagnosis. Clinical and laboratory values were used according to Child–Pugh classification or MELD score. Mainly macroscopic changes of liver parenchyma, ascites, jaundice, or signs of portal hypertension were used for clinical evaluation. In more severe disease, possible tremor and encephalopathy were also assessed. Histologic proof was not a prerequisite for the diagnosis of cirrhosis in the majority of patients. Of 123 patients with cirrhosis, 23 (19%) had histologic proof of cirrhosis in our hospital. In patients where the diagnosis was made only after intraoperative macroscopic findings (26 out of 123; 21%), Child and MELD scores had to be retrospectively derived from preoperative data. Partially subjective parameters such as amount of ascites or encephalopathy were classified to the best of knowledge.

Surgery

For further subgroup analyses, surgical procedures were classified as minor (isolated abdominal wall procedures, cholecystectomy, and other limited intra-abdominal procedures like shunt implantations or catheter insertions) or major (all resections of the gastrointestinal tract, with the exception of cholecystectomy, any kind of perforation, bleeding or peritonitis). Subgroup comparison was also performed between elective and emergent surgical procedures, the latter being defined as performed within 24 h after diagnosis.

Liver Function

Preoperative CTP scores were assessed according to the clinically widely used model including serum bilirubin, albumin, prothrombin time, ascites, and hepatic encephalopathy.[17] MELD scores were calculated using the following formula:

$$9.6 \times \log(\text{creatinine}[\text{mg/dl}]) + 3.8 \times \log(\text{bilirubin}[\text{mg/dl}]) + 11.2 \times \log(\text{INR}) + 6.4$$

as described before.[18] Results were then stratified into CTP groups A, B, and C and MELD scores <10, 10–15, and >15 points. In addition, results were correlated with the preoperatively assessed American Society of Anesthesiologists (ASA) score.[19]

Statistical Analysis and Definitions

Statistical analysis was done after entering all parameters into an SPSS Database (SPSS, version 15.0, Chicago, IL, USA). Subgroups were compared using chi-square, Fisher's exact, or Mann–Whitney *U* tests. Potential risk factors significant in univariate analysis ($p < 0.05$) were then entered into a binary logistic regression model (likelihood ratio statistics with forward selection strategy) to be tested for independent (multivariate) prognostic influence on perioperative mortality.

Mortality was defined as in-hospital mortality, independent on length of stay. None of the patients was transferred to other acute-care hospitals after surgery in our institution.

Results

Demographics

Median age was 61 years (range 21–87); 69% of the patients were male. During the study period, a number of 138 procedures were identified, done in 123 patients. One hundred ten patients underwent a single surgical procedure, 11 patients were independently operated on twice, and two patients had three unrelated surgical procedures, respectively. For further risk factor analysis in this study, each operation was noted as a single case ($n=138$).

Type and Extent of Liver Disease

Liver cirrhosis was preoperatively evident in 78%. In 22%, cirrhosis was unknown before surgery and discovered intraoperatively. The etiology of cirrhosis was alcoholic in 60% and cryptogenic/unknown in 22 (16%) patients. Viral hepatitis C was present in 15 (11%) and viral hepatitis B in nine (7%) cases. There were three patients with cardiac cirrhosis (2%) and two patients with either autoimmune hepatitis or combined hepatitis B and C (1.5% each). One patient had primary sclerosing cirrhosis, and one had primary biliary cirrhosis (0.7% each).

According to the CTP classification, 30% were CTP A, 43% CTP B, and 28% CTP C, respectively. Median MELD score was 13 (range 6–39).

Indications and Surgical Procedures

Complete listings of the procedures stratified into elective/emergent and minor or major operations are shown in Tables 1 and 2. Fifty-four percent of the procedures were classified as minor and 46% as major operations. Thirty-nine operations (28%) were isolated procedures of the abdominal wall and 99 (72%) were intra-abdominal procedures. Gastrointestinal procedures as such were done in 53 cases (40%); 15 (7%) cholecystectomies were investigated as a subgroup. A total of 12 (9%) surgical procedures were performed for not interventionally correctable bleeding. Perforations were treated in 21 (15%) of cases. In 70 (51%) patients, surgery was done as an elective procedure, leaving an almost equal amount of patients ($n=68$; 49%) having to undergo emergent surgery. Characteristics of preoperative parameters in elective and emergent surgery are given in Table 3. It is of note that patients requiring emergent surgery clearly had worse liver function (CTP and MELD scores), higher ASA scores, and (in median) deterioration of several preoperative laboratory parameters as compared to patients undergoing elective surgery (Table 3).

Mean operative time was 157 min (median 130 min, range 20–600 min). Sixty-two percent (86) of the surgical procedures lasted more than 2 h. One hundred twenty-six (91.3%) operations were performed with a primarily open approach. An overview of preoperative laboratory values is shown in Table 4.

Table 1 Operations performed stratified by emergent versus elective operations

	All (*n*)	Total (%)	Elective (%)	Emergent (%)
All	138	100	70 (51%)	68 (49%)
Type of surgery				
Major	63	46	20 (32%)	43 (68%)
Minor	75	54	50 (67%)	25 (33%)
Location of surgery				
Abdominal wall	39	28	29 (74%)	10 (26%)
Intra-abdominal	99	72	41 (41%)	58 (59%)
Subgroups				
Gastrointestinal	53	40		
Esophagus	1		1(100%)	0 (0%)
Stomach	9		3 (33%)	6 (66%)
Small bowel	15		3 (20%)	12 (80%)
Colon	23		11 (48%)	12 (55%)
Pancreas	5		4 (80%)	1 (20%)
Cholecystectomy	15		10 (66%)	5 (33%)
Perforation	21	15	0 (0%)	21 (100%)
Bleeding	12	9	0 (0%)	12 (100%)

Table 2 Description of all 138 procedures with classification as major or minor surgery

	n	Percent
Total	138	100
Minor	75	54
Laparotomy[a] (nonresectional procedures)	11	8
Abdominal hernia closure with mesh	38	28
Cholecystectomy	15	11
Gastrostomy/feeding tube placement	3	2
Diagnostic laparoscopy	2	1
Excision mesenteric gastrinoma	1	1
Closure of loop ileostomy	1	1
Laparoscopic gastric banding	1	1
Pneumoperitoneum	1	1
Appendectomy	1	1
Local adhesiolysis	1	1
Major	63	46
Laparotomy for bleeding (various causes)	9	7
Laparotomy/suture closure of perforated peptic ulcer	10	7
Gastric resection (Billroth II)	2	1
Gastroenterostomy	2	1
Small bowel resection	6	4
Ileocecal resection	3	2
Colectomy (left/right)	8	6
Colectomy (total)	3	2
Colectomy (sigmoid)	5	4
Rectal resection (including one TEM)	3	2
Pancreatic resections (PPPD or DPPHR)	5	4
Splenectomy	3	2
Hepaticojejunostomy	2	1
Esophageal resection (thoracoabdominal)	1	1
Portocaval shunt	1	1

TEM transanal endocopic microsurgery, *PPPD* pylorus preserving pancreatoduodenectomy, *DPPHR* duodenum preserving pancreatic head resection

[a] In some with minor nonresectional procedures, without opening of the gastrointestinal tract or violation of the portal venous system

Blood Loss and Transfusion

The mean number of transfused units of red packed blood cells (PRBC) was 1.85 (median 0, range 0–25). The mean number of intraoperatively transfused fresh-frozen plasma units was 1.76 per patient (median 0, range 0–25). For further risk factor analysis, the intraoperative administration of PRBC was classified as yes or no, and estimated blood loss was classified as below or above 1,000 ml.

Postoperative Complications

Any complication (overall complication rate) was recorded in 77% of the cases. Surgical complications occurred in 56% and other (nonsurgical, general) complications in 36%. In patients undergoing emergent operations, all complication rates were significantly higher than in patients having elective surgery (Table 5).

Mortality (All 138 Cases)

Overall in-hospital mortality was 27.5% (38 deaths in 138 cases). Stratified by CTP scores, overall mortality was the highest in CTP group C (63%), CTP group B patients had a mortality rate of 17%, and CTP group A had 10% ($p<0.001$; Table 6). The MELD score univariately also showed a comparable distribution of mortality (MELD <10 9%, MELD 10–15 19%, and MELD >15 54%; $p<0.001$). By analyzing categorized laboratory values, we also could identify a low sodium level, increased creatinine and prothrombin time, low thrombocyte count, low albumin level, increased bilirubin, hemoglobin <10 g/dl, and increased leukocyte count as univariate risk factors for mortality (Table 6). In addition, the groups of patients undergoing major surgery (vs. minor surgery), undergoing intra-abdominal procedures (vs. abdominal wall), and requiring blood or thrombocyte transfusions univariately were at significantly increased risk for mortality.

In multivariate risk factor analysis for mortality in all 138 cases (Table 7), the CTP classification (relative risk in total 2.4, relative risk CTP C versus CTP A/B 3.9), ASA classification, the need for intraoperative blood transfusion, and a preoperative sodium level <130 mmol/l were independent risk factors. The MELD score did not show a statistically significant influence on mortality by multivariate analysis, neither as a categorial nor as a continuous variable. These effects did not change relevantly after removing the ASA score (as a potentially subjective parameter) from the model.

Mortality (Elective Procedures)

Mortality after the 70 elective operations was 8.7%. In contrast to the entire study group (with almost half of the procedures performed as emergencies), only a preoperatively increased creatinine level was a (univariate) risk factor for mortality. Although in several factors (age group, diabetes, MELD groups, ASA score, sodium level) there was a trend for differences between the subgroups, this did not reach statistic significance (Table 8). Patients with increased creatinine had a significantly higher MELD score

Table 3 Preoperative parameters in patients undergoing elective or requiring emergent surgery

	Elective surgery (n=70)	Emergent surgery(n=68)	p
CTP score			
A (%)	50	9	<0.001
B (%)	41	44	
C (%)	9	47	
MELD score (median, range)	10 (6–27)	16.5 (6–39)	<0.001
ASA			
I–II (%)	33	7	<0.001
III–IV (%)	67	85	
V (%)	0	7	
Age (years, median, range)	62 (21–84)	58 (28.87)	0.02
Sodium (mmol/l, median, range)	138 (125–144)	137 (120–154)	0.17
Bilirubin (mg/dl, median, range)	1.1 (0.3–5.0)	2.3 (0.3–19.5)	<0.001
Albumin (g/dl, median, range)	3.6 (1.6–5.0)	2.8 (1.0–5.3)	<0.001
Creatinine (mg/dl, median, range)	0.9 (0.5–6.2)	1.0 (0.3–7.0)	0.07
Prothrombin Time (%, median, range)	88 (53–130)	59 (12–121)	<0.001
Hemoglobin (g/dl, median, range)	12.6 (7.7–16.4)	9.8 (2.3–17.2)	<0.001
Leucocytes (10^3/μl, median, range)	6.8 (1.8–28.7)	8.4 (1.0–30.3)	0.11
Thrombocytes (10^3/μl, median, range)	158 (36–652)	121 (7–445)	<0.01

(median 13) than patients with normal renal function (MELD median 8; p<0.01).

In 35 patients with cirrhosis classified as CTP A, four patients died after elective surgery: one after thoracoabdominal esophageal resection, two after hemicolectomy, and one after hernia repair. Out of 29 elective cases with CTP B, one patient died after hernia repair, and one patient in the CTP C group (six patients) died after elective laparotomy and Denver shunt implantation.

Table 4 Preoperative laboratory values (median, range)

Parameter	Median	Range
Hemoglobin (mg/dl)	11.7	2.3–17.2
WBC (×10^9/l)	7.1	1.0–30.3
Thrombocytes (×10^9/l)	146	7–652
Albumin (g/l)	3.1	1.0–5.1
Total protein (g/l)	6.3	0.9–8.9
Bilirubin (mg/dl)	1.5	0.3–19
Prothrombin time (%)	74	12–130
Partial thromboplastin time (s)	38	25–160
ALAT (U/l)	23	4–548
ASAT (U/l)	35	6–2,266
gamma-Glutamyl-transferase (U/l)	87.5	5–590
Creatinine (mg/dl)	0.9	0.3–7.0
Sodium (mmol/l)	137	120–155
Choline esterase (U/l)	2,475	328–7,762
Alkaline phosphatase (U/l)	143	44–1,206

Mortality (Emergent Procedures)

In contrast to elective procedures, mortality was very high in the 68 patients requiring emergent surgery (mortality 47%). As for the entire study group, mortality in emergent cases correlated with the CTP score (CTP C 72%, CTP B 30%, and in the CTP A group none of six patients died; p< 0.001; Table 9). The categorized MELD score univariately showed (compared to the CTP score) a lower but still significant correlation with mortality (MELD <10 20%,

Table 5 Postoperative morbidity after all 138 operations and in the subgroups of elective and emergent operations

Complication (type)	n	With complications (n)	With complications (%)	p
Any				
All operations	138	106	77	<0.001
Elective	70	45	64	
Emergent	68	61	90	
Surgical				
All operations	138	77	56	<0.001
Elective	70	33	47	
Emergent	68	44	65	
Medical				
All operations	138	49	36	<0.001
Elective	70	14	20	
Emergent	68	35	52	

Table 6 Univariate analysis of risk factors for mortality after 138 operations

Overall mortality (n=38/138), 27.5%	n total	n deceased	% mortality	p
Gender				
Male	95	24	25	0.38
Female	43	14	33	
Age group				
<60	63	21	33	0.16
≥60	58	17	23	
CTP				
A	41	4	10	0.0001
B	59	10	17	
C	38	24	63	
MELD				
<10	44	4	9	0.0001
10–15	48	9	19	
>15	46	25	54	
ASA score				
I–II	28	1	4	0.0001
III–IV	105	32	30	
V	5	5	100	
Sodium				
>130 mmol/l	107	20	19	0.0001
≤130 mmol/l	30	17	57	
Creatinine				
<1.1 mg/dl	89	18	20	0.010
≥1.1 mg/dl	49	20	41	
Prothrombin time				
≥70%	84	11	13	<0.001
<70%	54	27	50	
Partial thromboplastin time				
<36 s	59	12	20	0.08
>37 s	78	26	33	
Thrombocyte count				
$>100\times10^9$/l	93	16	17	<0.001
$\leq100\times10^9$/l	45	22	49	
Bilirubin				
≤1.2 mg/dl	57	9	16	0.005
>1.2 mg/dl	76	29	38	
Albumin				
>3 g/dl	48	9	19	0.044
<3 g/dl	82	29	35	
Preoperative hemoglobin				
>10 mg/dl	85	18	21	0.034
≤10 mg/dl	53	20	37	
Preoperative leukocytes				
$\leq10\times10^9$/l	71	11	16	0.001
$>10\times10^9$/l	67	27	40	
Diabetes mellitus				
Yes	32	9	28	0.93
No	106	29	27	
Cirrhosis known preoperatively				
Yes	107	31	29	0.48
No	31	7	23	
Operative blood loss				
≤1,000 ml	103	22	21	0.005
>1,000 ml	35	16	46	
PRBC transfusion intraoperatively				
Yes	55	30	55	0.0001
No	83	8	10	
Thrombocyte concentrate anytime				
No	131	31	2	0.0001
Yes	7	7	100	
Type of surgery				
Minor	75	9	12	0.0001
Major	63	29	46	
Type of surgery				
Abdominal wall	39	3	8	0.001
Intra-abdominal/others	99	35	35	
Duration of surgery				
≤2 h	52	16	31	0.51
>2 h	86	22	26	

MELD 10–15 33%, and MELD >15 60%; p=0.03). As for all patients, the categorized preoperative laboratory values sodium, prothrombin time and bilirubin, and the thrombocyte count were univariate risk factors for mortality. Again, the groups of patients undergoing major surgery (vs. minor surgery), having intra-abdominal procedures (vs. abdominal wall), and requiring blood or thrombocyte transfusions were at significantly increased risk for mortality (Table 9).

In multivariate risk factor analysis for mortality in the 68 emergent cases (Table 10), the CTP classification (relative

Table 7 Results of multivariate risk factor analysis for mortality in all 138 cases including CTP score or MELD score

	p	RR	95% CI
CTP model			
CTP classification	0.025	2.4	1.1–5.2
ASA classification	0.038	3.2	1.1–9.7
Intraoperative blood transfusion	0.003	5.8	1.9–18.4
Sodium (cutoff 130 mmol/l)	0.008	4.6	1.5–14.5
MELD model			
ASA classification	0.058	2.9	1.0–8.9
Emergent surgery	0.035	3.5	1.1–11.3
Intraoperative blood transfusion	0.006	5.1	1.6–16.3
Sodium (cutoff 130 mmol/l)	0.003	5.7	1.8–18.2

CTP or MELD scores were entered in two different models to exclude multicollinearity

Table 8 Univariate analysis of risk factors for mortality in elective surgery (n=70)

Mortality in elective surgery (n=6/70), 8.6%	n total	n deceased	% mortality	p
Gender				
Male	53	4	8	0.59
Female	17	2	12	
Age group				
<60	25	1	4	0.31
≥60	45	5	11	
CTP				
A	35	4	11	0.4
B	29	1	3	
C	6	1	17	
MELD				
<10	34	2	6	0.64
10–15	30	3	10	
>15	6	1	17	
ASA score				
I–II	23	1	4	0.15
III–IV	47	5	11	
V	0	0	0	
Sodium				
>130 mmol/l	59	4	7	0.22
<130 mmol/l	11	2	18	
Creatinine				
<1.1 mg/dl	53	2	4	0.01
≥1.1 mg/dl	17	4	24	
Prothrombin time				
≥70%	63	6	10	0.39
<70%	7	0	0	
Thrombocyte count				
$>100\times10^9$/l	57	6	11	0.22
$\leq100\times10^9$/l	13	0	0	
Bilirubin				
≤1.2 mg/dl	38	4	11	0.55
>1.2 mg/dl	31	2	7	
Albumin				
>3 g/dl	17	1	6	0.56
<3 g/dl	47	5	11	
Preoperative hemoglobin				
>10 mg/dl	53	5	6	0.65
≤10 mg/dl	17	1	9	
Preoperative leukocytes				
$<10\times10^9$/l	45	2	4	0.1
$>10\times10^9$/l	25	4	16	
Diabetes mellitus				
Yes	18	3	17	0.16
No	52	3	6	
Cirrhosis known preoperative				
Yes	58	5	9	0.97
No	12	1	8	
Operative blood loss				
<1,000 ml	60	5	8	0.86
>1,000 ml	10	1	10	
PRBC transfusion intraoperatively				
Yes	14	2	14	0.39
No	56	4	7	
Type of surgery				
Minor	50	3	6	0.22
Major	20	3	15	
Type of surgery				
Abdominal wall	29	2	7	0.67
Intra-abdominal/others	41	4	10	
Type of surgery				
Abdominal wall	29	2	7	0.61
GI tract	22	3	14	
Cholecystectomy	10	0	0	
Others	9	1	11	
Length of surgery				
<2 h	23	2	9	0.98
>2 h	47	4	9	

risk in total 6.2, relative risk CTP C versus CTP A/B 6.8), the need for intraoperative blood transfusion and a preoperative sodium level <130 mmol/l were independent risk factors for mortality. As in the entire study group, the MELD score did not show a statistically significant influence on mortality by multivariate analysis after emergent operations.

Discussion

Several studies have shown a high rate of overall mortality after surgery in patients with liver cirrhosis, ranging from 10% to 85% and depending on liver function.[12,15,20,21] This high mortality seems unacceptable by modern surgical standards. The mechanisms and reasons leading to such a high mortality are not completely understood. A clinically significant reduction of perioperative mortality in the past decades has not been reported[3,15,22]. Stratification of patients according to risk factor analysis on the other hand does seem to provide some means of predictability in terms of long-term outcome (Mayo score[1]). Whether those risk factors are universal or vary among the different kinds of surgical procedures remains to be elucidated.

While severity of cirrhosis and liver function have traditionally been described using the CTP score in cirrhotic patients,[12,15] the model for end-stage liver disease has been able to provide an even more accurate prediction of outcome (mortality) in various settings.[1] After cardiac surgery in patients with liver cirrhosis for instance mortality

Table 9 Univariate analysis of risk factors for mortality in emergent surgery (n=68)

Mortality in emergent surgery N=32 (68), 47.0%	n total	n deceased	% mortality	p
Gender				
Male	42	20	48	0.91
Female	26	12	46	
Age group				
<60	38	20	53	0.3
>60	30	12	40	
CTP				
A	6	0	0	0.0001
B	30	9	30	
C	32	23	72	
MELD				
<10	10	2	20	0.03
10–5	18	6	33	
>15	40	24	60	
ASA score				
I–II	5	0	0	0.001
III–IV	58	17	29	
V	5	5	100	
Sodium				
>130 mmol/l	48	16	33	0.001
<130 mmol/l	19	15	79	
Creatinine				
<1.1 mg/dl	36	16	44	0.65
≥1.1 mg/dl	32	16	50	
Prothrombin time				
≥70%	21	5	24	0.01
<70%	47	27	57	
Thrombocyte count				
$>100\times10^9$/l	36	10	28	0.001
$\leq100\times10^9$/l	32	22	69	
Bilirubin				
≤1.2 mg/dl	19	5	26	0.014
>1.2 mg/dl	45	27	60	
Albumin				
>3 g/dl	15	5	33	0.18
<3 g/dl	51	27	53	
Preoperative hemoglobin				
>10 mg/dl	32	13	41	0.32
≤10 mg/dl	36	19	53	
Preoperative leukocytes				
$<10\times10^9$/l	26	9	35	0.11
$>10\times10^9$/l	42	23	55	
Diabetes mellitus				
Yes	14	6	43	0.72
No	54	26	48	
Cirrhosis known preoperative				
Yes	49	26	53	0.11
No	19	6	32	
Operative blood loss				
<1,000 ml	43	17	40	0.1
>1,000 ml	25	15	60	
PRBC transfusion intraoperatively				
Yes	41	28	68	0.0001
No	27	4	15	
Thrombocyte concentrate anytime				
Yes	7	7	100	0.007
No	61	25	41	
Type of surgery				
Minor	25	6	24	0.004
Major	43	26	61	
Type of surgery				
Abdominal wall	10	1	10	0.011
Intra-abdominal/others	58	31	53	
Type of surgery				
Abdominal wall	10	1	10	0.03
GI tract	31	18	58	
Cholecystectomy	5	1	20	
Others	22	12	55	
Surgery for perforation or bleeding				
Yes	33	20	61	0.03
No	35	12	34	
Duration of surgery				
<2 h	29	14	48	0.86
>2 h	39	18	46	

risk was well predicted by MELD scores.[3,16,23] Befeler et al. have also been able to show the superiority of the MELD score for extrahepatic general surgery in a group of 53 patients.[5] While large populations of patients seem to be correctly classified using one of these scoring systems, it is evident that not all pathophysiologic conditions can be taken into account by a scoring system.

In our study, we retrospectively evaluated perioperative mortality in patients undergoing elective or emergent general surgical (nonhepatic) procedures. To assess potential risk factors other than CTP or MELD scores, we not

Table 10 Results of multivariate risk factor analysis for mortality in 68 cases undergoing emergent surgery including CTP score or MELD score

	p	RR	95% CI
Mortality			
CTP classification	0.006	6.2	1.7–22.7
Intraoperative blood transfusion	0.001	20.6	3.5–119.4
Sodium (cutoff 130 mmol/l)	0.014	9.1	1.6–52.6
MELD model			
Intraoperative blood transfusion	0.0001	19.1	3.8–95.8
Sodium (cutoff 130 mmol/l)	0.003	13.5	2.4–76.9

only examined the outcome with regard to these scoring systems but also analyzed the influence of various other variables in univariate and multivariate settings. Our analysis included patients who were treated in the last decade with full access to modern surgical, hepatology, and intensive care facilities at our university hospital.

It is clinically not surprising that mortality was more than five times higher after emergent (almost 50%) than after elective procedures (below 10%) in our series. Patients requiring emergent surgery had poorer liver function (CTP, MELD), lower hemoglobin, more major procedures, and (by definition of emergency) different indications for surgery. The rather high proportion of emergency procedures in our series (almost half of the cases) explains the high overall mortality in our analysis. Various other published series report on lower proportions of emergency operations (e.g., 10–40%).[1,6,12,13]

The need for blood transfusion was a strong independent (i.e., after multivariate analysis) predictor of perioperative mortality in the entire group or in patients requiring emergent operations.[24] The reasons for this are certainly manifold: While in emergent cases many patients are operated for bleeding, necessary blood transfusions do more likely reflect a strongly impaired coagulation rather than primary or surgical bleeding in the other cases. So clinically the need for blood transfusion does seem to subsume alterations in the coagulation cascade (due to liver dysfunction) and any preoperative blood loss making it a rather simple yet effective predictor of mortality. The need for blood transfusion was not correlated with mortality in the elective setting in our patients (20% of those required blood transfusion). This certainly is due to patient selection: Patients undergoing elective procedures had, in average, better liver function (i.e., CTP, MELD, laboratory values) and less major procedures.

A rather simple, but on multivariate analysis consistent predictor of mortality (in the entire patient group) was the ASA score. It is, in part, self-explaining that patients requiring emergent surgery often have higher ASA classification. In our multivariate analysis of risk factors for mortality in the entire group, however, ASA was an independent predictor of the outcome. With its limitations of being a "subjective score", it has even proven its superiority to well-established scores such as CTP and MELD for liver resection in a study by Schroeder et al.[25] More importantly, however, the ASA score proved to be one of three independent risk factors for early and late (i.e., after 1 year) mortality in the large series from the Mayo Clinic.[1] As already outlined in the study by Teh et al., the ASA score is influenced by the presence of liver cirrhosis itself and may therefore be lower in patients without preoperatively known cirrhosis.[1]

In our series, serum sodium levels did also prove to be a predictor of mortality in multivariate analysis (in the entire and emergency group, not in elective cases). This readily available parameter seems to reflect liver function, hepatorenal physiology as well as the other factors or models.[26–28] Hyponatremia has also been shown to predict mortality in nonsurgical settings for patients with liver cirrhosis in a recent study by Cárdenas and Ginès.[29] A new score including MELD and serum sodium (MESO index) has even been proposed to be superior in predicting mortality of cirrhotic patients.[26,27]

The CTP score was an independent predictor of mortality in the entire or emergency surgery group. In contrast to some other reports,[1,5] the MELD score was not superior to the CTP score in our series. This finding is in line with observations by Schroeder et al.[25] Other groups did find a correlation between CTP and MELD scores[6,16] or did consider them both reasonable[30] or equal.[13] Even after applying alternative multivariate models for CTP or MELD classes to exclude multicollinearity, other factors like sodium level, emergent indication, or the need for blood transfusion were stronger risk factors than the MELD score.

In our series, the risk factor profile in elective surgery was completely different to risk factor analysis in emergency situations. This is certainly due to a selection bias. In the elective setting, major surgery is reserved to patients with better liver function. CTP class C cirrhosis is generally a contraindication for major surgery in our institution. In addition, we always attempt to preoperatively ameliorate poor liver function in the elective setting together with our hepatologists. In contrast, major surgery is performed for life-threatening conditions (e.g., bleeding, perforation) also in patients with poor liver function.

In the 70 elective cases in our study, only the preoperative presence of impaired renal function, but not liver function or other laboratory values, influenced mortality rate. Mortality was six times higher in patients with an increased creatinine although only two of those 17 patients had a creatinine level above 2 mg/dl. Mild or moderate renal dysfunction has also been shown to influence postoperative complication rates or even mortality in various other settings like pancreatic surgery,[31,32] cardiac surgery,[33] liver resection,[34,35] or vascular surgery.[36]

Potential Limitations of Our Study

Although most factors were completely documented in the patient charts, the retrospective evaluation may bear a bias regarding the subjective CTP classification (i.e., encephalopathy, amount of ascites). In addition, as in most comparable published reports, there is certainly a relevant selection bias of patients with liver cirrhosis at least in the

elective setting: Although we always apply the CTP score preoperatively if cirrhosis is known or suspected, additional subjective parameters like age, underlying disease, other co-morbidities, or the individual surgeons' judgment may lead to a contraindication to elective surgery (and non-appearance of such cases in surgical series).

Conclusion

Our results demonstrate that perioperative mortality remained high in patients with liver cirrhosis undergoing general surgery, especially in emergent situations. Patients with poor liver function and/or need for blood transfusions even had a very high mortality. In our experience, the Child score (together with other variables like ASA score and preoperative serum sodium level) independently correlated with mortality in emergent operations whereas the MELD score was inferior in predicting the outcome. In the selected patients with liver cirrhosis requiring elective surgery, not liver function but mild to moderate renal dysfunction relevantly determined postoperative mortality. A strategy to improve liver and kidney function preoperatively (especially in elective cases) and to reduce the risk of bleeding or the need of blood transfusions may be advocated.

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2010 SSAT POSTER PRESENTATION

Mechanisms of Action of the Gasotransmitter Hydrogen Sulfide in Modulating Contractile Activity of Longitudinal Muscle of Rat Ileum

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Abstract
Aim This study aims to determine mechanisms of action of the gasotransmitter hydrogen sulfide (H_2S) on contractile activity in longitudinal muscle of rat ileum.
Methods Ileal longitudinal muscle strips were prepared to measure isometric contractions. Effects of sodium hydrosulfide (NaHS), a donor of H_2S, were evaluated on spontaneous contractile activity and after enhanced contractile activity with bethanechol. L-cysteine was evaluated as a potential endogenous donor of H_2S. We evaluated involvement of extrinsic nerves, enteric nervous system, visceral afferent nerves, nitric oxide, and K_{ATP}^+ channel and K_{Ca}^+ channel activity on the action of H_2S using non-adrenergic/non-cholinergic conditions, tetrodotoxin, capsaicin, L-N^G-nitro arginine (L-NNA), glibenclamide, and apamin, respectively, as well as electrical field stimulation.
Result NaHS dose-dependently and reversibly inhibited spontaneous and bethanechol-stimulated contractile activity ($p<0.05$). L-cysteine had no inhibitory effect. Non-adrenergic/non-cholinergic conditions, tetrodotoxin, capsaicin, L-NNA, glibenclamide, or apamin had no major effect on total contractile activity by NaHS, although both tetrodotoxin and apamin decreased the frequency of bethanechol-enhanced contractile activity ($p<0.05$). We could not demonstrate H_2S release by electrical field stimulation but did show that inhibition of cystathionine β synthase, an endogenous source of H_2S, augmented the inhibitory effect of low-frequency electrical field stimulation.
Conclusion H_2S inhibits contractile activity of ileal longitudinal muscle dose-dependently but not through pathways mediated by the extrinsic or enteric nervous system, visceral afferent nerves, nitric oxide, K_{ATP}^+ channels, or K_{Ca}^+ channels.

Keywords Intestinal motility · Gasotransmitter · Hydrogen sulfide · Longitudinal muscle · Physiology · Motility · Contractile activity · Ileum longitudinal smooth muscle

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Introduction

Contractile activity of the small intestine is regulated or modulated by many factors, such as the central nervous system, the enteric nervous system (ENS), gut hormones, mechanical factors, and the local neurohumoral milieu. Neural modulation by the classic neurotransmitters acetylcholine and norepinephrine, or the non-adrenergic, non-cholinergic neurotransmitters, such as vasoactive intestinal peptide (VIP), substance P, and many other neuropeptides, transmit the neural signal by binding to receptors on the post-synaptic cell membrane, inducing the intracellular release of a second messenger, which then leads to alteration of contractile activity. It has become increasingly clear that endogenously-produced biologic gasses called "gasotransmitters" can also play an important role in the signal transduction from nerves in the control of small intestinal motility. The two more commonly appreciated gasotransmitters, nitric oxide (NO) and carbon monoxide (CO), are freely permeable across the cell membrane, diffuse into the target cell, and affect intracellular pathways directly; these gasotransmitters are released following "on demand" enzymatic synthesis.[1] Thus, mechanisms of signal transduction by gasotransmitters are different from that of the classic neurotransmitters.

Hydrogen sulfide (H_2S), which is known more commonly as a toxic pollutant, is the newest member of the gasotransmitter family.[2] Endogenous H_2S is produced from the substrate L-cysteine by two enzymes, cystathionine beta (β) synthase (CBS) and cystathionine gamma (γ) lyase (CSE). The mechanism of action of H_2S is well studied in vascular smooth muscle,[3] where H_2S opens ATP-sensitive potassium channels (K_{ATP}^+ channels) leading to hyperpolarization of the membrane potential, closing of voltage-gated Ca^{2+} channels, and subsequent vasorelaxation.

Very few studies have explored the role and mechanisms of H_2S in the control of small intestinal motility. Prior work in our laboratory demonstrated that the enzymes that generate endogenous H_2S, CBS, and CSE, are expressed in the enteric nerves of the small intestine (Kasparek et al., under review). Moreover, two prior studies reported that exogenous sodium hydrosulfide (NaHS), an H_2S donor, caused a dose-dependent inhibition of jejunal and ileal contractile activity.[4–6] This inhibitory effect was independent of K_{ATP}^+ channel activity.[5] Moreover, mechanisms of action and functional roles of H_2S in the modulation of small intestinal contractile activity remain poorly understood.

The aim of our study was to determine the effects and mechanisms of action of H_2S applied either exogenously and released endogenously on contractile activity in the longitudinal muscle of rat ileum. By using inhibitors which block specific potential pathways of signal transduction, we studied the involvement of the enteric nervous system, primary afferent nerve fibers, NO, and two different types of K^+ channels (K_{ATP}^+ channel and K_{Ca}^+ channel) in the response to H_2S. By using exogenously applied L-cysteine, the substrate for endogenous production of H_2S, and electric field stimulation in an attempt to release H_2S from enteric nerves, we sought to investigate the effects of endogenous release of H_2S. Our hypothesis was that H_2S released from enteric nerves acts as an endogenous inhibitor of contractile activity of the longitudinal smooth muscle of rat ileum by a direct effect on smooth muscle contractile activity via opening of K_{ATP}^+ channels.

Materials and Methods

Preparation of Animals

Procedure and animal care were performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Mayo Foundation in accordance with the guidelines of the National Institutes of Health and the Public Health Service Policy of the Human Use and Care of Laboratory Animals and was approved by the IACUC of the Mayo Clinic.

Recording of Contractile Activity

Male Lewis rats (Harlan–Sprague–Dawley, Indianapolis, IN, USA) weighing 275–350 g were used in the experiments. Rats were anesthetized initially with inhalation of 2% isoflurane (Abbott Laboratories, North Chicago, IL, USA) and maintained by intraperitoneal sodium pentobarbital (30–50 mg/kg; Ampro-Pharmacy, Arcadia, CA, USA). Via a midline celiotomy, a segment of ileum 10 cm proximal to the ileocecal valve was harvested and kept in chilled, modified Krebs-Ringer's bicarbonate solution (concentrations in mmol/L: NaCl 116.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 23.8, calcium disodium edentate 0.26, and glucose 11.1) pre-oxygenated with 95% oxygen/5% carbon dioxide (Praxair, Burr Ridge, IL, USA). After opening the ileal segment along its mesenteric border, full-thickness muscle strips (2×8 mm; width×length) were cut in the direction of the longitudinal muscle layer. Purposely, we did not remove the mucosa and submucosa to maintain the transmural anatomy and all enteric neural connections. Both ends of the muscle strip were tied with 5–0 silk and suspended vertically in 10-ml tissue chambers filled with modified Krebs-Ringer's bicarbonate solution kept at 37.5°C and bubbled continuously with 95% oxygen/5% carbon dioxide. One end of the muscle strip was connected to a fixed hook; the other end was attached to a metal hook connected to a noncompliant force transducer (Kulite Semiconductors Products, Inc., Leonia, NJ, USA) to measure the isometric force generated by the muscle strip. Contractile activity was monitored by an eight-channel recorder (Grass 7D polygraph; Grass Instrument Co, Quincy, MA, USA) in real-time while in parallel being displayed and stored digitally on a personal

computer using dedicated software (MP-100A-CE and Acq-Knowledge; Biopac Systems, Inc., Goleta, CA, USA) for detailed computer analysis later. Our system has been well described previously.[7,8] At the end of each experiment, the muscle strips were blotted on filter paper and weighed to standardize the contractile data as per milligram tissue weight.

Experimental Design

Muscle strips were equilibrated for 60–90 min with washout of the bath solution every 15 min to allow development of stable, spontaneous contractile activity. Thereafter, the optimal length (L_O) of each muscle strip was achieved by incremental stretching at 5–10-min intervals to a length beyond which further stretching no longer increased either amplitude or frequency of spontaneous contractile activity. All subsequent experiments were performed at L_O. Muscle strips not developing a stable and characteristic spontaneous contractile pattern were excluded from the study. Each experimental condition was carried out in at least two muscle strips per rat in a minimum of six rats per condition.

To determine the effect of exogenous H_2S, we chose to use the well-established H_2S donor NaHS. At pH of 7.4 and temperature of 37.5°C, 18.5% of NaHS exists as H_2S in solution.[9] To determine a dose–response curve, four different escalating concentrations of NaHS (10^{-5}, 10^{-4}, 5×10^{-4}, 10^{-3} M) yielding concentrations of H_2S in solutions of about 1.8, 18, 90, and 180 μM, respectively, were added to eight muscle strips per rat in eight rats with washout of the bath solution between each dose. We used NaHS as an exogenous donor of H_2S purposely, because not only is it easier technically, but use of NaHS is also more reliable in attaining an accurate concentration of H_2S in the bath solution than preparing a solution by bubbling of H_2S through the bath. The effect of NaHS (10^{-4} and 10^{-3} M) on precontracted muscle strips was studied by application of NaHS 90 s after exposure of the muscle strips to the muscarinic agonist bethanechol at a dose of 3×10^{-6} M; this dose of bethanechol caused an increase in the frequency and amplitude of contractions rather than a tonic contraction.[10] We applied these two doses of NaHS cumulatively at an interval of 5 min without washout between doses to determine a dose response during bethanechol-enhanced contractile activity.

Next, non-adrenergic and non-cholinergic (NANC) conditions were established by adding atropine (10^{-7} M), phentolamine (10^{-5} M), and propranolol (5×10^{-6} M) to the bath of two muscle strips to investigate the role of adrenergic and cholinergic neurons in mediating the effect of NaHS as we have reported before.[7,8] The effect of NaHS was studied at two doses (10^{-4} and 10^{-3} M) beginning 30 min after establishment of NANC conditions; because the muscarinic receptor antagonist atropine was used to establish NANC conditions, we did not study the effect of NaHS after administration of bethanechol. Thereafter, the effect of the global neural inhibitor tetrodotoxin (TTX; 10^{-6} M) on baseline activity was studied in the same two muscle strips as for the NANC conditions as reported before.[7,8] TTX inhibits voltage gated, fast sodium channels in nerve cell membranes to prevent depolarization of the cell membrane and the subsequent release of neurotransmitters from virtually all nerves within the muscle strip. After exposure of the muscle strips to TTX for 30 min, the effect of NaHS was evaluated at two doses (10^{-4} and 10^{-3} M). Thereafter, the effect of NaHS was studied again at two doses (10^{-4} and 10^{-3} M) after bethanechol (3×10^{-6} M) in the ongoing presence of TTX.

In two other muscle strips from six rats, we used capsaicin to investigate the role of visceral afferent nerves in mediating the effect of NaHS. Capsaicin is an agonist of transient receptor potential vanilloid receptor 1 (TRPV-1) and is a well-established method of desensitizing primary afferent nerve fibers *in vitro*[11–15]; we confirmed the effectiveness of capsaicin in preliminary experiments by observing a tachyphylaxis, i.e., a lack of an immediate contractile response (which occurred on first exposure to capsaicin) on subsequent doses of capsaicin. After exposure to capsaicin at two separate doses of 10^{-5} and 10^{-4} M for 30 min, the effect of NaHS was studied at two doses of 10^{-4} and 10^{-3} M. Thereafter, the effect of NaHS at two doses (10^{-4} and 10^{-3} M) was studied again after bethanechol (3×10^{-6} M) in the ongoing presence of capsaicin.

In two other muscle strips from six rats, we used the NO synthase inhibitor L-N^G-nitro arginine (L-NNA) at two separate doses of 10^{-4} and 10^{-3} M to investigate the effect of the NO pathway and/or any interaction with NO in mediating the effect of NaHS. L-NNA inhibits endogenous production of NO at these doses.[8] After exposure to L-NNA at two doses (10^{-4} and 10^{-3} M) for 30 min, the effect of NaHS was studied at two doses (10^{-4} and 10^{-3} M). Thereafter, the effect of NaHS was studied again at two doses (10^{-4} and 10^{-3} M) after bethanechol (3×10^{-6} M) in the ongoing presence of L-NNA.

In the last two muscle strips from six rats, we used glibenclamide at two doses of 10^{-4} and 10^{-3} M to investigate the involvement of K_{ATP}^+ channels in the effect of NaHS. Glibenclamide blocks the K_{ATP}^+ channel at these doses.[4] After exposure to glibenclamide at two doses of 10^{-4} and 10^{-3} M for 30 min, the effect of NaHS was studied at two doses (10^{-4} and 10^{-3} M). Thereafter, the effect of NaHS was studied again at two doses (10^{-4} and 10^{-3} M) after bethanechol (3×10^{-6} M) in the ongoing presence of glibenclamide.

In two different muscle strips from six rats, the effect of L-cysteine was studied at three doses of 10^{-4}, 10^{-3}, and 10^{-2} M with washout between each dose. Because L-cysteine is a substrate for endogenous enzymatic production of H_2S,[8] we hypothesized that L-cysteine would increase endogenous production of H_2S, and the H_2S released would have some effect on contractile activity. We did not study the effect of L-

cysteine after bethanechol. In these same two muscle strips, we used apamin to investigate the involvement of K_{Ca}^{+} channels in the response to NaHS because apamin blocks the K_{Ca}^{+} channels.[4] After exposure to apamin at two doses of 10^{-6} and 5×10^{-6} M for 30 min, the effect of NaHS was studied at two doses (10^{-4} and 10^{3} M). Thereafter, the effect of NaHS was studied again at two doses (10^{-4} and 10^{-3} M) after bethanechol (3×10^{-6} M) in the ongoing presence of apamin.

In six other muscle strips from six rats, the response to electrical field stimulation (EFS) was evaluated at 6 and 50 Hz using a constant voltage (20 V), pulse width (0.5 ms), and duration of stimulation (10 s) similar to our previous work.[7] We chose 6 Hz as an inhibitory EFS and 50 Hz as an excitatory EFS based on prior work.[7] All EFS studies were performed under NANC conditions with atropine (10^{-7} M), phentolamine (10^{-5} M), and propranolol (5×10^{-6} M) in the bath to exclude adrenergic and cholinergic effects induced by EFS. Between each EFS, 10 min were allowed for spontaneous contractile activity to recover before the next EFS was applied; the bath solution was changed after each series of stimulations. First, we evaluated the response of spontaneous contractile activity to EFS under NANC conditions in all six muscle strips as control conditions. After completing these control conditions, we evaluated EFS separately after inhibition of the endogenous H_2S-producing enzymes CBS and CSE, an inhibitor of NO synthase, and a competitive inhibitor of VIP. First, we used the CBS inhibitor aminooxyacetic acid (AOAA) at a dose of 10^{-4} M. After exposure to AOAA for 30 min in two muscle strips from six rats, the effect of AOAA on baseline contractile activity was evaluated for 15 min. Thereafter, the response to EFS was studied in the presence of AOAA. In two other muscle strips, we used the CSE inhibitor DL-propargylglycine (PPG) at a dose of 2×10^{-3} M.[16] After exposure to PPG for 30 min, the effect of PPG on baseline contractile activity was evaluated for 15 min. Thereafter, the response to EFS was studied in the presence of PPG. After washout of the bath solution, we evaluated the effect on baseline contractile activity of the combination of AOAA (10^{-4} M) and PPG (2×10^{-3} M) for 30 min and, thereafter, the response to EFS was evaluated in the presence of both AOAA and PPG.

In two other muscle strips from six rats, we evaluated the effects of inhibiting the dominant NANC inhibitory neurotransmitters NO and VIP in an attempt to reveal any more subtle effects of the release of endogenous H_2S by EFS. We used L-NNA at a dose of 10^{-3} M. After determining the response of baseline contractile activity to L-NNA exposure for 30 min, the response to EFS was studied in the presence of L-NNA. After washout of the bath solution, we evaluated the combination of L-NNA (10^{-3} M) and the VIP antagonist [D-p-Cl-Phe^{6},Leu^{17}]-VIP (10^{-6} M) for 30 min. Baseline contractile activity and the response to EFS were studied in the presence of L-NNA and the VIP antagonist. After washout of the bath solution, we then used the combination of all four inhibitors/antagonists, L-NNA (10^{-3} M), VIP antagonist (10^{-6} M), AOAA (10^{-4} M), and PPG (2×10^{-3} M). After exposure for 30 min, the effect of these four inhibitors on baseline contractile activity and EFS was evaluated.

Data Analysis

Phasic changes in force (total contractile activity) measured as area under the contractile curve (AUC) were analyzed by a data acquisition system (AcqKnowledge, Biopac Systems, Inc., Goleta, GA, USA). We set the baseline tone before each intervention as zero when we calculated the AUC, which enabled us to analyze the phasic contractile activity. In addition to the measurements of AUC, we also measured and analyzed changes of mean amplitude, baseline tone, and frequency under each condition. Thereafter, the effects of each of the drugs NaHS, L-cysteine, NANC conditions, TTX, capsaicin, L-NNA, glibenclamide, and apamin on spontaneous activity were measured for 5 min and compared to the baseline contractile activity for 5 min measured immediately before each drug was administered. This technique allowed us to control for any effects on baseline contractile activity by any of the drugs tested. In contrast, the effect of administration of AOAA and/or PPG and L-NNA alone or in combination with the VIP antagonist on spontaneous contractile activity was measured for 15 min after exposure of the muscle to these agents for 30 min and was compared to the 5 min immediately before administration of the antagonists. When muscle strip contractile activity was enhanced with bethanechol, the subsequent response to NaHS was measured for 5 min and compared to the 90 s of pre-contraction immediately before administration of NaHS and adjusted for a 5-min interval. For the dose responses to NaHS, the responses after pre-contraction, and the responses to the various inhibitors/antagonists, the mean value of individual muscle strips per rat were meaned, and the mean responses across the six rats were calculated. Drug responses are given as% change from baseline contractile activity (defined as 0%), with positive values representing an increase and negative values a decrease in contractile activity.

The response to EFS was studied for the 10 s of EFS in all experiments; the "off contraction" that occurred immediately after termination of EFS was not evaluated. According to the findings from our previous studies, we used 6 Hz as an inhibitory frequency, and 50 Hz as a non-inhibitory EFS frequency.[7,10] Because our previous work suggested differences in the first 4 s and the last 6 s of the total 10 s of EFS, we analyzed separately the effects of EFS for the whole 10 s, the first 4 s, and the last 6 s.[8,17–19] Contractile activity was expressed as the percent of baseline contractile activity for an equally long interval (4, 6, or 10 s) measured during the 20 s immediately before EFS.

All data are expressed as mean±SEM. Analysis of variance was used to analyze the effects of a dose–response

to NaHS, while paired student's *t* tests were used to compare the effects of different drugs and EFS; when individual comparisons were made, we used the conservative Bonferroni correction to correct for the multiple comparisons. In addition, we also used Wilcoxon rank sums when the values were not distributed normally.

Drugs

Apamin, AOAA, atropine sulfate, bethanechol chloride, capsaicin, L-cysteine, glibenclamide, L-NNA, phentolamine hydrochloride, PPG, DL-propranolol hydrochloride, NaHS, TTX, [D-p-Cl-Phe^{6},Leu^{17}]-VIP were purchased from Sigma-Aldrich, St. Louis, MO, USA. For the stock solution, capsaicin and glibenclamide were dissolved in dimethylsulfoxide (Sigma-Aldrich, St. Louis, MO, USA). L-NNA and DL-propranolol hydrochloride are dissolved in 0.5 N hydrochloric acid (HCl), and 0.1 N HCl was used for further dilutions to 10^{-4} M of L-NNA. Preliminary experiments showed that 0.5 N HCl and dimethylsulfoxide had no effect on spontaneous contractile activity or pH of the bath solution. All other drugs were dissolved in purified water.

Results

Response to NaHS (Exogenous Donor of H_2S)

NaHS at all doses inhibited spontaneous basal activity in a dose-dependent manner ($p<0.05$; Figs. 1a and 2a). NaHS at

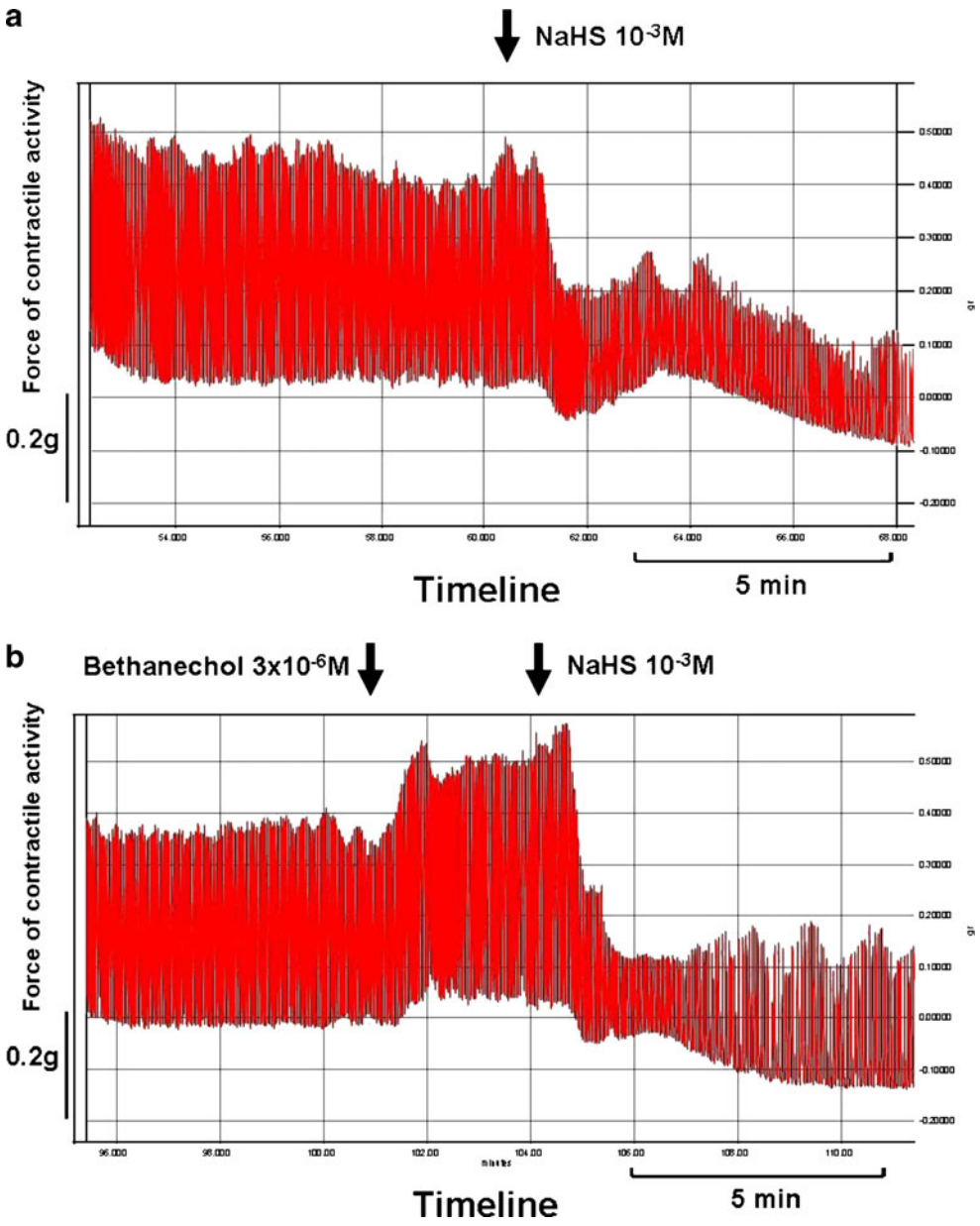

Fig. 1 Effects of NaHS on **a** spontaneous contractile activity. NaHS at 10^{-3} M inhibited contractile activity by decreasing amplitude, baseline tone, and frequency, and **b** after precontraction with bethanechol (3×10^{-6} M). Bethanechol increased amplitude and baseline tone. NaHS at 10^{-3} M inhibited contractile activity after bethanechol by decreasing amplitude, baseline tone, and frequency

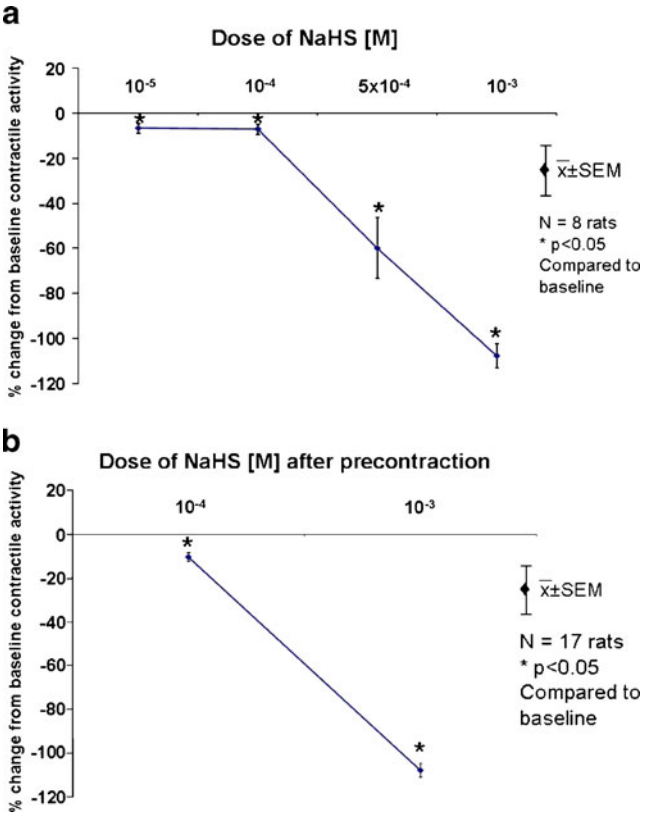

Fig. 2 Effect of NaHS on spontaneous and bethanechol-stimulated contractile activity. **a** Spontaneous contractile activity measured by area under the contractile curve for 5 min was defined as 0%; therefore, negative values represent inhibitory effects on contractile activity. NaHS inhibited spontaneous contractile activity in a dose-dependent manner; *$p<0.05$ (after Bonferroni correction) compared to spontaneous contractile activity. **b** The area under the contractile curve for 5 min of the bethanechol-stimulated pre-contracted condition was defined as 0%. NaHS at both doses inhibited contractile activity after precontraction with bethanechol; *$p<0.05$ (after Bonferroni correction) compared to baseline contractile activity

the doses of 10^{-4} and 10^{-3} M also inhibited the contractile activity enhanced by bethanechol (3×10^{-6} M; $p<0.05$) to the same percentage as for spontaneous activity (Figs. 1b and 2b). These effects of NaHS on spontaneous (Table 1) and enhanced contractile activity (Table 2) occurred by inhibiting total contractile activity (area under the contractile curve) but also by decreasing amplitude, baseline tone, and frequency of contractions (at greater dose of 5×10^{-4} M on spontaneous contraction).

Table 2 Effect of NaHS after precontraction with bethanechol (percent change from baseline contractile activity; mean ± SEM; $n=17$ rats)

	10^{-4}M	10^{-3}M
Total contractile activity (AUC[a])	−10±2*	−108±3*
Average amplitude	−6±1*	−53±2*
Baseline tone	−21±3*	−65±3*
Frequency	−5±1*	−37±1*

[a] Area under the contractile curve

*$p<0.05$ compared to baseline contractile activity defined as 0% (ANOVA)

To investigate the involvement of neural pathways in the response to NaHS, NaHS at the doses of 10^{-4} and 10^{-3} M was used under NANC conditions and after pretreatment with TTX. There were no significant changes in the inhibitory effects of NaHS on total contractile activity under NANC conditions or after pretreatment with TTX (Table 3). The presence of TTX decreased the inhibitory effect of NaHS on the frequency of contractions in bethanechol-treated tissues, but had no effect on total contractile activity, average amplitude, or baseline tone (Table 4).

To investigate the involvement of primary afferent nerve fibers in the response to NaHS, primary afferent nerve fibers were defunctionalized with capsaicin (10^{-5} and 10^{-4} M) as described previously.[11–15] The lesser dose of capsaicin caused a short-lasting excitatory response immediately after administration, but when evaluated 30 min later, contractile activity had returned to baseline levels, and capsaicin had no persistent effect on spontaneous contractile activity for the next 5 min baseline interval. In contrast, the greater dose of capsaicin decreased spontaneous contractile activity that persisted for the duration of the NaHS experiments (at least 45 min); thereafter, when the tissue chamber was washed, spontaneous contractile activity returned. There was no change in the inhibitory effect of NaHS on total contractile activity after desensitization of

Table 1 Effect of NaHS on spontaneous contraction (percent change from baseline contractile activity; mean ± SEM; $n=8$ rats)

	10^{-5}M	10^{-4}M	5×10^{-4}M	10^{-3}M
Total contractile activity (AUC[a])	−7±2*	−7±2*	−60±13*	−108±5*
Average amplitude	−3±1*	−3±1*	−20±5*	−35±3*
Baseline tone	−6±1*	−5±2*	−32±6*	−46±5*
Frequency	1±1	0±0	−17±3*	−38±2*

[a] Area under the contractile curve

*$p<0.05$ compared to baseline contractile activity defined as 0% (ANOVA)

Table 3 Effect of NaHS (10^{-3} M) on spontaneous contraction in the presence of specific inhibitors (percent change from baseline contractile activity; mean ± SEM; n=6 rats)

	Without inhibitor	NANC	TTX	Capsaicin		L-NNA		Glibenclamide		Apamin	
			(10^{-6}M)	10^{-5}M	10^{-4}M	10^{-4}M	10^{-3}M	10^{-4}M	10^{-3}M	10^{-6}M	5×10^{-6}M
Total contractile activity (AUC[a])	−108±5	−124±13	−128±13	−122±8	−183±24	−147±19	−125±14	−140±21	−158±18	−122±14	−135±12
Average amplitude	−35±3	−45±4	−47±4	−41±4	−33±2	−48±4	−49±5	−31±3	−40±4	−47±4	−46±5
Baseline tone	−46±5	−51±7	−53±8	−40±5	−28±4	−52±8	−52±7	−32±6	−36±10	−49±6	−51±9
Frequency	−38±2	−30±2	−32±2	−42±4	−38±7	−34±2	−33±3	−36±4	−39±3	−37±2	−24±2

[a] Area under the curve

primary afferent nerves with capsaicin on total contractile activity either during spontaneous contractile activity (Table 3) or after bethanechol (3×10^{-6} M) (Table 4).

To investigate any interaction between H_2S and the release of NO, L-NNA was used to block NO production. At the doses of 10^{-4} and 10^{-3} M of NaHS, there was no change in the inhibitory effect of NaHS on total contractile activity after pretreatment with L-NNA on either spontaneous contractile activity (Table 3) or after bethanechol (3×10^{-6} M; Table 4).

Finally, to investigate the involvement of K_{ATP}^{+} and K_{Ca}^{+} channels in the response to NaHS, glibenclamide and apamin were used. Glibenclamide alone at both doses inhibited spontaneous contractile activity ($p<0.05$); however, the inhibitory effects of NaHS on total contractile activity either during spontaneous bethanechol-enhanced contractile activity were unchanged (Tables 3 and 4). Apamin had no effects on baseline spontaneous activity, and pretreatment with apamin also had no effect on the inhibitory effects of NaHS either on total contractile activity, average amplitude, or basal tone either during spontaneous or enhanced activity (Tables 3 and 4). The greater concentration of apamin did, however, significantly decrease the inhibitory effect of NaHS on the frequency of contractions in bethanechol-enhanced tissues (Table 4) but not on baseline spontaneous activity (Table 3).

Effect of Endogenous Substrate of H_2S

Administration of L-cysteine did not alter spontaneous contractile activity at any of the doses evaluated (10^{-4}, 10^{-3} and 10^{-2} M; data not shown). We did not evaluate the effect of L-cysteine on bethanechol-enhanced activity.

Response to EFS

EFS at 6 Hz did not alter total contractile activity (AUC) for the entire 10 s in NANC conditions but did inhibit total contractile activity during the first 4 s of EFS. In the presence of all of the inhibitors we evaluated (PPG, PPG and AOAA, L-NNA, L-NNA and VIP_{antag}, and all four), there was no significant difference in the effect of EFS compared to control conditions (NANC conditions) for the entire 10 s, first 4 s, or last 6 s (Fig. 3a) except for an augmentation of inhibition with AOAA alone at each time duration tested. This effect of AOAA was not seen when AOAA was combined with PPG; when mean amplitude, baseline tone, and frequency were analyzed, no changes were noted, similar to total contractile activity (data not shown).

EFS at 50 Hz increased total contractile activity for the entire 10 s and for the last 6 s in NANC conditions ($p<0.05$) and caused an inhibition of total contractile activity during the first 4 s of EFS ($p<0.05$). In the presence of

Table 4 Effect of NaHS (10^{-3} M) after precontraction with bethanechol in the presence and absence of specific inhibitors

	Without inhibitor	TTX	Capsaicin		L-NNA		Glibenclamide		Apamin	
		(10^{-6}M)	10^{-5}M	10^{-4}M	10^{-4}M	10^{-3}M	10^{-4}M	10^{-3}M	10^{-6}M	5×10^{-6}M
Total contractile activity (AUC[a])	−108±3	−126±15	−109±3	−144±20	−112±9	−100±7	−130±23	−210±48	−121±9	−111±9
Average amplitude	−53±2	−50±2	−55±3	−40±4	−56±6	−54±5	−46±5	−56±3	−56±4	−53±6
Baseline tone	−65±3	−64±6	−58±3	−45±7	−65±7	−59±7	−49±4	−53±8	−66±6	−60±10
Frequency	−37±1	−24±2*	−33±2	−35±3	−30±2	−29±2	−27±1	−33±6	−27±1	−20±1*

[a] Area under the contractile curve

*$p<0.05$ compared to control response (without inhibitor; ANOVA)

these same inhibitors we used, there were no significant differences in the effects of EFS compared to control conditions (NANC conditions) for the entire 10 s, first 4 s, or last 6 s (Fig. 3b). When mean amplitude, baseline tone, and frequency were analyzed, no changes were noted, similar to the lack of effect on total contractile activity (data not shown).

Discussion

The aim of our study was to determine the effects and mechanisms of action of exogenous and endogenously released H_2S on contractile activity in the longitudinal muscle of the ileum in rats. We studied ileal longitudinal muscle as part of our ongoing, comprehensive approach to characterizing inhibitory neurotransmitters in the small intestine.[8,17–19] By using several targeted inhibitors which block different potential pathways of signal transduction, we showed that H_2S inhibited reversibly the spontaneous and cholinergically stimulated contractile activity in rat ileal longitudinal muscle. This effect was not mediated via the enteric nervous system, primary visceral afferent nerve fibers, production of NO, K_{ATP}^+ channels, or K_{Ca}^+ channels. Our experiments with EFS attempted to uncover an inhibitory effect of endogenously released H_2S from intrinsic nerves. Although we were unable to show a convincing effect of inhibiting CSE, preventing the release of NO, or antagonizing VIP, we did demonstrate an augmentation of the initial inhibitory effect of EFS at 6 Hz by inhibiting CBS.

To the best of our knowledge, there are few reports that describe the effects of H_2S on contractile activity in the longitudinal smooth muscle of ileum in any species. Several reports have shown that exogenous H_2S manifests an inhibitory effect on spontaneous contractile activity in a dose-dependent manner in the ileum of rabbit,[5] as well as in pre-contracted ileal circular muscle of guinea pig.[6] We also demonstrated a dose-dependent, reversible inhibitory effect of NaHS on the presence of spontaneous and stimulated contractile activity as well as on mean amplitude, baseline tone, and frequency in the longitudinal muscle of rat ileum. Our data are in large part compatible with the limited data reported previously, although our experiments more comprehensively evaluate the potential mechanisms of action of H_2S.

We used10^{-5} M to 10^{-3} M NaHS as an exogenous donor of H_2S; 18.5% of NaHS in solution exists as H_2S. This approach led to estimated concentrations of H_2S of 1.85–185 μM. The concentrations of NaHS we used are similar to those in other reports[4–6] that also cause apparent physiologic effects, which suggests that responses to micrometer concentrations reproduce physiologic responses. This type of experiment tries to reproduce local concentrations of a "gasotransmitter" near the site of action rather than a general tissue concentration; this approach is similar to the approach used for neurotransmitters. Indeed, the local concentrations of H_2S may be greater than the general tissue or serum concentrations, because H_2S is released locally; local effects on synthesis and metabolism of H_2S may be very different near the site of release and the site of action.[20] There was no evidence that these concentrations caused any tissue toxicity, because the recovery of contractile activity after washout of the bath solution was rapid and complete even after repeated applications of the greatest concentration of NaHS (10^{-3} M).

The ENS plays an important role in modulating gastrointestinal motility. Much of our previous work and that of many others have focused on the ENS as the site of release of inhibitory neurotransmitters. We explored whether H_2S might induce the release of inhibitory neurotransmitters from intramural neurons. We showed that blocking NANC nerves selectively or blocking neural depolarization with TTX (and thus release of presynaptic vesicles non-selectively) were unable to prevent the inhibitory effect of H_2S on total contractile activity; however, TTX did block partially the inhibitory effect of NaHS on contractile frequency during bethanechol-enhanced contractile activity. These grouped

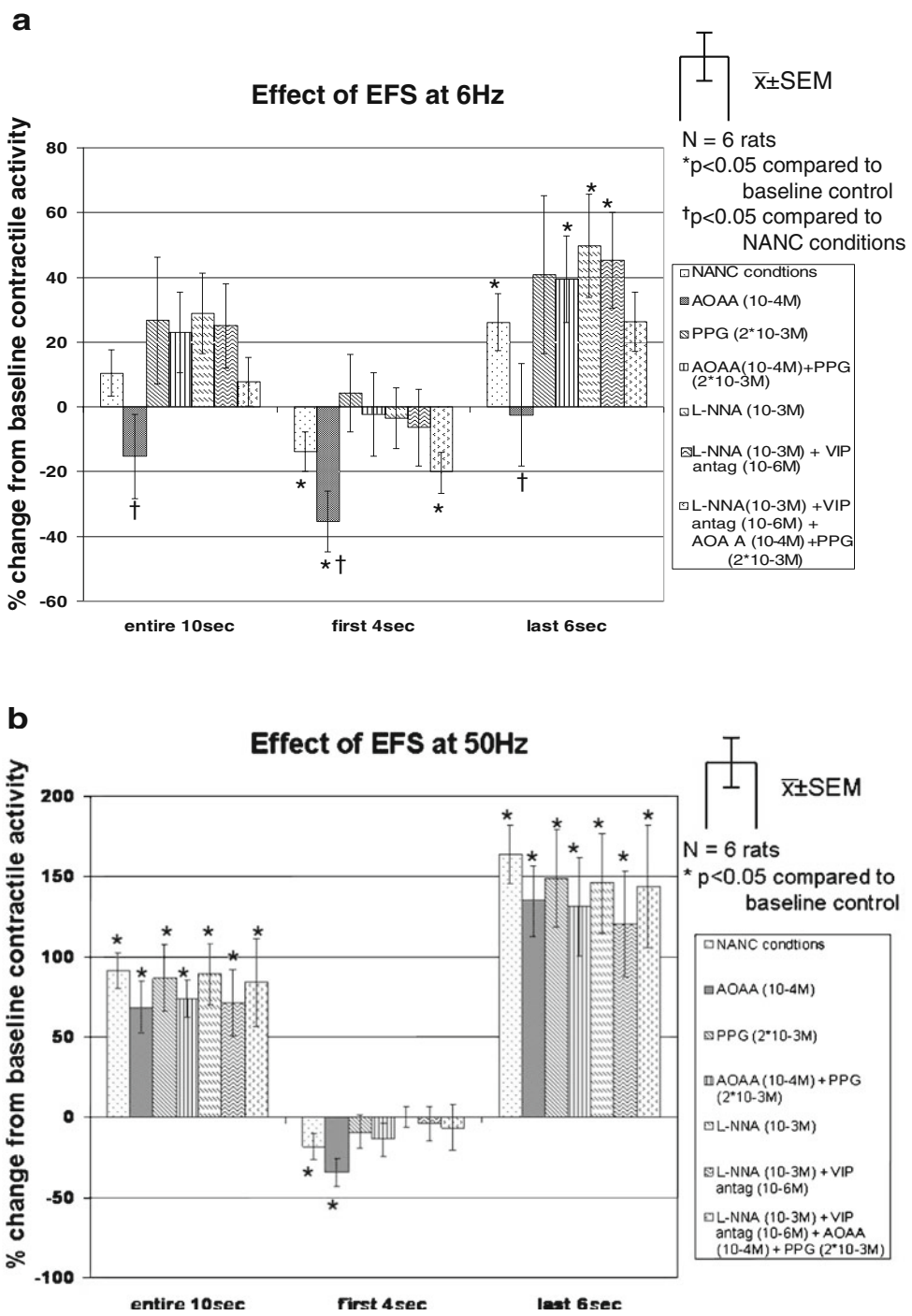

Fig. 3 Effect of EFS at **a** 6 Hz and at **b** 50 Hz for the entire 10 s, first 4 s, and last 6 s of EFS in the presence of inhibitors. The area under the contractile curve immediately before EFS was defined as 0%; positive values represent a stimulatory effect on contractile activity, while negative values represent an inhibitory effect. In the presence of these inhibitors, there was no significant difference of the effect of EFS compared to baseline control condition for the entire 10 s, first 4 s, or last 6 s in total contractile activity except for an augmentation of inhibition at 6 Hz by AOAA

observations suggest that the inhibitory effect of H_2S is not mediated primarily through modulation of enteric neural activity or by pathways stimulating enteric nerves, although H_sS may alter the increased frequency of contractions in response to a cholinergic agonist via a neural pathway.

A recent report has shown that H_2S acts to increase mucosal secretion via stimulating TRPV-1 receptors on primary afferent nerve fibers.[11] In the present study, there was no change of the inhibitory effect of H_2S on contractile activity after pretreatment of capsaicin. This observation suggests that the inhibitory effect of H_2S on contractile activity is not mediated via activation of primary visceral afferent nerve fibers; we cannot, however, exclude the possibility that the decrease in baseline spontaneous contractile activity after exposure to the greater dose (10^{-4} M) capsaicin might have impacted some of the inhibitory effect of H_2S.

The interaction of NO and H_2S in vascular smooth muscle has been well investigated.[21] In vascular smooth muscle,

exposure to NO increases the expression and activity of CSE,[22] while decreasing the expression of NO synthase.[23] In gastrointestinal (GI) smooth muscle, few reports have explored any interaction between NO and H_2S. In rat colon, the effect of H_2S is dependent on NO production,[24] but in guinea pig ileum, inhibition of NO production had no effect on the response to exogenous H_2S.[5] We also demonstrated that the inhibitory effect of NaHS in ileal longitudinal muscle of rat was not itself mediated via an NO pathway by using the inhibitor of NO synthase, L-NNA; these findings are compatible with the previous report.[5] We did not, however, explore any interaction between NO and H_2S using an exogenous NO donor, so we cannot exclude potential interactions of H_2S on NO released independently by a non-H_2S-mediated effect.

Almost all the primary effects of action of H_2S in vascular smooth muscle are mediated by K_{ATP}^+ channels, the opening of which induces hyperpolarization of the cell, closing of voltage-gated calcium channels, and muscular relaxation.[22] In GI smooth muscle, the importance of K_{ATP}^+ channels is more controversial and may vary with anatomic location. K_{ATP}^+ channels play an important role in the effect of H_2S in rat colon[4,24]; however, in guinea pig ileum, blocking of K_{ATP}^+ channels had no effect on the inhibitory effects of H_2S.[5] In our study, we show clearly using glibenclamide pretreatment that the inhibitory effect of H_2S in rat ileal longitudinal muscle was not mediated via K_{ATP}^+ channels. We did show, however, that baseline spontaneous contractile activity after exposure to glibenclamide was decreased, implicating K_{ATP}^+ channels in the modulation of spontaneous contractile activity.

We also explored the role of K_{Ca}^+ channels. In rat colon, Gallego et al.[4] reported that K_{Ca}^+ channels play an important role in mediating the effect of H_2S.[4,24] In contrast, Dhaese et al.[25] reported that the inhibitory effect of H_2S on contractile activity in rat colon was independent of K_{Ca}^+ channels.[25] Gallego et al. studied transmural segments of rat colon, while Dhaese et al. evaluated muscle strips. Our results studying transmural strips of longitudinal muscle showed that the inhibitory effect of H_2S in rat ileum was not mediated in great part via small conductance, K_{Ca}^+ channels as blocked by apamin. Apamin did, however, decrease the inhibitory effect of NaHS on contractile frequency during bethanechol-enhanced contractile activity, similar to TTX. Because K_{Ca}^{+2} channels are involved in the release of neurotransmitters, it is possible that NaHS may have a minor effect on neurotransmitter release. We cannot exclude the possibility that intermediate or large conductance K_{Ca}^+ channels may play an important role in the inhibitory effect of H_2S in ileal longitudinal muscle of rat.

Our last two experimental conditions were designed to try to release H_2S endogenously. Endogenous H_2S is believed to be produced from L-cysteine by CBS and CSE. Although both enzymes have been demonstrated in exist in rat ileum,[6] and we have shown previously that both enzymes can be imaged in rat small intestinal enteric nerves by immunohistochemistry (Kasparek et al., under review), we were not able to demonstrate any inhibitory effect on contractile activity when we exposed the muscle strips to L-cysteine, the presumed substrate for CBS and CSE. Linden et al.[16] reported that H_2S can be produced endogenously by mouse colonic muscle harvested carefully without exposure to the mucosal surface.

We tried to evaluate the role of intrinsic neurons in producing and releasing H_2S endogenously by means of delivering EFS at different frequencies. We used a low frequency (6 Hz) to investigate a relative inhibitory stimulus and a greater frequency (50 Hz) known to induce a net contractile effect. Although we used inhibitors or antagonists of known inhibitory neurotransmitters to block potential pathways which may be associated with the action of H_2S, there were no consistent differences in the contractile activity as measured by area under the contractile curve, amplitude, and baseline tone, between control and other conditions with inhibitors under NANC conditions. Although Teague et al.[5] reported that PPG caused an increase of contractile activity during EFS in guinea pig ileum, our results in rat ileal longitudinal muscle failed clearly to show that PPG increased significantly the contractile activity of ileal longitudinal muscle. Our different results from the study of Teague et al. may be related to the different species (rat vs guinea pig) or the experimental conditions of EFS, such as frequency, voltage, and/or exposure time to EFS. In contrast, our findings with AOAA were of interest, because the inhibitory effect of the first 4 s of EFS at 6 Hz was potentiated by AOAA. This effect suggests that AOAA, either by its likely inhibition of H_2S release or by its inhibition of another opposing transulfuration metabolic pathway, may somehow alter the release or inhibitory effects of another inhibitory neurotransmitter. Our experiments cannot further elucidate this question. From these results, we conclude that endogenous production and/or release of H_2S may be regulated or mediated by activation of intrinsic (enteric) neurons under the conditions of our EFS experiments. The amount of H_2S released during EFS, however, may have been too low, such that any major effects of H_2S were not detectable in our experiments.

In conclusion, we have demonstrated that H_2S in physiologic concentrations appears to be an inhibitory gasotransmitter in the ileal longitudinal muscle of rat. The inhibitory effect of H_2S did not appear to be mediated by the extrinsic or enteric nervous systems, primary visceral afferent nerve fibers, NO pathways, or K_{ATP}^+ or K_{Ca}^+ channels. This work suggests that the inhibitory effect of H_2S on smooth muscle contractile activity in the longitudinal muscle of rat ileum appears to involve other undetermined pathways. Possible

mechanisms by which H_2S may inhibit small intestinal contractile activity include the possibility of biochemical sulfhydration of cysteine residues of membrane or intracellular proteins or by effects on heme in enzymes such as guanylyl cyclase to augment cGMP.[26,27]

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SSAT POSTER PRESENTATION

The Impact of Scoliosis Among Patients with Giant Paraesophageal Hernia

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Abstract
Background Kyphoscoliosis is seen in approximately 1.4–15% of the octogenarian population of the US. We hypothesized that patients with kyphoscoliosis are affected with a reduced intra-abdominal volume and progressive laxity of the diaphragmatic hiatal sling musculature leading to an increased risk of hiatal hernia formation and progression over time.
Methods We retrospectively reviewed the clinical history and roentgenographic data of 320 paraesophageal hernia patients from 2003 to 2007. The prevalence of kyphoscoliosis among this patient cohort and the outcomes of surgical management were compared to paraesophageal hernia patients without kyphoscoliosis.
Results Ninety-three of the 320 patients (29.1%) were found to have significant K/S (mean age 74; 83% female). Laparoscopic repair of paraesophageal hernia with fundoplication was performed in 91% of these patients. There was one death (1.1%; aspiration pneumonia) and 17.2% major postoperative morbidity. Mean length of hospital stay was 8 days (median=4; range 2–71). Prolonged stays were related mainly to marginal pulmonary status. Kyphoscoliosis was associated with increased peri-operative pulmonary morbidity (16.1%) compared to patients without kyphoscoliosis (7.0%, $p=0.02$).
Conclusion Kyphoscoliosis may contribute to the development and progression of paraesophageal hernias. Surgeons approaching paraesophageal hernia repair should be aware of the increased pulmonary morbidity and the postoperative care required in managing these patients.

Keywords Scoliosis · Paraesophageal hernia · Fundoplication

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Introduction

Kyphoscoliosis is a frequently encountered condition involving curvature of the thoracic and/or lumbar spine that can develop during adolescence or adulthood. More common in women, the prevalence of scoliosis increases with age, ranging from 2% of the adolescent population [1] to as high as 15% among the elderly.[2] Untreated, significant kyphoscoliosis can be associated with increased mortality secondary to the development of impaired respiratory mechanics and the sequelae of chronic back pain.[3]

Interestingly, a clinical association between kyphoscoliosis and giant paraesophageal hernia formation has been noted historically by several authors.[4,5] To date, however, there is little published data available to further characterize this relationship. In the current study, the prevalence of kyphoscoliosis in patients undergoing repair of giant para-

esophageal hernia is evaluated, and the clinical impact of this condition is compared to those patients undergoing hernia repair without kyphoscoliosis.

Materials and Methods

Patients and Preoperative Evaluation

Approval for this study was provided by the Institutional Review Board of the University of Pittsburgh. We performed a retrospective review of 320 patients undergoing paraesophageal hernia repair at the University of Pittsburgh from 2003 to 2007. Patient chest radiographs (CXR) obtained perioperatively were evaluated for the presence of scoliosis—defined by a Ferguson Angle of ≥10°.[6] All films were reviewed by the investigators, and the diagnosis was independently confirmed by a radiologist. The diagnosis of paraesophageal hernia was confirmed preoperatively by barium swallow or CT scan.

Perioperative Course

Giant paraesophageal hernia repair was performed as described previously.[7–9] Generally, the authors prefer to perform a Nissen Fundoplication (JDL) or modified Toupet fundoplication (RJL) in this setting.[9,10] Other surgical procedures or adjuncts (e.g., Collis gastroplasty, gastropexy, cruroplasty) were performed at the discretion of the operating surgeon based upon surgeon preference and judgment, as well as individual clinical and anatomic patient characteristics. Patients were typically extubated on the day of surgery. Patients were mobilized out of bed into a chair following extubation. The patient's pulmonary hygiene was encouraged and ambulation initiated on the first postoperative day. A barium swallow was typically performed on the first postoperative day and, if satisfactory, a clear liquid diet was instituted. After 3 days of clear liquids, the patient's diet was advanced to full liquids for an additional 3 days, then to a post-Nissen soft mechanical diet. Patients were typically discharged by the 4th postoperative day. Perioperative endpoints analyzed in the current study include length of stay, morbidity, and mortality.

Statistical Analysis

Statistical comparisons were analyzed for significance utilizing t tests and Fisher's exact test. Results were considered significant at a p value<0.05.

Results

Ninety-three (29.1%) of the 320 patients undergoing giant paraesophageal hernia repair were found to have significant scoliosis (mean age=74; 83% female). Demographics and operative data comparing patients with and without scoliosis are detailed in Table 1. Patients with scoliosis were significantly older compared to those without scoliosis (75 vs. 67; $p<0.0001$), were more commonly female ($p=0.012$), and had a higher prevalence of diabetes mellitus (11.8% vs. 4.4%, $p=0.02$). There were no significant differences in operative approach or fundoplication type. The use of fundoplication adjuncts (e.g., Collis gastroplasty, cruroplasty, or gastropexy) was more commonly required in patients with scoliosis (68.8% vs. 55.9%, $p=0.034$) (Table 2).

There were two conversions among patients with scoliosis (bleeding, poor exposure).There was one perioperative death due to aspiration pneumonia (1.1%) in the scoliotic group, compared with four deaths in patients

Table 1 Patient demographics and co-morbid conditions

	Patients with scoliosis (n=93)	Patients without scoliosis (n=227)	P value
Median age (Range)	75 (18–89)	67 (32–89)	<0.0001
Gender	16 M, 77 F	71 M, 156 F	0.012
Co-morbidities			
COPD	22 (23.7%)	57 (25.1%)	0.89
Diabetes mellitus	11 (11.8%)	10 (4.4%)	0.02
Coronary artery disease	12 (12.9%)	29 (12.8%)	1.00
Congestive heart failure	5 (5.4%)	6 (2.6%)	0.31
Peripheral vascular disease	1 (1.1%)	3 (1.3%)	1.00
Peptic ulcer disease/GERD	19 (20.4%)	49 (21.6%)	0.88
Dementia	4 (4.3%)	8 (3.5%)	0.75
Renal insufficiency	2 (2.2%)	2 (0.9%)	0.33
Prior cancer history	4 (4.3%)	11 (4.8%)	1.00

Table 2 Operative data

	Patients with scoliosis (n=93)	Patients without scoliosis (n=227)	P value
Approach			0.114
Laparoscopic	87	221	
Open	6	6	
Fundoplication			
Nissen	63	142	0.442
Toupet	18	68	0.053
Dor	4	6	0.484
None	8	11	0.201
Adjuncts			
Collis Gastroplasty	53	111	0.218
Cruroplasty	5	24	0.197
Gastropexy	8	9	0.104
None	29	100	0.034

without scoliosis (1.8%, p=1.00). There was no significant difference in length of stay or overall morbidity between the two groups (Table 3). The overall complication rate was 30.1% in patients with scoliosis. Complications are detailed in Table 4. Importantly, patients with scoliosis were noted to have increased perioperative pulmonary morbidity (16.1% vs. 7.0%, p=0.02), with a significantly higher rate of postoperative respiratory failure [9.7% vs. 3.1%, p=0.02] compared to those patients without scoliosis. Deep vein thrombosis (4.9% vs. 0.4%, p=0.02) and acute hernia recurrence rates (3.2% vs. 0%, p=0.009) were also more common in patients with scoliosis. When considering all cases, complications were associated with increased patient age (72.9 vs. 66.4, p=0.00001) and female gender (28.3% vs. 13.8%, p=0.006). Complication rates were not found to correlate with operation type, approach, or the use of adjuncts (Table 4).

At a mean follow-up of 36.7 months, there were 29 (9.3%) documented radiographic hernia recurrences. Median time to recurrence was 17.9 months in patients without scoliosis, and 13.2 months in patients with scoliosis. There was no significant difference seen in freedom from recurrent hernia between groups. Reoperation was required in 16 (5%) patients (one, scoliosis; 15, no scoliosis; p=0.047), suggesting that scoliosis is not associated with increased recurrence risk.

Discussion

The association of hiatal hernia with kyphosis and scoliosis was first described by Comte in 1953.[4] Galvala and Matejcic and their associates were among the first to suggest that kyphoscoliosis may represent a causative factor in the development of hiatal hernias.[11,12] Axial deviation of the spinal canal at the level of the hiatus has been proposed to lead to distortion of the hiatal sling mechanism, thus promoting reflux and hiatal herniation (Fig. 1).[13–16] Other contributing factors include decreased intra-abdominal volume and increased intra-abdominal pressures seen in patients with kyphoscoliosis.[5,17,18]

In adults, scoliosis can be degenerative or idiopathic in nature.[1] The prevalence of scoliosis in the adult population ranges from 1.4% to 15% in the published literature[19–21] and increases with age.[2] Interestingly, 29.1% patients undergoing repair of a giant paraesophageal hernia in the current study were found to have scoliosis—a rate much higher than that seen in the general population. In our study, the association of paraesophageal hernia and kyphoscoliosis was much more common in women (p=0.012). This correlation has been noted previously, where size of hiatal hernia was found to correlate with degree of scoliosis among women, but not men.[22] In women with paraesophageal hernias the severity of scoliosis also correlated with patient age.[22] Indeed, in the current analysis, patients with scoliosis were significantly older (on average) compared to those patients without scoliosis (p<0.0001).

Little data currently exists regarding the clinical impact of kyphoscoliosis in the management of patients with hiatal hernia. Kyphoscoliosis is associated with restrictive impairment of pulmonary function,[23–26] chronic pain[27,28] and decreased vitality.[3,29] Curvature of the thoracic spine is specifically associated with decreased FEV1, FVC and pO2, and worsens with increasing curve magnitude.[30] The development of pulmonary hypertension and cor pulmonale has also been loosely associated with long-standing scoliosis.[31] In the current study, there was a statistically increased risk of pulmonary morbidity in patients with scoliosis undergoing repair of giant paraesophageal hernia (p=0.003). The most common complication type in patients with scoliosis was respiratory failure [9.7% vs. 3.2%, p=0.02] and pneumonia (4.3% vs. 2.3%, p=0.46), compared to patients without scoliosis.

Patients with paraesophageal hernias are frequently deemed poor operative candidates secondary to increased

Table 3 Postoperative outcomes

	Patients with scoliosis (n=93)	Patients without scoliosis (n=227)	P value
Conversions	2 (2.2%)	3 (1.3%)	0.630
Length of stay	4 (2–71)	4 (1–57)	0.237
Overall morbidity	28 (30.1%)	49 (21.6%)	0.115
Major morbidity	16 (17.2%)	26 (11.5%)	0.201
Mortality	1 (1.1%)	4 (1.8%)	1.000

Table 4 Perioperative complications in patients with scoliosis

Complication type	Patients with scoliosis (n=93)	Patients without scoliosis (n=227)	P value
Cardiac	2 (2.2%)	11 (4.8%)	0.36
Atrial fibrillation	1 (1.1%)	8 (3.5%)	0.46
Supraventricular tachycardia	0 (0%)	1 (0.4%)	1.00
Ventricular tachycardia	0 (0%)	1 (0.4%)	1.00
Cardiac arrest	1 (1.1%)	1 (0.4%)	0.50
Pulmonary	15 (16.1%)	16 (7.0%)	0.02
Respiratory failure	9 (7.1%)	7 (3.1%)	0.02
Tracheostomy	5 (4.9%)	1 (0.4%)	0.009
Pneumonia	4 (4.3%)	6 (2.6%)	0.48
Pneumothorax	1 (1.1%)	3 (1.3%)	1.00
Bronchoscopy	1 (1.1%)	0 (0%)	0.29
Empyema	1 (1.1%)	1 (0.4%)	0.50
Infections	9 (9.7%)	8 (3.5%)	0.05
Pneumonia	4 (4.3%)	5 (2.2%)	0.29
Leak/Abscess	3 (3.2%)	3 (1.3%)	0.36
Empyema	1 (1.1%)	1(0.4%)	0.50
Clostridium difficile	1 (1.1%)	3 (1.3%)	1.00
Wound infection	1 (1.1%)	1 (0.4%)	0.50
Other	15 (16.1%)	16 (7.0%)	0.02
Deep vein thrombosis	5 (4.9%)	1 (0.4%)	0.009
Acute hernia recurrence	3 (3.2%)	0 (0%)	0.02
Conversion	2 (2.2%)	1 (0.4%)	0.20
Ileus	2 (2.2%)	0 (0%)	0.08
Pulmonary embolism	1 (1.1%)	3 (1.3%)	1.00
Seizure	1 (1.1%)	1 (0.4%)	0.50
Renal insufficiency	1 (1.1%)	1 (0.4%)	0.50
Gastric distension—EGD	1 (1.1%)	2 (0.9%)	1.00
Hemorrhage—Re-Op	0 (0%)	4 (1.8%)	0.33
Encephalopathy	0 (0%)	2 (0.9%)	1.00
Myotomy	0 (0%)	1 (0.4%)	1.00

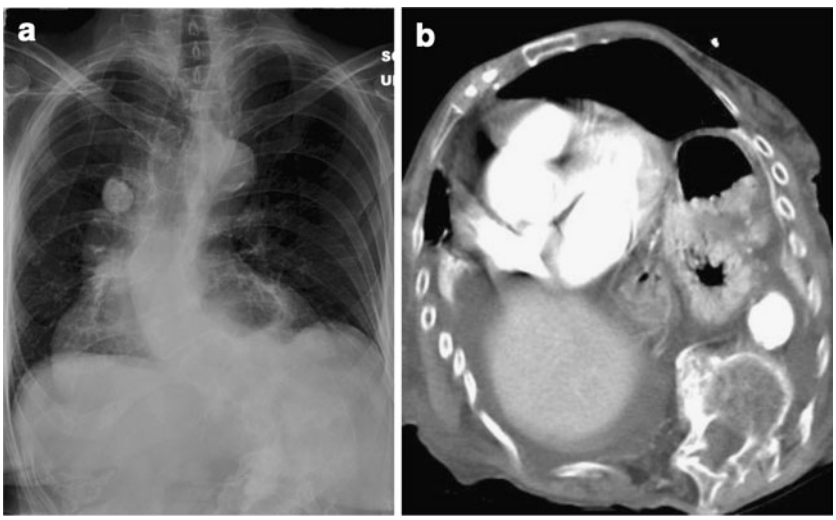

Fig. 1 Giant paraesophageal hernia and scoliosis. **a** CXR and **b** CT scan demonstrate marked curvature of the spine and giant paraesophageal herniation with associated chest wall and cardiovascular distortion

age and compromised pulmonary function.[32] A standardized approach is undertaken in the pre-operative assessment of patients with paraesophageal hernia in the setting of scoliosis, in a fashion similar to those patients without scoliosis.[33] Though pulmonary function tests were not routinely performed in the current study, this information may be useful in patients with scoliosis to assist in stratifying the increased risk of postoperative pulmonary insufficiency. In addition, repair of the paraesophageal hernia itself may lead to measured improvements in pulmonary function, thus providing additional impetus for paraesophageal hernia repair in patients with underlying scoliosis.[34,35] Specifically, paraesophageal hernia repair has been shown to provide significant improvement in spirometry values, dyspnea index, and quality of life scores.[36,37] Prospective studies will aid in delineating the physiologic impact of paraesophageal hernia repair on pulmonary function.

Limitations of this study include its retrospective nature, and the potential for selection and treatment bias. Another potentially confounding feature is that patients with scoliosis were older on average compared to patients without scoliosis (median age=75 vs. 67, $p<0.0001$). In addition, there was a higher percentage of female patients with kyphoscoliosis (82.8% vs. 68.7%, $p=0.012$). Despite these limitations, the findings of the current analysis support a clinical association of scoliosis and giant paraesophageal hernia formation, given the much higher than expected rate of scoliosis in patients with giant paraesophageal hernias compared with the general population. Though this analysis defines an association between scoliosis and giant paraesophageal hernias, no firm conclusions can be drawn regarding a causative relationship. Studies are in progress to evaluate genetic and connective tissue influences that may be involved in the pathogenesis of these conditions.

Though scoliosis was first described by Hippocrates, the specific pathogenesis of idiopathic and degenerative scoliosis remains obscure to this day. A number of hypotheses have been put forth to explain this phenomenon including physical changes related to musculoskeletal overuse/disuse, chronic improper posture, and imbalance of paraspinal musculature and its innervation.[38,39] At a molecular level, the development of scoliosis has been loosely associated with abnormal collagen distribution,[40,41] reduced serum melatonin levels,[42] altered growth factor expression (TGFβ and b-FGF),[43] and genetic polymorphisms involving IL-6 and MMP-3[44] as well as estrogen receptors.[45] The relationship of scoliosis and paraesophageal hernia leads to the hypothesis that these entities might arise from similar pathogenetic abnormalities. To date, however, the pathogenetic relationship between scoliosis and paraesophageal hernias remains to be elucidated.

Conclusion

The prevalence of kyphoscoliosis was 29.1% among patients undergoing giant paraesophageal hernia repair, significantly higher than that seen in the general population. The presence of kyphoscoliosis, or underlying conditions that predispose to it, may contribute to the development and progressive enlargement of hiatal hernias. Kyphoscoliosis is associated with increased perioperative pulmonary morbidity following giant paraesophageal hernia repair. Further studies are in progress to evaluate genetic and connective tissue influences that may be involved in the pathogenesis of these conditions. Surgeons approaching paraesophageal hernia repair should be aware of the increased pulmonary morbidity and postoperative care required in managing these patients.

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ORIGINAL ARTICLE

Detection of Lymph Node Involvement by Cytokeratin Immunohistochemistry is an Independent Prognostic Factor After Curative Resection of Esophageal Cancer

Goran Marjanovic · Markus Schricker · Axel Walch · Axel zur Hausen · Ulrich T. Hopt · Andreas Imdahl · Frank Makowiec

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Abstract
Background Involved lymph nodes (LN) are a negative prognostic factor in esophageal cancers. To assess the role of nodal micrometastases, we performed immunohistochemical analyses of LN after resection of node-negative esophageal cancers and correlated the results with survival.
Methods Seventy patients with esophageal cancer after curative resection and conventionally negative nodes were included. The LN were examined with six consecutive sections (three hematoxylin and eosin (HE) stained and three stained immunohistochemically with the cytokeratin (CK) antibodies AE1/AE3). Survival was evaluated uni- and multivariately. Median follow-up was 4.1 years.
Results Immunohistochemical analysis showed CK-positive LN in 16 (23%) patients. Of those 16 cases with CK-positive LN, nine had aviable macrometastases, ten had CK-positive scars/fibrosis and five had viable micrometastases. All patients with aviable macrometastases or CK-positive scars/fibrosis had undergone neoadjuvant chemoradiation. Five-year survival was 48% in all patients. In univariate analysis, survival was worse in patients with CK-positive LN (5-year survival of 30% vs. 54% in CK-negative LN; $p<0.02$) and in patients with squamous cell carcinoma (5-year survival of 38% vs. 75% in adenocarcinoma; $p=0.05$). Multivariate analysis revealed CK-positive LN ($p=0.02$) and (borderline) squamous cell carcinoma ($p=0.06$) as negative prognostic factors.
Conclusions The immunohistochemical analysis of LN may detect (viable or non-viable) tumor cells in lymph nodes after resection of conventionally node-negative esophageal cancers. Conventional pathological analysis by HE, therefore, understages esophageal cancer in these cases. The detection of CK-positive cells in resected LN is an independent prognostic factor in otherwise LN-negative esophageal cancer.

Keywords Esophageal cancer · Lymph node metastasis · Micrometastasis · Survival · Prognosis

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Introduction

The only potentially curative therapy for esophageal cancer is margin-negative esophageal resection with appropriate lymphadenectomy.[1] The nodal status is a strong prognostic factor in patients with esophageal cancer. Five-year-survival rates for completely resected node-negative (pN0, R0) patients were reported as high as 80%[2–5] whereas in patients with lymph node metastases (pN1, R0) survival rates are significantly lower with high local recurrence rates up to 25%.[5–7] However, even in patients with completely

resected and by conventional histology node-negative tumors, local recurrence rate can exceed 10%.[8] This phenomenon supports a hypothesis that some tumor cells (especially in lymph nodes) are not detected by conventional histopathology.

Conventional assessment of lymph nodes by hematoxylin and eosin (HE) staining is performed by one to two slices of each lymph node. Since routine histopathological examinations only detect metastases larger than 2 mm, micrometastatic cell clusters are not found during conventional HE staining.[9] Immunohistochemical (IHC) and molecular examinations facilitate the detection of single tumor cells or micrometastases smaller than 2 mm as defined by Hermanek et al.[10] Cytokeratine antibodies are widely used to detect and differentiate small epithelial tumor cell clusters with sufficient tissue contrast. Cytokeratines belong to a family of water-soluble proteins forming the cytoskeleton of epithelial cells.[11] Since healthy lymph nodes do not contain epithelial cells, CK positivity in lymph nodes in patients with esophageal cancer may indicate the presence of (viable or non-viable) tumor cells.

However, due to differing definitions of immunohistologically detectable tumor cells, cell clusters or micrometastases, the current literature is still controversial about their inherent prognostic relevance.[12–20]

Neoadjuvant chemoradiation may lead to downsizing and downstaging of the tumor, but without clear advantage in long-term survival in patients with locally advanced esophageal cancer.[21,22] The effect of neoadjuvant chemoradiation on the incidence of nodal tumor cells detected by IHC remains unknown.

The aim of our study was to identify the incidence and the prognostic relevance of immunohistologically detectable nodal tumor cells as described by *Hermanek* et al.[10] on long-term survival after esophageal resection in conventionally node-negative and completely resected patients.

Patients and Methods

A total of 162 patients with esophageal cancer underwent esophageal resection in our institution from 1991 to 2003. All patients had at least cT1-tumors, none had evident distant metastases. Staging routinely included endoscopy, endoscopic ultrasound (when technically possible) and thoracoabdominal computed tomography.

Since 2000, positron emission tomography has also been performed during staging. In general, lymph nodes were preoperatively classified as malignant if >1 cm by computed tomography (CT) or endoscopic ultrasound. To exclude other malignancies and to stage potential tracheal/bronchial infiltration, bronchoscopy was performed in patients with cancers of the upper or middle esophagus.

Study Patients

Routine postoperative pathology revealed negative lymph nodes and free resection margins (pN0, R0) in 87 of the 162 operated patients. For the purpose of our study, all specimens were reviewed by one experienced pathologist. Seven patients were then reclassified as R-1 ($n=5$) or pN1 ($n=2$). Ten of the remaining 80 patients died postoperatively or were without sufficient follow-up. Therefore, 70 patients with a (confirmed) pN0 R0 situation after resection of esophageal cancer could be included in our analyses.

The median age of the 70 patients (81% men) was 59 years (range, 36–75). Fifty patients (71%) had squamous cell cancer (SCC), 20 patients (29%) had adenocarcinoma (Adeno-Ca). The location of cancer was in the proximal esophagus in 16%, in the middle esophagus in 46% and in the lower esophagus in 37%. One patient had synchronous squamous cell cancer of the upper and lower esophagus. After resection, the final T stages were as follows: pT0, 29%; pT1, 17%; pT2, 29%; and pT3, 26%. None of the patients had a pT4 stage. The median number of assessed lymph nodes was 15 (interquartile range, 8 to 25).

Neoadjuvant Chemoradiation

In the 53 patients (76%) undergoing neoadjuvant chemoradiation, 36 Gy were applied for radiation (1.8 Gy/day, days 1–5, weeks 1–4). Additionally, those patients received 5-flurouracil (500 mg/m^2 body surface; days 1–5, weeks 1–4) and Cisplatin (20 mg/m^2 body surface; days 1–5, weeks 1 and 4).[23] After an interval of approximately 4 weeks the patients were restaged (endoscopy and CT) and resection was performed if feasible.

Definition of Tumor Remission

Tumor remission by chemoradiation was assessed by comparing clinical (CT and endoscopic ultrasound) tumor staging before neoadjuvant therapy and pathohistological findings after resection. If final pathohistological staging was identical to the pretherapeutic staging, or in the case of even higher TN staging after chemoradiation, we assumed a "no-remission/tumor progress" situation. Partial remission was defined as reduction of tumor size, T stage or N stage. Complete remission was defined in the absence of any detectable residual tumor cell after resection (i.e. pT0 pN0). Newer definitions of response to neoadjuvant therapy assessing the proportion of viable tumor cells in the resected specimen are used in our institution only since a few years and were, therefore, not included in this study.

Operative Procedure and Lymphadenectomy

Sixty-three (90%) of the 70 esophageal resections were performed through a thoracoabdominal approach (right thoracotomy), the remaining seven (10%) through a transmediastinal approach (including further cervical anastomosis). In general, a two-field lymphadenectomy was performed. Reconstruction consisted of a gastric conduit in almost all patients (n=68); two patients underwent colonic interposition.

Immunohistochemistry and Definition of CK Positivity

For the purpose of our study, the lymph nodes of all patients were examined immunohistochemically and after HE staining. A total of 1,211 LN (mean, 17 per patient) were assessed by two pathologists. The examination of each LN was carried out in six consecutive sections (thickness of the section, 2 μm; distance between the sections, 150 μm) (Fig. 1). Every 1st, 3rd and 5th section was HE-stained, every 2nd, 4th and 6th was stained immunohistochemically with a cytoceratine antibody cocktail AE1/AE3 (dilution, 1:200; DAKO, Hamburg, Germany). This antibody cocktail recognizes cytokeratine subclasses 1–8, 10, 13, 14, 15, 16, 19 and reacts with human epithelial cells like adenocarcinoma and squamous cell carcinoma cells without any known exception.

Immunohistochemical staining was done by the labelled avidin-biotin-peroxidase complex technique (Dako, Hamburg, Germany). After proteolytic pretreatment with proteinkinase K (Sigma, Steelze, Germany), the slides were incubated with the AE1/AE3 antibody cocktail for 30 min following incubation with the secondary antibody (ChemMate Detection Kit, DAKO-Cytomation, Hamburg, Germany) for 15 min. After adding the enzyme alkaline phosphatasis (ChemMate Detection Kit, DAKO-Cytomation, Hamburg, Germany) and its substrate neufuchsin, a typical red color reaction was induced. Counterstaining of the nuclei was performed with haemalaun solution (Merck, Darmstadt, Germany).

The exact definition of cytokeratine positivity (CK^+) is given in Table 1. In our analyses, CK^+ included the presence of aviable macrometastases and micrometastases, scarred areas and fibrosis (former tumor cells) and viable micrometastases. False-positive cytokeratine immunostaining, including that of plasma cells, interstitial reticulum cells and mesothelial cells, could be clearly distinguished by cell morphology and immunohistochemistry. Final agreement between the two pathologists was obtained using a two-head microscope.

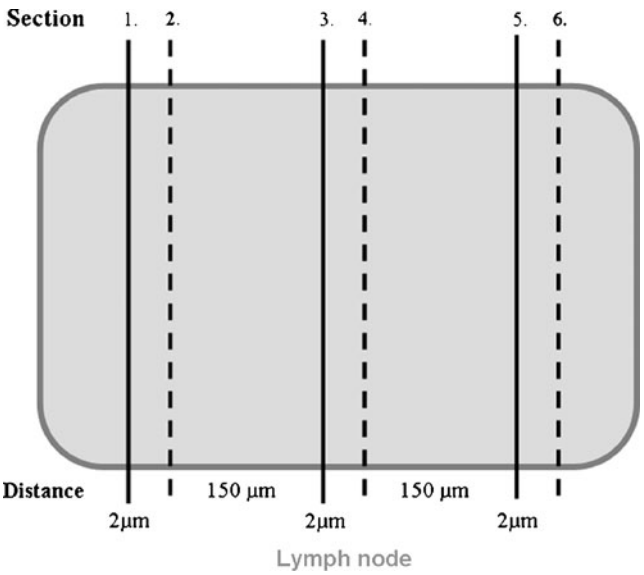

Fig. 1 Pattern draft of the histologic section procedure of each examined lymph node. Six consecutive sections were examined (thickness of the section, 2 μm; distance between the sections, 150 μm). Every 1st, 3rd and 5th section was HE-stained, every 2nd, 4th and 6th (*dashed line*) was stained immunohistochemically with cytokeratine antibodies AE1/AE3

Statistical Analysis

Patients' demographic, perioperative, routine pathology and survival data were gained by retrospective analysis of our esophageal surgery database (database and analysis with SPSS for Windows™, version 15.0, SPSS Institute, Chicago, IL). The survival status is received yearly from the tumor registry of the Comprehensive Cancer Center of our university hospital. Actuarial survival was estimated univariately using the Kaplan–Meier analysis, a log-rank test was applied to test for group differences. The Cox proportional hazard regression model (with forward likelihood-ratio statistics) was used for multivariate survival analysis. Median postop-

Table 1 Definition of cytokeratine positivity and subordinated viable micrometastases (adapted from *Hermanek* et al.[10])

Cytokeratine positivity	Viable micrometastasis
Viable micrometastases	With HE-staining not detectable
Aviable micrometastases	<2 mm in largest diameter
Aviable macrometastases	Cells with cytokeratin-positive cytoplasm
Scars and fibrotic areas	Stromal reaction of lymphatic tissue
	Large cells with large unshaped nucleus
	Large striking nucleolus
	Few cytoplasm

All nodal cells and areas positive for AE1/AE3 staining were subsumed as nodal cytokeratine positivity. Aviable macrometastases do not contain viable tumor tissue and are all larger than 5 mm in diameter, thus visible on HE-staining, too

erative follow-up was 4.1 (interquartile range, 1.4–6.9) years (time until death or last follow-up).

Results

CK Positivity

CK^+ cells in lymph nodes were detected in 16 of the 70 (23%) patients. CK^+ LN were found in ten of 50 (20%) patients with SCC and in six of 20 (30%) patients with Adeno-Ca (n.s.). Of the 16 patients with CK^+ LNs, five had viable micrometastases, nine had aviable micro- or macrometastases and ten had CK^+ fibrosis or scars. Examples of CK^+ metastases are shown in Fig. 2. A detailed listing of the findings in the 16 patients with CK^+ LNs is given in Table 2. The median (interquartile range) number of examined nodes was slightly but not significantly higher in patients with CK-positive nodes (18.5; 11.25–26.75) than in patients with CK-negative nodes (14.5; 7–22), $p=0.48$.

Nodal Status at Initial Staging

Only patients with a postoperative pN0 stage (by conventional histology) were included in our study. At the time of initial staging before surgery or neoadjuvant chemoradiation, however, 37 of 70 patients (53%) were classified as node positive (cN1). Thirty-three of the 53 patients (62%) later undergoing neoadjuvant chemoradiation were initially staged as cN1. Four patients initially staged node positive did not undergo neoadjuvant chemoradiation. Postoperatively, all four had tumor-free lymph nodes (conventionally and by immunohistochemistry). A summary of the initial nodal staging, neoadjuvant treatment and postoperative immunohistochemical results (CK positivity) of all 70 patients is given in Fig. 3.

Correlation of Neoadjuvant Chemoradiation with CK Positivity in LN

Seventy-six percent (53/70) of the patients had received neoadjuvant chemoradiation (36 patients with SCC and 17 patients with Adeno-Ca). Of those 53 patients, 20 (38%) had complete remission, 30 patients (57%) had partial and three patients (6%) had no remission of the primary esophageal tumor.

The rates of CK^+ LN in patients with neoadjuvant chemoradiation (13 of 53 patients; 25%) and in patients without neoadjuvant chemoradiation (three of 17 patients; 18%) were comparable. However, aviable nodal metastases ($n=9$) or CK^+ fibrosis/scars ($n=10$) in LN were only found in patients after chemoradiation, whereas all three CK^+ patients without neoadjuvant chemoradiation presented with viable micrometasases and without any scars/fibrosis in the lymph nodes.

Univariate Survival Analysis

During the median follow-up of 4.1 years, 43 of the 70 patients died. Median survival in the entire study group was 4.8 years. Overall actuarial survival was 69% after three and 48% after 5 years. Detailed subgroup survival analysis is shown in Table 3. Patients with SCC had a significantly poorer actuarial survival than patients with Adeno-Ca (38% after 5 years in SCC vs. 75% in Adeno-Ca, $p=0.05$; Fig. 4). Survival was also worse in the 16 patients with CK^+ LNs (30% after 5 years) compared to the 54 patients with CK-negative LNs (54%; $p<0.02$; Fig. 5). The univariate effect of CK positivity on survival was very strong in the subgroup of patients with Adeno-Ca ($p<0.01$; Table 3). Of the 14 patients with resected adenocarcinoma and CK-negative nodes, the first died more than 3 years after surgery, with a calculated five-year survival of 93% (median survival in this subgroup >5 years). However,

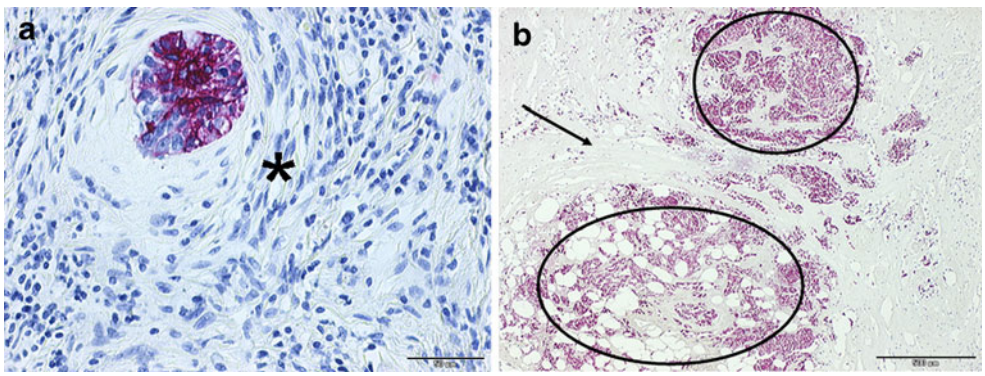

Fig. 2 **a** Immunohistochemical demonstration of a viable micrometastasis: cluster of tumor cells surrounded by a stromal reaction (*black star*; magnification ×20). **b** Immunhistochemical staining of an aviable macrometastasis (two ellipses). All tumor cells are destroyed—thus leading to pN0 staging—but staining with AE1/AE3 antibodies is positive. As a result of neoadjuvant chemoradiation, a fibrotic scar (*black arrow*) is visible surrounding the necrotic tumor tissue (magnification ×2.5)

Table 2 Demographic and tumor-related data of all 16 pN0 CK^+ patients with completely resected esophageal cancer

Patient	Age	Sex	Tumor type	Neoadjuvant RCTx	cTx stage	pTx stage	cNx stage	Viable MiM	Aviable MiM	Aviable MaM	Scars or fibrosis
1	53	F	SCC	No	1	1	0	yes	No	No	No
2	36	M	AC	Yes	3	3	1	No	No	Yes	No
3	61	M	AC	No	2	3	0	Yes	No	No	No
4	55	M	AC	Yes	3	0	1	No	No	Yes	No
5	56	F	AC	Yes	2	2	0	No	No	No	Yes
6	44	F	AC	No	3	3	0	Yes	No	No	No
7	69	M	SCC	Yes	3	3	1	No	No	No	Yes
8	60	F	SCC	Yes	3	2	1	No	Yes	No	Yes
9	74	M	SCC	Yes	3	0	1	No	No	Yes	Yes
10	52	M	SCC	Yes	4	3	1	Yes	No	No	No
11	64	F	SCC	Yes	3	0	1	Yes	No	Yes	Yes
12	59	M	SCC	Yes	2	0	1	No	No	Yes	Yes
13	71	M	SCC	Yes	3	0	0	No	No	Yes	Yes
14	55	M	SCC	Yes	3	0	0	No	No	Yes	Yes
15	62	M	SCC	Yes	3	2	1	No	No	Yes	Yes
16	65	M	AC	Yes	3	0	1	No	Yes	Yes	Yes

M/F male/female, *SCC* squamous cell carcinoma, *AC* adenocarcinoma, *RCTx* chemoradiation, *cTx/cNx/pNx* clinical/pathological T and N stage of the (primary) tumor, *MiM* micrometastasis, *MaM* macrometastasis.)

CK status showed only a tendency but no significant difference in patients with SCC (5-year survival of 30% in CK-positive nodes vs. 40% in patients with CK-negative nodes; $p=0.16$; Table 3). A further strong univariate difference of survival regarding CK status was found in the subgroup of 37 patients initially staged as node positive (cN^+): five-year survival was only 30% in the ten cN^+/CK-positive patients whereas it reached 65% in the 27 cN^+/CK-negative patients ($p<0.001$; Table 3). Further parameters like age, gender, pT stage, neoadjuvant chemoradiation, response to chemoradiation, number of examined LN (≤15 vs. >15) and tumor location did not influence survival in the entire patient group (Table 3).

Multivariate Survival Analysis

Multivariate analysis showed only the presence of CK positivity in the lymph nodes to be an independent factor significantly influencing survival ($p<0.02$, relative risk 2.2; Table 4). Unlike in univariate analysis (where it just reached statistical significance), the histological tumor type showed 'only' borderline significance in the Cox regression model ($p=0.06$).

Discussion

The presence of lymph node metastases is one of the most important prognostic factors in patients with esophageal cancer.[4] The detection of nodal (micro-) metastasis by methods other than routine HE-histopathological examination indicates higher tumor stages and may alter the prognosis. This fact has already been described in several gastrointestinal[24–26] and also extra-gastrointestinal malignancies like lung or breast cancer.[27–29]

In our study, we performed extensive analyses of more than 1,200 conventionally tumor-negative lymph nodes using cytokeratin antibody staining. Our results demonstrate that detection of viable or non-viable tumor cells by immunohistochemical techniques has a negative prognostic impact on long-term survival in patients after curative (i.e.

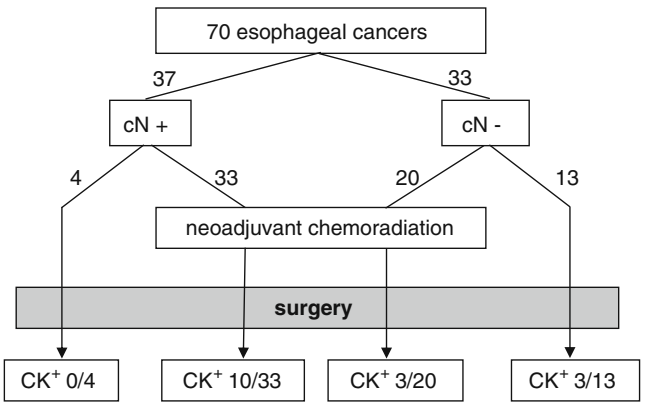

Fig. 3 Flow chart demonstrating pretherapeutic nodal staging (*cN*), neoadjuvant chemoradiation and final immunohistochemical cytokeratine (*CK*) status of the lymph nodes in 70 patients undergoing resection of esophageal cancer (pN0 and free margins)

Table 3 Univariate actuarial survival after margin-negative resection of esophageal cancer in conventionally node-negative patients

Parameter	Number	3-year survival	5-year survival	*p*
Histologic type				
SCC	50	64%	38%	0.05
Adeno-Ca	20	80%	75%	
CK-positive lymph nodes				
Yes	16	44%	30%	<0.02
No	54	76%	54%	
Gender				
Female	13	69%	34%	0.98
Male	57	68%	51%	
Age				
<60 years	37	68%	50%	0.23
≥60 years	33	70%	47%	
cN				
Positive	37	70%	55%	0.28
Negative	33	69%	42%	
Postoperative T stage				
pT0+pT1	32	66%	40%	0.25
pT2+pT3	38	71%	55%	
No of examined LNs				
≤15	36	72%	45%	0.28
>15	34	65%	52%	
Neoadjuvant chemoradiation				
Yes	53	68%	49%	0.70
No	17	71%	46%	
Tumor location (esophagus)[b]				
Upper	11	55%	36%	0.51
Middle	32	75%	48%	
Lower	26	65%	57%	
Subgroup analysis				
Response to chemoradiation[a]				
Complete	20	70%	51%	0.91
Partial	30	70%	49%	
Nodal CK status in cN-positive patients				
CK-positive	10	50%	30%	<0.001
CK-negative	27	78%	65%	
Nodal CK status in SCC				
CK-positive	10	50%	30%	0.16
CK-negative	40	68%	40%	
Nodal CK status in Adeno-Ca				
CK-positive	6	33%	–[c]	<0.001
CK-negative	14	100%	93%	

[a] Three patients without response or with progress under chemoradiation were excluded from this analysis (group too small)

[b] One patient with two simultaneous cancers (upper+lower esophagus) was excluded from this analysis

[c] No patient at risk at 5 years

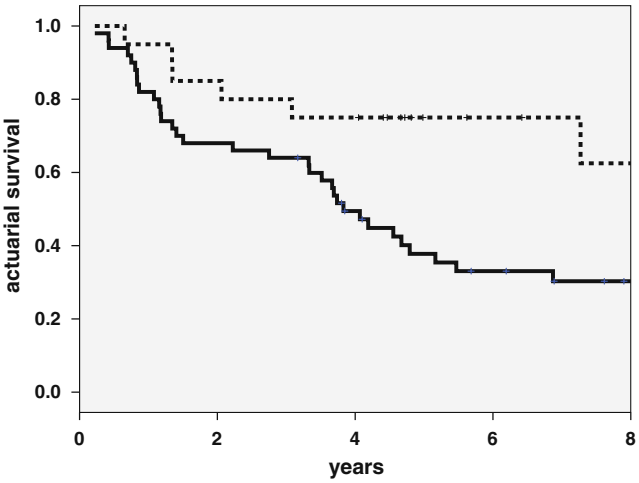

Fig. 4 Actuarial survival after margin-negative resection of conventionally node-negative esophageal cancers in relation to histology. Adenocarcinoma (n=20), *dashed line*; SCC (n=50), *solid line*; p=0.05

margin-negative) resection of esophageal cancer with a conventionally lymph node-negative stage. Survival in patients with those micrometastases is in the range of the survival in patients with 'conventionally' positive lymph nodes. The prognostic effect of CK-positive lymph nodes was especially marked in the subgroups of patients with adenocarcinoma and in patients initially staged as node positive, respectively. A positive effect of neoadjuvant therapy was not demonstrated directly in our analyses. However, since survival was even slightly (not significantly) higher in the patients initially staged node positive, and 89% of those patients underwent neoadjuvant chemoradiation, we believe that this represents a positive effect of chemoradiation on survival.

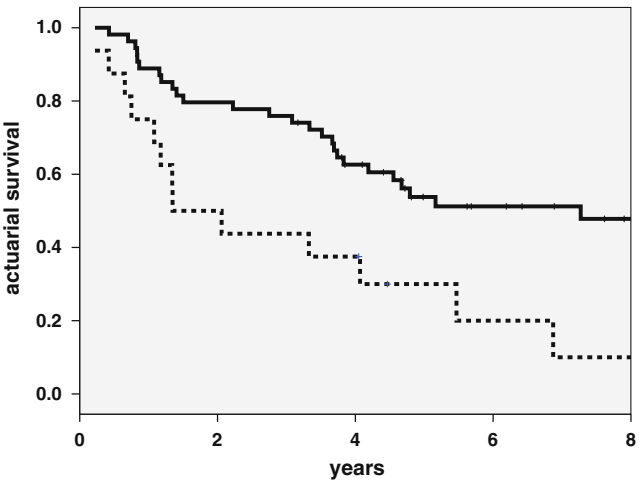

Fig. 5 Actuarial survival after margin-negative resection of conventionally node-negative esophageal cancers in relation to cytokeratin positivity (CK^+). CK-positive nodes (n=16), *dashed line*; CK-negative nodes (n=74), *solid line*; p<0.01

Table 4 Multivariate actuarial survival (Cox regression) analysis after margin-negative resection of esophageal cancer in 70 conventionally node-negative patients

Parameter	*p*	Relative risk	95% confidential interval
CK-positive lymph nodes	0.02	2.2	1.2–4.2
SCC (vs. Adeno-Ca)	0.06	2.1	0.9–4.5

Problem of HE Examination and Benefit of IHC Staining

A relatively high recurrence rate in conventionally (HE) node-negative patients might be explained by histopathological understaging. Usually, only a single section of each lymph node is examined during conventional assessment, representing only 1% of the submitted tissue.[9] In addition, isolated tumor cells or tumor cell residuals smaller than 2 mm, called micrometastases,[10] are routinely missed on HE series. By contrast, IHC staining directed against epithelial or tumor-associated antigens such as cytokeratines facilitates the detection of one viable or aviable tumor cell among up to 10^6 normal cells.[30] In our evaluations, we examined six consecutive sections of each lymph node, three after conventional and three after IHC staining. Every IHC positive cell or cell cluster was controlled in HE staining to differentiate between viable or aviable tumor tissue. Furthermore, potentially false-positive IHC staining could always be differentiated either by morphology or by additional immunohistochemistry. Performing six consecutive sections in each lymph node is very time consuming and expensive. It may be discussed whether doing more sections may reach even higher accuracy. However, *Izbicki* et al. have already demonstrated that examining three levels seems to be sufficient.[13]

Prognostic Relevance of "Micrometastases"

Immunohistochemical examination for lymph node micrometastases in esophageal cancer with conventionally negative nodes has generally been recommended by *Komukai* et al.,[14] but not yet implemented as a routine procedure. An important factor for this seems to be the variation of conclusions drawn from different retrospective studies on the prognostic significance of single tumor cells or cell clusters. Several studies included both patients with metastatic lymph nodes on conventional HE (pN1) examination and node-negative patients.[15,17,18,31,32] Others even included patients with distant metastases.[13,33] In our study, we excluded all patients with viable macrometastases (pN1 by conventional histopathology) which enabled us to evaluate the specific effect of micrometastases alone.

However, even the studies including only pN0 patients[12,14,16,20,34,35] showed controversial results regarding the prognostic relevance of micrometastases in esophageal carcinoma. This might be explained (at least in part) by a great variety of definitions of micrometastases. In our study, we used a definition of viable micrometastasis adapted from the definition proposed by *Hermanek* et al.,[10] which strongly distinguishes 'viable' micrometastases from isolated or disseminated tumor cells. In contrast to isolated or disseminated tumor cells, viable micrometastases show signs of growth in the lymph node as demonstrated by a stromal reaction in the direct surrounding tissue (Fig. 2) thus representing an occult metastatic potential.[10] Due to our strict definition of viable micrometastases, we detected those in only five patients (7%). Because of this low occurrence, a reliable conclusion of their inherent prognostic relevance remains impossible in our analysis.

In our study, we found a relatively high rate of tumor-associated overall nodal CK^+. Beyond viable micrometastases, this CK positivity subsumes non-viable tumor cells/tissue like aviable micro- and macrometastases, isolated cells, scars or fibrosis. The majority of our patients had received neoadjuvant chemoradiation, with most patients either having a partial or complete response on final pathological evaluation. We suppose that former viable tumor cells in pretherapeutic node-positive-staged patients were destroyed by neoadjuvant therapy, leaving aviable tumor cells or tumor cell remnants still expressing cytokeratine antigens of the cytoskeleton. Most of the studies mentioned above do not describe the presence of cytokeratine positivity other than micrometastases.[14,16,34–36] Since those studies did not evaluate CK positivity in patients after neoadjuvant chemoradiation, it might be possible that CK positivity other than by micrometastases was an effect of chemoradiation in our study.

However, *Doki* et al.[20] presented the results of cytokeratine evaluation in 41 node-negative patients with esophageal carcinoma, of whom 11 were treated with neoadjuvant chemotherapy. They described a novel pathologic entity other than micrometastases and called it 'cytokeratine deposits', which were only associated with preoperative chemotherapy. They suggested that cytokeratine deposits arose from dead tumor cells through either apoptosis or necrosis. In contrast to our results, the overall cytokeratine positivity did not affect long-term survival. This difference might be explained by the smaller patient number and the lack of radiation therapy in Doki's study. In a further study, the same group performed cytokeratine staining in esophageal squamous cell cancer with metastatic lymph nodes (pN1) in 107 patients, of whom 70% had undergone neoadjuvant chemotherapy.[37] Interestingly, the frequency of immunohistochemically detected viable micrometastases was significantly higher in the surgery alone group (no

neoadjuvant chemotherapy), whereas the frequency of cytokeratine deposits (according to non-viable tumor cells) was significantly higher in the pretreated group. They concluded that the disappearance of (viable) micrometastases and the presence of cytokeratine deposits indicate the eradication of micrometastases by neoadjuvant chemotherapy. Our data support this hypothesis, since in all cases with aviable micro- and macrometastases and/or scars/fibrosis, the patients had been pretreated with chemoradiation.

Role of Neoadjuvant and Possible Adjuvant Treatment

Although a pN0 stage is an independent prognostic factor for better survival, the survival of former node-positive patients (cN1), who were potentially downstaged by neoadjuvant chemoradiation[21] but proven to be CK^+, seems to be similarly poor as survival of definitely node-positive patients (pN1). And even if neoadjuvant chemoradiation, compared with surgery alone, may improve long-term survival and reduce locoregional tumor recurrence[38,39], its impact on postoperative morbidity and mortality should not be underestimated. The identification of patients with good response to neoadjuvant treatment may be a key question to be solved in the future[40,41]. In addition, adjuvant treatment for pN0 patients who are found to be nodal cytokeratine positive may be discussed.

Conclusions

In conventionally node-negative patients with esophageal cancer, immunohistochemical examination using cytokeratin antibodies may detect viable tumor cells, aviable tumor cells or tumor cell remnants in the resected lymph nodes in a relevant proportion of patients. Since patients with immunohistochemically proven viable or non-viable lymph node metastases have a significantly poorer prognosis, their disease is clearly understaged by routine histopathological examination. The immunohistochemical detection of nodal micrometastases, therefore, can be used as a further important prognostic factor. The strong prognostic influence of nodal cytokeratine-positive cells may suggest the routine use of IHC in the postoperative examination of conventionally negative lymph nodes. In addition, possible adjuvant treatment analogous to conventionally node-positive patients may be discussed in those cases.

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ORIGINAL ARTICLE

Direct Costs of Care in a Randomized Controlled Trial of Endoscopic Sclerotherapy versus Emergency Portacaval Shunt for Bleeding Esophageal Varices in Cirrhosis—Part 4

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Abstract
Background Emergency treatment of bleeding esophageal varices (BEV) in cirrhotic patients is of prime importance because of the high mortality rate surrounding the episode of acute bleeding. Nevertheless, there is a paucity of randomized controlled trials of emergency surgical therapy and no reports of the costs of any of the widely used forms of emergency treatment. The important issue of direct costs of care was examined in a randomized controlled trial that compared endoscopic sclerotherapy (EST) to emergency portacaval shunt (EPCS).
Methods Two hundred eleven unselected consecutive patients with *ultimately* biopsy-proven cirrhosis and endoscopically proven acute BEV were randomized to EST (n=106) or EPCS (n=105). Diagnostic workup was completed, and EST or EPCS was initiated within 8 h. Criteria for failure of EST or EPCS were clearly defined, and crossover rescue treatment was applied, when primary therapy failed. Ninety-six percent of patients underwent more than 10 years follow-up, or until death. Complete charges for all aspects of care were obtained continuously for more than 10 years.
Results Direct charges for all aspects of care were significantly lower in patients treated by EPCS than in patients treated by emergency EST followed by long-term repetitive sclerotherapy. Charges per patient, per year of treatment, and per year in each child's risk class were significantly lower in patients randomized to EPCS. Charges in patients who failed endoscopic sclerotherapy and underwent a rescue portacaval shunt were significantly higher than the charges in both the unshunted sclerotherapy patients and the patients randomized to EPCS. This result was particularly noteworthy given the widespread practice of using surgical portacaval shunt as rescue treatment only when all other forms of therapy have failed.
Conclusions In this randomized controlled trial of emergency treatment of acute BEV, EPCS was significantly superior to EST with regard to direct costs of care as reflected in charges for care as well as in survival rate, control of bleeding, and incidence of portal-systemic encephalopathy. These results provide support for the use of EPCS as a first line of emergency treatment of BEV in cirrhosis (clinicaltrials.gov #NCT00690027).

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Keywords Direct costs · Cirrhosis · Varices · Shunt · Sclerotherapy

Abbreviations

BEV	Bleeding esophageal varices
TIPS	Transjugular intrahepatic portosystemic shunt
EST	Endoscopic sclerotherapy
EPCS	Emergency portacaval shunt
PCS	Portacaval shunt
UGI	Upper gastrointestinal
ICU	Intensive care unit
PRBC	Packed red blood cells
PSE	Portal-systemic encephalopathy
EVL	Endoscopic variceal ligation
QOL	Quality of life
RCT	Randomized controlled trial

Introduction

Extensive data reported during the past 60 years have provided clear evidence that bleeding esophageal varices (BEV) is a common and highly lethal complication of cirrhosis of the liver.[1] The period surrounding the episode of acute bleeding has been reported to account for much of the mortality rate associated with BEV.[1] A number of modalities of emergency treatment of BEV are in use including pharmacologic measures, endoscopic therapy, transjugular intrahepatic portosystemic shunt (TIPS), and surgical portal decompression. There is no agreement which of these modalities is most effective, but there is general agreement that emergency treatment of BEV is of prime importance. Nevertheless, few randomized controlled trials of the various modalities of emergency treatment have been reported. In particular, no randomized trials involving emergency surgical therapy have been described. Moreover, little is known about the costs associated with emergency treatment of BEV, an important measure of the effectiveness of therapy.

From April 8, 1988, to December 31, 2005, we conducted a randomized controlled trial (RCT) in 211 unselected consecutive patients with cirrhosis and acute BEV in whom emergency and long-term repetitive endoscopic sclerotherapy (EST) was compared with emergency direct portacaval shunt (EPCS). The trial was a community-wide endeavor and was known as the San Diego Bleeding Esophageal Varices Study. In two recent publications, we described the study in detail and reported the outcomes first with regard to control of bleeding and survival,[2] and second with regard to development of portal-systemic encephalopathy (PSE).[3] In a third publication, we compared EPCS to rescue PCS following failed EST.[4] This report focuses on direct costs of care.

Patients and Methods

Design of Randomized Controlled Trial

Our two recent publications[2,3] described our RCT and provided full information on the protocols and methods. These include (1) design of study; (2) patient eligibility; (3) definitions (BEV, unselected patients ("all comers"), emergency EST, long-term EST, EPCS, failure of emergency primary therapy, failure of long-term therapy, rescue therapy, informed consent); (4) randomization; (5) diagnostic workup; (6) quantitative child's classification; (7) initial emergency therapy during workup; (8) endoscopic sclerotherapy; (9) emergency portacaval shunt; (10) posttreatment therapy; (11) lifelong follow-up; (12) quantitation of PSE; (13) data collection. The study protocol and consent forms were approved before the start of the study and at regular intervals thereafter by the UCSD Human Subjects Committee (Institutional Review Board). Figure 1 is a Consort Flow Diagram that shows the overall design and conduct of the RCT.[5, 6]

Direct Costs of Care

The EST and EPCS groups were compared with regard to direct costs of care. Complete UCSD charges were obtained for every patient entered in the RCT. In addition, all referring hospitals and referring physicians signed agreements to provide complete records of charges as they occurred. Prior to initiation of the study, UCSD Medical Center agreed to promptly provide copies of all hospital and outpatient charges on all patients at the time when the patients and insurance carriers were billed. Similarly, the UCSD Medical Group, which does the professional fee billing for all physicians who care for patients at UCSD Medical Center, agreed to promptly provide copies of all professional fee bills. Direct cost of care data were obtained from April 8, 1988 to November 11, 1999, after which there were too few survivors of EST to permit a valid comparison of direct cost data in the two study groups. In the final analysis, cost of care data from referring hospitals and non-UCSD physicians were not obtained for 30 of the 627 hospital readmissions (5%) and for 203 of the 4,757 readmission days (4%), equally divided between the EST and EPCS groups. This small deficiency in data collection had no influence on the overall results of the analysis.

Sources of healthcare funding or non-funding of all patients were identified at the time of entry in the study and

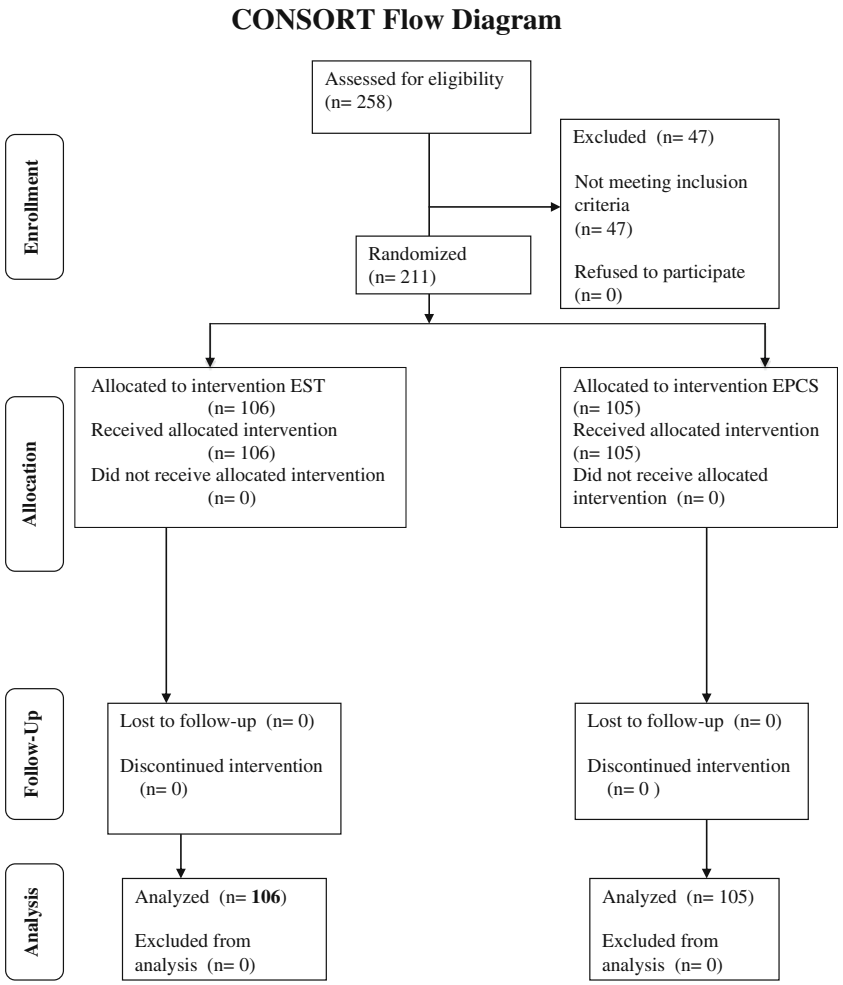

Fig. 1 The overall design and conduct of the prospective randomized controlled trial is shown in a Consort Flow Diagram [5,6]

continuously thereafter. Our analysis of direct costs of care has been accepted for oral presentation at a national meeting.

Statistical Analysis

The survival times were computed using the Kaplan–Meier method and were compared between arms using the log-rank test. Quality of life indices were compared between arms using Fisher's exact test (for categorical outcomes) and Wilcoxon rank-sum test (WRT; for continuous outcomes). For the charges for care in each category and for each treatment arm, the mean, standard deviation, and range (minimum–maximum) were computed. The comparison between arms used the nonparametric WRT. The charges per day for index admission were computed by dividing the index admission charges by the number of days of hospitalization. The charges per year for post-index admission and total were obtained by dividing the charges by the number of years of follow-up. The comparison between arms used the WRT. The sources of healthcare funding were compared between arms using Fisher's exact test. At the beginning of the study, it was decided in advance not to perform an interim statistical analysis of the data, so as not to diminish the power of the final analysis.

Results

Overall Outcome Data of EST versus EPCS

Our recent publications described the clinical characteristics of the 211 patients, findings on liver biopsy and initial upper endoscopy, results of laboratory blood tests, data on rapidity of therapy, data on control of bleeding, operative and endoscopic data, data on PSE, and data on survival.[2,3] On entry in the RCT the two groups were similar in every important characteristics of cirrhosis and BEV. Histologic proof of cirrhosis was obtained in all patients. Mean and median times from onset of bleeding to entry in the San Diego BEV Study were less than 20 h in both groups of patients, and from onset of bleeding to start of EST or

EPCS were less than 24 h. Excluding indeterminate deaths within 14 days unrelated to bleeding, EST achieved permanent long-term control of bleeding in only 20% of patients. In contrast, EPCS promptly and permanently controlled bleeding in every patient. Patients in the EST group required significantly more units of PRBC than those in the EPCS group because of continued or recurrent BEV. Survival rates at all time intervals and in all Child's classes were significantly higher after EPCS than after EST ($p<0.001$). Moreover, EPCS resulted in substantial long-term survival of patients in child's risk class C who had the most advanced cirrhosis of the liver. The incidence of recurrent PSE following EST was 35%, which was more than twice the 15% incidence following EPCS ($p<0.001$). EST patients had a total of 146 PSE-related hospital admissions, compared with EPCS patients who had 87 such hospital admissions ($p=0.003$). Recurrent UGI bleeding was a major causative factor of PSE in almost one half of the EST patients.

Direct Costs of Care

Table 1 summarizes the funding sources for all patients in the San Diego BEV Study. Medi-Cal, which in California is the form of Medicaid for low-income individuals, was the most frequent third-party carrier, accounting for 44% of third-party insurance coverage. A combination of Medi-Cal and Medicare provided healthcare insurance for 16% of patients, mainly those whose income was low and who were age 62 years or older, or had been declared permanently disabled. Patients whose income was below the poverty line and had no other healthcare insurance received coverage in 11% of the cases from San Diego County Medical Services. Fourteen percent of the patients had no healthcare insurance and 8% had private insurance. There were no significant differences in the funding sources of the two treatment groups. It is important to recognize that both the charges and actual costs of care were unrelated to the type of healthcare insurance or non-insurance held by each patient. For example, all EPCS patients received identical charges for a portacaval shunt unrelated to healthcare insurance or non-insurance. Similarly in the EST group the standard charges for a session of endoscopic sclerotherapy were not affected by a patient's health insurance or non-insurance.

Table 2 and Fig. 2 show the charges for hospitalization and outpatient care in thousands of US dollars in the EST and EPCS treatment groups. As expected, the charges for all aspects of the index admission in patients who underwent EPCS were significantly greater than the charges for initial EST ($p<0.001$). However, these charges were offset by significantly greater charges for post-index care in the EST group, in large measure because of recurrent BEV and the need for repeated readmissions to the hospital. In the final analysis, the total post-index charges were significantly greater in patients who were treated by EST compared to those who underwent EPCS ($p<0.001$), and the total overall charges for emergency and long-term care required over a number of years were greater in patients who received emergency followed by long-term repetitive EST, but the difference was not statistically significant ($p=0.08$). Note however that the charges in the EPCS group were spread over a significantly longer period of time.

Table 3 and Fig. 2 show the important relationship between charges and days or years of required care. When related to length of survival and, therefore, days or years during which care was required, EPCS was significantly less expensive than EST in every aspect of care except for the index admission. Charges for post-index care per year in the EST and EPCS groups, respectively, were a mean \$108,500 versus \$25,100 ($p<0.001$). Total overall charges for care of patients who entered the RCT were a mean \$168,100 per year in the EST group, versus \$39,400 per year in the EPCS group ($p<0.001$).

Table 4 shows the charges according to Child's risk classes assigned at the index admission. Total overall

Table 1 Funding sources for patients in San Diego BEV study

	EST ($N=106$)		EPCS ($N=105$)		Total ($N=211$)		P value (EST vs. EPCS)
	N	%	N	%	N	%	0.39
Medi-Cal	41	39	52	50	93	44	
Medicare	3	3	6	6	9	4	
Medicare/Medi-Cal	18	17	15	14	33	16	
Medicare/Private insurance	3	3	3	3	6	3	
County medical services	11	10	12	11	23	11	
Private insurance	9	8	7	7	16	8	
No insurance	20	19	9	9	29	14	
VA plus other insurance	1	1	1	1	2	1	

Table 2 Cost of care charges for hospitalization and outpatient care

Charges in $1,000	EST			EPCS			*P* value
	N	Mean (SD)	Range	*N*	Mean (SD)	Range	
Index admission	106			105			
Hospital charges		51.3 (52.9)	6.9–433.9		69.1 (56.1)	23.1–352.6	<0.001[a]
Physician charges		6.8 (6.6)	1.1–50.4		11.1 (5.4)	3.3–34.8	<0.001[a]
Total charges		58.1 (57.3)	8.1–458.5		80.2 (60.0)	33.7–380.5	<0.001[a]
Readmission post-index	93			88			
Hospital charges		104 (146.2)	0–911.4		56.6 (71.3)	0–262	<0.001[a]
Physician charges		15.4 (17.2)	0–89		8.6 (10.5)	0–49.2	<0.001[a]
Total charges		119.4 (157.5)	0–926.1		65.2 (80.6)	0–284.2	<0.001[a]
Outpatient post-index	93			88			
Hospital charges		16.0 (29.7)	0–267.3		8.4 (4.9)	0–27.7	0.17
Physician charges		8.0 (6.6)	0–25.1		6.3 (3.6)	0–12.8	0.29
Total charges		24.0 (33.7)	0–286.9		14.7 (7.6)	0–33.2	0.22
Total post-index	93	143.4 (159.9)	6.2–958.4	88	79.9 (79.8)	0–302	<0.001[a]
Total charges	93	194.5 (164.1)	27.5–982.8	88	150.4 (100.8)	41.4–682.5	0.08

After index admission, patients who died during index admission (13 in the EST arm and 17 in the EPCS arm) were excluded

[a] Statistically significant

charges per patient were lower in the EPCS group, but the differences from the EST group were not significant except in Child's class C. However, when charges were related to the more meaningful index of years of required treatment, mean total charges per year were significantly lower in patients randomized to EPCS than in those randomized to EST in all Child's classes (p=0.004 to <0.001). Since the EPCS patients lived much longer than the EST patients, the direct costs were spread over significantly more years and, therefore, the direct cost per year were much lower.

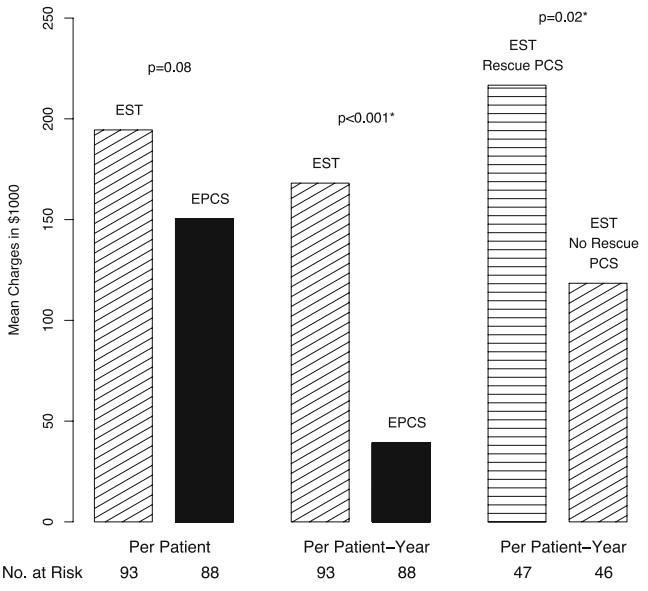

Fig. 2 Total charges for patients randomized to endoscopic sclerotherapy (EST; n=106) or emergency portacaval shunt (EPCS; n=105) treatment of acutely bleeding esophageal varices

Table 5 and Fig. 2 compare the charges made to the 50 patients who failed EST and then underwent a rescue PCS, with the charges made to EST patients who did not have a rescue PCS and, additionally, with the charges made to patients in the EPCS group. Compared to EST patients who did not undergo a rescue PCS, mean total charges in patients who needed a rescue PCS were significantly higher per patient (p<0.001) and per year (p=0.02). Moreover, rescue PCS in the EST group was significantly more costly than EPCS per patient (p<0.001) and per year (p<0.001).

Discussion

In the San Diego BEV Study, we did not determine actual costs of care but used charges as an indicator of costs. After meetings with hospital administrations at UCSD Medical Center and the referring hospitals, it was made clear that obtaining actual cost data over the long follow-up period would not be possible. It was recognized that, as Finkler has pointed out, "on average charges must exceed costs because of the need for expansion and replacement of equipment and facilities,...to cover care to the indigent and courtesy care; costs of community service; and items disallowed by Blue Cross, Medicare, and Medicaid".[7] However, the purpose of our RCT was not so much to determine the true costs of emergency treatment but to

Table 3 Cost of care charges related to time

Charges per day or per year in $1,000	EST			EPCS			*P* value
	N	Mean (SD)	Range	*N*	Mean (SD)	Range	
Index admission (per day)	106			105			
Hospital charges		3.941 (2.45)	0.83–16.98		5.60 (5.85)	1.98–52.06	<0.001[a]
Physician charges		0.66 (1.06)	0.17–10.61		1.05 (1.21)	0.16–7.28	<0.001[a]
Total charges		4.60 (2.99)	1.04–19.28		6.65 (6.83)	2.41–58.11	<0.001[a]
Readmission post-index (per year)	93			88			
Hospital charges		88.4 (210.7)	0–1642		20.4 (48.2)	0–262.3	<0.001[a]
Physician charges		12.4 (26.8)	0 180.6		2.6 (5.9)	0–35.8	<0.001[a]
Total charges		100.8 (235.7)	0–1832		23.0 (53.6)	0 298.1	<0.001[a]
Outpatient post-index (per year)	93			88			
Hospital charges		4.9 (6.1)	0–34.3		1.3 (1.2)	0–7.5	<0.001[a]
Physician charges		1.9 (2.9)	0–14.7		0.8 (0.5)	0–2.7	<0.001[a]
Total charges		7.8 (8.4)	0–48.4		2.1 (1.5)	0–9.5	<0.001[a]
Total post-index (per year)	93	108.5 (236.8)	1.5–1824.0	88	25.1 (54.0)	0–302.1	<0.001[a]
Total charges (per year)	93	168.1 (320.2)	2.9–1954	88	39.4 (70.4)	2.6–374.5	<0.001[a]

After index admission, patients who died during index admission (13 in the EST arm and 17 in the EPCS arm) were excluded

[a] Statistically significant

compare EST versus EPCS. In that comparison, use of charge data was valid and meaningful. Patients in both groups received identical charges for all given items of care such as room rate, ICU rate, laboratory tests, endoscopy, and so on.

Another aspect of the cost of care which we did not measure was in the important category of indirect costs due to mortality and morbidity. These consist mainly of loss of earnings due to premature death and loss of earnings due to days lost from work, including days lost from full-time housekeeping for women. Indirect costs represent a substantial fraction of the costs of illness and can be measured by using life tables and data produced by the U.S. Bureau of the Census and the US Bureau of Labor Statistics as we have done in the past.[8,9] Because EST, compared to EPCS in our RCT, had a significantly lower survival rate, shorter length of survival, and poorer quality of life due to recurrent BEV, recurrent PSE, and the need for repeated hospitalization, there can be no doubt that indirect costs of care were substantially higher in patients treated by EST than in those who underwent EPCS.

Only four studies of the presumed direct costs of emergency treatment of BEV have been reported, all of them involving small numbers of selected patients who underwent short follow-up. None of the studies determined the actual costs of care attributable to each individual patient. In 1984 and 1987, Cello and associates reported the results of a short-term RCT of emergency treatment of BEV conducted at a county general hospital.[10,11] EST (n=32) was compared with EPCS (n=32) in highly selected patients with what they defined as Child's class C cirrhosis. We have commented previously on this trial.[2,3,12] Almost half of the patients died during the index hospitalization. Charges for healthcare were used as a surrogate for costs, and details for obtaining charge data were sketchy. Cello and associates reported that healthcare charges were similar in the EST and EPCS treatment groups.

In 1997, Cello and associates reported the results of a short-term RCT of emergency treatment of BEV in which EST (n=25) was compared to TIPS (n=24) in highly selected patients admitted to three hospitals, namely, a county general hospital, a veterans administration hospital, and a university teaching hospital.[13] Follow-up information was obtained through face-to-face interviews, telephone interviews, or retrospective chart reviews. The costs of healthcare were determined but, considering the mode of follow-up, the data were incomplete. Moreover, the three hospitals differed in the assessment of hospital costs and professional fees, if any. The authors concluded that healthcare costs did not differ significantly between the two treatment groups.

In 1997 and again in 2003, Rosemurgy and colleagues reported the results of a RCT in which TIPS and H-graft portacaval shunt were compared in selected patients, most of whom were treated electively.[14,15] In the 1997 report only eight patients underwent emergency therapy and in the 2003 report, which was restricted to patients in Child's class C, only 13 patients were randomized to emergency care. Charges were used as a proxy for costs and a number of significant charges were excluded from the analysis. The

Table 4 Cost of care charges by Child's class

Charges per day or per year in $1,000	EST			EPCS			*P* value
	N	Mean (SD)	Range	*N*	Mean (SD)	Range	
Index admission—total							
Child's class A	32	42.6 (31.7)	13.3–145.0	26	57.6 (21.2)	36.2–146.5	<0.001[a]
Child's class B	46	48.9 (37.6)	8.1–177.5	50	81.8 (62.8)	33.7–380.5	<0.001[a]
Child's class C	28	91.0 (87.7)	21.1–458.5	29	97.5 (72.8)	37.7–337.7	0.25
Post-index—total							
Child's class A	31	151.1 (158.6)	13.5–693.8	25	87.5 (74.6)	12.5–283.5	0.16
Child's class B	44	144.4 (183.4)	6.2–958.4	45	77.9 (84.3)	0–302.0	0.015[a]
Child's class C	18	127.8 (91.8)	15.9–295.6	18	74.1 (78.8)	10.0–281.6	0.037[a]
Total charges per patient							
Child's class A	31	193.5 (159.0)	42.9–712.5	25	145.9 (76.5)	50.3–336.3	0.56
Child's class B	44	187.8 (182.2)	27.5–982.8	45	149.2 (111.9)	41.4–682.5	0.32
Child's class C	18	212.6 (129.5)	66.5–563.4	18	159.6 (105.7)	63.4–387.4	0.17
Index admission total/day							
Child's class A	32	3.75 (1.07)	2.25–6.27	26	4.69 (1.20)	2.41–7.61	0.003[a]
Child's class B	46	4.55 (3.28)	1.04–19.28	50	5.94 (4.49)	2.89–33.44	0.001[a]
Child's class C	28	5.65 (3.68)	2.11–16.33	29	9.64 (11.09)	2.86–5811	0.080
Post-index total per year							
Child's class A	31	53.1 (85.0)	1.8–352.3	25	16.1 (25.5)	1.4–125.1	0.003[a]
Child's class B	44	103.3 (159.9)	3.3–736.4	45	28.0 (56.7)	0–251.8	<0.001[a]
Child's class C	18	216.7 (456.1)	1.5–1824.0	18	30.5 (74.3)	1.5–302.1	<0.001[a]
Total charges per year							
Child's class A	31	71.2 (100.1)	2.9–437.6	25	26.0 (34.5)	4.9–170.8	0.004[a]
Child's class B	44	187.2 (320.2)	8.0–1674.0	45	44.0 (76.3)	2.6–337.9	<0.001[a]
Child's class C	18	288.3 (494.3)	10.5–1954.0	18	46.3 (90.9)	5.5–374.5	<0.001[a]

[a] Statistically significant

authors concluded in both reports that there was no significant difference in charges for care between the two forms of treatment.

In 1999, Gralnek and colleagues reported the economic impact of endoscopic therapy of BEV in a RCT that compared EST versus endoscopic variceal ligation (EVL) in selected patients who had only 1 year of follow-up.[16] Only 21 patients in the study underwent emergency treatment, and three of these were lost to follow-up, leaving only 16 patients for analysis of the direct costs of emergency care. The patients were treated at a veterans hospital and a university teaching hospital, but the distribution of patients among these two facilities in which the costs of care were undoubtedly different, was not provided. Direct costs for all 16 patients were estimated from the "UCLA estimated institutional combined fixed and variable costs for each of the services or procedures adjusted to the 1995–96 rate." Professional fee reimbursement was estimated using the 1996 AMA CPT codes and Medicare fee schedule. The actual costs engendered by individual patients were not determined. The authors concluded that median total direct costs and resource utilization were similar between EST and EVL.

At least 15 studies have been reported in which hypothetical models have been used to estimate cost of care of elective treatment aimed at primary prevention or secondary prophylaxis of BEV. Several recent publications have summarized these hypothetical studies.[17–19] None of these studies have included emergency treatment or therapy by surgery or TIPS. The Markov model and an event simulation model have been used most widely to calculate costs of healthcare.[20] The validity of these studies as a means of determining costs of healthcare is questionable because the calculations are based on a number of assumptions extracted from selected studies in the literature performed by other workers. Conclusions are the result of calculations, not personal observations, and are dependent on the accuracy of the reported observations of others.

There has been one recent RCT conducted by Henderson and colleagues that compared TIPS and distal splenorenal shunt and included cost of care calculations.[21,22] The RCT involved 140 highly selected patients with well-

Table 5 Cost of care charges in EST patients who had rescue portacaval shunt

Charges in $1,000	EST (rescue shunt)			EST (no rescue shunt)			EPCS			*P* value (EST vs. rescue)	
	N	Mean (SD)	Range	*N*	Mean (SD)	Range	*N*	Mean (SD)	Range	No rescue	EPCS
Charges per patient											
Index admission—total	50	76.7 (70.9)	9.4–458.5	56	41.6 (34.6)	8.1–172.6	105	80.2 (60.0)	33.7–380.5	<0.001[a]	0.20
Post-index—total	47	192.6 (198.5)	11.2–958.4	46	93.1 (82.7)	6.2–394.9	88	79.9 (79.8)	0–302.0	0.002[a]	<0.001[a]
Total charges per patient	47	263.6 (192.9)	27.5–982.8	46	100.7 (83.6)	32.3–411.7	88	150.4 (100.8)	41.4–682.5	<0.001[a]	<0.001[a]
Charges per day or year											
Index admission total/day	50	4.799 (2.813)	1.04–17.70	56	4.421 (3.156)	1.94–19.28	105	6.653 (6.831)	2.44–58.11	0.052[a]	0.009[a]
Post-index total per year	47	148.1 (308.4)	1.50–1824.0	46	68.1 (118.7)	1.8–736.4	88	25.1 (54.0)	0–302.1	0.057	<0.001[a]
Total charges per year	47	216.7 (397.1)	8.0–1954.0	46	118.4 (208.3)	2.9–1041.0	88	39.4 (70.4)	2.6–374.5	0.021[a]	<0.001[a]

Assessed for eligibility (n=258)

[a] statistically significant

compensated cirrhosis (Child-Pugh score of nine or less) who were admitted to five centers that were geographically distant from each other. The patients underwent TIPS or distal splenorenal shunt aimed at preventing variceal rebleeding. Nine hundred nine patients (85%) were excluded from the RCT and 33 refused to participate. Actual costs of care, or charges, or reimbursements were not determined. Cost analysis used diagnosis-related groups (DRG)-based costs for inpatient events and current processing terminology procedures (CPT)-based costs for outpatient events. National Medicare reimbursements from year 2003 were used for each DRG, inflated to year 2004 costs using the medical care inflation index. It was noted that a specific DRG for TIPS did not exist and had to be estimated based on Cleveland Clinic data applied to all five centers. Clearly, all costs of care were not included in the calculations. The authors concluded that there was no overall significant difference in the cost of managing these selected good-risk patients with either TIPS or distal splenorenal shunt.

The results of our RCT of emergency treatment of acute BEV followed by 9.4 to 10 years or more of follow-up indicate that direct costs of care as reflected by charges for all aspects of care were significantly lower in patients treated by EPCS than in those treated by EST. Overall charges per patient, charges per year of treatment, and charges per year in each Child's risk class were significantly lower in patients with BEV randomized to EPCS than in patients randomized to emergency followed by long-term repetitive EST.

Of particular note were the charges in patients who failed EST and underwent a rescue PCS. Charges for such patients were significantly higher than the charges required by EST patients who did not have a rescue shunt as well as by the patients who underwent EPCS. This finding is noteworthy because the main use of surgical shunts in recent years in the USA and abroad has been as elective rescue treatment for failure of endoscopic therapy and other forms of treatment of esophageal varices. Our study indicates that such use of surgical shunts is not only substantially less effective than EPCS, but also is much more costly.

The reasons why EPCS was less costly than EST are very likely a consequence of differences in effectiveness of emergency treatment of BEV. The most important determinants of effectiveness of therapy are survival rate, control of bleeding, and incidence of recurrent PSE. As we have observed in our recent reports, compared to EST, EPCS produced a significantly greater survival rate, was much more effective in controlling bleeding, and was followed by less than one half the incidence of PSE.

The effects of EPCS and, therefore, the significantly lower charges for care, were the result of several critical aspects of care in our RCT: (1) simplification of the diagnostic workup so that emergency diagnosis was

accomplished entirely at the bedside in a mean 4.4 h; (2) development of an organized system of pre- and post-therapy care; (3) a rigorous, lifelong program of follow-up with intensification of efforts to obtain dietary protein control and abstinence from alcohol; and (4) permanent (99%) long-term patency of the portacaval shunt.

As a final note, comment is warranted regarding the use of EST rather than EVL in this RCT, and the absence of TIPS. Our use of EST has received strong support from studies published in 2003, 2005 and 2006 that have questioned replacement of EST by EVL.[23–26] We discussed this issue and the justification for our use of EST in our recent publications.[2,4] It is noteworthy that currently, in our four-county community of 8.5 million people, gastroenterologists with whom we have had regular and frequent contact use EST more frequently than EVL. At the time when EVL was introduced at our institution we were well into our RCT and made the decision not to change from EST to EVL.

With regard to TIPS, which was popularized long after our RCT was initiated, it has become the most widely used procedure of choice when it is believed that portal decompression is needed. However, as we have pointed out previously, TIPS has a high rate of stenosis and occlusion, a resultant high incidence of PSE, and limited durability. The TIPS occlusion rate has been reduced by the recent introduction of the polytetrafluorethylene-coated stent, but the rates of occlusion and PSE are still much higher than the incidences of these serious complications following portacaval shunt in all of our studies. Recently, we completed a RCT comparing TIPS and EPCS and are in the process of analyzing the data for publication.

Conclusions

In conclusion, in this RCT of emergency treatment of acute BEV in 211 unselected, consecutive patients with cirrhosis of all grades of severity, EPCS resulted in significantly lower charges for all aspects of care, even when failure of EST to control bleeding was treated by rescue PCS as salvage therapy. Charges for EPCS were substantially lower overall, as well as in relation to days or years of survival, and in each Child's class. While charges are not identical to actual costs, and indirect costs were not determined, it is reasonable to conclude that the actual costs of EPCS, both direct and indirect, were significantly lower than the costs of EST. When added to the other demonstrated benefits of EPCS, specifically a higher and longer survival rate, markedly better control of bleeding, and significantly lower incidence of recurrent PSE, the results of our analysis of healthcare charges provide support for the use of EPCS as a first line of emergency treatment of BEV. Moreover, the results of this RCT raise questions about the widespread practice of using surgical portal-systemic shunt mainly or only as salvage therapy for failure of other modalities of therapy.

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ORIGINAL ARTICLE

Functional Polymorphisms Associated with Disease-Free Survival in Resected Carcinoma of the Esophagus

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Abstract
Purpose The aim of this study was to determine whether clinical outcome after surgical resection of esophageal adenocarcinoma (EAC) or esophageal squamous cell carcinoma (ESCC) could be predicted by functional polymorphisms in different proto-oncogenes and tumor suppressor genes.
Experimental Design Six single nucleotide polymorphisms (SNPs) in the *AURKA* (rs2273535), *ERBB2* (rs1136201), *MDM2* (rs2279744), *CDH1* (rs5030625), *CDKN2A* (rs11515), and *TP73* (rs2273953) genes were genotyped in a consecutive cohort of 346 esophageal cancer patients, who had underwent surgical resection with curative intent. Associations with disease-free survival (DFS) were analyzed with Kaplan–Meier curves and Cox regression, adjusting for potential confounders.
Results Univariate analysis showed no significant associations between the tested polymorphisms and DFS in patients with EAC or ESCC. However, in a multivariate analysis, patients with EAC carrying the heterozygous *MDM2* (rs2279744) T/G genotype had significantly improved DFS compared with patients carrying the wild-type genotype (adjusted hazard ratio (AHR), 0.63; 95% confidence interval (CI) [0.45–0.88]). Patients with EAC harboring the homozygous *CDH1* (rs5030625) GA/GA genotype had a significantly reduced survival as compared with patients carrying the wild-type genotype AHR 4.0, 95% CI [1.4–11].
Conclusions In a large cohort of esophageal cancer patients, the *MDM2* T/G and *CDH1* GA/GA genotype confer risk of death in patients with EAC. These data suggest that inter-individual differences in germ-line DNA have an impact on DFS in patients with EAC.

Keywords Esophagus · Adenocarcinoma · Squamous cell carcinoma · Polymorphism · SNP

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Introduction

Many therapeutic options are used to treat esophageal cancer, but traditionally, surgery is used most frequently to obtain loco-regional control and long-term survival.[1,2] Comprehensive preoperative staging has improved selection of patients for potentially curative surgery; however, many patients present with recurrent disease within 2 years after operation. The majority of these patients develop not only loco-regional recurrences, but also distant metastases (such as liver, lung, pleural, and/or peritoneal disease recurrences) are common.[3–5] Despite attempts to improve the outcome of patients with esophageal cancer, prognosis remains poor with a 5-year overall survival of 20–30%.[5,6]

Well-known prognostic factors for esophageal cancer are summarized in tumor node metastasis (TNM) staging.[7,8] Although TNM parameters have the advantage of simplic-

ity, they do not seem to completely reflect the biologic diversity of esophageal cancer.[9,10] The true drivers of this clinical biologic diversity include the molecular aberrations of the cancer and the genetic make-up of the patient. In this respect, the study of host genetic variability offers worthwhile potential to identify individuals that may have the best chance of survival. Single nucleotide polymorphisms (SNPs) in the germ-line are the most common type of host genetic variations. Gene-related functional SNPs can potentially lead to differences in protein expression and/or function. In this way, SNPs in proto-oncogenes and tumor suppressor genes can potentially alter the risk for metastatic or aggressive tumor, resulting in differences in clinical outcome.

Altered expression of the AURKA, ERBB2, MDM2, CDH1, CDKN2A, and TP73 proteins has been correlated to disease progression and clinical outcome in patients with esophageal cancer.[11–17] In addition, polymorphisms with effects on protein function have been identified in these proto-oncogenes and tumor suppressor genes.[18–23] Based on these results, we postulated that functional SNPs in the *AURKA* (*AURKA*_NM_003600.2; rs2273535 c.449 T>A), *ERBB2* (*ERBB2*_NM004448.2; rs1136201 c.655 A>G), *MDM2* (*MDM2*_NM002392.2; rs2279744 309 T>G), *CDH1* (*CDH1*_NM004360.3; rs5030625 -347 G>GA), *CDKN2A* (*CDKN2A*_NM000077.3; rs11515 c.712 C>G), and *TP73* (*TP73*_NM005427.1; rs2273953 81 G>A) genes may serve as molecular markers for clinical outcome in patients with esophageal adenocarcinoma (EAC) or esophageal squamous cell carcinoma (ESCC) who underwent surgical resection.

Patients and Methods

Patients Between 1996 and 2001, a total of 632 consecutive patients with esophageal cancer were evaluated for surgery with curative intent at the Erasmus University Medical Center (Fig. 1). Outcome for all patients with esophageal cancer referred to our hospital are collected

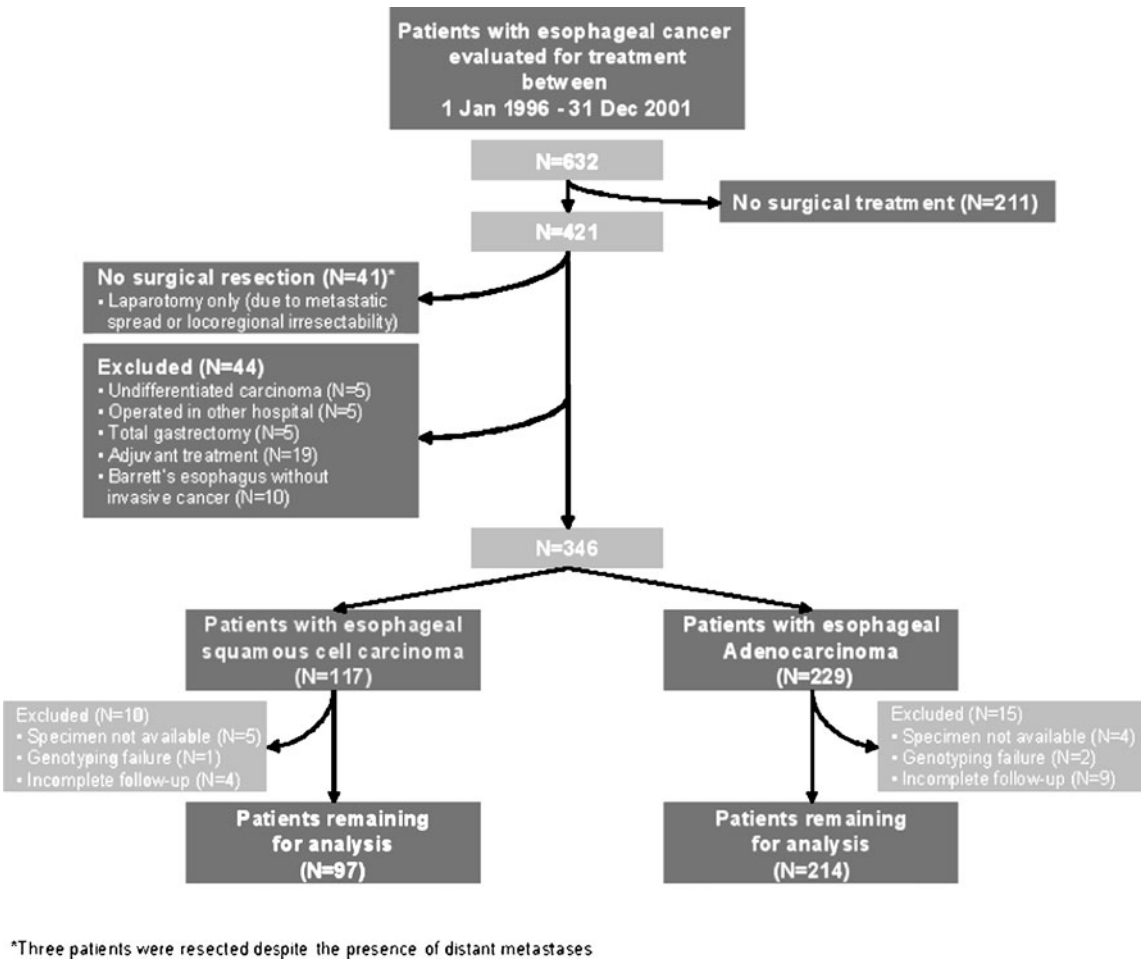

Fig. 1 Flowchart of patients with esophageal cancer referred to the Erasmus MC for treatment between January 1996 and December 2001. Patients excluded from the present study are shown

prospectively and stored in a database by a data manager. The data collected encompassed all relevant diagnostic tests, scheduled treatments, and pathology. All patients were staged using esophago-gastroscopy with biopsies, ultrasonography of the cervical and upper abdominal region, and computed tomography of the thorax and abdomen. Endoscopic ultrasonography for evaluation of T-stage and nodal status was routinely performed.

Surgery For carcinomas of the upper half of the intrathoracic esophagus, a right-sided thoracotomy was performed. For carcinomas of the lower half of the intrathoracic esophagus, a transhiatal esophagectomy was preferred. The tumor and its adjacent lymph nodes were dissected en bloc; however, no extended lymph node dissection was performed. The continuity of the digestive tract was restored by means of a gastric tube reconstruction or colonic interposition with a cervical anastomosis. Resections were considered radical (R0) if microscopic examination revealed no tumor tissue at or less than 1 mm from the circumferential, proximal, or distal margins. Pathological staging was done according to the Union for International Cancer Control (UICC) sixth edition. The tumor stage after resection was classified according to the TNM classification of the International Union Against Cancer.

SNP Genotyping To determine the individual genotype for each SNP, genomic DNA was extracted from frozen or formalin-fixed and paraffin-embedded (FFPE) tissues. Normal tissue was obtained from the resection specimens (i.e., tumor-negative lymph nodes or tumor-negative resection margins). All the archival tissue samples were used according to the code for adequate secondary use of tissue, code of conduct: "Proper Secondary Use of Human Tissue" established by the Dutch Federation of Medical Scientific Societies (http://www.federa.org).

Polymerase chain reactions (PCR) were carried out in a volume of 15 μl containing genomic DNA, 8.3 μl H_2O, 5 μl Mg^{2+}free buffer, 25 mM $MgCl_2$, 0.3 μl of 10 mM deoxynucleotide triphosphates, 20 pmol of each primer, and 1 U Taq polymerase (Promega, Madison WI, USA). PCR conditions were standardized at 35 cycles of 95°C for 45 s, 61°C for 45 s, 72°C for 30 s, with a 10-min extension at 72°C for 10 min following the last cycle. PCR primers for each SNP are shown in Table 1. For the polymorphism in *CDH1* (rs5030625), amplified PCR products were visualized on a denaturing polyacrylamide gel. For detection of the restriction length polymorphisms in *ERBB2* (rs1136201) and *AURKA* (rs2273535), PCR products were digested for 16 h at the appropriate temperature with10 U of restriction endonuclease BsmAI, MspI, or APOI (Promega, Madison, WI, USA), respec-

Table 1 Description of the polymorphisms located in different oncogenes and tumor suppressor genes

Gene	refSNP ID[a]	Change	Minor allele	Minor allele frequency[b]	Potential effect on protein function	Forward primer	Reverse Primer
AURKA	rs2273535	c.449 T>A (Phe>Ile)	A-allele	0.25	Preferentially amplified; faster growth of cultured cells[17]	5′-TCCATTCTAGGCTAC AGCTC-3′	5′-AAGAATTTGAAGGA CACAAGAC-3′
ERBB2	rs1136201	c.655 A>G (Ile>Val)	G-allele	0.16	Increased dimerization, autophosphorylation of HER-2 and tyrosine kinase activity[18]	5′-AGCCCTCTGACGTC CATC-3′	5′-CTGCAGCAGTCTCC GCATC-3′
MDM2	rs2279744	c.309 T>G	G-allele	ND	Associated with higher levels of MDM2 expression[19]	5′-GCGGAGGTTTTGTT GGACTG-3′	5′-CTGAGTCAACCTG CCCACT-3′
CDH1	rs5030625	c.-347 G>GA	GA-allele	0.14	Associated with decreased transcriptional activity[20]	5′-GGCCAGAGGACCG CTTGAG-3′	5′-GTTTGTTCGTTTT GGAGA-3′
CDKN2A	rs11515	c.712 C>G	G-allele	0.13	Potential detrimental effect on RNA stability[21]	5′-CCCCGATTGAAAGA ACCAGAGA-3′	5′-AGGACCTTCGGTGA CTGATGAT-3′
TP73	rs2273953	c.-30 G>A	A-allele	0.19	Influences the p73 translation[22]	5′-GAGCACGAGTTCCC AGGGTG-3′	5′-CCAAGCGCACTCA CAGAGAG-3′

ND not determined

[a] refSNP ID (http://www.ncbi.nlm.nih.gov/SNP)

[b] Minor allele frequency according 102 controls of the SNP500CANCER cohort (http://snp500cancer.nci.nih.gov/snp.cfm)

Table 2 Survival according to patients' and tumor characteristics

Variable	Patients with EAC (*N*=214)			Patients with ESCC (*N*=97)		
	No. (%)	Median DFS	*P* values	No. (%)	Median DFS	*P* values
Age in years			0.23			0.33
<65 years	111 (52)	19		64 (66)	21	
≥65 years	103 (48)	12		33 (34)	12	
Gender			0.41			0.44
Male	182 (85)	16		58 (60)	15	
Female	32 (15)	11		39 (40)	20	
Weight loss before operation			0.013			0.47
No loss or <5%	127 (59)	19		46 (47)	15	
5–10%	35 (16)	11		31 (32)	27	
>10%	32 (15)	8		17 (18)	10	
Not recorded	20 (10)	10		3 (3)	5	
Smoking status			0.98			0.70
Current smoker	54 (25)	14		43 (44)	16	
No current smoker	146 (68)	15		47 (49)	20	
Not recorded	14 (7)	8		7 (7)	4	
Location of tumor			0.150			0.008
Upper one third thoracic esophagus				3 (3)	4	
Middle one third thoracic esophagus	3 (1)	11		39 (40)	12	
Lower one third thoracic esophagus	68 (32)	24		45 (47)	20	
GEJ	86 (40)	13		10 (10)	60	
Gastric cardia	57 (27)	12				
Tumor length (cm)			0.028			0.27
0–2	34 (16)	41		8 (8)	37	
3–4	52 (24)	15		24 (25)	10	
4–5	67 (31)	14		31 (32)	24	
≥6	46 (22)	9		25 (26)	9	
Not recorded	15 (7)	16		9 (9)	11	
Barrett's epithelium			0.086			
No	127 (59)	12				
Yes	87 (41)	24				
Treatment			0.83			0.18
Surgery alone	180 (84)	14		28 (29)	8	
Chemotherapy+surgery	23 (11)	15		65 (67)	15	
Chemoradiotherapy+surgery	11 (5)	21		4 (4)	Not reached	
Resection type			0.83			0.042
Transhiatal	187 (87)	15		53 (55)	37	
Transthoracic	27 (13)	9		44 (45)	11	
Post-operative UICC stage			<0.001			<0.001
Complete response	8 (4)	40		13 (13)	Not reached	
I	24 (11)	98		13 (13)	86	
IIA	43 (20)	37		34 (35)	12	
IIB	8 (4)	15		4 (4)	26	
III	74 (34)	11		17 (18)	8	
IV	57 (27)	7		16 (17)	4	
Radicality of resection			<0.001			<0.001
R0	141 (66)	34		65 (67)	41	
R1	70 (33)	7		29 (30)	6	
R2	3 (1)	9		3 (3)	5	

tively. The DNA fragments were separated using 3% agarose gels. The polymorphisms in *CDKN2A* (rs11515), *MDM2* (rs2279744), and *TP73* (rs2273953) were genotyped by bi-directional sequencing.

Statistical Analysis Data on follow-up were collected from the prospective database and the medical charts. All patients were followed at an interval of 3 to 4 months during the first year, every 6 months for the second year, and then at the end of each year until 5 years after treatment. Recurrence or disease progression was diagnosed on clinical grounds. Whenever a relapse was suspected, radiologic, endoscopic, or histologic confirmation was sought. Recurrent disease was classified as local–regional (occurring in the upper abdomen or mediastinum) or distant (including cervical recurrences).

Study end-point was disease-free survival (DFS) that was defined as the time from surgery until recurrent disease or death from any cause. The Kaplan–Meier survival function and log-rank tests were used to assess clinical outcome in relation to patient's characteristics and individual polymorphisms. Cox proportional hazard ratios for patients with EAC were adjusted for weight loss prior to operation, tumor length, presence of Barrett's epithelium, radicality of resection, and pathological tumor stage. For patients with ESCC, Cox proportional hazard ratios were adjusted for location of tumor, resection type, post-operative TNM stage, and radicality of resection. Statistical significance was set at the 5% level. We did not adjust for multiple testing since each gene outcome was prespecified and of interest in itself.

Results

Patients A total of 346 esophageal cancer patients underwent surgical resection with curative intent. Of these, 25

Table 3 Polymorphisms and clinical outcome in patients with resected EAC

Genotype	Disease-free survival in EAC patients					Disease-free survival in ESCC patients				
	N	MPS (months)	Log-rank *P*	AHR	[95% CI][a]	N	MPS (months)	Log-rank *P*	AHR	[95% CI][b]
*AURKA*_rs2273535										
T/T	129	15	0.83	Reference		62	12	0.72	Reference	
A/T	75	14		1.1	[0.76–1.4]	29	20		0.60	[0.17–2.1]
A/A	9	21		0.92	[0.42–2.0]	5	55		0.63	[0.18–2.1]
*ERBB2*_rs1136201										
A/A	113	14	0.25	Reference		66	12	0.66	Reference	
A/G	86	15		0.92	[0.67–1.3]	23	26		0.73	[0.41–1.3]
G/G	14	12		0.68	[0.33–1.4]	6	8		1.3	[0.49–3.2]
*MDM2*_rs2279744										
T/T	100	11	0.076	Reference		40	10	0.63	Reference	
T/G	84	19		0.63	[0.45–0.88]	45	21		0.98	[0.59–1.6]
G/G	24	12		0.95	[0.58–1.6]	7	16		0.81	[0.28–2.4]
*CDH1*_rs5030625										
G/G	166	17	0.14			68	11	0.13	Reference	
G/GA	41	11		1.2	[0.78–1.7]	18	27		0.63	[0.32–1.3]
GA/GA	4	7		4.0	[1.4–11]	1	Not reached			
*CDKN2A*_rs11515										
C/C	162	13	0.79	Reference		74	12	0.67	Reference	
C/G	47	19		0.94	[0.65–1.3]	20	20		0.67	[0.36–1.3]
G/G	4	19		1.7	[0.52–5.6]	1	Not reached			
TP73_rs2273953										
G/G	138	16	0.44	Reference		62	24	0.48	Reference	
G/A	69	13		0.98	[0.71–1.4]	32	10		1.1	[0.66–1.8]
A/A	5	11		1.1	[0.41–3.1]	2	4		1.7	[0.40–7.3]

[a] Adjusted hazard ratio for weight loss prior to operation, tumor length, presence of Barrett's epithelium, post-operative TNM stage, and radicality of resection

[b] Adjusted hazard ratio for location of the tumor, type of resection, post-operative TNM stage, and radicality of resection

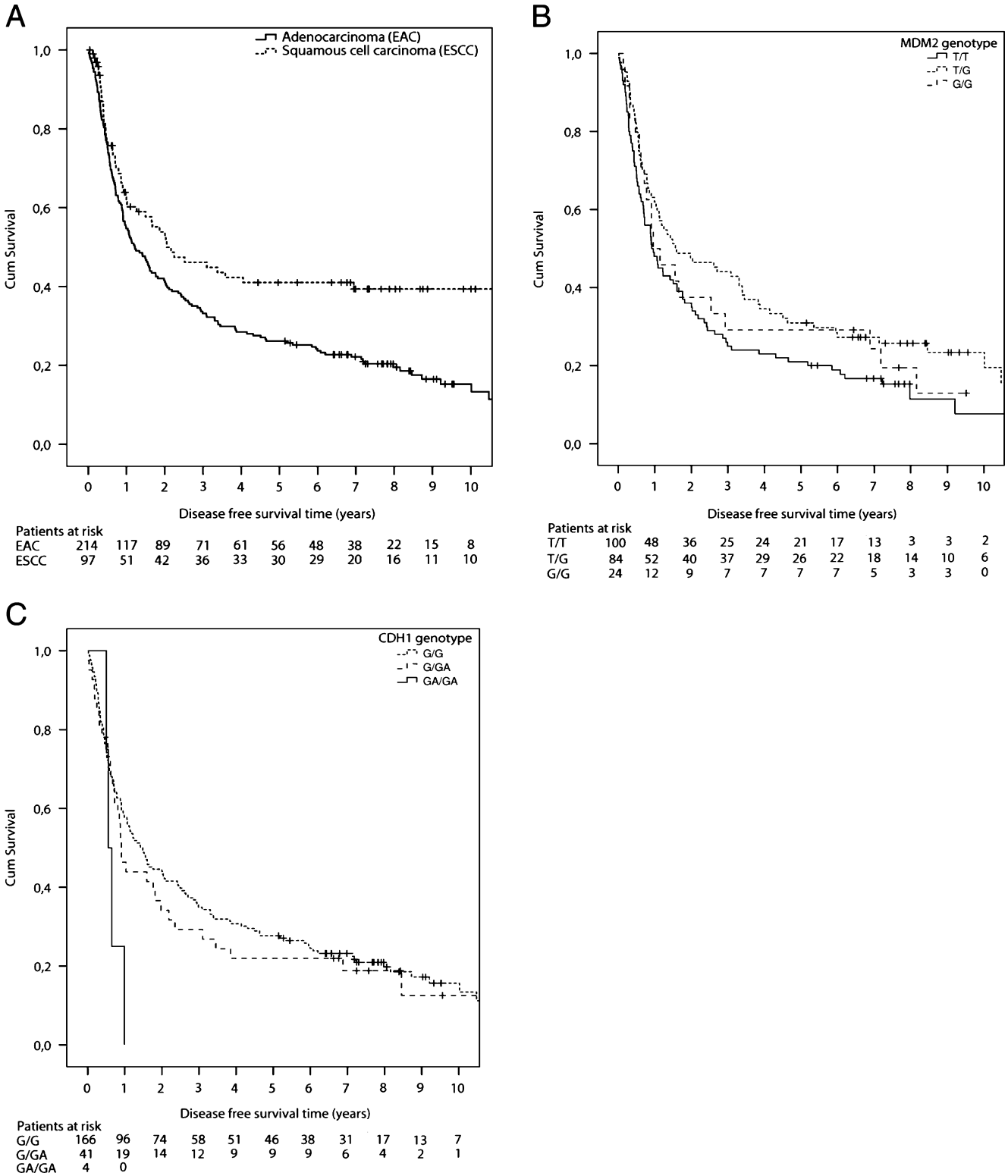

Fig. 2 **a** Kaplan–Meier analysis of DFS in patients with esophageal cancer, by histological subgroup. **b** Kaplan–Meier analysis of DFS in patients with EAC, by MDM2 (rs2279744). **c** Kaplan–Meier analysis of DFS in patients with EAC, by CDH1 genotype (rs5030625)

patients were excluded from the current follow-up study because no tissue samples were available (N=9), genotyping failure (N=3), or incomplete follow-up (N=13) (Fig. 1). Of the 214 EAC and 97 ESCC patients remaining for analysis, the majority were male, 85% in EAC and 60% in ESCC. Median age at time of diagnosis was 64 and 61 years, respectively. Of all patients with EAC, 84% underwent primary surgery and 16% received preoperative

chemotherapy with or without radiotherapy. In contrast, 71% of patients with ESCC received preoperative chemotherapy with or without radiotherapy and 29% underwent primary surgical resection (Table 2).

SNP Genotyping Genotyping was complete in 95% to 100% of EAC and ESCC patients. The genotype distributions did not deviate from HWE ($P>0.05$). The genotype distribution of each SNP is listed in Table 3. Tumor stage distributions were similar across all SNP genotypes, and there was no association between genotypes and age at diagnosis, sex, weight loss, smoking status, or preoperative treatment.

DFS and Pattern of Disease Recurrence The median DFS of EAC patients was 14 months (range, 0.07–138 months) and for ESCC patients 16 months (range, 0.5–148 months). At the time of analysis, 37 (17%) EAC and 27 (28%) ESCC patients were alive with a median DFS time of 93 months (range, 62–138 months) and 104 months (range 79–148 months), respectively (Fig 2A).

The pattern of disease recurrence is depicted in Table 3. Loco-regional recurrences were mediastinal or abdominal lymph node metastases and recurrences in the gastric tube. Distant metastases were found in liver, lung, brain, bone, adrenal gland, pleura, peritoneum, and skin.

Recurrent disease after surgery was found in 138 (78%) EAC patients; 40 patients had loco-regional recurrence, 51 had distant metastasis, and 46 had both loco-regional recurrence and distant metastasis. One patient had disease recurrence, but the site of failure was not recorded. Diseases recurrences were found in 51 ESCC patients; 28 patients had loco-regional recurrence, 10 had distant metastasis, and 10 had both loco-regional recurrence and distant metastasis. Three patients had disease recurrence, but site of failure was not recorded.

SNP Genotype and DFS Univariate analysis showed no significant associations between DFS in patients with EAC or ESCC and the genotype distributions of the *AURKA*, *ERBB2*, *MDM2*, *CDH1*, *CDKN2A*, and the *TP73* gene polymorphisms (Table 4; Fig 2B and C). However, in a multivariate analysis, patients with EAC carrying the heterozygous *MDM2* (rs2279744) T/G genotype had significantly improved DFS compared with patients carrying the wild-type T/T genotype (adjusted hazard ratio (AHR) 0.63, 95% confidence interval (CI) [0.45–0.88], $P=0.007$). The post-operative TNM stage of the tumor and the radicality of resection were also found important factors for DFS (HR 1.4, 95% CI [1.2–1.6], $P<0.0001$ and HR 2.3, 95%CI [1.7–3.1], $P<0.0001$ respectively).

Also, patients with EAC harboring the homozygous *CDH1* (rs5030625) GA/GA genotype had a significantly reduced survival as compared with patients carrying the wild-type G/G genotype AHR 4.0, 95% CI [1.4–11], $P=0.008$. In multivariate analysis, the post-operative TNM stage of the tumor and the radicality of resection were found as important factors for DFS (HR 1.4, 95% CI [1.2–1.5], $P<0.0001$ and HR 2.4, 95%CI [1.7–3.2], $P<0.0001$ respectively).

Table 4 Pattern of failure

	EAC (N=214)	ESCC (N=97)
Alive	37 (17)	27 (28)
Nature of first failure		
Local recurrence	40 (29)	28 (55)
Distant metastases	51 (37)	10 (20)
Local recurrence and distant metastases	46 (33)	10 (20)
Disease recurrence but site of failure not reported	1 (1)	3 (5)
Total deaths	177 (83)	70 (72)
Cause of death		
Cancer-related	138 (78)	51 (71)
Surgery-related	14 (8)	7 (11)
2nd Primary	5 (3)	6 (8)
Death from other cause (not cancer-related)	20 (11)	6 (8)

Discussion

In the present study, we determined the relationship between inter-individual DNA variations in six bona fide proto-oncogenes and tumor suppressor genes and DFS in a large cohort of Caucasian patients with esophageal cancer. After adjustment for potential confounders, the variant genotypes of SNPs located in the promoter region of the *MDM2* and *CDH1* gene were significantly associated with DFS in patients with EAC.

The results of the present study showed a significant survival benefit for patients harboring the *MDM2* T/G as compared with patients carrying the wild-type T/T genotype. The MDM2 protein is a nuclear phosphoprotein that binds and inhibits the tumor suppressor TP53 as part of an autoregulatory negative feedback loop. The most intensively characterized *MDM2* polymorphism is the T309G promoter SNP located in the first intron.[20] The G variant of this SNP is known to increase promoter-binding affinity, leading to up-regulation of MDM2 and consequent inhibition and down-regulation of the p53 pathway. Therefore, it could be expected that the variant *MDM2* genotypes (T/G and G/G)

are associated with adverse outcome in esophageal cancer patients (as shown in other cancer types).[24] However, the present study showed improved survival in patients with the *MDM2* T/G genotype compared with the wild-type T/T genotype. A possible explanation for our findings is provided by a large study in breast cancer patients that reported strong interaction between the *MDM2* SNP status and tumor TP53 status, which appeared to modify the association between TP53 status and breast cancer survival.[25] Among breast cancer patients with the wild-type *MDM2* genotype (T/T), a mutant TP53 status and aberrant TP53 expression in breast tumors were associated with poor survival. The tumor TP53 status was not associated with breast cancer survival among carriers of the variant *MDM2* allele (T/G or G/G). Since TP53 is the most frequently mutated gene in EAC, it could be hypothesized that the tumors of most patients with the T/T genotype harbor a TP53 mutation, which could lead to a reduced survival as observed in the present study. In a previously well-conducted study, the known *TP53* codon 72 Arg/Pro and *MDM2* polymorphisms were genotyped in 300 patients with EAC and 63 patients with ESCC.[26] As in concordance with the results of the present study, patients with EAC harboring the *MDM2* T/G genotype had a borderline improved overall survival as compared with patients carrying the wild-type genotype (AHR for death 0.70, 95% CI [0.50–0.99], $P=0.04$). But unlike the present study, the *MDM2* variant genotype did correlate with marked reduced survival in patients with ESCC. This could be due to differences in study samples size, population selection, tissue handling, and genotyping methods.

In this study, patients carrying the CDH1 GA/GA genotype had a significantly reduced survival as compared with patients with the wild-type G/G genotype. However, it should be noted that only four EAC patients carried the GA/GA genotype, which may represents a chance finding. Nevertheless, this -347 G/GA insertion polymorphism located in the promoter of the cell–cell adhesion gene *CDH1* has been reported to suppress *CDH1* gene expression and was found to be associated with familial gastric cancer and sporadic colorectal cancer.[27] The GA-allele has been associated with significant suppression of CDH1 promoter activity in colorectal and gastric cancer cell lines.[27] It can be hypothesized that the GA-allele might enhance the progression of esophageal cancer by reducing *CDH1* transcription resulting in a decrease in CDH1 protein expression and impairment of cell–cell adhesion. All four patients harboring the GA/GA genotype died of recurrent disease; three had loco-regional and distant metastasis, and one had only loco-regional disease recurrence.

To our knowledge, this is one of the first studies that investigated the relationship between polymorphisms and esophageal cancer outcome. Here, we studied (only) six polymorphisms, whereas SNP arrays can determine more than a million of SNPs in one sample. Although this technique is widely used on blood or fresh frozen samples, it is not very suitable for FFPE tissue samples (our series consisted primarily of FFPE samples). Therefore, collection of blood samples or fresh frozen tissue samples of esophageal cancer patients is necessary and should become standard procedure in order to perform genome-wide association studies. In this study, the majority of polymorphisms were not associated with DFS after esophagectomy. It could be well that our study, among a relatively large population ($N=346$) of esophageal cancer patients, failed to observe a difference due to under powering. Since esophageal cancer has a relatively low incidence, consortia (of multiple hospitals) are needed to validate these associations.

Recurrent cancer is the leading cause of death in patients undergoing surgical resection.[3–5] Although treatment options for esophageal cancer recurrences are limited, it could be proposed that early detection of recurrent disease is desirable because aggressive treatment may result in prolonged tumor-free survival or occasional cure. In this light, our findings could contribute to the identification of patients who are at high or low risk for the development of disease recurrences. It can also be proposed that patients with a certain genetic constitution that is associated with a high chance of (distant) disease recurrence should be given systemic adjuvant treatment after surgical resection. Furthermore, identification of polymorphisms associated with DFS could serve well as hypothesis generating for prospective studies that evaluate the prognostic significance of germ-line variants.

In summary, our results indicate that two of six investigated functional polymorphisms are associated with DFS in patients who underwent esophagectomy for EAC. Patients with EAC carrying the heterozygous *MDM2* T/G genotype had twofold reduced risk of disease recurrence, and patients with the homozygous *CDH1* GA/GA had a fourfold increased risk of disease recurrence. Additional prospective studies are necessary to validate both associations and to study the prognostic significance of both germ-line variants.

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Conflicts of Interest There are no conflicts of interest.

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ORIGINAL ARTICLE

A Case–Control Study of Laparoscopy-Assisted and Open Distal Gastrectomy for Advanced Gastric Cancer

Jianbo Shuang · Shengbin Qi · Jianyong Zheng · Qinchuan Zhao · Jipeng Li · Zhenghua Kang · Jin Hua · Jianjun Du

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Abstract
Background The application of laparoscopy-assisted gastric surgery has been increasing rapidly for the treatment of early gastric cancer. However, there were few reports of laparoscopic surgery in the management of advanced gastric cancer (AGC), especially with T3 depth of invasion. The aim of this study was to compare the technical feasibility and oncologic efficacy of laparoscopy-assisted distal gastrectomy (LADG) versus open distal gastrectomy (ODG) for advanced gastric cancer.
Methods A retrospective case–control study was performed comparing LADG and ODG for AGC. Thirty-five consecutive patients with AGC undergoing LADG between August 2005 and December 2007 were enrolled and these patients were compared with 35 AGC patients undergoing ODG during the same period.
Results Forty-two (60.0%) patients were T3 in terms of depth of invasion. Tumor location and histology were similar between the two groups. Operation time was significantly longer in the LADG group than in the ODG group. Estimated blood loss was significantly less in the LADG group. Hospital length of stay after LADG was significantly shorter than in the open group. Postoperative pain was significantly lower for laparoscopic patients. There were no significant differences in postoperative early and late complication and in the number of lymph nodes retrieved between the two groups, and the cumulative survival of the two groups was similar.
Conclusion Our data indicate that LADG for AGC, mostly with T3 depth of invasion, yields good oncologic outcomes including the similar early and late complication and the cumulative survival between the two groups after 50 months of follow-up. To be accepted as a choice treatment for advanced distal gastric cancer, well-designed prospective trial to assess long-term outcomes is necessary.

Keywords Laparoscopy-assisted distal gastrectomy · Open distal gastrectomy · Advanced gastric cancer · Case–control study

Introduction

Laparoscopic gastrectomy is revolutionizing surgery in the world, especially in the east for the high incidence of gastric cancer. Reports of laparoscopic techniques for treating patients with early gastric cancer in the world literatures have shown oncologic equivalency to the open technique, with the known benefits of minimally invasive approach, including less pain, earlier recovery, shorter hospital stay, and better quality of life.[1–3] However, application of laparoscopic techniques for advanced gastric cancer (AGC) remains controversial because of the technical difficulty of extraperigastric lymphadenectomy, possibility of peritoneal or port site seeding of malignant cells, and insufficient data related to the procedure's oncologic adequacy, especially for patients with T3 depth of invasion.[4,5] Moreover, according to reports about laparoscopic surgery for AGC, the depth of invasion was mostly limited to T2 and T3 was rarely concerned.[6,7]

In the present study, we described our experience with laparoscopy-assisted distal gastrectomy (LADG) in the

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treatment of AGC, most of which had T3 depth of invasion, and evaluated the oncologic safety of this approach through a case–control study.

Patients and Methods

From August 2005 to December 2007, we performed 364 gastrectomies for gastric cancer at our hospital. Of these patients, laparoscopy-assisted technique for D2 radical distal gastrectomy was carried out in 36 patients with AGC (9.6%). Surgical procedures were performed after obtaining informed consent following the explanation of the advantages, disadvantages, and any possible outcomes of LADG and open distal gastrectomy (ODG) in detail, and the surgical procedure (LADG or ODG) was chosen by the patients. Inclusion criteria were as follows: histologically confirmed adenocarcinoma of the stomach, performance status of ECOG 0-1, location of the tumor in the lower third of the stomach, no evidence of distant metastasis or invasion to adjacent organs, and confinement in the serosal layer (T3). We assessed the depth of invasion preoperatively by means of endoscopy and endoscopic ultrasonography and assessed the presence or absence of lymph node metastases using extracorporeal ultrasonography and computed tomography. One patient was excluded because of conversion to open gastrectomy due to T4 depth of invasion. Thus, 35 patients who underwent LADG were enrolled and compared with 35 patients who underwent open distal gastrectomy for AGC during the same period. We selected these controls from our computer database and matched them with the laparoscopic group for age, gender, and stage of gastric cancer. Follow-up data were obtained from patients' office records, and computed tomography and endoscopy were performed every 6 months after surgery.

All patients were subjected to follow-up. Statistical analysis was performed using SPSS.v16.0 for Windows (SPSS Inc., Chicago, IL). The statistical analysis was done using Student's t test or chi-square test, and cumulative survival was compared by Kaplan–Meier method and log rank test. Values of $p<0.05$ were considered to indicate significance.

Surgical Procedures

The surgical approach was as follows: Patients in both the lower and middle thirds underwent distal gastrectomy with D2 dissection (Fig. 1),[8] followed by Billroth II reconstruction. Patients were placed in the supine position and subjected to a 20° head-up tilt. After the establishment of a pneumoperitoneum at 12 mmHg, one initial 10-mm camera port was introduced below the umbilicus. The stomach and the peritoneal cavity were inspected to rule out adjacent organ invasion and peritoneal seeding using a 30° forward oblique laparoscope. A 10/12-mm port was inserted percutaneously in the left upper quadrant as a major hand port. A 5-mm trocar was placed at the contralateral site. Another two 5-mm trocars were respectively inserted in both the left and right lower quadrants.

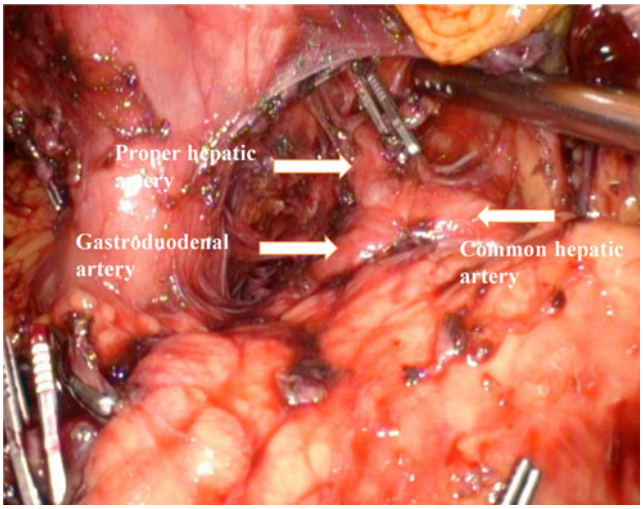

Fig. 1 Laparoscopic image around the proper hepatic artery, common hepatic artery, and gastroduodenal artery after lymph node dissection

The gastrocolic ligament was divided using an ultrasonically activated shear along the border of the transverse colon, thus including the greater omentum in the specimen to be resected. The dissection moved to the hepatic flexure and the pylorus. The right gastroepiploic vein was divided between titanium clips flush with the Henle's trunk and ended up in the Fredet area, where group 14v was removed. Right gastroepiploic artery was vascularized and cut at its origin from the gastroduodenal artery with titanium clips, just above the pancreatic head, to dissect group 6. The stomach was lifted headward to expose the gastropancreatic fold. The left gastric vein was carefully prepared and separately divided at the upper border of pancreatic body and then the left gastric artery was vascularized to remove group 7. The lymph nodes along the proximal splenic artery (group 11p) were removed. Subsequently, the dissection was continued rightward along the artery to remove the nodes along the celiac axis and the common hepatic artery (group 9, 8a) by retraction on left artery. The left gastric artery was cut between titanium clips at its origin from the celiac axis. The right gastric artery was divided at its origin from the common hepatic artery to dissect group 5. Along the border of liver, the lesser omentum was dissected and the lymph nodes of the anterior region of the hepatoduodenal ligament (group 12a) were dissected and removed. The first part of the duodenum was dissected and then

transected 2 cm below the pylorus with a 45-mm laparoscopic cartridge linear stapling device (endo-GIA, US Surgical Corporation, Norwalk, CT, USA) through a major hand port. The dissection of the gastrocolic ligament was continued toward the spleen with the removal of group 4sb; all short gastric vessels (group 4sa) were divided close to the spleen. Before gastric transection, the cardiac nodes were dissected en bloc including right cardiac nodes (group1) and left cardiac nodes (group 2). Two surgical instruments were used for such bit and precise dissection involved in ultrasonic devices (Ultracision–Harmonic Scalpel; Ethicon Endo-Surgery, Cincinnati, OH) and electrocautery. A small laparotomy incision was made under the xyphoid (5–7 cm). Gastrectomy and Billroth II anastomosis were extracorporeally performed using hand-sewn method. The specimen was pulled out of the peritoneal cavity through the small laparotomy incision.

For open procedure, approximately 15- to 20-cm length incision was made from falciform process to periumbilical area. Distal gastrectomy and D2 lymph node dissection were performed basically. Billroth II method was used for gastric reconstruction.

Results

Patient Demographics

Of the 70 case-matched patients evaluated, ten patients (14%) were women, with median age of 59 years. Median body mass index (BMI) for the laparoscopic group was 21 kg/m^2 (range 18–30 kg/m^2) compared with 23 kg/m^2 (range 16–28 kg/m^2) among the open surgery group. Patients from the laparoscopic group underwent a lower number of prior abdominal operations compared with the open group (14.3% versus 25.7%). All patients of the two groups did not undergo neoadjuvant chemotherapy (Table 1).

Operative Characteristics and Complications

The patients in both groups underwent distal gastrectomy with a Billroth II anastomosis. The median operative time was 320 min (range 260–570 min) for the laparoscopic procedure compared with 210 min (range 138–300 min) for the open procedure ($p<0.01$). The median blood loss in the laparoscopic group was 200 ml (range 100–600 ml) compared with 300 ml (range 100–1,000 ml) in the open group ($p<0.05$). The patient hospital length of stay after laparoscopic gastrectomy was 12 days (range 5–36 days) compared with 17 days (range 8–45 days, $p<0.01$). Postoperative pain, as measured by number of days of IV narcotic use, was significantly lower for laparoscopic patients, with a median of 3.0 days (range 0–5 days) compared with 4.0 days (range 1–6 days) in the open group ($p<0.01$, Table 2). No significant difference was observed between the two groups in the postoperative early complications (up to 30 days). Total early complications in the laparoscopic group were found in 2 of 35 patients, including wound infection ($n=1$) and pancreatitis ($n=1$). In the open group, complications occurred in 3 of 35 patients, including wound infection ($n=2$) and wound dehiscence ($n=1$). There were no late complications observed in the two groups.

Pathologic Characteristics

Pathology analyses were reviewed by a pathologist specializing in gastrointestinal diseases. Reports revealed no differences in histological type and tumor location among patients of both groups. Twenty-six patients of the laparoscopic group have tubular adenocarcinoma compared with 28 patients of the open group. In the laparoscopic group, the tumor locations of 21 and 14 patients were located in the body and antrum, respectively, compared with 22 and 13 patients in the open group. No positive resection margins were found in all of the resected specimens. The median number of lymph nodes resected following D2 dissection for laparoscopic surgery was 35 (range 7–63) compared with 38 (range 6–66) resected through open surgery (p=NS, Table 3). There was no significant difference between the two groups in depth of invasion, and 24.3%, 15.7%, and 60.0% of all the patients were T2A, T2B, T3, respectively (Table 4). There were no significant differences in extent of tumor invasion, pN stage, and TNM stage between the two groups. Twenty-eight patients (40.0%) were T2 (T2A or T2B) and 20

Table 1 Patient demographics for patients undergoing laparoscopic and open gastrectomy for adenocarcinoma

	Laparoscopic	Open	Total
Total cases (n)	35	35	70
Gender female	5 (14%)	5 (14%)	10 (14%)
Median age (range, years)	58 (36–78)	59 (24–78)	59 (24–78)
BMI median (range, kg/m^2)	21 (18–30)	23 (16–28)	22 (16–30)
Prior abdominal surgery	5/35 (14.3%)	9/35 (25.7%)	14/70 (20%)
Neoadjuvant chemotherapy	0	0	0

All comparisons not significantly different

Table 2 Operative characteristics and complications for laparoscopic versus open gastrectomy patients

Median (range)	Laparoscopic (n=35)	Open (n=35)	p value
Procedure			
BII	35	35	NS
Op time (min)	320 (260–570)	210 (138–300)	<0.01
EBL (cc)	200 (100–600)	300 (100–1,100)	<0.05
LOS (days)	12 (5–36)	17 (8–45)	<0.01
IV narcotic use (days)	3.0 (0–5)	4.0 (1–6)	<0.01
Early complications (<30 days)	2 (5.7%)	3 (8.6%)	NS
Late complications	0	0	NS

BII Billroth II, *LOS* length of stay, *EBL* estimated blood loss

(60.0%) patients were T3. According to the International Union against Cancer (UICC) classification of gastric cancer,[9] 23 cases (32.9%) were at stage Ib, 23 cases (32.9%) at stage II, 16 cases (22.9%) at stage IIIa, and 8 cases (11.4%) at stage IIIb (Table 4).

Median follow-up for the LADG group was 36.5 months (range 23–50 months) and for the ODG group was 38.5 months (range 27–50 months). There was no significant difference in the cumulative survival rate between the two groups after 50 months of follow-up (median follow-up of 35 months, p=0.399; Fig. 2), and the cumulative survival of T2 and T3 in the LADG group was similar (p=0.316, Fig. 3).

Discussion

Since Kitano et al. [10] performed the first laparoscopy-assisted distal gastrectomy by a Billroth I reconstruction for a patient with gastric cancer, the use of laparoscopic gastrectomy for gastric cancer has been generally attempted in Japan and Korea, and the popularity of laparoscopic gastrectomy with lymph node dissection has increased rapidly.

For distal AGC, the Japanese Gastric Cancer Association has presented complete D2 lymphadenectomy including lymph nodes 11p, 12a, and 14v as the standard therapy. Nevertheless, laparoscopic D2 lymph node dissection has not been widely investigated since it is considered to be technically difficult and was performed only in a few institutes by highly experienced surgeons.[11–14] Despite the ongoing controversy about whether laparoscopic gastrectomy with D2 lymph node dissection for gastric cancer was safe and effective, the most recent clinical trials showed that laparoscopic D2 lymph node dissection was a safe procedure for advanced gastric cancer if the surgery was performed by experienced surgeons.[11,15–17] Moreover, the most objective index of lymphadenectomy for gastric cancer is the comparison of the number of nodes obtained between open and laparoscopic surgery. According to the UICC TNM classification,[9] surgical removal of at least 15 lymph nodes is advocated in gastric cancer. Many authors have reported no major difference between the LADG and ODG procedures in terms of the number of retrieved lymph nodes.[12,18,19] We performed D2 lymph node dissection in both laparoscopic and open groups, and there was no significance between the two groups in the number of resected lymph nodes (median number 35 in the laparoscopic group versus 38 in the open group). This number is comparable to that reported by other authors who performed laparoscopic surgery for AGC.[18,20,21]

Although LADG has several advantages over conventional open surgery including less invasiveness, less pain, and earlier recovery, the question exists whether complications are prevalent with it. According to data from a nationwide questionnaire survey in Japan, the complica-

Table 3 Pathologic characteristics after laparoscopic versus open gastrectomy

	Laparoscopic (n=35)	Open (n=35)	p value
Histology			
Tubular adenocarcinoma	26	28	
Signet ring cell carcinoma	3	1	
mucinous adenocarcinoma	6	6	
Tumor location			
Body	21 (16%)	22 (23%)	NS
Antrum	14 (84%)	13 (77%)	
Positive resection margins	0	0	NS
No. resected lymph nodes (range)	35 (7–63)	38 (6–66)	NS

Table 4 TNM of patients According to the 1997 AICC pTNM staging system

	Laparoscopic (n=35)	Open (n=35)	Total (n=70)
pT stage			
T2A	10	7	17 (24.3%)
T2B	5	6	11 (15.7%)
T3	20	22	42 (60.0%)
pN stage			
N0	23	19	
N1	5	11	
N2	7	5	
Stage distribution			
Ib	10	9	19
II	15	13	28
IIIa	6	9	15
IIIb	4	4	8

All comparisons not significantly different

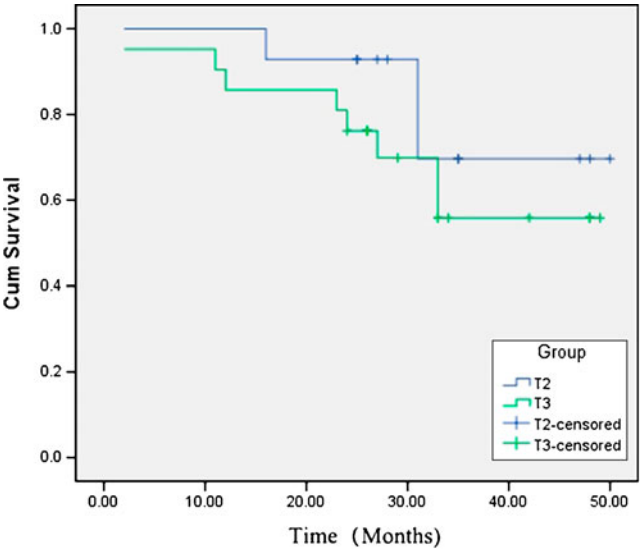

Fig. 3 Comparison of cumulative survival rate of T2 and T3 in the LADG group by log rank test (p=0.316)

tion rate was 8.71% and the mortality rate was 0.083%.[22] In the present study, the complication rate for all cases was 7.1% (5 in 70)—four of them were wound problems and one was mild pancreatitis—but these problems were resolved by conservative management. No patient died during hospitalization.

The majority of the studies about laparoscopic surgery for gastric cancer were focused on early gastric cancer, and only few reports have addressed the application of a laparoscopic procedure to patients with AGC and evaluate its safety in terms of clinicopathologic surgical outcomes and long-term follow-up results.[6,13,17,20,23] Moreover, most of these reports mainly concerned cases with T2 or lower

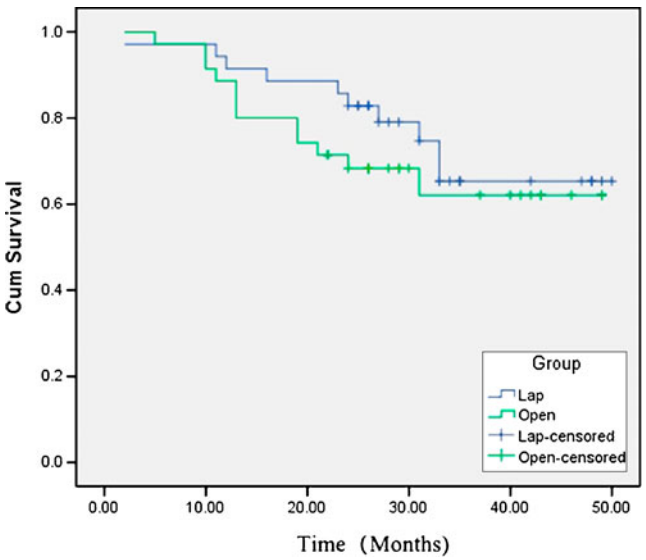

Fig. 2 Comparison of cumulative overall survival rate according to operation methods during a 50-month interval by log rank test (p=0.399)

depth of invasion, and the number and proportion of T3 cases in these literatures were very small. Of the cases in the present study, 42 patients (60.0%) were diagnosed as having T3 gastric cancer. Though some surgeons thought that laparoscopic curative surgery for T3 AGC was not yet acceptable, for there could be peritoneal seeding of malignant cells in dealing with possible metastatic lymph nodes or gastric lesion or there could be a risk of port site recurrence,[24] the results of the present study and other reports [7,19,20] showed good outcomes. There were few case–control studies on the outcome of LADG for AGC mainly with T2 and T3 depth of invasion published before. Strong et al.[19] reported a case–control study about totally laparoscopic surgery for subtotal gastrectomy. They compared 30 patients undergoing laparoscopic gastrectomy with 30 matched open gastrectomy, and their results indicated technical feasibility and equivalent short-term recurrence-free survival of laparoscopic subtotal gastrectomy for gastric cancer when compared with open procedure. However, 33 patients (55%) in their study had early-stage disease (Ia/Ib).

Although the present study was not a randomized controlled study and the follow-up period was not long enough, the survival rate of patients with AGC who underwent LADG was shown to be good. In conclusion, this study showed that LADG for AGC has several advantages over ODG, and LADG yielded similar oncologic outcomes including early and late complication and cumulative survival after 50 months of follow-up. To be accepted as a choice treatment for advanced distal gastric cancer, well-designed prospective trial to assess long-term outcomes is necessary.

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ORIGINAL ARTICLE

Aberrant Expression of miR-203 and Its Clinical Significance in Gastric and Colorectal Cancers

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Abstract
Background MicroRNAs (miRNAs) are small, non-coding RNAs of endogenous origin, they have been increasingly shown to have aberrant expression in many tumor types. miR-203 has not been comprehensively investigated in gastric and colorectal cancers.
Methods Total RNA was extracted from tissues of 212 patients with gastric or colorectal cancer as well as from seven gastric and colorectal cell lines. We determined the expression of miR-203 by real-time PCR and calculated using the $2^{-\Delta\Delta Ct}$ method. Then, we assessed miR-203 expression and clinicopathologic characteristics. Finally, we studied the effect of miR-203 on cell proliferation in SGC-7901 cells by MTT.
Results miR-203 has significantly low expression in colorectal cancer tissues ($p<0.001$, paired t test) and cancer cell lines compared to non-tumor counterparts. Moreover, low expression of miR-203 was correlated with tumor size ($p=0.015$, non-parametric test) and pT stage ($p=0.005$) in colorectal cancer. Although expression of miR-203 was not significant in gastric cancer tissues ($p=0.124$), interestingly, miR-203 was correlated with tumor size ($p=0.023$), macroscopic type ($p=0.045$), and pT stage ($p=0.013$). Furthermore, we found miR-203 can inhibit the cell proliferation in SGC-7901 cells.
Conclusion miR-203 may be related to the proliferation and invasion of gastric and colorectal cancers.

Keywords microRNA · miR-203 · Gastric cancer · Colorectal cancer · Clinicopathologic characteristics

Introduction

MicroRNAs (miRNAs) are an abundant class of small, endogenous, non-coding RNAs, and the miRNA products are small single-stranded RNAs of 19–22 nucleotides with a primary role of regulating the translation of many genes.[1] Many studies have shown that miRNAs can regulate a variety of cellular processes including cell proliferation, differentiation, and apoptosis.[2] In 2002, the first evidence of miRNA expression was provided in human B-cell chronic lymphocytic leukemia and results showed absence or down-regulation of miR-15 and miR-16 in patients with this disease.[3] Since then, an increasing number of studies have shown aberrant expression of miRNAs in many tumor types.[4,5] Moreover, much evidence has indicated that miRNAs are thought to function as tumor suppressors or oncogenes in the pathogenesis of cancers.[2]

In recent studies, expression levels of several miRNAs have been found to be correlated with gastric and colorectal cancers. In 2006, Volinia et al.[5] analyzed miRNA profiles in 363 primary cancer tissues and 177 normal tissues of six solid tumors, including 41 gastric cancer tissues and 54 colon cancer tissues by microarray. The results showed six down-regulated and 22 up-regulated miRNAs in gastric cancer, and one down-regulated and 21 up-regulated miRNAs in colon cancer. Chen et al.[6] also found the low expression of miR-148a and miR-152 in 101 gastric cancer tissues and 101 colorectal cancer tissues relative to matched

Yeunpo Chiang and Yongxi Song contributed equally to this work.

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non-tumor adjacent tissues (NATs) by real-time PCR, and indicated low expression of miR-148a and miR-152 were correlated with increased tumor size and more advanced pT stage. Furthermore, Chan et al.[7] study expression levels in 37 pairs of gastric cancer tissues compared with NATs by real-time PCR. The results showed expression level of miR-21 has significant up-regulation in gastric cancer, and suggested miR-21 could be considered as a diagnostic marker in gastric cancer.

In the present study, we examined the expression levels of miR-203 in a large number of gastric and colorectal cancer tissues and six cancer cell lines and analyzed the results relative to their non-tumor counterparts. Moreover, we also found an interesting correlation between low expression of miR-203 and clinicopathological characteristics of these cancers.

Material and Methods

Tissues Samples

Two hundred and twelve pairs of gastric and colorectal cancer tissues and their corresponding NATs were obtained from patients who underwent radical resection at the first hospital of China Medical University (Shenyang, China) between 2007 and 2009 and were subsequently diagnosed with gastric and colorectal cancers based on histopathological evaluation. Fresh samples were snap-frozen, put in liquid nitrogen immediately after surgery, and were stored at −80°C until used. Corresponding NATs were obtained from a part of the resected specimen that was the farthest distance from the tumor. One section of each sample was stained with hematoxylin–eosin (H&E). No previous local or systemic treatment had been conducted on these patients before the operation. The characteristics of patients consisted of 105 patients with gastric cancer (age range 26–84 years; mean age, 60.2 years; median age, 61 years) and 107 patients with colorectal cancer (age range 17–82 years; mean age, 62.1 years; median age, 63 years). The histological grade of cancers was assessed according to the standard of the World Health Organization. Cancers were classified using the TNM staging system of the American Joint Committee on Cancer (2010) and the International Union against Cancer. All patients in the study gave written informed consent and approval from the Research Ethics Committee of China Medical University (Shenyang, China) was obtained.

Cell Lines and Culture Conditions

Human gastric cancer cell lines (MGC-803, BGC-823, and SGC-7901), one normal gastric epithelial cell line (GES-1, as control), and colorectal cancer cell lines (HT-29, HCT-116, and SW-620) were obtained from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). All the selective gastric cancer cell lines were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA); GES-1 (as control) in Dulbecco's Modified Eagle medium (Invitrogen, Carlsbad, CA, USA); the HT-29 and HCT-116 were cultured in McCoy's 5a medium (Invitrogen, Carlsbad, CA, USA), and SW-620 was cultured in Leibovitz's L-15 medium (Invitrogen, Carlsbad, CA, USA). Colorectal cancer cell lines were compared to normal colorectal tissues (randomly selected three NATs from previous 107 cases of CRC as controls).[6] All of the cell lines were cultured at 37°C in a humidified atmosphere of 5% CO^2. Media were supplied with 10% fetal bovine serum.

Extraction, Polyadenylation, and Reverse Transcriptase Reaction

Using a mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA), we isolated the total RNA according to the manufacturer's instruction. The concentration and purity of RNA were controlled by UV spectrophotometry using a NanoPhotometer UV/Vis spectrophotometer (Implen, Schatzbogen, München, Germany).

Escherichia coli poly(A) polymerase (E-PAP) was used for polyadenylation of total RNA in a 37°C water bath for 30 min following the manufacturer's instructions using the poly(A) Tailing Kit (Ambion, Austin, TX, USA).[8] RNAs were purified by phenol-chloroform and ethanol. Then, they were dissolved in diethyl pyrocarbonate (DEPC)-treated water.

We completed the reverse transcription with a superscript III First-Strand Synthesis System for a reverse transcriptase–polymerase chain reaction kit (Invitrogen, Carlsbad, CA, USA). First, a 10 μL reverse transcriptase reaction mixture containing 1 μg of the RNA sample, 1 μL RT-primer, 1 μL 10 mM deoxyribonucleotide triphosphate mix, and DEPC-treated water at 65°C was incubated for 5 min. Then, a 10 μL mixture containing 2 μL 10× RT buffer, 4 μL 25 mM $MgCl_2$, 2 μL 0.1 M DTT, 1 μL RNaseOUT (40 U/μL), and 1 μL SuperScript III RT (200 U/μL) was added. The total reaction mixture was incubated in a 96-well plate of GeneAmp PCR 9700 Thermocycler (Applied Biosystems, Hayward, CA, USA) for 50 min at 50°C, 5 min at 85°C, and 20 min at 37°C after adding 1 μl RNase H to the mixture and held at 4°C.

Real-Time PCR

Real-time PCR was performed using the SYBR Premix Ex Taq™ IIKit (TaKaRa, Bio, Kyoto, Japan) according to the

manufacturer's instructions with a Rotor-gene 6000 system (QIAGEN, Valencia, CA, USA).[8] The 25 μl mixture of PCR consisted of 12.5 μl SYBR Green supermix, 8.5 μl RNase-free water, 1 μl forward primers, 1 μl reverse primers, and 2 μl reverse transcribed product. Threshold cycle data were determined by setting a default threshold. The reactive condition was 45 amplification cycles of 95°C for 5 s, 58°C for 20 s, and 72°C for 30 s in a 36-well optical plate using a Rotor-gene 6000 system. We adopted the U6 RNA as an endogenous reference compared to the expression level of miR-203, and used the method of $2^{-\Delta\Delta Ct}$ to calculate the relative expression levels of miR-203 in cancerous samples compared with their non-tumor counterparts.[9] All samples were performed in triplicate. The products of real-time PCR were confirmed by TA cloning and a sequencing assay. Primers are shown in Table 1.

Cell Transfection and MTT Assay

miR-203 mimics was a RNA duplex (Table 1) designed as described previously.[35] Non-specific sequences were used as a negative control RNA duplex (named as NC); it was nonhomologous to any human genome sequences (Table 1). All pyrimidine nucleotides in the miR-203 mimics or NC duplex were substituted by their 2-*O*-methyl analogs to improve RNA stability for MTT assay in vitro. We transiently transfected the miR-203 mimics (50 nM) and NC in cultured SGC-7901 cells at 30–50% confluence using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). All the RNA oligoribonucleotides were chemically synthesized by GenePharma (Shanghai, China).

The capacity for cellular proliferation was measured with the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Twenty-four hours after transfection, SGC-7901 cells with miR-203 mimics, SGC-7901 cells with NC, and SGC-7901 cells (about 0.5×10^4) were seeded into 96-well microtiter plate for 24, 48, 72, and 96 h, respectively. Then, the cells were incubated with 20 μL of MTT (5 mg/mL) for 4 h at 37°C and 150 μL of dimethl sulfoxide was added to solubilize the crystals for 20 min at room temperature. Optical density (OD) was measured at a wavelength of 490 nm by a spectrophotometer-Multiskan MK3 (Thermo, Waltham, MA, USA). All experiments were performed three times and were calculated using average results. Growth inhibition rate was calculated as following: (AC−AT)/AC×100% (AC = absorbance value of the NC and AT = absorbance value of the experimental group).[36]

Statistical Analysis

We used the method of $2^{-\Delta\Delta Ct}$ to analyze the association between gastric cancer tissues and their matched NATs as well as colorectal cancer tissues. First, the threshold cycle of fluorescence (C_t) for each sample was determined. ΔC_t indicates the difference of the expression level with the C_t value between miR-203 and U6 ($\Delta C_t = C_{t\mathrm{miR-203}} - C_{t\mathrm{U6}}$) and $\Delta\Delta C_t$ indicates the difference in the ΔC_t value between cancer tissue and the corresponding control ($\Delta\Delta C_t = \Delta C_{t\ \mathrm{cancer}} - \Delta C_{t\ \mathrm{control}}$). Finally, the $2^{-\Delta\Delta Ct}$ value (fold value) was calculated. When the $\Delta\Delta C_t$ value is zero, the expression level of miR-203 in cancerous samples and the corresponding controls is equal, and the fold value is onefold (2° equals one).[9] If the fold value is less than onefold, there is low expression of miR-203 in cancer tissues and cancer cell lines compared with their non-tumor counterparts.[10] By comparing the values of $\Delta C_{t\ \mathrm{cancer}}$ and $\Delta C_{t\ \mathrm{control}}$, we could compare the expression levels of miR-203 in cancer tissues and cancer cell lines with their non-tumor counterparts. We used paired *t* test to analyze the statistical differences in the

Table 1 RT-PCR primers for amplification of miR-203 expression and the sequence of miR-203 mimics, NC

Primer	Primer sequence (5′-3′)
RT-primer-1	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTTTTTTTTTA
RT-primer-2	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTTTTTTTTTG
RT-primer-3	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTTTTTTTTTC
miR-203-F[a]	GTGAAATGTTTAGGACCACTAGAA
miR-203-R[b]	GCTGTCAACGATACGCTACGT
U6 RNA-F[a]	CGCTTCGGCAGCACATATAC
U6 RNA-R[b]	TTCACGAATTTGCGTGTCAT
miR-203-mimics	GUGAAAUGUUUAGGACCACUAG
	AGUGGUCCUAAACAUUUCACUU
NC	UUCUCCGAACGUGUCACGUTT
	ACGUGACACGUUCGGAGAATT

[a] Forward primer

[b] Reverse primer

expression of miR-203 in cancer tissues and cancer cell lines relative to non-tumor counterparts as well as the effect of miR-203 on cell proliferation in SGC-7901 cells. Moreover, the association between miR-203 expression and clinicopathologic parameters was analyzed by non-parametric test (Mann–Whitney *U* test between two groups and Kruskal–Wallis H test for three or more groups). $p<0.05$ was considered significant. Statistical analysis was performed using Statistical Program for Social Sciences (SPSS) software 16.0 (SPSS Incorporated, Chicago, IL, USA).

Results

Expression of miR-203 in Gastric and Colorectal Cancers

We compared the ΔC_t between cancer tissues and NATs in gastric and colorectal cancers. Among 107 patients with colorectal cancer, the value of ΔC_t (mean ± SD) is 7.956±2.690 in colorectal cancer tissues as well as 6.053±2.748 in their matched NATs, and 76 (71%) cases showed a significant low expression of miR-203 in colorectal cancer tissues compared with NATs ($p<0.001$, paired *t* test, Fig. 1a, b). Moreover, we also found a significant low expression of miR-203 in colorectal cancer cell lines (HT-29, $p=0.005$; HCT-116, $p=0.027$; SW-620, $p=0.008$; paired *t* test) compared to normal colorectal tissues (Fig. 2a). On the other hand, the value of ΔC_t (mean ± SD) is 6.560±3.319 in gastric cancer tissues and is 6.088±2.597 in their corresponding NATs, but the low expression of miR-203 was not significant in 105 pairs of gastric cancer tissues compared with their matched NATs ($p=0.124$, Fig. 1c, d). However, 56 (53%) cases showed a significant low expression of miR-203 in 105 patients with gastric cancer. Furthermore, a significant low expression of miR-203 was found in SGC-7901 ($p=0.002$, Fig. 2b) compared to GES-1, but there was no significant difference for MGC-803 ($p=0.318$) and BGC-823 ($p=0.193$).

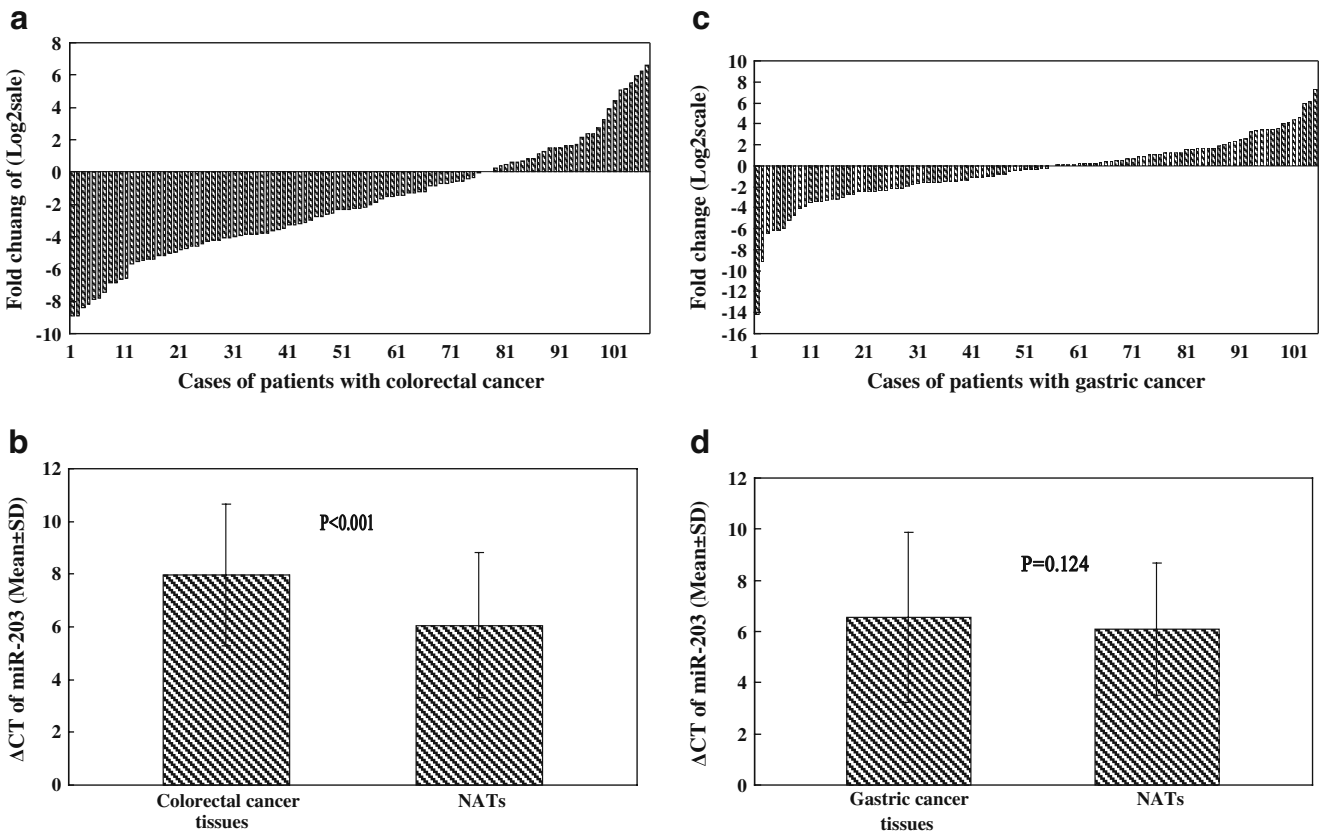

Fig. 1 Expression of miR-203 in 107 patients with colorectal cancer and 105 patients with gastric cancer. **a**, **c** Quantification of miR-203 was measured by SYBR Premix Ex Taq™ II. Each sample was analyzed in triplicate and repeated three times. Data were presented as log2 of fold-change of gastric and colorectal cancer tissues relative to matched NATs. **b**, **d** miR-203 was differentially expressed between gastric cancer tissues and matched NATs as well as between colorectal cancer tissues and matched NATs. miR-203 was normalized by U6RNA. $\Delta C_t = C_{t\mathrm{miR-203}} - C_{t\mathrm{U6RNA}}$. The ΔC_t of miR-203 was significantly higher in colorectal cancer tissues than NATs ($p<0.001$, paired *t* test). But there was no significance in gastric cancer tissues relative to NATs ($p=0.124$)

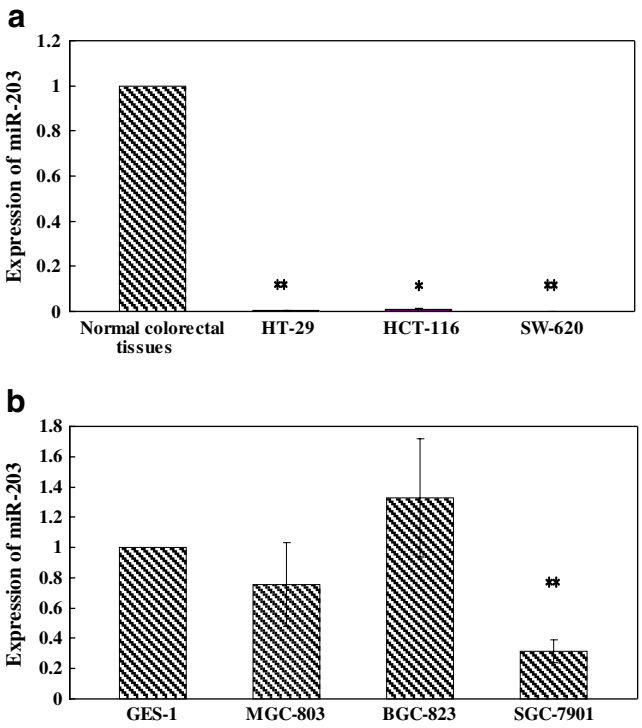

Fig. 2 Expression of miR-203 in three colorectal cancer cell lines (HT-29, HCT-116, and SW-620) and three gastric cancer cell lines (MGC-803, BGC-823, and SGC-7901). Quantification of miRNAs was measured by SYBR Premix Ex Taq™ II. Data were presented in colorectal cancer cell lines relative to normal colorectal tissues (randomly selected three NATs from previous 107 cases of CRC as control) as well as gastric cancer cell lines relative to GES-1 (normal gastric epithelial cell line). **a** Expression of miR-203 in three colorectal cancer cell lines (*$p<0.05$; **$p<0.01$). **b** Expression of miR-203 in three gastric cancer cell lines (**$p<0.01$)

Association Between Clinicopathological Characteristics and Low Expression of miR-203 in Gastric and Colorectal Cancers

In our study, there was a significant correlation between low expression of miR-203 and clinicopathologic characteristics in gastric and colorectal cancers. Patients with lower expression of miR-203 tended to have increased tumor sizes ($p=0.023$, Mann–Whitney U test), advanced Borrmann type ($p=0.045$, Kruskal–Wallis H test), and advanced pT stage ($p=0.013$, Mann–Whitney U test) of gastric cancer (Table 2). On the other hand, a significant low expression of miR-203 in colorectal cancer was associated with increased tumor size ($p=0.015$) and an advanced pT stage ($p=0.005$, Table 3). There was no significant difference between low expression of miR-203 and other clinicopathological characteristics such as sex, age, tumor location, histologic grade, pN stage, clinical stage, lymph node of metastasis rate, and lymphatic vessel invasion.

Table 2 Association between the expression of miR-203 with clinicopathological features in patients with gastric cancer

Gastric cancer	*n*	miR-203[a]
Sex		
Male	79	0.779 (0.175–2.403)
Female	26	1.035 (0.340–2.556)
p		0.431
Age (years)		
≤65	65	1.028 (0.314–2.330)
>65	40	0.586 (0.173–2.840)
p		0.522
Tumor size (cm)		
<6	73	1.112 (0.314–3.095)
≥6	32	0.380 (0.153–1.225)
p		0.023*
Tumor location		
Upper stomach	12	1.096 (0.216–2.160)
Middle stomach	25	0.681 (0.182–3.120)
Lower stomach	67	0.803 (0.265–2.308)
Entire stomach	1	0.175 (0.175–0.175)
p		0.753
Macroscopic type		
Early stage	3	4.019 (3.242–5.489)
Borrmann I+II	9	1.613 (0.333–8.438)
Borrmann III+IV	93	0.715 (0.192–2.196)
p		0.045*
Histologic grade		
Well/moderately well differentiated	21	1.149 (0.258–2.316)
Poorly differentiated	84	0.805 (0.230–2.840)
p		0.701
pT stage		
T1+T2+T3	45	1.320 (0.369–3.432)
T4	60	0.513 (0.161–1.773)
p		0.013*
pN stage		
N0	25	0.701 (0.280–2.670)
N1	14	1.080 (0.200–4.392)
N2	20	1.070 (0.347–2.410)
N3	46	0.791 (0.173–3.009)
p		0.867
pTNM stage		
I	10	2.071 (0.722–3.721)
II	22	0.406 (0.219–1.928)
III	73	0.807 (0.187–2.356)
p		0.293
Invasion into lymphatic vessels		
Negative	76	0.931 (0.241–3.063)
Positive	29	0.474 (0.166–1.797)
p		0.289

*$p<0.05$

[a] Median of relative expression with 25th–75th percentile is recorded in parentheses

Table 3 Association between the expression of miR-203 with clinicopathological features in patients with colorectal cancer

Colorectal cancer	*n*	miR-203[a]
Sex		
Male	65	0.205 (0.034–1.360)
Female	42	0.291 (0.066–1.418)
p		0.453
Age (years)		
≤65	64	0.234 (0.054–1.721)
>65	43	0.204 (0.035–1.032)
p		0.529
Tumor size (cm)		
<6	79	0.311 (0.063–1.551)
≥6	28	0.077 (0.021–0.676)
p		0.015*
Tumor location[b]		
Proximal colon	25	0.167 (0.056–1.196)
Distal colon and rectum	82	0.214 (0.051–1.550)
p		0.825
Histologic grade		
Well/moderately well differentiated	85	0.212 (0.054–1.550)
Poorly differentiated	22	0.236 (0.046–0.764)
p		0.553
pT stage		
T2+T3	75	0.374 (0.082–1.551)
T4	32	0.067 (0.022–0.612)
p		0.005*
pN stage		
N0	65	0.199 (0.056–0.919)
N1	30	0.448 (0.051–2.487)
N2	12	0.682 (0.051–3.747)
p		0.403
pTNM stage		
I	16	0.176 (0.048–0.422)
II	49	0.199 (0.056–1.451)
III	42	0.465 (0.051–2.487)
p		0.371
Invasion into lymphatic vessels		
Negative	95	0.212 (0.054–1.042)
Positive	12	0.721 (0.053–8.551)
p		0.413

*$p<0.05$

[a] Median of relative expression with 25th–75th percentile is recorded in parentheses

[b] Distal includes tumors located in or distal to the descending colon, sigmoid, and rectum. Proximal tumors include tumors in or proximal to the splenic flexure

Effects of miR-203 on Cell Growth of SGC-7901 Cells

After transfection, we found the cells which transfected with miR-203 mimics in SGC-7901 cells had an obviously growth inhibition compared to matched NC and SCG-7901 cells by MTT assay (Fig. 3). At the time point of 24, 48, 72, and 96 h posttransfection, the inhibition rates were 9.58%, 14.70%, 21.32%, and 13.96% in SGC-7901 cells, respectively.

Discussion

In recent years, studies have shown that aberrant expression of miRNAs contributes to the initiation and progression of cancers.[11] Moreover, the physiological and pathological roles of miRNAs have also been demonstrated in most tumor types[2,12] and miRNAs may play an important role in the diagnosis and treatment of cancer.[11,12] Therefore, the correlation between miRNAs and cancers has become a focus of cancer studies.

Aberrant expression of miR-203 has been found in human cancers: down-regulation of miR-203 was described in oral squamous cell carcinoma,[13] esophageal cancer,[14] hepatocellular carcinoma (HCC),[15] T cell lymphomas, chronic myelogenous leukemia, B-cell type acute lymphoblastic leukemia, [16] and central nervous system tumor cell lines,[17] whereas its up-regulation was found in lung cancer,[18] pancreatic cancer,[19] bladder cancer,[20] breast cancer,[21] and ovarian cancer.[22] In the present study, miR-203 has significantly low expression in colorectal cancer tissues and cancer cell lines compared to non-tumor counterparts, but there was no significance in gastric cancer. Taken together, we think the differential expression of miRNAs may be the result of tissue-specific differences. Just as Baffa et al.[23] suggested it, the different levels of miRNA expression were found in different organs of origin and they found that the expression of miRNAs were markedly tissue-specific.

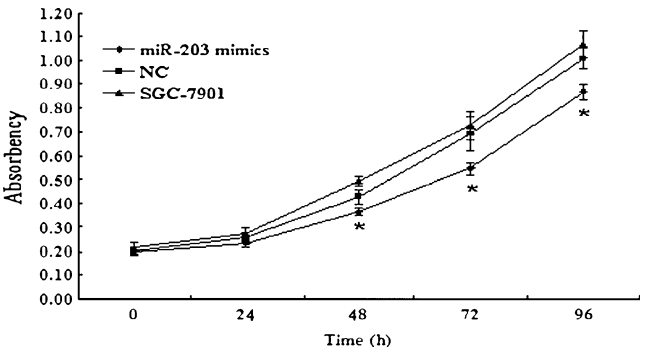

Fig. 3 miR-203 significantly inhibited cell proliferation in SGC-7901 cells by MTT assay (*$p<0.05$)

In previous study, Lu et al.[4] analyzed the expression levels of miR-203 in ten colorectal cancer tissues relative to six normal colorectal tissues (obtained from Boston and New York, USA) using a bead-based flow cytometric method and results indicated down-regulation of miR-203 in colorectal cancer (consistent with our investigation). However, Volinia et al.[5] reported the up-regulation of miR-203 in 46 colorectal cancer tissues relative to eight normal colorectal tissues (obtained from Italy) using microarray. The up-regulation of miR-203 was also found in 84 colorectal cancer tissues (obtained from Maryland, USA) using microarray and 113 colorectal cancer tissues (obtained from Hong Kong) using quantitative RT-PCR compared to their corresponding NATs.[24] Therefore, analysis of expression levels of miR-203 has been controversial in colorectal cancers to date. Possibly the different expression levels of miR-203 is due to different populations and different environments.[25] As in previous studies, Volinia et al.[5] reported the down-regulation of miR-155 in 39 pancreatic cancer tissues relative to 12 normal pancreatic tissues (obtained from Italy). However, Lee et al.[26] indicated that miR-155 was up-regulated in 28 pancreatic cancer tissues compared to 21 non-tumor tissues (obtained from Oklahoma and Ohio, USA). On the other hand, the small sample sizes may have contributed to this conflict. Therefore, considering these variations in results, we think the different expression of miRNAs may be caused by various combinations of factors, such as tissue-specificity, different populations, different environments, and small sample sizes. Further investigation is needed to resolve the issue.

The role of miR-203 remains unclear in the progression of gastric and colorectal cancers. If low expression of miR-203 is causal to the progression of gastric and colorectal cancers, it may be correlated with clinicopathologic characteristics of the disease. Our investigation showed that low expression of miR-203 was correlated with increased tumor size and advanced pT stage in gastric and colorectal cancers. Moreover, the low expression of miR-203 was also associated with advanced Borrmann type in gastric cancer. Previous studies had suggested that the increased tumor size and advanced pT stage in gastric cancer were important prognostic factors.[27,28,32] And Yokota et al.[32] indicated the macroscopic type in gastric cancer was also an important prognostic factor. On the other hand, some studies suggested the tumor size in colorectal cancer was an important prognostic factor.[33] And Xi et al.[29] also concluded the tumor size and invasion depth in colorectal cancer were important prognostic factors. On the other hand, the results of MTT showed miR-203 mimics can significantly inhibit the cell proliferation in SGC-7901 cells. Therefore, we think miR-203 plays an important role in gastric and colorectal cancers. In the future study, we will provide more information to explain the functions of miR-203 in gastric and colorectal cancer.

Expression of miRNAs could be reduced by many factors, including mutations,[30] transcription factors,[31] deletions[3], and methylation.[34] In previous study, Furuta et al.[15] reported that miR-203 was silenced through CpG-island methylation, and they suggested it was a novel tumor suppressor in HCC. Furthermore, Bueno et al.[16] also indicated that miR-203 was a tumor suppressor and inactivated in specific hematopoietic malignancies by both genetic and epigenetic mechanisms. They found that miR-203 was silenced by the loss of one allele and promoter CpG hypermethylation in the remaining DNA copy. And restoration of miR-203 expression might have therapeutic benefits in specific hematopoietic malignancies. Therefore, considering these reasons, we presumed that deletion and promoter methylation of miR-203 might be mechanisms for low expression of miR-203 in human gastric and colorectal cancers.

Conclusion

We found a significant low expression of miR-203 in colorectal cancer tissues relative to their corresponding NATs in a large number of samples as well as in three colorectal cancer cell lines. And the significant low expression of miR-203 was associated with increased tumor size and advanced pT stage in colorectal cancer. Furthermore, the low expression of miR-203 was also associated with increased tumor size, advanced Borrmann type, and advanced pT stage in gastric cancer. The present study is a basis for further studies on target genes and functions of miR-203 in gastric and colorectal cancers. Large-scale and long-term follow-up studies are needed to confirm the significance of miR-203 in gastric and colorectal cancers.

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ORIGINAL ARTICLE

Laparoscopic Resectional Gastric Bypass in Patients with Morbid Obesity: Experience on 112 Consecutive Patients

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Abstract
Introduction Gastric bypass, without gastric resection of the distal excluded stomach, is the surgical treatment more frequently performed for morbid obesity. Several postoperative complications related to the "in situ" distal stomach have been described, and few cases of undetected gastric carcinoma located in this segment of stomach have been published. In this paper, we present our early postoperative results in patients submitted to laparoscopic gastric bypass with resection of distal stomach in patients with morbid obesity.
Methods One hundred twelve consecutive patients were included in this study. The mean body weight was 112.15±5.1 (range 78–145), and BMI was 40.5±6.9 kg/m^2 (32.9–50.3). Patients were submitted to resectional gastric bypass by laparoscopic approach. The operative time was 133.7±29.1 min (range 120–240).
Results Postoperative complications occurred in 12 patients (10.7%) without any mortality. Early complications were observed in 11 patients while one patient presented a late complication, four patients were re-hospitalized, three of them without operation and other four of them were re-operated due to early (three patients) or late complication (one patient). One hundred patients (89.2%) were discharged at fourth postoperative day, seven patients remained in hospital between 5 and 10 days, and four patients after the tenth day due to complications. Leaks were observed in three patients. The histological study of the resected specimen was normal in only 8.9%.
Conclusions Laparoscopic resectional gastric bypass presents very similar results compared to classic gastric bypass, without significant increase of morbidity, mortality, early and late postoperative results, and therefore, it is an option for the surgical treatment of morbid obesity in countries with high risk of gastric carcinoma.

Keywords Gastric resection · Bypass · Laparoscopy

Introduction

Gastric bypass, without resection of the distal excluded stomach, is the surgical treatment more frequently performed for morbid obesity. The results of this procedure have been extensively reported and discussed on the literature.[1–3] Several postoperative complications related to the in situ distal stomach have been described, and few cases of undetected gastric carcinoma located in this segment of stomach have been published.[4–10]. In addition, in Chile, Japan, Korea, and Colombia, countries which present high rate of gastric cancer,[11–13] it is valid to postulate bariatric surgery with resection of the distal stomach. The criticism to this operation is that it could represent a more prolonged operation and could be associated with increased rate of postoperative complications.

In this paper, we present our early postoperative results in patients submitted to laparoscopic gastric bypass with resection of distal stomach in patients with morbid obesity.

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Patients and Methods

One hundred twelve consecutive patients were included in this study, 80 women and 32 men with a mean age of 39.4± 10.7 years. All patients had completed the protocol of preoperative evaluation including blood and metabolic tests, upper gastrointestinal endoscopy, abdominal ultrasound, nutritional and psychological visit in order to agree the surgical indication. The mean body weight was 112.15±5.1 kg (range 78–145), and BMI was 40.5±6.9 kg/m^2 (32.9–50.3). Sixteen patients had BMI less the 35 and corresponded to patients with esophagitis with Barrett's esophagus or diabetes type 2, and one patient with antral GIST. Fifty-eight patients had BMI between 35 and 39.9, and 38 patients had BMI more than 40 kg/m^2, all of them with associated co-morbidities. Table 1 shows the clinical characteristics and associated co-morbidities diagnosed preoperatively. All patients gave their informed consent for resectional gastric bypass.

Surgical Technique

After a small learning curve period, we adopted the Brazilian technique for laparoscopic gastric bypass,[14] introducing the addition of resection of the distal segment of stomach. The patient is placed in French position (with legs in abduction position). Pneumoperitoneum with Veress needle was working at 15 mmHg of intra-abdominal pressure and placement of 5 trocars for liver retraction, optic system, assistant, and surgeon instruments (Fig. 1). When the greater curvature is exposed using a Ligasure® device (Covidien, Cincinnati, USA), the gastroepiploic gastric branches are divided starting from 2 cm beyond the pylorus until the His angle, cutting the short gastric and posterior fundic vessels in a similar way when performing sleeve gastrectomy. Division of the adhesions of the posterior antral wall to the anterior pancreatic face and exposing the posterior wall of the duodenal bulb is performed. The gastrohepatic ligament is opened at the avascular membrane, and identification and division of the right gastric artery with Ligasure®, (Covidien, Mansfield, MA, USA) is performed. Division of the duodenum with a Duet-Endogia blue cartridge (Covidien, Mansfield, MA, USA) was introduced by the 15 mm port located at the right quadrant. Division of the fatty tissue, vessels, and Latarjet nerve of the lesser curvature exposed in this fashion the gastric wall just in front of the cardiotuberositarian vessels, 3 cm below the cardia. Then, a 45-mm Endogia device 4.8 mm stapler (blue cartridge) is introduced by the same port located at the right quadrant in order to start the division of the stomach 3 cm below the cardia. Gastric transection is completed with 2–3 additional 60 mm blue cartridge Endogia addressed up to the His angle in order to perform the gastric pouch guided by gastric tube 36French introduced by the anesthesiologist, leaving a gastric pouch

Table 1 Patients' demographic characteristic and co-morbidities

BMI	Mean, 40.5±6.9 kg/m^2 (range, 32.9–50.3)
<35	*n*=16
35.1–39.9	*n*=58
>40	*n*=38
Co-morbidities	
Diabetes	30
Fatty liver	26
Esophagitis without Barrett	24
Hypercholesterolemia	28
Hyperinsulinism	22
Barrett's esophagus	18 (with esophageal ulcer 2)
Arterial hypertension	18
Hiatal hernia	3
Asthma	1
Knee arthrosis	2
Infertility	1
Hypothyroidism	4
Cholelithiasis	6
Obstructive sleep apnea syndrome	1
Varicose veins	2
Failed sleeve	3
Failed lap band	2

-5mm Sub-xiphoid for liver retraction
-15mm upper right quadrant for left hand operator
-10mm supra-umbilical for optical system
-12mm upper left quadrant for hand operator and assistant
-10mm left sub-costal for hand operator or assistant

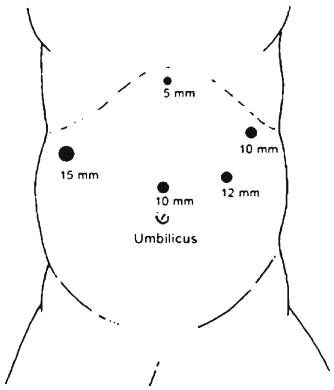


Fig. 1 Trocars site distribution: 5 mm subxiphoid for liver retraction, 15 mm upper right quadrant for left hand operator, 10 mm supra-umbilical for optical system, 12 mm upper left quadrant for hand operator and assistant, 10 mm left sub-costal for hand operator or assistant

of 60 ml capacity, measured by the instillation of methylene blue through the tube. The great omentum is retracted in order to localize the Treitz angle and identification of the biliary loop which is elevated to the gastric stump in order to approximate it and to perform gastrojejunostomy with 45 mm blue or white cartridge Endogia. Reinforcement of the stapler line and closure of the orifice of the entrance of the instrument is done with Monocril® 000 sutures (Ethicon, Cincinnati, USA). Afterwards, we perform latero-lateral jejunojejunal anastomosis, 170 cm distally, with white cartridge 45 mm endogia in the same fashion. In order to exclude any leak of the suture line, we transitorily block the flux to the jejunum with a long intestinal forceps and the anesthesiologist introduces 60 to 80 ml of methylene blue. Finally, we divided the biliary loop, 2 cm from the gastrojejunostomy, in order to complete the Roux-en-Y gastrojejunostomy (Fig. 2). A drain is placed close to the gastrojejunostomy and duodenal stump was exteriorized by the right quadrant port.

Postoperative Care

After the operation, patients were sent to a surgical intermediate care unit, and they stayed there for 1 day, being then discharged to the regular room. During the 3 days, patients receive intravenous therapy, and at third or fourth postoperative day, patients were submitted to radiological evaluation with barium sulfate in order to evaluate the anatomy of the gastric pouch, to exclude leaks, anastomotic strictures, or bowel obstruction.

Patients were followed-up monthly during the first 6 months by surgeons and nutriologist in order to evaluate the body weight decrease, nutritional indications, and vitamin supplement. After this, patients are controlled each 6 months.

In this paper, we analyze the operative time, early and late postoperative evolution, histological findings of the resected stomach, early postoperative complications, and follow-up regarding the loss of weight and BMI decrease during the first year after operation.

Results

Table 2 shows the early evolution after the operation. The operative time was 133.7±29.1 min (range 120–240). The more prolonged operations correspond to patients with adhesions of the posterior gastric wall and anterior pancreatic surface probably due to previous healed gastric ulcer (one patient), bleeding of the dissection of gastrohepatic ligament (three patients), and difficulties in performing the jejuno-jejunoanastomosis (two patients). No patients needed intraoperative transfusion. After the operation, patients received intravenous glucosaline solution with electrolytes, antibiotic prophylaxis for 12 h (Cefazolin®, 1 g/8 h), antithrombotic prophylaxis (Fragmin® 5,000 Us/c/day), early kinesic respiratory and body exercises, prokinetics, and pain management with ketoprofen and morphine PCA (patient-controlled analgesia) in demand.

At third postoperative day, all patients were controlled radiologically with barium sulfate swallow. In patients without complication, the intra-abdominal drain was retired at fourth postoperative day. After this, patients start with oral intake with semisolid foods for 2 weeks. One patient had to be converted to open surgery (0.9%) due to kinking

A Line of gastric division and resection

B 1 = gastrojejunal anastomosis

C 2 = jejuno-jejuno anastomosis

3 = Jejunal division below gastrojejunal anastomosis

D Final aspect of resectional gastric bypass

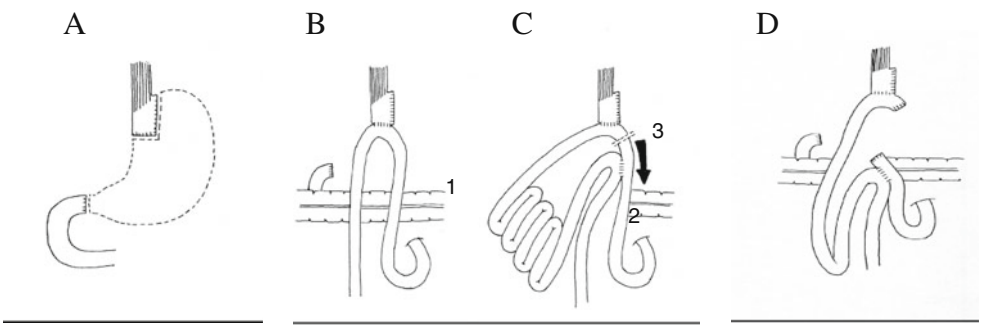


Fig. 2 Operation steps and anastomosis performance. **a** Line of gastric division and resection. **b** *1*=gastrojejunal anastomosis. **c** *2*=jejuno-jejuno anastomosis. *3*=jejunal division below gastrojejunal anastomosis. **d** Final aspect of resectional gastric bypass

Table 2 Operative and early postoperative evolution

Operative time (min)	133.7±29.1 (120–240)
Gastric capacity (ml)	38.4±14.1 (20–85)
Hospital stay (days)	5.8±0.97 (4–45)
Conversion	1 patient (kinked jejunojejunostomy)
Complications	12 patients
Re-operation	4 patients
	3 early
	1 hemoperitoneum trocar site+intestinal injury
	1 bowel obstruction
	1 early leak of duodenal stump
	1 late
	1 late leak of duodenal stump
Re-hospitalization	4 patients
	1 sub-phrenic abscess
	1 pneumonia
	1 spleen injury
	1 late duodenal stump leak (2 months later)
Mortality (30 days)	0

of the jejuno-jejunoanastomosis. Postoperative complications occurred in 12 patients (10.7%) without any mortality. Early complications were observed in 11 patients while one patient presented a late complication (late duodenal stump leak). Four patients were re-hospitalized, three of them without re-operation and one for re-exploration due to sub-hepatic abscess secondary to duodenal stump leak. Four patients were re-operated, three early after the operation and one due to late complication (2 months after the operation). The majority of patients were discharged at fourth postoperative day, but the mean hospital stay was 5.8+ 0.97 days (range 4–45) (Table 2).

Leaks were observed in three patients, two early leaks and one patient presented sub-hepatic abscess 2 months after the operation due to a minimal duodenal stump leak. The early leaks were minimal and localized in 1 patient at the gastric pouch suture line which appeared at third postoperative day and in1 patient at the duodenal stump diagnosed very early at the first postoperative day. The first one was treated conservatively with suction through the drain which was left in situ until no bile or gastric juice flux was observed and radiological control confirmed the leak closure. The second one was re-operated 24 h after the initial operation for suture and reinforcement of the stapled line by laparoscopic route. Patient with late duodenal stump leak was re-operated in order to drain the abscess and suture the orifice of the leak. The evolution of this patient with sub-phrenic abscess was successful once it was drained percutaneously, and fever and blood tests were normalized. This patient remained for 45 days hospitalized. Patients with intraluminal bleeding of the stapled suture line were successfully treated endoscopically with epinephrine submucosal injection and discharged between at sixth and eighth days. Patient with hemoperitoneum due to trocar bleeding was re-operated at 12 h after the operation by laparoscopy for peritoneal clean and hemostasis but after this a bowel perforation was suspected and he was re-operated again and remained in intensive care unit for 19 days and definitively discharged from the hospital at the 22nd day (Table 3).

One hundred patients (89.2%) were discharged at fourth postoperative day, seven patients remained in hospital between 5 and 10 days, and four patients after the tenth day due to complications. Among patients re-hospitalized, the first one, in order to drain percutaneously a left sub-phrenic abscess under tomographic visualization, also received complementary antibiotic treatment (metronidazole and cefuroxime) with complete clinical and tomographic resolution. The second one was re-hospitalized in

Table 3 Hospital stay and complications

	Patients (n=112)	Type of complication (n=12)	Hospital stay (days)
Less than 4 days	100	No	
5 to 10 days	7	2 bleeding suture line	7
		2 atelectasis	4
		1 pneumonia	9
		1 duodenal stump leak	8
		1 intestinal obstruction	8
More than 10 days	4	1 spleen injury	11
		1 hemoperitoneum	25
		1 gastric suture leak	
		1 sub-phrenic abscess	12
More than 1 month	1[a]	1 sub-hepatic abscess	
		Duodenal stump leak	45[a]

[a] Patient discharged at 4 postoperative day, re-hospitalized 2 months later during 45 days

Table 4 Postoperative complication and management. (n=12)

Complication	Number	Management
Early	11	
Leaks	2	1 Drainage and suction
		1 Laparoscopic duodenal stump closure
Sub-phrenic abscess	1	Puncture
Atelectasis	2	Kinesic
Pneumonia	1	Kinesic, antibiotics
Intragastric bleeding (suture line)	2	Endoscopic injection
Hemoperitoneum	2	1 Re-operation trocar hemostasia+Intestinal suture
		1 Spleen embolization
Bowel obstruction	1	Laparoscopic distorsion
Late	1	Open drain and duodenal suture 45 days

order to proceed to angiographic embolization of a branch of the spleen artery to stop a bleeding secondary to spleen injury during the operation; the third one presented fever at 21st postoperative day due to pneumonia, which was treated with antibiotics and kinesic therapy, and the fourth was re-operated by open approach in order to drain a late sub-hepatic abscess due to a minimal late duodenal leak 2 months later. Among the other three re-operated patients, the first one was due to early duodenal fistula at first postoperative day in order to perform closure of the leak and reinforcement of the stapler suture, the second one was due to 12 mm of paraumbilical trocar bleeding with hemoperitoneum, and the third one was due to intestinal obstruction due to adhesion near to the jejunojejunostomy. The details of the complications observed and the management indicated are shown in Table 4.

The late evolution regarding body weight is presented in Fig. 3. The body weight decreased from 112.15±15.1 kg (range 78–145) to 67.09+10.2 kg at 1 year after surgery. At the first month, the weight loss was 12.9±3.1 kg, at the

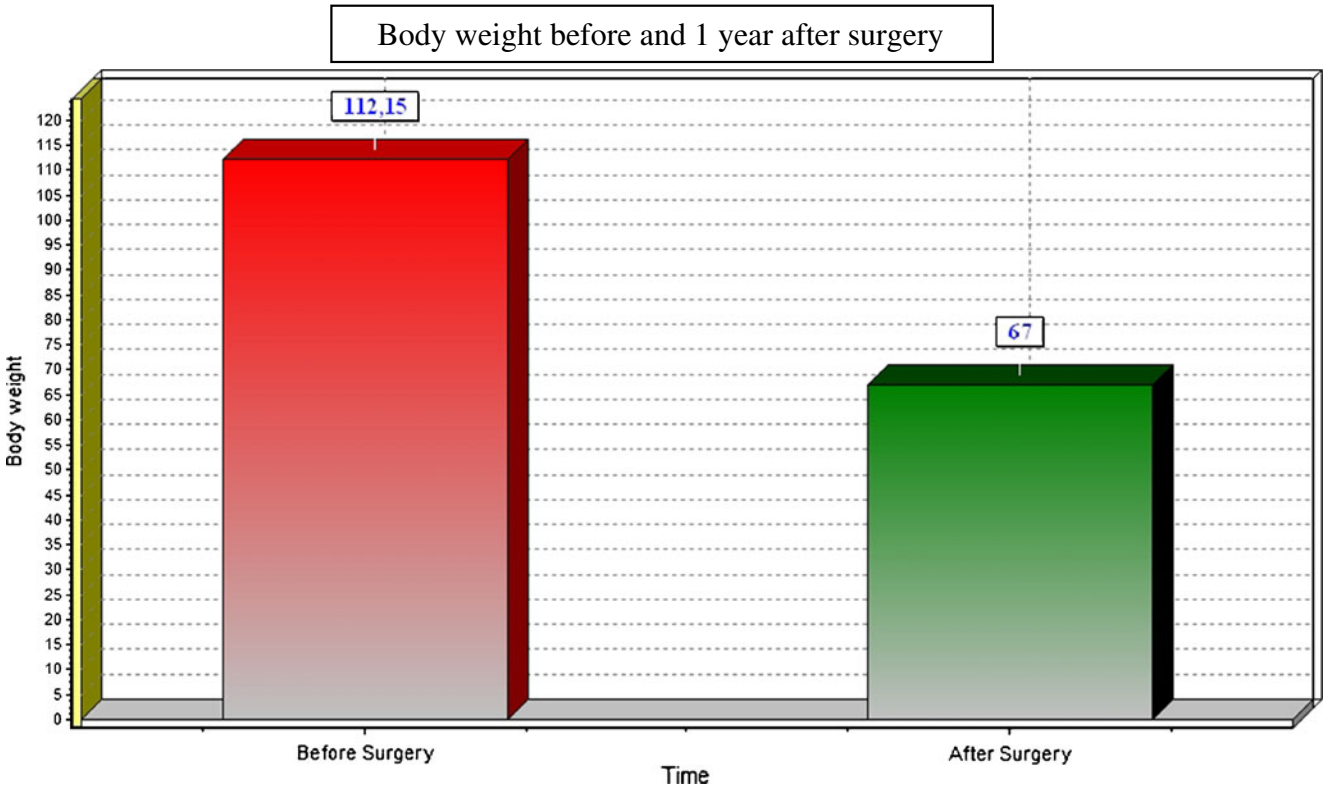


Fig. 3 Late evolution of body weight after surgery

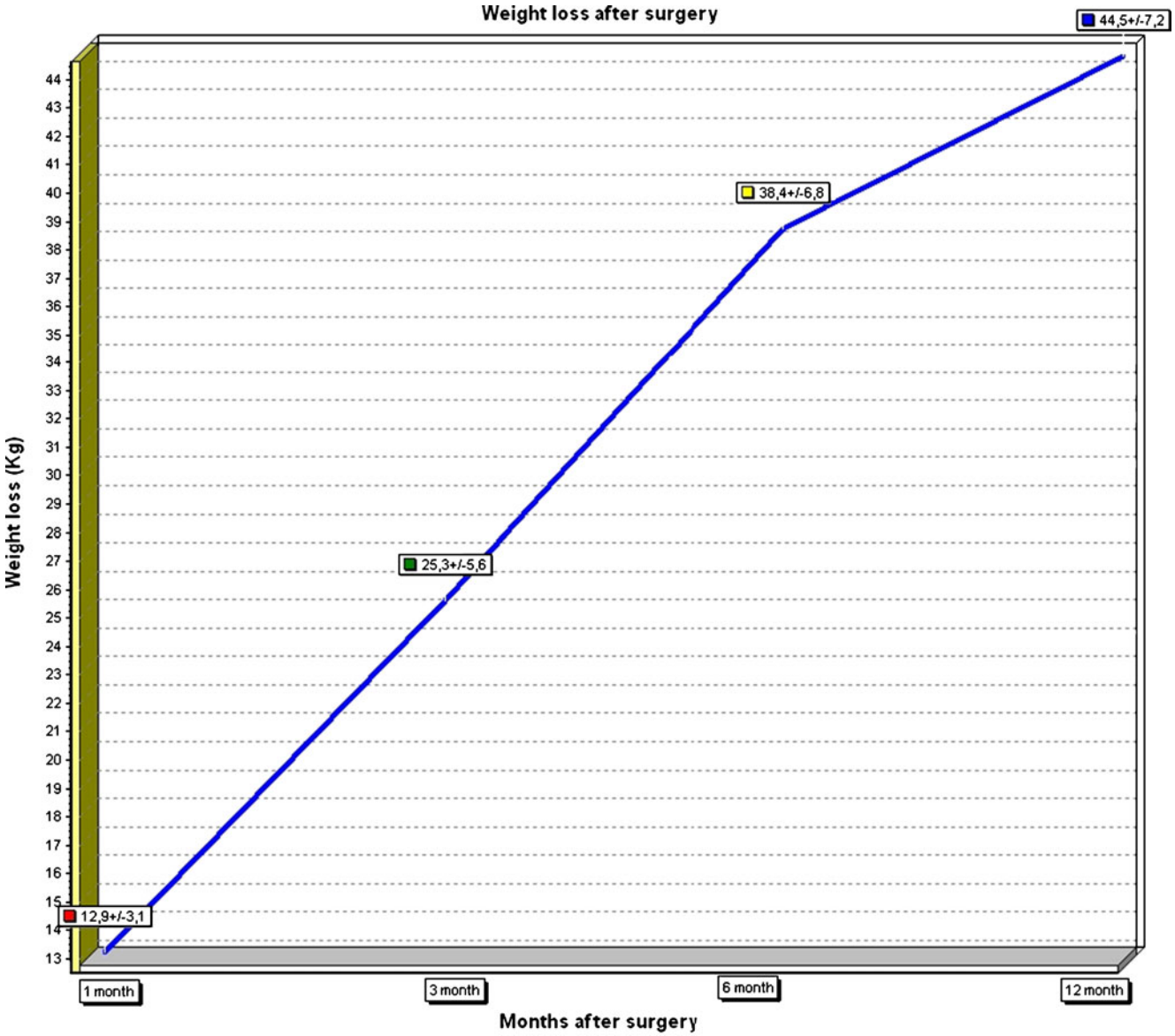


Fig. 4 Body weight loss after 1, 3, 6 and 12 months after surgery

third month, it was 25.3±5.6 kg, at the sixth month, it was 38.4±6.8 kg, and at 1 year after surgery, it was 44.5±7.2 kg (Fig. 4). Body mass index (BMI) reduction is presented in Fig. 5, which decreased from 40.5±(32.9–50.3) to 30.4±3.2 at the third month to 27.54±2.8 at the sixth month and to 24.69±2.4 1 year after surgery.

The results of the histological study of the resected specimen is shown in Table 5. Normal mucosa was found in ten patients (8.9%). The other 102 patients (91%) had different type of histological chronic gastritis. Concomitant findings were found in 34 patients (30.3%). Presence of *Helicobacter pylori*, despite its preoperative eradication, was found in five patients.

Discussion

The incidence rate of gastric cancer in the last decade in Chile is 27/100,000 inhabitants, but in the southern provinces, this incidence reaches to 29.2/100,000 inhabitants. Countries such as USA and Western Europe have low rates of gastric cancer, showing a decrease in the last decade, while in other countries with high rate of gastric cancer, such as Japan, Korea, and Latin America countries (Chile, Argentina, Brazil, Colombia, and Mexico), minimal changes have been reported in the last years.[11–13] For this reason, we and other Asian authors have postulated surgical procedure for morbid obesity which involve resection of the

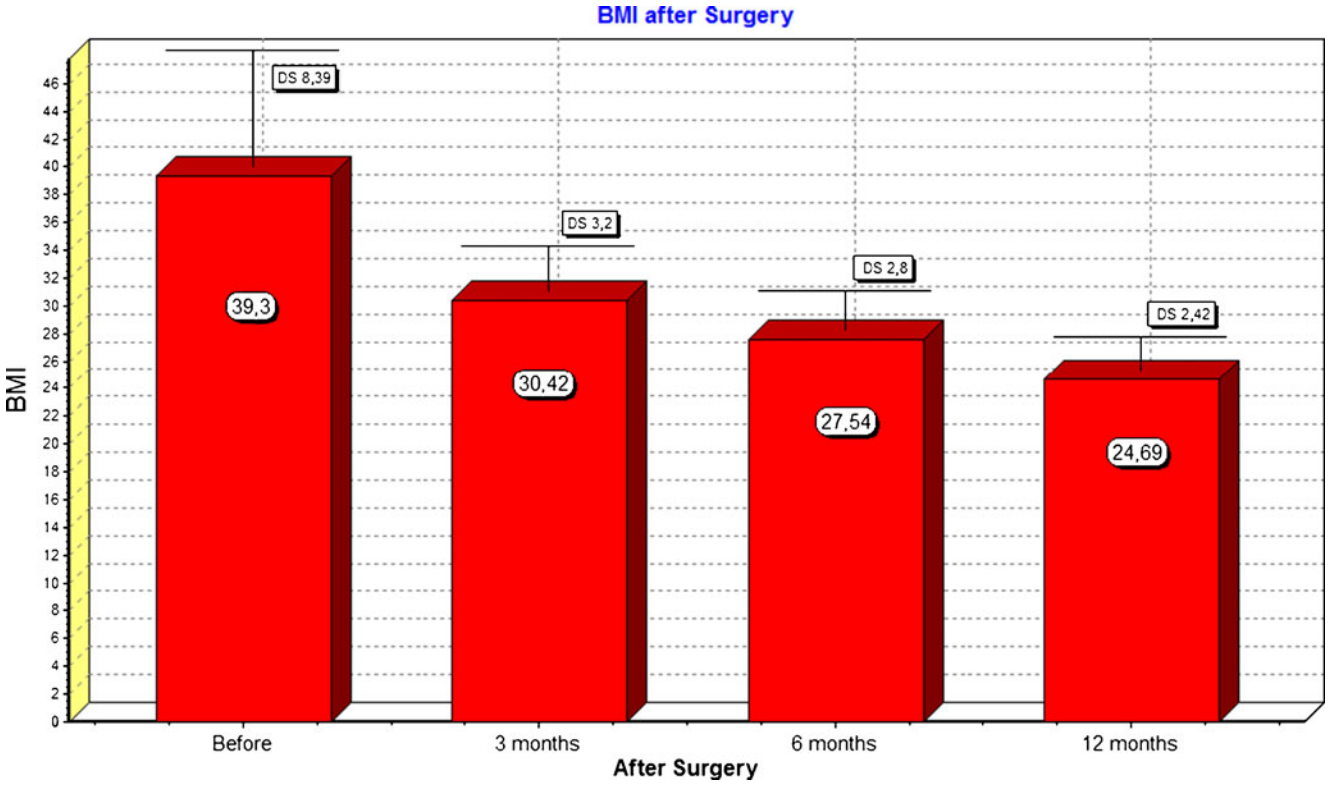


Fig. 5 Body mass index (BMI) reduction after the operation

stomach.[5,15] In our institution, the surgery more frequently performed in the last decade was resectional gastric bypass by open approach. Csendes et al.[5] published the results and the arguments concerning why gastric resection of distal stomach is a valid procedure in Chile and other countries with high incidence of gastric cancer. Kasama et al. propose to perform sleeve gastrectomy plus duodeno-jejunal bypass for morbid obese patients in Asia.[15] In the last 3 years, we started to perform gastric bypass with resection of the distal segment of stomach in patients with moderate obesity with Barrett's esophagus or diabetes associated or in patients with proper morbid obesity. Most surgeons can criticize our strategy; however, the results obtained have been very successful. Regarding the technical point of view, resection of the stomach creates a large space below the proximal gastric stump which facilitates enormously the performance of gastrojejunal anastomosis.

The first conclusion of this paper is that gastric resection of distal stomach during gastric bypass does not increase morbidity or postoperative mortality. After a short period of learning curve, the duration of the operation is only

Table 5 Histological findings in the resected stomach $n=112$

	Number	Percent
Normal	10	8.9
Gastritis		
Atrophic	11	9.8
Lymphoid	15	13.4
Follicular	33	29.4
Interstitial	38	33.9
Erosive hemorrhagic	5	4.4
Concomitant findings:		
Intestinal metaplasia	19	8.9
Lymphoid nodular hyperplasia	5	4.4
Polyps	5	4.4
Dysplasia	4	3.5
GIST	1	0.9
Helicobacter pylori +	5[a]	4.4

[a] Despite preoperative eradication

Table 6 Main early postoperative complications after gastric bypass: literature review (9,930 patients in 21 selected series)

Complications	Mean (range)
Leaks	4.2% (0.6–6.6)
Bowel obstruction	2.4% (0.4–5.5)
GI bleeding	1.9% (0.3–3.7)
Stomal stricture	3.3% (0.5–6.6)
Pneumonia	0.14% (0.06–0.4)
Pulmonary embolism	0.4% (0–0.8%)
Mortality	0.5% (0–1.1%)

15–20 min more prolonged compared to non-resectional gastric bypass by laparoscopic approach (LGBP). The operative time reported for non-resectional LGBP ranges from 91 to 277 min.[16–19] For open resectional gastric bypass, the duration of the operation ranged between 2 and 3 h.[5] The results obtained in this study regarding postoperative complications are no different to the reported results published by Csendes et al. for open resectional gastric bypass.[5]

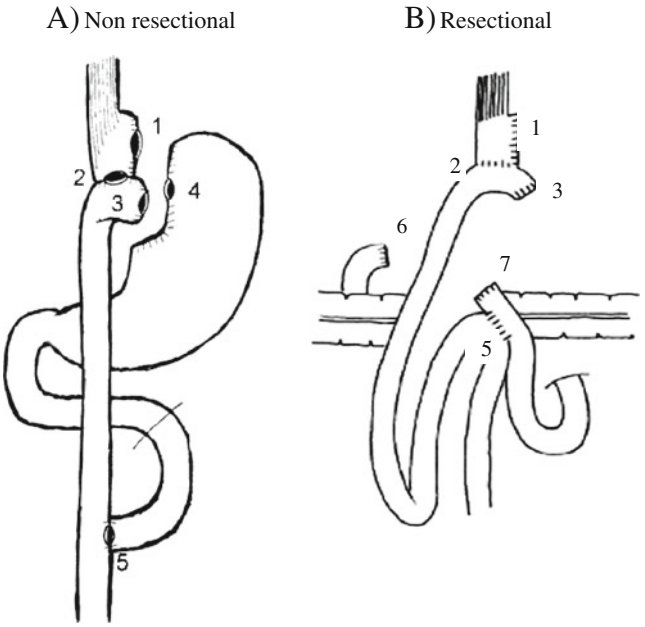
Our convertion rate is similar to the reported results in patients submitted to LGBP. The mean conversion rate for classic gastric bypass is 2.2% (range 0–3.0%) according to several selected papers analyzed.[4,19]

Regarding early postoperative morbidity, no increased complications due to resection of the distal stomach have been observed. Although there may be some patients with duodenal stump leak, which is managed medically by drainage, this complication is equivalent to complications when leaving the stomach in situ such us leaks from residual excluded stomach, bleeding from stomach and duodenum, and mostly producing gastro-gastric fistulas in 1% to 8% of the cases. Therefore, resection of the excluded stomach has several benefits. Besides, the early complications after laparoscopic Roux-en-Y gastric bypass without resection for distal stomach range between 3.3% and 15%.[4,18–21] After open and more recently, after laparoscopic non-resectional gastric bypass, several complications related to the in situ gastric remnant have been described. Bleeding from erosive gastritis or peptic ulcers (1–4%), gastro-gastric fistulas (6%), and perforated gastric ulcers with intra-abdominal abscess have been reported and discussed in the literature. In Table 6, we showed a summary of the reported complications after gastric bypass considering the more recent reports.[4,18–22] Nyugen et al. reported recently 21.6% of complication after gastric bypass.[4,18] The early complications include anastomotic leaks, bleeding, venous thromboembolism, anastomotic strictures being so frequent as 36% after hand-sewn anastomosis and being so critical that need dilatation in 10% of cases.[21–24] Maclean and others reported anastomotic ulcer reaching 16%, and more recently, in the last IFSO European Chapter Congress, similar results were reported.[2,6,7,25–36] In the present series, there are very few complications.

Anastomotic leaks are related to the learning curve but it occurs in 1–3% of patients even in experienced clinical centers.[4,25,37] Csendes et al.[28] published the experience in 557 patients submitted to open resectional gastric bypass. In this study, 12 patients (2.1%) developed an anastomotic leak at the gastrojejunostomy. All were managed medically, with antibiotics if necessary, enteral or parenteral feeding and frequent control by imaging procedures. One of the 12 (8%) patients died, 32 days after surgery from septic shock, without any abdominal collection secondary to the leak. The potential sites of leaks after surgery comparing gastric bypass with or without gastric resection are almost the same (Figs. 5 and 6). Duodenal stump leak is the additional site of leak after resectional gastric bypass, but it is very rare and easily treated by drainage if it is diagnosed early.

Gastrointestinal bleeding ranged between 0.26% and 3.7%.[29,30,35] Pulmonary embolism is a rare complication because of the prophylactic treatment with anticoagulant early after surgery; however, it represents the main cause of postoperative mortality.[4,26,30]

The mean hospital stay ranged from 1.6 to 4.0 days, excluding the patients who were converted to open surgery.[4,19,26] In the present experience, we have observed no increase of hospital stay, and cases more than 4 days correspond to few cases with postoperative complications.



1) Gastric stump (both)
2) Gastrojejuno anastomosis (both)
3) Proximal Jejunal stump (both)
4) Distal stomach (Only non resectional)
5) Jejuno-jejuno anastomosis (both)
6) Duodenal stump (Only resectional)
7) Distal jejunal stump

Fig. 6 Possible sites of leaks comparing non-resectional with resectional gastric bypass. **a** Non-resectional. **b** Resectional. *1* Gastric stump (both), *2* gastrojejuno anastomosis (both), *3* proximal jejunal stump (both), *4* distal stomach (only non-resectional), *5* jejuno-jejuno anastomosis (both), *6* duodenal stump (only resectional), *7* distal jejunal stump

Postoperative mortality ranged between 0% and 1.5%. However, data from Washington state register and Medicare systems reported 30-day mortality rate reaching to 2.0%. On the contrary, date from academic centers reports low postoperative mortality after bariatric surgery.[4,19,38,39] Morbidity and mortality are very low in our experience and exactly similar to the classic Roux-en-Y gastric bypass.

If we compare these data, we can conclude that resectional laparoscopic Roux-en-Y gastric bypass does not increase operative time, hospital stay, morbidity, and postoperative mortality.

Endoscopic/histologic findings or risk of gastric cancer in gastric segment have been studied and discussed in the literature. Kuga et al.[40] endoscopically studied the main gastric chamber after gastric bypass without resection. Only 25.7% of patients presented normal mucosa, 74.3% had erythomatous or erosive/hemorrhagic gastritis or atrophic gastritis. We observed almost the same findings studying histologically the resected specimen of distal stomach. Therefore, an important proportion of morbidly obese patients have pathologic gastric mucosa at moment of surgery. The controversy on the appearance of gastric cancer mainly refers to countries with a high incidence of gastric cancer (Asian and Latino-American countries). In countries with a very low incidence of gastric cancer, such as USA (six times less than Chile) obviously, it is not an important topic for discussion. In our department, we have found three cases of early gastric cancer during the preoperative evaluation by endoscopy, which is included in our study protocol for obese patients candidates to surgical treatment, and one patient was re-operated after sleeve gastrectomy and submitted to total gastrectomy because in the histological examination of the resected stomach, in situ gastric cancer in the line of resection was found.

Besides, few cases of gastric cancer cases have been published.[41–48] However, it is probably that other cases have not been reported. An experimental study suggested that Roux-en-Y gastric bypass reduces the risk of development of gastric cancer due to the lack of contact of with carcinogens' lower bile reflux and fewer bacterial in the proximal gastric pouch. However, this mechanism is not applied to the in situ distal segment because presence of chronic bile reflux, antral intestinal metaplasia (12%), bacterial overgrowth, and carcinogens could promote the development of gastric cancer in this segment, which is difficult to detect until the tumor has advanced disease.[49,50]

Other point of discussion is the necessity of late reconnection of gastric bypass performing re-gastrogastroanastomosis due to complications or later sequelaes of malnutrition or vitamins deficiencies.[51] We have never observed the necessity of reconnection of gastric transit due to this situation in more than 1,500 open or laparoscopic gastric bypass performed in our department. On the contrary, at the late follow-up, patients tend to regain weight. Therefore, it is not an argument to consider for leaving the distal stomach in situ for these eventual late complications.

We agree with all the arguments discussed by Csendes et al. in his previous report[5] in order to suggest gastric resection in these patients in order to avoid all these complications mentioned above.

The potential increase in cost is nil because the procedure is quite similar and we use only one additional blue cartridge in order to close the duodenal stump.

Finally, we believe that laparoscopic resectional gastric bypass presents very similar results concerning the operative performance of the procedure, without significant increase of morbidity, mortality, early and late postoperative results, and therefore, it is an option for the surgical treatment of morbid obesity in countries with high risk of gastric carcinoma.

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ORIGINAL ARTICLE

Minimally Invasive Total Gastrectomy for Gastric Cancer: A Pilot Series

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Abstract
Background Minimally invasive surgery for select gastrointestinal disease has gained worldwide acceptance. However, laparoscopic total gastrectomy for cancer remains controversial. The purpose of this study was to examine an initial experience with laparoscopic total gastrectomy.
Methods Medical records of 16 consecutive patients who underwent laparoscopic total gastrectomy between September 2007 and December 2009 were reviewed in a retrospective manner. Esophagojejunostomy was completed using a transorally delivered anvil, with double-stapled esophageal anastomosis.
Results There were no conversions to open procedures. Two patients (12.5%) required extended resections with en bloc distal pancreatectomy and splenectomy, one of whom also underwent transverse colectomy. The median lymph node count for patients who underwent D2 lymphadenectomy ($n=12$) for gastric adenocarcinoma was 31. There were no perioperative deaths and the median length of stay was 8 days. There were no anastomotic leaks, but three patients developed anastomotic strictures amenable to dilatation.
Conclusions Minimally invasive total gastrectomy can be performed safely and with adequate lymphadenectomy. The procedure provides an excellent short-term outcome with potential for improved patient outcome.

Keywords Laparoscopic gastrectomy · Gastric cancer · Total gastrectomy

Introduction

Minimally invasive techniques for colorectal cancer are being used with increasing frequency because they can be performed with lower morbidity and equivalent oncologic outcome to open techniques.[1] However, laparoscopic gastrectomy for cancer is not widely performed because the technique can be challenging and because concern exists regarding the adequacy of resection. A number of small randomized studies have compared laparoscopic to open gastrectomy, and adequacy of resection and oncologic outcomes appear to be similar.[2–5] Preliminary reports from the USA have established the feasibility of laparoscopic distal gastrectomy for cancer, and oncologic outcomes appear to be similar to those seen with open techniques.[6,7] While initial reports of laparoscopic total gastrectomy suggest that the procedure is possible,[8–11] few surgeons perform the procedure. Laparoscopic total gastrectomy poses a number of additional challenges, and this probably accounts for its slower acceptance by the surgical community. The procedure involves extending the dissection performed for distal gastrectomy and introduces the additional technical challenge of constructing an esophagojejunal anastomosis. Recently, techniques developed for bariatric gastrojejunal anastomoses have been adapted to esophagojejunal anastomoses.[12] The anastomosis has been simplified by the use of a transorally delivered anvil of a circular stapler. The Orvil™ device anvil is connected to a detachable oral delivery tube which is pulled through an enterotomy adjacent to the stapled end of the esophagus.[13,14]

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Following the introduction of the Orvil™ device and an initial experience with minimally invasive distal subtotal gastrectomy for cancer,[7] we initiated a laparoscopic total gastrectomy program. Our objective here was to report the initial experience with laparoscopic total gastrectomy.

Materials and Methods

Surgical Cohort

From September 2007 to December 2009, 16 consecutive patients who underwent laparoscopic total gastrectomy were identified from a prospective database at an NCI Designated Comprehensive Cancer Center. Patient characteristics, operative details, complications, and pathology parameters were extracted from the database. All surgical procedures were performed at City of Hope Medical Center. This retrospective research study was approved by the City of Hope Institutional Review Board.

Operative Technique

Following induction of general anesthesia, the patient is placed in supine, modified lithotomy position in slight reverse Trendelenburg (15–30°). The abdomen is entered at Palmer's point (i.e., left hypochondrium), using a Veress needle technique, and insufflated with CO_2. Port placement is similar to that used for laparoscopic distal gastrectomy as we have previously described.[15] A total of five ports (three 5-mm and two 10/12-mm ports) are placed in a V-shaped arrangement around the umbilicus. A right lateral 5-mm port is used for the placement of a fixed liver retractor, while right upper quadrant and left upper quadrant 5-mm ports are used for dissection. A supraumbilical 10/12-mm camera port is used and a left mid abdomen 10/12-mm port is used for linear stapling (Fig. 1).

The technique for total gastrectomy was modified based on the preoperative diagnosis. When the procedure is performed for gastric cancer, a complete omentectomy and D2 lymphadenectomy is performed. Dissection begins with complete omentectomy, accomplished by reflecting the greater omentum above the colon, followed by division of the gastrocolic ligament and entrance into the lesser sac. Mobilization of the stomach proceeds along the greater curvature up to the level of the left crus, with division of the short gastric vessels at the splenic hilum. The gastroepiploic vessels are ligated and divided at their origin and the first portion of the duodenum is transected with a linear stapler. With the stomach reflected to the left upper quadrant, the lymphadenectomy is completed either laparoscopically or with the use of the da Vinci® Surgical System (Intuitive Surgical Inc., Sunnyvale, CA) as described.[15] The D1 dissection involves removing the perigastric lymph nodes, including those around the lesser curvature (stations 1, 3, and 5) and greater curvature (2, 4, and 6). An extended, or D2, lymphadenectomy includes removal of the D1 nodes as well as those along the named branches of the celiac axis. Following dissection of the lymphatic tissue along the common hepatic artery (station 8), distally to the porta hepatis, and the left gastric artery (station 7), the left gastric vein is transected. The left gastric artery is transected at its origin and the celiac lymph node dissection is completed by skeletonizing the splenic artery from its origin out to the splenic hilum (stations 9–11). The greater omentum and the lesser omentum to the hepatoduodenal ligament are removed en bloc. The right and left crura of the diaphragm are dissected, and the distal esophagus is transected using a linear stapler. The 10/12-mm stapling port is then enlarged to 5 cm and a wound protector is placed for specimen extraction and for extracorporeal construction of an antecolic Roux-en-Y limb.

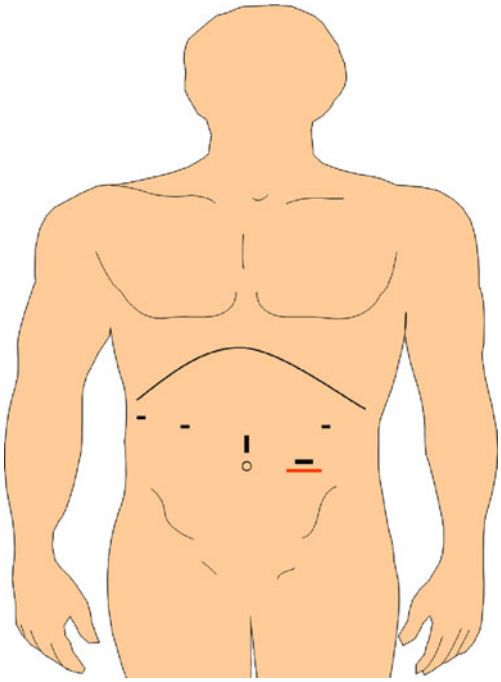

Fig. 1 Port placement for minimally invasive total gastrectomy

Following confirmation of the esophageal margin, the OrVil™ device (Covidien, Mansfield, MA) is passed through the mouth by the anesthesiologist and advanced to the distal esophagus. A small esophagotomy is made adjacent to the stapled esophageal stump and the orogastric tube is pulled into the peritoneal cavity and out through a 10/12-mm port placed in the partially closed wound protector.[16] The OrVil™ delivery tube is then detached from the anvil, leaving the anvil in position in the distal esophagus. A circular stapler (EEA XL 25 mm, Covidien) is advanced, through the partially closed wound protector,

into the blind end of the Roux limb. The EEA spike is advanced through the jejunum and connected to the EEA anvil which is secured in position using a janrick anvil grasper (Ethicon Endosurgery, Cincinnati, OH). The EEA stapler is closed and fired, completing the anastomosis; the open end of the jejunum is closed with a linear stapler. The mesenteric defects are left open. A feeding jejunostomy tube is placed laparoscopically using a percutaneous kit with T-fasteners to fix the bowel to the abdominal wall (Ross Laboratories, Columbus, OH). A nasogastric tube placed across the anastomosis is insufflated with air while the upper abdomen is filled with fluid to examine for air leak. A drain is placed adjacent to the esophagojejunal anastomosis and removed the day after a regular diet is resumed. The nasogastric tube is removed on postoperative day 1 and a radiographic swallowing study is performed on postoperative day 3, prior to initiation of a diet.

Results

Patient Characteristics

Between September 2007 and December 2009, 16 consecutive patients underwent minimally invasive total gastrectomy, seven males and nine females, with a median age of 62 (range 19–83). The characteristics of the patients are listed in Table 1. The mean BMI was 27.1 (range 16.4–69.5), reflecting a typical Western population. Patients underwent total gastrectomy with curative intent for gastric adenocarcinoma (N=12), for palliation (N=3), or prophylaxis (N=1) in a patient with family history of gastric cancer and a germline E-cadherin mutation. Patients who underwent total gastrectomy for cure also had a D2 lymphadenectomy, seven of which were DaVinci robot-assisted. The total gastrectomy for prophylaxis was completed with a D1 lymph node dissection.

Multi-organ resection was performed in two patients with T4 disease. One patient underwent splenectomy and completion gastrectomy for recurrent gastric adenocarcinoma. The other patient had tumor extension to the pancreas, requiring distal pancreatectomy, splenectomy and transverse colectomy, all of which was completed laparoscopically.

Table 1 Characteristics of the patient cohort

Factor	Median ± SD	Range	N (%)
Age (years)	62.0±17	19–83	
Gender			
Male	60		7 (44)
Female	62		9 (56)
BMI (kg/m^2) at surgery	24.9±12.8	16.4–69.5	
Histology			
Gastric adenocarcinoma			14 (87.5)
Other[a]			2 (12.5)
Tumor size (cm)	7.2±5.7	0.0–22.0	
LN status			
Positive			10 (62.5)
Negative			6 (37.5)
Stage			
I			5 (31.3)
II			1 (6.2)
III			4 (25)
IV			5 (31.3)
Other[b]			1 (6.2)

BMI body mass index, *LN* lymph node, *EBL* estimated blood loss, *LOS* length of stay

[a] Rhabdomyosarcoma (N=1) and prophylactic gastrectomy (N=1)

[b] Prophylactic gastrectomy

Table 2 Operative and postoperative data

Factor	Median ± SD	Range	N (%)
Multi-organ resection			
Yes			2 (12.5)
No			14 (87.5)
LN dissection			
D0	5	–	1 (6.2)
D1	29±10.6	25–45	3 (18.8)
D2[a]	31±15.4	17–76	12 (75)
Operative time (h)			
Gastrectomy only	6.3±1.4	2.8–7.4	
Multi-organ resection	8.2±0	8.2	
EBL			
Gastrectomy only	225±241	50–1,000	
Multi-organ resection	800±566	400–1,200	
LOS (days)	8±3.7	5–20	
Complications			
Esophageal leak			0 (0)
Esophageal stricture			3 (18.8)
Delayed emptying			0 (0)
Others			4 (25)
None			10 (68.8)
Follow-up (months)	7.4±7.2	0.4–29.6	

[a] Seven were performed with assistance of the da Vinci surgical robot

Peri- and Postoperative Data

The peri- and postoperative data are listed in Table 2. All procedures were completed laparoscopically, with a median operative time of 6.3 h (range 2.8–7.4) in those requiring gastrectomy alone, without the need for conversion to an open technique. The median estimated blood loss was 250 ml (range 50–1,200), 225 ml in those undergoing

gastrectomy alone and 800 ml for those who required multi-organ resection.

Resection margins were free of tumor in all except two patients, one of whom underwent palliative resection for a bulky metastatic rhabdomyosarcoma. The other patient presented with pan-gastric linitis plastica and had a positive distal margin after resection of the first portion of the duodenum. The median tumor size was 7.2 cm (range 0–22). When D2 lymphadenectomy was performed, a median of 31 (range 17–76) lymph nodes were detected. Final pathology showed five patients (37.5%) with early tumors, extending no further than the lamina propria, eight (50%) with T2 or T3, and two patients (12.5%) with tumors invading adjacent structures. Ten patients (62.5%) had positive lymph nodes.

There was no perioperative death or anastomotic leak. All patients were evaluated by esophagogram or computed tomography scan on postoperative day 3 or later. Four patients had perioperative complications (25%). These included supraventricular tachycardia, pancreatic fistula, and pneumonia. In three patients, esophageal strictures developed postoperatively. These were successfully managed with endoscopic dilatation. During an overall median follow-up period of 7.4 months (range 0.4–29.6), there was one death, from metastatic disease to the lung, in the patient with rhabdomyosarcoma. One patient with stage IV, and another with stage IIIB, gastric cancer developed peritoneal metastasis 4 months after gastrectomy.

Discussion

Minimally invasive techniques are gaining increasing acceptance in the cancer field. In colorectal cancer, level 1 evidence supports the use of a minimally invasive approach and suggests that it is an acceptable alternative to open surgery, with short- and long-term outcomes similar to those of open colectomy.[1,17,18] Important advantages gained over open surgery include reduced intraoperative blood loss, reduced postoperative pain, accelerated recovery, earlier return of normal bowel function, shorter hospital stay, and, ultimately, lower financial costs.[19–22] A laparoscopic surgical approach to gastrectomy was first introduced in the early 1990s.[23] While initially reserved for benign disease, it began to gain wider acceptance for gastric cancer.[24] Kitano et al.[4] published the first laparoscopic distal gastrectomy for gastric carcinoma in 1991, with a Billroth I reconstruction. Goh et al.[23] published the first laparoscopic distal gastrectomy with Bilroth II reconstruction for benign chronic ulcer in 1992, while Azagra et al.[25] published the first distal gastrectomy with Billroth II reconstruction for cancer in 1993.

A small prospective randomized trial of laparoscopic versus open gastrectomy performed in Italy demonstrated similar survival rates.[3] Laparoscopic-assisted distal gastrectomy, performed with an extracorporeal anastomosis, has been shown to result in improved quality of life for both pain and global health aspects.[26] Early small series of laparoscopic-assisted distal gastrectomy suggested that the technique would result in fewer pulmonary complications compared with the open approach.[4,5] However, larger laparoscopic-assisted gastrectomy studies do not support this assertion. The KLASS trial, a randomized, prospective multicenter study, showed no significant difference in morbidity or mortality between open and laparoscopic distal gastrectomy.[27]

Our approach has been to exploit recent advances in surgical stapling technology to enable an entirely laparoscopic approach with intracorporeal anastomosis.[15] Using the laparoscopic approach, we demonstrated a decrease in intraoperative blood loss, as well as a shorter hospital stay, when compared to patients who underwent open gastrectomy.[7] Our results were similar to those published by Strong et al.[6] who noted similar short-term survival rates for laparoscopic and open distal gastrectomy.

In contrast to laparoscopic distal gastric resection, there are few reports of laparoscopic total gastrectomy for gastric cancer, particularly in North America.[9,10] Laparoscopic total gastrectomy for gastric cancer was first described in 1999.[8,25] Initial reports suggest that gastric resection and lymphadenectomy can be performed with oncologic parameters comparable to open surgery.[11] However, in order for laparoscopic total gastrectomy to gain wider acceptance for the treatment of gastric cancer, it will be important to demonstrate that the outcomes are equivalent, or better than those seen with open procedures. Although overall survival remains the gold standard, it is widely accepted that the extent of lymphadenectomy is a surrogate marker of adequacy of resection.[28] According to the Japanese Gastric Cancer Association (JGCA), in early mucosal gastric cancer, D1 dissection is appropriate,[29] while D2 dissection, with a minimum of 25 lymph nodes harvested, meets the JGCA and Western criteria for lymphadenectomy in advanced gastric carcinoma.[30] We were able to meet these criteria with laparoscopic and, in a subset of patients, robotic extended lymphadenectomy. We have shown elsewhere that the use of the robot for extended lymphadenectomy is safe and allows for adequate lymph node retrieval during gastrectomy.[7,15,31]

Our approach to laparoscopic gastrectomy with lymphadenectomy is similar to that established by others.[16,32–34] One modification we have employed is the construction of the Roux-en-Y jejunojejunostomy by exteriorizing the proximal jejunum through the left abdominal specimen extraction site. This procedure is aided by the use of a wound protector which provides wound traction. The wound protector also facilitates the introduction of a circular stapler.[16]

The construction of an intracorporeal esophagojejunal anastomosis has been key to the success of a totally laparoscopic approach to total gastrectomy.[11] Difficulty with this step of the procedure, which was highly technically demanding, has been proven one of the greatest deterrents to the wider use of a totally laparoscopic approach to total gastrectomy. Recently, the use of an intracorporeal circular stapled esophagojejunostomy, using a transorally inserted anvil (OrVil™), has enabled a more straightforward reconstruction following laparoscopic total gastrectomy.[16] We elected to use the specimen extraction site for the introduction of the EEA stapler as the size of the EEA stapler is larger than the largest available laparoscopic port. By locating the extraction site in the left mid-abdomen, the EEA stapler can reach the gastroesophageal junction through the same incision and it is also possible to complete the jejunojejunostomy by exteriorizing the proximal jejunum without ever having to enlarge the incision. Extracorporeal jejunojejunostomy takes less time than an intracorporeal approach and does not result in a larger incision. Although it could be argued that this modification would render this technique laparoscopic-assisted, we would argue that it does not since it was performed through an existing incision. We have found this approach to be technically straightforward and safe. In our series, there were no postoperative leaks.

Internal hernia complicating laparoscopic Roux-en-Y gastric bypass is documented in the literature, but is rare after gastrectomy. It has been postulated that this may have something to do with the length of the Roux limb. One report showed that with closure of the mesenteric defects, the incidence of internal hernia in the retrocolic approach to Roux-en-Y gastric bypass was 2.6% compared with 0% with the antecolic technique ($P<0.025$).[35] However, Bauman and Pirrello,[36] in a retrospective series of 1,047 patients, found an incidence of 6.2%, using the antecolic method, without closure of the mesenteric defect. He concluded that leaving the defects open did not increase the incidence of internal hernia when the antecolic approach was used. We have not had any institutional experience of this complication to date.

The median length of stay (LOS) was 8 days. This is longer than that following laparoscopic distal gastrectomy, with an average LOS of 7.2 days.[26] The primary reason of the extended length of stay is that the ability to tolerate a diet adequate for hospital discharge is longer after esophagojejunal than after gastrojejunal anastomosis. The 8-day median LOS is significantly shorter than that for open total gastrectomy, with reports ranging from 11 to 23 days. Other small series of laparoscopic total gastrectomy also report longer LOS, ranging from 11 to 18 days.[11,16] We found that delayed anastomotic stricture does occur in a minority of patients. Fortunately, it has been managed by a single endoluminal esophageal dilatation.

The small series we report here represents our early experience with laparoscopic total gastrectomy. The small sample size suggests that we are still within our learning curve for the procedure. Nonetheless, we have demonstrated that laparoscopic total gastrectomy can be performed using a straightforward intracorporeal technique. Furthermore, the procedure can be performed with few complications and a very low conversion rate. This can be accomplished with luminal and nodal clearance similar to that seen with standard open techniques. This preliminary report supports the further evaluation of minimally invasive total gastrectomy for cancer in prospective studies.

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ORIGINAL ARTICLE

Meranzin Hydrate Induces Similar Effect to Fructus Aurantii on Intestinal Motility through Activation of H_1 Histamine Receptors

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Abstract This experiment studied the potential effect of meranzin hydrate (MH) and decoction of herb Fructus Aurantii (FA) on rat gut motility. It also investigated the prokinetic mechanism of MH. Experiments were performed on male Sprague–Dawley rats (200–220 g). The study included: (1) qualitation of MH and four other known compounds in FA and jejunum after oral administration of FA decoction to rats; (2) in vitro experiment of MH on rat jejunum contractions; (3) in vivo experiment of FA and MH in rats. Dose-dependently, MH (1–100 μM) increased amplitude in longitudinal and circular jejunum muscles. Pretreatment of jejunum longitudinal strips with benzhydramine (1 μM) remarkably inhibited the contractions induced by histamine (1 μM) and MH (10 or 30 μM). Pretreatment of jejunum longitudinal strips with atropine (1 μM) reduced the contractions induced by acetylcholine (1 μM) but did not influence the contractions induced by MH (10 or 30 μM). Interestingly, the antagonism of benzhydramine to MH was also verified in vivo. MH can be absorbed into the jejunum following oral administration of FA decoction. In healthy rats, MH (7, 14, and 28 mg/kg) and FA (3.3, 10, and 20 g/kg) both promoted intestinal transit and gastric emptying in a dose-dependent manner when gavaged acutely. In cisplatin model rats, MH (14 and 28 mg/kg) significantly reversed cisplatin-induced delay in gastric emptying. Meranzin hydrate can induce similar effect to Fructus Aurantii on intestinal motility and it was, at least in part, mediated by stimulation of H1 histamine receptors.

Keywords Meranzin hydrate · Intestinal motility · Jejunum · Fructus Aurantii

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Introduction

Functional dyspepsia (FD), recently defined as persistent or recurrent postprandial distress syndrome (early sensation or postprandial fullness) and epigastric pain syndrome (pain and discomfort or burning in the epigastrium), is a common pathology of the gut.[1] The prevalence of FD has been noted to vary between 11–29.2% globally.[2] Although FD is not life-threatening and it has not been shown to be associated with any increase in mortality,[2] but, the impact of this condition on patients and health care services has been shown to be considerable. It is reported that people with FD have a significantly reduced quality of life when compared to the general population.[3] The main pathophysiology is gut motor dysfunction.[4,5] To date, the prokinetic effect of

cisapride, mosapride citrate, and domperidone is far from satisfactory due to side effects.[5–7]

Some traditional Chinese medicines (TCM) have produced relatively favorable effect to FD in China, Japan, and Korea. The representative formulae are FM (Fructus Aurantii and Magnolia bark) and XSJ (Xiaoyao-San-Jiawei).[8] Both contain Fructus Aurantii (FA). FA is considered as a prokinetic herb [9,10] and it mainly contains hesperidin, narirutin, naringin, neohesperidin, and meranzin hydrate (MH; Fig. 1). The first one stimulates the gastrointestinal movement,[11] the middle three components have antioxidant and/or anti-inflammatory effects which are beneficial in the treatment of FD,[12] and the function of the last one has been little studied. Its previously reported activity referred to anticancer.[13] MH, located in the peel of Citrus maxima fruit[14] and *Murraya paniculata*,[15] was recently isolated from FA by us for the first time.

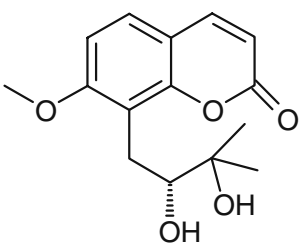
We were strongly interested in whether MH could induce similar effect to FA on intestinal motility. Actually, bioactivity-guided isolation is a popular strategy for isolation of new lead compounds nowadays.[16] Its major drawbacks are the frequent isolation of known metabolites [16] and the poor absorption of new compounds, which often resulted in the failure of new drug development.[17] We should focus on the effect of a new compound whose effect has been little researched.

Due to the causes mentioned above, we intended to ascertain whether MH can be absorbed into the jejunum following oral administration of FA decoction. If so, we will further investigate its effect on intestinal motility and prokinetic mechanism. The effect of MH, in selected doses, on cisplatin-induced delay in gastric emptying in rats was also investigated. Mosapride had the effect of reversing delay in gastric emptying in rats.[5] It was used for comparison. Study on this problem will not only guide the clinical applications but also provide an experimental basis for new drug development.

Materials and Methods

Experiments were performed using male Sprague Dawley rats (200–220 g) provided by Animal Experimental Center in Kaifu District (Changsha, China). All experiments conformed to the Regulations for the Administration of Affairs Concerning Experimental Animals (1988), which were approved by the Animal Experimental Center for Central South University (Changsha, China). Animals were housed in a temperature-controlled facility with a 12-h light/dark cycle. They had unlimited access to food and water for 14 days.

Fig. 1 The structure of meranzin hydrate



Merazin hydrate

Preparation of Fructus Aurantii Decoction

Fructus Aurantii was purchased from LBX pharmacy (Changsha, China) and identified. Voucher specimen (No.20090601) was deposited at the Laboratory of Ethnopharmacology in Xiangya Hospital, Central South University (Changsha, China). FA was boiled twice in distilled water (1:10, *w/v*) for 30 min. The blended supernatant was then lyophilized. The yield of lyophilized powder of FA was 22.56% (*w/w*).

Experimental Design

The study included: (1) qualitation of MH and four other known compounds in FA and jejunum after oral administration of FA decoction to rats; (2) in vitro experiment of MH on rat jejunum contractions; (3) in vivo experiment of FA and MH in rats.

Qualitation of MH and Four Other Known Compounds in FA and Jejunum

Qualitation of MH and Four Other Compounds in FA

The reference compounds are naringin, hesperidin, neohesperidin, narirutin, and meranzin hydrate. Naringin and hesperidin were purchased from Organic Herb Company (Changsha, China), narirutin was purchased from Sikehua Biochemical Technology Company (Chengdu, China), meranzin hydrate was purchased from DIAO Company (Chengdu, China), neohesperidin was purchased from Fukete Biochemical Technology Company (Changsha, China). Methanol was LC-grade (Tedia, USA), acetic acid was from Sinopharm Chemical Reagent Co.Ltd. (Shanghai, China). All water was triple-distilled with silica glass equipment in the Laboratory of Ethnopharmacology in Xiangya Hospital. The purity of all reference compounds was >99%.

Analysis was performed using a Waters Acuity UPLC BEH 2.1×100 mm, 1.7 μmC_{18} column system (Waters Corporation, Milford, USA). The mobile phase was composed of A, acetonitrile; B, water; and C, acetic acid (the amount of acetic acid is kept constant at 0.5% during the entire method) with gradient elution (0–10 min, 13–18%A; 10–20 min, 18–25%A; 20–25 min. 25–60%A). The flow rate of the mobile phase was 0.3 ml/min, and the temperature was maintained at 25°C. The concentration of FA before injection was 0.31 mg/ml.

Qualitation of MH and Four Other Compounds in Jejunum

The rats were fasted for 24 h with free access to water before drug administration. FA (20 g/kg) was orally administered 30 min before cervical dislocation. Segments of jejunum were carefully removed and placed in 0.9% sodium chloride prior to sample preparation. A 1-cm long segment of jejunum was then transferred to an inverted Petri dish placed in an ice box containing small volumes of ice-cold 0.9% sodium chloride. The tissue sections were then cut open along the mesenteric border and laid flat with the mucosal layer uppermost. The mucosal layer was scraped off from approximately 1-cm^2 segment of jejunum tissue. This mucosal tissue was homogenized in 500 μl of ice-cold 0.9% sodium chloride and centrifuged (Sigma 2–16 k, German Sigma Centrifuge) at 15 000 rpm at 4°C for 15 min, 100 μl of the supernatant was transferred into the glass autosampler vial and then added 100 μl methanol, vortexed for 10 min and then centrifuged at 15 000 rpm at 4°C for 15 min. 3 μl of the sample was then automatically injected into the UPLC system for analysis. The UPLC system and mobile phase were the same with 2.3.1..

In Vitro Experiment of MH on Rat Jejunum

Chemicals

Atropine sulfate (atrop), acetylcholine (ach), histamine (hista), benzhydramine (benzh) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Isolated Male Sprague Dawley Rat Jejunum

Tissue Preparation Male Sprague Dawley rats (200–220 g) were fasted for 24 h, and then sacrificed by stunning and cervical dislocation. Two-centimeter pieces of the jejunum segment were dissected from the jejunum. Luminal contents were washed out with Krebs-bicarbonate buffer (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11 mM Glucose, pH 7.4, at 37°C). Each segment was then opened along the mesenteric border, cut into 10×4 mm strips approximately along the circular and longitudinal axis individually and transferred into cold Krebs-bicarbonate buffer. The mucosa was removed to expose the muscularis externa.

Measurement of Contractile Activity Contractile activity was measured using a computerized integration system (BIOPAC MP150; BIOPAC system, Inc., USA). One side of the jejunum strip was connected to an electrode in a bath. The other side was connected via a thread to a tension transducer. The contractile responses of the strips to MH were measured according to the previously described method.[18] After mounting the isolated jejunum strips in organ bath, the strips were allowed to equilibrate for 30–60 min with washout every 10 min and oxygenated with 95% O_2 and 5% CO_2 at 37°C. Tension of 0.5 g for circular muscles and 1.0 g for longitudinal muscles was slowly applied to the tissues before treating drugs. MH was administered at increasing concentrations (1–100 μM) without washing between the administrations. The mean amplitude was measured for each concentration in the same way.

In order to determine MH's site of action, atropine sulfate (1 μM) and benzhydramine (1 μM) were used to mediate the blockage of muscarinic receptors and H_1 histamine receptors respectively. Ach (1 μM) and histamine (1 μM) were used as positive controls in comparison with MH-induced contractile change of jejunum. All antagonists used were pretreated for 5 min before adding MH, ach, or histamine. The basal levels (before treatment of drugs) served as control, so the values of drug-induced contractile responses are represented as a percentage of the control, means±S.E. of eight experiments.

In Vivo Experiment of FA and MH in Rats

Chemicals

Evans blue, charcoal, aqueous tragacanth, mosapride, and hydroxypropylmethyl cellulose were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Intestinal Transit Rate in Rats

Gastrointestinal transit was measured using a charcoal propulsion test.[19] The test compound and saline (control) were administered orally. Rats were randomly distributed into groups (eight each). Group 1 received 0.9% saline (10 ml/kg, p.o.; control); groups 2–4 were treated with FA in three doses (3.3, 10, or 20 g/kg, p.o.); group 5–8 were treated with MH in four doses (3.5, 7, 14, or 28 mg/kg, p.o.); group 9 received benzhydramine (10 mg/kg, i.p.). Group 10 received benzhydramine (10 mg/kg, i.p.) 50 min after oral administration of MH (14 mg/kg). Group 11 was given mosapride citrate (10 mg/kg, p.o.) as a positive control. Mosapride has been shown to accelerate upper GI motility.[20] One hour after FA, MH, or mosapride administration, each rat was orally administered 1 ml charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth). Rats were killed 30 min later by cervical dislocation. The extent of charcoal propulsion in the small intestine was measured (distance traveled by the charcoal head from the pylorus as well as total length of the small

intestine). Intestinal transit rate%=distance travelled by charcoal head/length of the small intestine×100.

Gastric Emptying Rate in Rats

The rate of gastric emptying of a non-nutrient semisolid meal was measured by SpectraMax M5 (Molecular Devices Company, USA), the method was adapted from the measurement of gastric emptying in mice previously described[21,22] with minor modifications. Male Sprague Dawley rats were fasted overnight with water ad libitum. A test meal of Evans blue (50 mg/ml in 0.9% NaCl with 0.5% methylcellulose) was given (1.5 ml/rat) by gastric tube. Thirty minutes after the meal was given, the animals were sacrificed. The stomach of each individual rat was cut just above the lower esophageal sphincter and the pyloric sphincter. Evans blue remains largely in the lumen of the stomach, a part of the Evans blue is trapped in the mucus layer of the stomach and a very small amount of Evans blue is resorbed in the mucosa after 30 min .[23] The stomach and its contents were then put in 15 ml 0.1 N NaOH. These samples therefore contain the total amount of Evans blue present in the stomach (mostly luminal and within the mucus layer). The stomach was minced and homogenized (GERRESHEIMER, USA) during 30 s. The samples were further diluted to 30 ml with 0.1 N NaOH and left at room

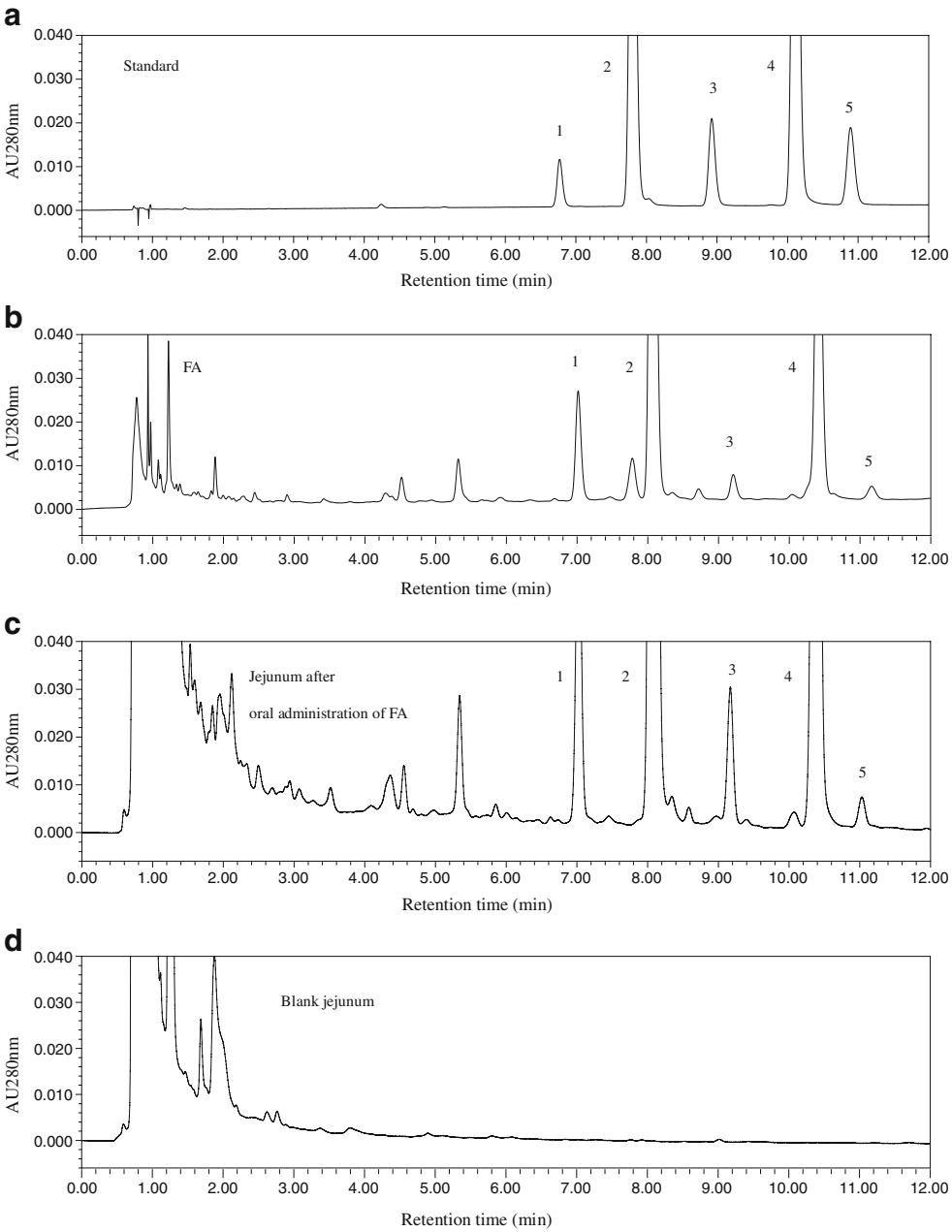


Fig. 2 Typical chromatograms of the standard mixture (**a**), FA (**b**), the five compounds in jejunum at 30 min after oral administration of FA decoction (**c**) to rats and blank jejunum (**d**) at 280 nm. (*1*) narirutin; (*2*) hesperidin; (*3*) naringin; (*4*) neohesperidin; (*5*) meranzin hydrate

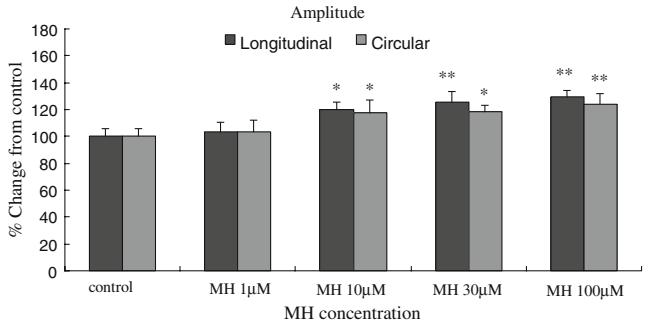


Fig. 3 The amplitude of longitudinal and circular muscle contraction in jejunum. The average amplitude were measured by isometric force transducers before (5 min) and after (15 min) treatment of MH. The values are represented as a percentage of the measurement before administration of MH (% of control, means±S.E.). Graph showing the concentration-related stimulant effects of MH (1–100 μM) on the contractions of jejunum. MH (1–100 μM) significantly increased the mean amplitude of contraction in jejunum compared with untreated controls (n=8, *one asterisk* (*) P<0.05 or *two asterisks* (**) P<0.01)

temperature for 1 h. Five milliliters of the supernatant were then centrifuged at 3,000 rpm for 15 min at 4°C. Samples were further diluted (1/50) with 0.1 N NaOH and the absorbance was read at a wavelength of 565 nm (A565) with SpectraMax M5. The stomach and its contents obtained from a rat sacrificed immediately after orogastric administration of Evans blue served as standard (reference stomach). Percent gastric emptying was calculated as [(A565reference–A565sample)/A565reference]×100.

Tested drugs

Effect of FA	FA at three different doses (3.3, 10, or 20 g/kg) was orally administered 30 min before the test meal.
Effect of MH	MH at four different doses (3.5, 7, 14, or 28 mg/kg) was orally administered 30 min before the test meal.
Effect of Mosapride	Mosapride (10 mg/kg) was orally administered 30 min before the test meal.
Effect of cisplatin	Animals were given the test meal, and simultaneously injected with cisplatin (10 mg/kg, i.p.).
Effect of MH on cisplatin-induced delay in gastric	Effect of MH (14 and 28 mg/kg) was respectively

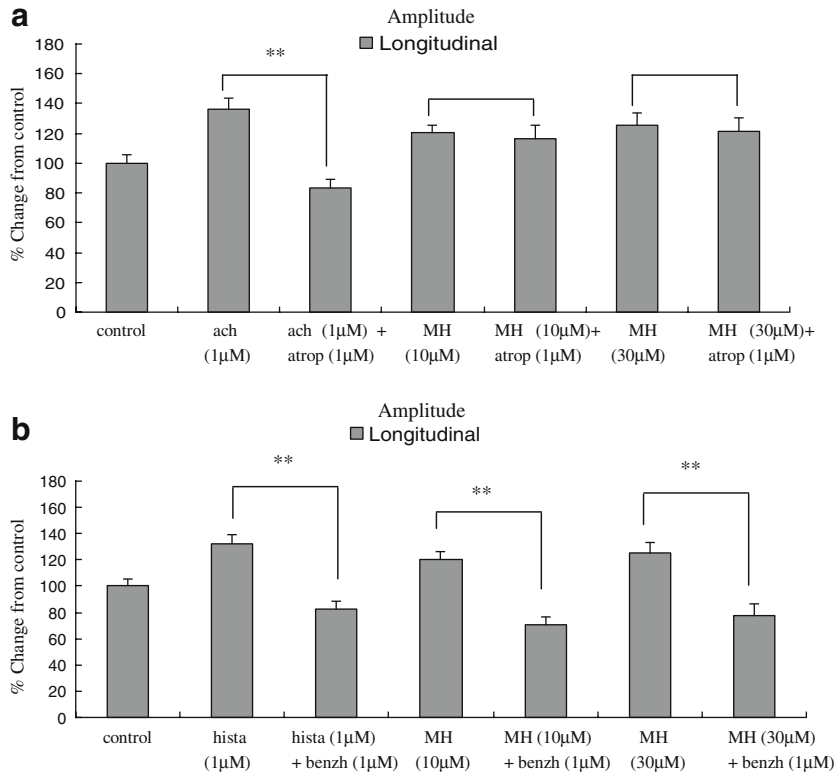


Fig. 4 Effects of antagonists on MH (10 or 30 μM)-induced contractions of jejunum longitudinal muscles. After pretreatment of the longitudinally mounted jejunum strips with atropine (**a**) or benzhydramine (**b**) for 5 min, MH (10 or 30 μM), ach (1 μM), or histamine (1 μM) was treated for 20 min, respectively. The average amplitude of muscle contractility were measured by isometric force transducers before (5 min) and after (20 min) treatment of agonists (n=8, *two asterisks* (**) P<0.01)

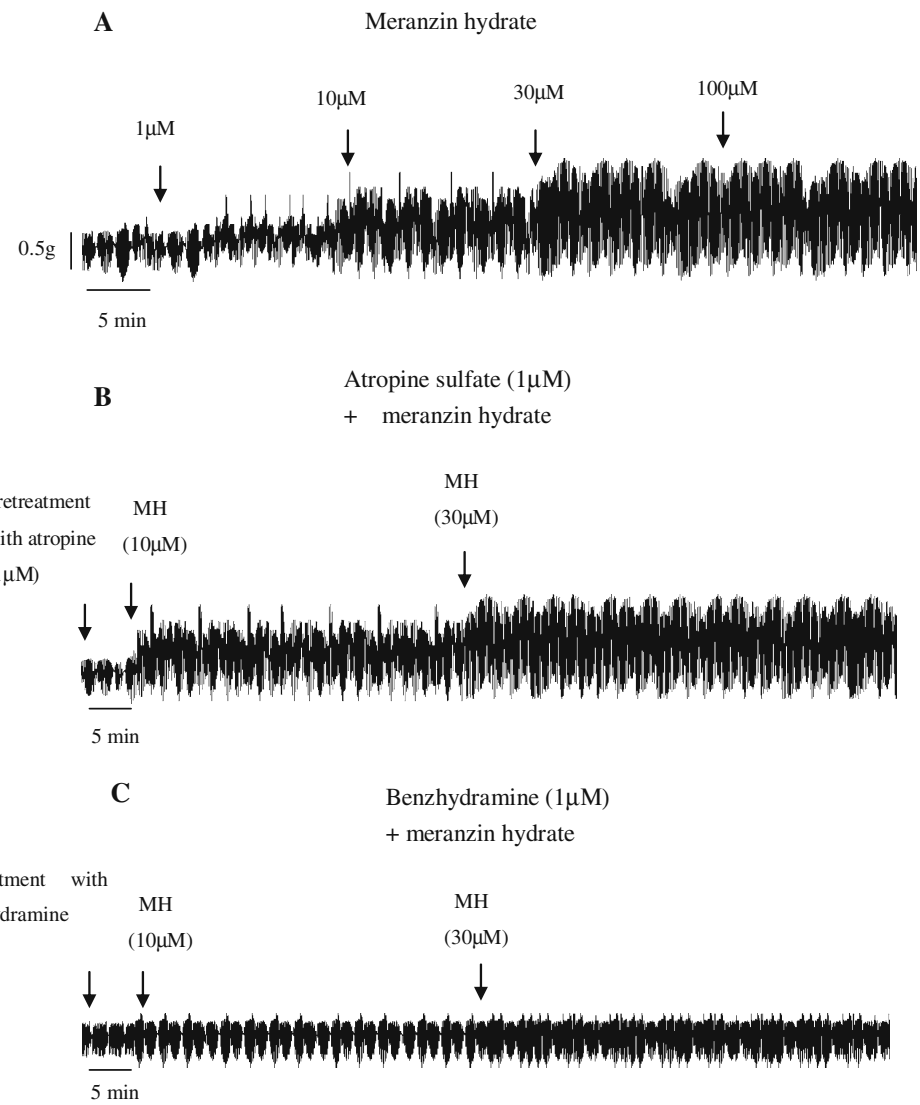


Fig. 5 Representative tracings. **a** Effect of MH (1–100 μM) on spontaneous activity of longitudinal muscle of jejunum. Cumulatively administered molar doses of agents caused a dose-dependent increase in contractile activity. **b** Pretreatment with atropine (1 μM) did not affect the response of jejunum longitudinal muscles to MH. **c** Pretreatment with benzhydramine (1 μM) abolished the effect of MH on jejunum longitudinal muscles

emptying

investigated on the delay of gastric emptying induced by cisplatin (10 mg/kg, i.p.). Animals were given the test meal at 30 min after MH (14 and 28 mg/kg, p.o.) or mosapride (10 mg/kg, p.o.) administration, and simultaneously injected with cisplatin (10 mg/kg, i.p.). The effect of MH was compared with mosapride.

Statistical Analysis

Data are expressed as means±S.E., and n indicates the number of replications for each data point or refers to the number of animals used. For the comparison of data, paired or unpaired Student's t tests or analysis of variance (ANOVA) were used where applicable. Differences were considered to be significant when $p<0.05$.

Results

Qualitation of MH and Other Four Compounds in FA and Jejunum

According to the UPLC method used, MH and the four other components in FA were rapid and successfully separated in less than 12 min. Chromatographic conditions were optimized to obtain good separation of the target compounds and avoid endogenous substances interference. Following oral gavage of FA decoction to rats, the five components particularly MH were absorbed by jejunum. Using PDA, UV spectra of the bioactive

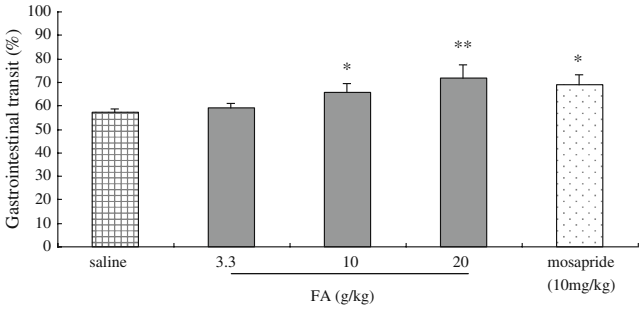


Fig. 6 Effect of FA on gastrointestinal transit rate of charcoal in rats. Rats were treated with 0.9% saline (10 ml/kg, p.o.; control), FA (3.3, 10, or 20 g/kg, p.o.), and mosapride (10 mg/kg, p.o.). *One asterisk* (*) $P<0.05$ or *two asterisks* (**) $P<0.01$ vs control group

constituents can be compared with those of the authentic standards. Typical chromatograms of the authentic standards, FA and the jejunum recorded at 280 nm are shown in Fig. 2.

In Vitro Experiment of MH on Rat Jejunum

Isolated Male Sprague Dawley Rat Jejunum MH (1–100 μM) dose-dependently increased the mean amplitude of contractions in the longitudinal and circular strip compared to untreated controls ($n=8$). In longitudinal strips, the percentage of control values were 103.3±7.15% (1 μM), 120.16±5.65% (10 μM), 125.80±7.74% (30 μM), 129.30±5.20% (100 μM). For circular strips the percentage of control values were 103.47±8.48% (1 μM), 117.20±9.14% (10 μM), 118.63±4.68% (30 μM), 124.30±7.80% (100 μM; Figs. 3 and 5).

On the basis of above results, submaximally effective concentrations (10 and 30 μM) of MH was used to investigate the site of its stimulant effect. Pretreatment of jejunum longitudinal strips with benzhydramine (1 μM), for 5 min dramatically inhibited the amplitude of histamine (1 μM) and MH (10 or 30 μM) induced longitudinal muscle contractions, whereas atropine (1 μM) did not affect MH-induced increases of longitudinal muscle contractions. The stimulant effect of MH may be mediated at least partly via stimulation of H_1 histamine receptors (Figs. 4 and 5).

In Vivo Experiment of FA and MH in Rats

Intestinal Transit Rate Induced by FA in Rats FA dose-dependently propelled the charcoal travel. The distance traveled by the vehicle control (saline) was 57.10±1.36%. FA at the low dose of 3.3 g/kg failed to accelerate charcoal transit. At the doses of 10 and 20 g/kg moved the charcoal to 65.70±3.66% and 72.01±5.50% and the effects were comparable to that of mosapride of 10 mg/kg (69.22±3.81%; Fig. 6).

Intestinal Transit Rate Induced by MH in Rats MH induced a dose-dependent increase in the distance traveled by charcoal in the gut of rats at 3.5, 7, 14, or 28 mg/kg. At the dose of 14 and 28 mg/kg, the ITRs were 68.21±4.67% and 74.86±6.01%. Mosapride (10 mg/kg) moved the charcoal to 69.22±3.81% of the small intestinal length. The accelerating effect of MH (14 mg/kg) was significantly reduced after pretreatment with benzhydramine (10 mg/kg). Benzhydramine (10 mg/kg) alone significantly reduced ITR as compared with the control group (a decrease of 20.07%). MH (14 mg/kg) significantly increased the ITR of the benzhydramine-treated group (by 31.81% compared with the benzhydramine group and 5.36% compared with the control group; Fig. 7).

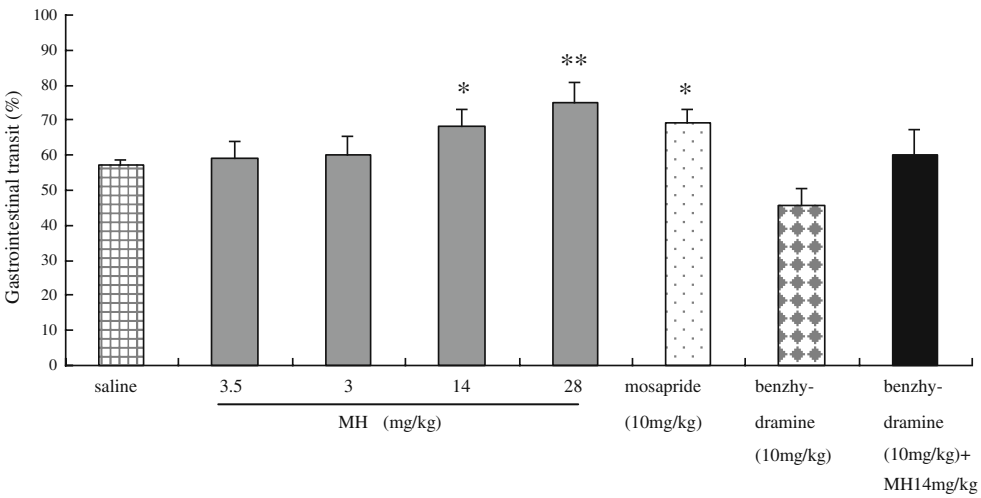


Fig. 7 Effect of MH on gastrointestinal transit rate of charcoal in rats. Rats were treated with 0.9% saline (10 ml/kg, p.o.; control), MH (3.5, 7, 14, or 28 mg/kg, p.o.), mosapride (10 mg/kg, p.o.), benzhydramine (10 mg/kg, i.p.), benzhydramine (10 mg/kg, i.p.+MH 14mg/kg, p.o.), *one asterisk* (*) $P<0.05$ or *two asterisks* (**) $P<0.01$ vs control group

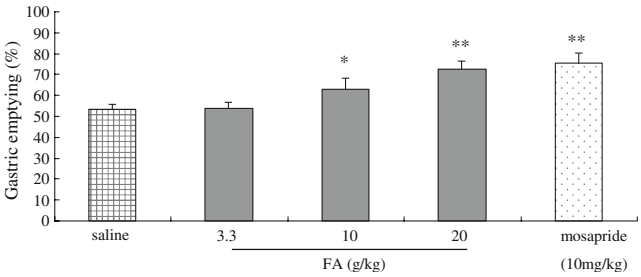


Fig. 8 Effect of FA on gastric emptying of Evans blue in rats. Rats were treated with 0.9% saline (10 ml/kg, p.o.; control), FA (3.3, 10, or 20 g/kg, p.o.), mosapride (10 mg/kg, p.o.), *one asterisk* (*) $P<0.05$ or *two asterisks* (**) $P<0.01$ vs control group

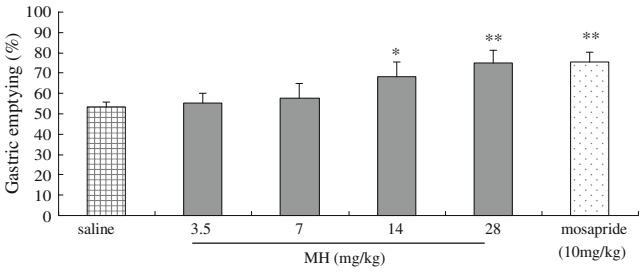


Fig. 9 Effect of MH on gastric emptying of Evans blue in rats. Rats were treated with 0.9% saline (10 ml/kg, p.o.; control), MH (3.5, 7, 14, or 28 mg/kg, p.o.), mosapride (10 mg/kg, p.o.). *One asterisk* (*) $P<0.05$ or *two asterisks* (**) $P<0.01$ vs control group

Gastric Emptying Rate

Effect of FA	Compared to saline, FA at a dose of 10 and 20 g/kg significantly accelerated gastric emptying of Evans blue from 53.20±2.41% to, respectively, 63.09±5.38% and 72.77±3.46%. Lower dose (3.3 g/kg) of FA failed to accelerate gastric emptying (*$P<0.05$ or ** $P<0.01$ vs control group; Fig. 8).
Effect of MH	MH increased gastric emptying dose-dependently between 3.5 and 28 mg/kg with significant effects at 14 mg/kg (68.33±4.7%) and 28 mg/kg (76.21±3.9%), and the effects were comparable to that of mosapride of 10 mg/kg (69.87±4.3%; Fig. 9).
Effect of MH on cisplatin-induced delay in gastric emptying	Pretreatment with oral MH at doses of 14 and 28 mg/kg, increased gastric emptying to 40.56±5.98% and 48.19±6.1%, respectively as compared to cisplatin (10 mg/kg) alone. The reversal of delayed gastric emptying was statistically significant at both doses (Fig. 10).

Discussion

As shown in Figs. 2, 6, 7, 8, and 9, five components particularly MH were absorbed into the jejunum following oral administration of the FA decoction and MH could produce similar effect to Fructus Aurantii on intestinal motility.

In vitro experiment demonstrated that MH had a significant dose-dependent stimulant effect of the amplitude of isolated rat jejunum (Fig. 3 and 5). At the highest MH concentration (100 μM), the amplitude of rat longitudinal jejunum strip could be multiplied for 1.29 times by MH compared to the control and circular strip for 1.24 times. This provides direct evidence for FA's enterokinetic effect. Muscarinic and H_1 histamine receptors are largely expressed in the muscle layers of GI tracts.[24–27] GI motility could be influenced by muscarinic and histaminergic modulation.[24,28] Cholinomimetic mechanisms are involved in the regulation of excitatory action of GI smooth muscles.[24,27] Atropine, a non-selective muscarinic receptor antagonist can block the rat intestine muscle contractions, both tone and amplitude, caused by acetylcholine.[29] Histamine, being an important cellular messenger of the gastrointestinal tract[25] can stimulate various smooth muscles including the gut tissues through activation of H_1 receptors.[26] Benzhydramine, a histaminergic (H_1) antagonist, can block H_1 receptors-mediated channels. In this study, mechanistic studies targeting muscarinic, and histamic pathways were performed challenging the effects of MH on jejunum peristalsis and intestinal transit against atropine and benzhydramine. Complete blockage of muscarinic receptors by atropine (1 μM) did not affect the

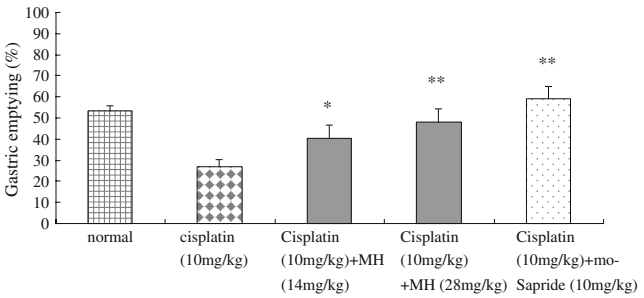


Fig. 10 Effect of MH and mosapride on cisplatin-induced delay in gastric emptying in rats (means±S.E., $n=8$, *one asterisk* (*) $P<0.05$ or *two asterisks* (**) $P<0.01$ vs cisplatin group)

response of jejunum to MH. The stimulant effect of MH on rat jejunum was abolished by benzhydramine (1 μM). Interestingly, we also find that the effect of MH (14 mg/kg) on intestinal transit was attenuated by benzhydramine (10 mg/kg) as shown in Fig. 7. The observation that benzhydramine could reduce the facilitative effect of MH further indicates the involvement of H_1 histamine receptors in mediating the stimulant effect of MH.

The in vivo experiment showed that MH (7, 14, and 28 mg/kg) and FA (3.3, 10, and 20 g/kg) promoted intestinal transit and gastric emptying in a dose-dependent manner when gavaged acutely (Figs. 6, 7, 8, and 9). Following gavage, MH (14 mg/kg) and FA (10 g/kg) promoted transit by 68.21±4.67% and 65.70±3.66%, respectively, compared with control. Their effects were similar to mosapride as indicated in Figs. 6 and 7. Mosapride, a widely accepted prokinetic agent ,[20,30] served as a positive control. To our surprise, MH was not only absorbable but also can induce similar prokinetic effect to FA, this suggested that a potential absorbable bioactive compound might exist in FA. Cisplatin, an antineoplastic agent with severe side effects of nausea and vomiting[31–33], can lead dose-related inhibition in gastric emptying.[33] In cisplatin model rats, MH (14 mg/kg and 28 mg/kg) significantly reversed cisplatin-induced delay in gastric emptying (Fig. 10). The effect of MH was concentration-dependent. The beneficial effect of MH could be owed at least partly to its stimulant effect on gastrointestinal tract. An agent that can reverse cisplatin-induced delay in gastric emptying implies that it may have favorable prokinetic effect in clinic.[5,34]

On the whole, it is conceivable that MH maybe an important prokinetic component in FA. MH, a new compound isolated from FA, was proven for the first time to have prokinetic effects similar to FA on intestinal motility. In this experiment, FA was also demonstrated to induce apparent prokinetic effect. This was consistent with prior literature.[35] All our data indicated that MH might have great potential as a safe and effective prokinetic agent capable of lessening FD symptoms and increasing quality of life in FD patients. This agent may be useful in reducing cisplatin-induced emesis and improve gastrointestinal symptoms such as chemotherapy-induced abdominal discomfort. It also provided experimental evidence for clinical application of FA. Further studies on the prokinetic mechanism of FA and MH on gastrointestinal motility may be helpful in determining the therapeutic values of FA and MH in gastrointestinal motor disorders.

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ORIGINAL ARTICLE

Mesenteric Vascular Thromboembolism in Inflammatory Bowel Disease: A Single Center Experience

Christian S. Jackson · Jonathan Fryer · Silvio Danese · Aryvdas Vanagunas · Sharon Polensky · Alan L. Buchman

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Abstract
Background Vascular thrombotic complications in inflammatory bowel disease (IBD) are well recognized, although mesenteric vascular thrombotic disease is rare.
Methods We describe nine patients in a tertiary care center with IBD that developed thrombosis of the mesenteric arterial or venous vasculature (e.g., mesenteric thrombosis, MT).
Results Eight subjects developed mesenteric venous thrombosis (five located in the superior mesenteric vein and three located in a branch of the portal vein) and one had a mesenteric arterial embolus, located in the splenic artery. Five subjects had Crohn's disease (CD), and four had ulcerative colitis. The one subject diagnosed with an arterial thrombosis had CD. Mean time from diagnosis of IBD to diagnosis of thrombosis was 24.6±13.5 years. Five of the nine subjects developed mesenteric venous thrombosis while their IBD was clinically in remission. Seven of nine subjects were symptomatic from the development of MT, including bowel infarction that led to development of short bowel syndrome.
Conclusion Mesenteric thrombosis is a rare complication of IBD and may develop during clinical remission, suggesting a potential role for factors other than clinically significant inflammation in its pathogenesis.

Keywords Mesenteric arterial thrombosis · Mesenteric venous thrombosis · Crohn's disease · Ulcerative colitis · Inflammatory bowel disease

The authors report no potential conflicts of interest.

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Introduction

The initial report of thromboembolism as a complication of inflammatory bowel disease (IBD) was described in a patient with ulcerative colitis (UC) in 1936.[1] Since this initial observation, multiple reports have suggested an association between both arterial and venous thromboembolism in the setting of IBD.[2–4] The frequency of thromboembolic events has varied between 1% and 8% of patients with IBD,[5] whereas in the postmortem period, the incidence has been reported as high as 41%.[5] Although lower extremity deep venous thrombosis (DVT) and pulmonary embolism are the most common thromboembolic phenomena encountered in patients with IBD,[2,4,6] mesenteric vascular thromboembolism (MT) has become more frequently recognized.[7,8] The majority of reported

cases of mesenteric thrombosis in association with IBD have been arterial, although venous thromboembolism has also been described.[9,10]

MT is a potentially devastating clinical sequela of IBD because it may lead to acute mesenteric ischemia and subsequent catastrophic mesenteric infarction, leading to death if not recognized early. Acute mesenteric ischemia in the non-IBD patient population is often lethal and in-hospital mortality rates have remained at 60–80% over the past 20 years.[11]

Methods

A retrospective chart review of patients with records from our institutions' IBD and Intestinal Rehabilitation Centers identified those with IBD and MT as well as those with mesenteric venous (MV) and short bowel syndrome (SBS) from March 2000 to December 2006. Evaluation for the presence of a hypercoaguable state included the testing for factor V Leiden, prothrombin and methytetrahydrofolate gene mutations, measurement of serum protein C and S, antithrombin III activities, plasma homocysteine concentration, the presence of antiphospholipid antibody, lupus anticoagulant titers, and plasma concentration of soluble (s) CD40 ligand (L). Smoking and contraceptive use was queried in all subjects. “Active” IBD was defined at the time of diagnosis of mesenteric vascular thromboembolism if the subject had pertinent gastrointestinal symptoms (abdominal pain, vomiting, diarrhea, or hematochezia), in conjunction with endoscopic or radiographic evidence of disease activity at that time and was thought by their treating physician to manifest “active IBD” that warranted medical or surgical therapy. An elevated erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) was considered and used to help guide that decision but was not sufficient alone; absence of an elevated ESR and/or CRP did not preclude the diagnosis of active IBD.

For the sCD40L measurements, venous blood was collected prospectively from subjects and one control in EDTA-containing blood collection tubes, placed immediately on ice and centrifuged at 1,000×*g* for 15 min, and plasma was stored at −80°C until analysis. sCD40L was measured by immunoassay (Quantikine, R&D Systems, Minneapolis, MN) with absorbance measured at 450 nm as previously reported.[12]

Results

Nine subjects developed MT (Table 1). Eight had mesenteric venous thrombosis (five located only in the superior mesenteric vein (SMV) and three located in a branch of the portal vein, PV), and one had a mesenteric arterial thrombosis located in the splenic artery (SA). Five subjects had Crohn's disease (CD) and four had UC. The one subject with an arterial thrombosis had CD. Mean time from diagnosis of IBD to thrombosis was 24.6±13.5 years. Although there were no deaths in our group, five patients had catastrophic consequences that led to SBS. A potential underlying risk for hypercoaguability was identified in four of the nine subjects (one had lupus anticoagulant detected and was also a heterozygote for the factor V Leiden mutation, one subject was a heterozygote for the factor V Leiden gene mutation alone, and one subject had a previously diagnosed DVT), and one subject had a plasma sCD40L plasma concentration raised compared with the other thrombotic patients, as well as normal controls (337 vs 110±32 pg/ml).[13] No other biochemical risk factors were found. One subject was an active cigarette smoker. No

Table 1 Subjects with mesenteric vascular thrombosis

Subject	Age (years)	Sex	IBD	Location of MT	SBS	Clinical symptoms of MT	IBD remission	Risk factor(s) for MT	Time between IBD diagnosis and MT
1	36	M	CD	PV	No	Yes	No	No	9
2	51	M	CD	SA	No	Yes	No	No	20
3	27	F	UC	PV	No	Yes	Yes	No	14
4	22	F	CD	PV	No	No	Yes	LA, factor V Het	12
5	49	F	UC	SMV	Yes	Yes	No	Pre DVT, CD40Ls	31
6	63	M	CD	SMV	Yes	Yes	Yes	No	40
7	28	M	UC	SMV	Yes	Yes	No	Factor V Het	11
8	65	F	UC	SMV	Yes	Yes	Yes	Smoker	40
9	62	M	CD	SMV, SV	Yes	No	Yes	No	45

MT mesenteric thrombosis, *PV* portal vein, *SA* splenic artery, *SMV* superior mesenteric vein, *SV* splenic vein, *LA* lupus anticoagulant, *Factor V Het* heterozygote for factor V gene mutation

patients had received anticoagulation prior to diagnosis of thrombosis. All of the patients with UC had pancolitis, and all of the patients with CD had ileal disease (two had extensive colonic disease in addition). Five of the nine subjects developed MT while their IBD was clinically in remission. Seven of nine subjects had symptomatic MT and required acute intervention. Four of the five subjects that developed SBS required an exploratory laparotomy for diagnosis of MT. Seven of the nine subjects received anticoagulation following diagnosis of MT and none have experienced symptomatic recurrent thrombotic events.

Discussion

Our retrospective review of IBD patients referred to our hospital or already followed at our IBD and Intestinal Rehabilitation Centers identified nine subjects who were diagnosed with MT, several of whom developed SBS. Although mesenteric thrombosis is a rare event, the striking finding in our series was that the majority (56%) of patients were perceived to have been in clinical remission at the time of their thrombotic event. This suggests that clinically detectable systemic inflammation had little role in the pathogenesis, although undetected inflammation cannot be excluded. Our observations are in congruence with those of Talbot et al., who observed that 77% of their patients with IBD developed peripheral venous thromboses during a period of clinical remission.[14] Irving et al. also described four patients with ulcerative colitis, all in clinical remission, who developed MT.[15] We cannot, however, ascertain the exact time at which thrombosis developed in our subjects who were clinically asymptomatic at the time of diagnosis. Four of our subjects did have potentially contributory risk factors other than IBD itself, although five had no known risk factors other than IBD. Although hyperhomocysteinemia is commonly encountered in both patients with CD or UC, we found no evidence of this abnormality in our patients. However, hyperhomocystemia has not been associated with thromboembolic events in patients with IBD.[16]

All our subjects with CD had ileocolonic disease, and all subjects with UC had pancolonic disease. Similar to one of the conclusions by Solem et al., these results may imply that the extent and distribution of colonic disease may correlate with thromboembolic risk.[6] The perioperative period was not a risk factor for development of MT in our subjects, in contrast to Hatoum et al. and Fichera et al.[17,18]

Increased platelet aggregation may be responsible, at least in part, for MT that occurs in the setting of active inflammation.[15] Webberly et al. found that spontaneous platelet aggregation occurred in vitro in platelets isolated from 30% of patients with active IBD but not in platelets from control subjects.[19] Mesenteric arterial and venous blood samples from patients with CD and UC showed increased platelet aggregation in mesenteric arterial and venous vasculature as compared with controls. Platelet aggregation was even greater in venous samples as compared with mesenteric arterial samples.[20] However, whether or not increased platelet aggregation occurs in patients that are in clinical remission with no evidence of systemic inflammation is unknown. The increased platelet aggregation in active IBD may be related to an enhanced CD40/CD40L system which is a key regulator and amplifier of immune reactivity and is activated in IBD.[12,21–24] Some investigation has shown that both CD and UC patients, especially those with clinically active disease, have significantly greater expression of this immunoregulatory and pro-inflammatory molecule when compared with healthy controls,[12,24] although that was not apparent in our case series. Our population of patients would suggest the CD40 pathway does not play a role in thrombosis in IBD patients.

Thrombosis in IBD appears to be a multivariate process with multiple potential risk factors; the etiology remains to be fully characterized. It is important to recognize that even in the absence of clinically significant inflammation devastating thrombosis may occur. Whether or not to anticoagulate patients with IBD and MT is controversial: certainly, anticoagulation in the setting of active IBD may result in increased hemorrhage risk. Given the substantial consequences of MT, however, we believe that all patients whose IBD is in remission but develop MT should receive life-long anticoagulation. Those patients whose MT developed in the setting of significant systemic inflammation should be treated on an individual basis.

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ORIGINAL ARTICLE

Neuroendocrine Tumors of Meckel's Diverticulum: Lessons from a Single Institution Study of Eight Cases

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Abstract

Introduction Endocrine tumors of Meckel's diverticulum are rare. Their clinical and pathological characteristics are not well known, making it difficult to assess the best strategy for therapeutic management.

Materials and Methods Eight cases of endocrine tumors of Meckel's diverticulum, submitted to surgical resection in our institution between 1977 and 2009, were studied. Clinical charts were reviewed; classification, grading, and staging were performed according to recent international recommendations. Five cases, including two associated with the carcinoid syndrome, were revealed by mesenteric mass or liver metastases; three cases were diagnosed incidentally at laparotomy or laparoscopy.

Results All cases presented as typical well-differentiated midgut endocrine tumors. Five cases were associated with mesenteric lymph node metastases; three presented with liver metastases. Seven cases were classified as well-differentiated endocrine carcinomas, one as well-differentiated endocrine tumor of benign behavior.

Discussion All tumors >1 cm, but one, had regional or distant disease. All patients had complete surgical resection of the primary. One patient deceased after 25 months; the others were alive after 12–101 months.

Conclusion In conclusion, endocrine tumors of Meckel's diverticulum are rarely symptomatic and often diagnosed at an advanced stage. All tumors measuring more than 1 cm in diameter must be resected according to oncological principles.

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Keywords Neuroendocrine tumors · Meckel's diverticulum · Surgery · Pathology · Carcinoid syndrome

Introduction

Meckel's diverticulum is the most common developmental abnormality of the gastro-intestinal tract.[1,2] Corresponding to a vestigial remnant of the omphalomesenteric or vitelline tract, it is located about 60 cm from the ileocecal valve, on the antimesenteric side of the small intestine. Meckel's diverticulum is known to be a rare but important location of primary endocrine tumors. Less than 200 cases have been described in the world literature, including the very first case for which the term "carcinoid" has been coined.[3] Most have appeared as case reports and have been published much prior to the recent efforts made in identifying and validating histoprognostic factors for gastroenteropancreatic endocrine tumors.[4–7] This paucity of information hampers a clear delineation of the clinical and pathological characteristics of these endocrine tumors and, in turn, makes it difficult to assess the best strategy for therapeutic management and follow-up. To date, several important issues remain controversial, including the following: (a) is there any relation between endocrine tumorigenesis and the possible occurrence of ectopic gastric and pancreatic tissues in Meckel's diverticulum[1,8] as suggested by some authors?[9] (b) Are endocrine tumors of Meckel's diverticulum more similar to appendiceal tumors as suggested by some reports,[10] or to ileal tumors as suggested by more recent analyses?[3] (c) Which is the actual risk of malignancy of the endocrine tumors of Meckel's diverticulum and their prognosis?

Addressing these issues is essential to determine the optimal therapeutic strategy and surveillance program in patients presenting with these rare and challenging tumors. This is particularly important for the cases discovered incidentally and/or post-operatively, especially since recent changes in the surgical practice, such as the increasing proportion of simple diverticulectomy under laparoscopy, may increase the risk of incomplete resection for tumors overlooked at pre- or per-operative stages.

We were therefore prompted to report eight cases of endocrine tumors of the Meckel's diverticulum, diagnosed and treated in our own center. This comparatively large series, the largest published so far, offers the opportunity: (a) to illustrate the full range of clinical and pathological features susceptible to be displayed by these tumors, (b) to reevaluate their risk of malignancy and to assess their prognosis, taking into account the recent proposals made for classifying, grading, and staging gastroenteropancreatic endocrine tumors,[4–7] (c) to discuss their optimal management, especially in light of the surgical techniques currently used for the resection of a Meckel's diverticulum discovered incidentally.

Material and Methods

Study Group

Hôpital Edouard Herriot, Lyon, is a large university hospital with an important activity in primary care and emergency surgery; it is also a referral center for a number of digestive tumors, including neuroendocrine tumors. For the purpose of the study, all the records from the Department of Surgery and Department of Pathology have been verified and the database containing informations about the patients referred to our institution for a gastro-entero-pancreatic neuroendocrine tumor has been checked. All patients submitted to surgical resection at Hôpital Edouard Herriot for a neuroendocrine tumor of Meckel's diverticulum between 1977 and 2009 were retrieved from these various sources and included in the study.

The study group consisted of eight patients. During the same period, in our institution, 112 Meckel's diverticula were resected in adult patients and 987 patients have been referred for the diagnosis and/or the treatment of a gastro-entero-pancreatic endocrine tumor.

Clinical charts and biological data were available for all the cases included in the study. The original pathological reports and all the available histological material were reviewed; additional immunohistochemical studies were performed when necessary. Complete follow-up was obtained for all patients; the end of follow-up period was 2010, June 1.

Clinical and Pathological Data

The following clinical parameters were recorded: sex, age at diagnosis, circumstances of diagnosis, evidence of regional and distant metastases, type of treatment, duration of follow-up, and status at the end of follow-up period. Urinary levels of 5-HIAA and serum levels of serotonin were recorded when available.

The following pathological features were noted: maximum diameter of the tumor, location of the tumor (basis or proximal half, tip or distal half, whole length), degree of morphological differentiation, extent of local invasion, evidence of angioinvasion and/or perineural invasion. The presence of ectopic gastric or pancreatic tissues was searched for in the adjacent mucosa. Immunohistochemical studies were performed in all cases to detect the following markers: chromogranin A (clone DAK-A3, Dako, Glostrup, DK), synaptophysin (clone 27G12, Novocastra, Newcastle-upon-Tyne, UK), NCAM (clone C56-504, Novocastra),

S100 protein (polyclonal, Dako), CDX2 (clone CDX2-88, Biogenex, San Ramon, CA, USA), and to evaluate the expression of the following hormones: serotonin (clone 5HT-H209, Dako), somatostatin (polyclonal, INSERM U45, Lyon, F), gastrin (polyclonal, INSERM U45), insulin (clone 2D11-H5, Novocastra), glucagon (clone K79bB10, Sigma, St Louis, MI, USA), pancreatic polypeptide (polyclonal, INSERM U45), ghrelin (clone Ab57222, Abcam, Cambridge, UK), and calcitonin (polyclonal, Dako). The mitotic index and the Ki67 index were determined according to the current recommendations.[5,7]

Tumors were classified according to the World Health Organization (WHO) classification.[4] They were graded according to European Neuroendocrine Tumor Society (ENETS) proposals.[5] Their TNM stage was determined according to ENETS proposals[6] and Union Internationale Contre le Cancer (UICC) classification.[7]

The survival curve of the eight patients with a neuroendocrine tumor of Meckel's diverticulum was determined according to the Kaplan–Meier method and compared with those of patients with ileal and appendiceal neuroendocrine tumors submitted to surgical resection in our institution during the same period.

Results

Clinical Features

There were five male and three female patients, aged from 45 to 72 years (median±SD=58±8.3 years). The circumstances of diagnosis were variable. In two patients (#1 and #2), the diagnosis was made during the investigation of a typical carcinoid syndrome, including flushing, diarrhea, and, in one case, right cardiopathy due to tricuspid insufficiency. Abdominal imaging studies, including ultrasonography associated with abdominal computed tomography (CT) in one case (patient #1) and with entero-CT in the other (patient #2), revealed the presence of multiple liver metastases. In the patient in whom it has been performed, entero-CT showed the presence of a hypervascular nodule located in the distal part of the small intestine (Fig. 1). In the other patient, no tumor was detected by CT scan but celio-mesenteric arteriography showed the presence of a hypervascular mesenteric mass. A pre-operative diagnosis of metastatic endocrine tumor was made by the histological examination of a guided liver biopsy in one patient (#1; Table 1).

In three patients (cases #3, 4, and 5), the diagnosis was made after the incidental discovery of multiple liver metastases (patient #3) or of a mesenteric mass (patients #4 and #5) at imaging studies performed for unrelated symptoms (hematuria in one patient, search for abdominal vascular lesions in two patients with hypertension and/or atheroma). Entero-CT, performed in two cases (patients #3 and #4), revealed the presence of a hypervascular lesion of the small intestine in both cases. In the remaining case (patient #5), no intestinal tumor was identified by abdominal CT scan. In two cases, a pre-operative diagnosis of metastatic endocrine tumor was made by the histological examination of a guided biopsy of a liver metastasis (patient #4) or of a mesenteric mass (patient #5).

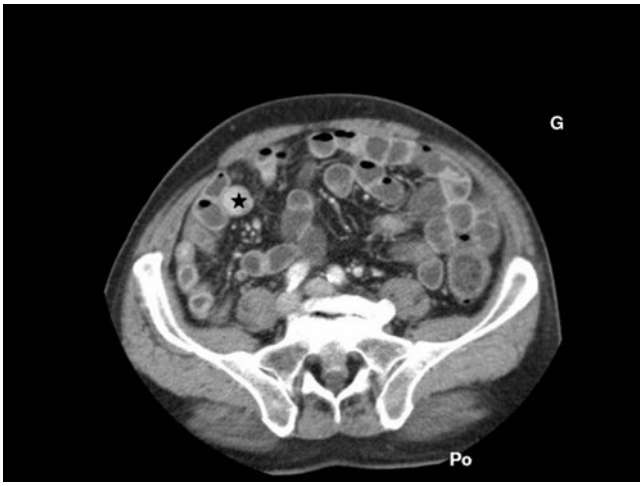

Fig. 1 Radiological features. CT scan (patient #2) showing a hypervascular mass located in the small intestine (*star*)

In the last three cases (patients #6, #7, and #8), the diagnosis was made post-operatively after the resection of a Meckel's diverticulum discovered incidentally at laparotomy (patient #6) or at laparoscopy (patients #7 and #8), during rectocolectomy for rectal adenocarcinoma in one patient (#6), cholecystectomy for cholelithiasis in the second patient (#7) and hysterectomy for uterine leiomyoma in the third case (#8). The resection of Meckel's diverticulum was performed by wedge resection in one patient (#6) and by stapler transverse diverticulectomy, in the two others (#7 and #8).

Octreoscan was performed in six cases. Intense positivity was detected in the primary tumor and in metastatic sites in three out of the four cases in which the study was performed pre-operatively; in the remaining case, only liver metastases were positive whereas the primary tumor was not detectable. In the two cases in which the test was performed post-operatively, intense positivity was detected in mesenteric lymph node metastases.

Urinary 5-HIAA levels were increased in the four cases (from 70 to 1,988 μmol/24 h; N<42 μmol/24 h) in which they have been assayed. Serum serotonin levels were increased (from 2.3 to 8.1 μmol/L; N<0.5 μmol/L) in the six patients in whom they have been evaluated.

Histological and Immunohistochemical Features

In all cases, the tumor was unique; no evidence for multifocal endocrine tumors of the small intestine was found in any

Table 1 Clinical features, treatment, and clinical course

Patient number	Age (years)	Sex	Presentation	Regional lymph node metastases	Liver metastases	Initial treatment of the primary	Other treatments	Duration of follow-up and status at the end of follow-up
1	62	F	Carcinoid syndrome	+	+	Segmental resection	TACE, somatostatin analogs, interferon	25 months, deceased
2	66	M	Carcinoid syndrome	+	+	Segmental resection	Surgical treatment of liver metastases, somatostatin analogs	101 months, alive
3	53	M	Mesenteric mass	+	−	Segmental resection		85 months, alive
4	57	M	Liver metastases	+	+	Segmental resection	Surgical treatment of liver metastases, somatostatin analogs	77 months, alive
5	72	M	Mesenteric mass	+	−	Segmental resection		56 months, alive
6	54	F	Incidental discovery at laparatomy	−	−	Wedge resection		52 months, alive
7	58	M	Incidental discovery at laparascopy	−	−	Transverse diverticulectomy		23 months, alive
8	45	F	Incidental discovery at laparascopy	+	−	Transverse diverticulectomy	Segmental resection of the small intestine and mesentery, somatostatin analogs	12 months, alive

M male, *F* female, + present, − absent, *TACE* transarterial chemoembolization

patient. The maximum diameter of the tumors ranged from 4 to 27 mm (median±SD=19±6.8 mm). The tumor was located in the proximal part of the diverticulum in four cases (patients #1, #2, #3, and #4) and in its distal part in three cases (patients #6, #7, and #8; Fig. 2a); it involved the whole length of the diverticulum in one case (patient #5). All tumors were morphologically well differentiated (Fig. 2b and c). They were formed by nests of monomorphic endocrine neoplastic cells with abundant cytoplasm and a centrally placed nucleus containing small nucleoli; tumor nests were separated by connective septa containing numerous vessels (Fig. 2c). Cellular atypia was rare. Angioinvasion was present in three cases and perineural invasion in three. In one case (patient #6), the tumor was limited to the submucosa (Fig. 2a). All the other tumors invaded the whole thickness of the diverticulum wall, including the serous layer (Table 2).

All tumors strongly expressed chromogranin A and synaptophysin; NCAM was detectable in five cases. CDX2 was expressed in all cases. There was no evidence of sus-tentacular cells after immunolabelling with anti-S100 protein in any case. All tumors strongly expressed serotonin (Fig. 2d); none of the other hormones tested was detected, except somatostatin, found in scattered tumor cells in one case (patient #2).

The mitotic index was <2 in all cases. The Ki67 index was >2% in only one case (patient #3).

Regional lymph node metastases were present in five cases. Liver metastases were detected in three patients. Six out of the seven tumors measuring more than 1 cm were metastatic at diagnosis.

All tumors were classified as well differentiated endocrine carcinomas according to the WHO classification, except one (patient #6), which was classified as a well-differentiated tumor of benign behavior. According to ENETS recommendations, all tumors were considered grade 1, except one (patient #3), which was classified as grade 2. All tumors were staged as T3 according to both ENETS and UICC TNM classifications, except one (patient #6), classified as T1. According to ENETS recommendations, one patient was stage I, one stage IIb, three stage IIIb, and three stage IV.

The histological analysis of the peritumoral mucosa of the Meckel's diverticulum showed no evidence of ectopic, gastric, or pancreatic, tissues in any case. In all cases, the peritumoral mucosa was of small intestinal type (Fig. 2b). No endocrine cell hyperplasia was detected after labeling for chromogranin A and synaptophysin.

Treatment

Resection of the primary tumor was performed in all cases, including patients with liver metastases because of the risk of local obstruction or occlusion, either by resection of the

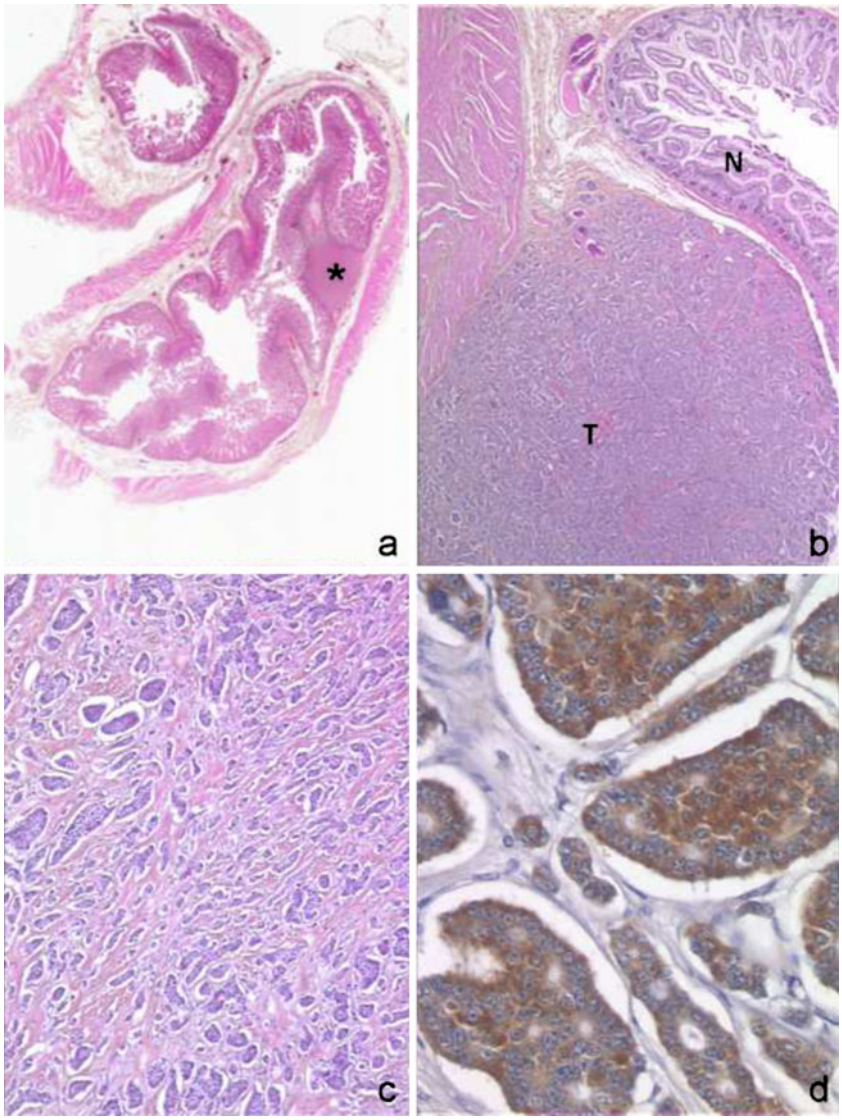

Fig. 2 Histological and immunohistochemical features. In **a**, a low power magnification of a submucosal endocrine tumor (*asterisk*; patient #7) discovered incidentally in a Meckel's diverticulum. **b** A closer view of the tumor (*T*) and of the adjacent normal mucosa (*N*), of ileal type. **c** A high power view of another tumor (patient #6), formed by nests of well-differentiated neoplastic endocrine cells, embedded in a dense fibrous stroma. **d** Homogeneous and intense serotonin expression by tumor cells (patient #8). **a**, **b**, and **c** Hematoxylin-eosin-saffron staining; original magnifications: **a** ×10, **b** ×120, **c** ×240. **d** Indirect immunoperoxidase with nuclear counterstaining; original magnification, ×310

Meckel's diverticulum (diverticulectomy or wedge resection) or by segmental resection of the small intestine (Table 1).

In two patients (#7 and 8), a simple diverticulectomy was performed. In one case (#7), no further treatment was performed, despite the presence of a large tumor invading the serous layer, since the patient refused a subsequent segmental resection of the distal ileum; at the date of the submission of the manuscript, after 20 months of follow-up, there is no evidence of recurrence or metastatic dissemination. In the other patient (#8), a 60-cm long segmental resection of the small intestine, with resection of a large mesenteric mass, was performed in a second step; there was no tumoral residue in the remaining portion of the diverticulum, which could be identified; the mesenteric mass corresponded to two large lymph node metastases.

In one patient (#6), in whom a wedge resection was performed during a rectocolectomy for rectal cancer, no further treatment of the endocrine tumor was done.

Segmental resection of the small intestine, including the Meckel's diverticulum and the mesentery, was performed in the five other patients. In two patients (#2 and 4), a surgical treatment of liver metastases was associated in the same time (left lobectomy in one case, left lobectomy and right metastasectomies in the other case). In one patient (#1), transarterial chemoembolization was performed pre- and post-operatively; in another patient (#2), it was performed post-operatively.

In addition to surgical treatment, somatostatin analogs were used in four patients (#1, 2, 4, and 8) and interferon in one (#1). No patient received chemotherapy.

Clinical Course

One patient (#1) deceased after 25 months, with progressive disease. All other patients were alive at the end of the follow-up period; the total duration of follow-up ranged

Table 2 Histological and immunohistochemical features

Patient number	Maximal diameter (mm)	Location	Differentiation	WHO classification	Mitotic index	Ki67 index	Grade	TNM (ENETS, UICC)	Clinical stage (ENETS)	Hormonal profile
1	15	Proximal	Well differentiated	WDEC	<1	0.5%	1	T3	IV	Serotonin, somatostatin
2	20	Proximal	Well differentiated	WDEC	<1	0.1%	1	T3	IV	Serotonin
3	20	Proximal	Well differentiated	WDEC	2	3%	2	T3	IIIb	Serotonin
4	27	Proximal	Well differentiated	WDEC	1	1%	1	T3	IV	Serotonin
5	20	Whole length	Well differentiated	WDEC	1	1%	1	T3	IIIb	Serotonin
6	4	Distal	Well differentiated	WDET	<1	0.25%	1	T1	I	Serotonin
7	18	Distal	Well differentiated	WDEC	1	0.25%	1	T3	IIb	Serotonin
8	12	Distal	Well differentiated	WDEC	<1	1%	1	T3	IIIb	Serotonin

WDEC well-differentiated endocrine carcinoma, *WDET* well-differentiated endocrine tumor, *WHO* World Health Organization, *ENETS* European NeuroEndocrine Tumor Society, *UICC* Union Internationale Contre le Cancer

from 12 to 101 months (median±SD=56±32.5 months) (Table 1). The 5-year survival was 80% for patients with regional disease and 75% for patients with liver involvement. The survival curve of the patients included in the study was compared to those of patients with respectively, ileal and appendiceal neuroendocrine tumors submitted to surgical resection in our institution during the same period (Fig. 3). In our experience, the survival probability of patients with neuroendocrine tumors of Meckel's diverticulum was somewhat intermediate between those of patients with respectively, ileal and appendiceal neuroendocrine tumors. Their mean survival was longer than in patients with ileal tumors but shorter than in patients with appendiceal tumors. The differences were statistically significant (log rank test, $p \leq 0.05$) but the low number of cases included in the study group prevents any definitive conclusion.

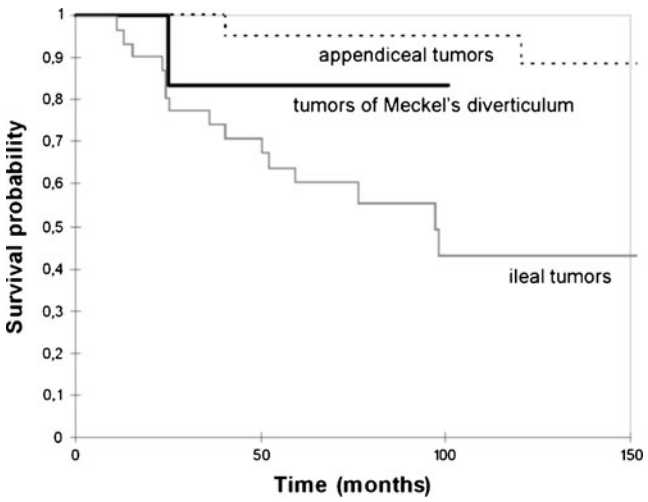

Fig. 3 Survival curves of patients with neuroendocrine tumors of Meckel's diverticulum as compared to patients with respectively, ileal and appendiceal neuroendocrine tumors submitted to surgical resection in the same institution during the same time period

Discussion

Our study, based on a series of eight patients, illustrates the various clinical presentations of neuroendocrine tumors of Meckel's diverticulum. It shows that these rare tumors are closely related to ileal endocrine tumors and that they are associated with a high risk of metastatic dissemination, even when the diagnosis is made incidentally. These findings have strong implications for patient management, and especially for the choice of the best surgical approach.

Our experience confirms the low incidence of the neuroendocrine tumors of Meckel's diverticulum: they represent less than 1% of the cases of digestive endocrine tumors referred to our institution over a 30-year period. However, in our department, about 10% of Meckel's diverticula resected in adult patients may contain an endocrine tumor. The incidence is therefore not negligible in this particular subset of patients.

In the literature, many cases of endocrine tumors of Meckel's diverticulum have been reported because they were discovered incidentally, during an abdominal surgical procedure for another indication, or because they were revealed by local symptoms, such as bowel obstruction, intussusception, or diverticulitis.[11,12] However, in our series, representative of the activity of a large primary and referral center in digestive surgery, none of the patients was symptomatic, only three cases were discovered incidentally and the five others were revealed by regional or distant metastases. In two out of these five cases, the diagnosis was made during the investigation of a typical carcinoid

syndrome related to the presence of liver metastases, as previously reported in a few patients;[13,14] in the three other cases, the diagnosis resulted from the incidental discovery of a mesenteric mass (two cases) or of liver metastases (one case) at imaging studies performed for unrelated symptoms. This underlines that endocrine tumors of Meckel's diverticulum are frequently diagnosed at an advanced stage; therefore, this location must be considered during the work out of a presenting liver metastasis and attentively searched for at imaging studies, even if the exact location of the lesion is usually difficult to assess.

A pathological review of the cases included in our group study was made in the light of the current classifications and of the recent grading and staging systems proposed for gastroenteropancreatic endocrine tumors.[4–7] All the tumors observed in our series looked morphologically very similar to the so-called "carcinoids" typically observed in the distal ileum and appendix. In keeping with the cases previously reported in the literature,[3] all the tumors included in our study were morphologically well differentiated and were found to synthesize and secrete serotonin (two cases were even functional and associated with the carcinoid syndrome). As expected, tumor cells expressed the transcription factor CDX2, a transcription factor characteristic of the distal intestine and strongly detected in midgut endocrine tumors.[15–17] Our findings therefore show that most neuroendocrine tumors of Meckel's diverticulum present as typical midgut endocrine tumors and share many morphological and functional features with appendiceal and ileal endocrine tumors. In some details, they are more similar to ileal than to appendiceal tumors: for instance, in our cases, there was no evidence for the presence of sustentacular cells expressing S100 protein, which have been described as typical for appendiceal, but not ileal endocrine tumors.[18] We found no evidence, neither functional nor immunohistochemical, of gastric or pancreatic differentiation among the cases examined in this study. This is in line with the absence of gastric or pancreatic ectopic tissues in the adjacent mucosa in all the cases included in our series. Our findings therefore do not lend additional support to the hypothesis that the occurrence of such ectopic tissues in Meckel's diverticulum is involved in the histogenesis of the endocrine tumors observed in this location, even if the association has been observed in a very few cases.[9,19,20]

In keeping with previous analyses of the case reports and short series published in the literature,[3,21,22] our findings, based on a comparatively large, monocentric series, confirm that the behavior of the endocrine tumors of Meckel's diverticulum is similar to that of ileal endocrine tumors. Seven out of the eight cases included in our study were large and locally invasive and six presented at diagnosis with regional lymph node and/or liver metastases. They were therefore classified as well-differentiated endocrine carcinomas according to the WHO classification;[7] only one was classified as well-differentiated endocrine tumor of benign behavior. The presentation of endocrine tumors of Meckel's diverticulum is therefore much more similar to that of ileal tumors than to that of appendiceal ones.

An important consequence is that the prognostic significance of several histological factors, such as size and local invasion, is different between tumors of the appendix and tumors of Meckel's diverticulum. In endocrine tumors of the appendix, the risk of metastatic dissemination is known to be negligible for lesions measuring less than 2 cm.[23] In contrast, in our series, six out of the seven tumors measuring more than 1 cm were metastatic. In this respect as in many others, endocrine tumors of Meckel's diverticulum therefore behave like ileal tumors, not like appendiceal tumors. In the same way, in endocrine tumors of the appendix, the invasion of the muscularis propria and/or serosa is not considered an objective sign of malignancy.[7,23] In contrast, in Meckel's diverticulum, as well as in the ileum, this feature is clearly associated with a malignant behavior: in our series, six out of seven tumors invading beyond the muscularis propria were objectively malignant, as shown by the presence of lymph node and/or liver metastasis.

There is no consensus about the treatment of endocrine tumors of Meckel's diverticulum, which are usually not included in the guidelines and recommendations for the management of ileal tumors.[24] In the current literature, it is widely accepted that endocrine tumors of Meckel's diverticulum larger than 2 cm, usually associated with regional lymph node metastases, require large resections of the small bowel and mesentery. The cut-off of 2 cm clearly derives from the experience with appendiceal tumors[23] but may not be justified in the case of tumors of Meckel's diverticulum: our experience suggests that the cut-off for a high risk of malignancy and disseminated disease is likely to be lower and, as for ileal tumors, may be as low as 1 cm. In such cases, as for ileal tumors, surgery of the primary should adhere to oncological principles, including clearance of lymph node metastases by dissection around the mesentery.[24,25] In addition, for metastatic tumors, complementary approaches are needed: our data show that an aggressive treatment of liver metastases, combining surgery and local treatments, such as embolization, may result in prolonged survival. This is especially important since our study, in keeping with previous reports,[26] confirms that the overall prognosis of patients with neuroendocrine tumors of Meckel's diverticulum, even with regional or distant disease, is significantly better than that reported for patients with ileal tumors at the same stages.

The treatment of smaller lesions, especially when discovered incidentally, is much more controversial. The

conventional surgical procedures recommended in this situation are wedge resection or even segmental resection, including the mesentery, especially when there is a doubt on the presence of a tumor within the diverticulum (Fig. 4).[27] Transverse diverticulectomy, frequently used in prophylactic resection, is recommended only for asymptomatic Meckel's diverticulum with no risk factor and negative per-operative examination, including the palpation of the basis of the diverticulum.[28] With the development of laparoscopic techniques, a tendency to extend the indications of transverse diverticulectomy, quick and simple to perform, has been observed.[2,29–31] However, due to the conditions of the surgical procedure, no direct examination, and in particular, no palpation of the diverticulum is possible. In these conditions, the risk to overlook the presence of a small tumor increases, as well as the risk of an incomplete resection of the lesion, especially because, with this technique, the resection of Meckel's diverticulum is necessarily incomplete (Fig. 4). This underlines the potential problems raised by prophylactic diverticulectomy. Our data are too limited to make it possible a full discussion of this issue. However, the potential risks of transverse diverticulectomy are well exemplified in our group study. The two cases of our series discovered at laparoscopy and treated by transverse diverticulectomy were classified as carcinomas: one because of evidence of local invasion beyond the muscularis propria and the other one because of evidence of regional lymph node metastasis. In both cases, a secondary segmental resection of the small intestine and mesentery was programmed. This underlines how important is the per-operative management of such lesions, in order to select the most adapted surgical approach.

Conclusion

Our results show that endocrine tumors of Meckel's diverticulum usually share many features with other midgut endo-

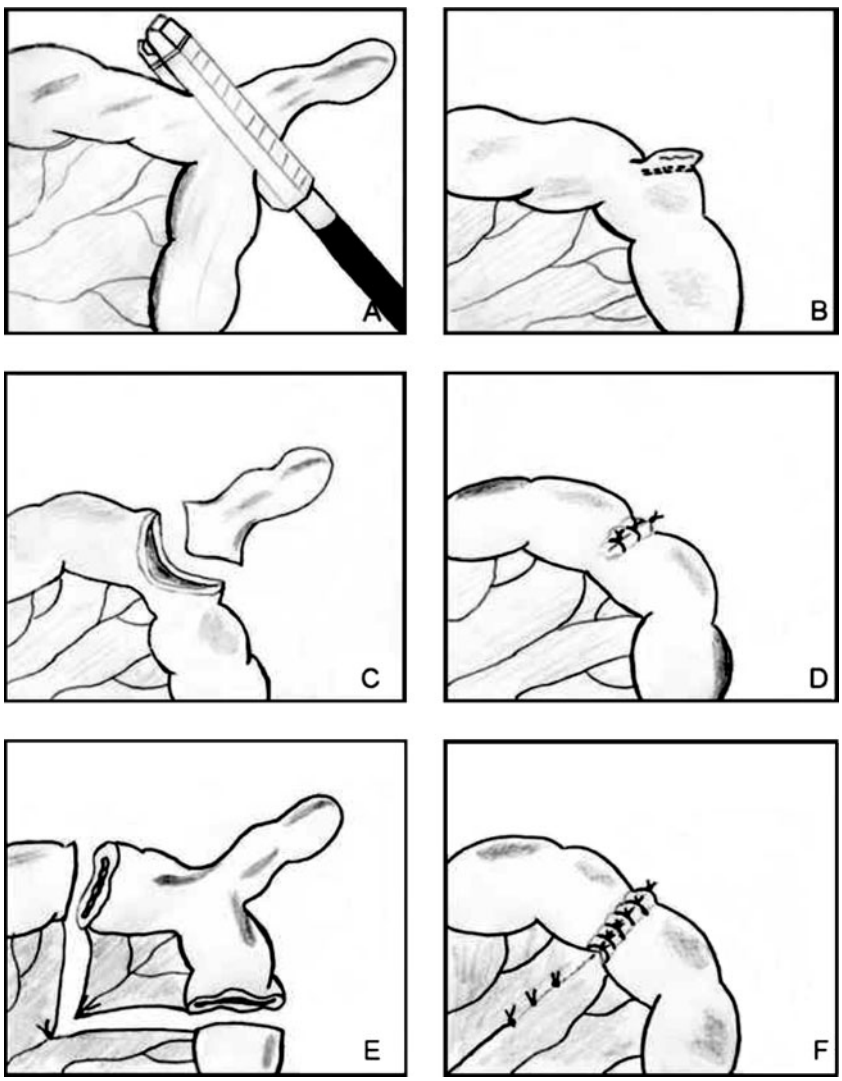

Fig. 4 Schematic representations comparing transverse diverticulectomy (**a**–**b**), wedge resection (**c**–**d**), and segmental resection (**e**–**f**) for Meckel's diverticulum. Note that with transverse diverticulectomy, resection of Meckel's diverticulum is necessarily incomplete

crine tumors, including their morphological characteristics and functional properties. They underline that, unlike appendiceal endocrine tumors but like ileal endocrine tumors, endocrine tumors of Meckel's diverticulum ususally behave as malignant tumors, with a high risk of regional and/or distant dissemination. This points out to the importance of the surgical management of the patients presenting these rare tumors, especially when Meckel's diverticulum is discovered incidentally at laparatomy or laparoscopy.

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ORIGINAL ARTICLE

Analysis of 230 Cases of Emergent Surgery for Obstructing Colon Cancer—Lessons Learned

Ahmet Kessaf Aslar · Süleyman Özdemir · Hatim Mahmoudi · Mehmet Ayhan Kuzu

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Abstract
Purpose We aimed to identify prognostic factors affecting clinical outcomes in emergent primary resection.
Methods A retrospective analysis of prospectively acquired data of 230 consecutive emergent patients between August 1994 and January 2005 were evaluated in this study. Sixty-nine patients applied with right colon obstruction and 161 patients with left. Resection and primary anastomosis was carried out in 128 patients and resection and stoma in 102 patients. The patients were divided into two cohorts: patients who developed poor outcome within 30 days after surgery and those who did not.
Results Major morbidity or mortality were reported in 60 (26.1%) patients. Analysis revealed that the most important prognostic factors for poor outcome were American Anesthesiology Association (ASA) grade ≥3, Acute Physiology and Chronic Health Evaluation II (APACHE II) score ≥11, age >60 years, presence of peritonitis, and surgery during on-call hours. Age >60 years and on-call surgery were determinant factors in right-sided obstructions, whereas ASA grade ≥3, APACHE II score ≥11, and presence of peritonitis were determinant factors in left-sided obstructions.
Conclusions All these factors but the timing of the operation emphasize the pivotal role of the patient's physiological condition on admission. Accurate preoperative evaluation might predict the clinical outcome and help in establishing the most appropriate treatment

Keywords Obstructing colon cancer · Emergency management · Prognostic factors

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Introduction

Malignancy remains the most common cause of large bowel obstruction.[1–3] Despite efforts to obtain an early diagnosis, 8% to 40% of patients with colorectal cancer present with intestinal obstruction.[4–7] In order to relieve this obstruction, emergent surgical therapy is usually required. Due to the paucity of prospective randomized trials, controversy still exists about the best surgical treatment. Because the subsequent anastomotic complications in an unprepared bowel are higher, some surgeons prefer resection and stoma (RS) for emergent cases. In contrast, recent results of studies of resection and primary anastomosis (RPA) for malignant colonic obstruction in an unprepared bowel are encouraging.[8,9] As well, the side of the obstruction can influence the choice of procedure, with RPA being considered safe for right-sided obstruction but a controversial choice for left-sided obstructions. Regardless of the type of procedure used, emergent colorectal surgery

in high-risk, elderly, and frail patients with distended, unprepared bowel has the potential for high morbidity and mortality rates. Physiopathological deterioration of the patient, co-morbidities, advanced age, and disease also contribute to this poor clinical outcome.[10–13]

Quantifying the risk of morbidity or mortality related with emergent colorectal surgery at admission has a crucial impact in surgical practice. In order to determine post-operative clinical outcome, there are a number of indices which can be used to assess patients presenting with large bowel obstruction, such as the American Anesthesiology Association (ASA) grade, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and C -POSSUM. However, there is an ongoing debate on the best method to predict the postoperative outcome. Our study aimed to identify prognostic factors that affect the adverse clinical outcome in patients who undergo emergent primary resection for this clinical presentation. A retrospective analysis of prospectively acquired data from 230 patients who underwent resection with or without anastomosis for obstructing colorectal cancer was made to determine surgical outcomes between the right- and left-sided obstruction or between the RPA and RS surgical options.

Patients and Methods

Patients and Inclusion Criteria

We studied prospective data on consecutive patients who underwent emergent colonic resection for obstructive colorectal cancer between August 1994 and January 2005 in Ankara Numune Teaching and Research Hospital and between June 1998 and January 2005 in the Department of General Surgery, University of Ankara, Turkey. Approval for the study was obtained from the local ethics committee, and all patients included in the study gave their informed consent.

All patients who underwent resection with or without primary anastomosis for histopathologically proven malignant bowel obstruction within 24 h of admission were included. Patients who were treated palliatively with stoma and/or by-pass procedures (without resection of the tumors) or who underwent preoperative decompression were excluded from the study. Patients undergoing RPA with covering stoma were also excluded from the study.

Preoperative and Postoperative Procedures

Preoperative evaluation of patients included clinical examination, blood tests, and plain abdominal and chest radiograms. Abdominal ultrasonography or computed tomography was performed to assess the extent of the disease and the location of the obstruction. Patient evaluation and the operative intervention were decided by the staff surgeon. The patients were operated on either by staff surgeons or by a resident under the supervision of the staff surgeon. Staff surgeons, residents or both were present in all operations. All patients were prepared for surgery in a routine fashion with naso-gastric decompression, adequate IV fluid and electrolytes resuscitation. None of the patients included in this study were treated with preoperative decompression techniques. All the patients received prophylactic antibiotics at the time of anesthesia induction. Some patients received therapeutic antibiotics, depending on intra-operative findings. The bowel was unprepared and on-table lavage was not performed in any of the patients. One-stage procedure (RPA) was carried out if the intestinal perfusion was adequate and there was neither tension on the anastomosis line nor generalized peritonitis. All of the anastomoses were in inverting and two-layered fashion. Sump drains were placed near all of the anastomoses. When primary anastomosis was not possible, a two-stage procedure (RS) was performed if there was generalized peritonitis, tension on anastomosis line or incongruity on colonic ends or according to the surgeon's experience. All of RS procedures in the left-sided obstruction were Hartmann's procedures.

The patients were assessed for postoperative complications in the hospital until discharge or death, and up to 30 days after an operation following successful discharge. Each patient was contacted on postoperative 30th day to further assess morbidity and mortality.

Variables

The following variables were recorded: age, sex, duration of symptoms, ASA grade, APACHE II score, concurrent illness, location of obstruction, type of operation (RPA, RS), time of operation (daytime vs on-call hours), surgeon's experience (resident vs staff), intra-operative findings (peritonitis, perforations), surgical site infections (SSI), major morbidities (intra-abdominal abscess, anastomotic leakage, stoma revision, reoperation), mortality, and length of hospital stay. Preoperative evaluations included treatment records.

The following definitions of *concurrent illnesses* were used. Cardiovascular disease was defined as a history of congestive heart failure, hypertension, myocardial infarction, angina, or cerebrovascular disease. Pulmonary disease was defined as chronic obstructive pulmonary disease, respiratory insufficiency, or bronchial asthma. Diabetes mellitus included both type I and II. Chronic renal failure was documented by biopsy, by persistently elevated serum creatinine levels, or by dialysis requirement. *Colonic obstruction* was defined as the total absence of flatus or bowel movements for at least 24 h, abdominal distention, and the presence of dilated colon on plain abdominal film.

With regard to procedural specifics, obstruction was confirmed by water-soluble contrast enema when necessary.

Patients with an obstructive lesion proximal to splenic flexure were recorded as *right-sided obstruction* and those with obstruction distal to splenic flexure were recorded as *left-sided obstruction*. The surgeon was defined as a *resident* if he or she had an experience of less than 5 years; otherwise the surgeon was defined as *staff*.

With regard to surgical outcomes, SSI was defined either on the basis of clinical criteria, such as purulent wound discharge, a wound that was open for treatment of presumed infection, or a wound breakdown/dehiscence with clinical evidence of infection, or on the basis of bacteriological criteria, such as a positive culture from a serous or sanguineous discharge. SSI was defined as *superficial* if the infection involved only the skin and the subcutaneous tissue within 30 days of surgery. Superficial infection was defined as any redness, swelling, heat with tenderness, pus in the wound, or a positive culture from any discharge that needed drainage and packing. SSI was defined as *deep* if the infection involved the fasciae and muscular layers. *Intra-abdominal abscess* was defined according either to clinical findings or the radiological evaluation and/or intra-operative findings on reoperation. *Anastomotic leakage* was diagnosed clinically on the basis of evidence of a fecal fistula or the appearance of feces from the drain, local or generalized peritonitis, evidence of anastomotic dehiscence at reoperation, or by water-soluble radiological studies (if necessary). *Stoma revision* was defined if there had been septic complications due to either stoma retraction or necrosis which require laparotomy. *Mortality* was defined as that occurring within 30 days postoperatively or before discharge if the patient stayed in the hospital more than 30 days. The length of *hospitalization* was calculated as the period from the admission and discharge in days.

Statistical Analyses

Differences between groups for non-normally distributed continuous or ordinal variables were analyzed by the Mann–Whitney *U* test. Chi-square test or Fisher's exact test was used for nominal variables. The degree of association between variables was evaluated by Spearman's correlation coefficient. In order to define risk factors of outcome variables, multiple logistic regression analysis was used. Statistical analyses were performed using SPSS for Windows 11.5. p values less than 0.05 were considered statistically significant.

Results

Demographics

During the study period, 3,214 patients with colorectal cancer were treated at both centers, 255 of whom were admitted for obstruction and emergency surgery. Of these 255 patients, 25 were excluded: 12 unresectable with stoma, seven with anatomosis and covering stoma, four with preoperative decompression, and two unresectable with stent decompression. The remaining 230 patients (137 male and 93 female) were included in this study. RPA was performed on 128 patients and RS was performed on 102 patients. The median age was 62 years (range, 18–90). Fifty-three percent of the patients were over 60 years (the life expectance in Turkey is 66.2 years for males and 68.2 years for females). Patient demographics, preoperative observations, procedural specifics, and surgical outcomes for all 230 patients are summarized in Table 1.

Physiological Status

Symptoms and Duration All patients had abdominal symptoms and findings, the most frequent being abdominal distension (n=209) and abdominal pain (n=186), followed by tenderness (n=173), change in bowel habits (n=220), nausea and vomiting (n=152), and peritoneal irritation (n=91). The average duration of obstructive symptoms prior to diagnosis was 5.3±2.1 days.

Concurrent Illness Medical history revealed cardiovascular disease in 83 patients, pulmonary disease in 38, diabetes mellitus in 14, renal failure in three, and various other diseases in five of the patients. Thirty-six patients had more than one co-existing disease.

Procedural Specifics

Location of Obstruction Obstructing tumors were most commonly located in the left colon (70% of patients). When the data was stratified according to the location of the obstruction (Table 2), we found that right-sided obstructions had an increased proportion of men (p=0.009), longer duration of symptoms (p=0.03), and were more likely to be treated with RPA, whereas left-sided obstructions were preferentially managed by RS (p<0.001).

Type of Operation Overall, RPA was performed on 128 patients and RS was performed on 102 patients. When the data were stratified according to the type of operation, peritonitis and perforation were significantly more common intra-operative findings in patients who underwent RS when compared with RPA (p<0.001 for each; Table 3). As also shown in Table 2, right-sided obstructions were more likely to be treated with RPA (p<0.001; Table 3).

Time of Operation Sixty-six of 128 patients in the RPA group and 62 of 102 patients in the RS group underwent surgery during on-call hours. Poor outcomes (major

Table 1 Population data

Variable	Number
Demographics	
Total	230
Age (years)	
Mean (SD)	59.8 (12.7)
Median (range)	62 (18–90)
Age >60 (%)	123 (53.5%)
Sex	
Male/Female	137/93
Physiologic status	
Duration of symptoms (days)	
Mean (SD)	5.3 (2.1)
ASA grade	
Mean (SD)	2.3 (1.03)
Median (range)	2 (0–5)
Grade ≥3 (%)	94 (40.9%)
APACHE II score	
Mean (SD)	8.5 (4.8)
Median (range)	8.0 (0–23)
Score≥11 (%)	78 (33.9%)
Concurrent illness	121 (52.6%)
Procedural specifics	
Location of obstruction	
Right/Left	69/161
Type of operation	
RPA/RS	128/102
Time of operation	
Day/On call	102/128
Surgeon	
Resident/Staff	151/79
Intra-operative findings	
Peritonitis (%)	68 (29.6%)
Perforations (%)	36 (15.6%)
Surgical outcomes	
SS I	
Superficial (%)	49 (21.3%)
Deep (%)	32 (13.9%)
Major morbidities	
Intra-abdominal abscess (%)	14 (6.1%)
Anastomotic leakage (%)	8 (3.5%)
Stoma revision (%)	6 (2.6%)
Reoperation (%)	18 (7.8%)
Mortality (%)	29 (12.6%)
Hospitalization (days)	
Mean (SD)	12.3 (86.1)
Median (range)	11.0 (1–36)

Table 2 Univariate analysis of variables stratified by location of obstruction

Variable	Right-sided (proximal)	Left-sided (distal)	*P*
Demographics			
Total	69	161	
Age (years)			
Mean (SD)	59.3 (15.8)	60.1 (11.2)	0.98
Median (range)	62 (18–88)	62 (23–90)	
>60	36	87	0.79
Sex			
Male/Female	50/19	87/74	0.009
Physiologic status			
Duration of symptoms (days)			
Mean (SD)	5.7 (1.8)	5.08 (2.1)	0.03
ASA grade			
Mean (SD)	2.3 (0.7)	2.3 (0.9)	0.052
Median (range)	2 (1–4)	2 (1–4)	
Grade ≥3	26	68	0.52
APACHE II score			
Mean (SD)	8.9 (4.8)	8.3 (4.8)	0.81
Median (range)	8 (0–23)	8 (0–22)	
Score ≥11	24	54	0.85
Concurrent illness	39	82	0.44
Procedural specifics			
Type of operation			
RPA/RS	53/16	75/86	<0.001
Time of operation			
Day/On call	32/37	70/91	0.69
Surgeon			
Resident/Staff	53/16	98/63	0.02
Intra-operative findings			
Peritonitis	16	52	0.16
Perforations	10	26	0.75
Surgical outcomes			
SSI			
Superficial	10	39	0.99
Deep	14	18	0.07
Major morbidities			
Intra-abdominal abscess	4	10	0.84
Anastomotic leakage	3	5	1.00
Stoma revision	0	6	0.59
Reoperation	4	14	0.59
Mortality	7	22	0.76
Hospitalization (days)			
Mean (SD)	11.6 (5.5)	12.6 (6.4)	0.47
Median (range)	10 (1–29)	11 (1–36)	

Table 3 Univariate analysis of variables stratified by type of operation

Variable	RPA	RS	*P*
Demographics			
Total	128	102	
Age (years)			
Mean (SD)	59.6 (12.7)	60.1 (12.8)	0.68
Median (range)	62 (18–88)	62 (21–90)	
>60	68	55	0.90
Sex			
Male/Female	77/51	60/42	0.84
Duration of symptoms (days)			
Mean (SD)	5.4 (2.02)	5.1 (2.08)	0.22
Procedural specifics			
Location of obstruction			
Right/Left	53/75	16/86	<0.001
Time of operation			
Day/On call	62/66	40/62	0.16
Surgeon			
Resident/Staff	87/41	64/38	0.41
Intra-operative findings			
Peritonitis	16	52	<0.001
Perforations	6	30	<0.001
Surgical outcomes			
SSI			
Superficial	23	26	0.16
Deep	14	18	0.14
Major morbidities			
Intra-abdominal abscess	6	8	0.06
Anastomotic leakage	8	NA	
Stoma revision	NA	6	
Reoperation	8	10	0.06
Mortality	14	15	0.39
Hospitalization (days)			
Mean (SD)	12.8 (6.4)	11.8 (5.8)	0.35
Median (range)	11 (1–36)	10 (1–34)	

NA, not applicable

complications and mortality) were significantly more frequent following the operations that took place during on-call hours when compared to the daytime hours (p=0.009; Table 4). However, when major complications were examined individually, there were no significant differences between on-call hours and daytime hours (anastomotic dehiscence, n=6 vs n=2; intra-abdominal abscess, n=8 vs n=6; reoperation, n=11 vs n=7), whereas the incidence of mortality was significantly different (n=21 vs n=8; p=0.05).

Surgeon Resident surgeons performed 65.6% of all the operations (n=151, 98/151 left-sided, 87/151 RPA). RPA was performed in 87 of 151 obstructions. There were no significant differences between the operations performed by resident and staff surgeons regarding demographics, ASA grade, APACHE II score, intra-operative findings (peritonitis or perforations) (data not shown) or morbidity and mortality (Table 4).

Table 4 Univariate analysis of variables stratified by outcome

Variable	Favorable outcome	Poor outcome	*P*
Demographics			
Total	170	60	
Age (years)			
Mean (SD)	58.7 (12.2)	62.9 (13.9)	0.04
Median (range)	60 (18–88)	65 (21–90)	
>60 (%)	79 (46.5%)	44 (73.3%)	<0.001
Sex			
Male/Female	103/67	34/26	0.59
Physiological status			
Duration of symptoms (days)			
Mean (SD)	5.38 (2.0)	5.1 (2.17)	0.23
ASA Grade:			
Mean (SD)	2.2 (0.8)	2.9 (0.8)	<0.001
Median (range)	2 (1–4)	3 (1–4)	
Grade ≥3 (%)	55 (32.4%)	39 (65%)	<0.001
APACHE II score			
Mean (SD)	7.68 (4.58)	10.81 (4.7)	<0.001
Median (range)	7 (0–23)	11 (0–22)	
Score ≥11 (%)	42 (24.7%)	36 (60%)	<0.001
Concurrent illness	81 (47.6%)	40 (66.7%)	0.011
Procedural specifics			
Location of obstruction			
Right/Left	52/118	17/43	0.74
Type of operation			
RPA/RS	103/67	25/35	0.01
Time of operation			
Day/On call	84/86	18/42	0.009
Surgeon			
Resident/Staff	112/58	39/21	0.90
Intra-operative findings			
Peritonitis (%)	39 (22.9%)	29 (48.3%)	<0.001
Perforations (%)	23 (13.5%)	13 (21.7%)	0.14
Surgical outcomes			
SSI			
Superficial (%)	31 (18.2%)	18 (30%)	0.046
Deep (%)	15 (8.8%)	17 (28.3%)	<0.001
Hospitalization (days)			
Mean (SD)	11.8 (4.9)	13.9 (8.6)	0.03
Median (range)	10 (5–30)	13.5 (1–36)	

Intra-operative Findings Peritonitis was documented in 68 of 230 patients, 36 of whom also had perforations. Peritonitis and perforations were most common in the left-sided obstructions, although this was not significant (Table 2). RS was significantly more frequently performed in the presence of both peritonitis and perforation ($p<0.001$; Table 3). Major morbidities and mortality were significantly more common in the presence of peritonitis ($p<0.001$; Table 4), and further examination of these variables individually found each to be more frequent in the presence of peritonitis (SSI, $p<0.001$; intra-abdominal abscess, $p=0.02$; reoperation, $p<0.001$; mortality, $p=0.01$).

Surgical Outcomes

Surgical Site Infection The most frequent complication was SSI, which occurred in 81/230 patients. Superficial SSI was detected in 49 patients whereas deep SSI in 32. No significant difference was found either between right- and left-sided obstruction (Table 2) or between RPA and RS (Table 3) with regard to SSI; however, SSI was significantly more likely to be present in patients with poor outcomes (Table 4). Superficial surgical site infection like cellulitis was managed with a single empirical antibiotic. Culture results were used to guide any antibiotic changes.

Intra-abdominal Abscess Intra-abdominal abscess developed in eight patients following RS (six left-sided obstruction). Five of these were treated by reoperation (four left-sided and one right-sided obstruction) and three were treated conservatively. Two patients in the reoperation group and one in the conservative treatment group died. Six patients had intra-abdominal abscess following RPA (four left-sided obstruction). Percutaneous drainage was performed in two patients with left-sided obstruction and four patients were treated conservatively (two in each side). There was no mortality in this group. There was no difference in the frequency of intra-abdominal abscess related to the side of obstruction (Table 2) or type of surgery (Table 3).

Anastomotic Leakage Anastomotic leakage occurred in three of 53 patients (5%) after right-sided RPA and in five of 75 patients (6%) after left-sided RPA. All of them, except one, in the left-sided anastomosis required reoperation to take down the anastomosis and to clean the peritoneal contamination. Anastomotic leakage resulted in mortality in six patients and three on each side.

Reoperation Reoperation was performed on ten patients following RS (9%) and in eight following RPA (6%). In the RS group, reoperation was performed on six patients because of the requirement for stoma revision due to septic complications (five stoma necrosis and one stoma retraction) and in four patients because of intra-abdominal abscess. Three of the patients who underwent stoma revision and two who underwent intra-abdominal abscess drainage died. In the RPA group, seven patients underwent reoperation because of anastomotic dehiscence (four left-sided (5%) and three right-sided (5%)). One further patient was operated for intra-abdominal abscess drainage. Two and three patients died following anastomotic leakage for left- and right-sided anastomosis, respectively. There was no difference in the frequency of reoperation related to the side of obstruction (Table 2) or type of surgery ($p=0.06$) (Table 3).

Mortality The overall mortality rate was 12.6% (29/230 patients). Out of 230 patients, 14 in the RPA group (six right- and eight left-sided) and 15 in the RS group (one right- and 14 left-sided) died within 30 days after the operation. Except for six patients, all the mortalities were over 60 years of age. Of the 29 deaths, 11 were attributable to pulmonary disease, 11 to intra-abdominal sepsis, four to myocardial infarction, and three to pulmonary embolism. Of the 11 patients who died from intra-abdominal sepsis, six patients had anastomotic leakage following RPA (three left- and three right-sided obstruction and five of whom were converted to stoma and one patient could not be operated due to septic shock); three patients died due to intra-abdominal abscess following RS for left-sided obstruction (two of whom had reoperation for abscess drainage and the third was drained percutaneously); and two patients who had undergone RS for left-sided obstruction died following reoperation for stoma complications.

We compared the survivors with the non-survivors regarding demographic characteristics, co-existing diseases, ASA grades, APACHE II scores, timing of the operation, surgeon's experience, intra-abdominal findings, localization of the lesion, and types of the operations and complications. For these groups results were respectively as follows: mean age—64.5 (13.6) vs 59.1 (12.4) year, $p=0.008$; ASA grade—3.1 (0.9) vs 2.2 (0.8), $p<0.001$; APACHE II score—12.8 (5.2) vs 7.8 (4.4), $p<0.001$; frequency of co-existing diseases—68.9% ($n=20$) vs 50.2% ($n=101$), $p=$not significant (n.s.); frequency of operations on on-call—72.4% ($n=21$) vs 53.2% ($n=107$), $p=0.052$; frequency of peritonitis—48.2% ($n=14$) vs 26.9% ($n=54$), $p=0.02$; frequency of anastomotic leakage—42.9% ($n=6$) vs 1.75% ($n=2$), $p<0.001$; frequency of stoma revision—20.0% ($n=3$) vs 3.4% ($n=3$), $p<0.001$; and frequency of reoperation—34.5% ($n=10$) vs 3.9% ($n=8$), $p<0.001$.

Prognostic Factors

Major morbidity (intra-abdominal abscess, anastomotic leakage, stoma revision, reoperation) and mortality were reported in 60 (26.1%) patients. Age over 60, ASA grade

≥3, APACHE II score ≥11, presence of concurrent illness, operations performed within on-call hours, RS, presence of peritonitis, and presence of SSI were significantly associated with poor outcome (Table 4). Multivariate analysis of these variables revealed that age >60, ASA grade ≥3, APACHE II score ≥11, operations performed within on-call hours, and presence of peritonitis were the most important prognostic factors which were related to poor outcome following emergent resection of obstructive colorectal cancer (Table 5) Multivariate analysis was also performed to assess which variables were significantly associated with poor outcomes specific to the location of the obstruction. This analysis showed that age over 60 and operations performed within on-call hours were the most important prognostic factors in right-side obstructive lesions whereas ASA grade ≥3, APACHE II score≥11, and presence of peritonitis were the most important prognostic factors in left-side obstructive lesions Moreover, both the ASA grade (Fig. 1) and the APACHE II score (Fig. 2) were significantly related to mortality and the poor outcome ($p<0.001$, for both). In addition to this, moderate correlation exists between the ASA grade and APACHE II score ($r=0.69$). Likelihood ratio revealed that APACHE II score was a more predictive value than the ASA grade ($p=0.02$).

Discussion

Colorectal cancer screening programs and the use of colonic stents are promising measures which have the potential for improving the clinical outcome by either reducing the number of urgent admissions or changing an emergent surgery to a semi-elective one .[14] Despite these advances, emergent management of obstructing colorectal cancer remains strongly associated with high morbidity and mortality rates. Our study showed a 26.1% (60/230) morbidity rate (having one or more of intra-abdominal abscess, anastomotic leakage in the case of RPA, requiring stoma revision in the case of RS, or other reoperation) and 12.6% mortality rate (29/230), and similar results have been reported by other investigators.[5,15–17]

The first objective of our study was to identify correlates of poor clinical outcomes as possible risk factors. Univariate analysis found that poor outcome (major morbidity or mortality) was associated with older age, ASA grade ≥3, APACHE II score ≥11, concurrent illness, surgery conducted during on-call hours, type of operation (RS), presence of peritonitis, and SSI. However, since the clinical outcome following emergent colorectal surgery was shown to be clearly multifactorial in origin, we used logistic regression analysis to test for the ability of all these collected parameters to predict the poor outcome. Age over 60 years, ASA grade ≥3, APACHE II score ≥11, surgery conducted during on-call hours, and presence of peritonitis were found to be the most important determinants of the poor outcome after emergent colorectal surgery. A further subgroup analysis found that age and the timing of the operation were the most significant parameters to predict the poor outcome in the right-sided obstruction, whereas the presence of peritonitis, ASA grade ≥3, and APACHE II score ≥11 were shown to be the best predictors of poor outcome for the left-sided obstruction. All these factors but the timing of the operation, underline the pivotal role of the physiological condition of the patient at initial evaluation prior to emergent surgery. These findings are consistent with some other studies that assessed the prognostic parameters in emergent colorectal surgery.[5,13,15–18]

The high morbidity and mortality rates reported in the literature can probably be attributed to co-morbidity [19] and many factors related with the emergency condition such as preoperative health status, age, the presence of peritoneal contamination, operating surgeon, timing of the operation, type of operation and obstruction site.[20–26] However, the findings of our study show that the physical status rather than the factors related to the surgical procedure, is one of the principal determinants of outcome after emergency surgery for obstructing colorectal cancer. Our study confirmed that the ASA grade and the APACHE II score were significantly worse in patients who had poor outcome and can be taken as strong predictors of perioperative morbidity and mortality. Furthermore, a correlation was shown to exist between both these scores and the outcome. It has been reported that ASA score of 3 or more, presence

Table 5 Multivariate analysis of risk factors for major morbidity and mortality

Variable	Total patients		Right-sided obstruction		Left-sided obstruction	
	Odds ratio (95% CI) (95% CI)	*P*	Odds ratio	*P*	Odds ratio (95% CI)	*P*
Peritonitis	5.8 (2.7–12.6)	<0.001			3.4 (2.6–8.8)	0.001
APACHE II score ≥11	3.6 (1.6–7.7)	0.001			3.2 (2.1–5.6)	0.005
On-call hours	2.9 (1.4–6.3)	0.004	2.1 (1.3–3.1)	0.04		
Age >60 years	2.4 (1.1–5.3)	0.04	3.9 (1.9–8.6)	0.001		
ASA grade ≥3	2.2 (1.1–4.8)	0.04			2.8 (2.4–5.6)	0.001

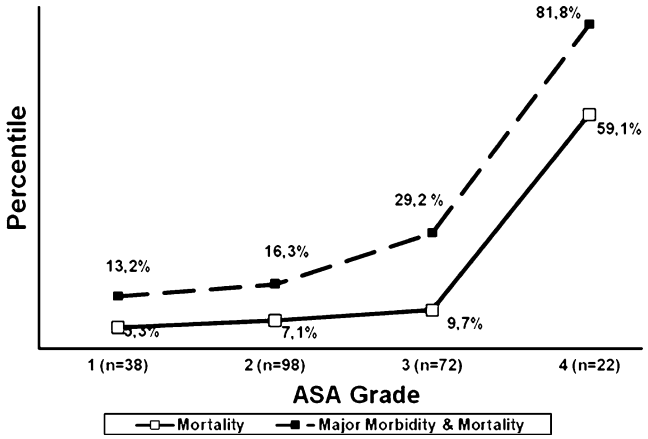

Fig. 1 Correlation of ASA grade and major morbidity and mortality. Patients (n=230) were assessed and given an ASA grade prior to emergent surgery for malignant colon obstruction. Major morbidities (intra-abdominal abscess, anastomotic leakage, stoma revision, and reoperation) and mortality were recorded, and the frequency (percent) of mortality or major morbidity+mortality was calculated

of proximal colon damage (any tear in colon or colonic distension or damage in vascular supply) and preoperative renal failure are associated with worse clinical outcome in large bowel obstruction of all causes.[15] In another study, Tobaruela et al.[27] reported a statistically significant association of higher mortality with ASA grading and acute physiology component of the APACHE II score following their review of 51 patients operated in emergency settings for colorectal cancer. The findings of these studies are quite consistent with ours. Since our centers are tertiary referral emergency units, most of the cases were transported from smaller local hospitals. Therefore, approximately 56% of cases were performed during on-call hours under the observation of the junior on-call staff surgeons.

The second objective of our study was to determine any differences in surgical outcomes between right- and left-sided obstruction and RPA and RS procedures. Right-sided tumors present with fewer clinical signs than left-sided ones do, as was the case in the present study – the duration of symptoms was significantly longer in the right-sided obstructions. Nevertheless, the clinical outcomes were similar with regard to the localization of the obstruction.

With regard to RPA vs RS, our results showed no significant difference in surgical outcomes (major morbidity, mortality, or length of hospitalization) when the data were stratified by procedure, but a significant difference as a result of procedure when the results were stratified by outcome. It is important to consider that the choice of procedure was not randomized, rather, RPA was only carried out when the surgeon believed that local conditions were appropriate. Over 50% of patients who had peritoneal contamination and approximately 30% of free perforation cases underwent RS whereas these percentages were 12.5% and 4.7% in the RPA, respectively. As a result of this preference, a higher proportion of patients with poor surgical outcome had undergone RS, but RS itself was not identified as a prognostic factor.

For emergent surgery in the unprepared bowel, RS is still one of the best operative alternatives, especially in the presence of peritonitis and for the left-sided obstructions, which is supported by the results of the present study. Significantly high number of patients underwent Hartmann's procedure in the left-sided obstructions. RS was performed in 26% of the right-sided obstructions, all of whom had peritonitis and 62.5% had free perforation. The higher incidence of anastomotic dehiscence than those with distal large bowel obstruction (13.8% vs 5.1%) led to a surgical decision change in Biondo et al.'s philosophy.[15] They also recommended protective or terminal ileostomy in high-risk right-sided obstruction patients as we did in our series.

Although RS has been preferred for emergent cases, recent results of studies of RPA for malignant colonic obstruction in an unprepared bowel are encouraging, and in fact, this procedure was used for 128/230 patients in our study. Our overall anastomotic dehiscence rate was 6.3%. No significant difference was detected when compared to the 5.7% and 6.7% leakage rates for right- and left-sided primary anastomosis, respectively. Similarly, comparison of one-stage resection and anastomosis of acute complete obstruction of left and right colon revealed no significant difference in postoperative mortality or anastomotic leak rates in Lee, Hsu and Alvarez series.[28–30] Our leak rate was, indeed, higher when compared to those found in the previous studies; however, none of the anastomosis was covered or decompressed prior to emergent surgery in our series. In addition to this, approximately 70% of the

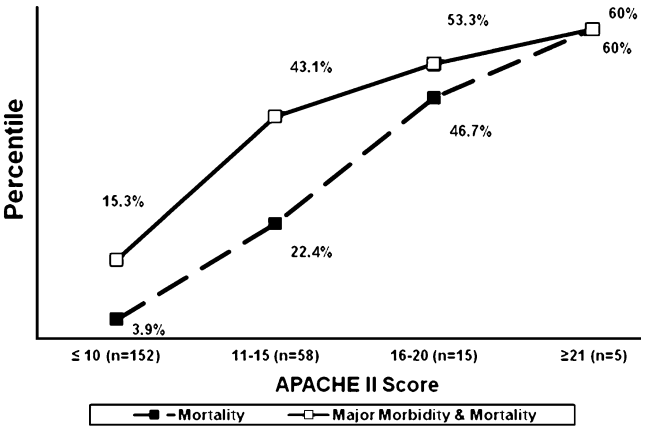

Fig. 2 Correlation of APACHE II score and major morbidity and mortality. Patients (n=230) were assessed and given a APACHE II score prior to emergent surgery for malignant colon obstruction. Major morbidities (intra-abdominal abscess, anastomotic leakage, stoma revision, and reoperation) and mortality were recorded, and the frequency (percent) of mortality or major morbidity+mortality was calculated

primary anastomosis was performed by surgery residents under the supervision of the on-call staff surgeons; and 65% of the major morbidities and mortalities occurred following the operations performed by resident surgeons, mainly during on-call hours. All of the anastomotic leaks, except one, required reoperation to take down the anastomosis and six of eight died due to intra-abdominal sepsis.

Though not included in this study and not widely available, it has been well documented that malignant obstruction is successfully decompressed by stents.[31–33] This intervention significantly reduces the need for emergency surgery, thus allowing an elective one.

Our study design has several important drawbacks. Firstly, the type of the surgical procedure was solely determined according to the surgeon's preference. An additional limitation is related both to the level of the skill and the heterogeneity of the surgeon s. More than 65% of the operations were performed by the trainees. Although all operations were performed under the supervision of the staff surgeon, only one of them had had colorectal surgery training. Nevertheless, this is a retrospective analysis of prospectively collected data of the prognostic factors that may influence the outcome of malignant large bowel obstruction in two surgical training centers. To test our results in a more robust fashion, randomized studies should be performed.

Conclusion

In conclusion, the findings of the present study have clearly documented that emergent colorectal surgery for malignant obstruction continues to have the risk of high morbidity and mortality. It is therefore essential to consider and choose the most appropriate treatment option relying on preoperative prognostic factors such as age, co-morbidities, duration of symptoms, presentation of the patient, intra-operative findings, and above all the skill of the surgeons. Accurate preoperative evaluation of these prognostic factors might allow us to predict the clinical outcome, and provides reliable assistance in the surgical decision making.

Conflicts of interest No conflict of interest.

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ORIGINAL ARTICLE

Anastomotic Leakage Contributes to the Risk for Systemic Recurrence in Stage II Colorectal Cancer

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Abstract
Purpose In stage II colorectal cancer (CRC), high-risk patient selection is required, but no candidate markers have been elucidated. Our concern was whether anastomotic leakage (Lk) is a potential available clinicopathological factor for selecting high-risk stage II.
Methods Two hundred seven patients with stage II CRC who underwent curative resection were analyzed. Clinical variables were tested for their relationship to survival.
Results The 5-year disease-free survival rate (DFS) was 87.0%. The univariable prognostic analyses indicated that Lk (P=0.003) was the only significant factor. The multivariable prognostic analysis revealed that Lk remained to be potently independent [hazard ratio (HR), 4.21, P=0.021), and the DFS was 58.3% in cases with Lk, while 88.7% in the counterpart. The multivariable logistic regression analysis revealed perioperative blood transfusion (P=0.001) was independently associated with Lk. Intriguingly, Lk was closely associated with hematogenic recurrence (P=0.003) rather than peritoneal or local recurrence. Although sustained increase of the serum C-reactive protein at 2 weeks after operation predicted poor prognosis, the mutitivariable analysis including the C-reactive protein level revealed that Lk still indicated the prognostic potential (HR, 3.70, P=0.075).
Conclusions The findings concluded that Lk may be a high risk for systemic recurrence in stage II CRC.

Keywords Colorectal cancer · Stage II · Prognosis · Anastomotic leakage

Introduction

Colorectal cancer (CRC) is the second most prevalent cancer,[1], and chemotherapy has dramatically improved prognostic outcome of CRC patients over the past decades.[2,3] Nevertheless, CRC remains the fourth leading cause of cancer death worldwide with about 530,000 deaths every year.[1] Recently, as the prognostic outcome of stage III patients has been dramatically improved due to prevalent use of adjuvant chemotherapy and improvement of chemotherapy regimens,[2,4] adjuvant chemotherapy is consented as standard therapy in stage III CRC. Similarly, application of adjuvant chemotherapy is under discussion for patients with high-risk stage II disease[5] although no selecting marker has been clinically identified at present. In stage II patients, approximately 20% of the patients have yet suffered from recurrence in spite of potentially curative resection.[6] Therefore, pre- or postoperative prognostic markers have been anticipated for selecting high-risk patients who may benefit from adjuvant

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chemotherapy after curative operation of stage II CRC. Several prognostic markers or predictors of chemosensitivity for stage II patients have been reported such as allelic imbalance,[7] gene expression profiling by cDNA microarray,[8] or microsatellite instability,[9] respectively. However, such molecular markers have been unsuitable for routine application at present because they have not been finally validated yet and are still costly and time-consuming.

Anastomotic leakage (Lk) is thought to occur at a rate ranged from 3% to 18% and has been reported to be a risk factor for local recurrences in curatively operated CRC patients.[10–12] In this meaning, at least patients with Lk may be potential candidate for adjuvant chemotherapy. However, these results were based upon curatively operated patients with CRC of several stages, and the impact of Lk on long-term survival remains controversial,[10–14] especially in stage II CRC. Accordingly, clinicopathological factors including Lk were prognostically analyzed within stage II patients to evaluate whether Lk could be a clinically available parameter for predicting long-term prognosis.

Patients and Methods

Characteristics of Patients with Stage II CRC

A total of 1,101 patients having electively undergone surgical resection of primary CRC at the Kitasato University Hospital from January 1, 1990 to March 31, 2000, were reviewed. Patients with colorectal multiple cancer, malignant disease of other organ, familial adenomatous polyposis, or inflammatory bowel diseases, patients who underwent resections without anastomosis, and patients undergone emergency resection for perforation or one-stage resection for obstruction were excluded. Among the remaining 946 patients of sporadic CRC, 207 patients were diagnosed (21.9%) as stage II CRC disease and were operated on with curative intent. Preoperative chemotherapy or radiation therapy had not been performed in this cohort. Patients without obstruction received mechanical bowel preparation with polyethylene glycol electrolyte solution the day before surgery, and patients with obstruction and patients with rectal cancer received bedside orthograde colorectal lavage with lukewarm water. Prophylactic intravenous antibiotics were administered at the induction of anesthesia and 3 h after the beginning of operation. Patients were followed up until the recurrence of cancer or end point (April 30 2007). All patients were followed up at least every 3 months for the first year and every 6 months thereafter. Follow-up assessment involved a medical history-taking, physical examination, biologic tests, measurement of the serum CEA and CA19-9 levels, colonoscopy, chest radiography, abdominal ultrasonography (US), and chest/abdominal computed tomography (CT). Serum CEA and CA19-9 were usually evaluated every visit, and abdominal US and CT were performed every 6 months. Chest CT and colonoscopy were examined every year. Recurrence was diagnosed on the basis of imaging and, if necessary, either cytologic analysis or biopsy was performed. Patient demographics, tumor characteristics, and postoperative course were recorded and analyzed. Perioperative transfusion was defined as allogeneic blood transfusion during surgery or in the first two postoperative days, as in previous press,[15] and was performed at the discretion of the treating surgeons and anesthesiologists. The number of total dissected lymph nodes was also classified according to previous press.[16] Pathological TNM classification was made according to the UICC (*Unio Internationalis Contra Cancrum*) staging system.

Patients who received adjuvant chemotherapy for more than 3 months were defined as adjuvant chemotherapy "Yes" group. Adjuvant chemotherapy was consisted of oral administration of 5-fluorouracil (5FU)-based regimens: 5FU, Tegafur/uracil (UFT), or Furtulon (5′-deoxy-5-fluorouridine) alone, or one of them plus PSK (protein-bound polysaccharide K). Although curative operation alone is a standard therapy in stage II CRC at present, oral adjuvant chemotherapy had been recommended to patients with stage II CRC during the term of this patient cohort if they fulfilled the following eligibility criteria: age of 20 to 75 years; the absence of prior chemo-immunotherapy or radiotherapy, and the absence of severe liver dysfunction, heart failure, renal dysfunction, or other severe systemic complications. Therefore, patients who received oral adjuvant chemotherapy reached 180 cases, and the remaining 27 patients declined or did not fulfill the above criteria.

Lk was defined as any clinical or radiological evidence of dehiscence of the anastomosis: the presence of peritonitis caused by anastomosis dehiscence, the presence of feculent discharge from the drainage tube, or the presence of abscess with demonstration of Lk. These were also confirmed by radiography from drainage tube, hydrosoluble enema, or CT-guided abscess drainage except the cases with obvious feculent discharge from the drainage tube (Supplemental Table 1). Anastomotic dehiscence, which was basically diagnosed by, later, routine imagings prior to closure of diverting ileostomy, was not included. We performed routine imagings only for patients with diverting ileostomy prior to ileostomy closure more than 3 months after primary operation. Four patients underwent diverting ileostomy, but no anastomotic dehiscence was detected in such routine diagnosis.

Statistical Analysis

The relationship between Lk and clinicopathological parameters were assessed by Pearson's chi-square test or

Fisher's exact test, as appropriate, and multivariate logistic regression analysis were performed to obtain an adjusted effect of each factor. The time of follow-up was calculated from the operation date for the primary lesion to the date of recurrence. Cumulative disease-free survival (DFS) of patients was estimated using the Kaplan–Meier method, and statistical significance of the difference of the survival rate between groups was tested using the log-rank test. For the Kaplan–Meier estimate of the survival curves, we truncated the data at a follow-up period of 5 years to avoid the number at risk to be too small. Those with a survival time of more than 5 years were reported to be 5 years, and events occurring after the end of the 5-year follow-up period were computed as censored data. Five-year cumulative DFS probability was estimated using the life table method with the interval length set at 1 month. Multivariable analysis was performed by employing the Cox proportional hazards model to examine the interaction between Lk and other clinicopathological variables and estimate the independent prognostic effect of Lk on survival by adjusting for confounding factors. For ordinal variable, when zero event was detected in the lowest exposure group, analyses was designed to be performed by grouping categories together, treating it as ordinal data to get an average effect, or by confounding sensitivity analyses excluding it from analysis. Within the present study population, there were 27 recurrences of stage II CRC which allows up to three variables to be included in a multivariable regression model. To avoid over-fitting, all potential confounding factors of Lk were reduced to one single composite characteristic by applying a propensity score.[17] The conventional P value of 0.05 or less was used to determine the level of statistical significance. All reported P values are two-sided. Analyses were performed independently at our clinical research center using SPSS version 17.0 software (SPSS Inc., Chicago, IL).

Results

Patients' Characteristics and Their Association with Lk

The clinicopathological characteristics were shown in Table 1. One hundred twenty-seven males and 80 females were analyzed with age being 61.0±11.1 years. Lk occurred in 12 (5.8%) cases, and, among them, only one patient had a particularly preoperative complication (diabetes mellitus). The diabetes of this patient was well-controlled by insulin from preoperation through postoperation. And, there was no patient with other factors for poor nourishment such as medication of steroids. Lk occurred in 22.2% of patients with perioperative blood transfusion and in 1.2% of those without perioperative blood transfusion. Lk was significantly related to perioperative blood transfusion ($P<0.001$, Fisher's exact test), followed by T4 factor (direct invasion into other organ; $P=0.071$), the elevation of preoperative CEA ($P=0.110$), and tumor position ($P=0.129$). Preoperative obstruction was observed in only one patient with Lk (Table 1). There was also no significance in relationship between Lk and obstruction in the present study population. Lk occurred in five cases (3.8%) in colon cancer and seven in rectal cancer (9.2%). Among them, two patients required ileostomy (reoperation) for Lk in colon cancer and five in rectal cancer, and one patient (colon cancer) underwent ileostomy before curative resection (two-stage operation) for obstruction, one patient (rectal cancer) underwent diverting ileostomy, and the remaining three patients were conservatively observed with percutaneous drainage and finally cured. The multivariable logistic regression analysis of these factors indicated that Lk was independently associated with perioperative blood transfusion ($P<0.001$).

Kaplan–Meier Estimate of 5-Year DFS

All the patients were included in the survival analysis. The overall follow-up period ranged from 2 to 207 months (median, 116 months), and the mean DFS was 55.4 months corresponding to a 5-year follow-up. Because a cumulative DFS probability of 50% was not yet reached by the end of 5-year follow-up, the overall median DFS time was not determined. The overall DFS rate was 87.0% (27 cases with recurrence and 180 cases without recurrence). Five-year cumulative DFS of patients with Lk was remarkably worse (58.3%), which corresponded to stage III CRC (63.2%), compared with those without Lk (88.7%; $P<0.001$, Fig. 1a). Lymphatic involvement (ly; $P=0.119$) and vascular involvement (v; $P=0.086$) tended to indicate poor prognosis (Supplemental Fig. 1a, b), and patients with both ly and v involvement ($n=28$) showed significantly poor prognosis (DFS, 84.9%) compared with the counterpart ($n=179$; 100.0%; $P=0.033$; Supplemental Fig. 1c).

When separately analyzed on tumor position, Lk still significantly affected adversely on long-term prognosis in both colon and rectum (Fig. 1b, c), and there was no significant difference between DFS of patients with Lk in colon cancer (60.0%) and that in rectal cancer (57.1%). In addition, Lk was the only significant prognostic factor, and there was no factor which had prognostic potential ($P<0.1$) both in colon and rectum when separately analyzed (data not shown).

Contribution of Lk to the Risk of Recurrence with Multivariable Analysis

Cox proportional hazards model was applied to estimate the effect of Lk on DFS. Lk was the only significant prognostic

Table 1 Characteristics and those in correlation with anastomotic leakage (Lk)

Variables	No. of patients	Percentage	Lk			
			Present	Absent	Present rate (%)	P^a values
Gender						
Male	127	61	10	117	7.9	0.13
Female	80	39	2	78	2.5	
Age (years)						
<60	94	45	4	90	4.3	0.55
>60	113	55	8	105	7.1	
Tumor position						
Ccolon	131	63	5	126	3.8	0.13
Rectum	76	37	7	69	9.2	
Differentiation						
Non-poor	194	94	12	182	6.2	0.36
Poor[b]	13	6	0	13	0.0	
T factor						
T3	199	96	10	189	5.0	0.07
T4	8	4	2	6	25.0	
Lymphatic involvement (ly)						
Negative	16	8	0	16	0.0	0.61
Positive	191	92	12	179	6.3	
Vascular involvement (v)						
Negative	19	9	1	18	5.3	0.92
Positive	188	91	11	177	5.9	
Preoperative CEA						
Normal (<2.5 ng/ml)	138	67	5	133	3.6	0.110
Elevated (>2.5 ng/ml)	69	33	7	62	10.1	
Preoperative CA19-9						
Normal (<37 ng/ml)	183	88	10	173	5.5	0.64
Elevated (>37 ng/ml)	24	12	2	22	8.3	
Obstruction						
Yes	16	8	1	15	6.3	0.94
No	191	92	11	180	5.8	
Lk						
Yes	12	6	n/a	n/a	n/a	n/a
No	195	94	n/a	n/a	n/a	
Number of total dissected lymph node						
<6	5	2	0	5	0.0	0.78
6–10	27	13	1	26	3.7	
11–15	34	17	3	31	8.8	
>15	141	68	8	133	5.7	
Laparoscopy-assisted operation						
Yes	8	4	0	8	0.0	0.47
No	199	96	12	187	6.0	
Adjuvant chemotherapy						
Yes	180	87	9	171	5.0	0.2
No	27	13	3	24	11.1	
Perioperative transfusion						
Yes	45	22	10	35	22.2	<0.001
No	162	78	2	160	1.2	

OR odds ratio, *LNDE* lymph node dissection extent, *n/a* not applicable

[a] Compared by Fisher's exact test or chi-square test

[b] Poor consists of poorly differentiated, mucinous, and undifferentiated types

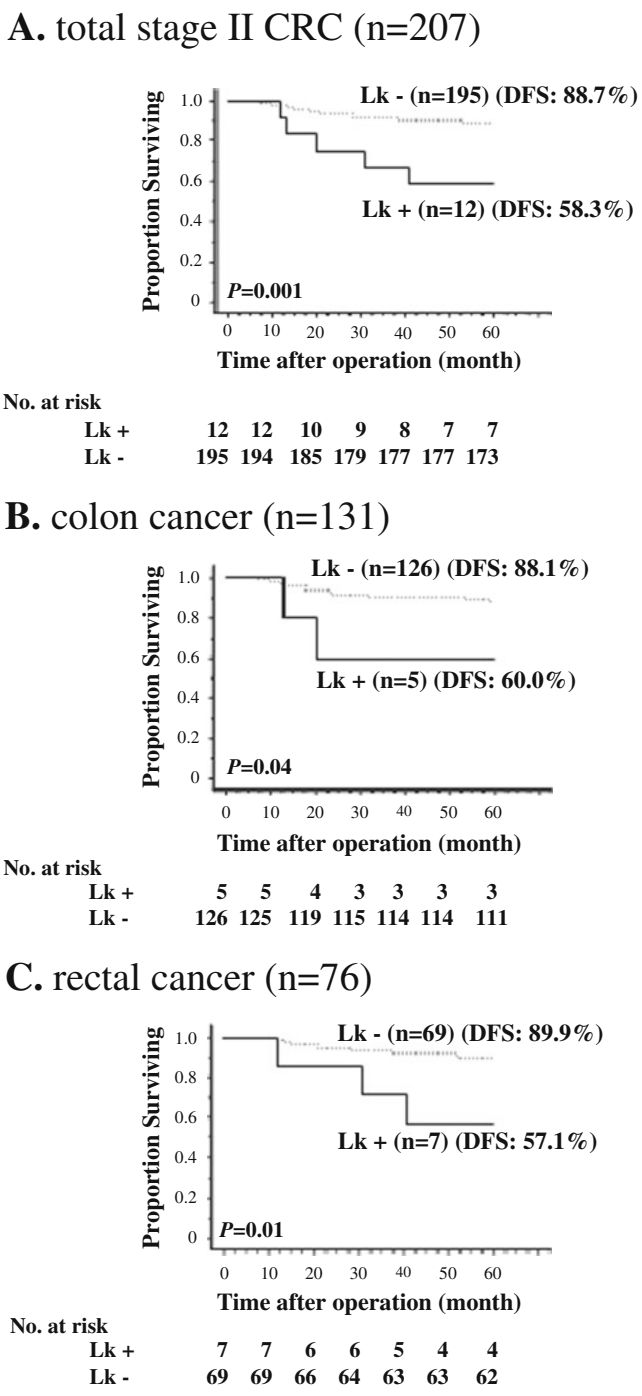

Fig. 1 Kaplan–Meier curve of 5-year DFS according to anastomotic leakage (Lk): **a** Total stage II CRC (n=207). **b** Colon cancer (n=131). **c** Rectal cancer (n=76)

factor, and there was no other factor which had prognostic potential (P<0.1). The crude hazard ratio (HR) of Lk-positive compared to Lk-negative was 4.38 (95% confidence interval (CI), 1.66–11.58; P=0.003), which indicated Lk increased the risk of recurrence of CRC and cancer-related death by more than four times that of without Lk. The effect of Lk on recurrence in colon and rectal cancer group gave similar results: crude HR (95%CI) was 4.1 (0.9–17.9) for the colon group and 4.9 (1.3–19.0) for the rectal group.

Before multivariable analyses were adopted to estimate adjusted effect of Lk on DFS, we further confirmed that there was no interaction effect between cancer position (colon or rectum) and Lk (P=0.874); taking into account that evaluation in each group would result in a small sample size and thus decrease the power of the study, we finally combined them together. Potential confounders of variables were included in the multivariable analysis (Table 2). The adjusted HR of Lk became 5.27 (95%CI, 1.54–18.10; P=0.008) in comparison to Lk-negative. We also performed an analysis by using propensity score to adjust the effect of Lk by transforming all other confounding variables into a single estimator and revealed that, after the adjustment, the HR of Lk became 4.21 (95%CI, 1.24–14.33; P=0.021). These findings suggested that Lk seems to be an independent and significant risk factor of poorer DFS (Table 2).

Lk was Associated with Hematogenic Recurrence Rather than Local or Peritoneal Recurrence in Stage II CRC

Next, first recurrence site in patients with stage II CRC was analyzed according to Lk. Interestingly, Lk was correlated with hematogenic recurrence (P=0.003 by Fisher's exact test) rather than local recurrence or peritoneal dissemination (P=0.605; Table 3). Therefore, Lk may cause systemic micrometastasis, leading to systemic recurrence.

Effect of Lk on DFS When Taking Systemic Inflammatory Response into Account

Recently, a systemic inflammatory response, as evidenced by raised circulating levels of C-reactive protein (CRP), has been reported to be associated with poor survival in patients who underwent potentially curative resection for CRC.[18] These reports may explain the above implication of Lk in systemic recurrences, hence circulating level of CRP was analyzed, which was measured as a part of routine blood examination either before or after potentially curative resection for stage II CRC. CRP level was classified as raised (≥1.0 mg/dl) or normal (<1.0 mg/dl) from a clinical practice view. Lk was significantly correlated with CRP level at 1 or 2 weeks after curative operation (P=0.018, 0.003, respectively, by Fisher's exact test; Supplemental Table 2). Moreover, the sustained elevation of CRP level at 2 weeks after operation predicted significantly worse prognosis (DFS, 75.0%) than its counterpart (89.3%; P=0.022, compared by log-rank test, Supplemental Fig. 2), while preoperative CRP and CRP at 1 week after operation did not show prognostic significance (data not shown). The multivariable prognostic analysis including CRP at 2 weeks

Table 2 Prognostic analysis of stage II patients according to 5-year DFS (n=207)

Variables	Univariable analysis		Multivariable analysis			
			Model 1		Model 2	
	HR (95%CI)	P^b values	HR (95%CI)	P^b values	HR (95%CI)	P^b values
Lk	4.38 (1.66–11.58)	0.003	5.27 (1.54–18.10)	0.008	4.21 (1.24–14.33)	0.021
Gender (male)	1.87 (0.79–4.43)	0.154	1.76 (0.71–4.34)	0.221	n/d	n/d
Age >60	1.26 (0.58–2.71)	0.559	1.24 (0.56–2.73)	0.603	n/d	n/d
Tumor position (colon)	0.99 (0.46–2.17)	0.988	1.12 (0.47–2.69)	0.797	n/d	n/d
Poor differentiation[c]	0.56 (0.08–4.14)	0.572	0.59 (0.07–5.29)	0.637	n/d	n/d
T factor (T4)	1.02 (0.14–7.51)	0.985	0.65 (0.07–5.66)	0.693	n/d	n/d
Lymphatic involvement (ly)	22.90 (0.05–9651.67)	0.310	n/d	n/d	n/d	n/d
Vascular involvement (v)	23.51 (0.09–6204.78)	0.267	n/d	n/d	n/d	n/d
Preoperative CEA elevation	1.21 (0.55–2.64)	0.636	1.13 (0.48–2.68)	0.783	n/d	n/d
Preoperative CA19-9 elevation	0.59 (0.14–2.48)	0.470	0.57 (0.13–2.55)	0.458	n/d	n/d
Obstruction	1.54 (0.46–5.11)	0.482	1.89 (0.47–7.56)	0.368	n/d	n/d
Number of total dissected lymph node					n/d	n/d
<6	reference		reference		n/d	n/d
6–10	1.60 (0.21–12.01)	0.649	0.50 (0.05–5.53)	0.570	n/d	n/d
11–15	1.26 (0.43–3.75)	0.674	0.48 (0.05–5.05)	0.542	n/d	n/d
>15	1.29 (0.48–3.50)	0.615	0.40 (0.04–3.68)	0.416	n/d	n/d
Laparoscopy-assisted operation	0.96 (0.13–7.05)	0.956	1.15 (0.15–8.79)	0.895	n/d	n/d
Adjuvant chemotherapy	0.90 (0.31–2.59)	0.838	0.95 (0.29–3.08)	0.928	n/d	n/d
Perioperative transfusion	1.28 (0.54–3.03)	0.575	0.70 (0.22–2.24)	0.547	n/d	n/d
Propensity score	n/d	n/d	n/d	n/d	1.16 (0.07–18.50)	0.918

DFS disease-free survival, *HR* hazard ratio, *CI* confidence interval, *n/d* not determined

[a] End-point: date of death or April 30, 2007, no patient was lost to follow-up

[b] Significance based on Cox's proportional hazard model

[c] Poor consists of poorly differentiated, mucinous, and undifferentiated types

There was no event in ly or v negative cases, so that these variables were excluded from multivariable analysis

Multivariable model 2 indicates the adjusted effect of Lk by applying propensity score which is a conditional probability of presenting Lk given by other clinicopathological factors including gender, age, tumor position, differentiation, vascular involvement, preoperative CEA elevation, and perioperative transfusion

after operation (n=175) showed that Lk still indicated prognostic potential (HR, 3.70, P=0.075; Table 4). This result suggests that Lk is more strongly associated with recurrence independent of sustained systemic inflammation.

Discussion

The present study showed that an anastomotic leakage (Lk) was closely associated with an adverse impact on long-term DFS (5-year DFS, 58.3%) in patients who underwent potentially curative resection for stage II CRC, and it was the most robust independent prognostic factor. This DFS was comparable to that of patients with stage III CRC. Although intramural vessel involvement may be available for the selection of low-risk patients (DFS, 100.0%), it was insufficient for the patient selection who have high risk of recurrence and would be rather low-risk selection (Supplemental Fig. 1). Therefore, with regard to patient selection, Lk alone may be potential classifier of stage II CRC. Lk has

Table 3 Association of Lk with first recurrence site in stage II patients

Lk	Local or peritoneal recurrence		P^a values	Hematogenic recurrence		P^a values
	Present	Absent		Present	Absent	
Yes	1	11	0.605	4	8	0.003
No	14	181		8	187	

[a] Significance based on Fisher's exact test

Table 4 Multivariate analysis of Lk effect on 5-year DFS in stage II CRC patients taken CRP into account (n=175)

Variables	Model 1		Model 2	
	HR (95%CI)	P^b values	HR (95%CI)	P^b values
Lk	3.05 (0.79–11.83)	0.106	3.70 (0.88–15.62)	0.075
Post-CRP (2w)	0.53 (0.21–1.35)	0.182	n/d	n/d
Gender (male)	1.97 (0.73–5.30)	0.178	n/d	n/d
Age>60	1.34 (0.59–3.14)	0.464	n/d	n/d
Tumor position (colon)	1.12 (0.43–2.91)	0.823	n/d	n/d
Poor differentiation[c]	1.02 (0.12–8.45)	0.986	n/d	n/d
T factor (T4)	0.53 (0.05–5.14)	0.583	n/d	n/d
Preoperative CEA elevation	1.30 (0.52–3.22)	0.572	n/d	n/d
Preoperative CA19-9 elevation	0.21 (0.03–1.66)	0.139	n/d	n/d
Obstruction	1.50 (0.33–6.90)	0.602	n/d	n/d
Number of total dissected lymph node			n/d	n/d
<6	Reference		n/d	n/d
6–10	6863.02	0.938	n/d	n/d
11–15	10138.02	0.935	n/d	n/d
>15	7343.4	0.937	n/d	n/d
Laparoscopy-assisted operation	1.17 (0.15–9.12)	0.884	n/d	n/d
Adjuvant chemotherapy	0.79 (0.23–2.75)	0.710	n/d	n/d
Perioperative transfusion	0.86 (0.26–2.84)	0.803	n/d	n/d
Propensity score	n/d	n/d	1.50 (0.16–13.88)	0.724

DFS disease-free survival, *HR* hazard ratio, *CI* confidence interval, *n/d* not determined, post-CRP (2w), *CRP* level at 2 week after operation

[a] End-point: date of death or April 30, 2007, no patient was lost to follow-up

[b] Significance based on Cox's proportional hazard model

[c] Poor consists of poorly differentiated, mucinous, and undifferentiated types

Variables with no event were excluded from multivariate analysis

Multivariable model 2 indicates the adjusted effect of Lk by applying propensity score which is a conditional probability of presenting Lk given by other clinicopathological factors and CRP level

been reported to be a risk factor of local recurrences in curatively operated CRC patients[10–12,19] which included several stage CRCs. However, to our knowledge, our study is the first report concerning Lk with high risk of recurrence limited in stage II disease. Interestingly, in our study, Lk was significantly implicated in systemic recurrence (P=0.003) rather than local recurrence in stage II.

In our study, there was no prognostic difference between colon cancer and rectal cancer. Although tumor position did not affect Lk and long-term prognosis in this study, anastomosis and prognosis in rectal cancer is thought to be affected by various factors compared with that in colon cancer.[10,20–23] However, even when separately analyzed on tumor positions, Lk was still significant prognostic factor (Fig. 1b, c).

Adjuvant chemotherapy for stage II CRC has been controversial at present because stage II patients show good prognosis and only a part of high-risk stage II patients may benefit in prognosis from previous studies.[6,24,25] Nevertheless, at present, standard chemotherapy is not recommended for stage II CRC patients because of excellent prognosis. Our current study included many such patients even with Lk who actually underwent adjuvant chemotherapy, but which did not include the most active agents such as oxaliplatin, CPT-11, bevacizumab, or cetuximab, suggesting that Lk anyway showed high risk for stage II CRC irrespective of adjuvant therapy. Therefore, our current result is worthy of further study on high-risk patient selection in stage II CRC and also on more powerful adjuvant chemotherapy such as FOLFOX in stage II patients with Lk in order to elucidate the benefit of adjuvant chemotherapy for these patients. In addition, neoadjuvant chemo-radiotherapy for locally advanced rectal cancer is now becoming standard. However, during the terms of this current study, we did not think that neoadjuvant treatment is really effective for such patients from a prognostic point of view. Thus, Lk in patients with neoadjuvant treatment should be also studied in the future.

Several parameters have been reported as independent prognostic factor or chemosensitive marker for patient selection allowing for the application of adjuvant chemotherapy in stage II CRC.[6,24,26] The number of evaluated lymph nodes,[27] T4 factor (direct invasion into adjacent structure),[16,28] tumor budding/infiltrating,[29] vascular involvement,[16,28] or perforation through the tumor[28] were such high-risk potential markers. In the present study, vascular involvement tended to be a prognostic factor, however, it was not insufficient to select high-risk patients. On the other hand, the number of evaluated lymph nodes and T4 factor did not indicate any prognostic significance in our current cohort of stage II CRC. Several molecular and genetic markers have also been reported to indicate poor prognosis of stage II CRC such as the DNA aneuploid,[30] 17p or 18q allelic imbalance,[7] gene expression profiling by cDNA microarray,[8] and micrometastasis detected by reverse transcriptase-polymerase chain reaction of CEA[31] or CK20.[32] In addition, microsatellite instability (MSI) has been reported as chemoresistant marker.[9] Actually, the largest stage II colon cancer trial (ECOG 5202, the US Gastrointestinal Intergroup including the National Cancer Institute of Canada) is ongoing, in which patients are now selected prospectively for adjuvant chemotherapy based on 18q loss of heterozygosity and MSI status.[33] Nevertheless, all such genetic and molecular tools are unsuitable for routine application at present because they are costly and time-consuming methods and have not been validated yet. In this meaning, Lk is easily available for patient selection at any minute.

Viable cancer cells in the lumen may be present at the site of the anastomosis at the time of surgery, which can be detected on suture or staple lines of anastomosis,[34] and on the occasion of Lk, those may be capable of implantation and subsequent local recurrence.[35] However, this theory alone did not explain the association of Lk with systemic recurrence in the present study. Systemic inflammatory response, as evidenced by raised circulating concentrations of CRP, has been reported to predict recurrence and disease-specific survival in curatively operated CRC patients.[18] Consistently, the sustained CRP elevation at either 1 or 2 weeks after operation was significantly associated with Lk, and especially, CRP at 2 weeks after operation per se predicted poor prognosis (P=0.022) in the present study. CRP may reflect the inflammatory response promoted by various cytokines which are presumably released from leukocytes in the malignant process.[36] On the other hand, a raised CRP level was thought to be related to the reduction of circulating lymphocytes.[37] In addition, the reduction of lymphocytes in the peripheral blood was shown to reflect the immune suppression in patients with malignant tumor,[38] and tumor-induced immune suppression adversely affects their prognosis.[39]

Perioperative allogeneic blood transfusion was reported to be an independent risk factor for Lk in a dose-dependent manner.[23] Also in the present study, perioperative blood transfusion affected Lk most robustly even when CRP was included in the multivariable logistic analysis (data not shown). Allogenic blood transfusion impairs the cell-mediated immune response[40] and predisposes to postoperative infectious complication,[41] and cell-mediated immune responses, which include mainly macrophage and T-lymphocyte, has been thought to affect the healing process.[42] Tadros T. et al. reported that perioperative blood transfusion impaired the healing of experimental intestinal anastomosis in an animal model using bursting pressure of anastomosis, in addition, cell-mediated immune response, as evidenced by exogenous IL-2, reversed the negative effects of blood transfusion on anastomotic repair.[43] Taken together, Lk may lead to systemic recurrences partly through cancer immune suppression together with sustained CRP elevation and perioperative blood transfusion. Conversely, we could also say that Lk is favored by a local depression of the immune system for the presence of undetected micrometastasis.

Recently, it has been suggested that tumor progression such as invasion and metastasis is coordinated by both cancer cells and host stromal cells, which consist tumor microenvironment.[44–46] A variety of host bone marrow-derived cells, which include inflammatory cells, cancer-associated fibroblasts, and endothelial progenitor cells compose of a tumor microenvironment.[47–49] Host inflammatory cells produce much more TGF-β than tumor cells, leading to inhibition of host tumor immune surveillance,[50,51] which may lead to cancer cell escape and intravasate into circulation. Local inflammation caused by Lk may additionally affect the above mechanism and may result in metastasis-prone phenotype. However, in order to answer the reason why Lk was associated with systemic recurrence, further experimental studies, such as comparison of circulating cancer cells or cytokines in both patients and experimental model, may be needed.

In conclusion, we showed that Lk was the most robust independent prognostic factor among the clinicopathological factors in stage II CRC. These results suggest that Lk may be appropriate for the selection of high-risk patients. And, Lk was associated with systemic recurrence in both colon and rectal cancer. Because Lk necessarily occurs at a given rate in spite of perioperative treatment with maximal attention and it is immediately available for clinical use from cost and technical point of view, Lk could be a factor for selecting high-risk patients. As only 12 patients (out of 207) had an Lk in this study, the prognostic impact of Lk should be validated in a larger study. On the other hand, because the DFS of patients without Lk was still 88.7%, further molecular tools would be necessary.

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ORIGINAL ARTICLE

Depth of Tumor Invasion Independently Predicts Lymph Node Metastasis in T2 Rectal Cancer

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Abstract
Objective The aim of this study was to identify risk factors of lymph node metastasis (LNM) for T2 rectal cancer.
Methods From a prospectively maintained single-institution database, we identified 346 consecutive pT2 rectal cancers treated with total mesorectal excision from 1998 to 2009. Univariate and multivariate analyses were performed to identify risk factors associated with overall and intermediate/apical LNM. The incidence of overall and intermediate/apical LNM was analyzed by tree analysis.
Results Age, tumor location, pathological features, and depth of invasion were independent predictors for overall LNM. Tumor location, pathological features, and depth of invasion were independent predictors for intermediate/apical LNM. Tree analysis showed that the incidence of LNM was 7.7% for upper rectal cancer with favorable pathological features, and 3.4% for mid/lower rectal cancer without other identified risk factors. The incidence of intermediate/apical LNM was 5.7% for superficial T2 rectal cancer with favorable pathological features, and 3.1% for deep T2 rectal cancer locating in upper rectum with favorable pathological features.
Conclusions Depth of invasion is an independent predictor for LNM in T2 rectal cancer. Using tree analysis, we identified a subset of patients with low risk of LNM who may be candidates of local excision.

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Keywords Depth of invasion · T2 rectal cancer · Lymph node metastasis · Tree analysis

Introduction

Radical resection with total mesorectal excision (TME) principle is the standard of care for rectal cancer. However, morbidity, mortality, and functional outcome (especially colostomy) are still the major issues for patients with old age, co-morbidity, or low-locating tumor.[1–4] Local excision has recently been increasingly used for early stage rectal cancer, especially for patients in the above setting in order to avoid a major procedure.[5,6] However, if the patients are not selected appropriately, the oncologic outcome may be compromised. As has been reported, local recurrence rates after local excision for T1 rectal cancer range from 3.4% to 18%.[7–10] After being strictly selected, patients with T1 cancer without high risk features are able to achieve favorable long-term oncologic outcome.[7,11] Thus, the role of local excision has been well established in this setting. However, for T2 cancer, the role of local excision remains controversial. There are great discrepancy in oncologic outcome after local excision for T2 rectal cancer with recurrence rates ranging from 0% to 67%,[12–14] which may be associated with the divergent criteria for patient selection. Recently, there are studies showing that T2 rectal cancer treated by local excision in combination with neoadjuvant or adjuvant chemoradiation can achieved satisfied long-term outcome, suggesting that there may be subset of patients who are eligible for local excision subjective to appropriate selection.[11,15]

One of the major causes of recurrence is the metastatic lymph nodes (LNM) which cannot be removed by local excision. Previous reports show that the incidence of LNM for T2 rectal cancer is about 17–43%.[16–18] Therefore, identification of risk factors associated with LNM would help in selecting proper patients for local excision. Unfortunately preoperative imaging, including magnetic resonance imaging, computed tomography, and endorectal ultrasonography have limited value in predicting LNM in early rectal cancer.[19] T stage has been well recognized as one of the most important predictor of LNM for colorectal cancer.[16,17,20] When looking specifically at T1 rectal cancer, the depth of invasion into the submucosa is also reported to be an independent predictor of LNM.[21] However, little is known about whether the depth of infiltration in T2 rectal cancer is associated with LNM. Currently, the TNM staging system defines rectal cancer that invades into, but not penetrate muscularis propria as T2.[22] However, according to the histology, muscularis propria can be further divided into inner or circular layer (superficial T2) and outer or longitudinal layer (deep T2), which can be determined without much effort under microscopy.[23]

The current study was to evaluate the depth of invasion in muscularis propria as well as other clinico-pathological features as the predictors of LNM in T2 rectal cancer using specimens resected with TME principle at a single institution with uniform surgical and pathological techniques.

Material and Methods

Patients Selection

From a prospectively maintained database at Sun Yat-sen University Cancer Center, we identified a study population of 346 patients with tumor invading into, but not penetrating muscularis propria in rectum (T2) from January, 1998 through April, 2009. We excluded patients with recurrent rectal cancer, patients who underwent transanal local excision or endoscopic mucosal resection, patients who have been treated with neoadjuvant therapy, and patients with benign diseases. Clinicopathological features of the patients included in this study are summarized in Table 1.

Pretreatment Evaluation

Pretreatment evaluation included digital rectal examination, chest X-ray or computed tomography (CT) scans, abdominal and pelvic CT scans, flexible endoscopy, and serum carcinoembryonic antigen. The distance of the inferior end of the tumor from the anal verge was determined by flexible endoscopy, and/or digital rectal examination. At the time of treatment, biopsies and operative pathological specimens were reviewed by pathologists at the Sun Yat-sen University Cancer Center. The sixth edition of the American Joint Committee on Cancer TNM system was used for staging.[22]

Treatment and Lymph Node Sampling

All patients received radical rectal resection according to the principles of TME. For patients with upper rectal cancer, removal of longer than 5 cm of the rectum and mesorectum distal to the lower margin of tumor were required. Lymph node (LN) sampling was categorized according to the number and sites in the mesorectum. LNs located along the mesorectal border (within 1 cm) of the rectum were categorized as perirectal LNs; while LNs located outside the field of perirectal LNs were categorized as intermediate/apical LNs.

Table 1 Clinicopathologic characteristics of patients

Clinicopathologic features	Number (%)
Age (years)	
≤65	217 (62.7)
>65	129 (37.3)
Gender	
Female	182 (52.6)
Male	164(47.4)
Tumor location (from the anal verge (cm))	
≤8	291 (84.1)
>8	55 (15.9)
Tumor size (cm)	
≤3	139 (40.2)
>3	207 (59.8)
Preoperative CEA (ng/ml)	
≤5	256 (74.0)
>5	62 (17.9)
N/A	28 (8.1)
High grade or mucinous/signet-ring cell type	
Absent	300 (86.7)
Present	46 (13.3)
Depth of infiltration in muscularis propria	
Inner (circular) layer	118 (34.1)
Outer (longitudinal) layer	228 (65.9)
Distribution of metastatic LN	
Perirectal LN only	37 (45.7)
Intermediate/apical LN±perirectal LN	44 (54.3)
Pathological stage	
I (T2N0M0)	265 (76.6)
IIIA (T2N1M0)	67 (19.4)
IIIC (T2N2M0)	14 (4.0)

Abbreviation: *N/A* not available, *CEA* carcinoembryonic antigen, *LN* lymph node

Statistical Analysis

We correlated eight demographic and histopathological characteristics of the 346 patients with the presence of LNM and the site distribution of LNM. Categorical variables are presented as frequencies (percentages) and continuous variables as means with standard deviations or medians with ranges. The Mann–Whitney *U* test was used to compare non-continuous variables as appropriate. Independent risk factors for LNM were determined using logistic regression analysis. Independent risk factors for site distribution of LNM were determined using ordinal logistic regression analysis. All variables that were significant in the univariate analysis were entered into a multivariate model by using an enter fashion. *P* value <0.05 was considered significant. Statistical analyses were performed with SPSS statistical software (SPSS Inc. Chicago, IL, version 15.0 for Windows).

Results

Clinicopathological Characteristics

There were 346 patients with T2 rectal cancer enrolled in the current study. The median age at diagnosis was 59 (range, 26–93) years old. The median distal tumor margin from anal verge was 5 (range, 1–15) cm. The median number of retrieved LNs was 11 (range, 3–52) per specimen. The patients with LNM had a median of 2 (range, 1–20) LNs involved. The demographic data are shown in Table 1.

Risk Factors for Overall LNM

Univariate analysis indicated that young patients (age, ≤65 years; $P=0.041$), mid and lower locating tumor (distance from anal verge, ≤8 cm, $P=0.024$), large tumor (diameter >3 cm, $P=0.052$), unfavorable pathological features (high grade or mucinous/signet-ring cell type, $P<0.001$), and deep T2 ($P=0.014$) were predictors for LNM in T2 rectal cancer (Table 2). Multivariate logistic regression analysis indicated that young patients ($P=0.045$), mid and lower locating tumor ($P=0.049$), unfavorable pathological features ($P=0.003$), and deep T2 tumor ($P=0.033$) were independent predictors for LNM in T2 rectal cancer (Table 2).

Risk Factors for Intermediate/Apical LNM

Of the patients with LNM, 37 patients (45.7%) had perirectal LNM only, while 44 patients (54.3%) had intermediate/apical LNM with or without perirectal LNM. Univariate analysis indicated that young patients ($P=0.036$), mid and lower locating tumor ($P=0.020$), unfavorable pathological features ($P=0.001$), and deep T2 ($P=0.011$) were the predictors for intermediate/apical LNM in T2 rectal cancer (Table 3). Multivariate ordinal logistic regression analysis indicated that mid and lower locating tumor ($P=0.049$), unfavorable pathological features ($P=0.004$), and deep T2 ($P=0.015$) were the predictors for intermediate/apical LNM in T2 rectal cancer (Table 2).

Number and Site Distribution of LNM

For patients with metastatic LN involving only perirectal group, the percentage of patients with one, two, three, and more than three LNM was 64.9%, 24.3%, and 8.1%, and

Table 2 Logistic regression analysis of predictors of LNM (univariate and multivariate analyses)

	Univariate analysis		Multivariate analysis	
	P	Odds ratio (CI)	*P*	Odds ratio (CI)
Gender (male vs. female)	0.984	0.98 (0.61–1.66)		
Age (>65 vs. ≤65 years)	0.041	0.56 (0.33–0.98)	0.045	0.55(0.31–0.99)
Tumor location (>8 vs. ≤8 cm)	0.024	0.36 (0.15–0.87)	0.049	0.40 (0.16–1.00)
Tumor size (>3 vs. ≤3 cm)	0.052	1.69 (1.00–2.87)	0.067	1.69 (0.96–2.96)
Circumferential involved (>1/4 vs. ≤1/4)	0.650	1.14 (0.65–1.98)		
Preoperative CEA (>5 vs. ≤5 ng/ml)	0.065	1.65 (0.97–2.81)		
High grade or mucinous/signet-ring cell type (+ vs. −)	<0.001	3.43 (1.80–6.55)	0.003	2.77 (1.42–5.40)
Depth of invasion in muscularis propria (outer vs. inner layer)	0.014	2.08 (1.16–3.71)	0.033	1.93 (1.06–3.54)

CI confidence interval, *CEA* carcinoembryonic antigen

2.7%, respectively; while for patients with metastatic LN involving intermediate/apical group, the percentage of patients with one, two, three, and more than three LNM was 43.2%, 18.9%, 13.5%, and 43.2%, respectively (Fig. 1). Patients with perirectal LNM had significantly fewer number of metastatic LNs than patients with intermediate/apical LNM (P=0.001).

Tree Analysis of the LNM

We hierarchized the patients for tree analysis according to the independent risk factors for overall LNM that had been identified (Fig. 2). As a result, a subset of patients with low risk of LNM were identified. The incidence of LNM was 7.7% for upper rectal cancer with favorable pathological features, and 3.4% for mid/lower rectal cancer without other identified risk factors.

We also hierarchized the patients for tree analysis according to the independent risk factors for intermediate/apical LNM that had been identified (Fig. 3). The incidence of intermediate/apical LNM was 5.7% for superfical T2 rectal cancer with favorable pathological features, and 3.1% for deep T2 rectal cancer locating in upper rectum with favorable pathological features.

Discussion

The main drawback of local excision in early rectal cancer is the lack of LN removal, which is the major cause of treatment failure.[6] Since the incidence of LNM in T2 rectal cancer is reported to be relatively high (17–43%),[16–18] it would be critical to identify the risk factors predicting LNM, which may aid in selecting appropriate patients for local excision.

In the current study, besides the previous noted variables such as age,[20] location of tumor,[21] and pathological features,[16] we identified deep T2 as one of the independent predictors of LNM in T2 rectal cancer. To our knowledge, this is the first report showing that risk of LNM in T2 rectal cancer is associated with depth of invasion in muscularis propria. Our results showed that the risk of LNM was significantly lower when tumor was confined within the inner layer (15.3%) compared with tumor infiltrated into

Table 3 Logistic regression analysis of predictors of intermediate/apical lymph node metastasis (univariate and multivariate analyses)

	Univariate analysis		Multivariate analysis	
	P	Odds ratio (CI)	*P*	Odds ratio (CI)
Gender (male vs. female)	0.985	1.00 (0.61–1.64)		
Age (>65 vs. ≤65 years)	0.036	0.56 (0.32–0.96)	0.067	0.59 (0.34–1.04)
Tumor location (>8 vs. ≤8 cm from anal verge)	0.020	0.35 (0.14–0.85)	0.049	0.41 (0.16–0.99)
Tumor size (>3 vs. ≤3 cm)	0.060	1.69 (0.97–2.88)		
Circumferential (>1/4 vs. ≤1/4)	0.699	1.11 (0.64–1.94)		
Preoperative CEA (>5 vs. ≤5 ng/ml)	0.112	1.64 (0.89–3.01)		
High grade or mucinous/signet-ring cell type (+ vs. −)	0.001	2.81 (1.51–5.25)	0.004	2.33 (1.23–4.42)
Depth of invasion in muscularis propria (outer vs. inner layer)	0.011	2.12 (1.19–3.78)	0.015	2.08 (1.15–3.74)

CI confidence interval, *CEA* carcinoembryonic antigen

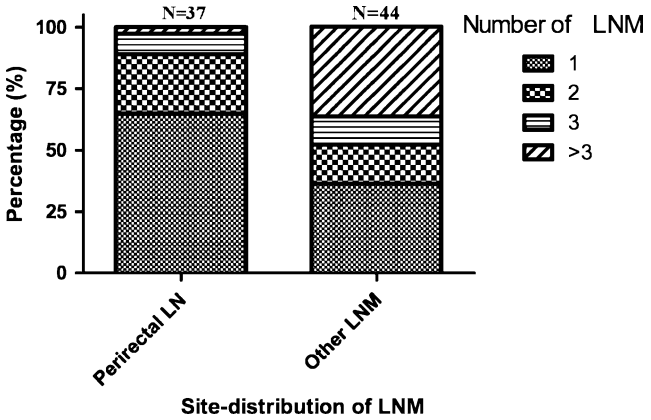

Fig. 1 Number of metastatic lymph nodes according to the site of metastasis. Patients with perirectal LNM had significantly fewer number of metastatic LNs than patients with intermediate/apical LNM (P=0.001)

outer layer (27.6%). The risk of LNM for tumor confined within inner layer was comparable to that of T1 rectal cancer,[17] whereas for tumor infiltrating into the outer layer, the risk of LNM approached that of T3 rectal cancer.[24] Considering the tremendous difference in risk of LNM between the two groups, it would be necessary to incorporate the depth of infiltration (circular or longitudinal muscularis propria infiltration) into pathological report for T2 rectal cancer resected by local excision. When final pathological report shows that longitudinal muscularis propria is invaded, salvage radical resection would be necessary. Although there is concern that such approach might violate the principles of oncologic surgery, recent data suggest that salvage operation right after (within 30 days) local excision does not compromise oncologic outcome.[25] Meanwhile, since the determination of superficial and deep T2 is based on the histological landmark-circular layer and longitudinal layer of muscularis propria which are well recognized by the pathologists,[23] the effort needed to train a pathologist to report the finding would be relatively small.

The current study demonstrated that young patients (≤65 years), mid and lower locating tumor (≤8 cm from anal verge), unfavorable pathological features, and deep T2 were the independent predictors for LNM in T2 rectal cancer. On the basis of these risk factors, we further identify a subgroup of patients with relatively low incidence of LNM by tree analysis. The results showed that the incidence of LNM was 7.7% for upper rectal cancer with favorable pathological features, and 3.4% for mid/lower rectal cancer without other identified risk factors, which is comparable to that of LNM in low risk T1 rectal cancer and may be ideal candidate for local excision.[17,21]

In the current study, we also looked into the site distribution of LNM with special interest in perirectal LNM only which might be removed by new local excision technique, transanal endoscopic microresection (TEM). Since TEM allows an excellent exposure of the operative field with a stereoscopic endoscopic vision, it is possible not only to perform a complete full-thickness excision with

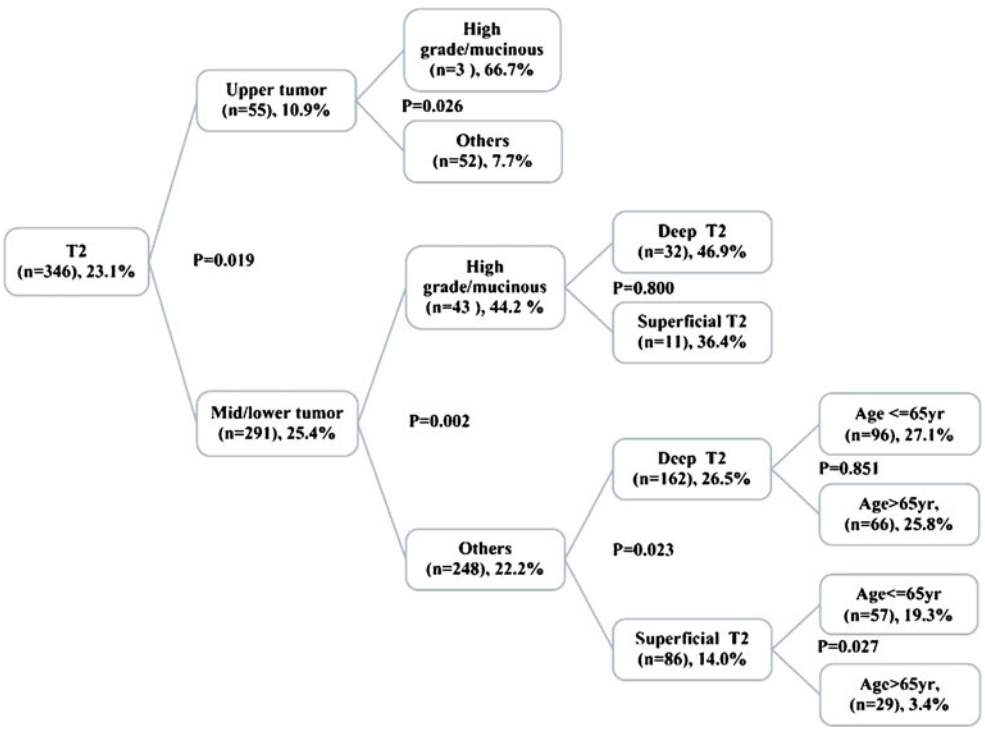

Fig. 2 Incidence of overall lymph node metastasis in patients with T2 rectal cancer hierarchized by identified risk factors. Tree analysis showed that the incidence of LNM was 7.7% for upper rectal cancer with favorable pathological features, and 3.4% for mid/lower rectal cancer without other identified risk factors

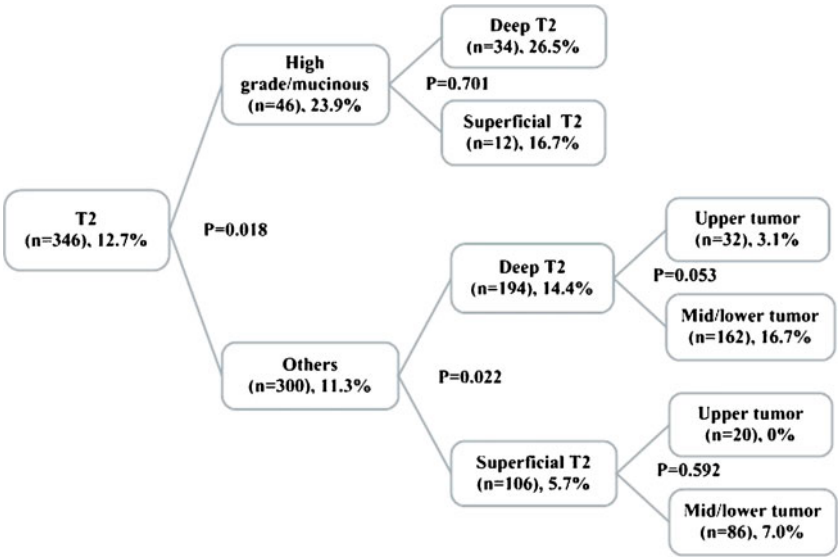

Fig. 3 Incidence of intermediate/apical lymph node metastasis in patients with T2 rectal cancer hierarchized by identified risk factors. Tree analysis showed that the incidence of intermediate/apical LNM was 5.7% for superficial T2 rectal cancer with favorable pathological features, and 3.1% for deep T2 rectal cancer locating in upper rectum with favorable pathological features

an appropriate margin, but also to remove all the adjacent perirectal fat making the same plane of dissection utilized in the TME procedure.[26] Our results showed that 45.7% of LMN occurred only in perirectal group. Moreover, about 90% of these patients have very limited number of metastatic LNs (64.9% with one LNM and 24.3% with two LNM), which might be removed by the TEM technique. Meanwhile, we also identified risk factors for intermediate/apical LNM in T2 rectal cancer, including mid and lower locating tumor (≤8 cm from anal verge), unfavorable pathological features (high grade or mucinous/signet-ring cell type), and deep T2. Moreover, tree analysis showed that the incidence of intermediate/apical LNM was 5.7% for superfical T2 rectal cancer with favorable pathological features, and 3.1% for deep T2 rectal cancer locating in upper rectum with favorable pathological features,

Currently, the application of local excision for T2 rectal cancer remains controversial. However, there is a interesting phenomenon that the prospective data [7,9,11,27] reveal much better outcome (4–5% locoregional recurrence rate for T1 and 14–16% for T2) comparing with retrospective data [6,9,14] (12–18% locoregional recurrence rate for T1 cancers and 22–47% for T2 cancers). A possible explanation for the discrepancy in outcome may be due to the different criteria in patient selection, which is far more rigid in prospective trials. As a result, it would be essential to strictly select the candidates for local excision procedure following the guidance of identified risk predictors. The present study showed that approximately 23% of T2 rectal cancer patients had relatively low risk of LNM, suggesting these patients might be proper candidates for local excision. When looking specifically at site distribution of metastatic LN and considering the LN confined in perirectal group as potentially resectable by TEM, approximately 40% of additional patients might be candidates for local resection if the present classification had been applied. However, one should be cautious to the latter concept, since there are no data showing the outcome for resection of metastatic perirectal LN using TEM technique in T1 or T2 cancer. Noteworthily, TEM technique should be reserved for early tumor (especially for T1) without risk factors. As for T2 tumor, if TEM is to be applied, besides strictly patient selection on the basis of risk model, neoadjuvant/adjuvant chemoradiation may be necessary.[7,11]

This study is subjected to several limitations. Currently, the NCI guidelines utilize rigid sigmoidoscopy to determine the location of rectal cancer.[28] However, in our practice, we do not use rigid scope routinely. Since most of the tumors locate within 8 cm from the anal verge and could be touched by digital examination, the impact of the variation of distance evaluated by digital examination and flexible scope on the result is limited. Meanwhile, since we did not measured the depth of invasion in muscularis propria using endorectal ultrasound (ERUS), we could not draw a conclusion with regard to the possibility of using ERUS for differentiation of superficial T2 and deep T2. Further study delineating the depth of invasion evaluated by ERUS and risk of LN metastasis in T2 tumor is warranted.

In conclusion, besides the previous noted variables (age, location of tumor, and pathological features), depth of infiltration is also an important independent risk factor for predicting LNM in T2 rectal cancer. Using the tree analysis models, we identified a subset of patients with low risk of LNM or low risk of intermediate/apical LNM, who may be candidates for TEM procedure.

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ORIGINAL ARTICLE

Early Detection of Anastomotic Leakage After Elective Low Anterior Resection

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Abstract
Background Colorectal anastomotic leakage is a serious complication leading to major postoperative morbidity and mortality. In the present study, we investigated the early detection of anastomotic leakage before its clinical presentation.
Method Fifty-six patients with rectal cancer were included prospectively in this study. All patients underwent elective low anterior resection. Peritoneal samples were collected from the abdominal drains at the first, third, and fifth days postoperatively for bacteriological study (quantitative cultures for both aerobes and anaerobes) and cytokines (IL-6, IL-10, TNF) measurement. Patients were divided into two groups: those without symptomatic or clinical evidence of anastomotic leakage (AL; group 1) and those with clinical evidence of AL (group 2). Study variables included hospital stay, wound infection, operative time, blood loss, height of anastomosis, *intraperitoneal* cytokines, and microbiological study of peritoneal fluid.
Result Clinically evident AL occurred in eight patients (14.3%) and diagnosed postoperatively on median day 6. Intraperitoneal bacterial colonization and cytokine levels were significantly higher in patients with clinical evidence of AL. Wound infection was significantly higher in anastomotic leakage group. The hospital stay for the patients with anastomotic leakage was significantly longer than those without AL (14±1.41 vs. 5.43±0.89 days). A significant difference among two groups was observed regarding operative time, blood loss, blood transfusion, and height of the anastomosis.
Conclusion The peritoneal cytokines levels and intraperitoneal bacterial colonization might be an additional diagnostic tool that can support the decision making of surgeons for early detection of anastomotic leak in colorectal surgery.

Keywords Anastomotic leakage · Cytokines · Bacterial colonization

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Introduction

Anastomotic leakage after large bowel resections remains one of the most serious and important complications, despite recent advances in colorectal surgery. Recently, with an increasing number of sphincter-preserving procedures, there are more patients at risk for possible leakage. The leakage rate varies from 0.5% to 30%.[1–5]

Clinical anastomotic leakage is associated with multiple morbidities, poor functional outcome,[6] increased mortality rate ranged from 10% to 15% and increased overall and local recurrence in patients who underwent resection for rectal cancer.[7,8]

Clinically, anastomotic leakage diagnosis has been reported to be on median postoperative days 7 to 11;[9,10]

some studies reported an even longer interval, up to 45 days postoperatively.[11,12] Such long intervals are associated with increased morbidity and mortality therefore exclusion or confirmation of the diagnosis of anastomotic leakage (AL) have to take priority in patients with any suspicion of AL after colorectal surgery.[13]

Several studies investigated early prediction of anastomotic leakage after colorectal surgery, to allow the treatment to be instituted before the patient develops serious complications such as organ failure and death.[9,14]

After abdominal surgery, proinflammatory cytokines such as TNF-α and IL-6 A are released into the peritoneal cavity and generate an inflammatory reaction, which is inhibited by other cytokines such as IL-10.[14–16] The increase in peritoneal cytokines can predict anastomotic complications, thus uncomplicated postoperative course is associated with decreasing peritoneal cytokine levels, whereas increasing levels indicate an unfavorable postoperative course.[14,17]

The clinical approach of bacterial quantitation to treatment has been emphasized during the last decades.[18] There is a correlation between the wound microbial load and the likelihood of infection as evidenced by delayed wound healing. [19–21] There are not many current researches that correlate the quantitative microbial load of specific bacteria to the clinical significance of specific isolates in causing anastomotic leakage.

So, this study aimed to investigate intraperitoneal bacterial colonization and cytokine (IL-6, IL-10, TNF-α) level in the early postoperative period that might possibly serve as an indicator of early prediction of anastomotic leakage in patients undergoing elective low anterior resection.

Patients and Methods

Patients

Consecutive patients who were treated for rectal cancer with low anterior resection at the Colorectal Surgery Unit of Mansoura University Hospital, Mansoura, Egypt, during the period from March 2007 to December 2009 were eligible for the study.

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

Pre-operatively, all patients underwent mechanical bowel preparation and were given cefuroxim and metronidazole for antibiotic prophylaxis. Low molecular weight heparin was used for deep vein thrombosis prophylaxis.

Low anterior rectal resections were performed for patients with rectal cancer. Anastomoses were handsewn or EEA stapler of appropriate size. After removal of the stapler, both doughnuts were inspected for integrity. The integrity of the anastomosis can be tested by transanal air insufflation after the true pelvis was filled up with saline solution. A leakage that was revealed during this procedure was treated by oversewing (small leakage) and performance of a new anastomosis (leakage over 25% of the circumference). A temporary loop ileostomy was constructed in cases where the presence of risk factors were thought to increase the risk of anastomotic leakage: comorbidity factors or adverse effect during operation. At the end of the operation, intraoperative irrigation was done and an 18-French drain was placed in the pelvis or abdomen. Postoperative complications were recorded in all patients.

Definition of Anastomotic Leakage

AL was defined clinically as gas, pus, or fecal discharge from the drain, fecal discharge from the operative wound, pelvic abscess, peritonitis, and rectovaginal fistula. All symptomatic anastomotic leakages were confirmed by one or more of the following methods: radiological contrast study, CT scan, digital rectal palpation.[13,14,17]

Patients were divided into two groups: those without symptomatic or clinical evidence of AL (group 1) and those with clinical evidence of AL (group 2).

Sampling

Peritoneal samples were collected from the abdominal drains at the first, third, and fifth days postoperatively for peritoneal microbiological study and cytokines (IL-6, IL-10, TNF) level measurement. Samples were centrifuged at 3,000*g* for 10 min at 4°C and stored at 20°C, until cytokines analysis.

Assays for TNF-α, IL-6, and IL-10

Tumor necrosis factor TNF-α, IL-6, and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA; Ray Bio^{R} Human IL6, IL10, and TNF ELISA Kit protocol). All kits were used according to the instruction of the manufacturers.

Microbiological Study

Sample Collection and Transport Specimens submitted to microbiology laboratory were obtained from the abdominal drain at first, third, and fifth days postoperatively by aspiration of the drained fluid by a syringe after evacuation of the air and capping of the needle. The samples were transported to the medical diagnostic and infection control unit as soon as possible without delay. The time between

collection of the material and inoculation of the specimens ranged from 30 min to 2 h at the most.[22]

Sample Processing Quantitative culture was done according to Collee et al.[23] A measure of 0.1 ml was obtained to make tenfold serial dilutions of bacterial suspension. A 0.1-ml measure from each dilution was pipetted and distributed widely with sterile glass spreader on sheep blood agar 5%, chocolate, and MacConkey *agar* plates for aerobes and facultative anaerobic organisms. An additional neomycin blood agar plate was inoculated for anaerobic cultivation. The plates were incubated aerobically at 37°C and examined at 24 and 48 h. While for anaerobic organism isolation, the plated media were incubated in GasPak Jars and examined at 48, 96 and 120 h.[22] The viable count was calculated from the plates with average colony count (30 to 300 *colonies*).

Microbial Identification Isolates were identified by Gram-stain and colonial morphology. Further identification of the isolates was done using API 20 E and API 20NE systems (bioMerieux, Marcy l'Etoile, France) for facultative anaerobic and aerobic organisms, respectively. With regard to organisms isolated anaerobically, identification was based on the cultural, microscopical, and antimicrobial sensitivity characteristics of the organism (sensitivity to metronidazole and resistance to penicillin and aminoglycosides).

Statistical Analysis

Statistical analysis of the data in this study was performed using SPSS software, version 10. For continuous variables, descriptive statistics were calculated and were reported as mean±standard deviation. Categorical variables were described using frequency distributions. The Student's t test for paired samples was used to detect differences in the means of numerical variables; Chi-square test was used for nominal variables, and Fisher's exact test was used in cases with low expected frequencies. P values<0.05 were considered to be significant.

Results

Of the 56 patients, with rectal cancer who underwent elective low anterior resection during the period from March 2007 to December 2009, clinically evident AL occurred in eight patients (14.3%; five men and three women). The mean age was 53.37±8.41 (range, 42–66) years; see Table 1.

Anastomotic leakage was diagnosed on median day 6 (range, 2–8) and all occurred before discharge from hospital. Of the eight patients who developed symptomatic leakage, one patient died of sepsis secondary to anastomotic leakage, one patient already had covering ileostomy passed conservatively, and the other six patients were urgently re-operated on and had loop ileostomy.

Wound infection was significantly higher in anastomotic group 7 (87.5%) versus seven (14.6%) in patients without anastomotic leakage. The hospital stay for the patients with anastomotic leakage was 14±1.41 days[12–16], which took significantly longer than those without AL at 5.43+ 0.89 days[4–8] (Table 1).

The operative details of the two groups are presented in Table 2. A significant difference among two groups was

Table 1 Demographic data of the patients operated on with anterior resection of the rectum

Variables	No anastomotic leakage	With anastomotic leakage	*P* value	95% confidence interval of the difference	
				Upper	Lower
Age	51.22±8.55 (35–67)	53.37±8.41 (42–66)	0.52	−8.6827	4.3910
Sex					
Male	35 (72.1%)	5 (62.5%)	0.55		
Female	13 (27.9%)	3 (37.5%)			
BMI	23.62±4.62 (19–35)	25.87±4.48 (21–33)	0.20	−5.7742	1.2742
Smoking	20 (41.7%)	3 (37.5%)	0.82		
Staging					
Stage A	9 (18.8%)	2 (25%)	0.59		
Stage B	23 (47.9%)	4 (50%)			
Stage C	16 (33.3%)	2 (25%)			
Level of tumor	11.06±2.85 (6–15)	11.62±2.19 (9–15)	0.59	−2.6893	1.5643
Pre-operative radiotherapy	12 (25%)	1 (12.5%)	0.44		
Hospital stay	5.43±0.89 (4–8)	14±1.41 (12–16)	0.0001	.3741	−9.3125
Wound infection	7 (14.6%)	7 (87.5%)	0.0001		

Table 2 Intraoperative data

Variables	No anastomotic leakage	With anastomotic leakage	*P* value	95% confidence interval of the difference	
				Upper	Lower
Operative time	149.89±20.35 (110–195)	175.25±34.37 (130–290)	0.0001	−66.3103	−24.3980
Blood loss	331.25±189.23 (100–750)	593.75±187.91 (250–800)	0.001	−407.2550	−117.7450
Blood transfusion	9 (18.8%)	4 (50%)	0.04		
Height of anastomosis	6.43±2.21 (2–10)	5±1.06 (4–7)	0.009	0.4024	2.4726
Stapled used	14 (29.2%)	3 (37.5%)	0.63		
Defunction stoma	16 (33.3%)	1 (12.5%)	0.24		

observed regarding operative time, blood loss, blood transfusion, and height of the anastomosis.

The causes of the anastomotic leak were anastomotic ischemia in two patients, staple failure in one patient, the lower level of anastomosis below 6 cm from anal verge in three patients, and AL also occurred in two patients with no obvious risk factors.

TNF-α, IL-6, and IL-10 Levels

The peritoneal cytokine response in both groups is shown in Table 3. Intraperitoneal IL-6 was increased postoperatively on first, third, and fifth day (P=0.0001; P=0.0001, P=0.0001, respectively) and IL-10 was increased on postoperative first, third, and fifth day (P=0.0001; P=0.04, P=0.0001, respectively). TNF-α was increased in AL group on third and fifth postoperative day (P=0.0001; P=0.0001, respectively) but not on the first day.

There was no a significant difference in the level of peritoneal cytokine response in patient with AL and already had a diverting ileostomy (one patient) from the rest of the patients with AL (seven patients).

Although peritoneal cytokine levels were significantly higher beginning on day 1, clinically evident anastomotic leakage occurred on day 6. All patients developed temperature above 38 C and absence of bowel movement. One patient died of sepsis secondary to AL; one patient already had covering ileostomy passed conservatively, and other six patients with proven AL were re-operated and defunctioning ileostomy was done.

Microbiological Result

Escherichia coli, *Klebsiella*, *Pseudomonas* species, and bacteriod micro-organism were significantly more in AL group in first, third, fifth days postoperatively as shown in Table 4.

E. coli was the most common micro-organism detected in patients with AL. In AL group starting from the first, third, and fifth postoperative day showed that CFU/

Table 3 Intraperitoneal cytokines level in both groups

Variables	No anastomotic leakage	With anastomotic leakage	*P* value	95% confidence interval of the difference	
				Lower	Upper
IL6					
First day	35482.50±9455.35 (22,100–50,700)	52482.50±14364.42 (32,100–75,900)	0.0001	−24828.9	−9171.06
Third day	28159.17±4839.05 (21,000–35,200)	115,450±34974.81 (35,200–145,400)	0.0001	−97532.9	−77048.9
Fifth day	22219.58±1825.13 (20,100–25,900)	148,125±50753.85 (23,600–175500.00)	0.0001	−139,957	−111,854
IL10					
First day	23,800±9687.80 (11,300–50,700)	41982.50±10974.88 (21,700–55,900)	0.0001	−25734.7	−10630.3
Third day	22209.17±6079.59 (11,200–35,200)	33,355±36141.13 (15,200–122,500)	0.04	−22013.7	−277.9923
Fifth day	19708.33±3919.36 (11,200–25,900)	77842.50±62181.47 (19,700–151,400)	0.0001	−75502.0	−40766.3
TNF					
First day	161.94±16.10 (142–200)	169.65±8.63 (154–182)	0.19	−19.4527	4.3060
Third day	141.31±7.39 (130+155)	511.25±54.61 (427–555)	0.0001	−385.8895	−353.9855
Fifth day	78.10±19.88 (47+121)	824.50±113.94 (617–945)	0.0001	−780.8636	−711.9281

Table 4 Intraperitoneal bacteriological study in both groups

Variables	No anastomotic leakage	With anastomotic leakage	*P* value
E. coli			
First day	2 (4.2%)	3 (37.5%)	0.002
Third day	5 (10.4%)	6 (75%)	0.0001
Fifth day	3 (6.3%)	8 (100%)	0.0001
Bacteroides			
First day	2 (4.2%)	3 (37.5%)	0.002
Third day	2 (4.2%)	5 (62.5%)	0.0001
Fifth day	2 (4.2%)	6 (75%)	0.0001
Pseudomonas			
First day	3 (6.3%)	3 (37.5%)	0.009
Third day	3 (6.3%)	3 (37.5%)	0.009
Fifth day	2 (4.2%)	4 (50%)	0.0001
Klebsiella			
First day	4 (8.3%)	2 (25%)	0.16
Third day	4 (8.3%)	3 (37.5%)	0.02
Fifth day	5 (10.4%)	4 (50%)	0.005

milliliters was $\geq 10^6$ for *E. coli*, *Pseudomonas* species, and bacteriod micro-organism.

In the patient who died of sepsis, in the fifth postoperative day, secondary to anastomotic leakage, we found that CFU/ml was $\geq 10^6$ for *E. coli*, *Klebsiella*, *Pseudomonas* species, and bacteriod micro-organism in all samples except the sample which taken after abdominal closure where CFU/ml was $\leq 10^5$ for *E. coli* with no detected other micro-organism

In patients without AL, 41 patients showed no growth all over the duration of the study, and seven patients showed CFU/ml$\leq 10_5$ for the *E. coli*, *Klebsiella*, *Pseudomonas* species, and bacteriod micro-organism.

Discussion

Anastomotic leak following colorectal surgery is a significant complication that can result in severe sepsis, is a requirement for further surgery and a stoma, and is associated with prolonged hospital stay, considerable cost, multiple morbidities, and poor functional results.[24–26] The mortality rate associated with symptomatic leaks is 6% to 22%. The highest mortality rate was reported by the West of Scotland and Highland Anastomosis Study Group.[27] Also, Anastomotic leak has been associated with a higher local recurrence rate after curative treatment of colorectal malignancies.[28,29]

The most important risk factor for leakage is height of anastomosis form the anal verge; the lower the anastomosis, particularly below 6 cm, the higher the risk.[11,30] Other risk factors that have been attributed to anastomotic leakage are patient-specific risk factors, such as chronic obstructive pulmonary disease, ischemic heart disease, and diabetes mellitus; ASA score≥3; systemic hypertension; tobacco and alcohol use; prolonged use of high-dose steroids; under-nutrition; obesity; and male sex[2,11,31], and operative risk factors as poor colonic preparation, presence of peritonitis, adverse effect during operation, intraoperative blood loss/transfusion, anastomotic ischemia or tension, presacral hematoma, or fluid collection with subsequent infection and pelvic drainage.[1,11,30–33] In addition, cancer itself has been reported as a risk factor of anastomotic leakage.[5,23] Nevertheless, anastomotic leakage also occurs in patients with no obvious risk factors.[9,34]

The role of a temporary diverting stoma has been a matter of controversy, whether it could reduce the incidence of anastomotic leakage or not. Some studies have demonstrated a reduction in leakage rates in patients with covering stoma;[5,35] however, others showed no clear benefit of using a diverting stoma.[3,10] Moreover, a protective stoma is also associated with increased hospital stay and cost. Also, stoma reversal can cause morbidity and even mortality.[1,3,10] The role of mechanical bowel preparation and prophylactic antibiotic therapy in preventing AL is unclear, despite some studies that describe a low incidence of AL.[14]

The early detection of these complications within the first postoperative day by clinical examination is difficult, and the exact laboratory biomarkers of early prediction of these complications are unknown. Dulk et al.[13] and Peel AL[36] described the clinical signs of AL which included fever, increased leukocyte count, and increased C-reactive protein (CRP) level Furthermore; Systemic Inflammatory Response Syndrome (SIRS) was indicated to be a sign of AL. The following signs could also occur with SIRS: changed mental status, oliguria, increased levels of serum

creatinine, and ileus. But, neither clinical signs and symptoms nor systemic analysis of parameters such as CRP and leukocytosis are specific for anastomotic leakage diagnosis.[37,38]

Patients with an anastomotic intramucosal pH<7.28 in the first 24 h postoperatively have 22 times more risk of anastomotic leak. Therefore, by measuring intramucosal pH using Tonometry in the early postoperative period, the risk of anastomotic leak can be more accurately predicted.[30] Also, oxygen-tension measurements can be an additional diagnostic tool that can support the early prediction of AL in colorectal surgery. So, adequate tissue oxygenation pre- and postoperatively (continuously for 7 days) showed clear benefit towards prevention of anastomotic leakage. Reduced blood flow induces a switch from aerobic to anaerobic metabolism; the level of lactate will rise and pyruvate will decrease, resulting in an increased lactate/pyruvate ratio and decreased glucose levels which may be early signs of symptomatic anastomotic leakage before clinical symptoms are evident.[9]

A number of recent studies have investigated the cytokine response within the peritoneal cavity after abdominal surgery.[14,15] Polymorphonuclear leukocytes, macrophages, and peritoneal mesothelial cells are all probably production sites for this local cytokine as a part of the peritoneal response to surgical and infectious injury.[39,40]

Some studies have shown increased levels of the peritoneal cytokines including TNF-α, IL-1, IL-6, and IL-10 in patients with postoperative complications, and its concentrations reflect the severity of stress caused by abdominal operations[14], whereas others have failed to demonstrate this.[9] More specifically, peritoneal IL-6, IL-10, and TNF-α levels were significantly higher in patients with AL compared with patients without AL.[14,15]

Burak Ugrasx et al. [14] investigated the early prediction of peritoneal IL-6, IL-10, and TNF-α levels in developing AL after colorectal surgery and found that peritoneal cytokine response was suitable for the early identification of AL. Peritoneal cytokine levels in the patients with AL were significantly higher than in the patients without AL. Postoperatively, peritoneal cytokine levels in patients without AL were decreased; however, peritoneal cytokine levels in patients with AL were increased.

In our study, peritoneal IL-6, IL-10, and TNF-α levels were significantly higher in patients with AL than in patients without AL. On the first postoperative day, cytokine level indicated severe local inflammation, which occurred days before the clinical signs of AL.

It has been shown that during the first postoperative day, the peritoneal concentrations of cytokines reflect the severity of stress caused by abdominal operations. It is suggested that decreasing peritoneal cytokine levels occurred in a normal postoperative course, whereas increasing levels indicate an abnormal postoperative course. The overexpression of peritoneal cytokines, as a local inflammatory response, in response to microbial invasion might be a very early event in the development of AL.[14,17,41–43]

Ruiter et al. [44] reported that the composition of the microbial flora present in the abdominal fluid of patients critically ill with abdominal sepsis varies depending on location of the perforation. In lower gastrointestinal perforation, the most frequently isolated aerobic organisms were *E. coli*, *Klebsiella*, and *Pseudomonas* species. The predominant anaerobes were *Bacteroides*.[22,44]

The first stage of microbial infection is colonization which is defined as the presence of a micro-organism in an internal organ that is normally sterile; failure to clear colonizing micro-organisms invariably leads to high concentrations of potentially pathogenic micro-organisms (PPMs).[45]

Infection is a microbiologically proven clinical diagnosis of inflammation, local and/or generalized. This includes not only clinical signs, but also the presence micro-organisms of $\geq 10^5$ colony forming units (CFU)/milliliters in diagnostic samples obtained from an internal organ or the isolation of a micro-organism from peritoneal fluid.[35,44–46]

Secretions from internal organs of healthy individuals are normally sterile. The main mechanism by which micro-organisms cause endogenous colonization/infection is migration which is the movement of live PPMs from one place, e.g., gut where they are present in overgrowth, to other sites, in particular, normally sterile internal organs.[47–49]

The vast majority of postoperative infectious complications after colorectal surgery are caused by colonic flora. The predominant bacteria are the anaerobic *Bacteroides* accompanied by a smaller amount of aerobic coliforms.[22,50]

The organisms that predominate in peritonitis are the endotoxin-generating facultative anaerobes such as *E. coli* and the obligate anaerobes such as *Bacteroides fragilis* which are involved in the later phases of the infection while *E. coli* is responsible for the acute peritonitis phase of infection.[35,44–46]

The bacterial flora in the human colon is normally a stable ecologic environment. After perforation or spillage of the colon, more than 400 different species of bacteria will contaminate the peritoneal cavity.[44,51] But, of the vast number of species of bacteria that invade the peritoneum, only few will survive outside their native intraluminal environment. If an infection results, it will be polymicrobial in nature.[22]

It has long been thought that cleaning the bowel preoperatively reduced the bacterial load. However, while reducing fecal mass, pre-operative bowel preparation does not alter the concentration of fecal organisms intraluminally.[52] Previous studies have shown that a vigorous 72-h mechanical

cleansing regimen only produced a significant reduction in coliforms, while the residual colonic microflora remained unchanged.[52–54] In unprepared colon, besides the possibility of wound infections, the bacteria can also lead to local infection of the anastomosis, causing leakage.[55,56]

In our study, we found higher frequency of *E. coli*, *Klebsiella*, *Pseudomonas* species, and *Bacteroides* in patients with anastomotic leakage after low anterior resection. The more types of bacteria isolated from the patients, the higher the postoperative morbidity.

We suggest that the estimation of the peritoneal cytokine levels might be an additional diagnostic tool that can support the decision making of surgeons for early detection of anastomotic leak in colorectal surgery. Our data will need confirmation to define precise cut-off values for the identification the patients with ongoing anastomotic leak after low anterior resection.

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ORIGINAL ARTICLE

Endoscopic Intraoperative Anastomotic Testing May Avoid Early Gastrointestinal Anastomotic Complications. A Prospective Study

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Abstract
Background Gastrointestinal anastomotic complications represent serious events; methods to evaluate anastomotic integrity seem to be suboptimal. Since endoscopic intraoperative anastomotic testing allows direct visualization of anastomosis, complication rates may be theoretically reduced by the use of this technique.
Methods A prospective study involving 118 consecutive oncologic patients undergoing endoscopically tested gastrointestinal stapled anastomoses was carried out. As controls, 148 historical patients without anastomotic testing were used for comparisons.
Results In the study group, anastomotic testing revealed 16 defects: 11 (9.3%) air leaks and five (4.3%) bleeding anastomoses. All leaks were oversewn and secured. Bleeding anastomoses were managed under direct visualization, and one non-patent anastomosis was redone. Forty-one (15.4%) postoperative anastomotic complications were observed: eight (3%) bleeding anastomoses, seven (2.6%) stenoses, and 26 (9.8%) clinical leaks. No early dehiscence or bleeding occurred if anastomoses were intraoperatively checked, while these complications were significantly more frequent in non-checked anastomoses (6.1% and 5.4%, respectively). Conversely, late leak and stenosis rates were similar between the two groups.
Conclusion Endoscopic anastomotic testing was a safe and reliable method to assess integrity of gastrointestinal anastomoses, to correct any defect under direct visualization, and to avoid early complications. However, this method seemed inadequate to predict late anastomotic complications.

Keywords Gastrointestinal anastomosis · Intraoperative endoscopy · Postoperative complications · Early and late dehiscences

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Introduction

Anastomotic complications still represent a serious postoperative event in patients undergoing gastrointestinal anastomosis.[1] In spite of recent advances in surgical techniques (such as stapling devices), anastomotic leakage, bleeding and stenosis occur with low but not negligible rates leading to significant morbidity and sometimes even to death.[2,3] Moreover, anastomotic leakage has recently been confirmed to be associated with a significant greater risk of poor outcome in oncologic patients.[4] Therefore, the availability of a reliable intraoperative anastomotic testing (IOAT) technique to evaluate the integrity of anastomosis and predict its outcome would be of outmost importance.[3,5] Lower colonic anastomoses have been traditionally checked by means of a rectal probe filled with air [6–8] or saline [9–11] or, more recently, by performing a methylene blue enema,[12]

with satisfactory results. However, even if upper gastrointestinal anastomoses could be tested as well by means of a nasogastric tube, these methods are suboptimal for predicting anastomotic complications because they do not allow direct visualization, thus potentially producing false negative results.[13] However in contrast, evaluation of anastomotic integrity by means of endoscopy (endoscopic IOAT) would have substantial advantages because of a complete visualization of the anastomotic line, adequate insufflation for air leak test, evaluation of patency, and, finally, management of any defect under direct inspection.[2,3] The first encouraging experiences with a sigmoidoscope or a flexible colonoscope, as well as more recent studies, supported evidence that endoscopic IOAT could be very useful to reduce leakage and bleeding rates.[2,3,14,15] Despite these premises, there is little, if any, literature addressing the role of endoscopic IOAT in colonic anastomoses or upper gastrointestinal anastomoses, respectively. In addition, some authors questioned this technique arguing that endoscopic IOAT could either weaken or damage the anastomosis, or provide a false sense of anastomotic integrity leading to a dangerous postoperative delay in patients with clinical leakage.[16–18] Finally, in a recent large series of rectal cancer patients, endoscopic IOAT was shown to be unable to reduce the rate of postoperative anastomotic leaks.[19] Therefore, evidence supporting wide use of endoscopic IOAT in gastrointestinal surgery was inconclusive.[3,20]

To evaluate the impact of this technique on the incidence of postoperative gastrointestinal anastomotic complications, we carried out a prospective, controlled, nonrandomized cohort study. Our study end points were: (1) the reliability of the method in the intraoperative detection of anastomotic integrity; (2) the incidence of postoperative anastomotic complications after endoscopic IOAT; and (3) the safety of the procedure (i.e., endoscopic IOAT-related complications).

Materials and Methods

Patients

Patients undergoing a gastric or colonic anastomosis were initially eligible for this prospective study. To produce comparable data with homogenous groups and to avoid interfering factors, patients undergoing radiation or chemotherapy were excluded from the study, and only oncologic patients who underwent elective surgery with stapled anastomosis were enrolled. Thus, gastroesophageal junction and distal gastric cancers, requiring either neo-chemotherapy–radiotherapy (RT/CT) with distal esophagectomy or subtotal gastrectomy with hand-sewn gastrojejunostomy, respectively, were excluded. Right colon and rectal cancers were considered ineligible because, at our institution, the former undergo hand-sewn ileocolic anastomosis, and the latter are treated with neo-RT/CT, if appropriate, with colorectal anastomosis protected by a defunctioning stoma.[21,22] Ultimately, only gastric cancer patients undergoing total gastrectomy with stapled esophagojejunostomy as well as left colon cancer patients undergoing stapled colorectal anastomosis were included in this study.

At our institution, no intraoperative anastomotic testing was used until December 2005. Anastomotic integrity was judged through inspection of the doughnuts and according to the surgeon's own appraisal. From January 2006 to July 2009, in a prospective consecutive fashion, endoscopic IOAT was performed in 118 histology proven adenocarcinomas of the stomach (n=62) and left colon (n=56) undergoing gastrointestinal anastomosis (group 1 or tested group). From January 2001 to December 2005, 148 gastric (n=80) and colon (n=68) cancer patients undergoing successful gastrointestinal anastomosis without technical failure, in whom no anastomotic testing had been performed, constituted the control group (group 2 or non-tested group).

The following parameters were recorded in all patients: age, sex, performance status according to ECOG scale, body mass index [BMI, calculated as weight/height^2 (Kg/m^2)], presence or absence of diabetes and other comorbidities, and degree of histological differentiation (well, moderate, or poor). Since TNM staging system for gastric and colon cancers is quite different thus precluding any comparison between these tumors, all cancers were arbitrarily divided into the following stages: stage I: T_1T_2 N_0, stage II: T_3T_4 N_0, and stage III: any T N+ tumors (metastatic gastric and colon cancers were excluded from the study). All patients gave their informed consent and the study was approved by the ethics committee at the Department of Clinical and Experimental Medicine and Surgery of the Second University of Naples.

Intraoperative Procedures

All patients underwent conventional open surgery. After total gastrectomy and closure of the duodenal stump with a linear stapler (GIA, Covidien Autosuture, Norwalk, CT, USA), a long Roux-en-Y jejunal limb was transposed in a transmesocolic fashion, and an end-to-side esophagojejunostomy was created with a 25-mm circular stapler (premium plus CEEA, Covidien Autosuture, Norwalk, CT, USA). The jejunal stump was closed with a linear stapler (GIA, Covidien Autosuture, Norwalk, CT, USA), and a hand-sewn jejunojejunostomy was performed. In colon cancer patients, after left colectomy, a side-to-end colorectal

anastomosis with a 31-mm circular stapler (premium plus CEEA, Covidien Autosuture, Norwalk, CT, USA) was performed. The proximal colonic stump was closed with a linear stapler (GIA, Covidien Autosuture, Norwalk, CT, USA). In all cases, proximal and distal doughnut integrity was carefully checked.

In group 1, a flexible endoscope was used to inspect the circular staple line for any defect, bleeding, gross tumor, and patency. Afterwards, the endoscope was slightly withdrawn and an intestinal clamp was positioned downstream of the anastomosis. Then, the operative field was filled with saline, keeping the anastomosis underwater, and air insufflation was performed checking for any air bubbles (air leak test). Air leaking, bleeding, and non-patent anastomoses were managed as appropriate, and always endoscopically re-checked.

Postoperative Outcome

A clinical anastomotic dehiscence was defined as the presence of signs of peritonitis or abdominal sepsis with or without evidence of luminal content and/or gas through the drain, and demonstrable anastomotic breakdown by endoscopic and/or radiologic examination.[13,23] Postoperative clinical dehiscences were distinguished in early and late leaks according to the time of appearance and severity (see below).[1] Postoperative staple line bleeding was considered in patients vomiting blood and/or with blood-stained stool and/or with progressive anemia requiring conservative or surgical treatment. All patients entered a follow-up program for oncologic disease with added adjuvant chemotherapy as appropriate. In this period, occurrence of anastomotic stenosis requiring treatment was recorded.

Statistical Analysis

Statistical analysis was carried out using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). In all analyses, the significance level was specified as $p<0.05$. The equality of group means and comparisons between proportions were analyzed using unpaired Student's t test and chi-square test, respectively. Stepwise multiple regression was used to analyze correlations between preoperative factors and postoperative anastomotic complications.

Results

Group Comparisons

The tested group and the non-tested group matched well (Table 1). Particularly, no factor potentially capable of influencing anastomotic performance, such as BMI, diabetes, and tumor site, was significantly different between the two groups. Subgroup analysis, according to the tumor site, confirmed that no substantial differences were present between patients with tested and untested gastrointestinal anastomoses (Table 2). BMI value was significantly lower in gastric than in colon cancer patients in both groups (22±2 and 25±2, respectively; $p=0.002$).

Intraoperative Findings

In all patients, the anastomotic procedures progressed regularly. In group 2, proximal and distal doughnuts were always shown to be complete, and the surgical team was sure of the integrity of the anastomosis. In addition, in group 1, intraoperative endoscopy was successfully performed in all cases without any trouble. One hundred and two (86.4%) anastomoses had no defect. However in contrast, 16 (13.6%) anastomoses displayed pathological findings: 11 (9.3%) positive air leak tests and five (4.3%) staple line bleeding. In one of the five bleeding cases, the colorectal anastomosis was shown to have a mucosal diaphragm due to incomplete cutting of the mucosa; its patency was unclear. Anastomotic failures were equally divided between gastric and colon cancers (12.9% and 14.3%, respectively; $p=0.844$; Fig. 1). All 11 positive air leak test anastomoses were successfully oversewn with interrupted sutures, with negative air leak tests on repeat endoscopy. All but one of five bleeding anastomoses were managed under direct endoscopic visualization. In two cases, hemostasis was obtained by prolonged anastomotic compression; in two other cases, manual compression was unsuccessful and hemostasis was achieved by means of additional sutures. The remaining case of bleeding was shown to be associated with an anastomosis of uncertain patency; for this reason, the anastomosis was taken down and redone.

Postoperative Outcome

Overall, 41 (15.4%) anastomotic complications were observed: 26 (9.8%) clinical dehiscences, eight (3%) bleeding, and seven (2.6%) stenoses. Nine leaks, occurring in the first four postoperative days (mean 2.6±1, range 1–4 days) and associated with severe clinical signs of abdominal sepsis, were classified as early leaks. The remaining 17 anastomotic dehiscences, associated with more subtle signs of infection and occurring from the seventh to the 11th postoperative day (mean 9.3±1), were considered as late leaks. No significant correlations between anastomotic failure and age, sex, performance status, BMI, diabetes, comorbidities, histological differentiation, and tumor stage were shown by multiple regression

Table 1 Characteristics of the series and comparisons between the tested group and the control group

	All patients $n=266$	Group 1 $n=118$	Group 2 $n=148$	p value
Age	57±15 (32–85)	57±13 (36–82)	56±15 (32–85)	0.773
Sex m/f	165/101	73/45	92/56	0.938
Performance status				
0	70	31	39	0.998
1	115	51	64	
2	81	36	45	
BMI	23±3 (18–31)	23±3 (18–31)	23±3 (18–30)	0.146
Diabetes no/yes	207/59	90/28	117/31	0.693
Comorbidities no/yes	176/90	73/45	103/45	0.233
Tumor				
Stomach	142	62	80	0.903
Colon	124	56	68	
Grading				
Well	12	5	7	0.970
Moderate	219	97	122	
Poor	35	16	19	
Stage				
I	45	20	25	0.549
II	97	39	58	
III	124	59	65	

Group 1 tested anastomoses, *Group 2* non-tested anastomoses (control group), *BMI* indicates body mass index

Comorbidities indicate presence of heart, and/or lung, and/or renal disease

Stage was classified as follows: stage I: T_1T_2 N_0, stage II: T_3T_4 N_0, and stage III: anyT N+ cancers

For age and body mass index, data are expressed as means±SD (range)

Table 2 Characteristics and comparisons between the tested group and the control group according to tumor location

	Gastric cancers			Colon cancers		
	Group 1 $n=62$	Group 2 $n=80$	p value	Group 1 $n=56$	Group 2 $n=68$	p value
Age	57±14 (36–80)	54±15 (32–84)	0.252	56±12 (37–82)	59±15 (33–85)	0.385
Sex m/f	35/27	46/34	0.964	38/18	46/22	0.866
Performance status						
0	13	17	0.999	18	22	0.999
1	31	40		20	24	
2	18	23		18	22	
BMI	23±2 (18–27)	22±3 (18–27)	0.124	26±3 (21–31)	25±2 (21–30)	0.138
Diabetes no/yes	48/14	67/13	0.461	42/14	50/18	0.984
Comorbidities no/yes	43/19	58/22	0.823	36/20	39/29	0.548
Grading						
Well	2	3	0.979	3	4	0.986
Moderate	51	66		46	56	
Poor	9	11		7	8	
Stage						
I	9	11	0.412	11	14	0.975
II	19	33		20	25	
III	34	36		25	29	

Group 1 tested anastomoses, *Group 2* non-tested anastomoses (control group)

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Stage was classified as follows: stage I: T_1T_2 N_0, stage II: T_3T_4 N_0, and stage III: anyT N+ cancers

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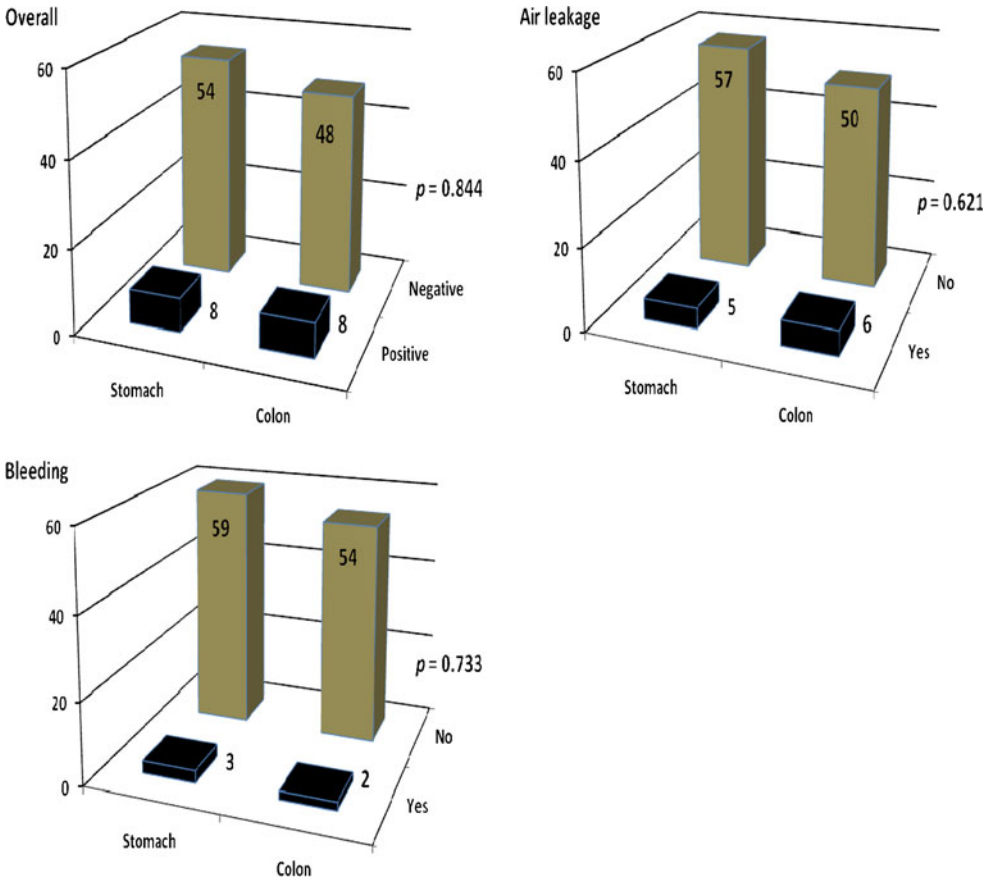

Fig. 1 Results of endoscopic IOAT in 62 gastric and 56 colon cancer patients undergoing gastrointestinal anastomosis. Total number of anastomotic failures, as well as anastomotic air leakage and bleeding rates were not significantly different between the two tumor sites

analysis. Complications were more frequent in gastric cancer patients, albeit without significant value (p=0.495).

In group 1, none of the patients had an early anastomotic dehiscence or bleeding. Five patients (4.2%) experienced a late anastomotic leak and three (2.5%) had a stenosis. Of note, in these eight patients, endoscopic IOAT failed to detect any defect. Conversely, in group 2, nine patients (6.1%) had an early anastomotic dehiscence, eight (5.4%) suffered from bleeding, and 12 (8.1%), and four (2.7%) had a late anastomotic leak and stenosis, respectively. Early postoperative anastomotic dehiscence and bleeding rates were significantly different between the two groups; however in contrast, the percentages of late complications were similar (Fig. 2).

The postoperative outcome according to tumor site is shown in Fig. 3. In gastric cancers, tested anastomoses showed three late leaks and one stenosis. However in contrast, non-tested anastomoses experienced six early leaks and as many bleeding, eight late dehiscences, and two stenosis. All patients but one with an early leak and sepsis underwent reoperation; three patients died (3.7%). The remaining patient with a low flow leak healed with antibiotics and total parenteral nutrition (TPN). Bleeding anastomoses were managed conservatively in four patients, while urgent endoscopic hemostasis was required in the remaining two patients. All patients recovered uneventfully. In both groups, late leaks were successfully managed by antibiotics and TPN; anastomotic stenoses required endoscopic dilations. Tested colonic anastomoses showed two late leaks and as many stenoses. Among patients with non-tested colonic anastomoses, three experienced early dehiscences, treated with immediate ileostomy (one death, 1.4%), and two suffered from bleeding requiring successful endoscopic hemostasis. In both groups, patients with a late leak underwent ileostomy (no death); anastomotic stenoses were successfully managed by endoscopic dilations. No endoscopic IOAT-related complications were recorded; particularly, prolonged postoperative ileus was not observed.

Discussion

Any gastrointestinal anastomotic failure may represent a life-threatening condition.[1,23] Anastomotic leakage, bleeding, and stenosis occur with low but still disappointing rates.[24,25] The cause of anastomotic defect is thought to be multifactorial, although an imperfect technique must play some part. Current evaluation methods not including direct visualization of the integrity of the anastomosis are considered to be suboptimal.[9,13] Therefore, a safe, reliable, and easily available method of intraoperative anastomotic testing is urgently needed.

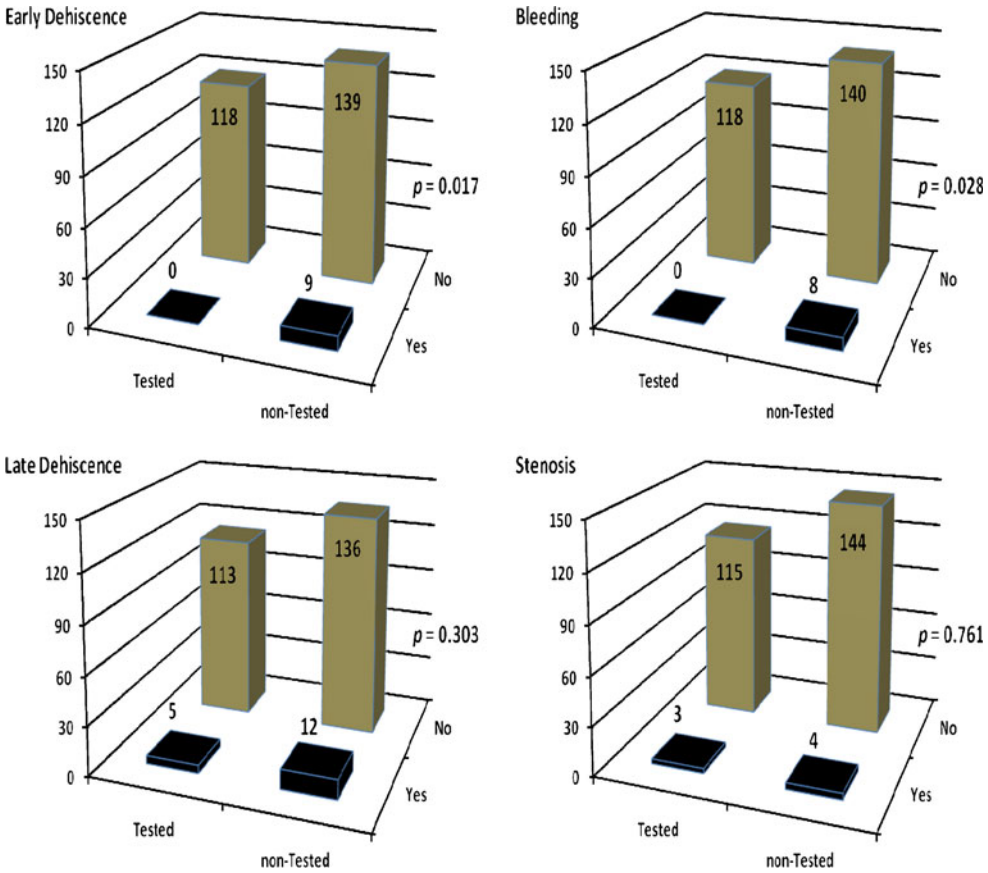

Fig. 2 Postoperative early and late anastomotic complications in 118 tested and 148 non-tested gastrointestinal anastomoses. Early anastomotic complications were significantly more frequent in non-tested anastomoses

In 2003, Schmidt et al. [19] reported that endoscopic IOAT was unable to reduce the postoperative leakage rate (10.4% vs. 11.1% in tested and untested anastomoses, respectively) in colorectal anastomoses. However in contrast, Ishihara et al.,[2] in their uncontrolled study on intraoperative colonoscopy for stapled colorectal anastomoses, concluded that the procedure could be safely performed and was useful for securing the anastomosis. These results were recently confirmed by two further studies.[3,13] Ultimately, this issue remains controversial and appears to be limited to colorectal anastomoses.

In this study, we performed endoscopic IOAT in 118 consecutive patients comparing intra and postoperative results with a control group of 148 patients who did not receive it. To maximize homogeneity and avoid bias, the study was designed with very strict entry criteria. As patients undergoing chemo-RT are known to have a higher risk of anastomotic leak they were excluded from the study.[26] Although hand-sewn and stapled anastomoses were not shown to have different postoperative leakage rates in colorectal anastomosis, little is known about upper gastrointestinal anastomoses; moreover, staple line bleeding was found to occur more often than bleeding after hand-sewn anastomosis.[3,13,27] Therefore, we chose to include only stapled anastomoses in our study. Together with a recent Japanese report, this is the first time that endoscopic IOAT has been investigated in esophagojejunal anastomoses.[28]

A major issue regarding analysis of postoperative outcome in studies similar to ours lies in the definition of what constitutes a clinical anastomotic leak and how outcome data are interpreted. There is no consensus definition of anastomotic leak, and many studies have used a broad definition based on clinical and radiographic features, leading to confusing results.[17,23] According to previous studies, leak was defined as a 'communication between the gastrointestinal lumen and the peritoneum at the anastomosis';[1,23] consequently, a clinical leak is an anastomotic dehiscence associated with clinical signs.[3] The intrinsic capacity of the body to isolate infection leads to the conventional belief that there is a substantial difference between early and late anastomotic leaks, the former being much more severe due to the frequent presence of diffuse peritonitis, and the latter showing vague symptoms of abdominal infection.[23,29] Surgeons are all too familiar with these different clinical aspects, and undoubtedly an early leaking gastrointestinal anastomosis may produce devastating consequences.[1,17] Thus, it is of paramount importance to make intraoperative airtight anastomoses to reduce or even avoid early leaks. Our results confirmed these assumptions. Tested anastomoses, including 11 oversewn

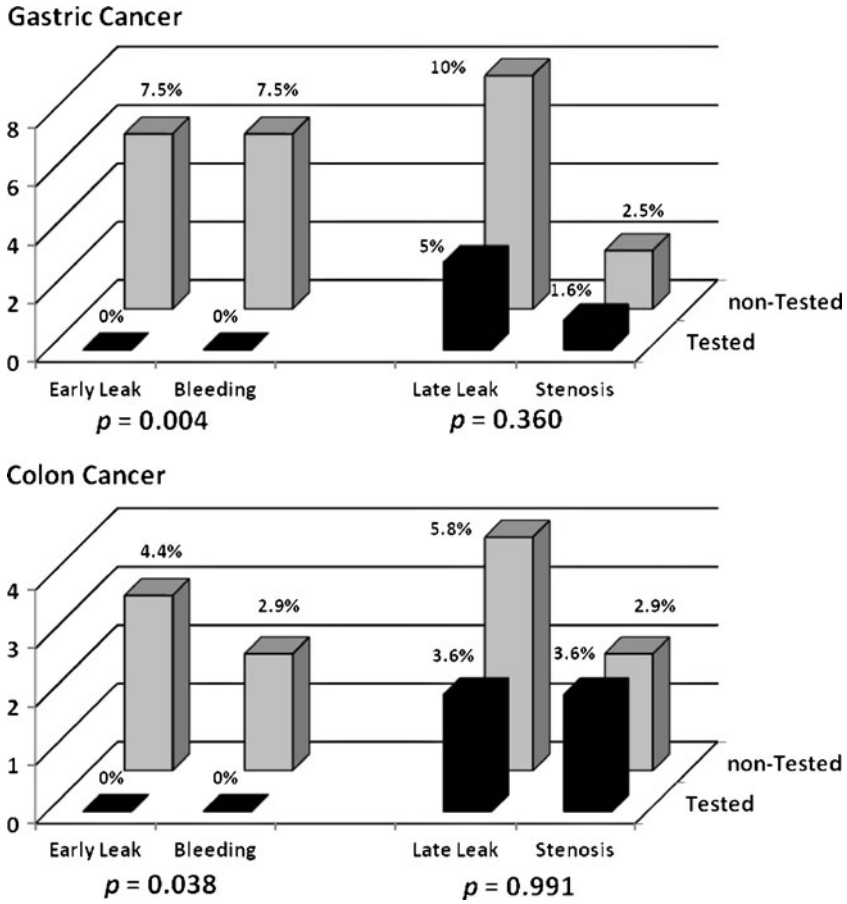

Fig. 3 Postoperative complications in tested and non-tested anastomoses according to the tumor site. Regardless of the tumor site, late anastomotic complications occurred with the same frequency; however in contrast, early complications showed significantly different rates between tested and non-tested anastomoses

because of positive air leak tests, were not associated with early dehiscence; conversely, patients with non-tested anastomoses experienced nine early dehiscences requiring in all cases but one urgent re-operation with four deaths. Thus, these data suggest that endoscopic IOAT was a reliable method to identify leaking anastomoses, and airtight anastomoses ensured early anastomotic integrity. Conversely, late leak rates were similar in tested and non-tested anastomoses, and had a successful outcome. This suggests, on one side, that early and late dehiscences have different pathogenetic mechanisms and clinical course, and, on the other hand, that an airtight anastomosis does not rule out the possibility of a late postoperative anastomotic disruption. This latter very important issue was previously stressed by other authors,[1,3,13] although postoperative early and late leaks were not clearly defined. Thus, this is the first study showing that endoscopic IOAT may prevent early but not late dehiscences.

No conclusive data are available on the best way to treat a positive air leak test anastomosis. Ricciardi et al. [3] found that postoperative clinical leak rates were 12.2% and 0% in oversewn anastomoses and either repeated anastomoses or proximal fecal diversion, respectively. Conversely, other authors did not report an increase in postoperative clinical leaks in oversewn anastomoses.[2,13,14] Our data are in agreement with these experiences; in our series, all leaking anastomoses were oversewn and no postoperative clinical leaks were experienced.

Bleeding following gastrointestinal anastomosis is infrequent but may have significant consequences,[24] and it seems to be more common following stapled anastomoses.[13,27] In our experience, endoscopic IOAT was unique because it allowed to pinpoint the bleeding point and to manage it under direct visualization. Conversely, postoperative staple line bleeding in non-tested anastomoses required medical treatment and, in three cases, urgent endoscopic hemostasis. Another great advantage of endoscopic IOAT was to identify a non-patent anastomosis that could be taken down and redone. Finally, late stenosis rates were identical in tested and non-tested anastomoses (2.5% vs. 2.7%, respectively), suggesting that endoscopic IOAT was inadequate to predict this complication.

In conclusion, endoscopic IOAT was a reliable method to assess the integrity of a gastrointestinal anastomosis, with the additional advantage of prompting correction of a possible defect under direct visualization and repeat assessment. Early postoperative complications were prevented and no patient in the tested group experienced a life-threatening course. However, endoscopic IOAT was shown to be a poor indicator of late complications, since even

flawless anastomoses were demonstrated to be associated with late leaks or stenoses. The procedure was not expensive, required no disposable instruments, took a very few minutes, and, above all, was safe.

However, it has to be emphasized that the current study showed some limitations due to comparisons to historical controls that could not lend credibility to its conclusions. A randomized controlled trial specifically addressing this issue should be warranted to lead the way toward providing evidence confirming these results.

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ORIGINAL ARTICLE

Chemotherapy, Liver Injury, and Postoperative Complications in Colorectal Liver Metastases

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Abstract
Background Systemic chemotherapy (CTx) is increasingly used before surgery for colorectal liver metastases (CRC-LM). However, CTx may cause liver injury like steatosis, steatohepatitis, and sinusoidal injury which may be associated with postoperative morbidity. Some recent data have even shown an increased mortality in patients with CTx-associated steatohepatitis. We, therefore, analyzed our recent experience with potential hepatic injury and its association with CTx and morbidity in patients undergoing surgery for CRC-LM.
Methods From 2001 to 2007, 179 patients underwent primary liver resection for CRC-LM. Sufficient non-tumorous liver parenchyma could be re-evaluated for this study in 102 patients. In these 102 patients (66% male, median age 62 years, median BMI 26, 8% diabetics (IDDM)), liver injury was classified using established criteria for steatosis and sinusoidal dilatation (SD) and then compared with preoperative CTx and postoperative outcome. Fifty-eight percent of the operations were (extended) hemihepatectomies (ExtRes), 42% segmental or wedge resections (LimRes). Before resection, 66% had received CTx (33% FU-based (FU), 19% oxaliplatin-based (Oxa), 12% irinotecan-based (Iri), and 3% Oxa+Iri). The interval between CTx and surgery was always ≥4 weeks.
Results Mortality was 3/102 (2.9%). Any complication occurred in 48%, hepatic insufficiency in 5.9%, and liver-related complications in 24%. Hepatic steatosis >20% was found in 37% (half of them with steatosis >50%). BMI correlated with the frequency of steatosis. Steatosis >20% was more frequent in patients with preoperative chemotherapy but did not depend on the chemotherapy regimen. No relevant risk factor for grades 2 and 3 SD was found. The specific use of Oxa or Iri did not significantly correlate with hepatic injury. Neither a CTx per se nor the different CTx regimens nor the extent of hepatic injury showed any negative influence on mortality, complication rates, or hepatic insufficiency. Patients with IDDM had a higher mortality (25% vs 1% without IDDM; $p<0.02$), increased complication rate (75% vs 46%; $p=0.11$), a higher rate of hepatic insufficiency (25% vs 4%; $p<0.02$), and more liver related complications (50% vs 21%; $p=0.06$). Patients undergoing ExtRes had a higher overall ($p<0.01$) and liver-related ($p=0.05$) complication rate compared to LimRes. None of the 34 patients with preoperative Oxa or Iri died or developed hepatic insufficiency.
Conclusions In our experience, hepatic injury (steatosis) was influenced by BMI and by preoperative CTx. Neither preoperative CTx nor liver injury increased perioperative morbidity. Patients with IDDM were at a rather high perioperative risk.

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Keywords Colorectal cancer · Liver metastases · Liver resection · Morbidity · Chemotherapy induced liver injury

Introduction

Hepatic resection is currently the best therapeutical option for cure or relevant prolongation of life in patients with liver metastases (CRC-LM) arising from colorectal cancer (CRC). Long-term outcome after resection of CRC-LM is steadily improving since the first larger series from the 1990s to survival probabilities of up to 58% after 5 years in recent reports from specialized centers.[1–5] More than a decade ago, many patients with node positive CRC and/or with metastatic disease were treated with 5-fluorouracil-based chemotherapeutic regimens. With the invention of newer chemotherapeutical agents like oxaliplatin or irinotecan, outcomes were improved in adjuvant[6] and metastasized (non-curative) situations.[7] In addition, it has been demonstrated since the mid-1990s that neoadjuvant chemotherapy with newer agents may provide higher response rates, rendering a subgroup of those patients candidates for (curative) liver resection.[8–11] Beyond that, a recent multicenter trial has even demonstrated a positive effect on progression-free survival after perioperative oxaliplatin-based chemotherapy (FOLFOX 4) in primarily resectable CRC-LM.[12]

The increased use of (preoperative) chemotherapy for colorectal liver metastases has led to a growing awareness of potential hepatotoxicity by some of these agents during the last years. The development of different types of histological liver damage like steatosis, sinusoidal injury (SI, "blue liver syndrome"), or steatohepatitis was attributed to the use of oxaliplatin (SI), irinotecan (steatosis) or even 5-fluorouracil.[13–16] In several surgical or surgical–pathological studies,[14,17–19] chemotherapy-induced liver damage was associated with a higher postoperative morbidity[14,17–19] or even mortality[14,20] whereas others could not demonstrate a correlation between chemotherapy (and chemotherapy-induced liver damage) and postoperative complications.[15]

The purpose of our study was to evaluate the effect of preoperative chemotherapy on liver histology in 102 patients undergoing their first liver resection for colorectal liver metastases. We further correlated the use of chemotherapy and the different types of liver damage with postoperative morbidity.

Patients and Methods

From 2001 to May 2007, 179 patients underwent primary resection of CRC-LM at the Department of Surgery of the University of Freiburg. One hundred two of those patients with sufficient information on preoperative chemotherapy, chemotherapy without the antibodies bevacizumab or cetuximab, and with adequate amounts of non-tumorous liver parenchyma in the original histological specimens could be included in our evaluations. The demographic data and tumor characteristics are given in Table 1.

Chemotherapy Before Liver Resection

Of the 102 patients in our study, 34 (33%) had never received any chemotherapy before liver resection. Thirty-four patients (33%) had received FU-based, 19 (19%) oxaliplatin-based, and 12 (12%) irinotecan-based chemotherapy. Three patients (3%) had preoperatively received oxaliplatin plus irinotecan (FOLFOXIRI). Thirty-five patients (34%) received chemotherapy more than 6 months, and 33 patients (32%) less than 6 months before liver resection. In part, chemotherapy was given as adjuvant therapy after resection of the primary CRC. Since chemotherapy was partially documented retrospectively and, in most instances, administered by oncologists outside of our university hospital, we could not always completely differentiate between adjuvant, initially palliative, or true neoadjuvant chemotherapy. However, the time interval between the last cycle of preoperative chemotherapy and surgery was well documented and enabled us to perform comparisons between groups receiving chemotherapy more or less than 6 months before liver resection. It is of note that in 74% of the 34 patients receiving oxaliplatin and/or irinotecan-based

Table 1 Demographic and oncological data of 102 patients undergoing liver resection for colorectal metastases

Age in years (median, range)	61 (35–8)
Gender	
Female (*n*, %)	35 (34%)
Male (*n*, %)	67 (66%)
Body mass index (median, range)	25.7 (17–39)
Primary CRC (*n*, %)	
Colon	64 (63%)
Rectum	38 (37%)
Nodal status primary CRC (*n*, %)	
Positive	68 (67%)
Negative	27 (27%)
Unknown	7 (7%)
Size largest metastasis (mm, median, range)	35 (3–155)
No of liver metastases (median, range)	2 (1–11)
Type of liver resection	
Wedge	10 (10%)
Segmental[a]	33 (32%)
Left hemihepatectomy	7 (7%)
Extended left hemihepatectomy	13 (13%)
Right hemihepatectomy	24 (24%)
Extended right hemihepatectomy	15 (15%)
Negative resection margin (*n*, %)	93 (91%)

[a] One or two segments

regimens, chemotherapy was terminated within 6 months before resection. In patients receiving chemotherapy, the median number of cycles was six (range 1–17).

Definitions

Mortality is documented as hospital mortality in our hepatic surgery database. Since we have follow-up information of all surviving patients, we certainly did not miss any early mortality after discharge. Hepatic insufficieny was defined as the presence of total bilirubin >6 mg/dl and/or hepatic encephalopathy. A bilioma was defined as symptomatic perihepatic bile collection (with or without need for drainage). For further risk factor analysis, the term "liver-related complication" was defined as the presence of hepatic insufficiency, bilioma, and/or symptomatic ascites requiring interventional or medical treatment.

Histopathological Evaluation

The assessment of non-tumorous liver parenchyma was performed by reviewing the original H&E- and Sirius-stained slides of the hepatic resection specimens by a senior pathologist with expertise in liver pathology (A.z.H.) and a trained scientific research assistant (S. M.). As mentioned above, only resection specimens containing sufficient non-tumorous tissue were included in this study. Steatosis was graded by the percentage of involved hepatocytes as absent, <20% of hepatocytes (grade 1), 20–50% of hepatocytes (grade 2), or >50% of hepatocytes (grade 3). Sinusoidal dilatation (SD) was graded according to Rubbia-Brandt[13] as absent (grade 0), grade 1 (centrilobular involvement limited to one third of the lobular surface), grade 2 (centrilobular involvement extending into two thirds of the lobular surface), or grade 3 (complete lobular involvement). Lobular neutrophil infiltration was classified as grades 0, 1, or 2 as described before.[14,21] Since ballooning of hepatocytes, as reported by Kleiner et al.,[21] was not evaluated in this series, we used a modified score for the description of steatohepatitis (sum of steatosis grade 0–3 and lobular neutrophil infiltration grade 0–2; maximum possible sum 5). Steatohepatitis (SH) was definded or examined as potential risk factor for complications in our analyses as score 4–5 or score 5. Parameters further documented in the re-analysis of the histological specimens were the grade of fibrosis (absent, portal fibrosis without (grade 1), with few (grade 2), or with numerous septa (grade 3), and cirrhosis (grade 4)) and the grade of periportal neutrophil infiltration (Figs. 1 and 2).

Data Collection and Statistics

The patient-related perioperative results of our study were gained by retrospective analysis of our prospective hepatic database. The results of histopathological assessment of liver damage were added to this database. In our hepatic database, only the type of preoperative chemotherapy was documented until 2008. We retrospectively could add data specifying date and number of cycles of chemotherapy in many patients. Following exploratory data analysis comparisons between groups were performed by χ^2, Fisher's exact, or Mann–Whitney U test were applicable. Multivariate risk factor analysis was performed using binary logistic regression (likelihood ratio forward selection strategy). All data analyses were performed using SPSS™ (SPSS for Windows™, Version 15.0, SPSS Inc., Chicago IL, USA).

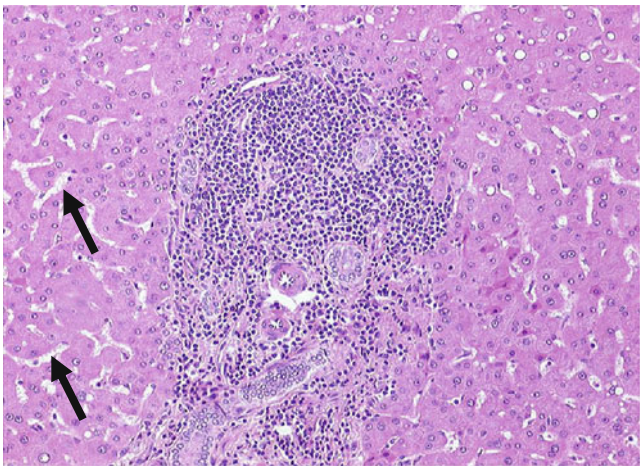

Fig. 1 H&E-stained non-tumorous liver parenchyma specimen showing sinusoidal dilatation (*arrows*) and intensive periportal lymphocytic infiltration (*center*)

Results

The groups of patients with (n=68) or without (n=34) preoperative chemotherapy showed no differences regarding median age (61.5 vs 63 years) and BMI (26 vs 25). In addition, gender (66% and 65% male) and the rate of major resections (55% vs 65%) were comparable in both groups.

Liver Injury

The overall frequencies and grades of histological injury in the non-tumorous liver parenchyma are shown in Table 2. A steatosis (any grade) was found in 88%, any sinusoidal dilatation in 81%, a steatohepatitis (score 4/5) in 23%. Seventeen percent of the livers presented with severe steatosis (> 50%), 15% had signs of severe (grade 3) sinusoidal dilatation and 8% had severe (score 5) steatohepatitis. Any grade of fibrosis was observed in 47% of the patients (Table 2). However, fibrosis grade 2 or higher was found in only 11%. One of the patients had

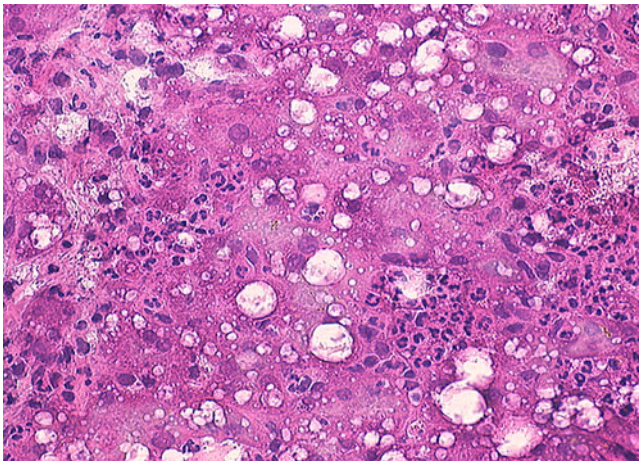

Fig. 2 Example of steatohepatitis with mixed-type (macro- and microvesicular) steatosis and several foci of neutrophil infiltration

grade 3 fibrosis, and another patient was found to have signs of cirrhosis in his non-tumorous liver parenchyma.

Risk Factors for Steatosis

Patients receiving preoperative chemotherapy had a significantly higher rate of steatosis >20% ($p<0.01$) but not (significantly) of severe steatosis (>50%; Table 3). Patients receiving at least six chemotherapy cycles had a higher risk of steatosis >20% but not of steatosis > 50%. The chemotherapeutic regimen, however, did not correlate with steatosis. Patients with a BMI>25 had an increased risk for steatosis >20% ($p<0.02$), whereas the small subgroup of patients with a BMI over 30 were at increased risk for severe steatosis ($p<0.03$). Further parameters like age, gender, or the presence of IDDM did not correlate with hepatic steatosis. After entering the significant univariate risk factors for steatosis > 20% into multivariate analysis, both chemotherapy ($p<0.01$) and BMI>25 ($p<0.03$) also were independent risk factors for steatosis.

Risk Factors for Sinusoidal Dilatation

Patients with a higher BMI had a lower rate of SD grades 2 or 3 (Table 3). Surprisingly, patients preoperatively receiving oxaliplatin-based chemotherapy had a low rate of grades 2 or 3 SI. The vast majority (73%) of patients with oxaliplatin-based chemotherapy, however, had sinusoidal dilatation grade 1 in histopathological evaluation.

Risk Factors for Steatohepatitis

As for steatosis, BMI and preoperative chemotherapy correlated with the presence of steatohepatitis (score 4 or 5 as defined in our study). In patients with a BMI>25, SH was observed in 30% whereas in patients with a BMI≤25, SH was found in only 13% ($p<0.04$). A BMI>30 ($n=13$) was even associated with SH in 46%. Patients with preoperative chemotherapy had an increased rate of SH (28% vs 12% in patients without chemotherapy; $p=0.065$). After entering both parameters (chemotherapy and classified BMI) into multivariate analysis "only" BMI ($p=0.05$) remained an independent prognostic factor for the presence of steatohepatitis.

Analysis of the most severe form of steatohepatitis (grade 5, $n=8$) revealed no significant risk factor, but this may be partially due to the low number. It is of note that neither chemotherapy per se nor the type of chemotherapy nor the number of chemotherapy cycles and the preoperative interval between end of chemotherapy and surgery showed any influence on the presence of grade 5 steatohepatitis, but patients with a BMI>30 had a slightly higher rate (15% vs. 7% in BMI <30).

Risk Factors for Fibrosis

By univariate analysis, BMI was a significant risk factor for relevant fibrosis: of 56 patients with a BMI>25, 11 (20%) had a fibrosis higher than grade 1 whereas none of the patients with a BMI below 25 had these higher grades of fibrosis ($p<0.01$). A preoperative chemotherapy with FU- (18%) or oxaliplatin-based (21%) regimens was associated with a fibrosis > grade 1 whereas none of the patients

Table 2 Liver injury in 102 patients with colorectal liver metastases

Type of liver injury	Number	Percent
Steatosis		
None	12	12%
<20%	53	52%
20–50%	20	20%
>50%	17	17%
Sinusoidal dilatation		
None	19	19%
Grade 1	43	42%
Grade 2	25	25%
Grade 3	15	15%
Steatohepatitis (score 0–5)		
None	5	5%
Score 1–3	74	73%
Score 4–5	23	23%
Score 5	8	8%
Fibrosis		
None	44	43%
Grade 1	47	46%
Grade 2	9	9%
Grade 3	1	1%
Cirrhosis	1	1%

Table 3 Risk factor analysis for liver injury

	Number	Steatosis > 20% n (%)	*p* Value	Steatosis > 50% n (%)	*p* Value	SD grade 2 or 3 n (%)	*p* Value
Chemotherapy							
Yes	68	46%	0.006	19%	0.35	34%	0.12
No	34	18%		12%		50%	
Chemotherapy type							
None	34	18%	0.07	12%	0.53	50%	0.026
FU-based	34	41%		15%		44%	
Oxaliplatin-based	19	53%		26%		11%	
Irinotecan-based	12	50%		25%		50%	
Irinotecan+Oxaliplatin	3	33%		0%		0%	
Chemotherapy							
None	34	18%	0.01	12%	0.63	50%	0.29
≥ 6 months	35	51%		20%		34%	
< 6 months	33	40%		18%		33%	
Chemotherapy cycles[a]							
0–5	64	28%	0.02	17%	0.95	39%	0.60
≥ 6	34	53%		18%		38%	
Diabetes							
Yes	8	38%	0.94	25%	0.51	38%	0.92
No	94	36%		16%		39%	
Gender							
Female	35	31%	0.46	14%	0.64	34%	0.46
Male	67	39%		18%		42%	
BMI 1							
≤ 25	46	24%	0.019	11%	0.15	50%	0.043
> 25	56	46%		21%		30%	
BMI 2							
≤ 30	88	35%	0.43	14%	0.024	43%	0.06
> 30	14	46%		39%		15%	
Age							
≤ 60 years	42	45%	0.12	19%	0.59	41%	0.83
> 60 years	60	30%		15%		38%	

[a] Data unavailable in four patients

receiving irinotecan and only one of 34 patients without chemotherapy showed fibrosis > grade 1.

Postoperative Morbidity and Mortality

Any postoperative complication occurred in 49 of the 102 patients (48%). A detailed summary of the complications is shown in Table 4. Preoperative chemotherapy (all regimens) did not lead to significantly increased overall or specific complication rates (Table 4). Liver-related complications (liver insufficiency, biliary leak/bilioma, symptomatic ascites) were documented in 24 patients (23.5%). A liver insufficiency as defined in our analysis occurred in six patients (5.9%).

Three of the 102 patients died, all after major resections (in-hospital mortality of 2.9%). The first patient not surviving liver resection had early bleeding after extended right hemihepatectomy, followed by liver failure, colonic perforation, and septic multiple organ failure. The second patient developed severe pneumonia and liver failure; the third patient had fatal (septic) liver failure plus non-occlusive mesenterial ischemia after an extended right hemihepatectomy. Only one of those three patients preoperatively had (FU-based) chemotherapy; two were diabetics. None of them had a BMI>30, severe hepatic steatosis, sinusoidal dilatation grade 3, steatohepatitis grade 4, or 5 or fibrosis higher than grade 1. The presence of IDDM was the only risk factor for mortality (25% in the eight patients with IDDM vs 1% in non-diabetics; $p<0.02$).

The results of univariate risk factor analyses for any complication or liver related complication are demonstrated in Table 5. Major resection (vs limited resection) was the only significant risk factor for overall complications. There was a tendency for more complications in patients with

Table 4 Postoperative complications after resection of colorectal liver metastases in the entire patient group and in the subgroups with or without preoperative chemotherapy

Complication	All patients (n=102)	Chemotherapy (n=68)	No chemotherapy (n=34)
Any complication	48%	50%	44%
Abdominal infection[a]	8%	7%	9%
Bleeding	1%	0%	3%
Wound infection[b]	19%	19%	18%
Pneumonia	7%	4%	12%
Urinary tract infection	6%	3%	12%
Other	20%	18%	24%
Liver related	24%	24%	24%
Hepatic insufficiency	6%	2%*	15%*
Bilioma	17%	18%	15%
Symptomatic ascites	9%	7%	12%
Hospital mortality	2,9%	2%	6%

Multiple complications per patient are possible. With the exception of hepatic insufficiency, there were no statistically significant differences between the subgroups

[a] Infected fluid collection, abscess or peritonitis

[b] Major and minor wound infections

*p<0.01

steatosis of at least 20% or steatohepatitis grade 5, but neither severe steatosis (> 50%) nor the extent of fibrosis showed any significant correlation with the overall complication rate. As for liver injury, neither a preoperative chemotherapy per se nor the number of chemotherapy cycles nor the regimen nor the preoperative time interval of chemotherapy showed any influence on complication rate.

Six patients (5.9%) developed liver insufficiency. Surprisingly, patients without preoperative chemotherapy (n=34) had a significantly higher risk of postoperative liver failure than the 68 patients with preoperative chemotherapy (15% vs. 1.5%; p<0.01). The single patient with chemotherapy developing liver insufficiency had received a 5-FU-based regimen and had IDDM. It is of note that none of the 34 patients with an oxaliplatin and/or irinotecan-based regimen (74% of those with chemotherapy terminated within 6 months of surgery) developed liver insufficiency. By univariate analysis, patients with diabetes also had a higher risk of liver insufficiency (25% vs. 4% in non-diabetics; p<0.02). Of the eight diabetic patients, seven had received chemotherapy before liver surgery. Liver insufficiency occurred in one of 43 patients (2%) after limited resection and in five of 59 patients (8.5%) after major resections (p=0.19). The different types of histological liver injury, BMI, and intraoperative blood transfusions did not show any influence on the occurrence of liver insufficiency. None of the 11 patients with fibrosis grade 2 or higher developed liver insufficiency.

As for the overall complication rate, the extent of resection and IDDM were the only parameters relevantly influencing the rate of liver-related complications (Table 5). In patients with major resections, liver-related complications occurred in 31% whereas limited resections were followed by those complications in only 14% (p=0.05). Patients with IDDM had a more than twofold higher rate of liver-related complications than non-diabetic patients (50% vs 21%, p=0.06). Again, neither preoperative chemotherapy nor liver injury nor BMI correlated with the occurrence of liver-related complications (Table 5). It is of note that only one of 14 patients with a BMI>30 postoperatively developed liver-related complications.

Risk Factor Analysis in 59 Major Hepatectomies

Since complications like liver insufficiency or other liver-related morbidities occur predominantly in major resections (i.e., less remaining functional liver volume after resection), we repeated the evaluation of potential risk factors for complications (any and liver related; liver insufficiency) in the subgroup of 59 patients undergoing major liver resection (hemihepatectomy or extended hemihepatectomy). Any liver-related complications occurred in this subgroup in 61% and 31%, respectively. The presence of IDDM was the only significant risk factor for both definitions of complications (Table 6). As already shown for the entire study group, neither chemotherapy nor liver injury significantly influenced these complication rates after major resection. However, the frequency of complications was higher in patients with grade 5 steatohepatitis, but the low number of those cases (and the low number of "events") anticipate potential statistical differences. It is of note, again, that none of the six patients with a BMI>30 developed liver-related complications. The few patients with fibrosis > grade 1 had a higher liver-related complication rate, but due to the small subgroup size, this showed no statistical influence (Table 6).

Discussion

Adjuvant chemotherapy is established since many years in the treatment of node positive colorectal cancer after curative resection. Originally based on fluorouracil and leucovorin, newer data suggest an even better outcome by adding oxaliplatin in the adjuvant setting.[6] In patients with

Table 5 Risk factor analysis of postoperative complications in 102 patients with liver resection

	Number	Any complication *n* (%)	*p* Value	Liver related n (%)	*p* Value
Steatosis ≥20%					
No	65	42%	0.08	22%	0.53
Yes	37	60%		27%	
Steatosis ≥50%					
No	85	46%	0.33	24%	1.0
Yes	17	59%		24%	
Sinusoidal dilatation					
Grade 0/1	62	47%	0.75	23%	0.78
Grade 2/3	40	50%		25%	
Steatohepatitis I					
Score 0–3	79	44%	0.16	24%	0.82
Score 4–5	23	61%		22%	
Steatohepatitis II					
SCORE 0–4	94	46%	0,.11	23%	0.26
SCORE 5	8	75%		38%	
Fibrosis					
Grade 0–1	91	47%	0.65	22%	0.29
Grade 2–4	11	55%		36%	
Chemotherapy					
Yes	68	50%	0.58	24%	1.0
No	34	44%		24%	
Chemotherapy type					
None	34	44%	0.66	24%	0.43
FU-based	34	53%		18%	
Oxaliplatin-based	19	37%		26%	
Irinotecan-based	12	58%		42%	
Irinotecan+Oxaliplatin	3	67%		0%	
Chemotherapy					
None	34	44%	0.66	24%	0.60
≥6 months	35	55%		29%	
<6 months	33	46%		18%	
Chemotherapy cycles [a]					
0–5	64	47%	0.83	27%	0.48
≥ 6	34	44%		22%	
Diabetes					
Yes	8	75%	0.11	50%	0.06
No	94	46%		21%	
Gender					
Female	35	49%	0.94	17%	0.27
Male	67	48%		27%	
BMI 1					
≤ 25	46	46%	0.66	24%	0.93
> 25	56	50%		23%	
BMI 2					
≤ 30	88	47%	0.65	26%	0.15
> 30	14	54%		8%	
Amount liver resected					
Limited	43	30%	0.002	14%	0.05
Major	59	61%		31%	
Age					
≤ 60 years	42	57%	0.12	17%	0.17
> 60 years	60	42%		28%	

Table 5 (continued)

	Number	Any complication *n* (%)	*p* Value	Liver related n (%)	*p* Value
Intraop. transfusion					
Yes	31	55%	0.36	23%	0.88
No	71	45%		24%	

[a] Data unavailable in four patients

colorectal liver metastases, chemotherapy regimens containing oxaliplatin or irinotecan are increasingly used for neoadjuvant treatment. During the last decade, several publications could demonstrate that about one fifth of patients with initially irresectable liver metastases may undergo curative resection after neoadjuvant chemotherapy, with acceptable long-term outcome.[8,10,11] In addition to these data, the recently published results of the EORTC Intergroup trial 40983 could show a benefit in progression-free survival in patients with initially resectable liver metastases after neoadjuvant FOLFOX4 chemotherapy.[12]

After the "invention" of neoadjuvant chemotherapy for colorectal liver metastases in the 1990s, published series from several centers reporting potential liver injury and its correlation to postoperative complications followed in the 2000s. However, most of these published reports differed in study design (inclusion criteria), definition of liver injury, and definition of complications. Some of the studies included all patients with available non-tumorous liver histology[14,15,20] or randomly assigned patients to evaluation[22] whereas others included only patients with preoperative chemotherapy.[11,18,23] In the study of Kooby et al.,[17] patients with any grade of steatosis were analyzed and compared to a matched group of patients with normal liver parenchyma.

For the purpose of our study, we initially defined and classified liver injury as presented by Karoui et al.[19] Cutoff values for steatosis were, therefore, 20% and 50% in our analyses whereas other groups used cutoff values of 30% and 60%. We also did not exactly use the definition of steatohepatitis as proposed by Kleiner et al.[21] which was used in several of the mentioned studies.[14,15,23] However, since steatosis and lobular neutrophil infiltration were well analyzed in our specimens, the definition and classifications of steatohepatitis applied in our analyses certainly do not differ relevantly from other reports.

In our study of 102 analyzed patients (two thirds preoperatively received chemotherapy), we could demonstrate a relationship between preoperative chemotherapy and steatosis in the non-tumorous liver parenchyma. However, we could not demonstrate a regimen-specific influence on the type of liver injury as reported in several other studies.[13–15] It is of note that in patients undergoing preoperative oxaliplatin-based chemotherapy, any grade of sinusoidal dilatation was observed in nearly all patients (as in the entire study group), but the proportion of advanced SD (grades 2 or 3) was even lower than in patients without or with other chemotherapy regimens. Steatohepatitis (grades 4 or 5 in our classification) was found in 23%, and 8% had the most severe form (grade 5). Since steatosis is a relevant part of (our) definition of steatohepatitis, it is not surprising that the same risk factors were found for steatosis and steatohepatitis.

Independent from preoperative chemotherapy, the BMI was the strongest predictor of liver injury in our study. Patients with a BMI higher than 25 were at increased risk of steatosis, steatohepatitis, and fibrosis. Patients with a BMI higher than 30 even had a risk of steatoheatitis grades 4 or 5 of almost 50%. In the two largest studies systematically analyzing chemotherapy-induced liver injury,[14,15] a significant correlation between chemotherapy regimen and type of liver damage was reported. In both studies, sinusoidal dilatation was associated with preoperative oxaliplatin-based chemotherapy. Pawlik et al.[15] also showed a significant correlation between irinotecan-based chemotherapy and steatosis/steatohepatitis whereas Vauthey et al.[14] found only a correlation of irinotecan with steatohepatitis (but not with steatosis). It is of note that the rates of patients with liver injury varied partially in these studies (steatohepatitis 1% and 8%) or were differently defined. In contrast to our results, Karoui et al.[19] found a correlation between preoperative chemotherapy (regimen not specifically analyzed) and sinusoidal injury but no influence of chemotherapy on the rate of hepatic steatosis. In a study including 90 patients with preoperative chemotherapy, Nakano et al. could demonstrate a higher rate of sinusoidal injury after oxaliplatin-based chemotherapy whereas steatosis was not influenced by the type of chemotherapy.[18] The BMI as a potential risk factor for liver injury was not evaluated in all of the mentioned studies. Kooby et al. demonstrated that BMI was a risk factor for steatosis in an analysis of almost 500 patients.[17] As in our evaluations, Pawlik et al.[15] could indentify BMI as an independent factor for steatosis in multivariate analysis. In addition, Brouquet et al. could even identify BMI as the sole independent predictive factor for the presence of liver steatosis or steatohepatitis.[23] In the large study by Vauthey et al., a possible direct association between BMI and steatosis was not evaluated. However, the authors stated that the association between irinotecan and steatohepatitis was significantly higher in patients with a BMI>25.[14] It is of note that, in a

Table 6 Risk factor analysis of postoperative complications in 59 patients with major liver resection

	Number	Any complication *n* (%)	*p* Value	Liver related *n* (%)	*p* Value
All	59	61%		31%	
Steatosis ≥ 20%					
No	39	56%	0.31	31%	0.95
Yes	20	70%		30%	
Steatosis ≥ 50%					
No	52	60%	0.55	31%	0.92
Yes	7	71%		29%	
Sinusoidal dilatation					
Grade 0/1	30	63%	0.71	27%	0.51
Grade 2/3	29	59%		35%	
Steatohepatitis I					
Score 0–3	46	59%	0.49	33%	0.51
Score 4 or 5	13	69%		23%	
Steatohepatitis II					
Score 0–4	56	59%	0.27	7%	0.23
Score 5	3	100%		33%	
Fibrosis					
Grade 0–1	55	60%	0.55	29%	0.38
Grade 2–4	4	75%		50%	
Chemotherapy					
Yes	37	62%	0.82	30%	0.87
No	22	59%		32%	
Chemotherapy type					
None	22	59%	0.96	32%	0.40
FU-based	16	63%		19%	
Oxaliplatin-based	7	71%		43%	
Irinotecan-based	11	55%		46%	
Irinotecan + Oxaliplatin	3	67%		0%	
Chemotherapy					
None	22	59%	0.90	32%	0.98
≥ 6 months	17	59%		29%	
< 6 months	20	65%		30%	
Diabetes					
Yes	5	100%	0.06	80%	0.01
No	54	57%		26%	
Gender					
Female	25	60%	0.89	24%	0.35
Male	34	62%		35%	
BMI 1					
≤ 25	32	50%	0.06	28%	0.67
> 25	27	74%		33%	
BMI 2					
≤ 30	53	60%	0.77	34%	0.08
> 30	6	67%		0%	
Age					
≤ 60 years	27	67%	0.41	22%	0.20
> 60 years	32	56%		37%	
Intraop. transfusion					
Yes	31	55%	0.36	23%	0.88
No	71	45%		24%	

recently published analysis, aspirin intake was independently associated with a reduced risk for sinusoidal lesions.[23]

As in some large studies examining risk factors for morbidity after liver resection,[24,25] the extent of resection was a strong predictor of postoperative complications in our study. Limited resections in patients with colorectal metastases, however, are rarely associated with severe complications. In addition, the proportion of patients requiring preoperative chemotherapy for initially unresectable liver metastase is certainly higher in patients later undergoing major resections. We, therefore, performed separate analyses in the subgroup of patients with major resections. As in the entire patient group (where it showed a trend), the presence of diabetes mellitus was a risk factor for any or liver-related complications in patients undergoing major resection. Since diabetes was not associated with liver injury in our evaluations, the reasons for the higher complication rates in diabetics are probably independent of liver injury.

With the exception of a distinct trend for a (nonsignificant) higher overall complication rate for patients with steatosis > 20% or with severe steatohepatitis, the other types/definitions of liver injuries did not show any correlation with postoperative complications. We especially could not demonstrate any significant influence of liver injury on liver-related complications. Preoperative chemotherapy did not influence postoperative morbidity in our patients, neither directly nor via potential liver injury. We could observe an even lower risk of liver insufficiency in patients with chemotherapy. None of the 34 patients with preoperative oxaliplatin- or irinotecan-based chemotherapy developed liver failure. Although patients with a higher BMI were at increased risk for steatosis, steatohepatitis, and fibrosis, the BMI itself did not influence postoperative morbidity. Patients with a BMI>30 had even a trend to a lower liver related complication rate.

Since "only" three of 102 patients died of postoperative complications, conclusions on risk factors for mortality are difficult to assess. However, since two of eight diabetic patients died postoperatively, diabetes statistically was a risk factor for mortality in our experience. The presence of diabetes mellitus is also a risk factor for mortality, for any, and for liver-related complications in our current analyses of 243 patients after primary liver resection for colorectal metastases since 2000 (data not shown).

In contrast to our results, several studies reported a significant association between chemotherapy (and/or chemotherapy-induced liver injury) and postoperative morbidity. Already more than a decade ago, Behrns et al. showed an increase in mortality and morbidity in patients with moderate to severe steatosis.[26] These data were later confirmed by Kooby et al. who also showed a higher morbidity and a trend to a higher mortality in patients with "marked" steatosis.[17] In a newer report with analysis of different chemotherapy regimens in a large number of patients, Vauthey et al. could demonstrate a significantly increased mortality in the subgroup of patients with steatohepatitis (which was significantly associated to irinotecan chemotherapy).[14] The authors, however, did not find influences of other chemotherapy regimens or of sinusoidal injury on morbidity and mortality. In further studies from France, the postoperative complication rates were variably increased in patients after more than six cycles of chemotherapy,[19] in patients with sinusoidal injury (which was associated with oxaliplatin > six cycles and female gender),[18] or after more than 12 cycles of chemotherapy (increased length of stay, more reoperations).[22] In the large randomized EORTC study on neoadjuvant chemotherapy, the rate of reversible postoperative complications was also significantly higher after preoperative FOLFOX4.[12]

As in our study, Pawlik et al.[15] could not find a correlation between preoperative chemotherapy and morbidity/mortality in their analysis of more than 200 patients, despite a regimen-specific association between chemotherapy and liver injury.

The findings of our study are potentially limited by the relative low number of patients in each chemotherapy group which may preclude a potential difference between the groups. However, we could not find any negative influence of chemotherapy on morbidity, especially in the 34 patients with oxaliplatin- and/or irinotecan-based chemotherapy. In this context, we have to mention that a "high volume" liver surgery program was initiated in our department only in 2001 (with a new team). Systematic "aggressive" preoperative/neoadjuvant chemotherapy, as for example administered in many patients in some specialized centers in the USA[14] or France[8,10] is given since a few years only in our institution. In addition, we generally perform surgery not earlier than 4 to 6 weeks after the end of chemotherapy and require a functional liver remnant of at least 30% in otherwise healthy livers. These factors might explain some of our results (lack of negative influence of chemotherapy on morbidity).

Another potential weakness is the fact that the significant worse perioperative outcome in diabetic patients is based on a rather small number (eight patients). Diabetes was not specifically examined as a potential risk factor for complications neither in some large perioperative studies[24,25,27] nor in most studies on preoperative chemotherapy. We, therefore, propose the inclusion of diabetes in future evaluations of perioperative outcomes after liver resection.

Conclusion

The major conclusion of our study is that factors other than preoperative chemotherapy may have a clinically relevant

influence on postoperative morbidity after resection of colorectal liver metastases. Major resections (a fact which is self-explaining) and IDDM were relevant risk factors for morbidity in our series. Although chemotherapy led to distinct features of liver injury, this did not significantly influence the postoperative course in the patients of our study. Liver injury (steatosis, steatohepatitis, fibrosis) was relevantly influenced by the body mass index, independent of chemotherapy.

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ORIGINAL ARTICLE

Adenosquamous Versus Adenocarcinoma of the Pancreas: A Population-Based Outcomes Analysis

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Abstract
Background Pancreatic adenosquamous carcinoma has historically been characterized as having a more aggressive clinical course than ductal adenocarcinoma. The natural history of this disease, however, is essentially unknown.
Methods We evaluated the clinical characteristics of all patients with pancreatic adenosquamous carcinoma recorded in the California Cancer Registry 2000–2007 and compared them to those of patients with ductal adenocarcinoma.
Results Ninety-five patients with pancreatic adenosquamous carcinoma and 14,746 patients with ductal adenocarcinoma were identified. Demographics were similar between subtypes ($p>0.05$). Disease stage at presentation was also similar; over 50% of each diagnostic group presented with metastatic disease ($p=0.62$). Surgical resection was more common among patients with locoregional adenosquamous carcinoma than adenocarcinoma ($p=0.0004$), but rates of adjuvant therapy administration were similar ($p>0.05$). The cohorts' median overall survival durations were similar in a Cox proportional hazards model ($p=0.45$); overall survival was also similar when only patients with resected disease were considered ($p=0.65$). Early stage, resection and receipt of radiation or chemotherapy were favorable independent prognostic factors among patients with adenosquamous carcinoma. The median overall survival duration of patients with resected adenosquamous carcinoma was 12 months (95% CI, 8–52).
Conclusions Adenosquamous carcinoma has a natural history similar to that of ductal adenocarcinoma when treated with prevalent clinical patterns of care.

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Introduction

Adenosquamous carcinoma (ASC) is a rare pancreatic cancer that is has been suggested to be distinct from pancreatic ductal adenocarcinoma (AC) both histopathologically and clinically.[1, 2] Histologically, ASC is distinguished from AC by the presence of both adenocarcinomatous and squamous components.[3] Clinically, the disease has been characterized by an extremely poor prognosis, even relative to that of AC—which itself is associated with median overall survival durations as low as 3–6 months among patients with metastatic disease and as high as 24 months among patients with resectable cancers.[4, 5] Indeed, the median overall survival duration of patients with localized ASC has been reported to be as low as 6 months following radical tumor resection, with 2-year

survival an infrequent event. Patients with advanced ASC treated with palliative intent have fared even worse.[5–7]

The histopathologic phenotype of ASC is well defined and thus ASC remains a unique diagnostic entity. The clinical significance of this diagnosis is unclear, however, because its natural history is poorly understood. Indeed, the demographics, treatment patterns, and oncologic outcomes of patients with ASC are essentially unknown because all clinical knowledge of the disease has been accumulated from case studies [8–26] and small, single-institution anecdotes—reporting patients compiled over a period of decades—the overwhelming majority of whom had localized disease and were treated with surgery alone.[2, 5, 7, 27–31] Given the time, stage, and treatment biases inherent in these previous reports, we hypothesized that the natural history of ASC has been mischaracterized and its clinical significance overstated. We sought to more completely establish the clinical profile of ASC relative to AC and to elucidate any unique characteristics that might influence the design of rational treatment strategies. To these ends, we examined a consecutive series of patients with ASC recorded in a large state cancer registry over a recent 8-year time period. We evaluated demographic and clinical features of ASC, including survival estimates after treatment with prevalent patterns of care, and compared these clinical parameters to those of patients with AC treated in the same recent time period.

Patients and Methods

Cancer Registry

We performed a historical analysis of cases in the California Cancer Registry database (CCR). The CCR is the largest contiguous area, population-based cancer registry in the world, collecting more than 130,000 new cases yearly. Standardized data collection and quality control procedures have been in place since 1988.[32, 33] The CCR is part of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) Program. Case reporting is estimated at 99% for the state, and follow-up completion rates exceed 95%.[34, 35] The CCR has received the highest level of certification from the North American Association of Central Cancer Registries.[36] Data were abstracted from medical records by trained registrars according to standardized protocols.[32, 33] Tumor site and histology were coded according to standardized criteria.[37]

Study Population

Histopathologic diagnoses recorded in the CCR were ascertained by examination of fine needle aspiration or surgical specimens by local pathologists. Pancreatic tumors were identified using the SEER primary site recode 21100. Pancreatic ductal adenocarcinomas were identified by ICDO (third edition) histology codes 8140, 8141, 8142, 8144, 8490, 8500, 8501, 8503, 8504, 8507.[37] Adenosquamous carcinomas were identified by histology code 8560. Other non-ductal cancers were expressly excluded. All incident cases recorded between January 2000 and November 2007 for whom complete follow-up data were available through November 2007 were included for analysis.

Recorded data included demographic information, histology, burden of disease at presentation, first treatment history, socioeconomic status, and vital status. Socioeconomic status is denoted as a single index variable using statewide measures of education, income, and occupation from census data, as described previously.[38, 39] Quintiles for the socioeconomic status score were used for analysis, with socioeconomic status 1 and 5 denoting the lowest and highest quintiles, respectively.

The criteria used for American Joint Commission on Cancer (AJCC) staging of pancreatic cancer underwent a dramatic revision between the fifth and sixth editions.[40] In the CCR, AJCC staging per seventh edition guidelines is available only for cases diagnosed in or after the year 2004. We therefore allocated cases by the SEER summary stage into cohorts with "localized" (no tumor extension or malignant regional lymphadenopathy regardless of tumor size), "regional" (based on the presence of either tumor extension to adjacent viscera or lymph nodes), or "metastatic" disease. Patients with localized or regional disease in whom pancreaticoduodenectomy, distal pancreatectomy, or total pancreatectomy were performed were considered to have undergone an oncologic resection; patients who underwent an oncologic resection who received either chemotherapy or radiation therapy in the first course of treatment were considered to have undergone adjuvant therapy. Hospital registrars contacted cases annually, and CCR staff annually reviewed state death certificates to identify deceased registry cases.

Statistical Analysis

Clinical characteristics were analyzed with Pearson's chi-square test or Fisher's exact test for categorical and dichotomous variables and the Student's *t* test for comparison of continuous variables. The overall survival duration (in months) was calculated using dates of diagnosis and either death from any cause or last contact. The Kaplan–Meier method was used to generate survival curves. The log-rank test was used to assess differences between survival curves. Multivariate survival analyses were performed using Cox proportional hazards ratios. Fifty-five

Table 1 Demographics and treatment of patients with pancreatic adenosquamous carcinoma and ductal adenocarcinoma reported in California, 2000–2007

	Adenosquamous	Adenocarcinoma	*p*
N, %	95 (0.39)	14,746 (59.9)	
Demographic variables			
Age, mean (SD)	68.5 (11.8)	68.6 (11.8)	0.9188
Sex, *n* (%)			0.1565
Male	55 (57.9)	7,462 (50.6)	
Female	40 (42.1)	7,284 (49.4)	
Ethnicity, *n* (%)			0.3740
White	72 (75.8)	9,760 (66.2)	
Black	5 (5.3)	1,108 (7.5)	
Hispanic	11 (11.6)	2,425 (16.5)	
Asian	7 (7.4)	1,365 (9.3)	
Other	0 (0)	88 (0.6)	
SES quintile, *n* (%)			0.3013
Lowest	10 (10.5)	2,083 (14.1)	
Second lowest	22 (23.2)	2,622 (17.8)	
Middle	14 (14.7)	3,153 (21.4)	
High	23 (24.2)	3,322 (22.5)	
Highest	26 (27.4)	3,566 (24.2)	
Clinical stage, *n* (%)[a]			0.6242
Localized	8 (8.9)	976 (7.0)	
Regional	34 (37.8)	4,864 (35.1)	
Metastatic	48 (53.3)	8,029 (57.9)	
Missing data, *n* (%)[b]	5 (5.3)	877 (5.9)	
Treatment variables			
Any surgery, *n* (%)[a]			**<0.0001**[a]
Yes	31 (32.6)	2,428 (16.5)	
No	64 (67.4)	12,300 (83.5)	
Missing data, *n* (%)[b]	0 (0)	18 (0.1)	
Any radiation, *n* (%)[a]			0.1515[a]
Yes	20 (21.1)	2,310 (15.7)	
No	75	12,422 (84.3)	
Missing data, *n* (%)[b]	0 (0)	14 (0.1)	
Any chemotherapy, *n* (%)[a]			0.6786[a]
Yes	42 (46.2)	6,296 (44.0)	
No	49 (53.8)	8,016 (56.0)	
Missing data, *n* (%)[b]	4 (4.2)	434 (2.9)	
Locoregional patients, *n* evaluated	42	5,838	
Onc. resection, *n* (%)			**0.0004**
Yes	26 (61.9)	2,071 (35.6)	
No	16 (38.1)[c]	3,750 (64.4)	
Type of resection, *n* (%)			0.1084
PD	18 (69.2)	1,690 (81.6)	
Distal panc.	5 (19.2)	181 (8.7)	
Total panc.	3 (11.5)	200 (9.7)	
Tumor diam. (mm); mean (SD)[a]	46.3 (19.0)	33.5 (15.1)	**0.0001**
Missing data, *n* (%)[b]	0 (0)	117 (5.7)	
Lymph nodes positive, *n* (%)[a]			0.8562
Yes	15 (57.7)	1,236 (60.2)	

Table 1 (continued)

	Adenosquamous	Adenocarcinoma	*p*
No	11 (42.3)	816 (39.8)	
Missing data, *n* (%)[b]	0 (0)	19 (0.9)	
Adj. chemotherapy, *n* (%)[a]			0.9902
Yes	13 (52.0)	1,037 (51.9)	
No	12 (48)	962 (48.1)	
Missing data, *n* (%)[b]	1 (3.8)	72 (3.5)	
Adj. radiation, *n* (%)			0.2876
Yes	12 (46.2)	747 (36.1)	
No	14 (53.8)	1,324 (63.9)	
Metastatic patients, *n* evaluated	46	7,832	
Pall. chemotherapy, *n* (%)			0.2397
Yes	24 (52.2)	3,411 (43.6)	
No	22 (47.8)	4,421 (56.4)	

The numbers in bold are those which are statistically significant, i.e. $p < 0.05$

For each variable, data was complete unless otherwise specified

SES socioeconomic status, *Onc.* oncologic, *panc.* pancreatectomy, *Adj.* adjuvant, *Pall.* palliative, *diam.* diameter, *PD* pancreaticoduodenectomy

[a] Percentage and *p* values refer to patients with complete data

[b] Percentage of total patients

[c] Among patients with locoregional ASC in whom the reason an oncologic resection was not performed was recorded, surgery was not recommended in 12 (two with localized cancers and ten regional), and one patient refused an operation

patients (all of whom had AC) in whom a diagnosis of cancer was made by review of an autopsy report or death certificate were excluded from all survival analyses. All analyses were conducted using SAS 9.2 (SAS Institute, Inc., Cary, NC). Statistical significance was assumed for a two-tailed p value<0.05.

Results

Demographics of Patients with ASC and AC

Between 2000 and 2007, 24,604 incident cases of pancreatic neoplasm were recorded in the CCR. Of these, 14,746 (59.9%) patients with AC and 95 (0.38%) patients with ASC were included in this analysis. Demographic data for these patients are reported in Table 1. The median age at diagnosis, sex, race, socioeconomic status, and clinical stage of patients with ASC and AC were similar ($p>0.05$). The majority of patients with each diagnosis were Caucasian; sex and socioeconomic status were evenly distributed. Over 50% of both groups were found to have metastatic disease upon presentation. In contrast, localized disease was identified in less than 10% of incident cases of each histopathologic subtype.

Treatment Patterns and Pathologic Variables of ASC and AC

Surgery was utilized more frequently for patients with ASC than those with AC, both overall (32.6% vs 16.5%, $p<0.0001$) and among patients with locoregional cancers (61.9% vs 35.6%, $p=0.0004$) (Table 1). Oncologic procedures performed for patients with ASC included pancreaticoduodenectomy ($n=18$), distal ($n=5$), and total pancreatectomy ($n=3$); the distribution of these operations was similar to that performed for AC ($p=0.11$). The mean tumor diameter in resected ASC specimens was larger than that in AC specimens (46.3 vs 33.5 mm, $p=0.0001$), but the frequency of positive lymph nodes was similar (57.7 vs 60.2%, $p=0.86$).

Overall, radiation ($p=0.15$) and chemotherapy ($p=0.68$) were administered to similar proportions of patients with ASC and AC. Twelve (46.2%) patients with ASC who underwent an oncologic resection were treated with adjuvant radiation and 13 (52.0%) received chemotherapy. Rates of administration of adjuvant radiation ($p=0.29$) and adjuvant chemotherapy ($p=0.99$) following resection for locoregional disease did not differ between groups. Likewise, among patients with metastatic disease, the rate of administration of palliative chemotherapy did not differ between patients with ASC and AC ($p=0.24$).

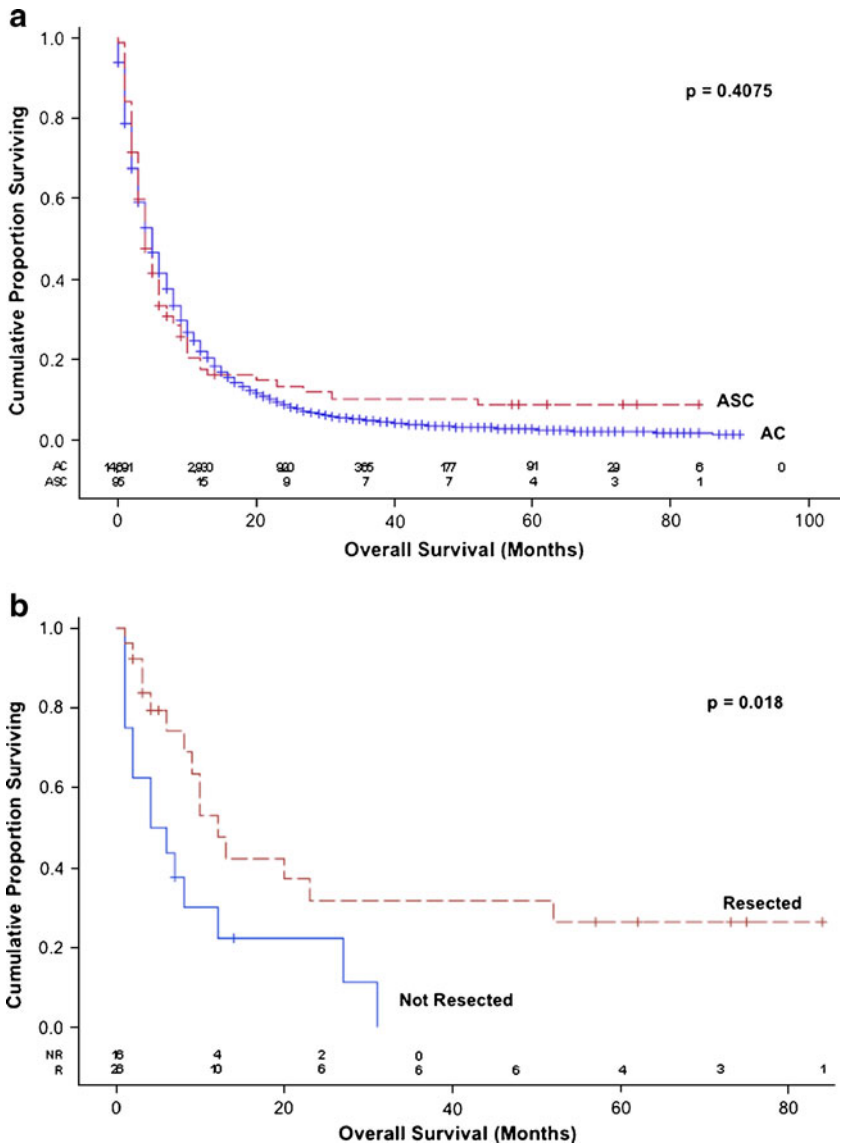

Fig. 1 **a** Overall survival of all patients with pancreatic adenosquamous carcinoma and ductal adenocarcinoma reported in California, 2000–2007. *Dashed line*, adenosquamous carcinoma (*ASC*); *solid line*, pancreatic ductal adenocarcinoma (*AC*). **b** Overall survival of patients with localized or regional adenosquamous carcinoma stratified by resection status. *Dashed line*, resected (*R*); *solid line*, not resected (*NR*)

Overall Survival of ASC and AC

As a group, the median overall survival duration of all patients with ASC was 4 months (95% CI, 3–6) and was similar to that of all patients with AC (p=0.41, Fig. 1a). The median overall survival duration of patients with ASC was also similar to that of patients with AC in subpopulations of patients stratified by age, sex, ethnicity, socioeconomic status, clinical stage, and the use of oncologic resection, radiation therapy, and chemotherapy on univariate analysis (p>0.05, data not shown). Furthermore, the median overall survival duration of all patients with ASC was similar to that of all patients with AC in a Cox proportional hazards model after adjustment for age, gender, ethnicity, socioeconomic status, stage of disease, and first treatment strategy [hazard ratio (HR), 1.091; 95% CI, 0.870–1.367; p=0.45] (Table 2). Finally, when only patients with locoregional cancers who underwent resection were considered, the median overall survival duration of patients with each histopathologic diagnosis were similar after adjustment for age, gender, ethnicity, socioeconomic status, clinical stage, tumor size, lymphatic involvement, and the receipt of adjuvant therapy (HR, 0.886; 95% CI, 0.530–1.482; p=0.65) (Table 3).

Favorable Prognostic Factors among Patients with ASC

Among all patients with ASC, favorable prognostic factors on univariate analysis included early clinical stage (p<0.0001), oncologic resection (p<0.0001), receipt of radiation (p< 0.0001), and receipt of chemotherapy (p<0.0233). In a Cox

Table 2 Cox proportional hazards model for overall survival of all patients with pancreatic adenosquamous carcinoma and ductal adenocarcinoma reported in California, 2000–2007

	HR	95% CI	*p*
Histologic subtype			
AC	1.000 (referent)		
ASC	1.091	0.870–1.367	0.4509
Age	1.010	1.009–1.012	**<0.0001**
Gender			
Male	1.000 (referent)		
Female	0.951	0.918–0.986	**0.006**
Ethnicity			
Caucasian	1.000 (referent)		
Black	1.035	0.966–1.108	0.3290
Hispanic	0.955	0.906–1.006	0.0802
Asian	0.922	0.866–0.981	**0.0108**
Socioeconomic status	0.966	0.953–0.980	**<0.0001**
Clinical stage			
Localized	1.000 (referent)		
Regional	1.275	1.177–1.382	**<0.0001**
Metastatic	2.293	2.117–2.484	**<0.0001**
Oncologic resection			
No	1.000 (referent)		
Yes	0.444	0.419–0.472	**<0.0001**
Any radiation			
No	1.000 (referent)		
Yes	0.887	0.840–0.936	**<0.0001**
Any chemotherapy			
No	1.000 (referent)		
Yes	0.508	0.488–0.528	**<0.0001**

The numbers in bold are those which are statistically significant, i.e. $p < 0.05$

HR hazard ratio for death, *95% CI* 95% confidence interval

proportional hazards model, each of these factors remained independently significant (Table 4).

Separate multivariate models were not constructed for patients with locoregional or metastatic ASC due to relatively small numbers in each of these subgroups. Among patients with locoregional ASC, however, those who underwent an oncologic resection had a median survival duration of 12 months (95% CI, 8–52) compared with 5 months (95% CI, 1–12) for those who did not, and the survival curves were significantly different ($p=0.018$) (Fig. 1b). A significant difference in survival could not be demonstrated between patients with resected locoregional ASC who did and did not receive adjuvant therapy ($p=0.09$ overall). Eight patients with locoregional ASC survived longer than 2 years, four of whom survived over 5 years. Each of these 5-year survivors underwent surgery and received adjuvant therapy.

Among patients with metastatic ASC, patients who received chemotherapy had a more favorable median survival duration (4.5 months; 95% CI, 3–6 months) than patients who did not (2 months; 95% CI, 1–3 months; $p=0.04$).

Discussion

ASC and AC share a similar histologic [3] and molecular [41] profile. ASC, however, has long been characterized as having a natural history distinctly more aggressive than that of AC. This has led some to question the role of aggressive treatment strategies for patients with this disease.[2, 5, 7, 27] The clinical significance of this rare diagnosis relative to AC is unclear, however, because the oncologic behavior of ASC has been described only by case studies and small, retrospective surgical series reporting patients with early

Table 3 Cox proportional hazards model for overall survival of patients with resected locoregional pancreatic adenosquamous carcinoma and ductal adenocarcinoma reported in California, 2000–2007

	HR	95% CI	*p*
Histologic subtype			
AC	1.000 (referent)		
ASC	0.886	0.530–1.482	0.6454
Age	1.008	1.003–1.014	**0.0043**
Gender			
Male	1.000 (referent)		
Female	0.998	0.893–1.116	0.9740
Ethnicity			
Caucasian	1.000 (referent)		
Black	1.095	0.865–1.387	0.4514
Hispanic	1.043	0.884–1.231	0.6150
Asian	1.007	0.818–1.241	0.9445
Socioeconomic status	0.945	0.905–0.987	**0.0112**
Clinical stage			
Localized	1.000 (referent)		
Regional	1.300	1.067–1.583	**0.0091**
Tumor diameter	1.009	1.005–1.012	**<0.0001**
Lymphatic involvement			
No	1.000 (referent)		
Yes	1.386	1.212–1.585	**<0.0001**
Adjuvant radiation			
No	1.000 (referent)		
Yes	0.791	0.679–0.922	**0.0027**
Adjuvant chemotherapy			
No	1.000 (referent)		
Yes	0.651	0.561–0.756	**<0.0001**

The numbers in bold are those which are statistically significant, i.e. $p < 0.05$

HR hazard ratio for death, *95% CI* 95% confidence interval

Table 4 Cox proportional hazards model for overall survival of all patients with pancreatic adenosquamous carcinoma reported in California, 2000–2007

	HR	95% CI	p
Age	1.012	0.986–1.038	0.3730
Gender			
Male	1.000 (referent)		
Female	0.905	0.536–1.528	0.7088
Ethnicity			
Caucasian	1.000 (referent)		
Black	0.703	0.244–2.023	0.5135
Hispanic	0.890	0.390–2.032	0.7815
Asian	0.655	0.244–1.755	0.3998
Socioeconomic status	0.936	0.765–1.145	0.5915
Clinical stage			
Localized	1.000 (referent)		
Regional	2.717	0.781–9.451	0.1161
Metastatic	4.690	1.445–15.216	**0.0101**
Oncologic resection			
No	1.000 (referent)		
Yes	0.369	0.183–0.747	**0.0056**
Any radiation			
No	1.000 (referent)		
Yes	0.474	0.242–0.927	**0.0292**
Any chemotherapy			
No	1.000 (referent)		
Yes	0.530	0.300–0.935	**0.0285**

The numbers in bold are those which are statistically significant, i.e. $p < 0.05$

HR hazard ratio for death, *95% CI* 95% confidence interval

stage cancers (Table 5). Moreover, no prior case–control studies or population-based analyses have been performed to definitively establish clinical differences between ASC and AC. In this, the largest study of ASC reported to date, we used a large cancer registry to evaluate the clinical features and oncologic outcomes of patients with this diagnosis. Using a relatively unbiased dataset, we characterize the natural history of ASC and show that ASC is no more inherently aggressive than AC. Indeed, we demonstrate that patients with these two diagnoses have a similar natural history when treated using prevalent patterns of modern clinical practice.

ASC has been reported to represent up to 4% of pancreatic neoplasms, but in the largest series of specimens analyzed at autopsy, ASC was identified in only 0.9%.[42, 43] In this analysis of a large tumor registry, we found a diagnosis of ASC in approximately 0.4% of 24,604 patients with newly documented pancreatic malignancies recorded between 2000 and 2007. This is remarkably similar to the rate of 0.5% identified in a recent 16-year survey of the State of Michigan Tumor Registry.[44]

Like patients with AC, most of the patients with ASC presented late in their natural history. Indeed, over 50% of patients analyzed in this study initially presented with synchronous distant metastases. Among patients treated surgically, those with ASC had larger tumors than those with AC; however, a larger proportion of patients with locoregional ASC underwent resection than that with AC, and resected ASC specimens were associated with a similar high rate of regional lymphatic involvement—approximately 60%—as AC tumors. Together, these findings reveal that—although considerably rarer—ASC presents at a similar (albeit advanced) stage as AC and suggest that the two diagnoses share a common biologic behavior prior to diagnosis and treatment.

Stage-specific treatment algorithms for patients with AC are reasonably well-established.[45] In contrast, the absolute infrequency of ASC has prohibited the development of standardized treatment protocols for this disease. Indeed, even the treatment of patients with early stage ASC remains controversial, due to reportedly dismal survival rates seemingly regardless of intervention.[5, 7] In a recent systematic review of prior reports, 39 patients with ASC who underwent surgery for non-metastatic disease had a median survival duration of 6.8 months (range, 4.6–9) and a 1-year survival rate of 25.5%.[6] In two recent single-institution series, overall survival of resected patients was somewhat more favorable. Among 38 resected patients from Johns Hopkins, the median overall survival duration was 10.9 months from diagnosis.[27] In another series from the Mayo Clinic, patients who underwent R0 or R1 resection had a median survival duration of 14.4 months and 8 months, respectively, compared to 4.8 months among patients treated without an operation.[7] The patients in each group were not described, however, suggesting that patients who did not undergo resection had advanced disease, prior comorbidities, a depressed performance status, or a combination of these factors.

The efficacy of non-operative therapies among patients with ASC has not been rigorously evaluated. Only one prior study has examined the utility of adjuvant chemoradiation for patients with this disease. In that small, retrospective series, 19 (50%) patients who underwent postoperative chemoradiation had a more favorable median overall survival than 19 (50%) patients who did not (13.6 months v. 8.6 months, $p=0.005$).[27] Although adjuvant chemoradiation was found to be the only significant prognostic factor with respect to overall survival on univariate analysis, the analysis suffered from clear selection bias. No studies have specifically studied the effects of systemic chemotherapy when administered in the adjuvant setting, nor its role as palliative therapy for patients with metastatic disease.

In this study, treatment of patients with ASC by surgical resection was associated with a more favorable overall survival relative to no resection, after adjustment for multiple clinical factors including disease stage. Moreover,

Table 5 Published case reports and clinical series of patients with pancreatic adenosquamous carcinoma, 1990–2010

Author (ref.)	Number	Resected, *n* (%)	Median age (years)	Adjuvant treatment, *n*	Median OS resected, months	Median OS unresected, months
Skafida[8]	1	1 (100)	70	1 CTX	6	NA
Lampropoulos[9]	1	1 (100)	72	1 CXRT	24	NA
Voong[27]	38	38 (100)	68	19 CTX 19 CXRT	10.9	NA
Kobayashi[10]	1	0 (0)	72	NA	NA	3
Smoot[7]	23	12 (52)	67[a]	5 CXRT	13.1	4.8
Hsu[5]	12	7 (58)	71	5 CTX	6.51	NR
Jamali[11]	1	1 (100)	75	1 CTX	6	NA
Alwaheeb[12]	1	1 (100)	45	NR	NR	NA
Inoue[13]	1	0 (0)	61	NA	NA	0.83
Murakami[14]	2	2 (100)	54	1 CTX 1 CXRT	4.5	NA
Rahemtullah[28]	14	2 (14)	70[a]	NR	13	4
Kardon[29]	25	13 (52)	65[a]	5 CTX	11.3	3.0
Yamaue[15]	1	1 (100)	63	1 CXRT	40	NA
Yavuz[16]	2	2 (100)	50	NR	36, NR	NA
Komatsuda[17]	1	1 (100)	67	0	6	NA
Aranha[18]	2	2 (100)	57	2 CXRT	13.5	NA
Madura[2]	6	6 (100)	64[a]	3 CXRT	5	NA
Nabae[19]	2	2 (100)	67	1 RT1 NR	6.5	NA
Lozano[20]	3	2 (67)	59[a]	3 CXRT[b]	NR	NR
Myung[21]	1	1 (100)	64	0	4	NA
Kuji[22]	1	1 (100)	73	0	2	NA
Campman[23]	1	1 (100)	65	NR	NR	NA
Onoda[24]	1	1 (100)	64	1 CTX	3	NA
Makiyama[25]	1	1 (100)	58	0	18	NA
Tanaka[26]	1	1 (100)	48	1 CTX	7	NA
Motojima[30]	6	3 (50)	67[c]	NR	7	NR
Yamaguchi[31]	8	8 (100)	56[a]	0	5.5	NA

NR not recorded, *NA* not applicable, *CTX* chemotherapy, *CXRT* chemoradiation, *RT* radiation, *OS* overall survival

[a] Mean

[b] Neoadjuvant chemoradiation

[c] Resected only

the overall survival duration of patients with locoregional ASC who underwent surgery was similar to that of patients with locoregional AC who underwent resection in the same time period. Together with the recent single-institution data from high-volume pancreatic treatment centers,[7, 27] these data suggest that resection is a reasonable therapeutic approach for patients with ASC in whom a margin-negative resection can be performed safely.

The role of non-operative therapies for patients with ASC is less clear. Although we could demonstrate no association between the administration of adjuvant radiation or chemotherapy on the survival of patients with locoregional ASC following resection, it is interesting that of the only six 5-year survivors with ASC reported to date (four in this series and two in the Johns Hopkins series [27]), all received surgery and adjuvant therapy. Among patients with metastatic ASC, patients who received chemotherapy had a longer overall survival duration (4.5 vs 2 months) than patients who did not. The significance of this finding is uncertain, however, because individual performance status—the most influential factor with regard to the administration of anticancer therapy among patients with advanced pancreatic malignancy—was not recorded in the CCR.[46] The absence of recorded performance status represents a fundamental limitation of this and other analyses of pancreatic malignancies using large, population-based datasets.

Two other limitations of this study are particularly noteworthy. Although attempts have been made to identify characteristic molecular fingerprints that may effectively distinguish between ASC and AC, the molecular profile of these two tumors are similar.[41] Therefore, ASC must be distinguished from AC histopathologically. A strict diagnosis of ASC requires that a malignant squamous component represent at least 30% of a routinely sectioned adenocarcinoma.[3, 29] This arbitrary cutoff has introduced ambiguity to the diagnosis of ASC that reflects both the absence of standardization in histopathologic methods used to process surgical specimens and the subjectivity with which they are evaluated. Indeed, when 38 surgical specimens initially diagnosed as ASC at Johns Hopkins were re-evaluated by

a single pathologist, 12 (32%) failed to meet strict criteria for the disease.[27] Significantly, although the presence of any squamous component was associated with poor prognosis in the Johns Hopkins study relative to a historic control group of patients with AC, the proportion of the squamous component was not associated with overall survival. The rationale for the strict 30% cutoff is therefore unclear, and several investigators have proposed eliminating this criterion altogether.[29]

It is also possible that some diagnoses were coded incorrectly in the CCR; however, all diagnoses recorded therein were validated by histopathologic or cytopathologic analysis. Moreover, accuracy of the histopathologic diagnoses recorded in large databases has been evaluated and compared with independent histologic review, with favorable results.[47, 48] Nonetheless, the accuracy associated with the diagnosis of ASC may not be as favorable due to the stringent diagnostic requirements for this disease. A further potential for misclassification may exist among patients with advanced cancer treated non-operatively, for whom a large surgical specimen for histopathologic evaluation is absent. The extent to which our conclusions are influenced by this issue is unknown.

In summary, we conclude that ASC is an extremely rare subtype of pancreatic cancer that shares many clinical characteristics—including biologic behavior and overall prognosis—with AC. In this population, the overall survival duration of all patients with ASC and AC were similar after adjustment for multiple clinical factors, including stage at presentation and first treatment strategy. These data therefore refute prior suggestions that ASC is inherently more aggressive than AC and imply that a nihilistic view toward patients with ASC must be avoided. Absent the ability to perform prospective studies to determine the response of ASC to individual therapies, and given the molecular, histopathologic and clinical similarity of these diseases, we recommend the use of aggressive, stage-specific, multidisciplinary treatment protocols developed for AC.

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ORIGINAL ARTICLE

Analysis of 6,747 Pancreatic Neuroendocrine Tumors for a Proposed Staging System

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Abstract
Objectives Currently, no reasonable staging system exists for pancreatic neuroendocrine tumors (PNET) to guide treating physicians. The aim of this study was to devise a staging system of relevant prognostic factors to better predict overall survival in PNET.
Methods A prospective 300 patient cohort and a review of the Surveillance Epidemiology and End Results database identified 6,447 patients with PNET from 1973 to 2008. Significant prognostic factors were created for an initial. Tumor: T (T1: ≤3 cm and localized to pancreas, T2: >3 cm and localized to the pancreas; T3: extension to adjacent organs and vessels), grade: G (G1: well/moderate and G2: poor/undifferentiated), and metastasis: M (M0: no distant mets, M1: distant mets) staging system.
Results Significant predictors of survival on multivariate analysis included age, size, grade, and metastasis. Based on the TGM staging system: stage 1 (T1–2, G1, M0), stage 2 (T1–2, G2, M0), stage 3 (T3G2M0, Tany, G1, M1), stage 4: (Tany, G2, M1) was created with survival being statistically different between stages ($p<0.0001$). Median survival rates were stage 1, 55 months; stage 2, 50 months; stage 3, 46 months; and stage 4, 25 months.
Conclusions Incorporation of this newly developed staging system into clinical practice will improve the ability to predict prognosis and aid in stratification of patients for clinical trials.

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Introduction

Pancreatic endocrine tumors still remain a rare and heterogeneous group of neoplasms reported to occur in fewer than one in 100,000 people per year.[1–3] These tumors have increased in overall incidence in recent years because of the increased use of screening CT scans and because of the relative ease to palliate and treat these patients when both local and metastatic disease is found.

The histopathologic criteria for the diagnosis of pancreatic neuroendocrine tumors (PNET) tumors have been widely established and validated in the current pathologic literature. The World Health Organization was the first organization to induce a system for both pathologic naming and classification of PNET tumors in 2000.[4,5] This classification was unique given the fact that it summarized clinical, molecular, and histopathologic features and attempted to define three types of biologically significant PNET tumors. More recently, there have been attempts to extrapolate the established TNM classification to pancreatic neuroendocrine tumors.[6–10] However, the TNM staging system does not take into consideration histologic grade or molecular subtypes such as mitosis and Ki-67 staging. Given the expansion of incidence and surgical diagnosis of PNET tumors, there is a growing need for all treating physicians to be able to appropriately risk stratify these patients to determine appropriate follow-up and the need for additional therapy based on their overall prognosis. Thus, the aim of this study was to devise a staging system of relevant prognostic factors to better predict overall survival in PNET tumors using a large national database and then validate this system with another large multicenter pancreatic dataset.

Materials and Methods

The Surveillance Epidemiology and End Results (SEER) database was queried for all cases of pancreatic neuroendocrine tumors as identified by International Statistical Classification of Diseases and Related Health Problems (ICD-0–3) and an extended ICD-0–3 International Classification of Childhood Cancer site recode of XII (a.1). All records in the current database were included, ranging from 1977 to 2006 for year of diagnosis. Demographics from each patient, including age at diagnosis, gender, and race were recorded, as were clinicopathologic factors for each tumor. The ability to undergo surgery was also reviewed; however, the type of surgery the patient undergoes is not recorded, and thus was not included in the staging criteria for the SEER dataset. These included size (in millimeters), grade, location, presence or absence of metastatic disease, and therapy received.

Clinicopathologic factors were analyzed to determine the effect on overall survival using the log-rank test (Table 1). Multivariate analysis was performed for statistically significant variables using the Cox proportional hazards model. All significant variables from univariate analysis were initially added to the multivariate model, and non-significant variables with p values greater than 0.05 were removed in a stepwise fashion.

For each patient with a primary tumor and no metastatic disease at the time of diagnosis, a tumor (T) and grade (G) stage was defined. T stage was defined by tumor size, and patients were dichotomized based on the cut-point that gave the greatest separation in survival, 30 mm. Tumors ≤3 cm and localized to pancreas were T1, >3 cm and localized to the pancreas T2, extension to adjacent organs and vessels were T3. In a similar fashion, G stage, based on histologic grade, was defined as G1 for grade I (low grade) or grade II (medium grade) tumors. Grades III (high grade) and IV (de-differentiated) comprised the G2 group. Individuals with presence of metastatic (including lymph nodes) disease at the time of diagnosis were classified as M1 and those without classified as M0. Kaplan–Meier curves were used to visualize survival for the groupings based on the T, G, and M stages, with differences between groups tested using the log-rank test.[11]

The T, G, and M categories were then combined in an attempt to form a standard four-tier staging system for PNET. Nodal status was not included as a separate factor in the proposed staging system, since it was not significant in the multivariate statistical model. However, because of the low propensity for regional lymph node disease with PNET and the poor prognosis this portends in other pancreatic tumors, we evaluated whether treating patients with positive nodal involvement as M1 disease improved the staging model. Further attempts beyond this to incorporate nodal status in the staging system failed to demonstrate predictive ability. Tumor size (T1 vs. T2) and grade (G1 vs. G2) were used to form groups that differentiated among the non-metastatic patients. This was accomplished by testing the various combinations of T stage and G stage using the log-rank test. The T and G stage groupings were combined with M stage to form an overall staging system. To assess the impact of the introduction of increased incidence for PNET, we further evaluated our staging system restricted to patients diagnosed in 6-year intervals.

The predictive ability of the individual T, G, and M stages were compared to the final TGM staging system using measures of predictive accuracy appropriate for survival data. Measures of prognostic separation,[12] explained variation,[13] and time-dependent receiver–operator characteristic (ROC)

Table 1 Clinicopathological characteristics of pancreatic neuroendocrine tumors

	Entire SEER dataset	Pancreatic Consortium Dataset
Total (*n*)	6,447	300
Age (years)		
Range	19–97	21–84
Mean	59.9	55.2
Median	61	55
Gender, *n* (%)		
Female	45%	48%
Male	55%	52%
Race, *n* (%)		
American Indian/Alaska Native	6%	2%
Asian or Pacific Islander	1%	0%
Black	10%	13%
White	83%	85%
Missing	0%	0%
Year of diagnosis, *n* (%)		
1973–1978	6%	0%
1979–1984	6%	0%
1985–1990	8%	0%
1991–1996	13%	1%
1997–2002	28%	28%
2003–2006	39%	35%
2007–2009	0%	36%
Surgery, *n* (%)		
Yes	25%	91%
Not recommended	40%	9%
Not recommended, contraindicated	35%	0%
Recommended, patient refused	5%	0%
Recommended not performed, unknown reason	18%	0%
Recommended, unknown if performed	0%	0%
Unknown	2%	0%
Histologic subtype, *n* (%)		
Carcinoid	6%	3%
Neuroendocrine	42%	60%
Islet cell	34%	20%
Insulinoma	2%	10%
Glucagonoma	1%	1%
Gastrinoma	2%	5%
Location, *n* (%)		
Head	58%	46%
Body	13%	11%
Tail	28%	43%
Size of tumor (mm)		
Range	7–240	7–250
Mean	52.9	43
Median	44	30
Missing	35%	0
Grade, *n* (%)		
Well differentiated—I	10%	72%
Moderately differentiated—II	7%	23%
Poorly differentiated—III	8%	10%
Undifferentiated—IV	6%	0%
Unknown	69%	0%
Extent of invasion, (%)		
Localized		69%
Duodenum, ampulla, bile duct		3%
Adjacent organs or vessel involved		3%
Unknown		25%
Lymph node involvement, *n* (%)		
Yes	13%	30%
No	12%	70%
Missing	75%	0%
Metastasis		
Yes	15%	14%
No	11%	86%
Missing	74%	0%
Follow-up duration (months)		
Range	1–401	10–311
Mean	34	43
Median	16	29

curves[14] were all calculated. Prognostic separation (D) in essence measures the separation of the Kaplan–Meier survival curves defined by the risk groups, with larger values indicating greater separation (and thus discrimination) between prognostic groups. Explained variation (V and V_W) gives the proportion of variation in survival that is accounted for by the staging system, with values closer to one indicating a greater predictive ability of the risk grouping. Time-dependent ROC curves plot values of the sensitivity and specificity calculated at specified times, with individuals still alive at time t serving as the 'control' group and those not alive at time t serving as the 'case' group. Time-dependent ROC curves were calculated at 5 and 8 years, and the area underneath the ROC curve (AUC) presented as a summary measure which gives the concordance of the risk grouping with the survival outcome at those times. The bootstrap percentile method was used to calculate 95% confidence intervals for all the measures in each case, using 1,000 bootstrap replicates.[15] The R package surev[16] was used for calculation of the V and V_W measures, package survivalROC[17] used for calculation of the time-dependent

ROC curves and AUC values, and the boot package[18] used for all bootstrap calculations. All statistical analyses for this study were completed using the R statistical software package, version 2.7.1.[19]

This staging system was then validated using another large prospective dataset collected through the collaboration of the Central Pancreatic Consortium.[20–22]

Results

Patient Demographics

Clinical pathologic characteristics of the patient population are displayed in Table 1. There has been a statistically significant increase in the number of patients diagnosed with pancreatic neuroendocrine tumors over the last three time intervals, with a near doubling of incidence from 1997 to 2002 (28%) and 2003 to 2006 (39%) when you compare to earlier years (Table 1).

The median size of tumor in the SEER database was 4.4 cm (range 0.7–24 cm.) with a limited information based on grade with only 31% of the patients in the dataset having a histologic grade that could be defined as well-differentiated (10%), moderately differentiated (7%), poorly differentiated (8%), and undifferentiated (6%). Lymph node metastases (13%) and overall distant metastases were seen in a small subset of the SEER dataset. In comparison to the pancreatic consortium dataset, median size of tumor was 3.0 cm with a range of 0.7–25.0 cm, with a much larger percentage having a complete dataset with a vast majority of patients having well-differentiated tumors (72%) and moderately differentiated tumors (23%) in comparison to the other later-grade tumors. The extent of the invasion was most commonly localized in the pancreas without extension in 69% of the patients. A similar small majority of patients presented with distant metastatic disease (14%) with a larger majority of patients having lymph node metastases (30%) in comparison to the SEER data set. Overall mean and median follow-up were fairly similar in both the SEER and pancreatic consortium datasets.

Survival Analysis

Multiple clinical pathologic characteristics were evaluated for their association with overall survival (Tables 2 and 3). In an evaluation of the SEER dataset, age greater than 65 years of age, year of diagnosis, surgery, pancreatic tail location, size of tumor, grade of tumor, lymph node involvement, and metastasis all were significant on univariate analysis (Table 2). Only gender and ethnicity were not found to be factors of overall survival on univariate analysis. For multivariate model, surgery, grade, and metastasis all remained significant (Table 3). For the Pancreatic Consortium dataset, none of these factors were found to be predictive related to the lack of power and the long overall survival seen in PNET tumors.

Development of a Staging System Using SEER Data

Statistically significant variables from our analysis were used to stratify patients and develop a staging system. Various cut-points of tumor size (2, 3, 5, and 10 cm) for non-metastatic patients were evaluated and compared via log-rank tests, with the cut-point at 3 cm giving the greatest separation in survival curves ($p<0.05$, HR 1.2, CI 1.01–4.5). These points were selected based on their familiarity in the TNM and WHO systems (2, 3, and 5 cm), as well as an approximation of the median non-metastatic tumor size (3–4 cm). However, size alone was not statistically different on multivariate analysis (Table 3) and there were even discordant effects of T1 vs. T2 stage, demonstrating the limited prognostic effect of just size alone and thus degree of extension was incorporated to define a more robust differentiation of T stage disease (Fig. 1a). Differences in survival based on tumor grades I and II vs. III and IV for non-metastatic patients were also significant (Fig. 1b; $p<0.01$, HR 2.2, CI 2.08–3.85), with a median survival of 60.5 months for G1 and 46.5 months for G2. After 80 months, patients with G2 tumors appear to have better survival than G1 (lower grade) tumors; however, this was not statistically significant and is related to the small number of patients with this long (6.5 years) follow up. Lymph node status was not significant in univariate. Thus, incorporation of lymph node status as an independent factor was not helpful in development of the staging system; however, nodal involvement was included as M1 disease in the final model. M classification was clearly significant in both univariate and multivariate analysis (Fig. 1c; $p<0.0001$, HR 2.13, CI 1.8–3.42).

The combinations of tumor size (T1 vs. T2) and tumor grade (G1 vs. G2) in non-metastatic patients were compared using the log-rank test to form groups that differed in survival. The survival curves of the three TG groupings are displayed in Fig. 2a. We used M1 and N1 patients to define stage IV disease. Defining stage IV as M1 alone versus M1 or N1 did not alter the risk stratification curves significantly; hence, we elected to define stage IV as either M1 or N1 status, which is in line with other soft tissue sarcomas. Thus, the final staging system was determined as follows: *stage I was defined as T1-2, G1, M0; stage II as T1-2, G2, M0; stage III as T3G2M0 or Tany, G1, M1; and stage IV as* $T_{any,}$ *G2, M1*. Since nodal involvement was included as M1 disease, the system was designated a TGM staging system. The proposed staging system for PNET is displayed in

Table 2 Univariate analysis of factors affecting survival

	SEER data					Pancreatic Cancer Consortium				
	No of deaths	No of censors	*P*	Hazard ratio	95% CI	No of deaths	No of censors	*P*	Hazard ratio	95% CI
Age										
less than 65	1,725		–	–	–	4	104	–	–	–
65 and older	1914		<0.001	1.8	1.55–2.02	17	170	0.08	0.8	0.6–1.34
Gender										
Female	1517	2,706	–	–	–	7	134	–	–	–
Male	1938	3,307	0.3	0.9	0.5–1.9	14	140	0.7	0.95	0.4–2.0
Ethnicity										
White	1,151	2,066	–	–	–	18	256	–	–	–
Black	164	294	0.02	0.77	0.6–0.95	3	35	0.3	0.87	
Asian/Pacific Islander	104	187	0.08	0.78	0.6–1.03	0	6	0.03	2.4	
Year of diagnosis										
1973–1978	276	387	–	–	–					
1979–1984	305	411	<0.001	4.4	3.2–6.1					
1985–1990	425	571	<0.001	5.1	3.8–7.1					
1991–1996	659	917	<0.001	5.2	3.9–6.9	3	13	–	–	–
1997–2002	1143	1,916	<0.001	4.5	3.6–5.7	5	76	<0.001	1.1	1.0–5.4
2002–2006	809	2,245	<0.001	2.6	2.2–3.1	13	134	<0.001	2.5	2.1–8.5
2007–2009						0	61	<0.001	9.5	2.3–15.5
Surgery										
Yes	850	2,287	–	–	–	18	258	–	–	–
No	2,693	4,010	<0.001	3.4	2.9–3.9	3	16	0.02	2.04	1.9–3.4
Unknown	75	150	0.4			–	–	–	–	–
Location (M_0 pts)										
Head	1,536	2,648	–	–	–	11	120	–	–	–
Body	292	605	0.8	1.02	0.8–1.3	1	33	0.04	1.04	1.01–2.3
Tail	614	1,301	<0.001	1.5	1.3–1.8	8	129	0.009	1.5	1.2–3.4
Size of tumor (M_0 pts)										
<=3			–	–	–	5	140	–	–	–
>3			0.05	1.2	1-4.5	15	126	0.2	1.2	0.8–2.3
Grade (M0 pts)										
Grade I	411	1,063	–	–	–	8	161	–	–	–
Grade II	624	887	<0.0001	3.8	2.9–4.9	5	50	0.04	1.2	1.0–1.5
Lymph node involvement										
No	170	749	–	–	–	5	121	–	–	–
Yes	253	842	0.01	1.4	1.07–2.0	8	52	0.0001	1.4	1.2–1.7
Metastasis										
No	10	232	–	–	–	12	230	–	–	–
Yes	712	1,968	<0.001	4.06	2.94–5.7	9	45	0.0001	1.5	1.1–2.0

Table 4, along with hazard ratios and confidence intervals for each stage relative to stage I. This stage discrimination was then validated using the pancreatic cancer consortium data for stage 1–3, given the fact that there were only five patients staged as stage 4, and the numbers were too small to validate this stage. In a multivariate model including age, surgery, and date of diagnosis, the hazard ratios associated with each stage did not change. Survival differences between stages were statistically significant ($p<0.0001$; Fig. 2b). Median survival rates were stage 1, 55 months;

Table 3 Multivariate analysis of factors affecting survival in PNET in the SEER and Pancreatic Consortium Dataset

	SEER Dataset			Pancreatic Cancer Consortium		
	P	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI
Age						
Less than 65	–	–	–	–	–	–
65 and older	0.8825	1.01	0.85–3.22			
Surgery						
No	–	–	–	–	–	–
Yes	0.0001	0.49	0.21–0.56	0.3	1.3	0.8–1.8
Size of tumor						
<=3	–	–	–	–	–	–
>3	0.78	1.23	0.67–4.21	0.5	0.96	0.85–1.09
Grade						
Grade I–II	–	–	–	–	–	–
Grade III–IV	0.01	2.2	2.08–3.85	0.5	0.7	0.4–1.9
Metastasis						
No	–	–	–	–	–	–
Yes	<0.0001	2.13	1.80–3.42	0.01	1.3	1.1–1.7
	N=987 missing			*N*=30 missing		

stage 2, 50 months; stage 3:46 months; and stage 4:25 months.

Evaluation of Predictive Accuracy

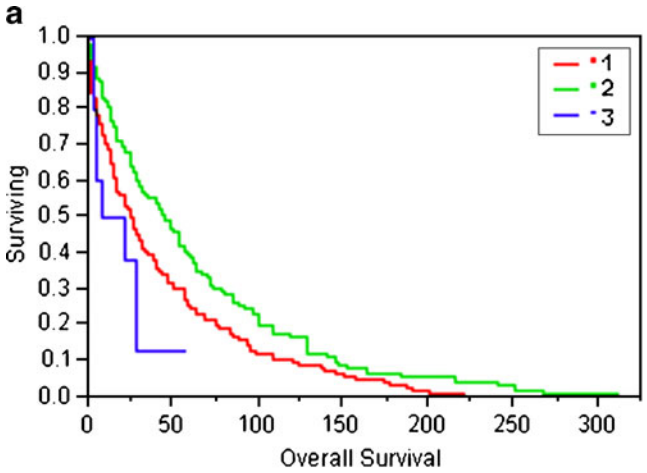
To evaluate how the predictive ability of the TGM staging system relative to the individual T, G, and M stages, we calculated summary measures of predictive accuracy specifically tailored for survival endpoints. The D statistic measures prognostic separation between risk groups,[12] the V statistic measures the proportion of variation which is explained by the risk groups,[13] and the AUC statistic gives a measure of concordance between survival outcome (evaluated here at 5 years) and the risk groups defined by the staging system.[14] Each measure along with 95% bootstrap confidence intervals is presented for the individual T (M0 pts), G (M0 pts), N, and M stages, along with the combined TG stages (M0 pts), TGM stages, and GM stages. For each measure, a higher value indicates greater predictive ability for survival. Among the individual T, G, N, and M stages, tumor grade and metastatic status are more predictive of survival compared to tumor size. The combined TG staging system offers improved predictive ability over the T stages along, although the improvement is somewhat slight (G, V=0.19 and AUC=0.69; TG, V=0.16 and AUC=0.69). The combined TGM staging system improves both the V (0.13) and AUC (0.77) statistics relative to the TG system, although the statistics are not directly comparable since the patient groups in each case do not directly overlap. The V (0.097) and AUC (0.69) statistics for the GM staging system are slightly lower compared to the TGM numbers for the whole sample, but still indicate decent predictive ability.

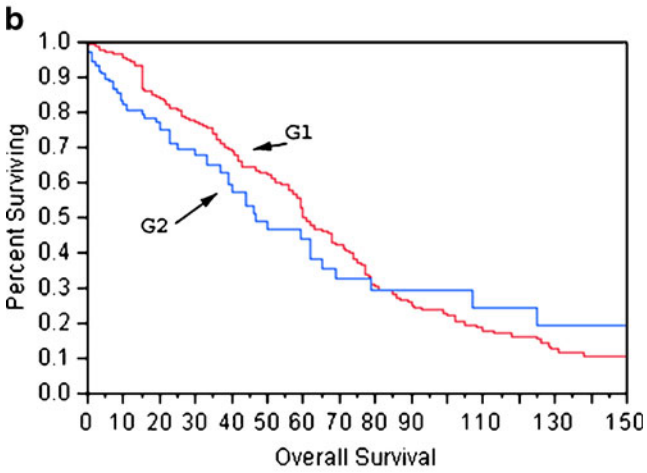
When both datasets are evaluated by the current American Joint Committee on Cancer (AJCC) staging version 6.0 TNM staging system, there is no stage discrimination (Fig. 2c), with similar survivals across all stages, median overall survival stage 1–16 months, stage 2–15 months, stage 3–18 months, and stage 4–16 months (p=0.5).

Discussion

Currently, there is not an accepted staging system in the USA for PNET. There has been a current recommendation to utilize the AJCC staging version 6.0 for pancreatic adenocarcinoma as a guide.[10] In this study, we evaluated the ability to initiate a predictive staging system for patients with PNET. There is good stage-specific survival discrimination with tumor size and invasion, grade,z and distant metastasis, all being independent predictors of survival.

A PNET pathologic and staging system has been adopted and optimized by the World Health Organization in 2000 and 2005, respectively.[4,9] This classification system accurately recognizes the clinical, molecular, and histopathologic characteristics of PNET tumors. It distinguishes between highly differentiated, mostly benign (carcinoid) endocrine tumors with an excellent prognosis, well-differentiated neuroendocrine carcinomas with a low





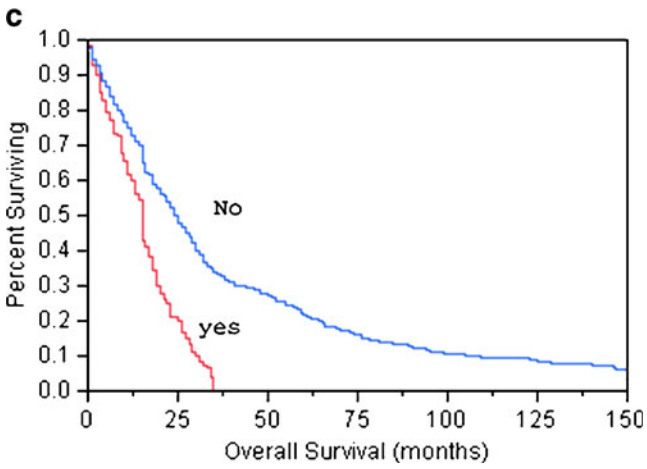
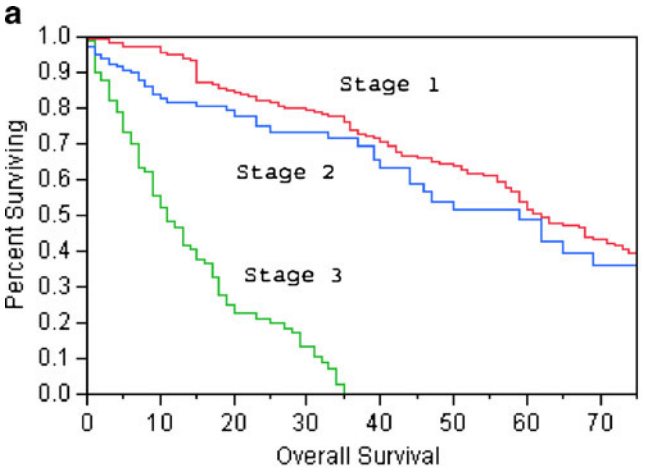
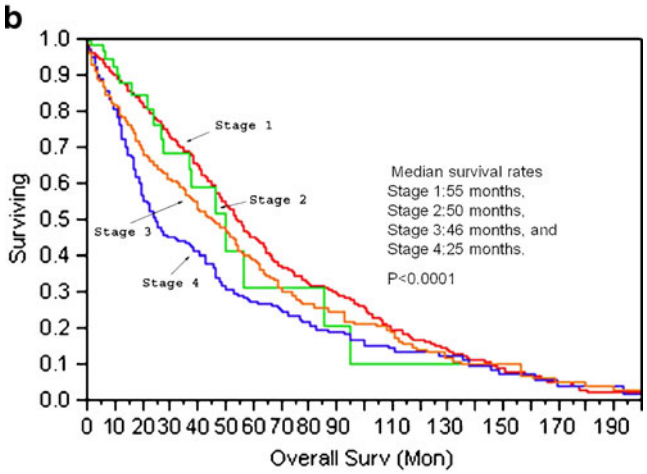


Fig. 1 **a** Overall survival by size and degree of invasion for T1, T2, and T3 staged tumors. **b** Overall survival by grade of tumor for G1 and G2 staged tumors. **c** Overall survival by presence of absence of metastatic disease





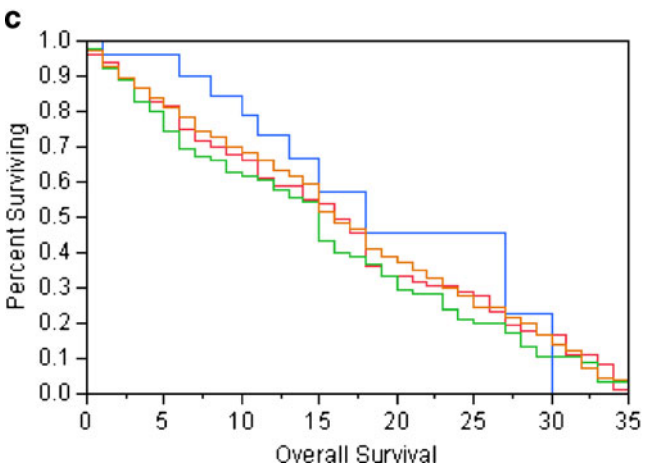


Fig. 2 **a** Overall survival by size, degree of invasion, and grade for non-metastatic PNET for stage 1, stage 2, and stage 3 tumors. **b** Overall survival of proposed staging system for stage 1 through stage IV PNET tumors. **c** Overall survival of PNET tumors staged by the proposed AJCC TNM version 6 staging system

malignant potential and favorable prognosis and poorly differentiated, mostly small cell highly malignant neuroendocrine carcinomas with a worse overall prognosis. There have been recommendations from the World Health Organization that this is an effective classification scheme and that clear guidelines do exist to begin to assist clinicians in the management of pancreatic neuroendocrine tumors. One of the criticisms of the World Health

Table 4 Multivariate Analysis Evaluation of the proposed TGM staging system for both the SEER and Pancreatic Consortium Dataset

Proposed TGM staging system for PNET by SEER dataset							
	T	G	M	Number (%)	*P*	Hazard ratio	95% CI
Stage I	T1–2	G1	M_0	560	–	–	–
Stage II	T1–2	G2	M_0	145	0.02	5.6	1.23–9.75
Stage III	T3 Tany	G2 G1	M_0 M1	1,160	<0.0001	1.7	1.3–6.51
Stage IV	Any T	G2	M_1	1,472	<0.0001	2.2	1.98–6.31
N=3,337							
Proposed TGM staging system for PNET by Pancreatic Consortium Dataset							
Stage I	T1–2	G1	M_0	222	–	–	–
Stage II	T1–2	G2	M_0	9	0.09	0.8	0.34–1.82
Stage III	T3 Tany	G2 G1	M_0 M1	55	0.03	1.2	1.06–2.6
Stage IV	Any T	G2	M_1	5	–	–	–
N=291							

Organization classification has been the histologic system that has been dependent on pathologic inter-observer interpretation that has the problems of having significant variability from hospital-to-hospital.[23] In addition in the past, the distinction between benign and malignant PNET was unclear and, thus, was not appropriately staged. A staging system that takes into account both the less ambiguous T and M stage in combination with a G (grade and degree of malignant potential) could be more easily adapted and, thus, applied university since tumor size, nodal, and distant metastasis are measured more objectively and grade in combination with mitoses per high powered field and Ki-67 staining could overcome these potential concerns. Currently, it is of utmost importance to establish a universal staging system because of the significantly growing interest in multi-institutional trials in which accurate staging across multiple centers is needed and required in order to appropriately analyze patients in both metastatic adjuvant and neoadjuvant studies.

Traditional predictors of outcome being tumor size, nodal status, and presence of distant metastasis have not been accurate and powerful predictors to define the biology of PNET tumors or most other neuroendocrine tumors.[24–29] Because of the relative rarity of PNET tumors, up to 1997, there were no current datasets that had the accurate power to establish a robust staging algorithm. As a result, earlier studies prior to that and, more importantly, using data from those earlier years, have led to conflicting data about the importance of tumor size, nodal status, and distant metastasis. Some studies have suggested that liver metastasis is the only independent predictor of survival and that tumor size is a potential surrogate marker of liver metastasis. In contrast, Kazanjian et al.[30] in an evaluation of 70 PNET patients reported that positive lymph nodes in the presence of liver metastasis could not affect survival. In our current staging system which controlled for patient gender, age, tumor location, metastasis incorporating both lymph nodes and liver metastasis was a strong independent predictor of survival. Similarly, tumor size alone or lymph node status alone was not a predictor of survival. However, when tumor size is incorporated with degree of invasion, a more robust and significant prognostic marker is obtained. Current pathologic standards do not obviate the requirement of the degree invasion to be commented upon which was one of the limitations in the SEER dataset that was evaluated is.

Limitations of the SEER-based registry should be taken into account when interpreting these results. Since there are limitations surrounding the SEER-based registry predominantly underreporting and incomplete data of adjuvant therapy, lack of information on patient co-morbidity, and whether the surgical margins were positive or negative in the patients treated with pancreatic neuroendocrine tumors. The lack of co-morbidities as well as margin status is a potential limitation related to this SEER-based registry that we believe that we have overcome with the more robust and more complete dataset related to the 300-patient validation model. However, with the lack of a statistical significant predictor of overall survival in this 300-patient dataset further confirms the challenges in PNET staging given the more favorable biology of this disease and the fact that a majority of this subset underwent resection.

Conclusion

In conclusion, our data indicate that a new clinical and pathologic staging system is needed for PNET tumors. Standardizing this staging system beyond just the recom-

mendation of the revised AJCC 6th edition is required in order to accurately and prospectively validates this initial version of a clinically relevant staging system. Standardization of pathologic evaluation and diagnosis is needed which has been easily achieved in other malignancies to where molecular markers have been incorporated into current AJCC staging systems. Having an effective staging system for PNET tumors is essential to assist in the adjuvant treatment decisions offered to patients to just importantly define patients who should be offered adjuvant therapy but more importantly to define patients who should not have to undergo adjuvant therapies with the potential risks that can occur in those treatments.

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ORIGINAL ARTICLE

Complex Pancreatic Surgery: Safety and Feasibility in the Community Setting

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Abstract
Introduction Advances in technology, innovative surgical procedures, and enhanced perioperative care have allowed more patients to be considered for complex pancreatic surgery. Published reports on the outcomes of pancreatic surgery performed at high volume tertiary referral centers have yielded excellent results. However, similar outcome and safety data from community hospitals is limited.
Material and Methods Consecutive complex pancreatic surgery performed by a single surgeon from December 2004 to December 2009 formed the study group. Factors analyzed included patient demographics, operative procedure, operative time, length of hospital stay, pathology, and 30-day morbidity and mortality.
Results One hundred and nine consecutive patients underwent pancreatic surgery, with a mean patient age of 62.4± 15.2 years. Eighty-three patients (76.1%) underwent definitive surgical procedure and 26 patients (23.9%) had palliative bypass after failed palliative biliary stenting. The mean operative time was 229±109 min, the mean length of stay was 8.6± 6.5 days and 24 (22.0%) patients had surgical complications.
Conclusion Complex pancreatic surgery can be performed safely at high-volume tertiary community hospitals with excellent outcomes comparable to tertiary academic centers. In the ongoing debate about the need for mandatory referral of complex surgical procedures, tertiary community hospitals with well-determined outcomes should be included.

Keywords Pancreatic surgery · Community hospital · Surgical outcomes

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Introduction

The natural history of pancreatic cancer, including its clinical quiescence and aggressive molecular epidemiology, plays an important role in its lethality. Surgical resection is the only curative option for patients with pancreatic cancer, yet it is associated with a high morbidity rate and disappointing 5-year survival rates of 10–29%.[1] Up to 85% of patients with pancreatic cancer are unresectable at the time of diagnosis, and the significant morbidity and limited survival rates associated with complex pancreatic surgery has led some to question the rationale of radical operations for pancreatic cancer.[2–8] In view of these facts, and given the complexity of surgical procedures required to treat pancreatic cancer, regionalization of complex pancreatic procedures such as pancreaticoduodenectomy (PD), has been proposed in an effort to optimize patient outcomes.[9–15] Previous reports have suggested that hospital procedure volume is associated with superior clinical outcomes in

patients undergoing complex operations, such as pancreatectomy and esophagectomy, as well as less complex procedures such as lumpectomy and colectomy.[12,16–18] Yet, contrary to these claims, others have reported no association between hospital volume and clinical outcome.[19] For example, Enzinger et al.[19] examined the relationship between hospital volume and clinical outcomes following gastrectomy among 306 US hospitals, and found no significant differences in 5-year overall survival or disease-free survival (short-term perioperative morbidity and mortality was not addressed) between low, moderate, and high volume hospitals.

Over the last two decades, significant advances in perioperative evaluation and patient selection, improved surgical techniques with combined regional and general anesthesia, and standard perioperative care and management have significantly reduced the mortality associated with pancreatic resection.[1] While sporadic reports of excellent clinical outcomes for PD performed at low volume hospitals have emerged, they are few in number.[9,19,20] Focusing exclusively on hospital volume, few studies have addressed the question as to whether patient outcomes are more dependent upon the surgeon's experience/training or the hospital setting in which they are performed. In order to address this question, we sought to analyze our early experience with complex pancreatic surgery performed by a single fellowship-trained hepatobiliary and pancreatic surgeon after the establishment of a community-based hepatobiliary and pancreatic surgery Center of Excellence.

Materials and Methods

Over 350 patients with complex pancreatic problems were evaluated by the surgical oncology service at the Saint Barnabas Medical Center (SBMC), Livingston, New Jersey, from December 2004 and December 2009. Patients with hepatobiliary diseases were excluded from this analysis. One hundred and nine consecutive patients requiring pancreatic surgery formed the study group. All patients who underwent pancreatic resection had preoperative imaging with triple phase contrast-enhanced computed tomography or magnetic resonance imaging, along with positron emission tomography scans when appropriate. Diagnostic laparoscopy was performed in all malignant cases in order to exclude the presence of occult or disseminated intra abdominal disease. Intraoperative ultrasound was used selectively.

SBMC is a 641-bed tertiary-care referral hospital for a network of six community hospitals. The hospital performs more than 29,000 surgical cases per year and has an Accreditation Council for Graduate Medical Education-approved general surgery residency program. SBMC offers a comprehensive cancer program including one of the largest radiation oncology programs in northern New Jersey, treating more than 1,000 patients yearly. Furthermore, the facility operates a highly specialized Gastrointestinal Cancer Program, offering advanced therapeutic alternatives including: selective internal radiation therapy for inoperable liver cancer, radiofrequency and microwave ablation, heated intraperitoneal chemotherapy for advanced tumors of the peritoneal cavity, and robotic surgery. All cases are subject to multi-discipline review and analysis prior to initiation of therapy.

Data from patients who underwent surgical intervention were collected prospectively from medical records, outpatient charts, lab records, and pathology reports and entered into a Microsoft Excel database (Microsoft Corporation™, Redmond, WA, USA). Pre-operative evaluation included physical examination, medical/cardiac clearance, pertinent laboratory and imaging studies, and tumor markers. The operative procedure, operative time, estimated blood loss, length of hospital stay, and 30-day morbidity and mortality were analyzed. Procedures involving pancreatic resection had surgical margin analysis reported as R0 (negative), R1 (microscopically positive), and R2 (grossly positive). The association of continuous variables was statistically analyzed by the Student *t* test.

Results

Demographics One hundred and nine patients underwent complex pancreatic surgical procedures for malignant, premalignant, or benign conditions. The mean age of all patients was 62.4±15.2 (range: 33–87) with a male to female ratio of 1.4:1. Sixty-seven (61.5%) patients had pre-existing comorbidities (Table 1) with hypertension being the most common (*N*=62, 56.9%). Additional common comorbidities included diabetes mellitus (*N*=30, 27.5%), coronary artery disease (*N*=20, 18.3%) and chronic obstructive pulmonary disease (*N*=3, 2.8%). Mean laboratory values for pre-operative bilirubin, pre-operative albumin, post-operative bilirubin, and post-operative albumin were 3.1±5.5, 3.9±0.9, 1.5±1.9, and 3.1±0.7 mg/dL, respectively.

Diagnoses Sixty-one patients (56.0%) had pancreatic pathology located within the head or uncinate process of the pancreas 20 patients (18.3%) had pathology in the ampullary/periampullary region, 19 patients (17.4%) had pathology in the body, and nine patients (8.3%) had pathology in the tail of the pancreas (Table 2).

The most common pancreatic pathology identified was 55 (50.5%) cases of ductal adenocarcinoma and 12 (11.0%)

Table 1 Comordities among 109 patients undergoing complex pancreatic procedures

All Patients (*N*=109)		PD Patients (*N*=40)	
Comorbidity	*N* (%)	Comorbidity	*N* (%)
HTN	62 (56.9)	HTN	24 (60.0)
DM	30 (27.5)	DM	9 (22.5)
CAD	20 (18.3)	CAD	8 (20.0)
COPD	3 (2.8)	COPD	1 (2.5)

Sixty seven patients (61.5%) undergoing complex pancreatic procedures had a comorbidity, with hypertension (*N*=62, 56.9%) and diabetes mellitus (*N*=30, 27.5%) being the most common. Twenty-six patients (65.0%) undergoing PD had a comorbidity, with hypertension (*N*=24, 60.0%) and diabetes mellitus (*N*=9, 22.5%) being the most common

PD Pancreaticoduodenectomy, *HTN* Hypertension, *DM* Diabetes mellitus, *CAD* Coronary artery disease, *COPD* Chronic obstructive airway disease

cases of intraductal papillary mucinous tumors. There were six cases (5.5%) of neuroendocrine tumor (NET), five cases (4.6%) of focal sclerosing pancreatitis, three cases (2.8%) of solid pseudo-papillary tumors of the pancreas, two cases (1.8%) each of lymphoepithelial cyst of the pancreas, pancreatic pseudocyst and duodenal villous adenoma, in addition to isolated cases (0.9%) of a mucinous carcinoma peritonei, necrotizing pancreatitis, and ruptured splenic artery aneurysm (Table 3). Ampullary/periampullary pathology (*N*=19, 17.4%) included nine cases (8.3%) of adenocarcinoma, four cases (3.7%) of tubulovillus adenoma, three cases (2.8%) of cholangiocarcinoma, two cases (1.8%) of D2-duodenal cancer, and a single case (0.9%) of duodenal ulcer with obstruction. The head of the pancreas was the most common site for pancreatic adenocarcinoma consisting of 19 (34.5%) cases. Among the six cases (5.5%) of NET, three involved the body (50.0%), two involved the head (33.3%), and one involved the tail (16.7%) of the pancreas.

Surgical Procedures Eighty-three patients (76.1%) underwent a definitive surgical procedure and 26 patients

Table 2 Location of the resected pancreatic pathology

Anatomical location	*N* (%)
Head/uncinate process	61 (56.0)
[a]Ampullary/periampullary	20 (18.3)
Body	19 (17.4)
Tail	9 (8.3)

The head of the pancreas (*N*=61, 56.0%) was the most common location for resected pancreatic lesions

[a] Includes duodenal, ampullary and distal common bile duct tumors

Table 3 Histopathology of pancreatic resections

Pancreatic pathology	*N* (%)
Adenocarcinoma	55 (50.5)
IMPT	12 (11.0)
NET	6 (5.5)
Focal sclerosing pancreatitis	5 (4.6)
Pseudo papillary tumor	3 (2.8)
Lymphoepithelial cyst	2 (1.8)
Pseudocyst	2 (1.8)
Adenoma	2 (1.8)
Necrotizing pancreatitis	1 (0.9)
Mucinous carcinoma peritonei	1 (0.9)
Ruptured splenic artery aneurysm	1 (0.9)
Ampullary/periampullary pathology	
Adenocarcinoma	9 (8.3)
Tubulovillus adenoma	4 (3.7)
Cholangiocarcinoma	3 (2.8)
D2-Duodenal cancer	2 (1.8)
Ulcer and stricture	1 (0.9)

The most common resected pathology were pancreatic adenocarcinoma (*N*=55, 50.5%), IMPT (*N*=12, 11.0%), ampullary/periampullary adenocarcinoma (*N*=9, 8.3%), NET (*N*=6, 5.5%), and focal sclerosing pancreatitis (*N*=5, 4.6%)

IPMT Intraductal papillary mucinous tumor, *NET* Neuro endocrine tumor

(23.9%) had palliative procedures and biopsy after failed palliative biliary stenting for obstructive jaundice or as a result the determination of unresectability at the time of laparotomy (Table 4). Forty patients underwent PD (36.7%) including three concomitant portal vein resections and a single case of concomitant partial hepatectomy (segments 6 and 8) for metastatic neuroendocrine tumor. Among 32 patients who underwent PD for malignant disease, 11 patients (34.4%) had stage I disease, 17 patients (53.1%) had stage II disease, and four patients (12.5%) had stage III disease. Four patients underwent total pancreatectomy (3.7%), with one patient undergoing a simultaneous resection of hepatic segments 2 and 6 for metastatic neuroendocrine tumor of the head and body. One patient undergoing total pancreatectomy had pancreatic cancer in the uncinate process and pre-existing dorsal agenesis of the pancreas. Fifteen patients underwent subtotal pancreatectomy (13.8%) and nine patients (8.3%) underwent distal pancreatectomy. Among this group, six patients underwent open resection and three patients underwent laparoscopic resection. Additional procedures included: diagnostic laparoscopy and pancreatic biopsy (*N*=7, 6.4%), enteric drainage of a pseudocyst (*N*=4, 3.7%), tumor enucleation for solid pseudopapillary tumor (*N*=2, 1.8%) and one case (0.9%) each of a Puestow procedure and pancreatic sparing duodenectomy. Among the 68 cases of pancreatic resection,

Table 4 Pancreatic procedures performed in 109 consecutive patients

Procedure	*N* (%)
Pancreaticoduodenectomy	40 (36.7)
Palliative bypass and biopsy	26 (23.9)
Subtotal pancreatectomy	15 (13.8)
Distal pancreatectomy	9 (8.3)
Diagnostic laparoscopy and biopsy	7 (6.4)
Total pancreatectomy	4 (3.7)
Internal Pseudocyst enteric drainage	4 (3.7)
Pancreatic Enucleation	2 (1.8)
Puestow procedure	1 (0.9)
Pancreas sparing duodenectomy	1 (0.9)

Pancreatic procedures performed included pancreaticoduodenectomy (*N*=40, 36.7%), palliative bypass and biopsy (*N*=26, 23.9%), subtotal pancreatectomy (*N*=15, 13.8%) and distal pancreatectomy (*N*=9, 8.3%)

67 patients (98.5%) achieved a R0 margin, one patient (1.5%) had R1 margins, and no patient had R2 margins of resection.

Peri-operative Results Mean operative time for all pancreatic resection patients was 229±109 vs. 326±64 min for the PD patients. Estimated blood loss for all pancreatic resection patients was 242±272 vs. 248±155 mL for PD patients. Hospital LOS for all pancreatic resection patients was 8.6±6.5 vs. 11.2±7.7 days for PD patients. There was one peri-operative 30-day mortality due to cardiac arrest on postoperative day 12 in a 75-year-old female who underwent a palliative double bypass procedure for unresectable pancreatic adenocarcinoma. Twenty-four (22.0%) of 109 patients suffered peri-operative complications (Table 5), with the most common complication being wound infection (*N*=6, 5.5%). Among the 40 patients undergoing PD, 15 patients (37.5%) suffered complications, with the most common complication being wound infection (*N*=5, 12.5%). Five patients (4.6%) underwent reoperation, three (7.5%) of which had undergone PD.

Discussion

The converse relationship between hospital volume and postoperative mortality among patients undergoing complex surgical procedures, including pancreatic resection, have been extensively examined and documented.[9,21,22] The Donabedian Model is a framework for quality-of-care,[23] developed to define, measure, and categorize quality in healthcare delivery. This model includes: structure (where the care is delivered), process (evaluating medical practice), and outcome (impact of care on health).[23] The outcome measure, which reflects how a unique patient fares following some form of medical intervention is the most difficult to measure. Although hospital volume is simple to measure and may be associated with improved patient outcomes for pancreatic resection and other procedures,[21–26] it may not be the sole determinant of outcome.

Table 5 Postoperative complications in 109 patients undergoing pancreatic surgery

All patients (*N*=109)		PD patients (*N*=40)	
Complication	*N* (%)	Complication	*N* (%)
Wound infection	6 (5.5)	Wound infection	5 (12.5)
Reoperation	5 (4.6)	Reoperation	3 (7.5)
Cholangitis/Sepsis	4 (3.7)	Cholangitis/sepsis	3 (7.5)
RS Infection/PE	3 (2.8)	RS infection/PE	2 (5.0)
Intra-abdominal abscess	2 (1.8)	Intra-abdominal abscess	1 (2.5)
Biliary leak	2 (1.8)	Biliary leak	2 (5.0)
Gastric outlet obstruction	2 (1.8)	Gastric outlet obstruction	1 (2.5)
Angina	1 (0.9)	Angina	0
Death	1 (0.9)	Death	0
Total	26 (23.9)[a]	Total	17 (42.5)[b]

Twenty-eight patients (25.7%) undergoing pancreatic surgery had 32 (29.4%) complications, with wound infection (*N*=6, 5.5%) being the most common. Sixteen patients (40.0%) undergoing PD had 19 (47.5%) complications, with wound infection (*N*=5, 12.5%) and cholangitis/sepsis (*N*=3, 7.5%) being the most common

PD pancreaticoduodenectomy, *RS* Respiratory infection, *GI* Gastro intestinal, *PE* Pulmonary embolism

[a] 26 complications occurring in 24 patients (22.0%)

[b] 17 complications occurring in 15 patients (37.5%)

Many high-volume pancreatic centers throughout the country have reported substantial reduction in hospital mortality over the last 10 years,[15,26] which is attributable to a variety of factors. Factors most often cited include the volume of procedures performed, perioperative care and nutrition, and strict adherence to critical care and perioperative pathways.[12,22,24] Mukherjee et al.[15] evaluated the impact of the UK Cancer Outcome Guidelines (COG) among 140 patients who underwent PD between 1999 and 2006. The COG was introduced in the UK in 1999 and was subsequently implemented in 2003. The institution of these guidelines led to the centralization of cancer services, including upper gastrointestinal cancer services and was restricted to tertiary referral centers.[27] In the pre-COG era (1999–2002) there were 41 PD performed compared to 99 performed in the post-COG era (2003–2006).[15] The authors reported a trend towards decreased mortality (9.7–5.0%, $p<0.448$) and morbidity (41.6–35.3%, $p<0.565$), concluding that the COG implementation lead to increased PD volume, higher staffing levels, and a trend towards better outcomes.[15]

Birkmeyer et al.[11] evaluated outcomes based on hospital volume among Medicare patients undergoing PD for pancreatic cancer and reported that more than 50% of these patients received care at hospitals performing fewer than two procedures per year. They evaluated outcomes based on four hospital volume categories, which included very low (<1 case per year), low (1–1.99 cases per year), medium (2–4.99 cases per year), and high volume hospitals (>5 cases per year). These authors reported that in-hospital mortality rates at low and very low-volume hospitals were three- to fourfold higher than at high-volume hospitals (12% and 16%, respectively, vs. 4%, $p<0.001$) and concluded that hospital volume is an important factor in surgical outcomes for PD.[11]

In an attempt to identify factors affecting outcomes after complex pancreatic surgery as well as to trace the evolution of a procedure, Cameron et al.[26] reported their 30-year experience involving 1,000 consecutive PD at the Johns Hopkins Hospital. They noted that advancements over the last three decades in imaging, intraoperative anesthesia, and peri-operative care were major factors resulting in improved patient outcomes and decreased perioperative morbidity and mortality. These authors also noted a 50% reduction in their operative time and a 30% reduction in estimated operative blood loss over the study period.[26]

Meguid et al.[28] conducted a retrospective analysis of 7,558 patients who underwent pancreatic resection from the Nationwide Inpatient Sample (20% sample of patients in the US from 1998 to 2003). This study reported a median annual institution pancreatic resection volume of 15 cases, mean in-hospital mortality of 7.6% and noted that based on a goodness-of-fit analysis, a minimum of 19 pancreatic resections per year is required to qualify as a high volume center.[28] However, they concluded that a volume cutoff for pancreatic surgery was arbitrary, as a difference in

Table 6 Comparative analysis of major tertiary academic centers and community hospital complex pancreatic surgical series

	Chamberlain et al. [8] SBMC	Hoshal et al.[30] SJMH	Mukherjee et al.[15] RLH	Schell et al.[25] ML	Cameron et al.[26] JHH
Number of Patients	109	134	140	301	1000
Time	12/2004–12/2009	1985–2002	1999–2006	1989–2003	1969–2003
Published	Present Study	2004	2009	2008	2006
Surgeon	Single	Single	Multiple	Multiple	Single
M:F	1.4:1	1.2:1	1.06:1	1:01	1.2:1
Mean Age	62.4±15.2	60	64	61	63.4
Comorbidity (%)	67 (61.5)	N/A	N/A	N/A	N/A
Mean EBL	242±272	950	N/A	1167±1411	700
Mean Operative Time (min)	229±109	348	N/A	402±120	330
Operative Mortality	0	0	N/A	N/A	0
30-Day Mortality	0.9%	3.7%	2.8%	4%	1%
Complications (%)	24 (22.0)	38 (28.0)	52 (37.1)	177 (58.8)	410 (41.0)
Reoperation (%)	5 (4.6)	5 (3.7)	N/A	22 (7.3)	21 (2.1)
Mean LOS (days)	8.6±6.5	9	16	16.1±23.5	9

Outcomes comparing community-based reports ([8] and [30]) and tertiary academic centers are provided ([15, 25], and [26]). Results between these five studies are comparable in all outcome modalities, including EBL, operative mortality, 30-day mortality, complications, and length of stay

SBMC Saint Barnabas Medical Center, Livingston, New Jersey, *SJMH* Saint Joseph Mercy Hospital, Anne Arbor, Michigan, *RLH* Royal London Hospital, London, UK, *ML* Moffit-Long Hospital, San Francisco, California, *JHH* Johns Hopkins Hospital, Baltimore, Maryland, *N/A* not available, *EBL* estimated blood loss, *LOS* length of stay

perioperative mortality was observed regardless of the volume cutoff used.[28] Riall et al.[29] reviewed the Texas Hospital Inpatient Discharge Database from 1999 to 2005 and identified 12 high-volume hospitals for pancreatic resection (>11 cases/year). Among these hospitals, there was significant variability in mortality, duration of stay, need for ongoing nursing care, operation within 24 h of admission, and hospital cost per patient visit.[29] They concluded that significant variability in outcomes occurred even among high-volume providers and reasoned that individual hospital differences likely accounted for much of the variability not explained by hospital volume.[29]

In addition to variability in outcomes at high-volume centers, a number of studies from low-volume and community-based hospitals have reported excellent outcomes. Schell et al.[25] performed a comparative outcome analysis of 369 patients who underwent PD at the University of California, San Francisco affiliated hospitals between October 1989 and June 2003. They noted that while high-volume centers did attain excellent surgical outcomes, smaller and lower-volume hospitals achieved similar surgical outcomes provided they import expertise and implement care pathways. The low-volume hospital group consisted of community-based hospitals and county general hospitals that performed an average of one PD per year, and a Veterans Affairs Medical Center that performed three PD per year. The high volume tertiary hospitals averaged 23 PD per year. They found no difference in regards to morbidity and complications between the groups (high volume, 58.8% vs. low volume, 60.3%; $p<0.579$). Moreover, the perioperative mortality rates for patients undergoing PD were approximately 4% in both groups, with no significant difference in 5-year survival rates (high-volume hospital 19% versus 18.3% for low-volume hospital group, $p<0.096$).[25]

The largest published community based study for complex pancreatic surgery to date is by Hoshal et al.[30] who reported their experience with 134 consecutive PD performed between 1985 and 2002. They reported an overall mortality of 3.7%, identified 60 major complications occurring in 38 patients (28%) and the need for reoperation in five patients (3.7%). The volume of pancreatic cases at the tertiary community hospital reported in the current report places us in a high volume group with an average of more than 20 complex pancreatic cases per year. All 109 cases in the current study were performed by a single surgeon (RSC), which provides for uniformity in operative technique and post-operative management. *We acknowledge that our surgical margin data may be greater than expected, however*, as Table 6 demonstrates, the results achieved are comparable to those published by high-volume university hospitals [15,25,26] and other community hospitals.[30] These results provide more data to support the notion that additional factors such as surgical experience and proper patient selection may be more determinant of outcome than the absolute number of cases and/or the size of the hospital where the cases are performed.

Although hospital volume is easy to measure, it is not reliable as the sole measure of quality or outcomes after pancreatic surgery. The idea that volume alone can be a proxy to define centers of surgical excellence is an imperfect rationale. Despite emerging reports of excellent surgical outcomes for many complex procedures performed at community based medical centers, a movement towards establishing volume-based referral centers for certain surgical (including pancreatic) procedures continue to be pushed.[20,31,32] The Leapfrog Group, comprised of health-care purchasers and providers representing 33 million patients, is perhaps the most vocal group promoting volume-based referral. In order to concentrate patient care in high volume hospitals, the Leapfrog initiative has set annual hospital volume thresholds for a number of different surgical procedures including: coronary artery bypass graft (450 cases), coronary angioplasties (400 cases), abdominal aortic aneurysm repairs (50 cases), aortic valve replacements (120 cases), esophagectomies (13 cases), pancreatic resections (11 cases), and bariatric surgeries (125 cases).[32] Whether there should be regionalization of major hepato-biliary–pancreatic procedures to academic centers of excellence is being similarly debated. Tertiary community based hospitals with excellent results should be included in any proposed mandatory referral system. To date, there remains no optimum combination of number of procedures, years of training, or other factors that assure good outcomes in surgery. Proper patient selection, in combination with a competent surgeon with adequate training, excellent critical care, and interdisciplinary support is the only means of optimizing patient outcome regardless of the hospital setting or procedure volume.

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ORIGINAL ARTICLE

Contemporary Single-Center Surgical Experiences in Redo Procedures of the Pancreas: Improved Outcome and Reduction of Operative Risk

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Abstract
Introduction Redo procedures of the pancreas are complex operations associated with significant morbidity and mortality rates. The operative risk may be minimised when indications for redo procedure are well reflected and operation is performed by an experienced surgeon. The aim of this study was to confirm this hypothesis evaluating our experiences with redo procedures.
Methods We reviewed 28 patients (mean age of 54 years; range 11–75 years) undergoing a redo procedure of the pancreas from January 2004 to June 2008 at our hospital. The term redo procedure was defined as a pancreatic reoperation that was carried out after preceding pancreatic surgery. Relaparotomies following acute complications after pancreatic surgery were not taken into consideration.
Results The following parameters were evaluated: median operative time 332 min (range 160–730 min), median intraoperative blood loss 625 ml (range 300–2,800 ml), median postoperative stay on Intensive Care Unit 20 h (range 0–112 h), median postoperative hospital stay 15 days (range 7–98), morbidity (14%), and mortality (3.6%).
Conclusions Redo procedures of the pancreas can be performed with low complication rates. In order to achieve a satisfactory outcome, the indication of redo procedures has to be well reflected, and operation may be performed by specialised and experienced surgeons.

Keywords Pancreatic surgery · Redo procedure · Chronic pancreatitis · Operative risk · Outcome

Introduction

Some patients who underwent previous pancreatic surgery may require reoperation for the following indications: malignant pancreatic lesion, persistent symptomatic chronic pancreatitis with intractable pain, undrained main duct segments, ductal or biliary stenosis, or an inflammatory mass.[1]

Redo procedures of the pancreas are very complex operations that are associated with significant morbidity and mortality rates. Adhesions and previous operations may create a hostile abdomen with an altered anatomy and consecutive difficulties accessing the pancreatic remnant. Moreover, the risk for intraoperative and postoperative complications is substantial. Therefore, the indication for reoperation has to be well reflected. Nevertheless, in some cases, reoperation is inevitable. Particularly, in patients with chronic pancreatitis, symptoms not responding to medical treatment can remain or develop again after drainage or resection surgery, indicating progress of the disease or failure of the primary operative procedures, respectively.

There exist several studies about re-explorations in patients with malignant tumours of the pancreas. In these

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patients, previous non-resectional surgery or palliative surgery (bilioenteric bypass, gastroenteric bypass) was carried out. Data demonstrate that in selected patients, reoperative pancreaticoduodenectomy can be performed safely and may result in prolonged survival.[2–5] Kleff et al.[6] reviewed 30 patients who underwent surgery for recurrent disease after initially curative (R0/R1) resection of pancreatic ductal adenocarcinoma. There was a tendency of increased median survival in these patients (17 months) compared with a group of patients who only received an exploration or bypass (9.4 months). However, this difference was not significant. The in-hospital morbidity and mortality rate of resected patients was 20% and 6.7% compared with 13.3% and 0% of patients who underwent only exploration or a palliative bypass.

Only few studies exist regarding the outcome of reoperative surgery of the pancreas in patients with chronic pancreatitis. Reviewing the current literature, 7–23% of patients with chronic pancreatitis, who underwent an operation of the pancreas, require reoperation due to complications of chronic pancreatitis in the pancreatic remnant.[7–9] Markowitz et al. [10] achieved acceptable outcomes in 14 patients who underwent reoperation following pancreaticojejunostomy for chronic pancreatitis. One patient died of pancreatic cancer. Ten of the other 13 patients had a satisfactory-to-excellent relief of pain, with resumption of a normal level of function. Of the ten previously euglycemic patients, eight remained free of diabetes mellitus. Prinz et al. [11] stated that in patients with recurrent pain and chronic pancreatitis after pancreaticojejunostomy, operative redrainage could provide pain relief with minimal loss of endocrine and exocrine function. In 14 patients, redo procedures were performed. Postoperatively, one patient died from hemorrhage, and four patients had complications. Schnelldorfer et al.[1] analysed the outcome of 74 patients with chronic pancreatitis, who underwent reoperation after failed prior pancreatic surgery. The overall complication rate, the incidence of major complications, and the postoperative mortality rate were similar compared to de novo procedure. Six patients died after reoperation (8%) due to major complications (four patients) or other complications that were not associated with reoperation (two patients).

In our opinion, the operative risk can be minimised when the indication for redo procedure is well reflected and operation is performed by an experienced surgeon. The aim of this study was to confirm this postulation evaluating our experiences with pancreatic redo procedures during the last 4 and 1/2 years after having launched a new pancreatic program in our hospital starting January 2004. Indications for redo procedures, intraoperative, and postoperative parameters as well as the postoperative outcome of each patient including endocrine and exocrine function as well as consumption of analgesics were evaluated.

Material and Methods

From January 2004 to June 2008, 950 patients underwent operations on the pancreas at our hospital, which is a specialised Pancreas Centre. In 28 of these patients (11 women; 17 men), in the mean age of 54 years (range 11–75 years), redo procedures of the pancreas were carried out. This corresponds to a relatively low rate of redo procedures of 3%.

Definition of the Term "Redo Procedure"

For this study, we defined the term redo procedure as follows: reoperation of the pancreas that was done several months or years after preceding pancreatic surgery irrespective of the primary diagnosis. Relaparotomies following acute complications after pancreatic surgery as, e.g., postoperative hemorrhage or anastomotic insufficiency as well as for recurrent pancreatic cancer were not taken into consideration.

Indication of Primary Pancreatic Operation

The majority of prior pancreatic operations were performed at other medical centers (21 operations; 75%). In only seven patients (25%), primary pancreatic surgery was carried out in our hospital.

The principal indication of the primary operation was chronic pancreatitis not responding to medical treatment or complicated by an inflammatory tumour, pseudocysts, chronic pain syndrome, or pancreaticolithiasis in 17 of 28 patients (60.7%). Moreover, in seven patients (25%) with malignant tumours (1× carcinoma of the ductus choledochus, 2× carcinoma of the pancreatic head, 1× metastasis of a renal cell carcinoma in the pancreas, 1× intraampullary carcinoma) oncologic R0 resections were performed. A benign tumour (2× cystic neoplasms of the pancreas, 1× adenoma of the papilla vateri) was resected in three patients (10.7%). One patient (3.6%) received pancreatic surgery (Whipple) due to a pancreatic leak after external cholecystectomy and insertion of a T-drain.

Diagnostic Procedures Preparing Redo Procedure

In all patients, gastroscopy, transabdominal and endoscopic ultrasound, computed tomography (CT), as well as laboratory tests including tumour markers, oral glucose tolerance test, and stool elastase were performed. Magnetic resonance imaging, magnetic resonance cholangiography, or endoscopic retrograde cholangiopancreatography were carried out when indicated.

Evaluation of Intra- and Perioperative Parameters

The following intra- and perioperative parameters were evaluated with regard to redo procedure: operative time,

intraoperative blood loss, necessity of red blood cell transfusions, postoperative stay on the Intensive Care Unit (ICU), and postoperative hospital stay. Morbidity and mortality included complications during operation, during postoperative hospitalisation or within 30 days of discharge. Complications were divided in minor and major complications. Following the classification of surgical complications[12–14], minor complications included grade 1 complications that are defined as minor risk events not requiring therapy (with exceptions of analgesic, antipyretic, antiemetic, antibiotic and antidiarrhoeal drugs). Major complications (grade 2 to 4 complications) are defined as potentially life-threatening complications.

Mortality rates were compared with de novo pancreatic operations (Whipple, distal pancreatectomy, pancreatectomy) that were performed at our hospital during the same period.

Follow-up

After discharge, patients were seen for follow-up examinations (gastroscopy, transabdominal ultrasound, CT, laboratory tests including tumour markers, oral glucose tolerance test, and stool elastase) in intervals of 3 or 6 months. In case of postoperative problems, patients could be presented at any time in our emergency room. Some patients who lived far away from our hospital received their follow-up examinations in a hospital near their home, and findings were transmitted. All patients received permanent pancreatic enzyme substitution. Follow-up data were complete for every patient (100%).

Results

Indications for Redo Procedure

Redo procedures were performed in large part within 1 to 3 years after the first operation on the pancreas (median 39 months; range 2–168 months). Six patients (21.4%) underwent redo procedures within 12 months, seven patients (25%) within 1 to 3 years, seven patients (25%) within 4 to 6 years, five patients (17.9%) within 7 to 9 years, and three patients (10.7%) after 10 or more years (Fig. 1).

Frequently, patients had more than one indication resulting in the decision for redo procedure (Fig. 2). The main indication for redo procedures was persistent chronic pancreatitis in the remnant pancreas with recurrent acute inflammations and a chronic pain syndrome (Figs. 3, 4, and 5). Figure 5 shows an intraoperative photograph of a protein plaque, which was located in the pancreaticojejunostomy. The plaque inhibited pancreatic secretion and supported the progress of chronic pancreatitis in the remnant pancreas. Also, interruptions in the flow of bile (e.g., stenosis of choledochojejunostomy) and recurrent cholangitis can constitute an indication for redo procedure. Other infrequent indications for redo procedures in our patient population were acute gastrointestinal bleeding and a malignant pancreatic lesion (one patient each).

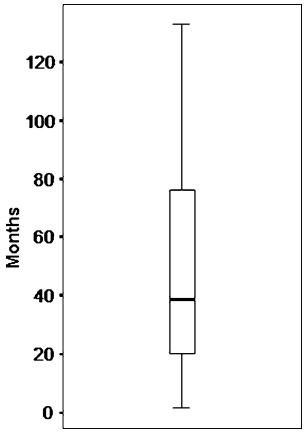

Fig. 1 *Boxplot* shows time interval between original operation and redo procedure. The *box* indicates the lower and upper quartiles; the *black bar* indicates the median. *Whiskers* represent 5th and 95th percentile

Different Forms of Redo Procedures

The most common redo procedure was the redo-Whipple operation (12 patients; 42.9%). This operation was performed in patients who underwent a classic Whipple operation (five patients) or a pylorus preserving Whipple operation (seven patients) in the past. During redo procedure, each anastomosis (gastroenterostomy, pancreaticojejunostomy, biliodigestive anastomosis) was resected and reconstructed. Whipple operation was also performed in eight patients (28.6%) after duodenum preserving pancreatic head resection (DPPHR), in one patient after pancreaticojejunostomy (3.6%), and in one patient (3.6%) after distal pancreatectomy. In this patient, a small remnant of the pancreas was left. Further redo

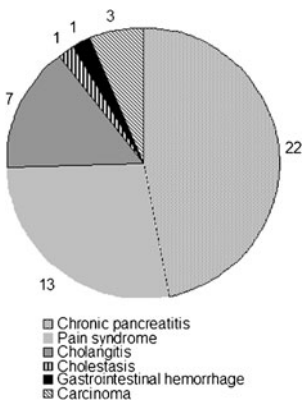

Fig. 2 Indications for redo procedure (*multiple nominations possible*)

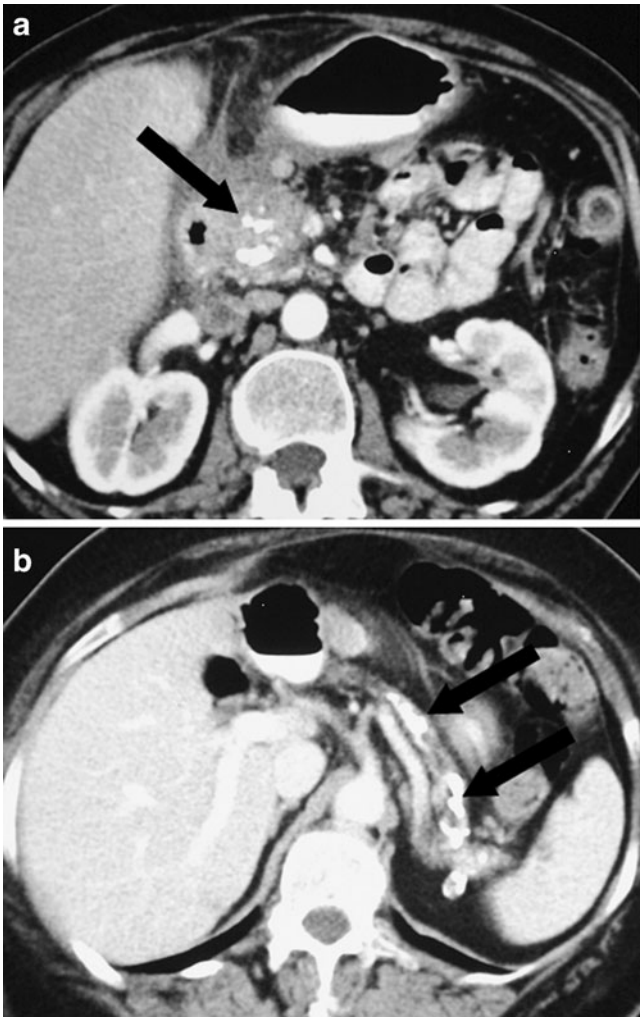

Fig. 3 Preoperative CT scans show indications for redo procedure. **a** 30 months after distal pancreatectomy, this patient underwent Whipple operation due to pancreatitis in the remnant pancreas with calcification of the head of pancreas (*arrow*). **b** 33 months after Whipple operation, this patient underwent redo-Whipple operation due to calcification and atrophy of the remnant pancreas with concrements in the pancreatic duct (*arrow*)

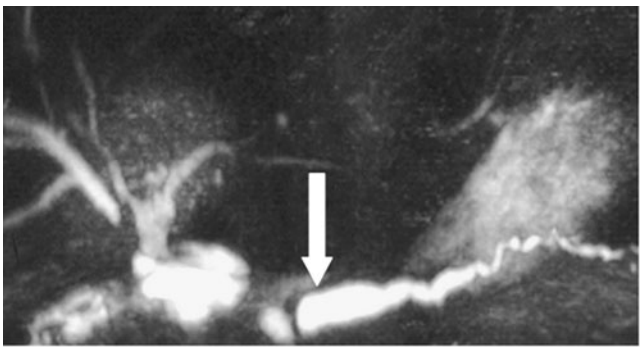

Fig. 4 Preoperative magnetic resonance cholangiography shows an indication for redo procedure. 33 months after Whipple operation, this patient underwent redo-Whipple operation due to dilatation of the pancreatic duct (*arrow*) and atrophy of the remnant pancreas

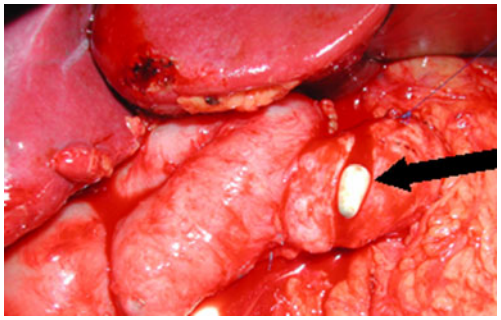

Fig. 5 Protein plaque (*arrow*) located in the pancreaticojejunostomy (*intraoperative photograph*)

procedures were completion pancreatectomy in four patients (14.3%), resection of the pancreatic margin in one patient (3.6%), and pseudocystojejunostomy in one patient (3.6%).

The following additional operative procedures were performed in four patients: synchronous transverse colon resection, extirpation of a benign liver tumour, segmental portal vein resection in combination with a left hemicolectomy, and segmental portal vein resection for tumour clearance in a patient with a mucinous cystadenocarcinoma.

Peri- and Postoperative Parameters

Intraoperative parameters such as operation time and intraoperative blood loss were reviewed for all redo procedures. The average operative time of redo procedures was 355 min (median 332; range 160–730 min). No operation lasted less than 2 h, and the majority of operations (18 operations, 64%) lasted 4 to 7 h. The average intraoperative blood loss was 811 ml (median 625 ml; range 300–2,800 ml). In 21 operations (75%), blood loss was less than 1 l (Table 1).

The following peri- and postoperative parameters were evaluated: postoperative stay on the ICU, intra- and postoperative red blood cell transfusions, morbidity, mortality, and duration of postoperative hospital stay (Table 1). The average postoperative stay on ICU was 29 h (median 20, range 0–112 h) (Table 1). Two patients (7%) did not need any intensive care after redo procedure. Twenty-four patients (86%) stayed 1 to 3 days on ICU. Only two

Table 1 Peri-and postoperative parameters

	Operative time (minutes)	Blood loss (ml)	ICU (hours)	Hospital stay (days)
Mean	355	811	29	21
Median	332	625	20	15
Range	160–730	300–2,800	0–112	7–98

patients (7%) spent more than 3 days after redo procedure on ICU.

In 28 performed redo procedures, three patients (10%) needed intraoperative red blood cell transfusions. Eight patients (26%) received postoperative red blood cell transfusions (Table 2).

The average postoperative hospital stay was 21 days (median 15, range 7–98) (Table 1). Twenty-one patients (75%) could be discharged from hospital within 3 weeks after operation. Only two patients (7%) had to stay more than 5 weeks.

Major Complications

Major complications had been observed in four patients (14%). All of them needed postoperative red blood cell transfusions. The following major complications occurred: anastomotic insufficiency of pancreaticojejunostomy and choledochojejunostomy, fascial dehiscence, severe subcutaneous bleeding, and anastomotic insufficiency and apoplexy of the remnant pancreas followed by arrosion bleeding (Table 2).

Minor Complications

Minor complications could be observed in nine patients (32%). All patients who developed major complications also developed minor complications. Some patients developed more than one minor complication. All of them responded well to medical treatment. The following minor complications were noted: gastric atony and delayed gastric emptying (5×), diarrhoea due to *Clostridium difficile* infection (2×), cholangitis (4×), chylous leak (2×), pneumonia (1×), pulmonary edema (1×), and urinary tract infection (2×) (Table 3). Aside from those patients who also developed major complications, the length of hospital stay was not affected by minor complications.

Mortality Rate

One patient died following complications after redo procedure; thus, mortality rate was 3.6%. In comparison, mortality rates of de novo pancreatic operations that were performed at our hospital during the same period were as follows: Whipple, 2% (5 of 281 operated patients died); distal pancreatectomy, 2% (2 of 102 operated patients died); pancreatectomy, 11% (5 of 46 operated patients died).

Follow-Up

The average follow-up period in this study was 20 months (median 20; range 1–46 months).

Table 2 Patients who received red blood cell transfusions or developed major complications

Patient	Gender (M/F)	Age	First operation	Redo procedure	Operative time	Intraoperative blood loss	Number of intraoperative blood transfusions	Number of postoperative blood transfusions	Stay on ICU (hours)	Duration of postoperative hospital stay (days)	Major complications
1	F	66	Whipple	Redo-Whipple	370	300	0	2	20	14	0
2	M	67	Whipple	Redo-Whipple	375	1,000	0	2	22	98	Insufficiency of PJ and CJ
3	F	45	Whipple	Resection/reconstruction of PJ	235	350	0	2	4	16	0
	M	51	DP	P	420	2,800	2	3	30	30	Fascial dehiscence
5	F	48	Whipple	P	480	2,000	3	2	68	24	0
6	M	40	DPPHR	Whipple	325	600	0	2	26	16	0
7	M	46	DPPHR	Whipple	485	600	2	0	21	11	0
8	M	40	DP	Whipple	440	1,200	0	2	13	13	Subcutaneous hemorrhage
9	M	71	Whipple	Redo-Whipple	400	600	0	6	20	58	Insufficiency+apoplexy of the remnant pancreas, arrosion bleeding

DPPHR duodenum preserving pancreatic head resection, *DP* distal pancreatectomy, *P* pancreatectomy, *PJ* pseudocystojejunostomy, *CJ* choledochojejunostomy

Table 3 Minor complications

	Gastric atony/delayed gastric emptying	Diarrhoea following *Clostridium difficile* infection	Cholangitis	Chylous leak	Pneumonia	Pulmonary edema	Urinary tract infection
Number of patients (*n*)	5	2	4	2	1	1	2

Postoperative Exocrine and Endocrine Function

In 23 patients (82%), exocrine function measured by stool elastase was already impaired before redo procedure had been performed. Exocrine function was normal before redo procedure and postoperatively impaired in two patients (7%). In three patients (11%), exocrine function remained in normal levels.

Fifteen patients (54%) who received redo procedures did not develop diabetes mellitus (DM) (performed redo procedures: 8× redo-Whipple, 5× Whipple after DPPHR, 1× Whipple after distal pancreatectomy, 1× resection of the pancreatic margin). One patient (4%) had latent diabetes that did not worsen after redo procedure (performed redo procedure: redo-Whipple). Four patients (14%) had insulin-dependent DM just before redo procedure (performed redo procedures: 1× redo-Whipple, 3× pancreatectomy).

Altogether, in four patients (14%), initial diagnosis of DM was established within few months after redo procedure. All of them developed insulin-dependent DM (performed redo procedures: 1× redo-Whipple, 2× Whipple after DPPHR, 1× redo-Whipple followed by pancreatectomy in course of major complication (see above)).

Another four patients (performed redo procedures: 1× redo-Whipple, 1× Whipple after DPPHR, 1× pancreatectomy, 1× pseudocystojejunostomy), who were treated with oral diabetes medications before, had to inject insulin few months after redo procedure (14%).

Postoperative Consumption of Analgetics

Due to chronic pain syndrome, many patients regularly consumed analgetics before redo procedure had been performed. Eleven patients (39%) consumed opioids, and 13 patients (46%) consumed non-steroid analgetics. Only four patients (14%) did not need any pain medication before redo procedure. After redo procedure, consumption of analgetics could be reduced in 18 patients (64%). Many patients, who needed continuous pain medication preoperatively, could reduce their consumption on demand. Following redo procedure, only seven patients (25%) consumed opioids, and no patient consumed non-steroid analgetics on a regular basis postoperatively. Just in one patient (4%), consumption of analgetics increased after redo procedure. This patient had an opioid abuse in his medical history.

Mortality During Follow-Up Period

During follow-up period, four patients died.

A 67-year-old man developed major complications (anastomotic insufficiency of pancreaticojejunostomy and choledochojejunostomy) and died 4 months after redo procedure.

A 62-year-old woman died 8 months after redo-Whipple operation for chronic pancreatitis. Before relaparotomy, this patient suffered from severe cachexia. After redo procedure, cachexia and pain syndrome did not ameliorate. Her death was a consequence of severe cachexia followed by immunodeficiency resulting in pneumonia and sepsis.

Two women at the age of 67 and 75 years, who underwent a redo-Whipple operation and Whipple after DPPHR for carcinoma of the pancreas, died 5 and 21 months later owing to recurrent pancreatic cancer.

Relaparotomy During Follow-Up Period

Two patients underwent relaparotomy after redo procedure. Six months after pancreatectomy, a 53-year-old man with persistent alcohol abuse presented with a perforated ulceration of the gastroenterostomy. Gastroenterostomy was resected and converted to a Roux-en-Y reconstruction.

The same conversion was performed in a 54-year-old male patient with stenosis of the gastroenterostomy 46 months after pancreatectomy.

Discussion

Redo procedures of the pancreas are rare and very demanding operations. In our hospital, which is a specialised Pancreas Centre, the incidence of redo procedures is 3%.

The most frequent indication for initial operation as well for redo procedure in our patient population was chronic pancreatitis. Chronic pancreatitis is a progressive, destructive inflammatory process leading to total destruction of the pancreatic tissue and results in malabsorption, diabetes mellitus, and severe unrelenting pain.[15] It is characterised by a progressive conversion of pancreatic parenchyma into fibrous tissue.[16] Due to intractable chronic pain, about 50% of the patients with chronic pancreatitis need surgical

intervention.[16] In addition, pancreatic surgery is indicated in the presence of complications of the disease not responsive to medical, endoscopic, or radiologic treatment.

Nevertheless, operative therapy remains a challenging problem since the pathogenesis of chronic pancreatitis and predictors of long-term success after operative treatment are poorly understood. Favourable outcomes, in particular long-term pain control, are difficult to achieve.[17] In our patient population, the incidence of a chronic pain syndrome was 79%. The causes for recurrent or persistent pain following pancreatic surgery are complex. Even after pancreatic surgery, chronic pancreatitis may persist and can progress in the pancreatic remnant. Neuropathic changes, residual or evolving pancreatic and biliary duct obstruction, and unrecognised pancreatic cancer can constitute an indication for redo procedure.[10]

In our series, the majority of previous pancreatic operations were performed at other medical centres (75%). Six patients (21%) underwent redo procedures already within 12 months. Markowitz et al.[10] and Imrie et al.[18] reported that this early failure is probably due to a faulty initial operative strategy. Lack of adequate drainage of the head of pancreas after lateral pancreaticojejunostomy, stricture of the pancreatic anastomosis after pancreatic head resection, obstruction of segments of the pancreatic duct after drainage procedure, and persistent disease within the remnant gland after distal pancreatectomy are potential explanations.[1,11] Otherwise, late failure after initial success of the operative treatment is more likely the result of disease progression.[10,18]

Usually redo procedures can be achieved by following a treatment strategy aimed at addressing identified residual disease while maximally preserving pancreatic tissue.[10] Redo procedures should be as simple and safe as possible and should preserve the remaining endocrine and exocrine functions of the pancreas. This is very important as patients who underwent redo procedures of the pancreas in our hospital were of young age (average 54 years). Also, the majority of patients suffered of benign diseases with potential long-term survival. Due to this, we believe that every effort should be undertaken to prevent the patient from an apancreatic state.

In our study, the main indications for redo procedures were persistent chronic pancreatitis, recurrent cholangitis, and a chronic pain syndrome. Redo-Whipple operation was the most common redo procedure (79%), and it was indicated in strictures and protein plaques of the pancreaticojejunostomy or stenoses of the choledochojejunostomy. Improvement of bile and pancreatic juice outflow resulted in acceptable outcomes; thus, completion pancreatectomies were infrequent. Nevertheless, in four patients (14%), total pancreatectomy had to be performed.

Our experiences have shown that even though redo procedures are complex and high-risk procedures, operative risk could be minimised. Average operative time (355 min), intraoperative blood loss (811 ml), postoperative stay on ICU (29 h), postoperative hospital stay (21 days), and morbidity (14%) rates were comparable to primary pancreatic operations. Also, mortality (3.6%) was comparable to de novo pancreatic operations (Whipple, 2%; distal pancreatectomy, 2%; pancreatectomy, 11%) that were performed at our hospital during the same period of time.

Requirements for such a satisfactory outcome are given in institutions with high expertise that provide an experienced multidisciplinary team. In this regard, Luft et al.[19] showed that hospitals that had higher volumes of specific surgical procedures had significantly lower impatient mortality rates than did their lower-volume counterparts. Also, van Heek et al.[20] documented an inverse relation between hospital volume and mortality. Furthermore, technical skill and experience of the individual surgeon is an important determinant of outcome. As described above, in several patients, extensive resections including portal vein resection, extirpation of liver lesions, resection of transverse colon, and hemicolectomy were required. For technical reasons or potential intraoperative complications, it is essential that the full armamentarium of reconstructive surgery is available. This underlines on one hand that redo procedures should be performed by in this field specialised and experienced surgeons. Birkmeyer et al.[21] postulated in this context the "surgeon-specific volume–outcome relation": an inverse relation between surgeon volume and operative mortality. High-volume pancreas surgeons achieve higher resectability rate, and their patients have higher long-term survival. Thus, the surgeon is an important prognostic factor. On the other hand, the availability of multidisciplinary teams composed of experienced surgeons, specialised nurses, intensivists, anesthesiologists, gastroenterologists, pathologists, and radiologists play a decisive role for a satisfactory outcome of redo procedures. In our opinion, redo procedures are very demanding operations that should be performed in high-volume departments or specialised Pancreas Centres. Profound experience with pancreatic surgery permits a favourable outcome of such a complex kind of surgery. This is reflected in an unchanged endocrine function (71%) and unchanged (32%) or reduced (64%) consumption of analgetics in a good portion of our patient population. Our observations comply with the experiences of Markowitz et al.[10] who reported about 14 redo procedures. One patient died of pancreatic cancer. Ten of 13 long-term survivors had satisfactory-to-excellent relief of pain, with resumption of a normal level of function. Of the ten previously euglycemic patients who underwent pancreatic head resection, eight remained free of diabetes mellitus. However, in view of the complexity of redo surgery, major complications occurred in four patients (14%), and one patient (3.6%) died of major complications.

Thus, surgeons have to be aware that even under optimal conditions, redo procedures remain high-risk operations. In order to minimise the operative risk, indication for redo procedure has to be reflected well in each patient. However, in some patients, redo procedure is the only therapeutic option. Under these circumstances, operative risk can be minimised, when surgery is performed in institutions with high expertise.

Conclusion

Our data revealed that redo procedures can be performed with low morbidity and mortality. Operative risk for the patients can be minimised when redo procedures are performed in institutions with high expertise as, e.g., specialised Pancreas Centres.

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ORIGINAL ARTICLE

Knockdown of microRNA-21 Inhibits Proliferation and Increases Cell Death by Targeting Programmed Cell Death 4 (PDCD4) in Pancreatic Ductal Adenocarcinoma

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Abstract
Objective This study aims to examine the expression of a panel of five microRNAs (miRNA) in pancreatic ductal adenocarcinoma (PDAC) and the functional effect of miR-21 inhibition in PDAC cell lines.
Background miRNA are short, non-coding RNA molecules, which play important roles in several cellular processes by silencing expression of their target genes through translational repression or mRNA degradation. They are often aberrantly expressed in cancer, and this dysregulation can promote carcinogenesis by altering the expression of tumour suppressor or oncogenes.
Methods miRNA expression levels were measured in 24 PDAC tumour/matched adjacent normal tissue samples and three PDAC cell lines using reverse transcription polymerase chain reaction. Levels of cell proliferation and death and expression of programmed cell death 4 (PDCD4; tumour suppressor) were studied in PDAC cells (MIA-Pa-Ca-2) in the absence or presence of a miR-21 inhibitor.
Results PDAC primary tissues and cell lines displayed a consistent upregulation of miR-21 ($P<0.0001$) and downregulation of both miR-148a ($P<0.0001$) and miR-375 ($P<0.0001$) relative to adjacent normal tissue. Furthermore, miR-21 levels in the primary tumours correlated with disease stage ($P<0.0001$). Inhibition of miR-21 in MIA-Pa-Ca-2 PDAC cells led to reduced cell proliferation ($P<0.01$) and increased cell death ($P<0.01$), with simultaneous increase in levels of the tumour suppressor, PDCD4 ($P<0.01$).
Conclusion miRNA expression profiles may be used as biomarkers for detecting pancreatic cancer. Moreover, miR-21 could be a predictor of disease progression and a possible therapeutic target in part by upregulating PDCD4 in pancreatic cancer.

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Keywords Pancreatic cancer *or* neoplasm *or* tumour · microRNA · miR-21 · PDCD4 · Carcinogenesis · Diagnosis · Prognosis · Biomarker · Cell culture

Introduction

Surgical resection is currently the only curative option for pancreatic cancer, but in most patients, the insidious nature of the disease results in a diagnosis being made when the cancer is unresectable.[1,2] Advances in chemotherapy have contributed to improved survival but mainly in the adjuvant setting.[3] The overall prognosis is poor with only 2% to 3% of patients expected to survive 5 years without surgical resection.[4,5] Such poor survival from

conventional treatments suggests a need to identify novel molecular targets.

A class of small ribose nucleic acid (RNA) molecules (microRNAs, miRNAs) have recently become the focus of intensive study in several cancer types, including pancreatic cancer.[6] miRNAs are short (19–24 nucleotides), endogenous, non-coding RNA sequences that regulate post-transcriptional gene expression. They function by binding to mRNA of target genes and prevent protein translation by the process of repression or degradation.[7,8]

More than 5,000 miRNAs have been discovered in 58 different species, with each having the potential to regulate hundreds of target mRNA indicating that a large proportion of the transcriptome is subject to miRNA-mediated regulation.[9,10] miRNAs can therefore play a role in a range of biological processes and have been implicated in differentiation, transformation and carcinogenesis.[11]

Aberrant expression of miRNAs has been shown to inhibit tumour suppressor genes or activate oncogenes inappropriately to initiate carcinogenesis.[12] Dysregulation of both miR-155[13] and miR-21[14] has been reported in pancreatic cancer precursor lesions (pancreatic intraepithelial neoplasia), which supports their role in the early stages of cancer development. Moreover, distinct miRNA expression profiles have been found in different tumour types,[8,15] indicating a potential role in both the diagnosis and treatment of cancer.[16]

Consistent upregulation of miR-21 in several different tumours suggests that it may be a tumour biomarker (Table 1). Furthermore, knockdown studies of miR-21 have confirmed its oncogenic role in cancer cell lines by inducing growth suppression[17–21] and upregulation of tumour suppressors such as phosphate and tensin homolog,[22] tropomyosin alpha-1 chain[23] and reversion-inducing cysteine-rich protein with kazal.[24]

Programmed cell death 4 (PDCD4) is a tumour suppressor which regulates multiple proteins that are involved in tumour progression, cell cycle control and differentiation[25] (Fig. 1). It has been identified as a novel tumour suppressor in several tumour types[26] including pancreatic cancer[27] and could be a potential target for treatment. Recent studies have identified that miR-21 targets PDCD4 in cancer cell lines[28–30]; however, this relationship has not been established in pancreatic cancer.

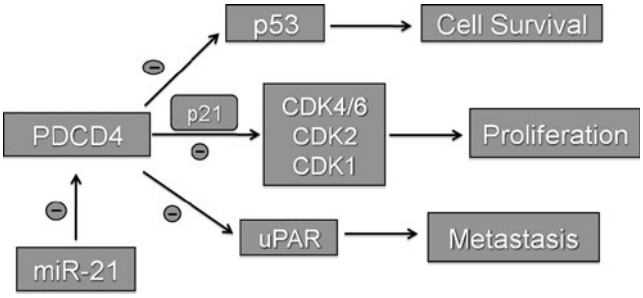

Fig. 1 Mechanisms through which PDCD4 acts to induce tumour suppression. ⊖ indicates inhibition. *CDK* cyclin-dependent kinase, *uPAR* urokinase receptor, *p53* tumour protein 53, *p21* cyclin-dependent kinase inhibitor 1

The aims of this study were to identify the expression of five key miRNAs (miR-21, miR-148a, miR-375, miR-181b and miR-151), previously reported to have been dysregulated,[31–33] in PDAC (tissue and cell line) and investigate the functional effect of miR-21 inhibition in MIA-Pa-Ca-2 PDAC cell line by measuring cell proliferation, cell death and PDCD4 levels.

Methods

Tissue Samples

Ethical approval for the study was obtained from the Derbyshire Research Ethics Committee for collection of PDAC and matched adjacent normal tissue from patients who underwent surgical cancer resection at Nottingham University Hospitals, Queen's Medical Centre, Nottingham, UK. Fresh tumour samples from 25 patients were collected, after obtaining informed consent between February 2009 and February 2010 (Table 2). A single specimen from the cohort reported to be benign following histopathological examination was excluded from analysis. No patient had been treated with neoadjuvant chemotherapy.

Resected specimens were transported on ice to the laboratory, where a 0.5×0.5-cm biopsy was taken from each tumour and macroscopically normal adjacent tissue. Histopathological examination confirmed that regions of the specimens where matched normal biopsies were taken had no

Table 1 Direction of change in miR-21, miR-148a and miR-375 expression in different gastrointestinal tumour types

	GI cancer tissue	GI cancer cell line
miR-21	↑Pancreatic,[32] ↑Colorectal,[57] ↑Oesophageal,[58] ↑Gastric,[59] ↑Cholangiocarcinoma[28]	↑Pancreatic,[60] ↑Gastric,[61] ↑Cholangiocarcinoma[28]
miR-148a	↓Pancreatic,[32] ↓Colorectal,[40] ↓Gastric[40]	↓Gastric,[40] ↓Colorectal[40]
miR-375	↓Pancreatic,[32] ↑Oesophageal,[62] ↓Gastric[63]	↓Gastric[63]

GI gastrointestinal, ↑ upregulated, ↓ downregulated, *miR-21* 8/8 studies demonstrated upregulation, *miR-148a* 5/5 studies demonstrated downregulation, *miR-375* 3/4 studies reported downregulation

Table 2 Patient details

Age[a]	70 (47–80)
Sex ratio (M/F)	11:13
UICC stage	
I	4
II	14
III	5
IV	1
Tumour differentiation	
Well	4
Moderate	13
Poor	7

[a] Values are median (range)

evidence of dysplasia. All samples were preserved in RNA later® (Sigma-Aldrich, Poole, UK) and stored at −4°C for 24 h prior to RNA extraction.

In three patients with unresectable tumours, intraoperative biopsies were taken and placed in RNA later® (stored at −4°C). As matched normal samples could not be retrieved in these cases, commercially available RNA from normal pancreas (Applied Biosystems, Foster City, CA, USA) was used.

Cell Lines and Culture

MIA-Pa-Ca-2, HUP-T3 and PSN-1 PDAC cell lines were obtained from European Collection of Cell Cultures (HPACC, Porton Down, UK). MIA-Pa-Ca-2 cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, Paisley, UK), PSN-1 cells in RPMI 1640 (GIBCO) and minimum essential medium (GIBCO) with 1% non-essential amino acids (Sigma-Aldrich) and 1% sodium pyruvate (Sigma-Aldrich) was used for HUP-T3 cell culture. Media were supplemented with 10% foetal bovine serum (Fisher Scientific, Loughborough, UK), 2 mM L-glutamine (Sigma-Aldrich), 50 U/ml penicillin (Sigma-Aldrich), 50 μg/ml streptomycin (Sigma-Aldrich) and 250 μg/ml of amphotericin B (GIBCO). Cells were cultured in T75 tissue culture flasks (TSZ Scientific, Framingham, MA, USA) in a humidified incubator at 37°C with 5% CO_2 (Sanyo, Osaka, Japan; model MCO-20AIC).

RNA Isolation

Total RNA from primary tissue and cell lines was isolated using TRI Reagent® (Applied Biosystems) according to the manufacturer's protocol.[34] RNA quality and quantity were determined with a spectrophotometer (Nanodrop 1000, Fisher Scientific). DNase-treated total normal human pancreatic RNA (1 mg/ml; Applied Biosystems, no AM7954) certified to contain miRNAs was used to compare against miRNA expression in tumour biopsies in which the disease was found to be unresectable and PDAC cell lines.

Real-Time Reverse Transcriptase Polymerase Chain Reaction for miRNA Expression with TaqMan® microRNA Assay

miRNA expression levels were quantified using TaqMan® miRNA assays (Applied Biosystems). A total of 10 ng of RNA was used to reverse transcribe specific miRNA of interest into cDNA using the TaqMan® miRNA reverse transcription kit (Applied Biosystems; no. 4367038). This was followed by real-time PCR using miRNAs specific TaqMan® probe assays (miR-21, ID 000397; miR-148a, ID 000470; miR-375, ID 000564; miR-181b, ID 001098; miR-151, ID 000596 and RNU44, ID 001094) in a Chromo4™ thermal cycler (Bio-Rad, Hemel Hempstead, UK).

Standard curves were examined in duplicate for both the miRNA of interest and the internal control gene RNU44. Sample reactions were all repeated in quadruplicate on three different PCR plates (Eppendorf, Hamburg, Germany). miRNA expression levels were normalised to RNU44 and calculated using the absolute quantification method.

Transfection of miR-21 Hairpin Oligonucleotide Inhibitor Studies

MIA-Pa-Ca-2 cells were seeded at 50,000 cells/well in a 12-well plate (PDCD4 expression and cell death studies) or 5,000 cells/well in a 96-well plate (cell growth studies). The cells were incubated overnight at 37°C with 5% CO_2 and then transfected with miRIDIAN negative control (100 nM) and miR-21 inhibitor (100 nM), combined with transfection reagent (DharmaFECT 2) according to the manufacturer's instructions[35] (Thermo Fisher Scientific, Dharmacon, Lafayette, CO, USA).

Real-Time Quantitative RT-PCR for PDCD4 mRNA Expression

Expression levels of PDCD4 mRNA were analysed by real-time reverse transcriptase polymerase chain reaction (RT-PCR) using TaqMan® PDCD4 Gene Expression Assay (Applied Biosystems, ID Hs00377253_m1). One micrograms of total RNA was reverse-transcribed following the TaqMan® high-capacity cDNA reverse transcription protocol (Applied Biosystems). The TaqMan® assay for the housekeeping gene B2M (ID Hs00187842) was used as normalization control, and PDCD4 mRNA levels were calculated using the absolute quantification method.

Protein Extraction and Analysis

MIA-Pa-Ca-2 cells were lysed using homogenisation buffer (25 mM Tris–HCl (pH 7.4), 300 mM sucrose, 10 mM monothioglycerol, 1 mM EDTA, 2.5% igepal, 1:500 protease

inhibitor (Sigma-Aldrich), 1:100 phosphatase inhibitor (Sigma-Aldrich)). Insoluble components were separated by centrifugation and protein concentration was measured using BCA assay. After boiling for 10 min in Laemmli buffer (Bio-Rad), proteins (50 mg) were resolved on 10% SDS polyacrylamide gels and transferred onto a nitrocellulose membrane. Western blotting was performed using anti-PDCD4 (1:2,000 dilution; Sigma-Aldrich) and anti-beta actin antibody (1:1,000 dilution; Abcam, Cambridge, UK) used as a protein loading control, and immunocomplexes were visualised by enhanced chemiluminescence (Bio-Rad) according to manufacturer's protocol. Quantity One® (Bio-Rad) software was used to quantify band intensities.

Cell Proliferation

Cell growth of MIA-Pa-Ca-2 cells was measured at 24, 48, 72 and 96 h of miR-21 inhibitor treatment (*vide supra*) using the Cell Titer® AQ_{ueous} One Solution Cell Proliferation MTS Assay, following the manufacturer's instructions[36] (Promega, Southampton, UK).

Cell Death Studies

Cell death was measured in MIA-Pa-Ca-2 cells at 42 and 78 h treatment with miR-21 inhibitor (*vide supra*) using the Calbiochem® cell death detection ELISA kit (Merck, Nottingham, UK) as per manufacturer's protocol[37] to perform relative quantification of nuclear matrix protein (NMP; a nucleus structural protein). Plates were analysed with a photocytometer at 450 nm (Wallac 1420 Victor™, Perkin-Elmer, Waltham, MA, USA).

Statistical Analysis

All data followed a non-Gaussian distribution and therefore was expressed as median, interquartile range (IQR) and range. The Mann–Whitney *U* test (unpaired data), Wilcoxon signed-rank test (paired data) or Kruskal–Wallis test were used for comparative analysis of data. Statistical significance was determined at $P \leq 0.05$. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

Results

Expression of miR-21, miR-148a, miR-375, miR-181b and miR-151 in Both Pancreatic Adenocarcinoma Tissue and Cell Lines

Expression of miR-21 was upregulated, whilst conversely downregulated in miR-148a and miR-375 in PDAC compared with normal pancreas in both tissue ($n=24$; $P<0.0001$) and cell lines ($P<0.001$; Figs. 2 and 3; Table 3). There was no correlation between tissue and cell lines for expression data for miR-181b and miR-151 (Figs. 2 and 3; Table 3). miR-181b and miR-151 demonstrated no significant change in expression between PDAC and adjacent normal tissue (Fig. 2; Table 3). miR-181b was significantly upregulated in one cell line (PSN-1; $P<0.001$), whereas miR-151 was upregulated in all three cell lines ($P<0.001$; Fig. 3). Levels of miR-21 were confirmed to be greater in patients with UICC stage III/IV ($n=6$) as compared to stage I/II ($n=18$) disease ($P<0.0001$) and with nodal disease ($P<0.0001$; Fig. 4; Tables 2 and 4).

Inhibition of miR-21 Upregulates the Expression of PDCD4 Message and Protein

miR-21 inhibition demonstrated upregulation of both PDCD4 message ($P<0.001$) and protein ($P<0.01$) relative to cells treated with a negative control or media alone at 48 h (Figs. 5 and 6a, b). The upregulation in PDCD4 message by knockdown of miR-21 suggests that the mechanism of action is through mRNA degradation rather than repression.

Suppression of Cell Growth and Increased Cell Death by miR-21 Inhibition

MIA-Pa-Ca-2 cells demonstrated elevated levels of miR-21 (Fig. 2). miR-21 inhibition significantly suppressed cell

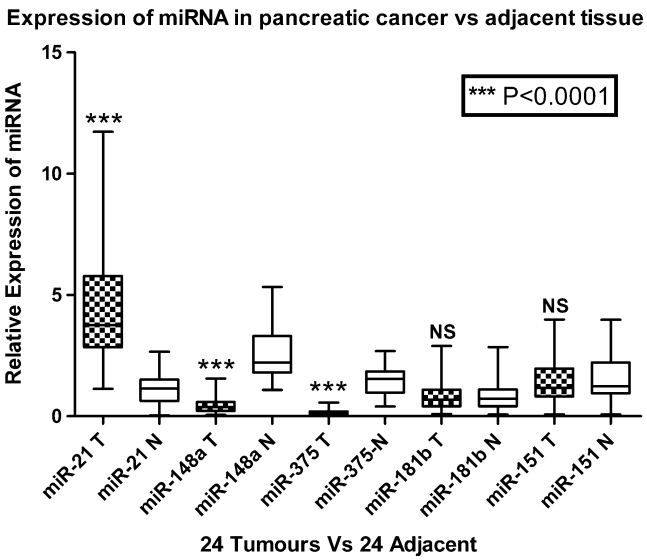

Fig. 2 Pooled expression analysis of miR-21, miR-148a, miR-375, miR-181b and miR-151 in both PDAC (*T*; $n=24$) and adjacent normal tissue (*N*; $n=24$). *NS* non-significant. *Box plots* indicate median with IQR and *whiskers* demonstrate the maximum and minimum levels of miRNA expression. Statistical significance comparing the expression of each miRNA between PDAC and adjacent normal tissue was calculated using the Wilcoxon signed-rank test

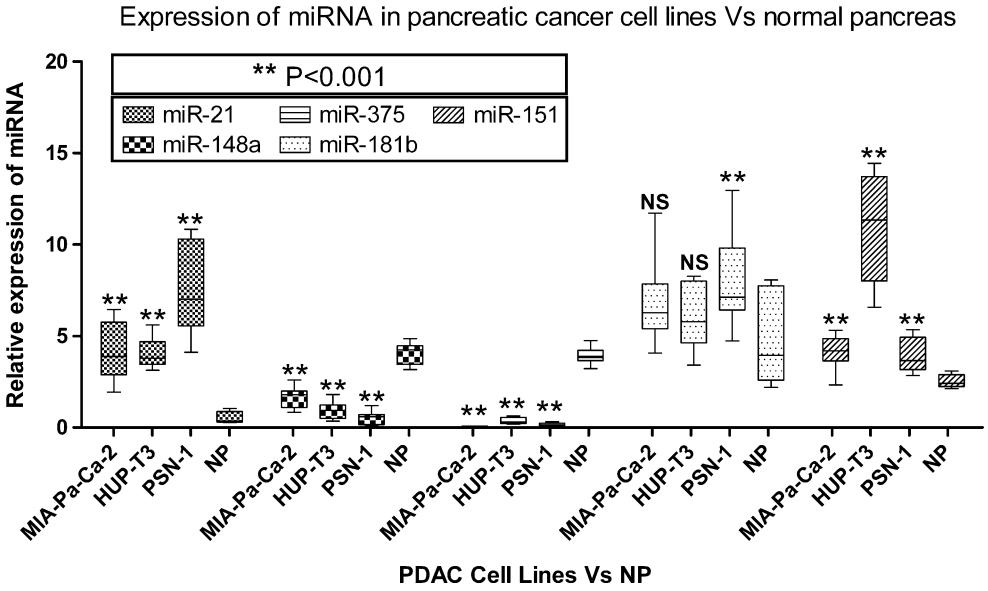

Fig. 3 Expression analysis of miR-21, miR-148a, miR-375, miR-181b and 151 in both PDAC cell lines (MIA-Pa-Ca-2, HUP-T3, PSN-1) and normal pancreatic RNA (*NP* applied biosystems). *Box plots* indicate median with IQR and *whiskers* demonstrate the maximum and minimum levels of miRNA expression. Statistical significance comparing expression of each miRNA between PDAC cell line and NP was calculated using the Mann–Whitney *U* test

proliferation ($P<0.01$) compared to untreated cells and those transfected with negative control (Fig. 7). NMP levels were found to be significantly elevated at both 48 and 72 h ($P<0.01$) in cells transfected with the miR-21 inhibitor (Fig. 8).

Discussion

The exact 'trigger' for the development of pancreatic cancer is yet to be revealed. However, at present, it is thought to be a multi-step process involving an accumulation of genetic mutations subsequently leading to their dysfunction. The discovery of miRNAs has resulted in significant advances in the understanding of cancer biology by providing additional mechanisms for genetic dysregulation. Emerging evidence has established that aberrant miRNA expression profiles are present in a variety of solid[38] and haematological malignancies.[39] RT-PCR was used to validate the differences in the expression of a panel of five miRNAs from PDAC and normal pancreas and investigate the functional effect of miR-21 knockdown in MIA-Pa-Ca-2 cells in search for novel biomarkers and therapeutic targets.

The miRNA expression data were combined from 24 tissue samples which demonstrated significant difference in expression between tumour and normal for miR-21, miR-148a and miR-375, although some overlap did exist with outlying expression data (Fig. 2). When examined individually, the expression of miR-21, miR-148a and miR-375 was significantly different between each tumour compared with its matched adjacent normal tissue with no overlap in outlying values. Therefore, it is possible that dysregulation of miRNAs may be defined at the individual level. Chen et al.[40] reported a similar observation and found consistent downregulation of miR-148a at differing levels in each

Table 3 Median expression of miR-21, miR-148a, miR-375, miR-181b and miR-151 in pancreatic ductal adenocarcinoma tumour samples ($N=24$) and adjacent normal pancreatic tissue

miRNA	Median miRNA expression (IQR)	*P* value
miR-21-T	3.77 (2.85–5.79)	<0.0001
miR-21-N	1.14 (0.64–1.51)	
miR-148a-T	0.37 (0.22–0.60)	<0.0001
miR-148a-N	2.22 (1.81–3.32)	
miR-375-T	0.11 (0.04–0.20)	<0.0001
miR-375-N	1.54 (0.99–1.85)	
miR-181b-T	0.74 (0.45–1.04)	NS
miR-181b-N	0.75 (0.49–1.06)	
miR-151-T	1.30 (0.85–2.14)	NS
miR-151-N	1.33 (0.97–2.28)	

NS non-significant

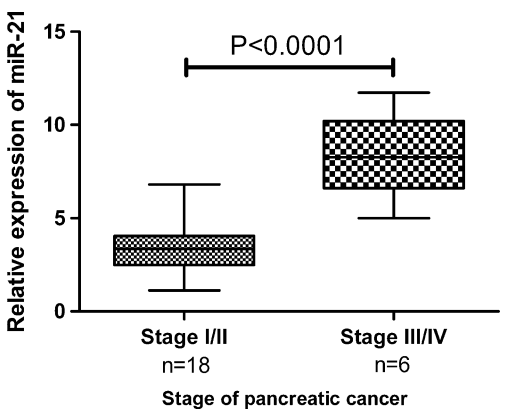

Fig. 4 Expression of miR-21 in stage I/II versus stage III/IV disease. *Box plots* indicate median with IQR and *whiskers* demonstrate the maximum and minimum levels of miR-21 expression. Statistical significance was calculated using the Mann–Whitney *U* test

Table 4 Correlation of miR-21, miR-148a, miR-375, miR-181b and miR-151 expression levels with disease grade, stage, nodal and resection status

Variable	*N* (%)	Median expression of miR-21 (IQR)	*P* value	Median expression of miR-148a (IQR)	*P* value	Median expression of miR-375 (IQR)	*P* value	Median expression of miR-181b (IQR)	*P* value	*N* (%)	Median expression of miR-151 (IQR)	*P* value
Grade												
1 and 2	17 (71)	3.61 (2.93–5.26)	NS	0.37 (0.21–0.61)	NS	0.09 (0.04–0.20)	NS	0.76 (0.50–1.33)	NS	17 (71)	1.33 (0.94–2.07)	NS
3	7 (29)	3.04 (1.95–4.49)		0.31 (0.25–0.48)		0.10 (0.05–0.16)		0.41 (0.26–0.56)		7 (29)	0.73 (0.54–0.93)	
TNM stage												
Stage I/II	18 (75)	3.36 (2.50–4.05)	<0.0001	0.40 (0.23–0.60)	NS	0.10 (0.05–0.19)	NS	0.65 (0.40–1.21)	NS	18 (75)	1.11 (0.78–1.77)	NS
Stage III/IV	6 (25)	8.97(6.72–10.31)		0.23 (0.13–0.37)		0.09 (0.01–0.21)		0.81 (0.69–1.19)		6 (25)	1.92 (1.32–2.87)	
Nodal status												
Negative	9 (38)	3.19 (2.38–3.96)	<0.0001	0.40 (0.23–0.83)	NS	0.08 (0.04–0.17)	NS	0.75 (0.41–1.25)	NS	9 (38)	1.14 (0.84–1.95)	NS
Positive	15 (63)	3.83 (3.07–5.94)		0.33 (0.19–0.55)		0.11 (0.06–0.21)		0.67 (0.46–1.19)		15 (63)	1.20 (0.81–1.85)	
Resection status												
R0	11 (52)	3.62 (3.02–4.55)	NS	0.41 (0.25–0.55)	NS	0.07 (0.04–0.16)	NS	0.56 (0.36–0.91)	NS	11 (52)	1.17 (0.81–1.82)	NS
R1/R2	10 (48)	3.47 (2.43–6.37)		0.30 (0.15–0.63)		0.14 (0.08–0.21)		0.81 (0.52–1.33)		10 (48)	1.25 (0.86–1.99)	

N number, *NS* not significant

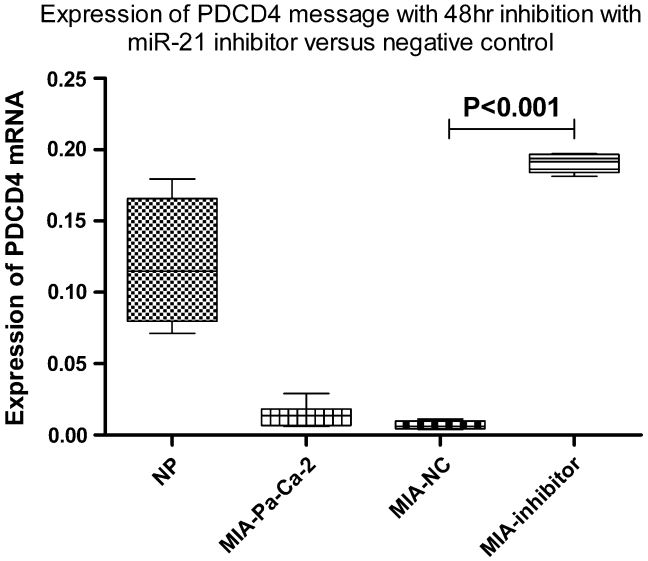

Fig. 5 Expression of PDCD4 mRNA in MIA-Pa-Ca-2 cell lines following 48 h of transfection with negative control or inhibitor. *NP* normal pancreas RNA (Applied Biosystems), *MIA-Pa-Ca-2* cells without treatment, *MIA-NC* MIA-Pa-Ca-2 cells transfected with negative control, *MIA-inhibitor* MIA-Pa-Ca-2 cells transfected with miR-21 inhibitor. *Box plots* indicate median with IQR and *whiskers* demonstrate the maximum and minimum levels of PDCD4 expression. Statistical significance was calculated using the Mann–Whitney *U* test

gastric (n=101) and colorectal cancer (n=101) specimen, five different gastric cancer cell lines (AGS, SGC-7901, MGC-803, BGC-823) and two colorectal cancer cell lines (HCT-116, SW-620) when compared with normal samples. The direction of change in the expression of these three miRNAs correlated with previous cancer studies (Table 1) as well as in PDAC cell lines (Fig. 3).

The expression of miR-181b (five—downregulation, five—upregulation and 14—no difference tumour and normal) and miR-151 (seven—downregulation, seven—upregulation and ten—no difference between tumour and normal) varied between each sample and did not show a significant difference when expression data were pooled for 24 samples (Fig. 1). Furthermore, there was no significant correlation for expression data between pancreatic cancer tissue and cell line (Figs. 2 and 3). Zhang et al.[33] examined the expression of 95 miRNAs in both primary pancreatic cancer tissue and cell lines using RT-PCR and reported a substantial difference in expression of 95 miRNAs in pancreatic cancer tissue and cell lines. However, in a proportion of these miRNAs, each individual tissue sample or cell type had differing expression with other cases or cell type, indicating the individual diversity in pancreatic cancer. It is possible that a few miRNAs may have unique profiling patterns at an individual level, whereas others may be consistently dysregulated sharing common pathways in pancreatic cancer pathogenesis. Diversity in miRNA expression among individual pancreatic

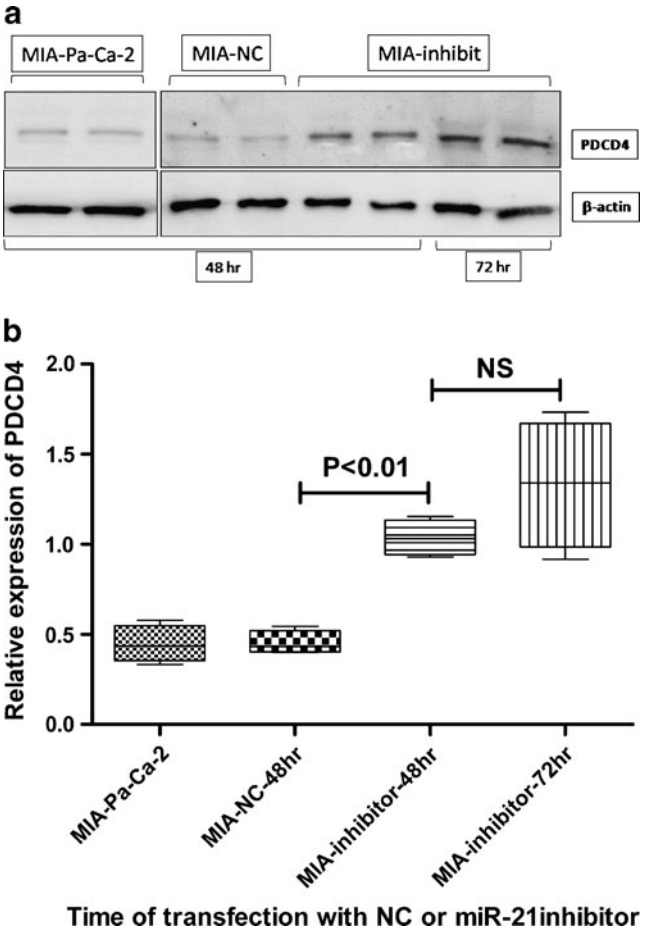

Fig. 6 **a** Western blot demonstrating the increase in expression of PDCD4 (56 kDa) at 48 and 72 h of miR-21 inhibition in MIA-Pa-Ca-2 cells. beta Actin shown below as a loading control. *MIA-Pa-Ca-2* untreated cells, *MIA-NC* MIA-Pa-Ca-2 cell treated with negative control, *MIA-inhibit* MIA-Pa-Ca-2 cell treated with miR-21 inhibitor. **b** Expression analysis of the average density of PDCD4 protein against beta actin in MIA-Pa-Ca-2 cells treated with 48 h of media, 48 h of *NC* negative control (100 nM) and 48 and 72 h of miR-21 inhibition. *Box plots* indicate median with IQR and *whiskers* demonstrate the maximum and minimum levels of PDCD4 protein expression. Statistical significance was calculated using the Mann–Whitney *U* test

cancer tissues may support the concept of personalized medicine in the care of these patients.

Bloomston et al.[32] reported a significant upregulation in miR-181b in archived formalin-fixed paraffin-embedded (FFPE) pancreatic cancer compared with adjacent normal tissue. FFPE samples have advantages of being readily available along with important prognostic data, but they should be used cautiously in the molecular setting due to reports of enzyme-related large RNA degradation and chemical modification from formalin fixation.[41,42] The survivability and expression level of miRNAs in FFPE tissue compared with fresh samples is still largely unknown.

Li et al.[43] examined the reliability of miRNA (n=160) expression with RT-PCR using TaqMan® assays in snap-frozen compared with FFPE thyroid follicular cell samples. They found comparable miRNA expression data between snap-frozen and FFPE samples exhibiting a strong correlation (r^2>0.95). However, few miRNA revealed poor correlation, the worst being miR-146 (decreased) and miR-302b (increased). They hypothesized that in cases of FFPE-related overexpression, precursors of miRNAs may have been cleaved from RNase to produce false-positive signals, which may be an explanation for differences seen from our study. Furthermore, reports have varied regarding the expression miR-181b: some demonstrating upregulation[44] whereas others discovering downregulation in both fresh cancer tissue[44] and cell lines.[45]

Only 10–20% of patients with pancreatic cancer undergo potential curative resection, and 90% of them will have recurrent disease within a year.[46] The use of computed tomography, positron emission tomography and endoscopic ultrasound has increased the sensitivity for predicting actual resectability towards 90%.[47] Despite this, 10% of patients assessed as having resectable disease undergo an unnecessary exploratory laparotomy. Expression profiles of miRNAs may act as new forms of diagnostic and prognostic markers. In situ hybridization demonstrated a significant upregulation of miR-21 in pancreatic cancers, but its expression correlated only with survival and not with tumour size or stage.[48] The present study is the first to identify that miR-21 may have the ability to predict disease stage (Fig. 4). This study was limited by the number of patients recruited, and future work is required to investigate miR-21 profiles in tissue or serum of patients with unresectable disease in order to assess its potential application in predicting disease resectability.

Recent evidence has suggested the role of miRNAs as regulators of cancer-related signalling pathways.[49] The relationship between aberrant expression of miRNAs in cancer and cell signalling pathways has been troubled by limited knowledge in target recognition. It has been reported that the miRNA ‘seed’ region (first two to seven nucleotide of 5′ untranslated region of miRNA) requires perfect complementarity for target mRNA binding,[50] although additional binding requirements are likely to exist. Therefore, although computational methods using ‘seed’ regions for target prediction give good guidance, they are not 100% accurate and experimental approaches through miRNA inhibition are needed to validate predicted targets.

Interesting reports of miRNA detection in serum have recently been established.[13,51,52] Specific expression patterns of serum miRNA have been demonstrated in lung cancer, colorectal cancer and diabetes.[51] The efforts to retrieve an adequate amount of tissue preoperatively for miRNA profiling would be difficult; however, EUS with fine needle aspiration (FNA) has recently emerged as a very specific and less invasive modality for preoperative

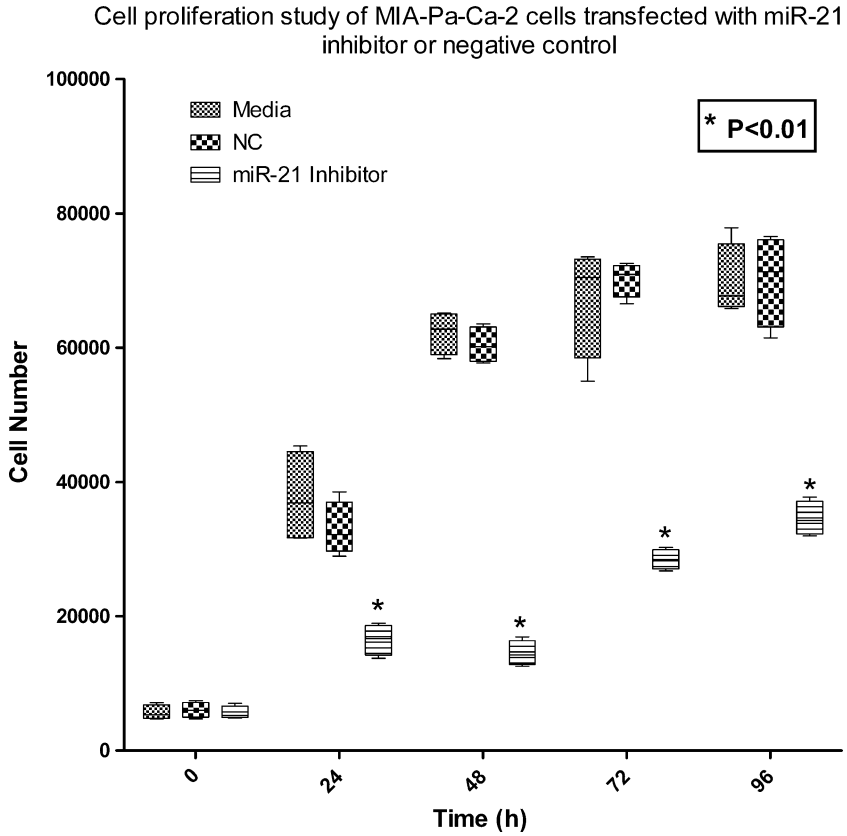

Fig. 7 Cell proliferation study for MIA-Pa-Ca-2 cells treated with media, *NC* negative control (100 nM) or miR-21 inhibitor (100 nM). *Box plots* indicate median with IQR and *whiskers* demonstrate the maximum and minimum MIA-Pa-Ca-2 cell number. Statistical test comparing the cell count of MIA-Pa-Ca-2 cells transfected with miR-21 inhibitor compared with NC or media was performed at each time point with the Kruskal–Wallis test

diagnosis and staging for pancreatic cancer. Szafranska et al.[53] isolated miRNA from FNAs of PDAC patients and controls to find that their expression differed from healthy pancreas, chronic pancreatitis and PDAC tissue. Nevertheless, venipuncture to obtain a blood sample would be least invasive and more acceptable to patients. Wang et al.[54] demonstrated 64% sensitivity and 89% specificity of PDAC diagnosis using the expression levels of a panel of four miRNAs (miR-21, miR-210, miR-155 and miR-196a) from patient serum samples. Development of blood-based biomarkers for PDAC is critical because patients remain asymptomatic until they present with locally advanced or metastatic disease. Therefore, detection of PDAC at a surgically resectable stage offers the best curative option. Further work investigating serum levels of miRNAs in patients with pancreatic cancer and age-matched controls would provide evidence for the potential of utilizing miRNAs for the blood-based detection, diagnosis and surveillance of cancer. This, less invasive method of obtaining patient samples would make large research studies possible in order to explore the use of serum miRNAs as potential biomarkers and prognostic indicators in pancreatic cancer.

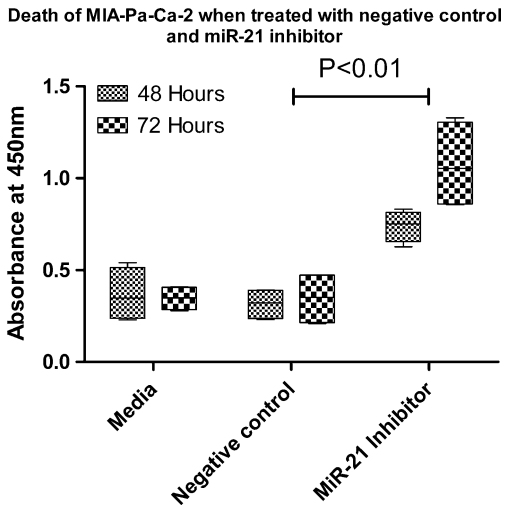

Fig. 8 Cell death study measuring nuclear matrix protein (NMP) in media of MIA-Pa-Ca-2 cells following treatment with media, negative control (100 nM) or miR-21 inhibitor (100 nM). *Box plots* indicate median with IQR and *whiskers* demonstrate the maximum and minimum absorbance. Statistical test comparing the NMP levels of MIA-Pa-Ca-2 cells transfected with miR-21 inhibitor compared with NC or media at both 48 and 72 h was performed using the Kruskal–Wallis test

Demonstrating the therapeutic potential of miRNA inhibition in PDAC models is far from its application in patients. There are some important issues which need to be resolved prior to consideration of any miRNA-based gene therapy which include the better understanding of in vivo miRNA molecule biostability, specificity, delivery and toxicity. LNAs are antimiR oligonucleotides (AMO), which function by blocking the interaction of miRNA and its target by

competition. A key feature of LNA is that it is a chemically modified AMO, which helps improve stability. Si et al.[55] formed xenograft carcinoma models using untreated MCF-7 cells or MCF-7 cells treated with antimiR-21 oligonucleotide. They found that tumours derived from MCF-7 cells treated with antimiR-21 were 50% smaller. Moreover, miR-21 inhibition in glioblastoma cells resulted in increased apoptosis.[17] Complete eradication of miR-21 was observed in LNA-antimiR-21-treated gliomas with the presence of neural precursor cells expressing tumour necrosis factor-related apoptosis in the murine brain.[56] These studies highlight the potential of antimiR oligonucleotides in studying in vivo miRNA function and for the development of miRNA-based therapeutics.

This study has demonstrated that PDCD4 is directly regulated by the miR-21, which is evident at the level of mRNA and protein. Furthermore, the reduced cell proliferation and increased death observed upon miR-21 inhibition suggests that PDCD4, amongst other tumour suppressors, is an important functional target in MIA-Pa-Ca-2 cells. The incomplete complementarity required for miRNA binding to mRNA supports the notion that miRNAs have multiple targets. Therefore, it is likely that the action of miR-21 must be through inhibition of many genetic targets. We were limited in this study to the investigation of PDCD4 in a single PDAC cell line. Therefore, further work is required to identify all miR-21 targets in various PDAC cell lines using DNA microarrays or quantitative proteomic strategies.

These findings contribute to a better understanding of the role of miRNAs in pancreatic carcinogenesis and demonstrate their possible use as biomarkers and treatment targets. The overall 5-year survival of 2% to 3% has prompted intensive searches for novel forms of treatment, and the recognition that miR-21 inhibition retards pancreatic cancer growth in vitro, in part through PDCD4 upregulation, provides a possible therapeutic approach which can be investigated further.

Conflicts of Interest None of the authors has a conflict of interest to declare.

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CASE REPORT

Emergent Orthotopic Liver Transplantation for Hemorrhage from a Giant Cavernous Hepatic Hemangioma: Case Report and Review

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Abstract
Introduction Cavernous hemangiomas represent the most common benign primary hepatic neoplasm, often being incidentally detected. Although the majority of hepatic hemangiomas remain asymptomatic, symptomatic hepatic hemangiomas can present with abdominal pain, hemorrhage, biliary compression, or a consumptive coagulopathy. The optimal surgical management of symptomatic hepatic hemangiomas remains controversial, with resection, enucleation, and both deceased donor and living donor liver transplantation having been reported.
Case Report We report the case of a patient found to have a unique syndrome of multiorgan cavernous hemangiomatosis involving the liver, lung, omentum, and spleen without cutaneous involvement. Sixteen years following her initial diagnosis, the patient suffered from intra-abdominal hemorrhage due to her giant cavernous hepatic hemangioma. Evidence of continued bleeding, in the setting of Kasabach-Merritt Syndrome and worsening abdominal compartment syndrome, prompted MELD exemption listing. The patient subsequently underwent emergent liver transplantation without complication.
Conclusion Although cavernous hemangiomas represent the most common benign primary hepatic neoplasm, hepatic hemangioma rupture remains a rare presentation in these patients. Management at a center with expertise in liver transplantation is warranted for those patients presenting with worsening DIC or hemorrhage, given the potential for rapid clinical decompensation.

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Keywords Hepatic hemangioma · Kasabach-Merritt syndrome · Liver transplantation

Introduction

Cavernous hemangiomas represent the most common benign primary hepatic neoplasm, with a reported prevalence of 3% to 20% based upon autopsy series.[1] These congenital vascular malformations are microscopically composed of cavernous vascular channels that are lined by single layers of flattened endothelium separated by fibrous septae. The majority of hepatic hemangiomas are less than 5 cm in size, solitary, and rarely symptomatic. There is a reported female-to-male ratio of up to 6:1, and most hepatic hemangiomas frequently present within the fourth to fifth decade of life. In 1970, Adam et al. reported a series of 106 hemangioma resections over a 30-year period and delineated hemangiomas >4 cm as "giant".[2] Giant hepatic hemangiomas represent <10% of all hepatic hemangiomas.[3] We describe here a report of emergent liver transplantation for a ruptured giant hepatic hemangioma in a patient with a unique syndrome of multiorgan cavernous hemangiomatosis.

Case Report

In September of 2003, a 32-year-old Caucasian female presented to an outside institution with a chief complaint of increasing abdominal fullness for 2 months. The patient reported mild abdominal discomfort during her daily activities, as well as decreased appetite and early satiety. Her past medical history was significant for an incidentally diagnosed liver mass in 1994 when she had undergone an abdominal computed tomographic (CT) scan as part of a workup for amenorrhea. At that time, the 10-cm well-marginated hypodense mass in the right lobe of the liver was confirmed to be a hemangioma by subsequent nuclear scan, and no further treatment was initiated. On her subsequent presentation in 2003, the patient was found to have massive hepatomegaly and thus underwent laparoscopic biopsy of the hepatic lesion. Pathology revealed a benign cavernous hemangioma, and the patient was then transferred to our institution for further evaluation.

On admission, the patient's laboratory values were notable for a total bilirubin of 2.1 mg/dL (normal range, 0.3–1.3) and an international normalized ratio (INR) of 1.4. Serum tumor markers were all within normal limits. CT scan of the abdomen and chest showed numerous, large hypervascular liver masses, the largest measuring 18× 23 cm (Fig. 1a), splenic hemangiomas, as well as innumerable bilateral pulmonary nodules (Fig. 1b). Given the multiorgan presentation raising concern for a metastatic neoplasm, the patient underwent video-assisted thoracoscopic surgery which demonstrated diffuse, purple, raised, subpleural nodules (Fig. 1c). The lung biopsy demonstrated pulmonary cavernous hemangiomas (Fig. 1d). Additional stains for both CD34 and CD31 highlighted the benign endothelial cell lining of the vascular spaces. In addition, HMB-45 staining was negative, ruling out lymphangioleiomyomatosis. The patient was discharged from the hospital and started on interferon alpha-2b in an attempt to shrink her hepatic and pulmonary lesions; this resulted in mild radiological and symptomatic improvement. Given the size and number of her lesions, it was felt that curative liver resection was not technically possible and that ultimately she might require liver transplantation. The patient was listed for transplantation, but over the ensuing years she remained stable.

In January of 2010, the patient developed severe mid-abdominal pain while riding a horse. She subsequently suffered a syncopal episode and was taken to the emergency room where she was found to be hypotensive with a hematocrit of 22%. She was transfused 4 units of blood and transferred to our facility. On arrival, she was in no distress and hemodynamically stable. Her exam was significant for markedly increased abdominal distension (Fig. 2a). Her admission weight was 84 kg. Laboratory values demonstrated a hematocrit of 24.6%, INR of 2.1, fibrinogen of 78 mg/dL, platelets of $99{,}000\times10^6$/L (decrease from her baseline of $180{,}000\times10^6$/L), creatinine of 0.61 mg/dL, and a total bilirubin of 4.4 mg/dL. Her admission Model for End-Stage Liver Disease (MELD) score was 20. A CT scan demonstrated a large amount of intra-peritoneal blood in addition to the known hepatic hemagiomas. Over the course of the subsequent 48 h, her abdominal distension worsened, and she developed progressive lower extremity edema. Despite resuscitation with blood products, as well as the use of aminocaproic acid, she continued to demonstrate a consumptive coagulopathy with evidence of ongoing bleeding. Her abdominal compartment syndrome worsened, with evidence of decreased urinary output and rising creatinine levels. A petition to the United Network for Organ Sharing Regional Review Board requesting a high initial MELD to ensure life-saving emergent transplantation was submitted and approved.

On hospital day 14, a suitable donor became available. The recipient was brought to the operating room and explored. Four liters of old blood, with no evidence of clot, were evacuated. There were no immediate signs of active bleeding. The liver occupied her entire abdomen, the majority of which showed the appearance of a

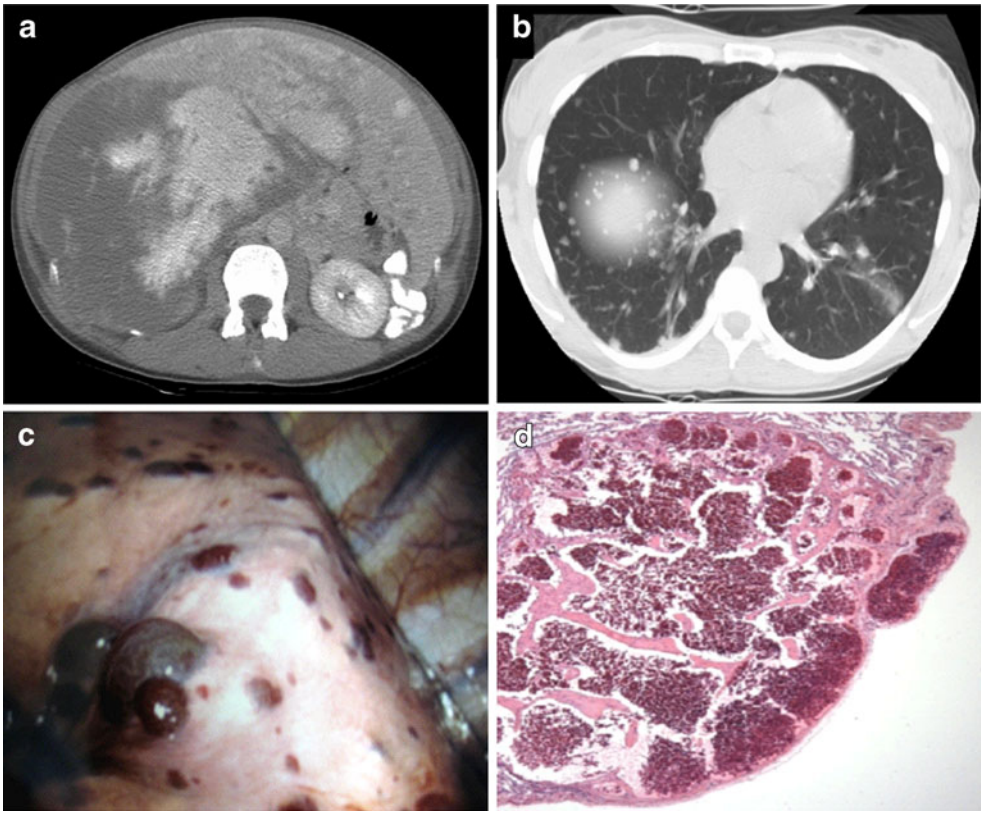

Fig. 1 **a** Initial abdominal CT at presentation in 2003 showing the largest lesion (18.1×15.9 cm) measured at level of the right portal vein and the second largest lesion was 4.3×3.5 cm. **b** Initial chest CT in 2003 revealing innumerable pulmonary nodules. **c** Video-assisted thoracoscopic surgery (VATS). Diffuse hemorrhagic, purple/red, raised nodules are seen on the surface of the lung. Biopsy of these lesions revealed benign cavernous hemangiomas. **d** Photomicrograph of an H&E-stained section of cavernous hemangioma in a lung biopsy specimen (40×). The lung lesions were small and well-circumscribed with benign endothelial cells and thin vessel walls and septa composed predominantly of fibrous tissue.

massive cavernous hemangioma (Fig. 2b). The omentum contained innumerable berry-sized hemangiomas. The patient had a conventional main hepatic artery, as well as replaced right and left hepatic arteries. These were individually ligated and divided. Clamps were then placed on the supra-hepatic inferior vena cava (IVC), infra-hepatic IVC, and portal vein. The liver was then drained of a large volume of blood to facilitate the completion of the posterior dissection under improved visualization. The liver was excised (Fig. 2c), and the donor allograft was brought to the operative field. A bicaval anastomosis, followed by a portal vein anastomosis, was performed. Given the small caliber size of each of the recipient's hepatic arteries, the arterial anastomosis was done directly to the supraceliac aorta. Biliary drainage was achieved by choledochocholedochostomy. Following completion of the liver transplant (Fig. 2d), the patient was extubated in the operating room and transferred to the intensive care unit. She was subsequently transferred to the transplant ward on post-operative day 1 and discharged home on post-operative day 11 following an uneventful post-operative course. On outpatient follow-up, the patient remains well and has begun to resume a normal level of activity.

The patient's weight at the time of discharge was 46 kg, a net loss of 38 kg from her pre-transplant weight. Histological examination of the liver revealed multiple areas of dilated blood-filled spaces lined by a layer of flattened endothelial cells, thus confirming the diagnosis of cavernous hepatic hemangioma. These changes occupied the entire right lobe and the majority of the left lobe of the liver.

Discussion

The majority of hepatic hemangiomas are asymptomatic and require no intervention. Indeed, advances in imaging technology, as well as the widespread application of diagnostic imaging, has resulted in the more frequent detection of hepatic hemangiomas.[4] For those with symptomatic lesions, right upper quadrant pain or fullness is the most common complaint. Whereas 40% of patients with 4-cm hemangiomas are symptomatic, 90% of patients with 10-cm hemangiomas are symptomatic.[5] Giant hemangiomas can present with abdominal fullness or pain, hemorrhage within the hemangioma or within the abdominal cavity with associated hemodynamic compromise, jaundice due to compression of the biliary tree, cardiac failure from massive arteriovenous shunting, or as a consumptive coagulopathy (Kasabach-Merritt Syndrome). The latter syndrome of profound thrombocytopenia, microangiopathic hemolytic anemia, a consumptive coagulopathy, and an enlarging vascular lesion was first reported in 1940 as a case of thrombocytopenic purpura in a 2-month-old child who presented with a rapidly growing cutaneous hemangioma.[6]

The optimal surgical management of giant hepatic hemangiomas remains controversial with resection, enucleation, and

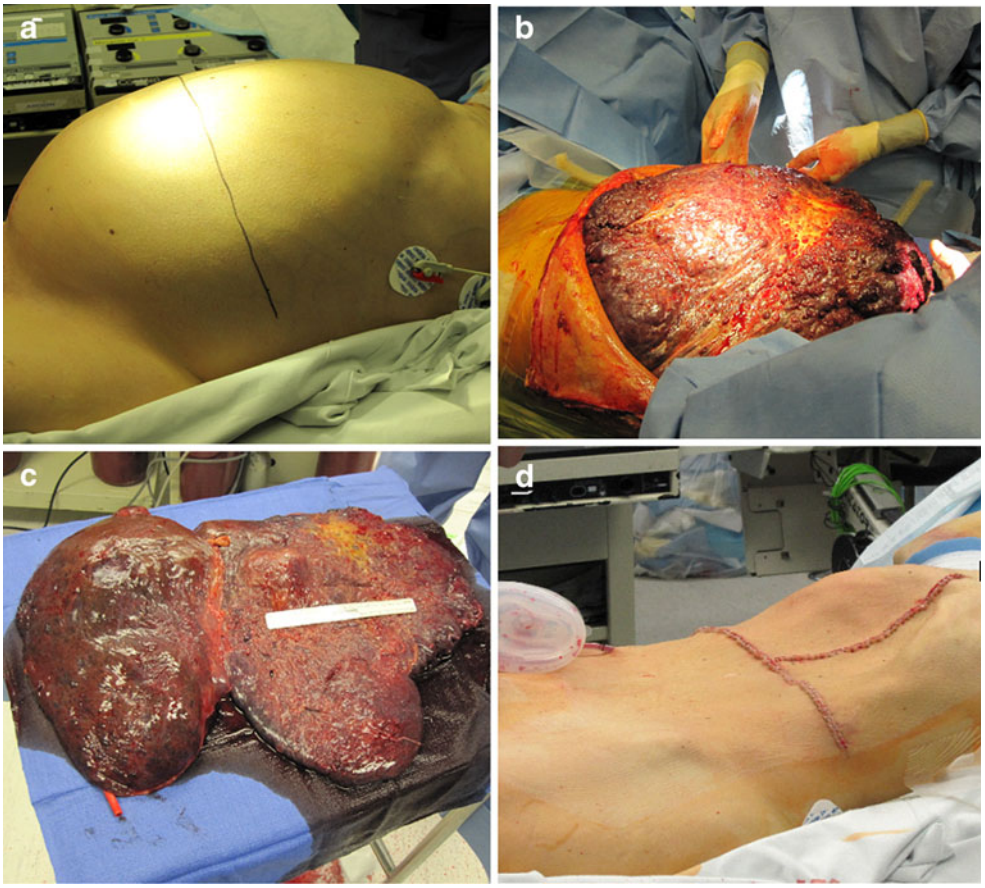

Fig. 2 **a** Intra-operative photo prior to start of liver transplantation demonstrating the patient's massive abdominal distension. **b** Giant cavernous hepatic hemangioma occupying the entire abdominal cavity. **c** Posterior aspect of gross liver specimen following resection and complete decompression (the *white ruler* on the specimen measures 15 cm in length and is on top of the left lobe). **d** Intra-operative photo at the completion of liver transplantation demonstrating the patient's abdomen.

both deceased donor and living donor liver transplantation having been reported.[7–13] Surgical resection or enucleation has often been reserved for symptomatic, single-lobe lesions. Symptomatic patients with unresectable lesions, multiple bilobar hemangiomas, or those involving the hepatic hilum, have often been referred for consideration for liver transplantation. Our patient's clinical course was unique as she was monitored for years without change in clinical presentation and without limitations in her daily activities. Furthermore, the size and diffuse nature of her hepatic hemangiomas precluded liver resection. Consideration for living donor liver transplant prior to her acute presentation was deferred given her continued stability as an outpatient. Furthermore, our patient's rapid decline following presentation prevented the ability for timely living donor evaluation and thus required petition for MELD exemption to allow for emergent liver transplantation. Patients with hepatic hemangiomas who present with evidence of worsening DIC and/or hemorrhage should be referred emergently to centers with expertise in liver transplantation, given the potential for rapid decompensation.

Although previous reports on liver transplantation for giant hepatic hemangiomas have documented reversal of Kasabach-Merritt Syndrome,[7–13] we believe this to be the first report of transplantation in the setting of hemorrhage from a giant hepatic hemangioma. Rupture of a hepatic hemangioma remains an extremely rare event, with a reported incidence of 1% to 4% [14] and a reported high rate of mortality (60–75%).[15] A report from 2003 identified 32 published cases of spontaneous hepatic hemangioma rupture in adults, of which 27 cases were available for review.[16] In the latter study, the mean hemangioma size was 14.8 cm. Four of the 27 patients died from hemorrhage without surgical intervention. Twenty-two of the remaining 23 patients underwent surgery (59% resection, 23% suture ligation, and 18% tamponade). Transcatheter arterial embolization was undertaken in 31% of patients prior to hepatic resection. Mortality for the different forms of surgical interventions was 23% for resection, 40% for suture ligation, and 75% for tamponade, with an overall mortality of 36%.[16] There were no documented cases of hepatic hemangioma rupture managed with emergent liver transplantation.

Non-surgical treatment of giant hepatic hemangiomas in stable patients has been reported with mixed results and includes interferon administration, transarterial catheter embolization, and hepatic radiation therapy. Interferon has known anti-angiogenic properties, and a few case reports have described its efficacy in the treatment of vascular malformations and neoplasms.[17] Our patient demonstrated minimal symptomatic and radiological improvement with

interferon-alpha-2b administration. Small case series have reported radiotherapy as an effective means to limit or regress hemangioma growth, as well as decrease associated symptoms. Radiotherapy has often been administered at a dose ranging from 15 to 30 Gy in 15 to 22 fractions over a few weeks with minimal morbidity.[18,19] It should be noted that the efficacy of radiation therapy in reversing the DIC associated with Kasabach-Merritt Syndrome has yet to be demonstrated. Hepatic arterial embolization has been reported to be the second most common form of treatment of symptomatic giant hemangiomas, with surgical resection being the most common.[20] Transarterial embolization has been reported to provide symptomatic relief, as well as a reduction in hemangioma size.[21,22] Indeed, the ability to perform catheter-directed therapy has circumvented the need for open hepatic artery ligation as a form of management for hepatic hemangiomas. However, the role of transarterial catheter embolization in the management of cavernous hepatic hemangiomas in the setting of Kasabach-Merritt Syndrome remains limited.[23] Complications associated with embolization include liver infarction with associated abscess formation, as well as treatment failure associated with vessel recanalization following embolization.[7] Superselective arterial embolization has been used for patients with hemorrhage from hepatic hemangioma rupture; however, this modality has been employed as a bridge to successful hepatic resection, rather than as a permanent treatment for ongoing hemorrhage.[24]

Conclusion

Herein, we describe a patient with massive hepatomegaly found to have what appears to be a unique syndrome of multiorgan cavernous hemangiomatosis of the liver, lung, omentum, and spleen without cutaneous involvement. Although the majority of hepatic hemangiomas remain asymptomatic, and thus require no intervention, symptomatic focal lesions can be addressed surgically by either enucleation or resection. More extensive symptomatic disease, as demonstrated in our patient, necessitates liver transplantation. Our patient suffered from ongoing intra-abdominal hemorrhage in the setting of Kasabach-Merritt Syndrome, with associated abdominal compartment syndrome. Her continued clinical decompensation required MELD exemption listing and thus allowed for emergent liver transplantation.

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HOW I DO IT

Laparoscopic Distal Pancreatectomy with or Without Splenectomy: How I Do It

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Abstract
Objectives Although the technique of distal pancreatectomy with or without en bloc splenectomy has been well described, the execution of this procedure may be technically challenging when performed laparoscopically. In this technical report, we aimed to describe the technique of laparoscopic distal pancreatectomy with or without splenic preservation.
Discussion Laparoscopic distal pancreatectomy with or without splenectomy is a safe and effective surgical approach for the correction of various conditions. It has been proven to be a feasible solution for the treatment of benign inflammatory conditions as well as neoplasms. Splenic preservation requires careful and meticulous dissection, but may be done safely.

Keywords Splenectomy · Distal pancreatectomy · Laparoscopy · Spleen preservation

Introduction

Laparoscopic distal pancreatectomy was initially described in 1994 by Gagner and Cuschieri.[1,2] Since that time, techniques have been developed for laparoscopic distal pancreatectomy with or without splenic preservation. While not yet the standard of care, laparoscopic pancreatic resection has become an accepted treatment for inflammatory conditions, benign tumors, and low-grade malignant tumors of the pancreas.[3] The purpose of this paper is to outline our method for laparoscopic distal pancreatic resection, with or without splenic preservation.

Operative Procedure

All patients undergoing laparoscopic pancreatic resection require a thorough pre-operative work-up, including full medical evaluation and cardiac evaluation as indicated. If splenectomy is anticipated based on tumor size or location, vaccinations against encapsulated organisms (*Streptococcus pneumoniae*, *Neisseria meningitides*, and *Haemophilus influenzae*) are administered 2 weeks prior to the date of surgery. If splenectomy is performed unexpectedly, these vaccines are administered postoperatively, prior to discharge from the hospital. Patients should ideally undergo routine hematologic tests including tumor markers and imaging such as computed tomography (CT) of the abdomen prior to surgery. Although an endoscopic retrograde cholangiopancreatography may be performed to assess ductal anatomy, a magnetic retrograde cholangiopancreatography can be performed. This technique has the advantage to provide similar information about the ductal anatomy, without the complications associated with pancreatic ductal injection of dye (e.g., pancreatitis). For smaller tumors, intraoperative laparoscopic ultrasound is useful for tumor localization.

Patient Positioning/Port Placement

We place patients on the operating table in lithotomy position. After a foley catheter is placed, a towel roll is placed under the patient's left side to elevate it to 15 to 20°. The operating surgeon stands between the legs of the patient with the first assistant on the right side of the operating table. Pneumoperitoneum is established after a 10-mm infraumbilical port is

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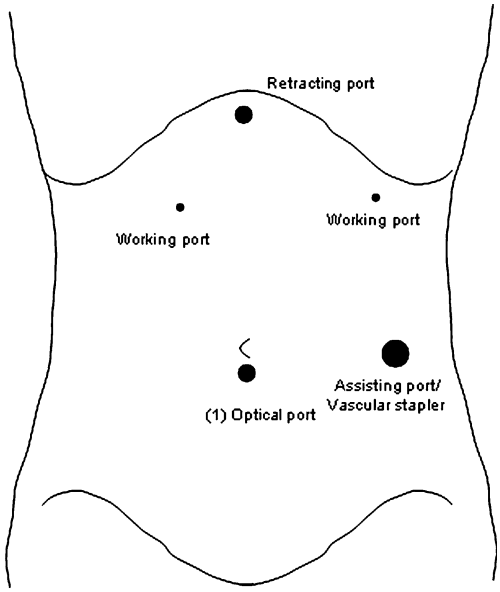


Fig. 1 Position of operative ports

placed. Subsequent ports are placed under direct visualization. A 10-mm subxiphoid port is placed in addition to a 5-mm port along the left anterior axillary line and a 5-mm port along the right midclavicular line just above the level of the umbilicus. A 12-mm port is placed along the left midclavicular line at the same level as the umbilicus for future use of the endovascular stapler (Fig. 1). The left midclavicular site is often extended for removal of the specimen at completion of resection.

Entry into Lesser Sac

Diagnostic laparoscopy is performed following placement of ports to ensure that no metastatic disease is present. Entry into the lesser sac is gained by dividing the greater omentum below the gastroepiploic arcade. This maneuver is facilitated by retraction of the stomach superiorly and lateral retraction of the omentum, thereby exposing the gastroepiploic arcade. The underlying gastrocolic ligament is then divided using the harmonic scalpel (Ethicon Endo-Surgery, Cincinnati, OH, USA) or alternatively, using LigaSure (Valleylab, Boulder CO, USA). This maneuver exposes the body and tail pancreas and localizes the pancreatic lesion (Fig. 2).

Dissection of the Pancreatic Body and Tail for Splenic Preservation

Distal pancreatic lesions are approached by dividing the left gastroepiploic vessel and mobilizing the splenic flexure. The short gastric vessels are preserved. The peritoneum along the inferior edge of the pancreatic tail is divided proximal to the tumor. During this step, care must be taken as the posterior surface of the pancreas is dissected off the retroperitoneal surface. This maneuver exposes the splenic vessels and their branches. The splenic vessels can be preserved, and their branches are most easily divided using the harmonic scalpel (Fig. 3). If the tumor invades the splenic vessels, these may be divided using the endovascular stapler. The spleen can still be preserved if the short gastric vessels are intact (Fig. 4).

Transection of Pancreatic Tail with Splenic Preservation

The key to successful splenic preservation in distal pancreatectomy was best described by Warshaw in 1988 and is easily applicable to laparoscopic techniques.[4] Successful splenic-sparing surgery involves maintaining adequate blood supply to the spleen via the short gastric vessels, or by preserving the splenic artery and vein (Fig. 4). Once the dissection is complete to a point where the pancreas can be transected proximal to the tumor margin, the pancreas is transected

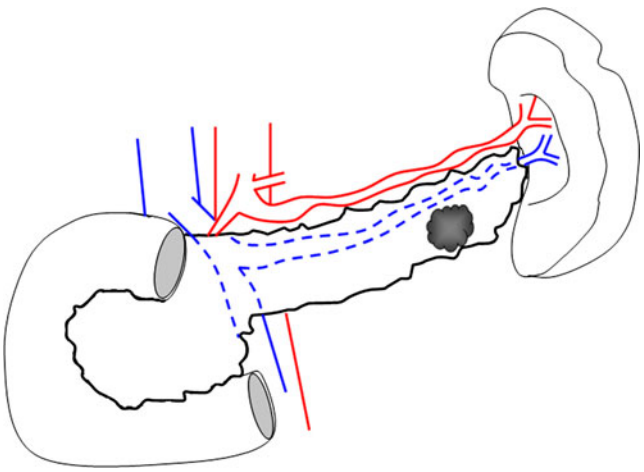


Fig. 2 Overview of the anatomy of the body and tail of the pancreas after entry into the lesser sac and localization of the pancreatic lesion

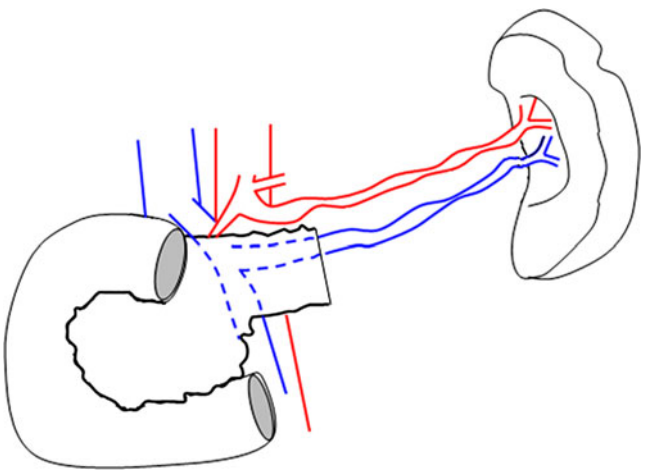


Fig. 3 Pancreatectomy with preservation of splenic vessels

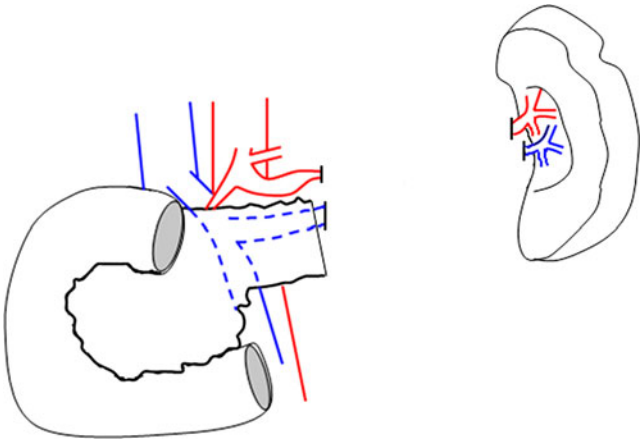


Fig. 4 Splenic-sparing pancreatectomy maintaining adequate blood supply via the short gastric vessels

using a 45-mm or a 60-mm Endo-GIA stapler (Covidien Healthcare, Norwalk CT, USA). The stapler is placed through the left-sided midclavicular port to allow a direct approach to the pancreas. The splenic artery and vein can be preserved if the stapler is used for the pancreas. If the pancreas is too thick to be safely stapled, we use either the TissueLink (Salient Surgical Technologies, Portsmouth NH, USA) or the laparoscopic Habib (AngioDynamics, Latham NY, USA). If the latter two approaches are used, we transect the splenic artery and vein with the stapler prior to transecting the pancreas. The dissection then continues to the splenic hilum. If the splenic vessels were transected proximally, they are transected again at the hilum. If the splenic vessels were preserved, then they are carefully dissected free with the harmonic scalpel at the splenic hilum.

Specimen Removal and Closure

The specimen is carefully removed via the left midclavicular incision using an Endo-Catch device. The incision used is usually enlarged transversely to allow for specimen removal. A closed-suction drain is placed through the subxiphoid trocar site and guided into the lesser sac. The incision used for specimen retrieval is then closed in standard fashion, as well as the 10-mm infraumbilical trocar site.

En-bloc Resection with Spleen

If the area of resection is too close to the splenic hilum, it may be necessary to perform en-bloc resection of the pancreatic tail and spleen (Fig. 5). Once the lesser sac is entered, the entire gastrocolic omentum is divided, including the short gastric vessels. The stomach may then be retracted anteriorly while the gastrocolic and gastrosplenic ligaments are separated.

The splenic artery is dissected circumferentially and transected with a vascular stapler to minimize risk of major bleeding during further dissection. The splenic flexure of the colon is then mobilized, usually aided by retracting the transverse colon inferiorly. After dissection along the inferior border of the pancreas, the pancreas is then lifted anteriorly to isolate and transect the splenic vein with the vascular stapler.

Once transection of the pancreas is complete, this larger specimen may be removed by extending the infraumbilical incision. The resected specimen is generally placed into an impermeable bag such as an Endo-Catch bag to avoid splenic rupture during specimen removal.

Postoperative Care

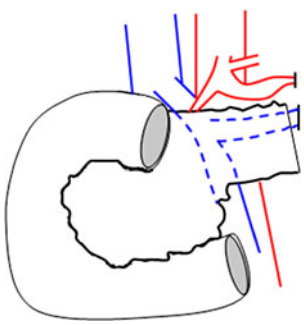
Following surgery, patients are admitted to the hospital and placed on the general nursing floor during the first postoperative day. Patients rarely require a stay in the intensive care unit postoperatively. During this time, patients are monitored for any signs of hemorrhage, a potentially devastating complication of this surgery. We do not place a nasogastric tube postoperatively, and most patients tolerate liquids on the first postoperative day. We usually advance patients to solid food for the second meal, and patients are discharged on the second postoperative day.

Pancreatic leak is a well-described complication in both laparoscopic and open pancreatic resection. Drain output should be monitored closely, and the fluid amylase of this output is checked after the second solid meal. The drain is pulled if the drain amylase is less than three times that of the serum amylase. If a leak is suspected based on high amylase content, management is generally conservative, and patients are discharged with the drain placed to a non-suction drainage bag. Patients who do not progress rapidly or those with fever and persistent abdominal complaints are assessed for intra-abdominal collections. Generally, these collections are diagnosed by CT scan, and the existing drain is repositioned, or a new catheter is percutaneously placed for drainage.

Conclusion

Laparoscopic distal pancreatectomy with or without splenectomy is a safe and effective surgical approach for the correction

Fig. 5 Distal pancreatectomy with en-bloc splenectomy



of various conditions. It has proven a feasible solution for the treatment of benign inflammatory conditions as well as neoplasms. Splenic preservation requires careful and meticulous dissection, but may be done safely if the location of the tumor being resected is amenable to this procedure.

Several studies have demonstrated that laparoscopic pancreatic resection has similar complication rates and long-term outcomes, with decreased intraoperative blood loss and length of hospitalization, as compared with open pancreatic resection. Baker et al. compared intraoperative and postoperative data of patients undergoing open or laparoscopic distal pancreatectomy. Though overall operative time, morbidity, and 30-day mortality were all comparable in both groups, and intraoperative blood loss and length of hospitalization were markedly improved in the laparoscopic group.[5] An earlier meta-analysis of 496 patients by Knaebel et al. also supports these findings in addition to demonstrating decreased overall mortality rates in patients undergoing laparoscopic distal pancreatic resection.[6] Similarly, a multi-institutional study of 667 patients following laparoscopic pancreatic resection demonstrated a statistically significant decrease in operative blood loss and hospital stay as opposed to patients undergoing open resection.[7] Complication rates following pancreatic resection, whether open or laparoscopic, remain high, nearing 50% in some studies. Finan et al. recently compared complication rates in patient groups undergoing laparoscopic versus open distal pancreatectomy and found that there was no significant difference in pancreatic fistula formation or clinically significant leaks between the two groups. Those undergoing laparoscopic resection, however, were noted to have significantly lower malignancy rates and smaller tumor sizes than the open resection group.[8]

Pancreatic fistula is one of the most common complications after pancreatic resection, with reported incidence of almost 30% in some series.[9–11] A large series by Harris et al. of 215 patients undergoing distal splenectomy demonstrated that transection via electrocautery in conjunction with oversewing of the pancreatic duct had the lowest rate of pancreatic leak.[12] In open surgery, oversewing the pancreatic duct is the standard procedure; however, this is not always easily performed during laparoscopic pancreatic resection, and a laparoscopic stapler is usually used for transection. Therefore, studies have been performed to evaluate the use of fibrin glue over the staple line or placement of bioabsorbable reinforcement over the staple line to prevent leak. A study by Jiminez et al. examined the use of ePTFE-buttressed staplers (Gore Bioabsorbable Seamguard, W.L. Gore, Newark, DE, USA) to enforce the staple line.[13] This series of 31 patients demonstrated a statistically significant decrease in the rate of pancreatic leak with the use of buttressed staplers. These results are also supported in a 2007 study by Thaker et al.[14] On the contrary, the use of fibrin sealant to occlude the pancreatic duct, thereby minimizing the risk of leak, has also been studied extensively with less encouraging results. Several studies have shown the use of Tisseel fibrin sealant (Baxter Pharmaceuticals, Deerfield, IL, USA) injected into the duct or over the pancreatic staple line, though not harmful, demonstrated no significant decrease in the rate of fistula formation.[15] Meticulous dissection and oversewing of the pancreatic duct remain the gold standard for optimal outcome to minimize pancreatic leak.

While there are several variations on the technique described above, this standard technique has proven successful in our patient series thus far for a variety of pancreatic pathologies.

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REVIEW ARTICLE

From Longitudinal Gastric Resection to Sleeve Gastrectomy—Revival of a Previously Established Surgical Procedure

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Abstract
Introduction Sleeve gastrectomy is becoming increasingly popular within bariatric surgery. Initially introduced as a component of complex interventions and later as part of a two-stage operation in high-risk patients, the procedure is now more common as one-stage operation and subject of avid scientific discussion. However, the concept of longitudinal gastric resection is not new. The procedure was already established in ulcer surgery but soon faded into insignificance. This article aims to trace the historical development of resection of the greater curvature with particular reference to its origin in ulcer and bariatric surgery. The contribution of ulcer surgery to modern sleeve gastrectomy is highlighted. Furthermore, the current value of sleeve gastrectomy within the spectrum of bariatric surgical procedures will be discussed. Relevant medical literature from PubMed to April 2010 was reviewed.
Discussion Besides bariatric surgery modern sleeve gastrectomy has one more so far largely neglected origin: segmental and later longitudinal gastric resection used in ulcer surgery. Experience and achievements from ulcer surgery simplified and facilitated development of sleeve gastrectomy which is not the desired universal procedure for bariatric surgery but certainly an attractive treatment option. It should be performed in a more standardized manner and with due regard to future long-term results.

Keywords Segmental gastric resection · Tube gastrectomy · Longitudinal gastric resection · Sleeve gastrectomy · Bariatric surgery

Introduction

Obesity is gradually turning into an epidemic condition throughout the world and has become a social, psychological, and economic burden of growing proportions.[1,2] It is associated with a large number of concomitant diseases (including type-2 diabetes, cardiovascular and respiratory diseases, dyslipidemia, and elevated risk of cancer) and also markedly shortens the obese person's life expectancy.[3,4]

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Due to the limited options and especially the poor long-term results of conservative treatment, the surgical approach of bariatric surgery has been established in the last few decades.[3]

A bariatric procedure is considered to be indicated in adult patients with morbid obesity (BMI≥40 kg/m^2) or a BMI≥ 35 kg/m^2 with additional comorbidities.[5,6] Long-term results of the surgical approach have been convincing in terms of reduced morbidity and mortality as well as enhanced quality of life.[7,8] Due to growing experience and the introduction of the endoscopic technique, the procedures have become increasingly safe and can be performed more easily by the use of modern stapling devices. Therefore, bariatric surgery is even considered in adolescents with a high-risk profile or in patients with BMI<35 kg/m^2.[9,10]

Several surgical procedures have been developed over time and nearly all of them are currently performed by the laparoscopic approach. A distinction has been made between restrictive, malabsorptive, combined restrictive and malabsorptive, and electrical procedures for gastric stimulation. This diversity and the ongoing modifications of

the procedures highlight the fact that there is no ideal procedure for widespread application. The quality of the respective procedures is no longer established by the previously used primary parameter of "excess weight loss," but by the procedure's potential to maintain sufficient weight reduction on a long-term basis while ensuring minimal mortality and morbidity.

In recent times, one procedure has become increasingly popular in obesity surgery, namely longitudinal gastric resection or sleeve gastrectomy. It was initially used as a part of complex interventions (including biliopancreatic diversion with duodenal switch) and later as a two-step bridging procedure in high-risk patients prior to final intervention, but was then established as a stand-alone procedure and is currently a subject of avid scientific discussion.

The current concept of tube gastrectomy by resection of the greater curvature is not new in bariatric surgery. It is largely neglected that segmental and especially longitudinal gastric resection were developed and used in ulcer surgery.[11–13] Following the introduction of adequate conservative drug therapies, ulcer surgery is now almost exclusively used as an emergency procedure. Subsequently longitudinal gastric resection faded into insignificance. However, longitudinal gastric resection can be regarded as precursor of modern tube gastrectomy, which is now known as sleeve gastrectomy and is experiencing a revival in obesity surgery.

The aim of the present review is to trace the historical development of the current longitudinal gastric resection on the basis of its origins in ulcer and bariatric surgery and to elucidate the subject with suitable illustrations. Furthermore the contribution of historic ulcer surgery to modern bariatric surgery and in particular sleeve gastrectomy is demonstrated. Finally, contemporary sleeve gastrectomy, its complications, and especially the current value of this procedure in the therapy spectrum of bariatric surgery will be discussed on the basis of major recent studies.

We conducted an extensive literature review and evaluation for this purpose.

(a) Development of longitudinal gastric resection in ulcer surgery

The advancing use of gastric surgery is a milestone in the evolution of abdominal surgery. The first gastric resection procedures were performed by J. Péan and L. Rydygier in 1879 and 1880, but with lethal outcomes.[14,15] T. Billroth is known as the pioneer of gastric surgery and its scientific foundation. In 1881, he performed the first successful gastric resection in a patient with a pyloric carcinoma.[16] His work served as the starting point for classical gastric resection procedures such as Billroth I and Billroth II (first performed on a human patient in 1885), depending on the manner of restoration of the gastrointestinal passage. These procedures became essential elements of every general surgeon's repertoire, particularly for ulcer treatment.

K. Schwarz's discovery of the concept of "no ulcers without acids" in 1910 had a decisive impact on the development of gastric resection procedures.[17] After this time, the aim of upcoming ulcer surgery was to reduce acid levels adequately in order to avoid recurrences. The purpose of the first segmental gastric resection procedures was to perform wedge- or V-shaped ulcer excision; these were conducted as early as 1897 by J. Mikulicz, 1904 by B. Riedel, and 1929 by F.G. Connell.[18–20] However, initially the outcome of these procedures was impeded due to considerable side effects like gastric emptying disorders.[11] Segmental gastric resection was greatly modified by O.H. Wangensteen who, in 1952, investigated the surgical procedures in great depth and resolved the problem of postoperative gastric emptying disorders by performing additional pyloroplasty.[21] The technique was developed further by D.J. Ferguson (1960), F. Largiadèr (1971), and T. Sekine (1975).[22–24] The outstanding aspect of these advancements was preservation of antral innervation in order to prevent the post-gastrectomy syndrome. Again it was O.H. Wangensteen who encouraged to make use of longitudinal resection of the greater curvature. Influenced by his experiences in segmental gastric resection with its undesirable side effects (among others dumping syndrome), he searched for an "acceptable operation" for ulcer treatment. He was aware of the fact that gastric parietal cells, which are responsible for the production of HCL, are most dense in the corpus region—lengthwise along the greater curvature.[11,25] When performing the previous classical resection procedures (including BI and BII resections and segmental gastric resection), portions of the stomach were removed at right angles to its conceived longitudinal axis. However, performing gastric resection along the longitudinal axis was considered even earlier by F. Neugebauer, A.A. Strauss, and V. Schmieden in 1921 and 1924.[26–28] In contrast to Wangensteen they performed resection along the lesser curvature to remove the ulcer itself, independent of acid reduction. However, Wangensteen performed the first experiments of longitudinal resection along the greater gastric curvature to reduce acidity of gastric juice in 1940. In some cases, he added a gastrojejunostomy at the antral gastric end (Fig. 1).[25,29] Based on promising results, he subsequently evolved his method of tubular gastric resection with additional transverse gastroplasty in order to accomplish a gastric reservoir function. Wangensteen started applying this technique in 90 patients with duodenal ulcers and reported convincing results.[11] However, after performing further animal experi-

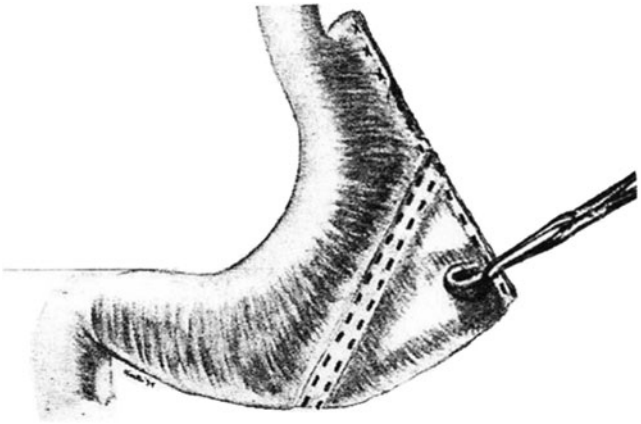

Fig. 1 As early as in 1940, O.H. Wangensteen performed longitudinal gastric resection to excise acid-producing regions of the stomach[29]

ments in 1957, he revised his initially positive verdict about the operation. The acid response of the stomach after test meals was many-fold higher in animals subjected to tubular resection than in those that had undergone segmental gastric resection. Wangensteen and colleagues attributed this phenomenon to the preservation of antral innervation and the resulting higher gastrin and acid secretion of the residual parietal cells. He concluded that tubular gastric resection should be viewed with caution and stopped using this technique.[30] It is noteworthy that L. Leger and L. Deloyers made use of tubular or longitudinal resection without supplementary transverse gastroplasty.[31,32]

In 1966, M. Saegesser introduced the theoretical construct of "ideal gastric resection" including resection of the corpus/fundus and the antrum, in combination with a selective post-branchial vagotomy and pylorotomy (Fig. 2).[33] By performing longitudinal resection of the greater curvature, he intended to reduce acid secretion while preserving gastric reservoir function and the natural food passage. In 1988–1990, J. Hauss and H.U. Spiegel focused on this construct and Wangensteen's results, and made further developments.[12,34] However, they dispensed with the vagotomy and pylorotomy demanded by Saegesser because they believed that partial resection of the antrum and postoperative reduction of parietal cells would achieve sufficient acid reduction. In animal experiments, they achieved a 70% reduction of acid secretion in the presence of a normal serum gastrin response (Fig. 3).[34] In 1993, subsequent animal studies using this model showed a linear correlation between the reduction of parietal cells and acid reduction levels.[35] Spiegel's model was based on longitudinal gastric resection on the side of the greater curvature using a stapler and a gastric probe as guide rail. Thus, he created a "modern" gastric tube (Fig. 4a, b).[35] Subsequently, the procedure was used in the clinical setting with promising results.[13,36]

The use of gastric resection procedures in gastroduodenal ulcer surgery entered a phase of stagnation and regression after this time. The decisive change which led to the renunciation of conventional resection procedures was the fact of advancing knowledge about the pathogenesis of ulcers, particularly the introduction of H_2 receptor antagonists at the end of the 1970s, the introduction of proton pump inhibitors at the end of the 1980s, and the discovery of *Helicobacter pylori* in 1982. These developments had an equally strong impact on various vagotomy procedures for denervation, which were used less, and less in ulcer surgery.

Currently, the use of gastroduodenal ulcer surgery is confined to classical ulcer complications (hemorrhage, perforation, penetration, pyloric stenosis) and to exclude malignant tumors in cases of ulcers refractory to conservative treatment. The clinical use of longitudinal gastric resection was therefore becoming increasingly insignificant soon after being established as a treatment option. This was accompanied by lack of sufficient data or further relevant publications.

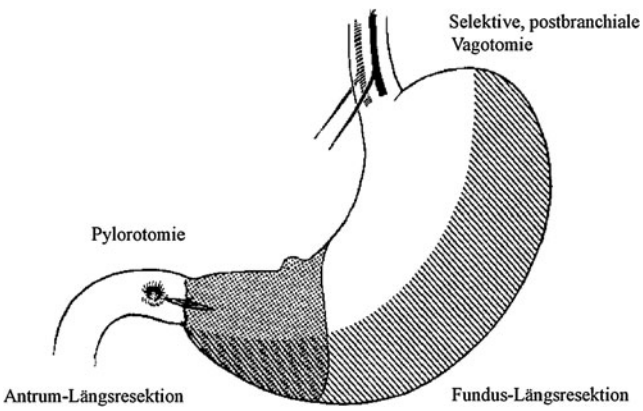

Fig. 2 In 1966, M. Saegesser propagated his construct of "ideal gastric resection" with longitudinal resection of the fundus and the antrum, selective post-branchial vagotomy, and pylorotomy[33]

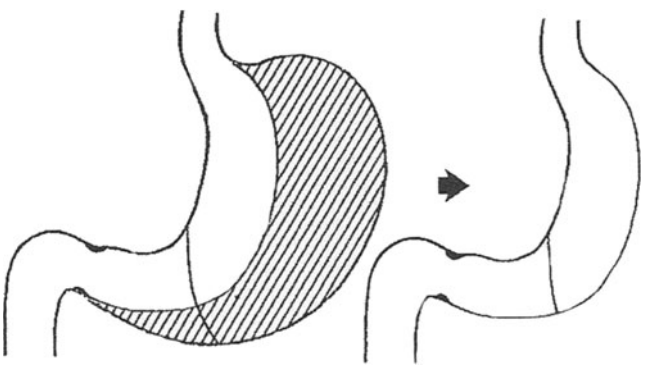

Fig. 3 In 1988, J. Hauss and H.U. Spiegel presented a modified longitudinal resection model without vagotomy and pyolorotomy and reported significant acid reduction[34]

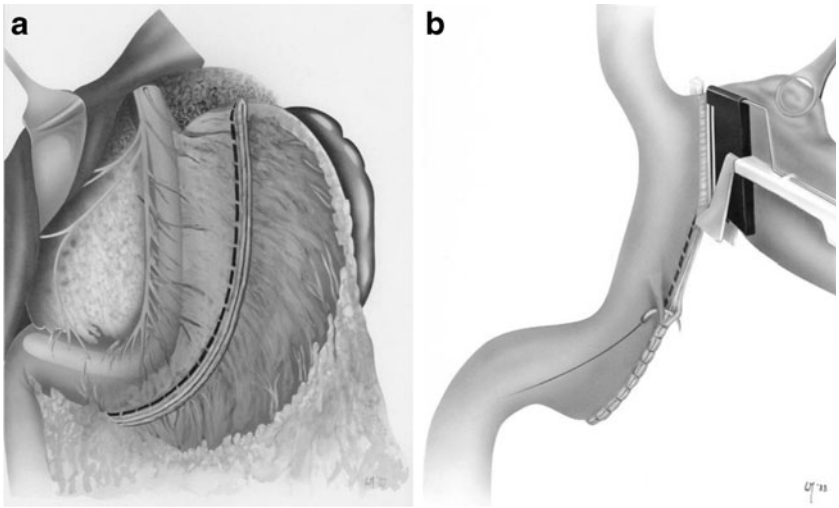

Fig. 4a and b In 1993, H.U. Spiegel used longitudinal gastric resection in a large study focused on the treatment of ulcers. He utilized a gastric probe as guide rail (**a**) and a linear stapler (**b**) to create a "modern" gastric tube[35]

(b) Development of longitudinal gastric resection in bariatric surgery

A review of the essential steps in the historical development of bariatric surgery is helpful in order to understand how longitudinal gastric resection appeared as sleeve gastrectomy within the modern therapy options. Obesity surgery started with purely malabsorptive procedures, moved on to combined malabsorptive and restrictive procedures, and finally consisted of mainly restrictive procedures. The first published bariatric intervention was a malabsorptive jejunoileal bypass performed by A.J. Kremen and co-workers in 1954.[37] Numerous modifications followed, particularly in respect of location and type of the anastomosis.[38] A significant reduction in weight was achieved. However, many of these procedures were accompanied by serious side effects (including diarrhea, hepatic cirrhosis, and electrolyte imbalance) and did not prevail in the long term.[2,39]

Gradually, bariatric interventions were increasingly focused on the stomach. Various methods were used to reduce gastric volume and stimulate satiety. Furthermore, a malabsorptive component was additionally employed to create a gastrointestinal bypass. In 1967, E.E. Mason submitted the first report of a gastric bypass after horizontal division of the stomach with re-anastomosis of its proximal portion by the use of a raised jejunal loop.[40] Again, numerous variations regarding pouch size or replacing division of the stomach by applying a horizontal row of clip sutures followed. The Roux-en-Y gastric bypass published by W.O. Griffen in 1977, using a gastrojejunostomy, and Y-Roux reconstruction, while avoiding bile reflux, provided the advantage of a tension-free anastomosis.[41] After further modifications (particularly in respect of placement of the pouch and the length of the respective loops), this technique evolved into a standard procedure in bariatric surgery, especially in the USA, because of its very favorable ratio between weight reduction and side effects.[42]

A further noteworthy milestone in the development of bariatric surgery is biliopancreatic diversion which was developed by N. Scopinaro in 1979. Biliopancreatic diversion is also a combination of a malabsorptive procedure and a restrictive component. Scopinaro combined horizontal gastric resection with closure of the duodenal stump and a gastrojejunostomy while creating a "common tract" by jejunoileostomy to exclude large portions of the small bowel (Fig. 5).[43] Scopinaro initially varied the lengths of the three segments of the small bowel. Subsequently a "common tract" about 50 cm in length and an "alimentary tract" about 250 cm length became established.[2,44] The disadvantages of the procedure include

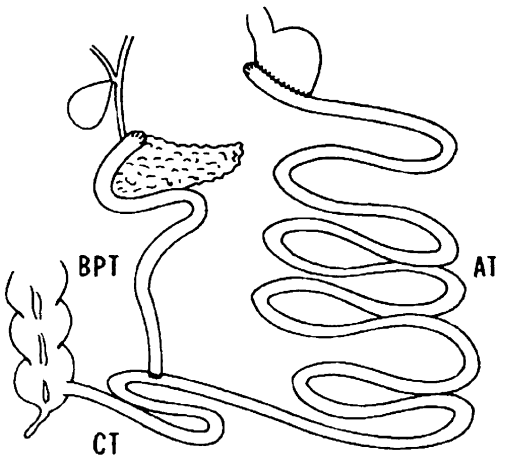

Fig. 5 In 1979, N. Scopinaro introduced his procedure of biliopancreatic diversion. He performed horizontal partial resection of the stomach with closure of the duodenal stump, gastrojejunostomy, and a jejunoileal anastomosis to create an "alimentary tract" (*AT*), a "biliopancreatic tract" (*BPT*), and finally, a "common tract" (*CT*)[99]

malassimilation of fat and deficiency syndromes such as those of protein, iron, or vitamins.[44,45]

In 1973, E.E. Mason and K.J. Printen reported the first purely restrictive procedure by incomplete horizontal division of the stomach while forming a conduit on the side of the greater curvature. However, the technique did not gain wide acceptance because of poorly sustained weight reduction.[46] Subsequent variations were used to achieve a reduction of gastric volume but were not successful due to dilatation of the gastric pouch.[2,38] This problem was finally resolved in 1982, again by E.E. Mason, who introduced vertical gastroplasty with creation of a pouch on the side of the lesser curvature by placing a vertical clip suture and providing additional reinforcement with a distal polypropylene mesh ring.[47] Finally, restriction of the stomach by the use of a gastric band was developed in 1978, initially without the option of being adjustable.[48] The adjustable gastric band initially introduced by L.I. Kuzmak in 1986 was modified further and is the second most commonly used procedure in obesity surgery these days.[38,49]

The modern procedure of longitudinal gastric resection or sleeve gastrectomy was incorporated quite late in the repertoire of obesity surgery. In 1993, P. Marceau and co-workers modified biliopancreatic diversion which had been introduced by N. Scopinaro and replaced horizontal gastric resection with longitudinal gastric resection on the side of greater curvature, combined with preservation of the pylorus, and additionally doubled the length of the "common tract" to 100 cm.[44] Initially the small bowel was anastomosed to the proximal duodenum with additional placing of a distal row of clip sutures without transsection of the duodenum. However, this procedure was frequently associated with insufficiency of clip sutures, followed by a renewed increase in weight.[44] The problem was resolved by the advancements made by D.W. and D.S. Hess, based on T.R. DeMeester.[50,51] In 1998, they introduced biliopancreatic diversion with placement of a duodenal switch under postpyloric transsection of the duodenum and subsequent anastomosis with the alimentary loop. The biliopancreatic loop was anastomosed in the region of the distal ileum by creating a 50- to 100-cm-long "common channel," again anastomosed to the alimentary loop. They also utilized tube gastrectomy as a restrictive component (Fig. 6).[51] In conjunction with the development of minimally invasive surgery, the first laparoscopic tube gastrectomy was performed in the course of biliopancreatic diversion with a duodenal switch in 2000 (Fig. 7).[52]

One of the milestones in the development of tube gastrectomy was the concept of the Magenstrasse and Mill operation. Developed with the aim of devising a physiological bariatric procedure while avoiding implant-related complications (such as those encountered with an adjustable gastric band or vertical banded gastroplasty) and reducing long-term metabolic complications, this procedure was described by D. Johnson in 1987.[53] A circular stapler is used to create a hole in the antrum region, and a linear stapler is used to create a gastric tube on the side of the

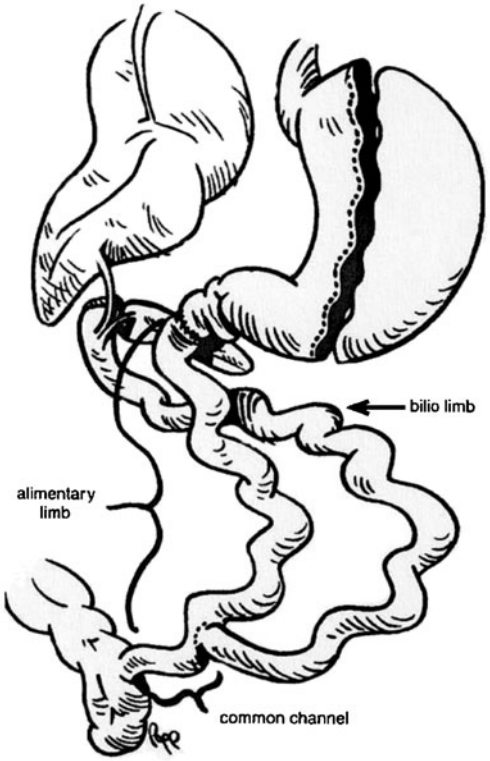

Fig. 6 In 1998, D.W. and D.S. Hess published biliopancreatic diversion with an additional duodenal switch. While preserving the pylorus they also created a biliopancreatic, an alimentary, and a common small bowel segment. Using longitudinal gastric resection, they established a combined restrictive-malabsorptive procedure[51]

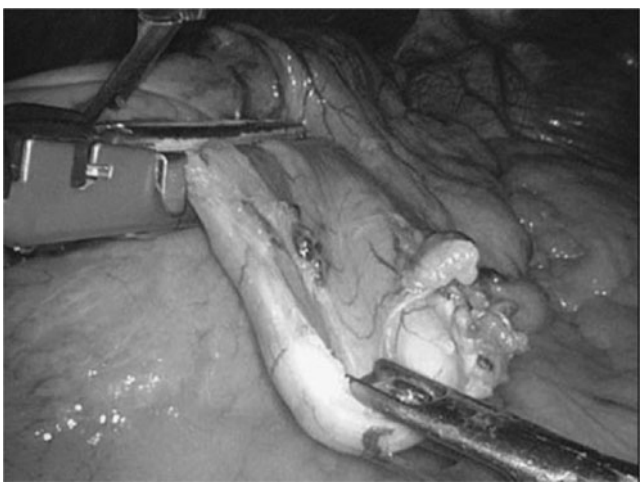

Fig. 7 Intraoperative view of contemporary laparoscopic sleeve gastrectomy. Resection is performed on the side of the greater curvature by the use of a linear stapler along a calibration probe[100]

lesser curvature (the Magenstrasse) while dividing the stomach longitudinally in cranial direction. In contrast to "modern" tube gastrectomy, no resection is performed. Because of the preserved antral mill of food, the method is known as the Magenstrasse and Mill operation. The procedure aroused a lot of interest because of its low side effects and marked weight reduction, particularly in the early postoperative phase.[53]

Thus, modern tube gastrectomy may also be regarded as continuation of the Magenstrasse in distal direction with subsequent resection (including the portions of the stomach that produce ghrelin; see the "Discussion" section). The use of tube gastrectomy as a "bridging" step in a two-step surgical procedure is probably one of the most recent developments. Tube gastrectomy is used as an initial intervention in high-risk patients in order to achieve a marked reduction of weight and risk factors and then perform the final intervention.[54–56]

Based on the positive experience gained from this concept and the technical simplicity of the procedure, gastric tube formation was eventually used as a stand-alone and single-step procedure. It is currently being applied to an increasing extent and is also extensively discussed.[54,56,57]

Discussion

Longitudinal gastric resection in ulcer and bariatric surgery was developed and established for different purposes and apparently, in a mutually independent manner. Nevertheless, achievements in historical ulcer surgery benefited the development of sleeve gastrectomy. Beyond this, resemblance in the development of longitudinal gastric resection and sleeve gastrectomy, respectively, can be demonstrated.

Significant experience with the procedures of segmental gastric resection contributed decisively to increase the understanding of antral innervation and pyloric function.[11] Side effects like malnutrition, gastric emptying disorders, dumping syndrome following segmental gastric resection, or classical Billroth procedures led to further development of pylorus-preserving gastrectomies providing knowledge of great value about the physiological consequences of resection procedures and vagotomy.[58,59] Gradually, the complex role of the stomach as storage unit (fundus and oral corpus) and as a mill (distal corpus and antrum) was recognized and the interference with surgical procedures evaluated.[60–62] Particularly the above-mentioned side effects encouraged O.H. Wangensteen to search for an acceptable operation for ulcer treatment and to introduce tubular or longitudinal gastric resection.[11] After Wangensteen turned away from the procedure research focusing on longitudinal gastric resection was not abandoned. His successors established a clinically applicable procedure for ulcer treatment despite preserving antral innervation by resection of the greater curvature and thus performing a sleeve gastrectomy.

However, after a brief period of clinical use this procedure faded into insignificance due to the upcoming and widespread conservative treatment options. On closer inspection of the circumstances during introduction of longitudinal gastric resection in bariatric surgery one is initially surprised about remarkable similarities to ulcer surgery. In his article focusing on the introduction of a "new type of gastrectomy" in 1993, P. Marceau described the ulcer genesis in biliopancreatic diversion due to absence of a buffer for gastric secretions. He demanded more physiological gastric emptying while preserving the antrum–pylorus–duodenum segment (in contrast to the procedures used until this time), as well as innervation. Thus, he developed the concept of acid reduction by longitudinal gastric resection.[44] D.S. and D.W. Hess also emphasized the role of ulcer reduction by longitudinal gastric resection.[51] Looking at the presented historic development particularly of longitudinal gastric resection and its underlying pathophysiological concept in ulcer surgery, it becomes evident that the procedure is not an entirely new concept of "gastrectomy." Rather, it is a revival of an established method in a different context.

In summary, it may be said that the contribution of ulcer surgery towards the understanding of the gastrointestinal system and particularly its innervation should not be underestimated. Even in recent times we benefit from this knowledge.[63] Especially bariatric surgery which focuses increasingly on the stomach as target organ obtains valuable information based on already discovered relations. Finally, technological achievements gained from the developing gastric surgery with its initially high mortality and serious complications should be taken into account. While open conventional suture procedures were used initially, surgeons were eager to acquire skills in performing stapler procedures which then became established and were eventually used on a routine basis. Candidates for bariatric surgery are subject to a massively increased risk of mortality and morbidity due to the presence of several obesity-associated concomitant diseases.[3] Peri- and postoperative risks could be markedly reduced by the introduction of minimally invasive techniques and the fact that they became established standard procedures over time.[5,52,64]

A closer look on longitudinal resection of the greater curvature is indicated. In ulcer surgery and early bariatric surgery, the resection was performed in order to reduce active parietal cells and not primarily as a restrictive step. However, already Wangensteen observed patients losing weight following tubular gastric resection even though he

tried to create a gastric reservoir by performing transverse gastroplasty.[11] Gradually the value of restriction was identified and especially one further important function of the resected gastric tissue. Resection of the fundus, which produces ghrelin, and additional reduction of gastric volume with dilatation of the antrum exert a marked positive impact on the sensation of satiety and reduction of calorie intake.[54,65,66] Ghrelin plays a central role in modulating appetite and the feeling of satiety, influencing gastrointestinal motility, particularly, body weight regulation. Consequently, both ghrelin agonists as prokinetics to treat gastroparesis and postoperative ileus and ghrelin antagonists in order to suppress appetite and to improve glycemic control are subject of intensive research.[67] The effects of longitudinal gastric resection on the gastrointestinal hormone interplay are far from being sufficiently discovered. Obviously, the hormonal effects are regulated in a complex manner involving among others AgRp, neuropeptide Y, and leptin.[68] The important role of these hormonal relations and their influence on metabolic disorders like diabetes is reflected by the increasing use of the term metabolic surgery.

Bariatric surgery is undisputedly one of the cornerstones of the treatment of morbid obesity and is subdivided into a number of different procedures.[3] Traditionally their success is measured in terms of "excess weight loss." Procedures with a malabsorptive component appear to be superior to others. However, the anticipated weight loss should not be the sole or even principal criterion for selection of a procedure. Complications related to the procedures of bariatric surgery are of substantial magnitude and must always be taken into account. The complexity of the surgical techniques and the potential surgical and metabolic complications of the various procedures are inversely related to the anticipated course of weight loss.[5] Especially malabsorptive and combined procedures are technically demanding and associated with increased rates of morbidity and mortality in high-risk patients. Postoperatively they are frequently associated with deficiency syndromes that require supplementation.[69–71] Gastro-gastric fistulas can now be largely avoided by complete division of the stomach. However, like leakage of the anastomosis, gastro-gastric fistulas still are a part of the spectrum of complications.[72,73] Depending on their severity and the time point of diagnosis, leakage of the anastomosis and strictures can be largely treated by the minimal-invasive approach and the use of stents. However, insufficiency of the duodenal stump after duodenal switch, relevant hemorrhage from clip sutures, or insufficiencies associated with concomitant cardiovascular reactions (particularly tachycardia as a warning sign) are serious complications that often require timely revision.[52,57,74] By modifications such as combined biliopancreatic diversion and sleeve gastrectomy, side effects like dumping syndromes or ulcers could be largely avoided over time, but still do occur especially in cases of gastric bypass.[51,75,76] Furthermore, extensive procedures favor the occurrence of obstructions, hernia, or inappropriate bacterial colonization of the intestines.[77,78]

Purely restrictive procedures such as laparoscopic insertion of an adjustable gastric band are convincing at first glance because of their low perioperative morbidity and mortality rates, but bear the risk of band dislocation (slippage), band migration, port complications, and also compliance-related late complications.[42,79]

Due to these numerous risks and complications, a procedure like sleeve gastrectomy which apparently can be performed easily and has a favorable risk–benefit ratio would appear to have arrived at the right moment. The renaissance, and the enormously rapid and widespread application of this method as a single-step procedure, is quite understandable.[80] Introduced as a stepwise mode of treatment, the procedure reduced the previously high mortality rates in high-risk patients (>6% with a BMI> 60 kg/m^2). As single-step procedure, it was convincing because of its low complication (about 9%) and mortality rates (< 1%), as well as its low rate of gastrointestinal long-term side effects.[81–83] Some authors give preference to sleeve gastrectomy as opposed to a gastric balloon as part of a stepwise treatment regimen in high-risk patients.[84] Analogous to the concept of the Magenstrasse and Mill operation, gastric tube formation avoids malabsorption and implant-related complications while ensuring physiological gastric emptying.[54,81] In contrast, sleeve gastrectomy involves irreversible resection of parts of the fundus and the corpus. The humoral aspect of the procedure seems to be important (see above). In trials, sleeve gastrectomy was found to achieve a mean excess weight loss of 33% to 83% 1 year after surgery.[54] Despite this wide range, it may be assumed that, even in the midterm, the procedure is associated with a similar marked reduction of weight as the usual procedures while reducing obesity-associated concomitant diseases.[85,86] If additional weight reduction is required subsequently, the procedure can be performed in a two-step manner with a malabsorptive component (gastric bypass or biliopancreatic diversion), either in a combined manner or a repeat sleeve gastrectomy can be conducted.[87,88]

Therefore, one is easily inclined to regard tube gastrectomy as the desired all-round procedure. However, sleeve gastrectomy is also not the sought-after ideal solution. When assessing the procedures carefully, one should consider the fact that longitudinal gastric resection on the side of the greater curvature is an irreversible step and is associated with placement of a long row of stapler sutures along a gastric wall of varied structure.[54,89] The most

frequent surgical complications of the procedure are leaks (about 0.9%), strictures (about 0.7%), and postoperative bleeding (about 0.4%). Revision rates are reported to be around 4%.[81,82] In addition to intraoperative inspection of the sutures, for instance by endoscopy or the use of methylene blue, several authors recommend oversewing the row of clip sutures or the use of clip reinforcement.[56,57,90] However, procedures of suture reinforcement or oversewing are controversially discussed. Some authors express apprehensions about suture weakening, do not necessarily attribute the reduction of insufficiency rates to suture reinforcement, or warn against strictures due to oversewing.[89,91] Other authors recommend laparoscopic greater curvature plication in order to avoid gastric resection and associated complications.[92]

Two factors deserve attention: firstly, a growing number of studies have been focused on the use of sleeve gastrectomy as a single-step procedure and report convincing results, although adequate evaluable long-term results (>5 years) are not yet available.[56,83,93] Secondly, sleeve gastrectomy is not performed in a standardized manner: various tube diameters and calibration probes (32 to 60 French) are used.[68,80,94] Besides, the extent of resection, particularly in respect of preservation/resection of the antrum varies.[57,95] Intraoperative measurement of the volume of the resected stomach is of great importance. A removed volume <500 cm^3 is apparently associated with an early weight regain.[57] Thus, the results of various workgroups must be compared with caution. Currently, the surgeon also is a substantial factor influencing the outcome of the procedure.

Any person involved in the treatment of obese patients should be aware of the fact that bariatric surgery is a domain of complex interventions in high-risk patients.[3] The ideal procedure does not exist. Rather, the key to successful treatment lies in a careful assessment of the individual risk jointly by the surgeon and the patient, as well as in providing intensive care and information before the operation and particularly in the long-term after a bariatric operation.[5,96] Eating habits, baseline weight, the anticipated weight loss, comorbidities, gender, age, and compliance are some of the numerous factors that must be taken into account.[5,97] A team experienced in handling a wide spectrum of bariatric operations with confidence is indispensable to perform successful obesity surgery with sustained enhancement of quality of life and life expectancy.[98]

Particularly sleeve gastrectomy should be viewed separately and not as a universal procedure. In view of the above-mentioned criteria, the procedure certainly is an attractive treatment option. However, it should be performed in a more standardized manner and with due regard to future long-term results.

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GI IMAGE

Radical Esophagectomy for a Patient with Reversed Intestinal Rotation and Complicated Vascular Anomalies in the Abdomen

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Abstract We report a patient of lower esophageal carcinoma with reversed intestinal rotation and major vascular anomalies including pre-duodenal pre-pancreatic portal vein, absence of the confluence of the splenic vein with the superior mesenteric vein, and deficiency of the common hepatic artery. We performed subtotal esophagectomy with three-field lymphadenectomy following reconstruction with the stomach. The postoperative course was uneventful. This might be the first case that had such complicated anatomical anomalies and radical esophagectomy was performed. As we had preoperatively recognized these anatomical anomalies on radiographic examinations, we could successfully perform esophagectomy and reconstruction without any complications.

Keywords Radical esophagectomy · Reversed intestinal rotation · Vascular anomalies

Introduction

Radical esophagectomy for esophageal carcinoma is a complicated and invasive surgical procedure with significant morbidity. Here, we report a case of esophageal carcinoma with intestinal malrotation and several major vascular anomalies including pre-duodenal pre-pancreatic portal vein, absence of the confluence of the splenic vein with the superior mesenteric vein (SMV), and deficiency of the common hepatic artery (CHA).This might be the first case which had such complicated anatomical anomalies and radical esophagectomy has been successfully performed.

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Case Report

A 62-year-old man, complaining dysphagia, underwent endoscopic examination which revealed an advanced carcinoma in the lower esophagus. He received two cycles of systemic chemotherapy with 5 FU and cysplatin as a neoadjuvant therapy. Preoperative examinations revealed several complicated anatomical anomalies. Barium radiographies of the upper and lower digestive tracts showed malrotation of the intestine. The duodenum did not form the C-loop and the horizontal duodenum existed ventrally on the stomach (Fig. 1a). The small intestine existed in the left side and the entire colon in the right side in the abdominal cavity (reversed intestinal rotation) (Fig. 1b). CT scan showed that the duodenum was not fixed by the ligament of Treitz and passed ventrally on the superior mesenteric artery (SMA) (Fig. 2a). A pre-duodenal and pre-pancreatic portal vein was observed (Fig. 2b). The pancreas was short, and its tail was absent. The stomach and the liver existed in normal positions. The spleen was unusually lobulated and an accessory spleen was detected. The splenic vein, joined by the inferior mesenteric vein and the left gastric vein, flowed independently into the lateral segment of the liver without confluence with the SMV (Fig. 2c). The CHA was deficient and the significant accessory hepatic artery, which flowed into bilateral lobe of the liver, was branched from

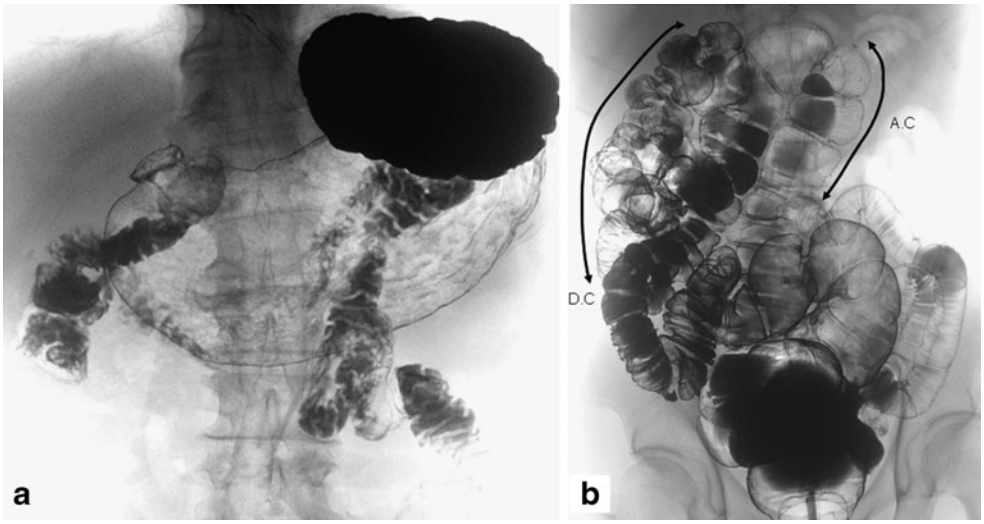

Fig. 1 **a** Upper gastrointestinal series showed that the duodenum did not form the C-loop, and the horizontal duodenum existed ventrally on the stomach. **b** Barium enema showed that the ascending colon existed in the midline and the descending colon in the right side in the abdominal cavity. *A.C* ascending colon, *D.C* descending colon.

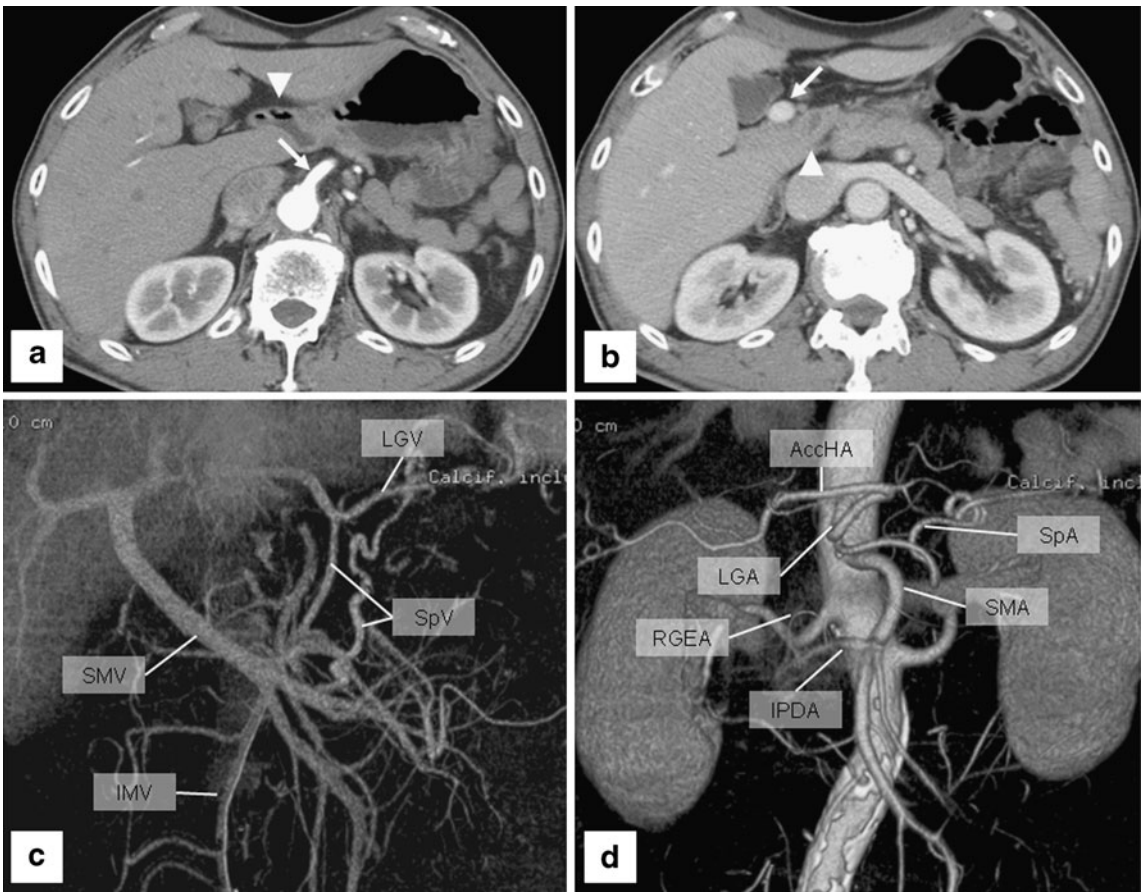

Fig. 2 **a** CT scan showed that the duodenum was not fixed by the ligament of Treitz and passed ventrally on the superior mesenteric artery. *Arrowhead* duodenum; *arrow* superior mesenteric artery. **b** The superior mesenteric vein lay on the duodenal bulb. *Arrowhead* duodenum; *arrow* superior mesenteric vein. **c** The splenic vein, joined by the inferior mesenteric vein and the left gastric vein, flowed independently into the lateral segment of the liver without confluence with the superior mesenteric vein. *LGV* left gastric vein, *SpV* splenic vein, *SMV* superior mesenteric vein, *IMV* inferior mesenteric vein. **d** The common hepatic artery was deficient and the significant accessory hepatic artery, which flowed into bilateral lobe of the liver, was branched from the left gastric artery. The right gastroepiploic artery was derived from the superior mesenteric artery via the inferior pancreaticoduodenal artery and the arterial communication of the head of the pancreas. *AccHA* accessory hepatic artery, *SpA* splenic artery, *LGA* left gastric artery, *SMA* superior mesenteric artery, *RGEA* right gastroepiploic artery, *IPDA* inferior pancreaticoduodenal artery.

the left gastric artery. The right gastroepiploic artery was derived from the SMA via the inferior pancreaticoduodenal artery and arterial communication of the head of the pancreas (Fig. 2d).

We planned to perform subtotal esophagectomy with three-field lymphadenectomy. At the operation, we first performed laparotomy prior to the thoracic esophagectomy to confirm whether the reconstruction of the digestive tract was safely possible. As we observed that the stomach had no abnormality, the pre-duodenal portal vein did not disturb the mobilization of the stomach, and the blood flow of the right gastroepiploic artery was enough for the reconstruction with the stomach, we decided to carry on the operation. After abdominal lymphadenectomy, thoracoscopic esophagectomy with mediastinal lymphadenectomy was performed. There was no obvious anatomical anomaly in the thoracic cavity and the mediastinum. Finally, reconstruction with the gastric tube simultaneously with cervical lymphadenectomy was performed. The stomach was pull up to the neck through the posterior mediastinum and cervical esophagogastrostomy was performed. The postoperative course was uneventful. The pathological diagnosis was esophageal squamous cell carcinoma; T3, N0, M0, Stage IIA on UICC TNM classification. He is now in good health 6 months after surgery.

Discussion

Intestinal malrotation in adults is usually asymptomatic and found incidentally on radiological examinations or at laparotomy for other diseases.[1,2] In our case, we first noticed his anatomical anomalies on CT scan performed during examination for esophageal carcinoma. Intestinal malrotation is classified into several types according to the position of the duodenum and colon.[3,4] In our case, both the duodenum and colon showed reversed rotation. Reversed intestinal rotation is the rarest condition and accounts for only 2–4% of all cases of intestinal malrotation.[5,6] Moreover, among the patients of the reversed intestinal rotation, most cases have reversed rotation of only the duodenum, that is the duodenum crosses in front of the SMA and the colon is arranged in a normal position except for the transverse colon lying behind the SMA.[1,4–6] Ones who have reversed rotation of both the duodenum and colon as our case, whose duodenum crossing in front of the SMA, the entire colon arranged in the right side and the descending colon fixed on the right side of the retroperitoneum, are less common and there have been only a few cases previously reported.[4,7] Intestinal malrotation can be associated with other various congenital anomalies including polysplenia, congenital heart diseases, interrupted inferior vena cava with azygos or hemiazygos continuation, pre-duodenal portal vein, and pancreatic anomalies.[1,2] Particularly, as the cases of reversed intestinal rotation associated with the anomalous mesenteric venous system have been reported,[8,9] a careful approach is necessary at the gastrointestinal surgery. Our case had polysplenia, short pancreas and several vascular anomalies including pre-duodenal pre-pancreatic portal vein, absence of the confluence of the splenic vein with the SMV, and deficiency of the CHA with a significant accessory hepatic artery from the left gastric artery as additional anomalies.

For successful radical esophagectomy for this case, the most principal problem was the reconstruction of the digestive tract after esophagectomy. Therefore, although we usually performed thoracoscopic esophagectomy following laparoscopic tubularization of the stomach and cervical esophagogastrostomy for the patients of thoracic esophageal carcinoma,[10] we started this operation with laparotomy and observation in the abdominal cavity to confirm the reconstruction of the digestive tract was safely possible. If we had judged that no organ could be pulled up as an esophageal substitute, we could have discontinued the operation and another therapeutic modality including definitive chemoradiotherapy should have been considered. It was important for treating this patient with such anatomical anomalies to recognize detailed anatomical features with sufficient preoperative examinations and to plan reliable surgical strategy.

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LETTER TO THE EDITOR

Biliary Tubes in Liver Transplantation

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We read with great interest the recent article by Nissen et al.[1] describing biliary reconstruction in deceased donor liver transplantation. With all the recent medical advances, biliary complications continue to remain the "Achilles heel" in liver transplantation. Biliary complications, including anastomotic bile leaks, strictures, and intrahepatic biliary strictures are encountered in 10–30% of liver transplants and lead to increased morbidity and mortality.[2] The incidence of biliary complications in livers from donors after cardiac death (DCD) is even higher and is up to 60% in some reported series.[3]

The authors describe placement of the T-tube for bile duct reconstruction through the recipient common bile duct. However, T-tubes have been associated with significant morbidity, particularly persistent bile leaks. At our center, we have successfully used transcystic duct placement of biliary tubes since 1998 in more than 1,000 liver transplants. Once the allograft gallbladder is removed, we place a 5-Fr ureteral stent (Bard polyurethane ureteral catheter, C. R. Bard, Inc, Covington, GA, USA) via the cystic duct. The stent is secured with a 5–0 Vicryl suture and a hemorrhoidal rubber band, Fig. 1a. The duct-to-duct biliary reconstruction is completed. The integrity of the reconstructed biliary anastomosis can be tested by gently injecting the biliary tube with 10 ml of saline. The biliary tube is externalized through the abdominal wall and secured. Posttransplant cholangiography is performed by our interventional radiologists. A typical cholangiogram is shown in Fig. 1b. After 3 weeks, once the cholangiogram via the biliary tube shows intact biliary anastomosis, the biliary tube is removed. By this time, the Vicryl suture is

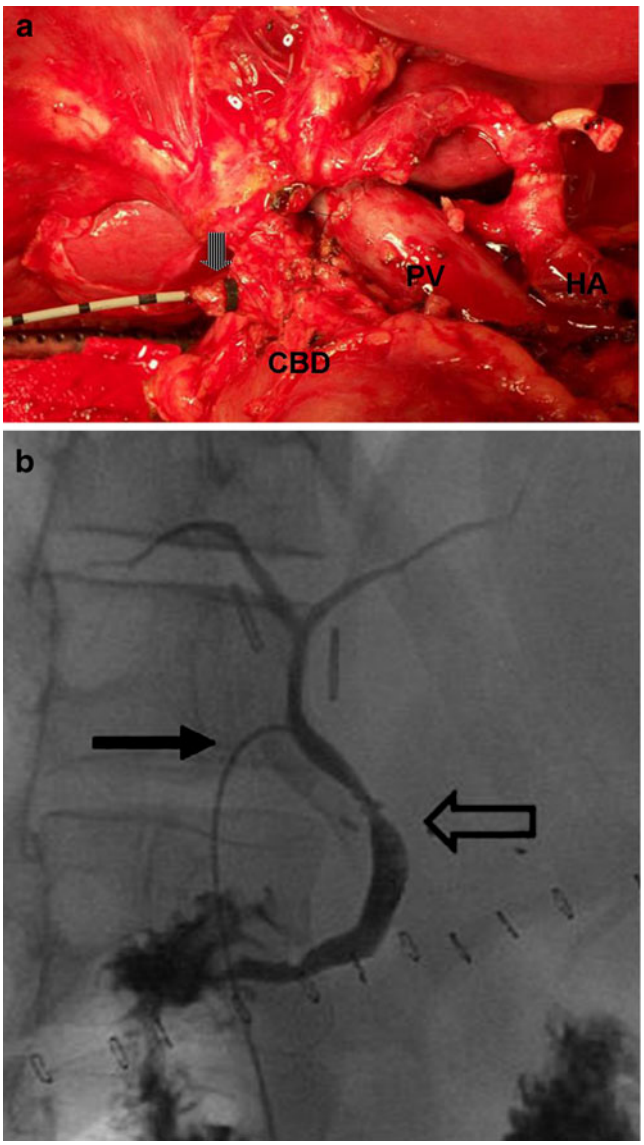

Fig. 1 **a** Transcystic biliary tube in a liver transplant graft. **b** Transcystic biliary tube cholangiogram

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mostly dissolved; thus, a gentle pull on the tube results in an easy extraction. The rubber band maintains a tight closure of the cystic duct, preventing a bile leak.

The authors place biliary T-tubes in patients who have an increased risk for bile leak, including those who undergo ductoplasty, those who have large size discrepancy, patients who have tight ampula, and patients who undergo split liver transplantation. We agree with the authors and also feel that the biliary tubes are invaluable in all liver transplant patients and even more so in patients receiving marginal donor grafts, particularly DCD grafts. Having a biliary tube offers multiple advantages, including noninvasive timely diagnosis of bile leaks as well as in the diagnosis and follow-up of the ischemic-type biliary strictures in the early period after a liver transplantation.

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The authors of the original manuscript were given the opportunity to respond to this letter and did not respond.