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Lack of Correlation Between a Self-Administered Subjective GERD Questionnaire and Pathologic GERD Diagnosed by 24-h Esophageal pH Monitoring

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Abstract
Introduction Self-reported reflux symptoms do not always correspond to pathologic gastroesophageal reflux disease (GERD). We evaluated whether GERD-related symptoms in the self-reported Mayo-GERD questionnaire (GERDQ) were correlated with current gold standard definitions of pathologic GERD.
Methods Three hundred thirty-six consecutive consenting individuals with GERD symptoms referred for 24-h esophageal pH monitoring completed a baseline GERDQ. Univariate and multivariate analyses identified questions that were most associated with percent total time pH<4 at distal probe (DT) >4% or DeMeester score (DS) ≥14.7, two accepted definitions of pathologic GERD. A risk score was created from these analyses, followed by generation of receiver operating characteristic curves and determination of *C*-statistics, sensitivity, and specificities at various cut points, with prespecified minimal values of each that would be required to meet the definition of "potential clinical utility."
Results Forty-nine percent of patients were found to have pathologic GERD; half the patients (not necessarily those with pathologic GERD) described suffering from severe or very severe heartburn or acid regurgitation in the past year. Univariate logistic regression analysis identified six of 22 key GERD questions that were significantly related to DT or DS, in addition to age and gender. Three questions (duration of symptoms, nocturnal heartburn, hiatal hernia) along with age and gender remained significant in multivariate analyses. A risk score (RS) was created from these five questions separately for DT and DS. For DT, the *C*-statistic for RS was 0.75, and at the optimal cut point of ≥6 that maximizes sensitivity (SS) and specificity (SP), SS was 68% and SP was 72%. For DS, the *C*-statistic was 0.73, and at the optimal cut point, SS was 82% and SP 60%. When considering other cut points, the rare extreme case of very low RS (≤2) was strongly predictive of lack of pathologic GERD: for DT, SS 100%/SP 18%, negative predictive value (NPV) 100%; and for DS, SS 97%, SP 25%, NPV 88%. However, only 10–15% of patients referred for pH testing had RS scores of ≤2.
Conclusion Self-reported prolonged history of GERD-like symptoms, nocturnal heartburn, history of a hiatus hernia, and male gender were associated with abnormal 24-h esophageal pH monitoring. However, these factors lack clinical utility to predict pathologic GERD in patients referred for pH testing. We found that 51% of patients with severe GERD symptoms do not have true pathological GERD on objective testing. The clinical implications of this study are significant in that treatment with acid-suppressing medication in such patients would be inappropriate.

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Keywords Gastroesophageal reflux disease · Esophageal 24-h pH test · Distal time percent · DeMeester score · Risk score

Introduction

Gastroesophageal reflux disease (GERD) is a common medical condition described as a chronic manifestation of acid exposure to the esophagus which causes a myriad of symptoms sufficient to impair quality of life.[1,2] In a systematic review of 77,671 patients, 25% of adults were reported to have an episode of heartburn at least once a month, and 12% had symptoms weekly, while 5% suffered from daily heartburn.[3] Typical symptoms associated with GERD are heartburn and acid regurgitation, which are highly specific but not sensitive for the diagnosis of GERD.[4] Temporally, acid reflux episodes variably corresponded to GERD symptoms in patients with and without pathologic GERD.[5,6] Thus, the correlation between symptoms and pathologic GERD has been variable, at best.

Symptom assessment through standardized questionnaires such as the Mayo-GERD questionnaire (GERDQ) allows patients to self-report GERD symptoms and enables clinicians to assess the impact of GERD-related symptoms on patients.[7] Assessing whether GERDQ can predict pathologic GERD is therefore an appealing extension of the use of this questionnaire. Predictive tools need to be concise, yet the design of the GERDQ is lengthy, consisting of 80 questions. To achieve clinical utility of such a predictive tool would require identifying a smaller subset of questions within the longer questionnaire.

Ambulatory 24-h esophageal pH monitoring has been long recognized as a standard for objectively measuring pathological GERD, with high sensitivity, specificity, and accuracy ranging between 84% and 100%.[8–10] The uniform pH scoring system identifies six important parameters as predictors of GERD symptoms.[10] In particular, percent total time pH<4 (distal time (DT)) and DeMeester score (DS) are widely accepted as two key quantitative parameters of GERD. DeMeester score is a sum of component scores of the six individual parameters (of which DT is one parameter). If DT>4% or if DS≥14.7, the test is considered diagnostic of pathologic GERD.[10–12]

We hypothesized that a specific subset of questions which addressed the typical symptoms of GERD within the GERDQ are highly correlated with the presence of pathologic GERD, as defined by DT and/or DS parameters. The primary goal of this study was to determine whether and which components of GERDQ accurately identified patients with pathologic GERD as defined by 24-h esophageal pH testing.

Materials and Methods

Study Design

The study was approved by the University Health Network Research Ethics Board. The study design was a prospective cross-sectional evaluation of consecutively consenting patients (February 2003 to February 2008) with clinical symptoms compatible with gastroesophageal reflux disease (GERD) who were referred to the Esophageal Function Laboratory at Toronto General Hospital for 24-h esophageal pH monitoring. All were naïve to pH testing. Patients eligible for inclusion were those patients with symptoms of GERD who were referred for 24-h pH testing who could read and understand English. Patients were excluded from the study if they had previous antireflux surgery, had a known esophageal motility disorder, or were under evaluation pre- or post-lung transplantation.

After consenting to participate in the study, patients were given the GERDQ questionnaire to complete over the course of the pH testing. Motility and ambulatory 24-h pH testing were performed as outpatients. Patients were required to fast for at least 4 h prior to the testing. All antireflux medications were discontinued 1 week prior to the pH testing. No restriction was placed on patients' daily activities, eating, drinking, or smoking habits. Data from GERDQ questionnaires were kept segregated from pH testing data until the time of analysis.

Mayo-GERD Questionnaire

Mayo-GERDQ is a validated self-administered questionnaire designed to measure symptoms of GERD consisting of 80 questions concerning patients' experience of acid reflux episodes.[7] The questionnaire addresses four major primary symptoms of GERD including heartburn, acid regurgitation, chest pain, and dysphagia, of which heartburn and acid reflux had the highest specificity for GERD.[4] In addition, GERDQ also asked questions about atypical symptoms, lifestyle, general quality of life, general medical history, and review of other symptoms. Twenty-two GERDQ questions related to cardinal symptoms of heartburn and acid regurgitation were selected for in-depth analyses. These 22 questions either utilized a Likert scale or were Yes/No dichotomized questions and were classified into eight major categories: duration since the first onset of symptoms, frequency of symptoms, severity of symptoms, nocturnal symptoms, antacid medication, duration of antacid administration, history of gastric or esophageal diseases, smoking and drinking habits.

Manometry and Esophageal pH Monitoring

Esophageal manometry was first performed on all patients to identify the level of the lower esophageal sphincter. An

eight-channel motility catheter was used to assess lower esophageal sphincter (LES) pressure when the patient was supine. Manometric LES values were identified through ten consecutive swallows with 5 cm^3 aliquots of water. The amplitude and activity of peristaltic contraction, the upper esophageal sphincter (UES) location, resting and contraction tone, and coordination were also measured.

After removal of the motility assembly, esophageal pH was measured using a COMFORTEC (Sandhill Scientific), two-channel pH probe with 15 cm spacing. The distal sensor was positioned at 5 cm above the manometrically defined LES, while the proximal one was located at 15 cm above the LES. Esophageal pH was monitored and recorded electronically for a 24-h period. Patients were asked to maintain daily normal activities and diet. Using the GERD pH monitoring device (Sandhill Scientific) and Bioview pH software, the number and duration of reflux episodes were measured. Upright and supine acid exposure times (in percentage) were also calculated.

Statistical Analysis

Summary statistics of demographic variables, GERDQ responses, and pH testing results were generated. Because both DT and DS have been used as the reference for defining pathologic GERD, we included both in our primary analyses. Patients were classified as having either pathologic GERD or not on the basis of the standardized cutoffs for DT(no GERD≤4%; Pathologic GERD>4%) or DS (no GERD, <14.7; pathologic GERD, ≥14.7). Univariate logistic regression analysis was used to test the association of each of the selected GERDQ questions to pathologic GERD status. Statistically significant predictors of pathologic GERD from univariate analyses were entered into a multivariate logistic regression model using stepwise selection with p value cutoffs of 0.20 and 0.15 to enter and remain in the model, respectively.

Using the estimated coefficients (β) to estimate relative weights of each predictor in the multivariate model, a risk score was created of the GERDQ questions in the multivariate model. Receiver operating characteristic (ROC) curves were generated for the risk score (Fig. 1). Sensitivity (SS) as well as specificity (SP) at different risk score cut points were considered, with the final cut points chosen to maximize potential clinical utility.

"Potential clinical utility" for the risk score was considered likely if any of the following conditions were met: (1) C-statistic from ROC≥0.85 or (2) having an optimal risk score cut point (which is the cut point which maximizes SS and SP together) such that both SP and SS are over 80%. We planned to pursue validation of the risk scores model using an independent set of samples only if "potential clinical utility" was met.

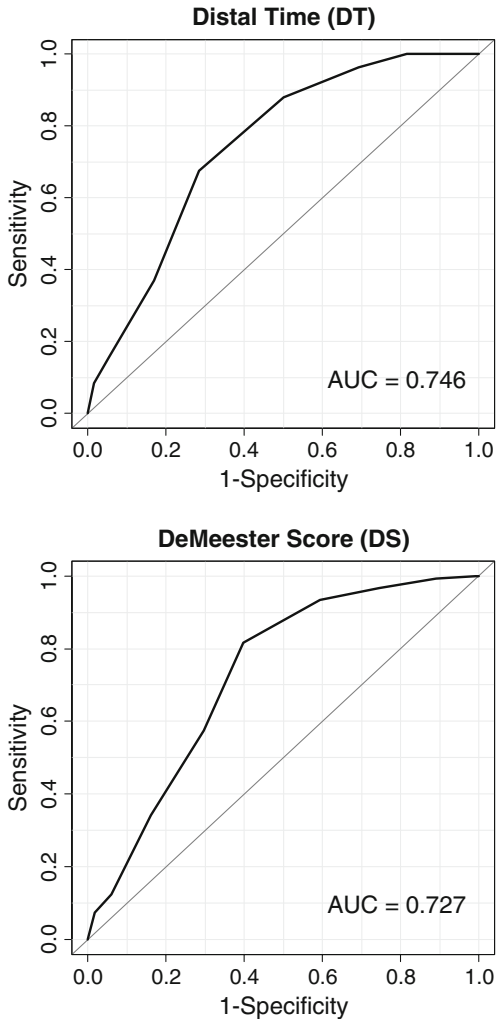

Figure 1 Receiver operator curves (ROC) for risk score, showing results for percent total time pH<4 at DT and DS separately.

Results

Demographics

Between February 2003 and February 2008, of consecutive patients referred for esophageal motility and pH monitoring, 374 met the inclusion criteria. Of these, 336 agreed to participate (90% annual recruitment rate) and completed both GERDQ and pH testing. Of patients completing GERDQ, 203 (60.4%) were females. The median (range) age was 49.8 (18–85) years (Table 1). Pathologic GERD was diagnosed in 49.4% based on the objective measurement of DT or DS, or both, on esophageal 24-h pH testing.

Symptoms

In this patient population, 48% had episodes of heartburn, and 41% had episodes of acid regurgitation for more than 5 years. Over half of all patients (51%) reported "severe or

Table 1 Demographic and Esophageal pH Study Data of Patients with GERD Referred for 24-H pH Monitoring

Characteristic	Total	Pathologic GERD[a]	No GERD[a]
Total number	336[b]	166	170
Age in years			
Median (IQR)	49.8 (18.9)	52.5 (18.5)	45.9 (20.1)
Gender			
Female	203 (60%)	89 (44%)	114 (56%)
Male	133 (40%)	77 (58%)	56 (42%)
DT			
Median (IQR)	3.60 (1.92)	7.5 (7.7)	1.0 (1.6)
DeMeester score (DS)			
Median (IQR)	13.1 (1.95)	32.7 (31.0)	4.5 (5.8)
Normal proximal upright time (%)	300/310 (96.8)	147/156 (94.2)	153/154 (99.4)
Normal proximal supine time (%)	261/309 (84.5)	114/156 (73.1)	147/153 (96.1)
Lower esophageal sphincter (LES) resting tone (mmHg)			
Median (interquartile range)	11 (6.0)		
Patients with normal result ≥16 to ≤30 (%)	51/306[c] (16.5)	12/152 (7.9)	39/154 (25.3)
Patients with abnormal result <16 (%)	255/306[c] (83.5)	140/152 (92.1)	115/154 (74.7)

IQR interquartile range

[a] Using combined definition of either DT>4% or DS≥14.7

[b] Denominator=336 unless otherwise specified

[c] Three patients had LES resting tone >30 mmHg

very severe" heartburn, but only 50% and 54% of these patients had DT>4% or DS≥14.7, respectively. Similarly, 48% complained of severe or very severe acid regurgitation, but only 52% and 56% of these patients had DT>4% or DS≥14.7, respectively. Over 80% of patients reported being awakened at night from heartburn, and 75% reported nocturnal regurgitation. Daily heartburn occurred in 39% of patients within the previous year, while 47% described heartburn occurring at least once a week in the past year. Symptom improvement with antacids was reported by 58%. Heartburn or acid regurgitation affected daily activities some of the time in 35% of patients and in most or all of the time in 21%. The 227 patients who had at least one episode of heartburn or acid reflux on 24-h pH testing had a median of seven (interquartile range 14) episodes of documented acid reflux based on DT or DS, but only 50% of GERD symptom episodes were correlated with DT>4% of DS≥ 14.7% (interquartile range 84%).

One in four patients had presented to their doctor's offices six times or more in the previous year for GERD symptoms. Ninety percent had already received some sort of diagnostic test by their physician prior to referral for pH testing. Ever-smokers accounted for only 45% of the patients, while fewer than 38% of this patient sample drank alcohol in the last year; however, almost 70% were coffee drinkers.

DT, DS, and Pathologic GERD

The median and interquartile range for DT and DS are presented, for the overall sample and separately by pathologic GERD status, in Table 1. As expected, DT and DS values were strongly correlated (Pearson correlation coefficient=0.98; $p<0.0001$). Because of this high correlation, we used either high DT or high DS to define pathologic GERD in our initial demographic comparisons. When comparing pathologic GERD to no-GERD individuals, males had a significantly higher prevalence of pathologic GERD ($p=0.01$, chi-square test), as did older individuals ($p=0.008$, t test), when using this combined DT/DS definition of pathologic GERD (Table 1).

Univariate and Multivariate Models

Although we utilized a combined definition of DT/DS for our demographic variables to allow convenient reporting in Table 1, we performed our primary analysis separately for DT and DS, given that each is considered a standard in its own right. Univariate logistic regression analysis identified six out of 22 GERD-related questions with the greatest statistical significance for either DT or DS. These questions, identified as (Q1) through (Q6), were: (Q1) When did heartburn first begin? (Q2) Has heartburn awakened you at night? (Q3) Have you had acid regurgitation in the past year? (Q4) Has acid regurgitation awakened you at night? (Q5) Have you ever had hiatus hernia? and (Q6) Have you ever had disease of esophagus or stomach? In addition to these GERDQ questions, being male (Q7: What gender are you?) and older age (Q8: How old are you at the time of your pH testing?) was also associated with abnormal DT or DS (Table 2).

In multivariate logistic regression analysis, (Q1) through (Q8) were assessed using stepwise selection. Five predictors remained statistically significant or near significant after stepwise selection, (Q1), (Q2), (Q5), (Q7), and (Q8), and these data are presented in Table 3. For (Q1), the original Likert categories were partially collapsed based on

Table 2 Summary of Univariate Analysis—Questions in GERDQ Most Associated with Pathologic GERD (as Defined by D or DS)

Question	Category	% Patients	DT		DS	
			OR (95% CI)	*p* value	OR (95% CI)	*p* value
1. When did heartburn first begin?	≤2 year ago	27	Reference	0.002	Reference	0.003
	>2 years and ≤5 years ago	25	1.96 (1.0–4.0)		2.06 (1.0–4.1)	
	More than 5 years ago	48	3.00 (1.6–5.6)		2.91 (1.6–5.4)	
2. Has heartburn awakened you at night?	No	17	Reference	0.004	Reference	0.02
	Yes	83	2.96 (1.4–6.2)		2.21 (1.1–4.4)	
3. Have you had acid regurgitation last year?	No	11	Reference	0.01	Reference	0.003
	Yes	89	2.81 (1.3–6.2)		3.35 (1.5–7.4)	
4. Has acid regurgitation awakened you at night?	No	25	Reference	0.01	Reference	0.02
	Yes	75	2.11 (1.2–3.7)		1.96 (1.1–3.5)	
5. Have you ever had a hiatus hernia?	No	42	Reference	0.0005	Reference	<0.0001
	Yes	58	2.36 (1.5–3.8)		3.15 (1.9–5.1)	
6. Have you ever had disease of esophagus or stomach?	No	78	Reference	0.05	Reference	0.02
	Yes	22	1.75 (1.0–3.1)		1.99 (1.1–3.5)	
7. Gender	Female	60	Reference	0.003	Reference	0.01
	Male	40	1.95 (1.3–3.0)		1.78 (1.1–2.8)	
8. Age	≤42 years old	35	Reference	0.005	Reference	0.002
	>42 and ≤55 years old	31	1.56 (0.9–2.7)		1.56 (0.9–4.6)	
	>55 years old	35	2.49 (1.4–4.3)		2.67 (1.5–4.6)	

the results of the univariate analysis and the frequency of each category (adjacent categories with few individuals were automatically collapsed together). For (Q8), the log-odds of the risk function for age approximated linearity; thus, dividing the sample into tertiles was chosen for convenience in developing the risk score model.

At least one question was not answered by 98 patients (29%), and therefore these patients could not be included in the multivariate analysis. However, using chi-square analysis, there was no difference between the group with missing questions and the group with complete answers in terms of the frequency of pathological GERD either by DT,

Table 3 Multivariate Analysis: Final Models

Questions	Category	Referent	*p* value	Odds ratio (95% CI)
DT Model				
(Q1) When did heartburn first begin?*	>2 years ago and ≤5 years ago	In the last 2 years	0.16	1.81 (0.8–4.1)
	>5 years ago		0.01	2.55 (1.2–5.3)
(Q2) Has heartburn awakened you at night?	Yes	No	0.01	2.92 (1.3–6.7)
(Q5) Have you had hiatus hernia?	Yes	No	0.02	2.05 (1.1–3.8)
(Q7). What gender are you?	Male	Female	0.0003	3.12 (1.7–5.8)
(Q8) How old are you?	>42 and ≤55 years	≤42 years	0.14	1.74 (0.8–3.6)
	>55 years		0.001	3.41 (1.6–7.2)
DS Model				
(Q1) When did heartburn first begin?**	>2 years ago and ≤5 years ago	In the last 2 years	0.11	1.90 (0.9–4.2)
	>5 years ago		0.02	2.36 (1.2–4.7)
(Q2) Has heartburn awakened you at night?	Yes	No	0.10	1.91 (0.9–4.2)
(Q5) Have you had hiatus hernia?	Yes	No	0.002	2.50 (1.4–4.5)
(Q7) What gender are you?	Male	Female	0.01	2.17 (1.2–4.0)
(Q8) How old are you?	>42 and <55 years	≤42 years	0.18	1.62 (0.8–3.3)
	>55 years		0.003	2.97 (1.4–6.1)

*DT model global *p* value for Q1 $p=0.04$; global *p* value for Q8 $p=0.005$

**DS model global *p* value for Q1 $p=0.06$; global *p* value for Q8 $p=0.01$

DS, or both. Also, answers to the eight questions were similar between the two groups at least for the questions that were answered (data not shown).

Risk Score Creation and Assessment of Risk Score Characteristics

Risk scores were weighted, with the weighting based on estimated β values of each question in the multivariate models (Table 4). The risk score developed for DS and DT using these criteria had a range from 0 through 9, but the weighting was slightly different for DS and DT (Table 4).

ROC curves were generated and yielded *C*-statistics for DT and DS of 0.75 and 0.73, respectively. Thus, this risk score fails criterion (1) for "potential clinical utility."

The optimal risk score cut point for DT was ≥5, and the optimal cut point for DS was ≥6. At these cut points, SS were 68% and 82% and SP were 72% and 60% for DT and DS, respectively. Thus, this risk score does not meet criterion (2) for "potential clinical utility" either (see Table 5).

Finally, when considering other risk score cut points, values for sensitivity and specificity for all possible cut points were well below 90%, except for the extreme risk score cut point of ≤2, which had SS values of 97–100% (range is reported as DS and DT analyses were performed separately) and negative predictive values of 88–100% in this population. As expected, SP values were very low for this cut point (below 50%). Furthermore, the percentage of individuals (in this population of referred patients for pH testing) that had risk score values ≤2 was only 10–15% (see Table 6).

Discussion

Despite a number of GERD questionnaires that were designed to provide reliable assessments of GERD symptoms, comparisons between questionnaires have been difficult, and interpretation of results have varied greatly.[13–18] Intuitively, clinicians have often assumed that GERD symptoms reported in a questionnaire would accurately represent pathologic GERD. If there is little or no association of GERD symptoms and objective esophageal 24-h pH measurements, misdiagnosis and inappropriate treatment for GERD may result. To our knowledge, our study is the first attempt to develop correlation between subjective self-reported GERD symptoms using GERDQ and objective quantification of key parameters used in 24-h pH testing.

Previous studies have reported that even if reflux symptoms are eliminated completely, it might not ensure normalization of esophageal pH reading.[19] Nor does persistence of GERD symptoms imply pathological GERD. In our study, we identified five key questions that were highly associated with the presence of pathologic GERD in this highly selected patient population who were referred for investigation of reflux symptoms. These questions asked about: (1) the duration since the start of heartburn (at least 2–5 years ago but especially if more than 5 years ago); (2) nocturnal symptoms of heartburn; (3) previous diagnosis of hiatus hernia; (4) being male; and (5) being older, all of which were shown to be statistically significant in the multivariate model. Yet with the rare exception of extremely low risk score values, the resultant risk score derived from these questions was not at all useful in discriminating pathologic GERD from

Table 4 Risk Score Index for the Significant GERDQ Questions from Multivariate Model

Variable	Category	DT		DS	
		Estimated β value from multivariate model	Risk Score Values*	Estimated β value from multivariate model	Risk score values[a]
Q1 When did heartburn first begin?	≤2 years ago	0	0	0	0
	>2 years but ≤10 years ago	0.59	1	0.64	1
	>10 years ago	0.94	2	0.86	2
Q2 Has heartburn awakened you at night?	No	0	0	0	0
	Yes	1.07	2	0.65	1
Q5 Have you ever had hiatus hernia?	No	0	0	0	0
	Yes	0.71	1	0.91	2
Q7 What gender are you?	Female	0	0	0	0
	Male	1.14	2	0.77	2
Q8 How old are you?	≤42 years	0	0	0	0
	>42 and ≤55 years	0.55	1	0.48	1
	>55 years	1.23	2	1.09	2

[a] β values from 0–0.25 were scored 0; from 0.25–0.75 were scored 1; and from 0.75–1.25 were scored 2. The interquartile cut points (0.25 and 0.75) were chosen for rounding purpose to the nearest 0.50

Table 5 Number of Patients with Risk Score Results by Pathologic GERD, as Defined by DT and DS, Separately Presented

	Risk Score										
	0	1	2	3	4	5	6	7	8	9	Total
DT											
DT≤4%	1	4	19	16	25	28	15	15	5	2	130
DT>4%	0	0	0	4	9	22	33	23	8	9	108
Total	1	4	19	20	34	50	48	38	13	11	238[a]
DS											
DS<14.7	1	12	17	18	23	12	16	12	5	2	118
DS≥14.7	0	1	3	4	14	29	28	26	6	9	120
Total	1	13	20	22	37	41	44	38	11	11	238[a]

[a] Values do not add up to 336 because of missing values in specific questions. A missing value in any of the five questions renders that sample unanalyzeable for risk score.

those with false-positive symptoms. Furthermore, despite self-reported severe symptoms, only approximately half the patients actually had pathologic GERD based on objective testing.

Similar to our study, Klauser et al.[4] found that GERD-related symptoms, particularly heartburn and acid regurgitation, were highly *associated* with pathologic GERD but were not particularly discriminatory for *predicting* pathologic GERD. Similarly, Schlesinger et al.[20] reported that 24-h pH monitoring was normal in half of the individuals with reflux symptoms and in 29% with erosive esophagitis. By all of our prespecified criteria (see "Statistical Analysis" section), our risk scores fell short of potential clinical utility for *predicting* pathologic GERD.

Various subjective diagnostic tools for GERD have been compared to objective 24-h pH monitoring. Klauser et al.[4] compared personal interview by gastroenterologists to pH testing, where there was some correlation between an experienced gastroentrologist's subjective assessment and pathologic GERD. Ghoshal et al.[21] compared another standardized questionnaire (Carlsson–Dent), esophageal biopsy findings, and treatment responses with omeprazole with pH testing and found some correlation between severity of symptoms and severity of pH findings. Our study focused on comparing a self-reported questionnaire of GERD symptoms, GERDQ, with pH testing results and found a general lack of clinical utility from GERDQ to predict pathologic GERD.

Table 6 Risk Score by Pathologic GERD, Using a Cut Point of ≤2, for DT and DS Separately

	Risk score		
	≤2	>2	Total
DT			
DT≤4%	24	106	130
DT>4%	0	108	108
Total	24	214	238
DS			
DS<14.7%	30	88	118
DS≥14.7%	4	116	120
Total	34	204	238

Nocturnal reflux symptoms are often considered one of the key symptoms of GERD, and this association was confirmed in our study. Weigt et al.[22] found that individuals with more typical symptoms of heartburn and regurgitation were associated with greater nocturnal esophageal acid breakthrough on pH testing in patients who were already on proton pump inhibitors. Another study reported low specificity (65%) of nocturnal heartburn and greater specificity using nocturnal acid regurgitation (88%) and cough at night (100%), and the study also reported low sensitivity with each of these symptoms.[23] It is likely that both nocturnal acid regurgitation and heartburn are associated with pathologic GERD, and both questions were strongly associated with pathologic GERD in our univariate analysis. However, the tight correlation between these two variables likely led to one of them dropping out of the multivariate model.

Our study found that patients with hiatus hernia were strongly associated with both abnormal DT and DS. This result corresponds to two studies. DeMeester et al.[24] reported that acid reflux episodes were found in greater proportion of patients who had a diagnosis of hiatus hernia compared with those without such a diagnosis (83% and 43%, respectively). Jenkinson et al.[23] reported that hiatus hernia alone could detect abnormal nocturnal acid reflux with 79% sensitivity and 76% specificity; furthermore, when hiatus hernia and nocturnal reflux symptoms (heartburn, acid regurgitation) were present together, specificity increased to 100%. Together, these data are all consistent with our present results.

We confirmed that men are significantly more likely to have pathologic GERD than women in findings previously reported.[25–27] Lin et al.[28] presented complementary data whereby, in men and women who had similar pH testing results, women reported greater severity of GERD symptoms (heartburn, acid regurgitation, nocturnal symptoms)

than men. Richter and DeMeester[29] theorized that a greater parietal cell mass in men leads to greater acid secretion in men but does not explain the differences in symptom perception. Lin et al.[28] suggested that higher symptom perception and lower pain threshold in women might account for some of these differences. In addition to gender, older men were found to experience longer episodes of reflux than either younger individuals of either gender in one study.[26] We confirmed the independent association between increasing age and higher rates of pathologic GERD but did not find an age–gender interaction described in this other study.[26]

Self-reported GERD questionnaires can be useful. Andersen et al.[30] found GERD-related questions to have high sensitivity. Symptom indicators successfully identified almost two thirds of patients with symptoms such as nocturnal heartburn, chest pain, and dysphagia. However, this study compared individuals having benign esophageal disease with individuals having angina pectoris, gastric and duodenal ulcers, or "normal" healthy populations which were vastly different from our underlying patient population. In addition, Shimoyama et al.[31] also evaluated nine questions from a 50-item questionnaire with a high sensitivity of 80% (compared to the original 50-item questionnaire); this study did not employ pH testing. While endoscopy may be useful to exclude non-GERD cases, there was also a wide variation to accurately diagnose pathologic GERD using the surrogate endoscopic marker of "mucosal breaks," and this variation depended greatly on endoscopists' experience.[32]

There are several limitations of this study. First, patients were all referred by their physicians for the esophageal motility and pH testing either because of poor response to drug therapy, referring physician's suspicions that the symptoms were *not* related to GERD, or prior to consideration of antireflux surgery. This would lead to potential selection bias towards both extremes: overrepresentation of severe pathologic GERD cases and overrepresentation of atypical GERD-symptom patients without pathologic GERD. However, under these circumstances, one would have expected a higher chance of identifying a clinical subset of questions that could discriminate pathologic GERD from no GERD, which was not what we found. Our results are further confounded by the fact that physician referral is typically based on the physician's assessment of a patient's GERD symptoms, and agreement between physicians' and patients' perceptions of GERD symptoms is often poor.[33] Secondly, we assessed only one questionnaire, GERDQ. Although this is a validated questionnaire in other settings, it is possible that other questionnaires could be more discriminatory for pathologic GERD in the setting of referral for pH testing. Despite these concerns, we chose GERDQ because it has been validated and assesses multiple dimensions of the most specific symptoms of GERD, heartburn and acid regurgitation. Thirdly, we assessed a very specific patient subgroup referred for pH testing as a result of our initial hypothesis. As shown in our results, our patients had a high prevalence of symptoms with specificity for pathologic GERD, including nocturnal symptoms, severe and frequent acid regurgitation, and/or heartburn symptoms often of prolonged duration. Thus, the usefulness of GERDQ in other settings, such as use as a general population screening tool or to correlate with impact on activities of daily living, cannot be generalized from this study. Finally, approximately one third of patients had at least one missing information question, which was probed to determine if results based on missing data were statistically different from the complete data. The potential discrepancy of the data might affect its validity for multivariate analysis. However, as patients with pathologic GERD evident by either DT>4% and/or DS≥14.7 were compared, none of these outcomes was statistically different from the missing and nonmissing groups. When the eight questions were first studied in the univariate analysis, the difference between the missing and nonmissing groups was found to be statistically significant only in the question, "Have you ever had acid regurgitation last year?" ($p=0.01$). Yet, this question was later discarded, and the overall difference between missing and nonmissing groups remained negligible for the multivariate analysis. It showed that these predictor questions carried equally valid beta coefficients for risk score development regardless of whether complete or missing information were used.

Conclusion

Using a self-administered standardized and validated questionnaire, GERDQ, our study found that abnormal 24-h esophageal pH monitoring was associated with the following characteristics: prolonged history of GERD-like symptoms, nocturnal heartburn, history of a hiatus hernia, and male gender. Despite statistically significant associations, these questions lacked clinical utility to predict pathologic GERD in patients referred for pH testing. Furthermore, pathological GERD as determined by 24-h pH testing was present only in approximately half of the patients despite severe self-reported symptoms.

The clinical implications of this study are significant in as much as patients with GERD symptoms are frequently treated with proton pump inhibitors or other acid-suppressing medications without objective evidence of pathological GERD. Our study demonstrates that 51% of patients with severe GERD symptoms do not have true

pathological GERD on objective testing, and treatment with acid-suppressing medication would be inappropriate. Similarly, patients who have had antireflux surgery who subsequently complain of GERD symptoms should have objective testing before prescribing acid-suppressing medications since symptoms do not correlate with actual acid reflux.

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Discussant

Dr Vic Velanovich (Detroit, MI): I would like to congratulate Dr. Chan and his colleagues for a well-presented study and thank them for getting their manuscript to me in advance of this meeting.

Eleven years ago, we actually did a similar study looking at symptom severity and comparing that to several physiologic measurements, including 24-h PH monitoring, esophagitis by manometry, and esophagitis as returned by endoscopy. And only the level of esophagitis was associated with the symptoms; nothing else was. So the results of your study really are not that surprising.

I do want to talk a little about what the purpose of the study was. It seems to me that you were trying to come up with a handful of questions that could be used to identify patients with pathologic reflux. And clearly, the GERDQ did not fit this bill, which is not surprising.

I do have one big-picture question and a couple of little-picture questions. If this endeavor was successful, that is if you could actually find a handful of questions to identify pathologic reflux, what do you perceive the clinical utility of such a questionnaire would be? Would it somehow change treatment or evaluation strategies?

And would it be more effective than a simple trial of proton-pump inhibitor (PPI) as that seems to be the standard of care now?

As far as the little-picture questions, there are many instruments available to measure GERDQ-related symptoms. So why did you choose this one?

And, more importantly, the instruments are validated usually in total, in complete questionnaires. So to cherry-pick a handful of questions does not necessarily confer that validity onto the questions you selected.

So did you compare the total score with the subset of the 22 questions that you actually ended up selecting and using?

Lastly, I noted that less than one-half of the patients in your study had pathologic reflux? Did you assess for non-acid reflux?

Once again, thank you for the opportunity to review the manuscript and discuss this, and congratulations on a well-done good study.

Closing discussant

Dr Kevin Chan: To answer the first question, I think if the study had been successful, identification of key questions that correlated with pathological GERD would have been useful to clinicians in helping to select patients for treatment whether medical or surgical or to direct further investigations.

The current practice of a trial of PPIs has resulted in many patients being treated with these medications inappropriately.

To answer your second question, we recognize and appreciate the fact that there are many validated questionnaires available. We chose the Mayo-GERDQ because of the breadth of information it captured regarding the patient's overall health. For the purpose of this study, we chose to analyze only the questions that had relevance to GERD. We did not exclude any questions that may have been related to GERD. The excluded questions related to past history, other nongastrointestinal medical conditions, general health, etc.

The last question is a very good one but the answer is no, we did not assess for non-acid reflux.

Discussant

Dr Tom DeMeester (USC): Dr. Chan's study is of particular interest to me because of the current acceptance that GERD is a chronic progressive disease that passes through distinct stages. The earliest of these stages is the non-erosive stage. These patients can have the symptoms of GERD but have normal esophageal acid exposure. I suspect that some of these patients were part of your study group.

The questions are, do such patients have GERD or just an acid-sensitive esophagus, and is an acid-sensitive esophagus an early stage of GERD before esophageal acid exposure becomes abnormal? Did you look specifically at this group of patients, i.e., those who had symptoms and were pH normal? Did you do any sensitivity testing on such patients? Did you investigate them further to see if they had an acid-sensitive esophagus or something of that nature? Did you assess how their questionnaire scores compare to those who had increased esophageal acid exposure on pH testing? Such patients would clearly affect your results.

Closing discussant

Dr Kevin Chan: Thank you, Dr Demeester. You make a very good point. We have not undertaken further evaluation of the patients with symptoms and negative pH testing. It would be useful to determine whether these patients had nonacid reflux or simply an acid-sensitive esophagus. It is also possible that 48-h pH monitoring may have shown more abnormalities.

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2009 SSAT QUICK SHOT PRESENTATION

Management Algorithm for Pneumatosis Intestinalis and Portal Venous Gas: Treatment and Outcome of 88 Consecutive Cases

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Abstract
Background Pneumatosis intestinalis (PI) and portal venous gas (PVG) historically mandated laparotomy due to the high mortality rate associated with mesenteric ischemia. Computed tomography (CT) can identify PI/PVG in patients with ischemic emergencies and benign idiopathic conditions.
Methods A consecutive series of patients with PI or PVG was reviewed from a single institution over 5 years. Eighty-eight cases of PI/PVG were studied: 74 initial patients (year 1–4) were used to generate a treatment algorithm and fourteen additional cases were used to test the algorithm.
Results PI and PVG were associated with three major clinical subgroups: mechanical causes (n=29), acute mesenteric ischemia (n=29), and benign idiopathic (n=26); four were unclassifiable. Patients with acute mesenteric ischemia were associated with abdominal pain (p=0.01), elevated lactate (≥3.0 mg/dL; p=0.006), small bowel PI (p=0.04), and calculated vascular disease score (p<0.0005). The three subgroups could be distinguished using the generated algorithm with a sensitivity of 89%, specificity of 100%, and positive predictive value of 100%.
Conclusions With greater sensitivity of modern CT scans, PI and PVG are being detected in patients with a wide range of surgical and non-surgical conditions. This clinical algorithm can identify subgroups to direct surgical intervention for acute ischemic insults and prevent non-therapeutic laparotomies for benign idiopathic PI and PVG.

Keywords Portal venous gas · Mesenteric venous gas · Pneumatosis intestinalis · Mesenteric ischemia · Exploratory laparotomy · Non-therapeutic laparotomy

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Introduction

Pneumatosis intestinalis (PI) and portal venous gas (PVG) are radiographic signs of underlying intra-abdominal pathology often associated with acute mesenteric ischemia. With the added sensitivity and increased utilization of modern computed tomography (CT), PI and PVG are being identified more frequently.[1–4] This poses a difficult clinical scenario because the clinical significance of PI and PVG, identified by modern CT, ranges from benign to catastrophic.[1–6] There is no definitive algorithm or accepted path of action in the surgical or medical community. New clinical management recommendations are needed in the medical and surgical literature to better distinguish those that require urgent intervention from those with benign causes.

Pneumatosis is characterized by collections of gas within the wall of the bowel that may involve the esophagus,[7–9] stomach,[7,10] small intestine, and colon.[1,3,5,6,11–13] In this paper, we will refer to PI as gas within the wall of any of these organs, and, where appropriate, will annotate the exact organ/organs involved. PVG is characterized by gas in the portal venous system either leading toward the liver within the superior mesenteric vein and tributaries (Fig. 1) or within the intrahepatic portal vein.[2,4,14,15] In our review, we identified patients with PI or PVG or both, and feel that they likely represent different phases of the same pathophysiology. This paper addresses PI of any portion of the GI tract and PVG with the expectation that we can better describe the clinical spectrum of this condition.

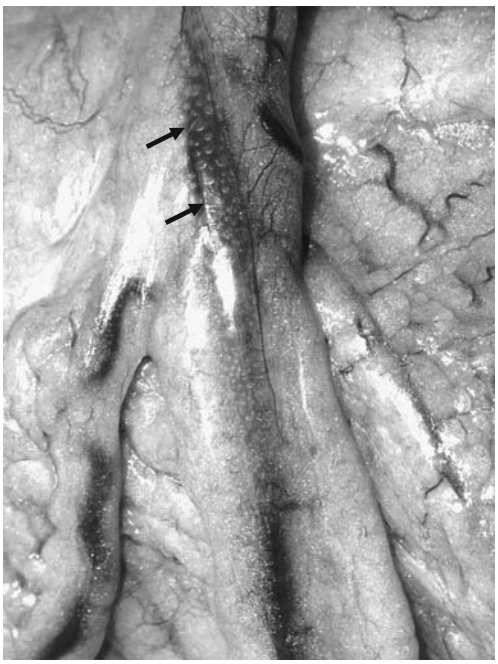

Figure 1 Intra-operative photo showing migrating gas bubbles in peripheral mesenteric veins (*arrows*). In this case, no intra-abdominal pathology was identified after primary and second-look operations.

We examined the presentation, management, and outcome (surgery, autopsy, or clinical course) of patients with PI and/or PVG detected by modern CT. Using the presenting history and physical exam, laboratory studies, and radiologic findings, we were able to identify three distinct clinical subgroups: mechanical diseases, acute mesenteric ischemia, and benign idiopathic. The goal of this study was to identify patients in need of urgent surgical intervention and distinguish them from those with benign idiopathic PI and PVG in order to prevent non-therapeutic laparotomies.

Methods

A consecutive series of patients with PI or PVG or both was identified over a 5-year timeframe at the University of Iowa Hospitals and Clinics. The medical records and radiology films were reviewed. Treatment and 30-day outcome from the initial diagnosis of PI or PVG were reviewed. All patients were identified through a search engine examining all CT radiology reports containing the words: *pneumatosis* and/or *portal venous gas/air*. Identified cases were then confirmed to be accurate by report and film review. All cases with patients less than 18 years of age were excluded. Patients were treated by surgical and medical specialists throughout the hospital; surgical consultation was not required for inclusion. This represents approximately 16 cases per year (2004, 21; 2005, 15; 2006, 15; 2007, 23; 2008, 14) treated at a tertiary care referral center with approximately 30,000 total admissions in fiscal year 2006–2007.[16]

Seventy-four patients identified from January 2004 to December 2007 were analyzed in the spring of 2008. This group comprised our *exploratory series* and was used to generate the proposed clinical algorithm. Between January 2008 and November 2008, 14 additional cases of patients with PI or PVG were identified. This group comprised our *confirmatory series* and was used to test the algorithm generated from the *exploratory series*. The information derived from this study was not presented or disseminated to the clinical services (surgery or medicine) during the study timeframe. No patients were treated using any preliminary data or the final algorithm. Since completion of the study, we have educated the surgical teams of our findings through educational talks. Institutional Review Board (IRB) approval was obtained prior to conducting this review.

We identified three distinct clinical groups of patients with PI or PVG. Characteristics of each group were summarized in tables. Student's *t* test was used to compare means of continuous characteristics (SPSS 15.0). Contin-

gency tables were created for individual characteristics with calculation of Fisher's exact tests and odds ratios to compare specific groups for statistically significant differences (SPSS 15.0). Differences were considered significant at p value<0.05. We report actual p values for all comparisons. Statistical adjustments for multiple comparisons were not performed.

Algorithms were generated using the presenting clinical, laboratory, and radiographic findings, based on the initial exploratory series. Sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) were calculated for the algorithms (Stata version 3). Algorithms were modified to maximize the aforementioned statistical values, while maintaining clinical sense and utility, until a final algorithm was achieved. The algorithm was then tested with the *confirmatory series*.

Results

Eighty-eight cases of PI or PVG were identified and reviewed in eighty-six patients. Two patients presented with PI or PVG twice and were counted as distinct episodes; events were greater than 3 months apart. Three distinct clinical subgroups were identified: Group 1 mechanical gastrointestinal (GI) causes, Group 2 acute mesenteric ischemia, and Group 3 benign idiopathic.

Four patients were unable to be classified into any of the above clinical subgroups. These cases are described here for completeness: all were female, average age 48 years, three had PI of the small bowel, three were on steroids, and one patient had HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) associated with eclampsia. These patients were unstable and rapidly deteriorating from progressive catastrophic clinical conditions. The CT was obtained at the terminal phase of each patient's disease and all died within 24 h. No distinct mechanical/structural injury or acute mesenteric event could be identified to explain PI/PVG. These cases were not included in the subsequent analysis leaving a total of 84 PI/PVG events: 70 in the *exploratory series* and 14 in the *confirmatory series*.

Exploratory Series

Mechanical Cause

Twenty-eight patients presented with a mechanical GI process to explain PI or PVG. The average age was 60 years (range 32 to 84) with an equal sex distribution (48% male and 52% female). All patterns of PI and PVG were found. PI was present in two-thirds of cases and PVG in one-third. When PI was noted, it involved either the small or large bowel, but seldom both, in keeping with the site of injury or disease. One-third of patients ($n=9$) had free intra-peritoneal air in addition to PI/PVG (Tables 1 and 3: Mechanical Group). Adhesion-related complications and hernias were common: prior abdominal surgery ($n=23$), complete or partial bowel obstruction ($n=13$), abdominal wall hernia ($n=4$), or volvulus ($n=3$). Other mechanical causes included: intussusception ($n=1$), trauma ($n=1$), unrecognized iatrogenic bowel injury ($n=1$), radiation ($n=1$), and diverticulitis ($n=1$). Endoscopic procedures were implicated as the source of PI/PVG in four cases. We defined this group carefully to avoid overlap with the ischemic group. The endoscopic procedures had to be performed less than 48 h prior to the diagnosis of PI/PVG and had to demonstrate no findings of ischemia. If bowel ischemia or necrosis was endoscopically identified or later identified, these cases were included in the ischemia group. Three of these cases were successfully managed non-operatively and one was taken to laparotomy secondary to an associated colonoscopic perforation.

Clinical presentation, laboratory studies, and associated co-morbidities were highly variable in this subgroup (Tables 2 and 3). All patients were treated according to standard medical or surgical management as dictated by the underlying cause. Two-thirds ($n=18$) underwent a therapeutic surgical procedure, for example, lysis of adhesions, hernia repair, or bowel resection. Findings at time of exploration included: ten bowel perforations including two duodenal perforations and one rectal perforation, 11 ischemic or necrotic bowels requiring resection, and six

Table 1 Characteristics: Exploratory Series

Characteristic	Mechanical	Ischemic	Benign
Number (% of total)	28 (38%)	23 (31%)	19 (26%)
Age, average	60 (32-84)	67 (40-89)	57 (28-80)
Pneumatosis intestinalis (PI)	17 (61%)	22 (96%)	15 (79%)
Small bowel	9	17	6
Large bowel	9	11	10
Portal Venous Gas (PVG)	10 (36%)	13 (57%)	9 (47%)
Both PI & PVG	3 (11%)	12 (52%)	7 (37%)
Free Air	9 (32%)	2 (9%)	1 (5%)

Table 2 Presenting Features: Exploratory Series

Characteristic	Mechanical ($n=28$)	Ischemic ($n=23$)	Benign ($n=19$)
Abdominal Pain	25 (89%)	21 (91%)	10 (53%)
Peritoneal Signs	4 (15%)	6 (35%)	2 (12%)
Lactate ≥3.0 mg/dL	6 (21%)	10 (43%)	1 (5%)
Mechanical Injury/Disease	24 (86%)	0	0
Endoscopic Procedure <48 hr	4 (14%)	0	0

Table 3 Characteristics: Mechanical Cases in Exploratory Series

Presentation	Past Medical History	Treatment	Outcome
49 y/o F PI	SBO and volvulus, s/*p* gastric bypass	Laparotomy-BR	Uneventful recovery
42 y/o F PI(G)/PVG	SBO s/*p* subtotal colectomy	Laparotomy-LOA	Uneventful recovery
66 y/o M PI	SBO s/*p* gastric bypass	Laparotomy-LOA	Small open wound
54 y/o M PI/PI(G)	SBO s/*p* gastric bypass, PUD	Laparotomy-BR	ICU, enterocutaneous fistula
78 y/o M PI	SBO, h/o GERD, HTN	NG decompression	Uneventful recovery
72 y/o M PI(E)/(G)	SBO s/*p* paraesophageal hernia repair	NG decompression	Uneventful recovery
70 y/o F PI(G)/PVG	Ventral hernia, obesity	Laparotomy-hernia repair	Hernia recurrence
35 y/o M PI(G)	PUD, von Hippel Lindau	Acid reducing medications	Uneventful recovery
57 y/o F PI/FA	LBO, h/o volvulus, bowel obstruction	Laparotomy-BR	Multisystem organ failure and death
82 y/o F PI(G)	Hiatal hernia	Hiatal hernia repair	Uneventful recovery
43 y/o F PI	Intussusception	Laparotomy-BR	Uneventful recovery
63 y/o M PI/PVG	Volvulus, h/o spine surgery	Laparotomy-BR	Readmission for dehydration
76 y/o F PI/PVG	Iatrogenic bowel injury due to retained biopsy needle	Laparotomy-BR	Multisystem organ failure and death
32 y/o M PVG/FA	S/*p* colonoscopy, h/o kidney-pancreas transplant	Laparotomy-BR	Uneventful recovery
77 y/o F PI/FA	S/*p* colonoscopy with cautery perforation	Laparotomy-BR	Uneventful recovery
63 y/o M PI	Pseudomembranous colitis, s/*p* colonoscopy	Antibiotics	Uneventful recovery
84 y/o M PI(G)/FA	Hiatal hernia, s/*p* EGD	None	Uneventful recovery
43 y/o M PI	Recent EGD/colonoscopy, T cell lymphoma	None	Uneventful recovery
36 y/o M PI	Trauma following MVA	Laparotomy-BR	Post-op abscess
68 y/o F PVG	Hepatic abscess, metastatic rectal cancer	Laparoscopic drainage	Uneventful recovery
65 y/o M PI	Colonic stricture, diverticulitis	Laparotomy-BR	Uneventful recovery
54 y/o F PVG	Active diverticulitis, HTN	Antibiotics	Uneventful recovery
42 y/o F PI/FA	PUD with perforation	Laparotomy-vagotomy, pyloroplasty	Uneventful recovery
62 y/o M PI/PVG/FA	LBO, PUD with perforation	Laparotomy-BR	Pneumonia
73 y/o F PI(G)/PVG	PUD	Acid reducing medications	Uneventful recovery
71 y/o F PI	Carcinomatosis	None	Hospice
50 y/o M PVG	Metastatic esophageal SCCa	None	Death
63 y/o M PI	XRT for rectal cancer	Diverting colostomy	Uneventful recovery

S/LBO small/large bowel obstruction, *BR* bowel resection, *(G)* gastric, *(E)* esophageal, *LOA* lysis of adhesions, *FA* free air, *PUD* peptic ulcer disease, *XRT* radiation

with adhesion-related complications amenable to correction or reduction without bowel resection (some patients had more than one finding). No patient underwent a non-therapeutic laparotomy in the mechanical group. There were three deaths, one in a patient with metastatic esophageal cancer and two in patients undergoing lysis of adhesions.

One-third of patients (n=10) were treated successfully using conservative means. Two had active peptic ulcer disease and gastric PI treated medically, one had acute diverticulitis treated with antibiotics and percutaneous abscess drainage. Two had advanced malignancies involving the GI tract: breast cancer causing duodenal obstruction and small bowel lymphoma. Other cases included: adhesion-related small bowel obstruction, post-EGD/colonoscopy, and post-operative partial gastric outlet obstruction all successfully managed with decompression or bowel rest. No patient required a subsequent surgery for delayed or missed diagnosis in the mechanical group treated conservatively.

Acute Mesenteric Ischemia

Twenty-three patients presented with PI or PVG associated with mesenteric ischemia. This group was older with an average age of 67 years (range 40 to 89) with a similar sex distribution (43% male and 57% female). PI was present in 22 of 23 cases: small bowel PI in 17, large bowel PI in 11, and both small and large bowel in 6. PVG was present in about half (n=13), but seldom seen without associated PI (n=1). Free air was identified in two patients (Table 4: Ischemic Group). Patients presenting with PI/PVG associated mesenteric ischemia had multiple cardiovascular risk factors. Documented coronary artery disease (CAD) was

Table 4 Characteristics: Ischemic Cases in Exploratory Series

Presentation	Vascular disease score	Treatment	Outcome
75 y/o F PI/PVG	7	Laparotomy with bowel resection	Recovered
77 y/o F PI/PVG	7.5	Laparotomy with bowel resection	Recovered
63 y/o M PI	4.5	Laparotomy with bowel resection	Recovered
64 y/o M PI/PVG	7	Laparotomy with bowel resection	Recovered
54 y/o F PI/PI(G)/FA	6	Laparotomy with bowel resection	Recovered
51 y/o F PI	7	Laparotomy with bowel resection	Recovered
55 y/o M PI	7	Laparotomy with bowel resection	Recovered
67 y/o F PI	5.5	Laparotomy with bowel resection	Recovered
76 y/o M PI/PVG	10	Laparotomy with bowel resection	Recovered
45 y/o M PI/PVG[a]	4	Laparoscopy converted to laparotomy, bowel resection	Recovered
89 y/o F PI[a]	6	Laparoscopy (entire small bowel ischemic/necrotic)	Died
40 y/o M PI/PVG	5	Laparotomy with bowel resection	Died
74 y/o F PI/FA	5	Laparotomy with bowel resection	Died
70 y/o M PI/PVG	7.5	Laparotomy with bowel resection	Died
68 y/o M PI	9.5	Laparotomy with bowel resection	Died
72 y/o F PI/PVG	7.5	Laparotomy with bowel resection	Died
57 y/o F PI	6.5	Laparotomy with bowel resection	Died POD39
66 y/o F PI/PVG	6.5	Laparotomy with LOA	Died
86 y/o F PI/PVG	8	Laparotomy with bowel resection	Died
84 y/o F PI	10.5	None	Died
72 y/o M PVG	7.5	None	Died
67 y/o M PI/PVG	5	None	Died
66 y/o F PI/PVG	6	None	Died

[a] Laparoscopy utilized, *FA* Free air

present in 52% and peripheral vascular disease (PVD) was present in 48%. Hypertension (HTN) was common (74%), followed by tobacco smoking (52%), hyperlipidemia (35%), and diabetes mellitus (DM) (30%). Although arterial embolic and atherosclerotic causes predominated, alternative causes of ischemia included: low-flow state secondary to sepsis or pressor support, venous thrombosis, and vasculitis.

Patients with acute mesenteric ischemia frequently presented with abdominal pain (91%), yet only one-third had peritoneal signs. Many were intubated or sedated which may explain this discordant finding. Alternatively, this is consistent with mesenteric ischemia often described as "pain out of proportion to physical examination." Lactate levels for the entire group were elevated with an average of 4.4 mg/dL (normal<2.2 mg/dL). Lactate levels were elevated in eleven, normal in ten, and not obtained in the remaining cases. Other measures of acidosis and infection (white blood cell count, base excess, pH, and bicarbonate) were highly variable and could not reliably predict which patients were suffering from acute mesenteric ischemia.

Treatment was predominately surgical resection of non-viable bowel: 18 underwent laparotomy and 100% demonstrated ischemia of the small or large bowel. Five patients were not offered surgery since it was deemed to be futile; mortality was 100%. Autopsy findings demonstrated acute mesenteric ischemia in those not taken emergently to the operating room. The mortality rate for the surgically treated subgroup was 39%. The mortality rate for this entire group was 52%.

Benign Idiopathic

Nineteen patients had benign idiopathic PI and/or PVG. The average age of this group was 57 years, with twice as many males as females. Benign idiopathic PI frequently involved the colon (n=10), the small bowel (n=6), and, rarely, the stomach (n=3). PI was an isolated finding in eight cases and seen in combination with PVG in seven. Abdominal pain was present in nine cases and not present in ten cases (three were sedated and/or intubated which could mask an accurate history and physical examination). This group had few cardiovascular risk factors and none was receiving pressor support. Recent surgery (5 to 30 days) was identified in some cases: trans-hiatal esophagectomy, resection of ileo-anal J pouch, and emergent sigmoid resection. PVG without PI was only seen in two cases and in both of these cases colonic pathology was identified: Crohn's colitis and colonic thickening secondary to Kayex-

alate use. Diarrhea was present in five cases yet no enteric pathogens or inflammatory diseases were identified in these cases. Half were on some form of immunosuppressive medication at the time of presentation: chemotherapy ($n=6$) or steroids ($n=3$). History of cancer treatment was frequently identified: esophageal cancer, recurrent uterine leiomyosarcoma, bladder cancer, B cell lymphoma, unresectable gastric cancer, stage IV ovarian cancer, and squamous cell carcinoma of the head and neck. One patient suffered from status epilepticus and had a non-therapeutic laparotomy for colonic PI. In eight cases, no identifiable past or current medical history could be associated with the PI, PVG finding (Table 5). Lactate levels were normal or not obtained in 17 cases (normal<2.2 mg/dL). One case had borderline elevation to 2.5 mg/dL. The other case had elevated lactate of 8.4 mg/dL. This patient underwent a non-therapeutic laparotomy with normal abdominal findings and had an uneventful recovery.

Emergent exploratory laparotomy was performed on nine patients. A "second look" laparotomy was performed in two cases. All laparotomies were non-therapeutic. One patient underwent right hemicolectomy with end ileostomy for pneumatosis coli. Pathology demonstrated normal vasculature and intact viable mucosa. One patient died on post-operative day five due to acute renal failure and an acute myocardial infarction. Autopsy demonstrated no signs of bowel injury, ischemia, or necrosis. Two major complications resulted from surgery: dehydration following end ileostomy and large incisional hernia. Ten patients were managed non-operatively. All patients treated in the non-operative group (without visual confirmation of presence or absence of ischemia or pathology) were alive and healthy 30 days after imaging.

Development of a Management Algorithm

Patients presenting with PI/PVG fell into three general subgroups: mechanical, ischemic, and benign idiopathic. As would be expected, those with acute mesenteric ischemia were oldest while those in the benign idiopathic outcome were youngest, but wide ranges existed. Previous abdominal surgery was common among all groups. Abdominal pain and peritoneal signs were seen most commonly in the mechanical and ischemic groups, but abdominal pain was also reported in half of those with benign idiopathic PI/PVG. Elevated lactate levels were commonly seen with mesenteric ischemia, but were often normal. Elevated lactate levels were seen in the mechanical and benign idiopathic subgroups though less frequently. The degree of gas present (minimal, moderate, extensive) was analyzed, but provided no discriminating information (data not shown). The distribution of PI within different anatomic regions was helpful to a degree. In the mechanical injury subgroup, PI was seen in organs corresponding to the site of injury. For example, strangulated hernia with small bowel demonstrated PI of the small

Table 5 Characteristics: Benign Idiopathic Cases in Exploratory Series

Presentation	Past medical history	Treatment	Outcome
50 y/o M PI/PVG	Alcohol abuse, cirrhosis	Laparotomy x 2	Incisional hernia
76 y/o M PI/PVG	Obesity, DM type II, transient ischemic attack (TIA)	Laparotomy x 2	Died POD#5
74 y/o M PI/PVG	HTN, CAD	Laparotomy	Uneventful recovery
44 y/o M PI	Prior motor vehicle accident (MVA) and head injury	Laparotomy	Uneventful recovery
80 y/o M PI/PVG	HTN, DM type II, chronic obstructive pulmonary disease (COPD)	Observation	Uneventful recovery
76 y/o M PI/PVG[a]	Esophageal cancer (POD #30)	Laparotomy	Developed pressure sore
78 y/o M PI/PVG[a]	Central nervous system (CNS) tumor	Laparotomy	Uneventful recovery
34 y/o M PI	Hx of drug abuse, CAD	Observation	Uneventful recovery
40 y/o F PI	Status epilepticus, phenobarbital coma	Laparotomy	Uneventful recovery
66 y/o F PI	Uterine sarcoma on chemotherapy, chronic diarrhea	Observation	Uneventful recovery
64 y/o M PI	Bladder cancer on chemotherapy	Observation	Uneventful recovery
67 y/o F PI	B cell lymphoma on chemotherapy	Observation	Uneventful recovery
32 y/o M PI (gastric)	Gastric cancer on chemotherapy	Observation	Uneventful recovery
63 y/o F PI (gastric)	Ovarian cancer on chemotherapy	Observation	Uneventful recovery
63 y/o M PI	Head and neck cancer on chemo-radiation, diarrhea	Laparotomy	Uneventful recovery
28 y/o M PVG	Crohn's on steroids, chronic diarrhea	Observation	Uneventful recovery
45 y/o M PVG	COPD on steroids and Kayexalate	Observation	Uneventful recovery
49 y/o M PI	POD #6 lysis of adhesions, hx of Crohn's, on steroids	Observation	Uneventful recovery
48 y/o F PI/PVG (gastric)	POD#5 sigmoid resection	Laparotomy	Uneventful recovery

[a] Vascular disease score greater than 4.0

intestine and PVG in the superior mesenteric vein. In the acute mesenteric ischemia subgroup, a large percentage of patients had combined large and small bowel PI. Clinically, this likely corresponded to an insult arising in the superior mesenteric artery or ileocolic distribution. Benign idiopathic PI was commonly associated with colonic PI and diarrhea, but numerous other patterns were present. We examined the radiographic patterns of PI and PVG looking at sites, patterns, and degree of PI/PVG, but were not able to distinguish these subsets with CT readings alone. Using individual clinical, laboratory, and radiographic findings, we could not demonstrate any safe method of segregating PI/PVG into its divergent clinical etiologies.

Several clinical and laboratory features were examined looking at the whole group, as well as three subsets. Selected characteristics were able to differentiate between the ischemic and benign subsets. Univariate analysis of distinguishing characteristics is noted in Table 6. Since vascular risk factors were most common in the mesenteric ischemia group and least common in the benign idiopathic group, a vascular disease score was created using weighted factors to achieve maximum differentiation between these two groups. The clinical items used to generate the vascular score are shown in Table 7. The point values were assigned initially based on univariate analysis, patterns in tabulated data, and clinical reasoning. They were subsequently adjusted in iterative fashion during algorithm development to achieve maximum sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The algorithm shown in Fig. 2 was developed by incorporating pathophysiological theories and clinical reasoning. Step 1 is designed to filter out patients with a rapidly progressing demise. We identified four patients who were unstable with PI/PVG. Step 2 filters out patients with mechanical or structural pathology. In most cases, the CT can provide a radiographic diagnosis with high degree of specificity: hernia, volvulus, or bowel obstruction. The most difficult patients to segregate were those with either mesenteric ischemia or benign idiopathic conditions. Step 3 identifies those with a higher probability of having a vascular ischemic etiology using a calculated vascular disease score. Sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) for each step are shown in Table 8.

Table 6 Univariate Analysis of Selected Characteristics Distinguishing Ischemic and Benign Subgroups

Characteristic	*p* value	Odds ratio	95% CI
CAD[a]	0.02	5.8	1.3-25.6
PVD[b]	0.005	16.5	1.9-145.0
Abdominal pain	0.01	9.5	1.7-52.1
Lactate ≥3.0 mg/dl	0.006	13.8	1.6-122.0
Small Bowel PI	0.04	5.1	1.2-21.4
Vascular Disease Score ≥4.0	<0.0005	–[c]	–[c]

[a] Coronary artery disease

[b] Peripheral vascular disease

[c] Cannot calculate due to presence of cells in contingency tables with value(s) equaling zero

Table 7 Calculation of Vascular Disease Score

Category	Characteristic	Points
History	Total vascular risk factors: smoking, diabetes, hypertension, hyperlipidemia	0.5 for each
	Coronary artery disease	2
	Peripheral vascular disease	2
	At risk for low-flow state to gut (moderate/severe CHF, arrhythmia, sepsis, IV pressors)	2
	Vasculitis or venous occlusion	2
Physical Exam	Abdominal pain or abnormal abdominal exam if intubated/sedated	1
Laboratory	Lactate ≥3.0 mg/dL	3
Radiology	Small bowel pneumatosis	1
	Total points possible	15

Confirmatory Series

The clinical algorithm was tested on all patients diagnosed and treated with either PI and/or PVG from January 2008 to November 2008. No clinicians had access to this algorithm or the results of this project during this timeframe. For unknown reasons, only one case of a mechanical PI/PVG was identified during this timeframe: strangulated hernia with non-viable small bowel. Surgical repair was performed and the patient made an uneventful recovery. The remaining cases were equally divided between mesenteric ischemia (n=6) and benign causes (n=7). In the mesenteric ischemia patients, the average vascular disease score was 7.3 (range 5–12) and 100% had a score greater than 4. Two were explored confirming the diagnosis of mesenteric ischemia; only one survived. Four were not taken to surgery due to either futility or patient and family wishes; all expired. In those categorized with benign idiopathic PI/PVG, the vascular disease score average was 1.8 (range 1–3) and 100% were less than 4. Fewer non-therapeutic laparotomies were performed, one out of seven. Two received hyperbaric treatments and four were observed. All made uneventful recoveries. Two have subsequently been admitted for similar events each diagnosed with benign PI/PVG and observed.

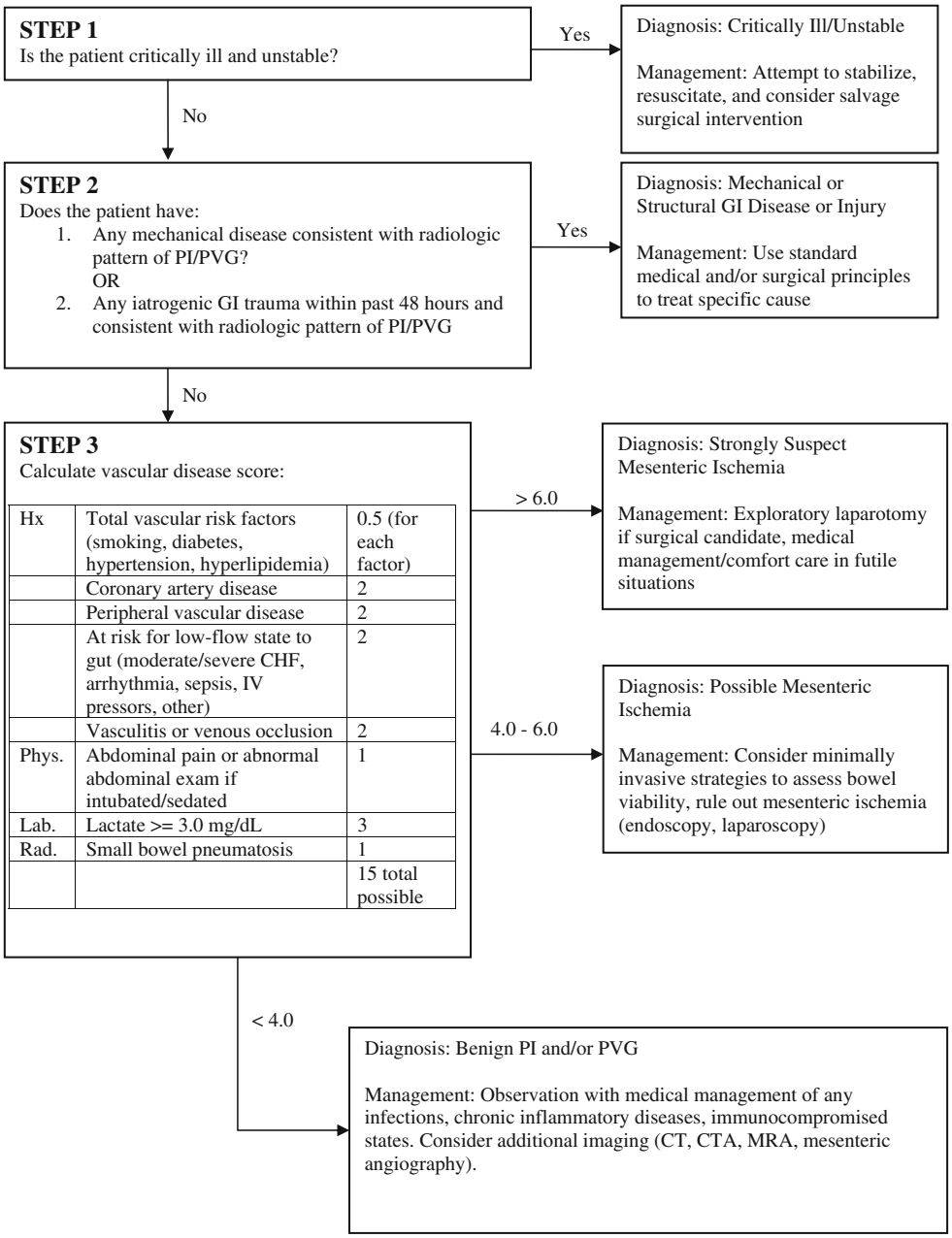

Figure 2 Management algorithm for pneumatosis and/or PVG identified on CT scan.

Discussion

PI and PVG are radiographic signs of underlying intra-abdominal pathology often associated with acute mesenteric ischemia. With the increased utilization of CT imaging, PI and PVG are being identified more frequently and can pose a difficult surgical dilemma.[1–4] Early and aggressive intervention can be lifesaving in patients with acute mesenteric ischemia.[3,5,15,17–19] But, early surgical exploration of any patient with PI and/or PVG would result in a non-therapeutic laparotomy rate of approximately 30%, based on our data. New clinical recommendations are needed in the medical and surgical literature to better distinguish patients with PI and PVG that require urgent intervention from those with benign causes.[4,5,18–21]

Reviews of PI and PVG exist in the medical literature (Table 9). Unfortunately, these historical series and case reports only add to the current misunderstanding of PI and PVG. Case reports and case series suffer from limited experience, variable imaging modalities, and long time intervals. Many of these reports have examined the radiographic findings or surgical outcomes in isolation. There is limited literature discussing both PI and PVG concurrently, despite the fact that they frequently overlap clinically.[22] With the inadequacy of current algorithms and the increased sensitivity of CT scans increasing the

Table 8 Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for Management Algorithm

Step	Sensitivity	Specificity	PPV	NPV
1	100%	100%	100%	100%
2	100%	100%	100%	100%
3	100%	96%	92%	100%
Final	89%	100%	100%	96%

detection of PI and PVG, a practical algorithm for managing this condition is needed. One previously published algorithm by Greenstein, proposed using emesis, WBC >12, age ≥60, PVG, sepsis, and acidosis to determine the need for surgical intervention.[5] We attempted to apply this algorithm to our study population and found it not to be clinically useful. We did not use emesis, WBC, age, or sepsis as criteria in our algorithm. By performing a large comprehensive review over the past 5 years, we have learned a great deal about PI and PVG. We have developed a new clinical algorithm based upon careful examination of our population. Testing of our algorithm was performed on only a limited number of cases (n=14). It remains to be determined if it will prove useful on a larger population.

There are two main theories regarding the fundamental pathophysiology of PI/PVG: mechanical and bacterial.[1,3,4,14,15,23] The mechanical theory postulates that PI develops when defects in the mucosa, in combination with increased intraluminal pressure, allow gas to infiltrate the GI tract wall (Fig. 3). A subgroup of patients with severe pulmonary conditions, may present with PI arising from pulmonary causes such as cough and rapid changes in intra-abdominal pressure. The bacterial theory postulates that PI develops when gas-producing bacteria gain entry to the GI tract wall and produce pockets of gas. Much of the supporting evidence for these two theories is derived from observational studies and one could argue that the mechanical and bacterial mechanisms may occur simultaneously. PVG can be seen in conjunction or in isolation from PI and represents a different phase of the same fundamental pathophysiology. In PVG, branching lucencies are seen within the mesenteric venous tributaries or in the liver often draining sites of PI (Fig. 4). Because many patients in our series presented with both PI and PVG simultaneously, we studied this presentation as one entity.

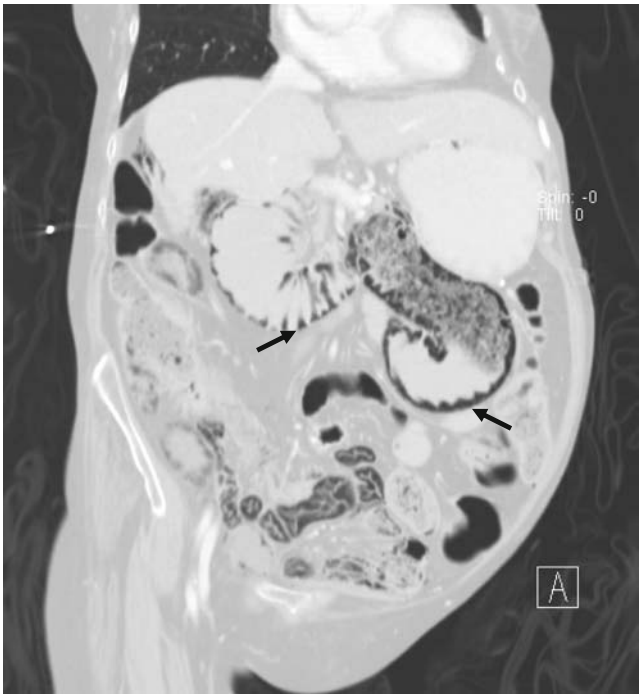

Figure 3 Pneumatosis intestinalis (PI) seen as curvilinear or circular lucencies within the wall of distended duodenum and proximal jejunum (*arrows*). Less extensive changes were noted throughout the entire small intestine.

Understanding the exact pathophysiology of PI/PVG, mechanical or bacterial, will remain difficult despite a large retrospective series, but important lessons can be learned. In our series, we did not identify any patients in the benign idiopathic group with positive blood cultures that would support the bacterial translocation theory. It is theoretically possible that the liver serves as an effective filter to bacteria in the bowel wall and venous tributaries, yet the benign clinical picture of several of these cases makes this unlikely. Until further study, we believe that PI/PVG arises primarily through the mechanical theory as outlined above.

Table 9 Published Literature on Pneumatosis and Portal Venous Gas

Author (yr)	Case mix	Time	Surgery	Ischemia	Mortality
Liebman (1978)[23]	64 literature review, PVG only	*n*/a	?	72%	75%
Kinoshita (2001)[4]	182 literature review, PVG only	*n*/a	51%	43%	39%
Iannitti (2003)[20]	26 case series, PVG only	8 yr	42%	27%	35%
Schindera (2006)[17]	11 case series, PVG only	8 yr	64%	54%	27%
Monneuse (2007)[18]	15 case series, PVG only	5 yr	100%	60%	47%
Greenstein (2007)[5]	40 case series, PI only	10 yr	35%	Not reported	20%
Morris (2008)[19]	97 case series, PI only	7 yr	33%	19%	22%
Current Report	88 case series	5 yr	50%	34%	29%

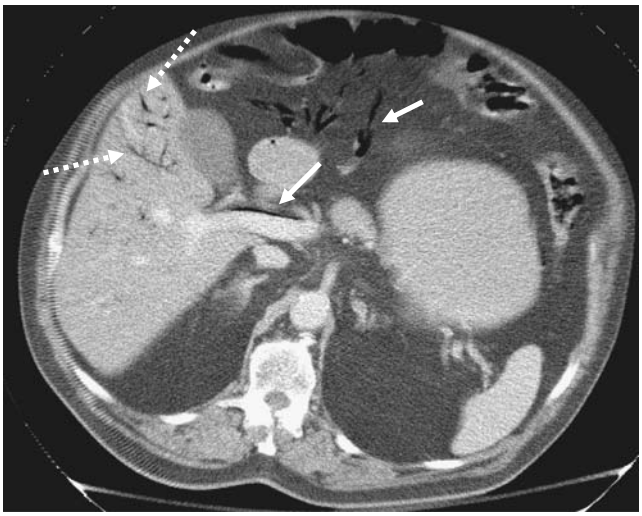

Figure 4 Portal venous gas seen as branching lucencies within the liver that extend within 2 cm of the liver capsule (*dashed arrows*). Gas extends to the periphery and is seen more prominently in the left hepatic lobe because of its ventral location in the supine patient. Mesenteric venous gas is seen as tubular branching lucencies in the mesenteric fat extending from the intestinal margin toward the mesenteric root and into the portal vein (*solid arrows*).

The more pressing clinical dilemma is deciding how to manage the patient with newly diagnosed PI/PVG.[5,19–21] Surgical exploration will be therapeutic in a large percentage of patients with mechanical and ischemic causes, as we clearly demonstrated. If the CT scan demonstrates pathology of a mechanical nature, standard surgical management is strongly advocated. This does not imply that laparotomy is the sole way to manage these patients. For example, endoscopic manipulation may introduce air into the extraluminal regions and may be safely observed. On the other hand, when an incarcerated hernia or bowel obstruction presents with either PI or PVG, early surgical exploration should be pursued to release strangulated bowel. In two-thirds of the mechanical cases of PI/PVG, surgery was able to treat the underlying pathology and no patient underwent a non-therapeutic laparotomy. In the conservative treatment group, no patients required a later laparotomy for a delayed or missed diagnosis. Due to the heterogeneity of the mechanical group, the proposed algorithm cannot be utilized to determine which patients in this group will require surgical versus medical intervention. For acute mesenteric ischemia, surgery is most often recommended. Unfortunately, aggressive surgical heroics often fail. Extremes of age and co-morbidities make many of these patients unsuitable for aggressive treatment such as revascularization or extended bowel resections. We explored 20 patients presenting with PI/PVG and confirmed acute mesenteric ischemia (combined exploratory and confirmatory series) and were able to salvage 12. Expectant supportive comfort care was employed in nine cases of mesenteric ischemia with a 100% mortality rate (Table 10). If expectant care is to be recommended, it is imperative for physicians to correctly discriminate between PI/PVG arising from acute mesenteric ischemia from benign PI/PVG.

Diagnostic laparoscopy provides a minimally invasive approach to evaluate for pathology while also creating a scenario for easy conversion to open laparotomy if necessary. We utilized laparoscopy infrequently in our series (n=3). In one case, laparoscopic intra-abdominal abscess drainage was both diagnostic and therapeutic. This patient had a peri-hepatic abscess and was classified in the mechanical group. In two other cases, laparoscopy was utilized to rule out mesenteric ischemia. One demonstrated ischemic bowel amenable to segmental resection which was done after converting to an open procedure. In the second case, the entire small and large bowel was non-viable and expectant care and death followed. In both cases, laparoscopy confirmed acute mesenteric ischemia accurately.

To be captured in this study, all patients underwent a CT scan showing either PI or PVG. A proportion of patients with acute mechanical or ischemic insults may be diagnosed clinically and never undergo CT scan. These patients may be explored surgically or treated expectantly. This series clearly did not capture all patients with mechanical bowel compromise or acute mesenteric ischemia and cannot be used to determine the overall incidence of PI/PVG for these conditions. It is also likely that many patients with

Table 10 Outcome of both Exploratory and Confirmatory Groups

	Mechanical causes	Mesenteric ischemia	Benign causes
Number (%)	29 (35%)	29 (35%)	26 (30%)
Vascular disease score	3.2 (0-9.5)	6.9 (4-12)	2.0 (0-6)
Vascular disease score ≥4.0	34%	100%	8%
Treatment	Surgery 66%	Surgery 69%	Surgery 38%
	Medical 34%	Futility 31%	Non-therapeutic 100%
			Observation 62%
Outcome	Recovered 90%	Recovered 41%	Recovered 96%
	Mortality 10%	Mortality 59%	Relapse 8%
			Mortality 4%

benign idiopathic PI/PVG never undergo evaluation and imaging for the condition.

The proposed algorithm has limitations. As seen in Table 10, 8% of cases in the benign PI subgroup had vascular disease scores greater than 4.0. These two cases are identified in Table 5 and both had a vascular disease score between 4.0 and 6.0, and one had a lactate of 8.5. Both of these cases underwent non-therapeutic laparotomies and both were discharged without complications. In the confirmatory series, seven cases of benign PI were identified and all had a vascular score less than 4.0. The algorithm identified these patients accurately. We utilized a low vascular score (less than 4.0) to ensure a margin of safety while accepting a slightly higher rate of non-therapeutic laparotomy.

Conclusions

Over the past 5 years, the surgical service at the University of Iowa has gained new insight into the management of PI/PVG but also new clinical dilemmas. We cannot simply explore all patients presenting with PI and PVG. Thirty percent of these patients will have benign idiopathic PI/PVG and should not be subjected to a non-therapeutic laparotomy. Surgery for this subset was associated with significant risks: one death and two complications. In the mechanical cause group, half were successfully treated with non-surgical means such as bowel rest and antibiotics. On the other hand, patients with PI/PVG and acute mesenteric ischemia require early and aggressive surgical management to have meaningful survival. We had a 60% survival rate with aggressive surgical management of mesenteric ischemia and 100% mortality with non-surgical approach. If patients with acute mesenteric ischemia are subjected to unnecessary delays because benign PI/PVG is suspected, patients will suffer greatly. The proposed algorithm utilizes standard admission history and physical examination findings and limited laboratory tests. None of the data collection points in the algorithm should delay treatment. Proper reading of the CT scan for mechanical mechanisms causing PI/PVG is critically important. This subset is extremely heterogeneous and poorly stratified if included in the latter steps of the algorithm. The remaining patients without mechanical causes segregate using the vascular disease score. The score is comprised of commonly identified vascular risk factors and other data available on initial consultation. The experienced clinician likely utilizes a similar stratifying system subconsciously. A conservative (low) vascular disease score cut-off was chosen to maximize the sensitivity of properly identifying patients with acute mesenteric ischemia. We incorporated the use of laparoscopy in borderline cases, but have not yet adopted this strategy widely at our institution. In the algorithm, costly and time-consuming adjuncts were kept to a minimum and only after some degree of certainty that benign PI/PVG was the correct diagnosis. These adjuncts could be considered confirmatory testing for the stable patient with an atypical presentation of benign PI/PVG, but should not delay prompt surgical exploration if mesenteric ischemia remains high in the differential diagnosis.

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Colonic Gene Expression in Conventional and Germ-Free Mice with a Focus on the Butyrate Receptor GPR109A and the Butyrate Transporter SLC5A8

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Abstract
Introduction Butyrate is a bacterial fermentation product that produces its beneficial effects on colon through GPR109A, a butyrate receptor, and SLC5A8, a butyrate transporter. In this study, we compared the expression of GPR109A and SLC5A8 between conventional mice and germ-free mice to test the hypothesis that the expression of these two proteins will be decreased in germ-free mice compared to conventional mice because of the absence of bacterial fermentation products and that colonization of germ-free mouse colon with conventional bacteria will reverse these changes.
Methods RNA was prepared from the ileum and colon of conventional mice and germ-free mice and used for RT-PCR to determine mRNA levels. Tissue sections were used for immunohistochemical analysis to monitor the expression of GPR109A and SLC5A8 at the protein level. cDNA microarray was used to determine the differential expression of the genes in the colon between conventional mice and germ-free mice.
Results In conventional mice with normal bacterial colonization of the intestinal tract, GPR109A and SLC5A8 are expressed on the apical membrane of epithelial cells lining the ileum and colon. In germ-free mice, the expression of GPR109A and SLC5A8 is reduced markedly in the ileum and colon. The expression returns to normal levels when the intestinal tract of germ-free mice is colonized with bacteria. The expression of the Na^+-coupled glucose transporter, SGLT1, follows a similar pattern. Microarray analysis identifies ~700 genes whose expression is altered more than twofold in germ-free mice compared to conventional mice. Among these genes are the chloride/bicarbonate exchanger SLC26A3 and the water channel aquaporin 4. The expression of SLC26A3 and AQP4 in ileum and colon is reduced in germ-free mice, but the levels return to normal upon bacterial colonization.
Conclusion Gut bacteria play an active role in the control of gene expression in the host intestinal tract, promoting the expression of the genes that are obligatory for the biological actions of the bacterial fermentation product butyrate and also the genes that are related to electrolyte and water absorption.

Keywords Gut bacteria · Dietary fiber · Colonic fermentation · Germ-free mouse · Microarray analysis · Electrolyte and water absorption

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Introduction

Based simply on cell number, adult humans can be considered more prokaryotic than eukaryotic as it is estimated that the cells in a human body are 90% microbial and only 10% human.[1,2] Mucosal surfaces in humans are colonized with a very complex and dynamic collection of microorganisms. The majority of these bacteria are housed in the gastrointestinal tract, reaching nearly 10^{14} microorganisms. This number is approximately tenfold greater than the total number of human somatic and germ cells in a human body.[1,2] Microbial densities are relatively low in the duodenum and jejunum (10^2 bacteria/ml of luminal contents), but a marked increase is found in the distal small

intestine (10^8/ml) and colon (10^{11}–10^{12}/ml).[3] These bacteria have long been considered as "commensal." Commensalism refers to a relationship between two organisms in which one benefits and the other is neither harmed nor helped. In this case, the bacteria benefit because the host provides them nourishment, while the host is neither helped nor harmed. This concept has changed dramatically in recent years. There is increasing evidence that the relationship between gut bacteria and the host is not "commensal" but "mutual." Mutualism refers to a relationship between two organisms where both benefit because of coexistence. The conventional bacteria in the gut (i.e., the bacteria that colonize the intestinal tract under normal conditions) are known to influence markedly the biology of the host in various processes, including energy balance, gene expression, immune function, and initiation/progression of specific intestinal diseases.[1,2,4–6]

The nourishment for gut bacteria comes primarily from dietary fiber. The components of dietary fiber are neither digested nor absorbed in the proximal small intestine. These components reach the distal bowel for subsequent fermentation by bacteria. This process generates high concentrations of short-chain fatty acids (SCFAs, i.e., acetate, propionate, and butyrate) in the colonic lumen. Though much less compared to the colon, SCFAs are generated to a significant extent also in the ileum. In addition, significant amounts of SCFAs generated in the colon may also enter the distal ileum through the ileocecal junction. SCFAs are believed to be responsible for the beneficial effects of gut microbiota on intestinal/colonic health.[7–9] These bacterial fermentation products provide metabolic fuel to the colonic/intestinal epithelium, modulate intracellular pH, cell volume, and other functions associated with ion transport, and regulate colonic/intestinal cell proliferation, differentiation, and gene expression.[7–9] Among the SCFAs, butyrate is of high importance as it has been shown to contribute to the differentiation of epithelial cells, enhancement of electrolyte and water absorption, promotion of angiogenesis, and modulation of the immune function.[10–12] The presence of butyrate and other SCFAs in the colonic/intestinal lumen is also linked to decreased incidence of colorectal cancer and inflammatory bowel disease.[10–12]

The molecular mechanisms by which the bacterial metabolite butyrate elicits its effects on colonic/intestinal epithelial cells are poorly understood. Recent studies have identified a Na^+-coupled transporter for butyrate and other short-chain fatty acids.[13,14] This transporter, known as SLC5A8 or SMCT1 (sodium-coupled monocarboxylate transporter 1), is expressed abundantly in the apical membrane of the ileum and colon.[15,16] SLC5A8 transports butyrate via a Na^+-dependent electrogenic process, and the expression of the transporter is reduced markedly in colon cancer.[17] Re-expression of the transporter in colon cancer cell lines leads to cell death in the presence of butyrate, and the process involves inhibition of histone deacetylases.[17] SLC5A8 functions as a tumor suppressor in colon, and the ability of the transporter to mediate the cellular entry of butyrate, an inhibitor of histone deacetylases, underlies its tumor-suppressive function.[18–21] More recently, we have demonstrated that butyrate can also elicit biologic effects on colonic epithelial cells without entering the cells.[22] This involves GPR109A, a G-protein-coupled receptor, which is expressed in the apical membrane of the ileum and colon where butyrate serves as its physiologic agonist. GPR109A also functions as a tumor suppressor in colon. Ectopic expression of the receptor in colon cancer cell lines by transfection with the receptor cDNA leads to cell death in the presence of butyrate without involving the inhibition of histone deacetylases.[22]

It has been shown previously that gut bacteria have marked influence on gene expression in the ileum.[23,24] The influence of gut bacteria on gene expression in the colon, where the bulk of gut bacteria reside, has not been studied. Butyrate is a major metabolite resulting from bacterial fermentation. With the recent discovery of SLC5A8 as a butyrate transporter and GPR109A as a butyrate receptor, which are expressed more prominently in the colon, we hypothesized that gut bacteria may control the expression of these genes in the colon. We tested this hypothesis by comparing the transcriptome of the colon between conventional mice with normal bacterial colonization of the intestinal tract and germ-free mice with no bacteria in the intestinal tract, placing a special emphasis on the genes coding for SLC5A8 and GPR109A. We also examined whether the changes in gene expression observed in germ-free mice revert back to normal when these mice are colonized with bacteria.

Material and Methods

Animals

Age-matched conventional and germ-free mice (Swiss Webster strain) were obtained from commercial sources (Taconic Farms, Inc., Petersburgh, NY) and used for experiments on the same day when they arrived at the Medical College of Georgia. The Medical College of Georgia does not have a germ-free facility, and therefore, the animals could not be acclimatized prior to the experiments. However, since the conventional mice as well as the germ-free mice were treated the same way, it was presumed that the lack of acclimatization of the animals would not be a confounding factor in the interpretation of the results. For studies involving colonization of germ-free mice with bacteria, age-matched conventional and germ-free mice

were kept at the Medical College of Georgia animal facility under conventional conditions for varying time periods (0–4 weeks). During this time, the animals had access ad libitum to tap water and regular unsterilized food. Mice were killed by cervical dislocation under isofluorane anesthesia. The terminal ileum (~3 cm) and the proximal colon (~3 cm) attached to the cecum were removed for preparation of RNA and tissue sections. Each experimental group consisted of four mice. Use of animals in these studies adhered to the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised in 1985) and was approved by the institutional Committee for Animal Use in Research and Education.

Reverse Transcriptase Polymerase Chain Reaction

RNA prepared from conventional, germ-free, and re-colonized mouse ileum and colon were used for reverse transcriptase polymerase chain reaction (RT-PCR). The PCR primers for gene-specific products were designed based on the nucleotide sequences available in GenBank (Table 1). Details of the conditions used for PCR such as the annealing temperature and cycle number are also given in Table 1. The levels of hypoxanthine phosphoribosyl transferase 1 (HPRT1) mRNA or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were used as the internal control in RT-PCR. PCR products were size-fractionated on agarose gels. Bands were visualized by ethidium bromide signals quantified using STORM phosphorimaging system. RT-PCR was carried out with three or four biological replicates, and PCR was repeated at least twice with each RNA sample. The band intensity of each PCR product was normalized using HPRT1 mRNA or GAPDH mRNA as an internal control.

Immunofluorescence Analysis

To examine the protein expression for GPR109A, SLC5A8, sodium-coupled glucose transporter 1 (SGLT1), and AQP4 in conventional, germ-free, and re-colonized mouse ileum and colon, immunofluorescence methods were used. Polyclonal antibodies against GPR109A and SLC5A8 were generated in rabbits. The specificity of these antibodies has been established in previous publications.[15,22,25,26] The polyclonal antibody specific for SGLT1 and the monoclonal antibody specific for AQP4 were obtained from commercial sources (SGLT1 antibody, Chemicon, Temecula, CA; AQP4 antibody, Santa Cruz Biotechnology, Santa Cruz, CA). Cryosections of mouse ileum and colon were fixed on ice in 4% paraformaldehyde for 10 min, washed with phosphate-buffered saline, and blocked with 1X Power Block for 60 min at room temperature. Sections were then incubated overnight at 4°C with 1:100 rabbit polyclonal anti-GPR109A, 1:250 anti-SLC5A8, 1:500 anti-SGLT1, or 1:250 anti-AQP4 antibody. Negative control sections were treated identically except that primary antibody was substituted with phosphate-buffered saline for overnight incubation. Sections were rinsed with phosphate-buffered saline and incubated for 30 min at room temperature with secondary antibodies. For detection of GPR109A, SGLT1, and AQP4 labeling, sections were incubated with 1:1,500 goat anti-rabbit IgG Alexa Fluor 568. For detection of SLC5A8 labeling, sections were incubated with 1:1,500 goat anti-rabbit IgG Alexa Fluor 488. Nuclei were counterstained with 1:10,000

Table 1 List of Primers used in this Study

Gene accession number	Primer sequence	Position	Product size (bp)	Annealing temperature and cycle number
GPR109A NM_177551	Sense: 5′-CGAGGTGGCTGAGGCTGGAATTGGGT-3′ Antisense: 5′-ATTTGCAGGGCCATTCTGGAT-3′	325–347 950–970	646	60°C, 30 cycles
SLC5A8 NM_145423	Sense: 5′-GGGTGGTCTGCACATTCTACT-3′ Antisense: 5′-GCCCACAAGGTTGACATAGAG-3′	371–392 700–721	351	60°C, 30 cycles
SGLT1 NM_019810	Sense: 5′-AGTATCTGCGGAAGCGGTTTGG-3′ Antisense: 5′-GTGAGACATGTTCTTGGCCGAGAG-3′	389–411 904–928	540	58°C, 30 cycles
FIAF AF_278699	Sense: 5′-CCCAGCAGCAGAGATACCTATCA–3′ Antisense: 5′-AGAGAGGCTCTTGGCACAGTTAAG-3′	383–406 1027–1051	669	58°C, 30 cycles
AQP4 NM_009700	Sense: 5′-ACTATTTTTGCCAGCTGTGATTCCAAACGA-3′ Antisense: 5′-TTCCCCTTCTTCTCTTCTCCACGGTCA-3′	517–547 912–939	423	61°C, 24 cycles
DRA NM_021353	Sense: 5′-CACAAATTCAGAAGACGAACATCGCAGACC-3′ Antisense: 5′-GCATCAGCATTCCCTTTAAGTTTCCGAGTG-3′	734–764 1310–1340	607	61°C, 24 Cycles
HPRT1 NM_013556	Sense: 5′-GCGTCGTGATTAGCGATGATGAAC-3′ Antisense: 5′-CCTCCCATCTCCTTCATGACATCT-3′	166–189 298–322	157	58–60°C, 30 cycles
GAPDH NM_008084	Sense: 5′-CTCTGGAAAGCTGTGGCGTGAT-3′ Antisense: 5′-CATGCCAGTGAGCTTCCCGTTCAG-3′	567–589 664–688	122	61°C, 24 cycles

Hoechst 33342, and the slides were coverslipped Vectashield Hardset mounting medium. The immunopositive signals were detected by epifluorescence using Zeiss Axioplan-2 microscope equipped with an Apotome (for optical sectioning), the axiovision program, and an HRM camera.

DNA Microarray Analysis of Gene Expression

Total RNA was prepared from tissue samples obtained from conventional, germ-free, and re-colonized mouse ileum and colon using RNA Trizol reagent (Invitrogen, Carlsbad, CA) and used for cDNA probe preparation. cDNA probes were synthesized using the FairPlay microarray labeling kit (Stratagene, La Jolla, CA). Two samples were pooled together for each experiment. The cDNA probes were then labeled with Cy3 or Cy5 monofunctional reactive dye (Amersham Biosciences, Piscataway, NJ). The appropriate Cy3- and Cy5-labeled probes were combined along with 10 μg mouse Cot-1 DNA (Invitrogen) and 4 μg yeast tRNA in a final volume of 15 μL and incubated at 98°C for 1 min. The denatured probes were mixed with 15 μL of 2X hybridization buffer (50% formaldehyde, 10X SSC, 0.1% sodium dodecyl sulfate (SDS)). The hybridization solution and cDNA probe mixtures were added to the processed National Cancer Institute mouse oligomicroarray slides which were then placed in hybridization chambers and incubated at 43°C for 16 h. The slides were then washed for 5 min in 2X SSC and 0.1% SDS, for 5 min in 1X SSC, and for 5 min in 0.2X SSC and then dried. Fluorescence images were captured using a Genepix 4000 (Axon Instruments, Union City, CA). Both image and signal intensity data were loaded into a database supported by the Center for Information Technology of NIH. Cy3/Cy5 intensity ratios from each gene were calculated and subsequently normalized to ratios of overall signal intensity from the corresponding channel in each hybridization. The normalized data were then extracted from the database as text files and analyzed using computer software JMP (SAS Institute, Cary, NC) to compare the gene expression profiles quantitatively. For clustering analysis, Cluster and TreeView programs[27] were used to analyze the gene expression patterns in a one-dimensional hierarchical clustering to generate gene dendrograms based on the pairwise calculation of the Pearson coefficient of normalized fluorescence ratios as measurements of similarity and linkage clustering. A twofold change was considered a significant difference. The clustered data were loaded into TreeView program and displayed by the graded color scheme as described previously.[28] The gene expression profiles were compared between conventional mice and germ-free mice to determine the changes that occur in colonic gene expression due to absence of gut bacteria. We also compared the gene expression profiles between germ-free mice and re-colonized germ-free mice to determine the changes that occur in colonic gene expression due to colonization of a previously germ-free intestinal tract. These experiments were carried out with two independent RNA samples from conventional mice, germ-free mice, and re-colonized germ-free mice.

Statistical Analysis

Statistical analysis was done using one-way ANOVA followed by Bonferroni multiple comparison test. The software used was Graph Pad Prism, version 5.0. A p value <0.05 was considered statistically significant.

Results

Expression of the Butyrate Transporter SLC5A8 and the Butyrate Receptor GPR109A in Conventional, Germ-Free, and Re-Colonized Mouse Colon and Ileum

We investigated whether the presence of gut bacteria influences the expression of the butyrate transporter SLC5A8 and the butyrate receptor GPR109A in the intestinal tract. The levels of SLC5A8 and GPR109A mRNA in colon and ileum were reduced markedly in germ-free mice compared to conventional mice (Fig. 1). For the butyrate transporter, the decrease in germ-free mice compared to conventional mice was 75% in the colon ($p<0.0001$) and 85% in the ileum ($p<0.0001$). The corresponding values for the butyrate receptor were 65% and 90% ($p<0.0001$). As a positive control, we monitored the expression of SGLT1 and fasting-induced adipocyte factor (FIAF) in these mice. It has been documented that the steady-state levels of SGLT1 mRNA in ileum are reduced, whereas the steady-state levels of FIAF mRNA in ileum are increased in germ-free mice compared to conventional mice.[23,24] Our studies confirmed these earlier findings in the ileum (Fig. 1). Interestingly, we found expression of these two genes also in the colon where their expression was altered in germ-free mice in a manner similar to changes that have been reported in the ileum. The steady-state levels of SGLT1 mRNA were reduced in germ-free mice compared to conventional mice by 65% in the colon ($p<0.0001$) and 75% in the ileum ($p<0.0001$). In contrast, the steady-state levels of FIAF were higher in germ-free mice compared to conventional mice by fivefold in the colon ($p<0.0001$) and by threefold in the ileum ($p<0.0001$). We then studied the effect of re-colonization of the intestinal tract in germ-free mice on the expression of GPR109A, SLC5A8, SGLT1, and FIAF. Germ-free mice were maintained in the Medical College of Georgia animal facility under conventional conditions for different time periods, and then the expression of these genes was

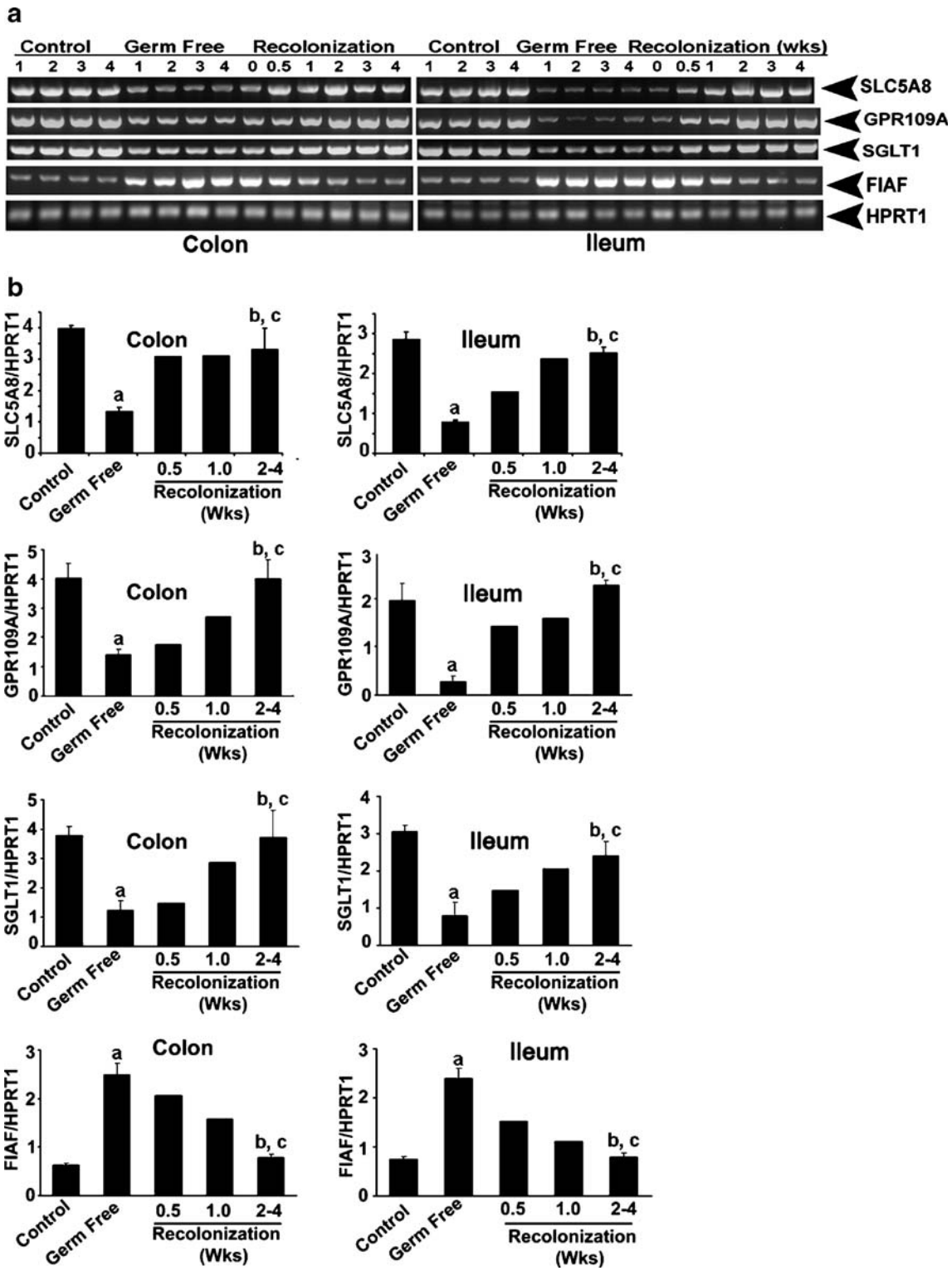

Figure 1 Levels of mRNA for SLC5A8, GPR109A, SGLT1, and FIAF in the colon and ileum of conventional (control) mice, germ-free mice, and germ-free mice whose intestinal tract was colonized by maintenance of the mice under conventional conditions (re-colonization). For re-colonization of germ-free mice with bacteria, age-matched conventional and germ-free mice were kept at the Medical College of Georgia animal facility under conventional conditions for varying time periods (0–4 weeks). During this time, the animals had access ad libitum to tap water and regular unsterilized food. **a** Representative RT-PCR data. **b** Quantification of RT-PCR products. *a* $p<0.0001$ for control versus germ-free; *b* not significant ($p>0.05$) for control versus re-colonized for 2–4 days; *c* $p<0.0001$ for germ-free versus re-colonized for 2–4 days.

evaluated. The changes observed in germ-free mice in terms of expression of the four genes were completely reversed when these mice were maintained under conventional conditions for 3–4 weeks (Fig. 1). There was no difference in mRNA levels for all four genes between conventional mice and germ-free mice that had been kept under conventional conditions for 2–4 weeks to colonize the intestinal tract ($p>0.05$). The reversal of the changes was evident within as early as 3–4 days of conventionalization of the germ-free mice.

We also monitored the protein levels for GPR109A, SLC5A8, and SGLT1 in conventional, germ-free, and re-colonized mouse intestinal tract (Fig. 2). All three proteins were expressed predominantly on the lumen-facing apical membrane of the ileal and colonic epithelial cells in conventional mice. The expression levels were drastically reduced in germ-free mice. These changes induced by the absence of gut bacteria were reversed when the intestinal tract of germ-free mice was re-colonized. The reversal of the changes was clearly evident within 1 week of conventionalization of the germ-free mice. Even though immunohistochemical analysis is not suitable for absolute quantification of the protein levels, the signal intensities for SLC5A8 and GPR109A in Fig. 1 seem to indicate that re-

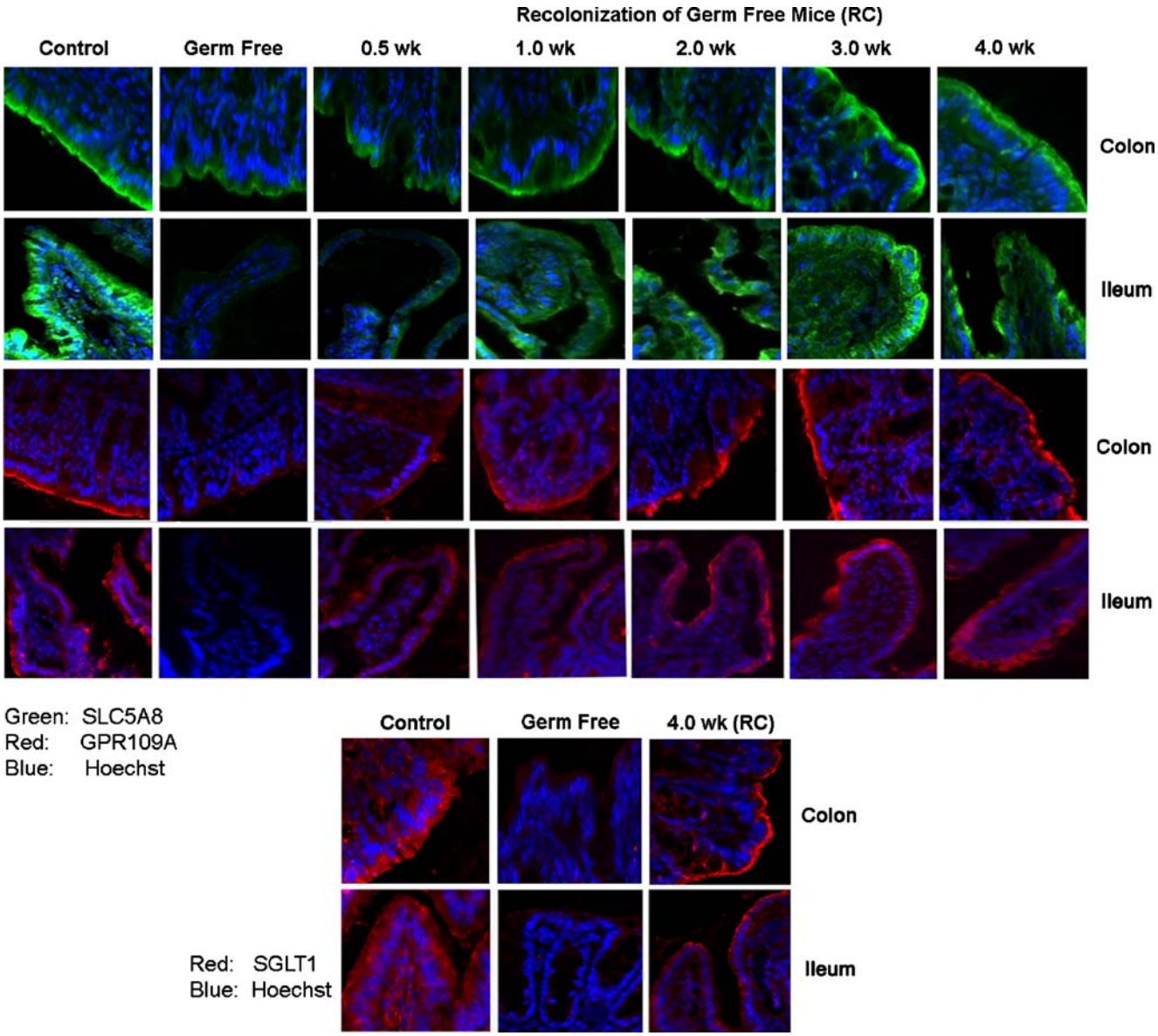

Figure 2 Levels of SLC5A8, GPR109A, and SGLT1 proteins and their localization in the colon and ileum of conventional mice, germ-free mice, and germ-free mice whose intestinal tract was colonized by maintenance of the mice under conventional conditions (re-colonization). Hoechst was used as a nuclear stain. The same animals described in Fig. 1 were used here as the source of tissue sections for immunohistochemical analysis.

colonization of the intestinal tract increases the expression of these two proteins in germ-free mice to levels comparable to those found in conventional mice. These data show that the presence of bacteria in the intestinal tract controls the levels of GPR109A and SLC5A8 mRNA and protein not only in the colon but also in the ileum.

Differential Gene Expression in Conventional, Germ-Free, and Re-Colonized Mouse Colon

It is known already that the expression of genes in the ileum is altered markedly in germ-free mice compared to mice raised under conventional conditions.[23,24] To determine whether this is also true in the colon, we analyzed the gene expression pattern in the colon of conventional mice, germ-free mice, and germ-free mice that were maintained under conventional conditions for 4 weeks to promote bacterial colonization of the previously germ-free intestinal tract. DNA microarray analysis indicated that ~700 genes were affected (increased or decreased) by more than twofold in colon from germ-free mice compared to colon from conventional mice (Fig. 3). These changes were reversed when the colon was re-colonized. Included among the genes that were upregulated in germ-free mouse colon compared to conventional mouse colon were those associated with cell cycle regulation and oncogenic signaling (e.g., cyclin D1, Cdk4, protein arginine *N*-methyltransferase 1, hepatoma-derived growth factor, guanine nucleotide exchange factor 2, eukaryotic translation initiation factor 4E binding protein 1, Rab 28, and LDL-receptor related protein 1), amino acid transport (e.g., the neutral amino acid transporter LAT1 and its heterodimeric partner 4F2hc), development (e.g., galactose-binding lectin, metallothionein 2, and α1A tubulin), and signal transduction (e.g., protein phosphatases, PAK1 interacting protein 1, cAMP-dependent regulatory protein kinase type IIβ, and Sprouty homolog 4; Table 2). Lactate dehydrogenase A, which is normally upregulated in tumor cells[21], was also among the genes that were upregulated in germ-free mouse colon. Most notable among the genes that were downregulated in germ-free mouse colon compared to conventional mouse colon were those involved in immune development and antimicrobial defense (Table 3). Some of these genes were downregulated more than 20-fold. This included immunoglobulins, angiogenin 4 (a Paneth cell protein with bactericidal activity), and CD52 and the protein kinase Adck1 that are related to the development of the immune system. There were also several transporters among the downregulated genes including the butyrate transporter SLC5A8, chloride/bicarbonate exchanger SLC26A3 (also known as downregulated in adenoma or DRA), calcium-activated chloride channel, and aquaporin 4 (AQP4). Similarly, certain metabolic enzymes or enzyme modulators were also downregulated (e.g., group IIA phospholipase A2 and serine peptidase inhibitor).

Expression of the Water Channel Aquaporin 4 and the Chloride/Bicarbonate Exchanger DRA in Conventional, Germ-Free, and Re-Colonized Mouse Colon and Ileum

AQP4 is responsible for water reabsorption in the gut, especially in the colon. DRA is an anion exchanger. It mediates chloride/bicarbonate exchange and plays an important role in electrolyte absorption in the intestinal tract. We found it very interesting and clinically relevant that the expression of aquaporin 4 and DRA was markedly downregulated in germ-free mouse intestinal tract (ileum and colon) compared to conventional mouse intestinal tract. This may suggest that the conventional bacteria in the intestinal tract play an active role in the control of water and electrolyte absorption. Because of the physiological and clinical significance of these findings, we wanted to confirm the microarray data by RT-PCR. These studies showed that the steady-state levels of DRA mRNA and AQP4 mRNA were decreased markedly in the colon and ileum in germ-free mice compared to conventional mice (Fig. 4a, b). For DRA, the decrease in expression levels in germ-free mice compared to conventional mice was 55% both in the colon and ileum ($p<0.0001$). The corresponding values for AQP4 were 85% in the colon ($p<0.0001$) and 30% in the ileum ($p<0.0001$). These data corroborate the microarray data. The changes in the expression of AQP4 were much more pronounced in the colon than in the ileum. The changes in the expression of DRA and AQP4 were, however, reversed completely, both in the ileum and colon, when the intestinal tract of germ-free mice was re-colonized. The reversal was evident within as early as 3–4 days of conventionalization of germ-free mice. There was no difference in the mRNA levels of AQP4 and DRA between conventional mice and germ-free mice that had been kept under conventional conditions for 2–4 weeks to colonize the intestinal tract ($p>0.05$). We also monitored the protein expression levels of AQP4 in conventional mice, germ-free mice, and germ-free mice that were re-colonized by maintenance under conventional conditions (Fig. 4c). AQP4 protein was expressed in the apical as well as the basolateral membrane of ileal and colonic epithelial cells in conventional mice. The expression levels decreased in germ-free mice, but the expression reverted back to normal levels in germ-free mice that were re-colonized.

Discussion

Gut bacteria play a critical role in the maintenance of colonic health, but the molecular mechanisms involved in

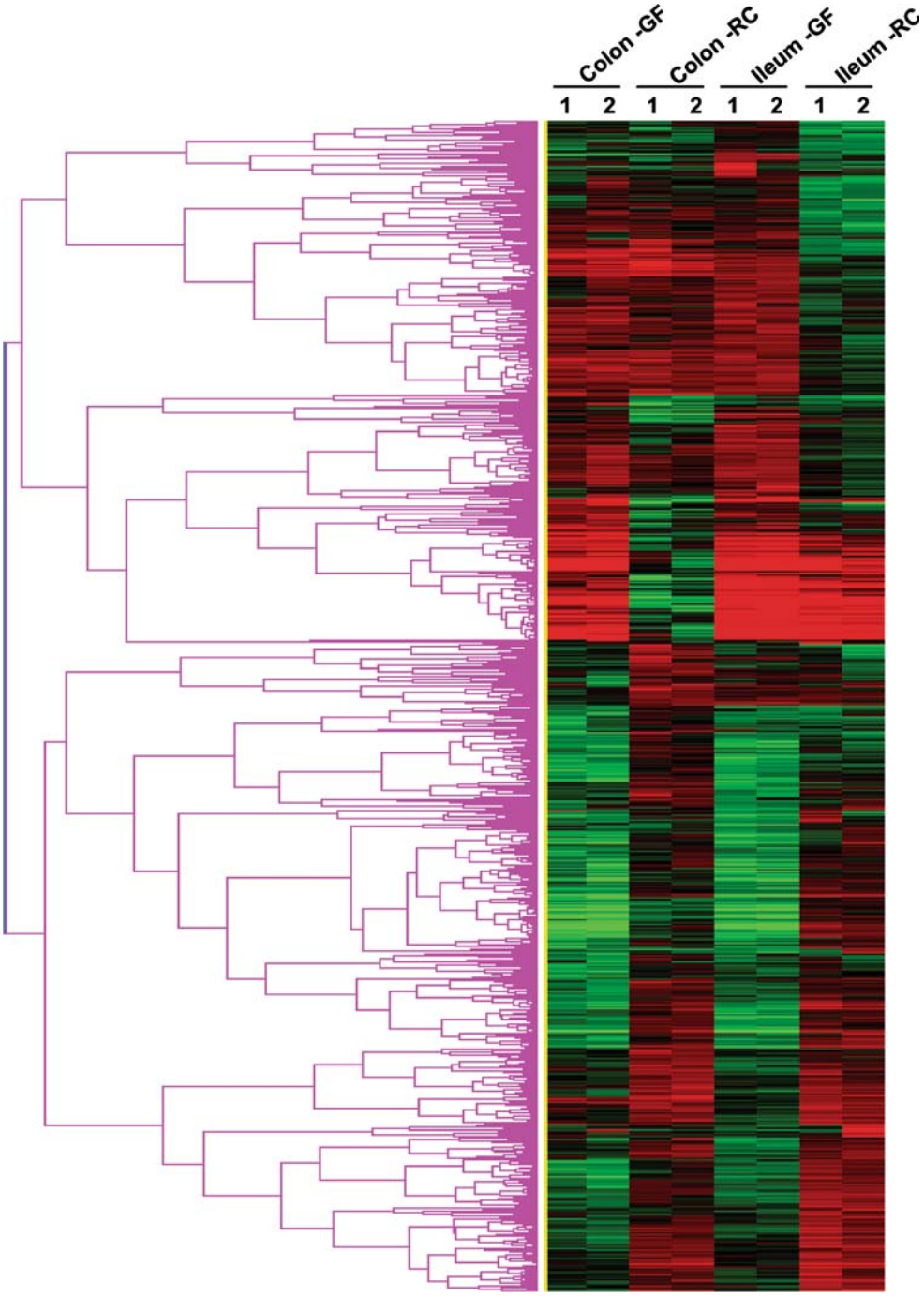

Figure 3 DNA microarray analysis of gene expression in the colon and ileum from age-matched conventional mice, germ-free mice (*GF*), germ-free mice whose intestinal tract was colonized by maintaining the mice for 4 weeks under conventional conditions (re-colonization, *RC*). The data are from two independent microarray experiments with two separate animals in each group. Expression levels of genes in germ-free mice were compared with those in conventional mice, whereas expression levels of genes in re-colonized mice were compared with those in germ-free mice. *Green signal* means downregulation of gene expression and *red signal* means upregulation of gene expression.

the process are not well understood. A considerable focus has been given to the SCFAs that are the products of bacterial fermentation of dietary fiber as the potential mediators of the communication between gut bacteria and the host.[7–12] Butyrate, one of the SCFAs, is an inhibitor of histone deacetylases (HDACs); it is therefore widely accepted that this bacterial metabolite has the ability to influence gene expression in the colon through HDAC inhibition. However, for the luminally produced butyrate to have its effect on the intracellular HDACs in the colonic epithelium, it has to enter the cells first to gain access to its target. SLC5A8 as the Na^+-coupled transporter for butyrate that is expressed in the lumen-facing apical membrane of colonic epithelial cells is likely to play an important role in this process.

SLC5A8 is not the only mediator of the biologic effects of butyrate in the colon. This bacterial metabolite elicits its effects on colonic epithelial cells also via the G-protein-coupled receptor GPR109A. Two independent groups of investigators, while searching for the mechanism underlying

Table 2 Upregulation of Select Genes with Known Function in Germ-Free Mice Colon and Intestine

Accession number	Gene name	Gene function	Fold change colon	Fold change intestine
Cell growth maintenance and oncogenic signaling				
NM_013749	Tumor necrosis factor receptor superfamily, member 12a (Tnfrsf12a)	Cell growth and/or maintenance	15	30
NM_007631	Cyclin D1 (Ccnd1) mRNA	G1 to S transition and oncogenic signaling	9	5
NM_007918	Eukaryotic translation initiation factor 4E binding protein 1 (Eif4ebp1)	mTOR signaling pathway	8	7
NM_024213	Anaphase promoting complex subunit 4 (Anapc4)	Cell cycle progression	7	6
NM_008722	Nucleophosmin 1 (Npm1), mRNA	Oncogenic signaling/ c-myc target	7	5
NM_019830	Protein arginine *N*-methyltransferase 1 (Prmt1)	Cell cycle regulation by methyltransferases	7	4
NM_009465	AXL receptor tyrosine kinase (Axl)	Oncogene and cell growth maintenance	5	11
NM_009870	Cyclin-dependent kinase 4 (Cdk4)	G1 to S transition and oncogenic signaling	5	5
NM_008512	Low-density lipoprotein receptor-related protein 1 (Lrp1)	Lipoprotein metabolism and oncogenic signaling	4	3
NM_027295	RAB28, member RAS oncogene family (Rab28), mRNA	Oncogene and cell growth maintenance	4	2
NM_008231	Hepatoma-derived growth factor (Hdgf), mRNA	Oncogenic signaling	2	4
Transporters				
NM_011404	Solute carrier family 7, member 5 (Slc7a5)	Transport system for arginine	6	7
NM_008577	Solute carrier family 3 member 2 (Slc3a2)	Cell spreading, migration and proliferation	3	N.S.
Metabolism, development, and transcriptional regulation				
NM_008630	Metallothionein 2 (Mt2)	Protection against oxidative damage	9	12
NM_008495	Lectin, galactose binding, soluble 1 (Lgals1)	Sugar-binding protein	7	15
NM_010699	Lactate dehydrogenase A (LDHA)	Catalyzes final step of anaerobic glycolysis	7	N.S.
NM_011653	Tubulin, alpha 1A (Tuba 1a)	Microtubule formation	5	7
Signal transduction				
NM_011158	Protein kinase, cAMP-dependent regulatory, type II beta (Prkar2b)	Role in mitosis and chromosome dynamics	4	5
NM_011898	Sprouty homolog 4 (Spry4)	Jak-STAT signaling pathway	3	3
NM_008913	Protein phosphatase 3, catalytic subunit, alpha isoform (Ppp3ca)	BCR signaling pathway	3	3

N.S. not significant

the lipid-lowering effects of nicotinate (niacin), discovered that nicotinate elicits its anti-lipolytic effect in adipocytes by activation of GPR109A/PUMA-G with subsequent inhibition of adenylyl cyclase.[29,30] Recently, Taggart et al.[31] have identified β-D-hydroxybutyrate (the major ketone body in circulation) as one of the physiologic agonists for GPR109A. In the same study, acetate and propionate showed no effect, but butyrate displayed significant interaction with GPR109A (EC_{50}, 1.6 mM). Since the luminal concentrations of butyrate in the colon are high enough to activate the receptor, we wondered if this receptor is expressed in the intestinal tract. Our subsequent studies showed that GPR109A is indeed expressed abundantly on the luminal (apical) membrane of mouse and human colonic epithelial cells.[22]

If SLC5A8 and GPR109A provide the molecular link between gut bacteria and the host through the fermentation product butyrate, it is important to know if the presence of bacteria in the gut influences their expression in the host. The present studies show unequivocally that gut bacteria are obligatory for optimal expression of the two genes. Absence of commensal bacteria leads to marked suppression of SLC5A8 and GPR109A expression in the colon. Interestingly, the same phenomenon is also seen in the ileum, indicating that the relatively smaller number of bacteria that colonize the terminal small intestine is sufficient to influence gene expression at that site as has been demonstrated by other investigators.[23,24] This effect is entirely reversible. Colonization of the intestinal tract in a previously germ-free mouse brings back the expression of the two genes to the levels comparable to those seen in a normal conventional mouse. In fact, maintenance of the germ-free mice under conventional conditions only for about 3–4 days seems to be sufficient to cause a significant reversal in the expression of these two genes, and the reversal appears to be complete within 4 weeks of re-

Table 3 Downregulation of Select Genes with Known Function in Germ Free Mice Colon and Intestine

Accession number	Gene name	Gene function	Fold change colon	Fold change intestine
Transporters				
NM_017474	Chloride channel calcium activated (Clca3)	Prevention of intestinal mucosa based lesions	17	11
NM_177296	Transportin 3 (Tnpo3)	Protein transport	8	31
NM_009700	Aquaporin 4 (Aqp4)	Membrane water transport and water homeostasis	7	2
NM_021353	Solute carrier family 26, member 3 (Slc26a3) DRA	Transporter and tumor suppressor	3	5
NM_145423	Solute carrier family 5, member 8 (Slc5a8)	Butyrate and pyruvate transporter and tumor suppressor	3	4
Defense response				
XM_001474025	Immunoglobulin heavy chain (lgh-VJ558)	Immune development	84	95
NG_005612	IgVk8-31 (IGKV8-31)	Immunoglobulin regulation	65	112
NM_152839	Immunoglobulin joining chain (Igj)	B cell development	61	95
NG_005612	Immunoglobulin kappa chain (Igk-V23)	B cell development	60	142
NG_005838	Immunoglobulin heavy chain complex (Igh-6)	B cell development	60	77
NM_177544	Angiogenin (Ang4)	Endogenous antimicrobial protein	37	6
X89106	Immunoglobulin heavy chain complex (Igh)	Natural killer cell mediated cytotoxicity	18	49
AY186205	Anti-human melanoma immunoglobulin light chain (HB8760)	Protection against melanoma	17	6
NM_028105	AarF domain containing kinase 1 (Adck1)	Immune development	15	29
NM_013706	CD52 antigen (cd52)	Immune development	8	18
Metabolism, development, and transcriptional regulation				
NM_011463	Serine peptidase inhibitor, Kazal type 4 (Spink4)	Endopeptidase inhibitor activity	8	7
NM_001082531	Phospholipase A2, group IIA (Plag2a)	Alpha-linolenic acid and arachidonic acid metabolism	5	21

colonization. We do not, however, have data on the extent of bacterial colonization or the luminal concentrations of the bacterial fermentation products during this time course of re-colonization.

The findings that gut bacteria influence the expression of SLC5A8 and GPR109A in the colon prompted us to examine the global gene expression pattern in the germ-free mouse compared to the conventional mouse. Such studies have been done in the ileum and have shown that gut bacteria influence the expression of hundreds of genes.[23,24] However, there are no reports of similar studies in the colon where the majority of bacteria resides. The present studies show that the expression of a large number of genes is altered in the colon in germ-free mice compared to conventional mice. These changes in the global gene expression are largely reversible since the expression pattern reverts back to normal in most cases upon colonization of the intestinal tract in the mouse which was previously germ-free.

The analysis of the global gene expression pattern in conventional mouse and germ-free mouse identified SLC26A3 and AQP4 among the genes that are silenced under germ-free conditions. We focused on these two genes for several reasons. SLC26A3 is a chloride/bicarbonate exchanger that plays a critical role in the absorption of chloride in the ileum and colon. AQP4 is important for water absorption. The same is true with SLC5A8 and SGLT1, the two transporters whose expression is silenced in germ-free mice. Since SLC5A8 functions as a Na^+-coupled transporter for butyrate with a Na^+:butyrate stoichiometry of 2:1, the transporter may promote Na^+ absorption in the colon in the presence of the bacterial fermentation product butyrate. Similarly, SGLT1 is a Na^+-coupled transporter for glucose (Na^+:glucose stoichiometry, 2:1) that plays an important role in Na^+ absorption in the intestinal tract in the presence of luminal glucose. It is generally assumed that SGLT1 is relevant only to the small intestine, but our present studies demonstrate the expression of this transporter not only in the ileum but also in the colon. Since the expression of SGLT1 is seen on the apical membrane of colonic epithelial cells, it is likely that that transporter participates in the absorption of glucose and hence promotes Na^+ absorption also in the colon. The silencing of SLC5A8, SGLT1, SLC26A3, and AQP4 in germ-free mice shows that gut bacteria play an active role in electrolyte and water absorption in the intestinal tract. The findings of the present studies show that conventional bacteria in the intestinal tract are obligatory for optimal

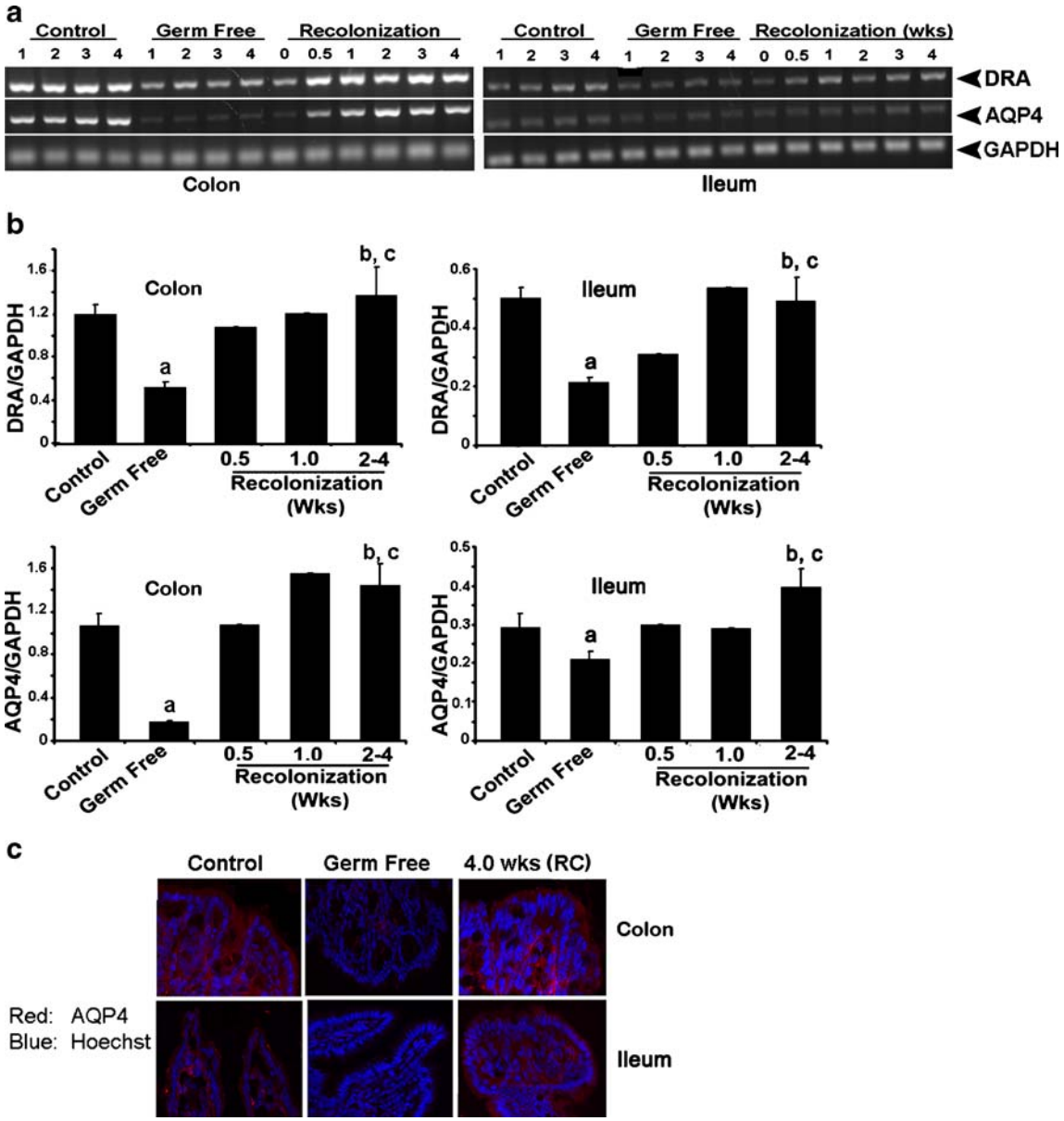

Figure 4 Levels of mRNA for DRA and AQP4, and expression of AQP4 protein in the colon and ileum of conventional mice, germ-free mice, and germ-free mice whose intestinal tract was colonized by maintenance of the mice under conventional conditions (re-colonization). The same animals described in Fig. 1 were used here as the source of RNA for RT-PCR and tissue sections for immunohistochemical analysis. **a** Representative RT-PCR data. **b** Quantification of RT-PCR products. *a* $p<0.0001$ for control versus germ-free; *b* not significant ($p>0.05$) for control versus re-colonized for 2–4 days; *c* $p<0.0001$ for germ-free versus re-colonized for 2–4 days. C Immunofluorescent detection of AQP4 protein. Hoechst was used as a nuclear stain.

electrolyte and water absorption. Furthermore, GPR109A that serves as a receptor for butyrate is coupled to Gi, the inhibitory G protein. Activation of the receptor by butyrate or other agonists leads to a decrease in intracellular levels of cAMP. This cyclic nucleotide is one of the major signaling molecules in the intestinal tract that control electrolyte and water absorption; elevation of intracellular levels of cAMP in the intestinal tract causes secretory diarrhea.[32–34] We speculate that activation of GPR109A by butyrate may have anti-diarrheagenic effects through its ability to reduce intracellular cAMP levels.

Though our studies show convincingly that the presence of conventional bacteria in the intestinal tract has marked influence on the expression of various genes in the ileum and colon, the exact molecular mechanisms involved in this process are not known. However, based on what is known on the biological actions of SCFAs and the recent discovery of SLC5A8 as the transporter for these bacterial metabolites

and GPR109A as the cell surface receptor for butyrate, we postulate that SCFAs, particularly butyrate, play an important role in the observed differences in gene expression between conventional mice and germ-free mice. But SCFAs represent only one group of metabolites generated in the intestinal tract by bacterial metabolism. Therefore, without additional studies, it is premature to conclude at this time that SCFAs are the sole mediators of gene expression in the intestinal tract in response to the presence of conventional bacteria.

Conclusion

Commensal bacteria in the mouse intestinal tract play an important role in the control of gene expression in the colon and ileum. In particular, these bacteria have marked influence on the expression of the genes involved in water and electrolyte absorption and in immune function.

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Discussant

DR. ROBERT MARTINDALE (Portland, OR): Thank you, Gail, for this very interesting work. This shows us that we truly are mutualistic with our bacteria as this paper and many others at this meeting have shown us.

There are several things you have very nicely shown through microarray and immunohistofluorescence data.

The question I have for you today is regarding the mechanism. Is it the bacteria themselves or some product of the bacteria? Can you speculate on the mechanism?

Discussant

GAIL CRESCI: Thank you for your very interesting question. We were interested in that question as well. We've done some preliminary work, and I don't have the data to show here, but because we saw down-regulation of the two genes that we are studying, SLC5A8 and GPR109A, and that we have previously shown that they are silenced in colon cancer by DNA methylation, we sought out to see if this might be the possible cause with germ-free animals.

Our preliminary work has actually shown that, in fact, for the silencing of these two genes, SLC5A8 and GPR109A, DNA methylation is definitely involved and DNMT1 seems to be the main isoform involved.

We also know butyrate alters gene expression as shown in other people's work. One may predict there to be an absence of butyrate in a germ-free mouse intestine as the production of butyrate results from bacterial fermentation of undigested polysaccharides. Thus with the absence of this molecule, gene expression may be altered.

I have attended many lectures here at this conference and have read many papers that lead to the thought that perhaps the bacteria itself may be influencing gene expression by secreting proteins or altering the lumen pH. So, without the presence of the gut microbiota, these proteins and other alterations would not exist. That is future work for us. Thank you.

Discussant

DR. ROBERT MARTINDALE (Portland, OR): Do you think having two mechanisms, including the receptor you've shown, as well as a transporter, shows the importance of butyrate? Thus, if one is knocked out, you still have another alternate way for butyrate to elicit its biologic effects on the cell?

Discussant

GAIL CRESCI: Yes. I think that's very important. We are very excited to see that both have a relationship with butyrate. We actually now have knockout mice for GPR109A as well as SLC5A8. So we are, in fact, going to start some studies looking at the potential mechanism there.

Discussant

DR. TEREZ SHEA-DONOHUE (Baltimore): I was interested that you have SGLT1 expression in the colon and one doesn't normally think of the colon as having a nutrient transporter like that. Can you comment on what you think the role of the transporter is there?

Closing discussant

GAIL CRESCI: We were surprised by that as well. Just speculating as I really wouldn't expect to find glucose in the colon. I've been to other presentations this weekend showing that some of these different micobiota rely on different nutrient sources. So perhaps the presence of glucose in the colon is for that or to help with sodium and water absorption as well.

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ORIGINAL ARTICLE

Association of C-Reactive Protein Levels and Long-Term Survival after Neoadjuvant Therapy and Esophagectomy for Esophageal Cancer

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Abstract
Background Preoperative C-reactive protein (CRP) levels have been shown to be prognostic markers of survival in patients undergoing esophagectomy for cancer. No study has evaluated the predictive value for survival of CRP levels after neoadjuvant chemoradiotherapy.
Methods Preoperative CRP levels were assessed in patients undergoing neoadjuvant therapy and esophagectomy for cancer. Groups were defined according to normal value cutoffs of the CRP measurements.
Results Seventy patients had normal CRP, and 20 patients had raised CRP. The groups did not differ in descriptives, comorbidities, white cell counts, pathological data, or morbidity. In-hospital death was higher in the raised CRP group (three versus one patient, $p=0.048$). The Kaplan–Meier survival analysis showed a significant survival advantage of patients with normal CRP compared to patients with raised CRP levels (median survival, 65.4 versus 18.7 months; log rank test, $p=0.027$). The Cox regression analysis identified three independent prognostic factors for survival: UICC stage (IIB/III versus I/IIA, HR 3.48, $p=0.007$), completeness of resection (HR 6.33, $p=0.002$), and CRP levels (raised versus normal, HR 5.07, $p=0.001$).
Conclusion Preoperative CRP levels are an independent prognostic marker for survival after neoadjuvant treatment in patients with esophageal cancer and may be of value in the re-staging process after neoadjuvant treatment.

Keywords Esophageal cancer · Outcomes · Neoadjuvant therapy · C-reactive protein

Introduction

Surgical resection, with or without neoadjuvant chemoradiotherapy (CRT), offers the best chance for cure in patients with esophageal cancer. Despite advances in staging, patient selection, and more aggressive treatment, 5-year survival rates are still poor at approximately 30–35%.[1,2]

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Patients receiving CRT followed by surgery have been shown to have a survival advantage compared to patients undergoing surgery alone.[3] Neoadjuvant CRT downstages tumors and allows higher rates of complete resection.[4] To assess the response to neoadjuvant CRT after neoadjuvant treatment and before surgery, abdomino-thoracic computed tomography (CT), endoscopic ultrasound (EUS), and ^{18}F-fluorodesoxyglucose positron emission tomography (PET) have been advocated.[5,6] In contrast to these histopathological and radiological methods, no reliable biomarker has been identified in esophageal cancer.[7] Ideally, a biomarker is reliable, readily available, easy applicable, and moderately priced. Biomarkers have been used in other tumors, such as breast cancer (Herceptin) or lung cancer (epidermal growth factor receptor).

C-reactive protein (CRP) has been demonstrated to be an independent prognostic marker of survival in esophageal cancer.[8–11] These studies either included only patients without CRT[8,9] or a mixed cohort with and without CRT.[10,11] Additionally, most studies included solely or

predominately squamous cell carcinomas (SCC).[8,10,11] Whether the CRP levels can be used as a marker of response to neoadjuvant treatment is unclear. One study showed an association between pre-neoadjuvant treatment CRP levels and pathological responders and suggested that pretreatment CRP levels might be indicative for sensitivity to CRT.[12] To our knowledge, no study has evaluated the predictive value for survival of CRP levels after neoadjuvant treatment and before surgery.

Hence, this study analyzes the validity of preoperative CRP levels in the re-staging process by analyzing the association of CRP with survival in patients undergoing neoadjuvant CRT followed by surgery.

Material and Methods

Patients and Data Acquisition

The study was approved by the responsible ethics committees. Two cohorts of patients from Switzerland and Australia undergoing esophagectomy for cancer were used to assess preoperative CRP levels. The Swiss cohort of patients consisted of a consecutive series of 220 patients who underwent esophagectomy at one large teaching hospital in Zurich from 2000 to 2008. The Australian cohort consisted of a consecutive series of 254 patients operated in Adelaide, South Australia, between 2000 and 2008 at two University hospitals and two affiliated private hospitals. From this total cohort of 474 patients, 279 patients were excluded because either no preoperative CRP levels were measured or the levels were measured too far in advance of surgery. The selection of patients to have CRP levels measure was based solely on the operating surgeon, with some using it routinely but others only sporadically. One hundred five patients proceeded directly to surgery. Ninety patients underwent neoadjuvant CRT and were primarily analyzed. Subanalysis of the patients proceeding directly to surgery was also performed.

In both locations, data were prospectively collected and stored in databases. Demographics, comorbidity, tumor pathology, morbidity, and mortality were retrieved from these databases. Any missing data were sought subsequently from the case records. Survival data were obtained either from State Cancer Registries or by direct contact with physicians or patients.

Staging, Neoadjuvant Treatment, and Surgery

In both countries, preoperative staging included upper endoscopy with biopsy and EUS, abdominal and thoracic CT, and PET scan. All patients were discussed at a multidisciplinary meeting involving surgeons, medical, and radiation oncologists, and surgical pathologists. Patients with advanced tumors (T3 or N+) usually received neoadjuvant treatment. This entailed two cycles of 5-fluorouracil (5-FU) and cisplatin in combination with 45–54 Gy of radiotherapy. Surgery was performed 6–8 weeks after the completion of pretreatment.

Despite the geographical difference, the surgical technique was comparable. All patients underwent surgery using an abdominal and transthoracic approach, and in all patients, the stomach was used as the conduit for reconstruction. The anastomosis was either performed with a stapled technique or hand sewn, according to the individual surgeon's preference. In a minority of patients, a minimally invasive approach was used, consisting of a thoracoscopic mobilization of the esophagus, followed by gastric mobilization either using a hand-assisted laparoscopic technique or an open technique. The anastomosis in minimally invasive esophagectomies was performed in the neck.

Specimens were classified according to the TNM classification (International Union against Cancer, UICC, sixth edition). In preoperatively treated patients, tumor regression grade (TRG) was determined in accordance with Mandard et al.[13]. Histomorphologic response was defined as follows: major response, TRG 1–2; minor response, TRG 3–5.

CRP Measurement

The CRP was measured preoperatively after completion of the neoadjuvant CRT. Only CRP measurements 2 weeks or less before surgery were eligible for analysis. CRP measurements were performed in serum samples with an automated immunoturbidmetric analyzer (Switzerland, Cobas ® Integra 800, Roche Diagnostics; Australia, Olympus AU 2700/5400, Olympus Diagnostics). Normal values were referenced by the manufacturer at <10 and <8 mg/L, respectively. According to the normal value cutoffs, the patient groups with normal CRP and raised CRP levels were defined.

Statistical Analysis

Statistical analysis was performed with MedCalc®, Version 9 for Windows. Means are presented with SD, medians with 95% confidence interval (95% CI). Comparison of data between the two patient groups was undertaken using chi-square tests for categorical data and Mann–Whitney *U* tests for continuous data. Survival was calculated with Kaplan–Meier and differences between groups with the log rank test. To determine the influence of different variables on outcome, Cox regression analyses were performed. The following variables were entered into the regression models: age (>65 versus ≤65 years), gender (male versus female), UICC stage (IIB/III versus I/IIA), histology (SCC

versus adenocarcinoma), completeness of resection (R1 versus R0), response to neoadjuvant treatment (major versus minor), surgical technique (minimal invasive versus open), and CRP groups (raised versus normal). For all long-term survival analyses, in-hospital deaths were excluded from analysis. A post hoc power analysis determined that the sample sizes were appropriate to assess influence of CRP levels on long-term survival (confidence level, 0.95; power, 0.80, calculated total sample size 85, normal/raised ratio 4/1; Boffin Software© 2006). A $p<0.05$ was considered statistically significant.

Results

Descriptives

There were 70 patients in the normal CRP group and 20 patients in the raised CRP group. Median CRP levels in the former were 4.0 (95% CI 3.8–4.0) and 19.3 (95% CI 12.1–37.8; $p<0.001$) in the latter. No significant difference in white cell counts (WCC), alanine aminotransferase (ALT) or international normalized ratio (INR) occurred (Table 1). The groups did not differ in descriptive variables, comorbidities, or American Society of Anesthesiologists (ASA) scores (Table 2).

Operative, Pathological, and Morbidity Data

Table 3 shows procedure-specific and pathological data. No differences between the groups occurred. In-hospital death was significantly higher in the raised CRP group (three versus one patient, $p=0.048$). In the raised CRP group, one patient died due to sepsis caused by an anastomotic leak and two patients due to pneumonia and respiratory failure. In the normal CRP group, the patient died because of sepsis and acute respiratory distress syndrome caused by an anastomotic leak. Surgical and medical morbidity did not differ between groups (Table 4).

Survival Analysis

The Kaplan–Meier survival analysis showed a significant difference between groups. Patients with normal preoperative CRP level had a significant survival advantage compared to patients with raised CRP levels (median survival 65.4 months and 5-year survival 52.1% versus median survival 18.7 months and 5-year survival 23.3%, log rank test $p=0.027$, Fig. 1). After stratifying groups according to UICC staging, there was still a difference between normal and raised CRP groups. However, this did not reach statistical significance (UICC I/IIA, normal CRP group: median survival not reached, 5-year survival 64.0% versus UICC I/IIA, raised CRP group: median survival 31.9 months, 5-year survival 35.7%, log rank test $p=0.109$; UICC IIB/III, normal CRP group: median survival 21.6 months, 5-year survival 29.4% versus UICC IIB/III, raised CRP group: median survival 11.5 months, 5-year survival 20.0%, log rank test $p=0.182$, Fig. 2).

The Cox regression analysis identified three independent prognostic factors for survival: UICC stage, completeness of resection, and CRP levels as demonstrated in Table 5.

Subanalysis of Patients without CRT

We performed a subgroup analysis of the 105 patients who proceeded directly to surgery. Eighty-three patients had normal CRP levels (median CRP, 4.0 mg/L), and 22 patients had raised CRP levels (median CRP level, 13.2 mg/L). No difference in descriptives, operative, pathological, or morbidity data between the groups occurred. There were three in-hospital deaths in each group. The Kaplan–Meier survival analysis showed no statistical difference between the CRP groups (normal CRP, median survival 43.5 months and 5-year survival 46.8%; raised CRP group, median survival 17.2 months and 5-year survival 48.6%, log rank test, 0.429).

Comparison of the patients with or without CRT and elevated CRP levels did not reveal any statistical differences between groups in term of long-term survival (CRP raised and CRT, median survival 18.7 months, 5-year survival 23.3%; CRP raised and no CRT, median survival 17.2 months, 48.6%, log rank test, 0.429).

The Cox regression analysis identified UICC stage and completeness of resection but not CRP levels as independent prognostic factors for survival (Table 6).

Table 1 Biochemical Results of Patients with Normal Versus Raised CRP

CI confidence interval, *CRP* C-reactive protein, *WCC* white cell count, *ALT* alanine aminotransferase, *INR* international normalized ratio

	Normal CRP, $n=70$	Raised CRP, $n=20$	p value
Median CRP (95% CI)	4.0 (3.8–4.0)	19.3 (12.1–37.8)	<0.001
Median WCC (95% CI)	5.8 (5.4–6.3)	7.2 (5.2–8.7)	0.064
Median ALT (95% CI)	21.0 (17.0–27.0)	18.0 (13.1–23.8)	0.209
Median INR (95% CI)	0.96 (0.9–1.0)	0.96 (0.9–1.1)	0.325

Table 2 Descriptive Variables, Comorbidities or American Society of Anesthesiologists (ASA) Scores

	Normal CRP, n=70	Raised CRP, n=20	p value
Median age in years (95% CI)	63.7 (59.0–67.1)	61.3 (51.3–63.6)	0.081
Gender			0.484
Male (%)	56 (80.0)	18 (90.0)	
Female (%)	14 (20.0)	2 (10.0)	
Mean preoperative BMI (SD)	24.5 (22.7–26.3)	25.6 (23.9–29.0)	0.335
Comorbidities			
Cardiac			0.863
No (%)	55 (78.6)	16 (80.0)	
Yes (%)	15 (21.4)	4 (20.0)	
Respiratory			0.723
No (%)	51 (72.9)	16 (80.0)	
Yes (%)	19 (27.1)	4 (20.0)	
Diabetes			0.673
No (%)	62 (88.6)	19 (95.0)	
Yes (%)	8 (11.4)	1 (5.0)	
Renal			0.632
No (%)	67 (95.7)	19 (95.0)	
Yes (%)	3 (4.3)	1 (5.0)	
ASA score (%)			0.872
ASA 1	2 (2.9)	1 (5.0)	
ASA 2	56 (80.0)	15 (75.0)	
ASA 3	12 (17.1)	3 (15.0)	

Table 3 Procedure Specific and Pathological Data

	Normal CRP, n=70	Raised CRP, n=20	p value
Open esophagectomy (%)	62 (88.6)	18 (90.0)	0.823
Minimal invasive esophagectomy (%)	8 (11.4)	2 (10.0)	
Median duration of surgery in minutes (95%CI)	259 (240–280)	275 (190–339)	0.685
Median blood loss in mls (95% CI)	500 (400–500)	400 (220–600)	0.279
Median length of stay (95% CI)	16.0 (14.0–21.0)	19.5 (15.7–28.8)	0.214
Localization of tumor			0.379
Mid third (%)	10 (14.3)	5 (25.0)	
Lower third (%)	37 (52.9)	11 (55.0)	
GEJ(%)	23 (32.8)	4 (20.0)	
Histology			0.459
Adenocarcinoma (%)	44 (62.9)	15 (75.0)	
SCC (%)	26 (37.1)	5 (25.0)	
UICC Stage			0.672
0 (%)	20 (28.6)	3 (15.0)	
I (%)	10 (14.3)	3 (15.0)	
IIA (%)	18 (25.7)	6 (30.0)	
IIB (%)	12 (17.1)	3 (15.0)	
III (%)	10 (14.3)	5 (25.0)	
Major response to CRT			0.722
No (%)	51 (72.9)	16 (80.0)	
Yes (%)	19 (27.1)	4 (20.0)	

Table 4 Mortality and Morbidity of Patients with CRT

	Normal CRP, n=70	Raised CRP, n=20	p value
Surgical morbidity			
Anastomotic leak			0.245
No (%)	62 (88.6)	15 (75.0)	
Yes (%)	8 (11.4)	5 (25.0)	
Chyle leak			0.667
No (%)	66 (94.3)	19 (95.0)	
Yes (%)	4 (5.7)	1 (5.0)	
Wound infection requiring surgery			0.805
No (%)	63 (90.0)	19 (95.0)	
Yes (%)	7 (10.0)	1 (5.0)	
Re-thoracotomy			0.667
No (%)	67 (95.7)	18 (90.0)	
Yes (%)	3 (4.3)	2 (10.0)	
Re-laparotomy			0.633
No (%)	67 (95.7)	19 (95.0)	
Yes (%)	3 (4.3)	1 (5.0)	
Medical morbidity			
Pneumonia			0.324
No (%)	49 (70.0)	11 (55.0)	
Yes (%)	21 (30.0)	9 (45.0)	
ARDS			0.452
No (%)	68 (97.1)	18 (90.0)	
Yes (%)	2 (2.9)	2 (10.0)	
Cardiac			0.958
No (%)	65 (92.9)	18 (90.0)	
Yes (%)	5 (7.1)	2 (10.0)	
Renal			0.632
No (%)	67 (95.7)	19 (95.0)	
Yes (%)	3 (4.3)	1 (5.0)	

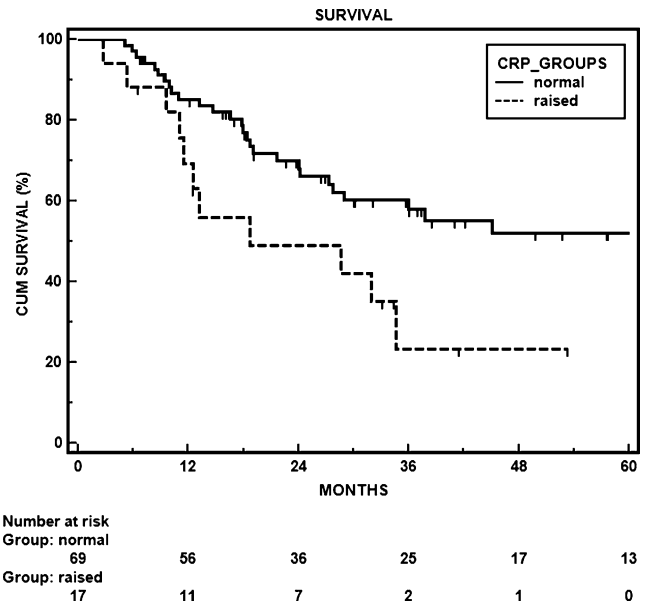

Figure 1 Kaplan–Meier curves normal versus raised CRP groups in patients undergoing CRT.

Discussion

The results of our study suggest that a raised CRP level after neoadjuvant CRT is an independent prognostic indicator for survival in patients with esophageal cancer. This is consistent with a number of articles that showed raised CRP levels to be indicative for poor survival in esophageal cancer.[8–11] However, no study analyzed exclusively patients after neoadjuvant CRT. Whereas two studies excluded patients with neoadjuvant treatment,[8,9] two other studies mixed patients with and without pretreatment.[10,11] Interestingly, CRP was not a significant prognostic indicator for survival in the patients who proceeded directly to surgery, although the median survival and 5-year survivals were less in the raised group—possibly a type I statistical error. Additionally, no difference between patients with raised CRP levels with or without CRT was observed. CRT might normalize the CRP levels in some tumors, indicating a favorable response to CRT and thus becoming a prognostic

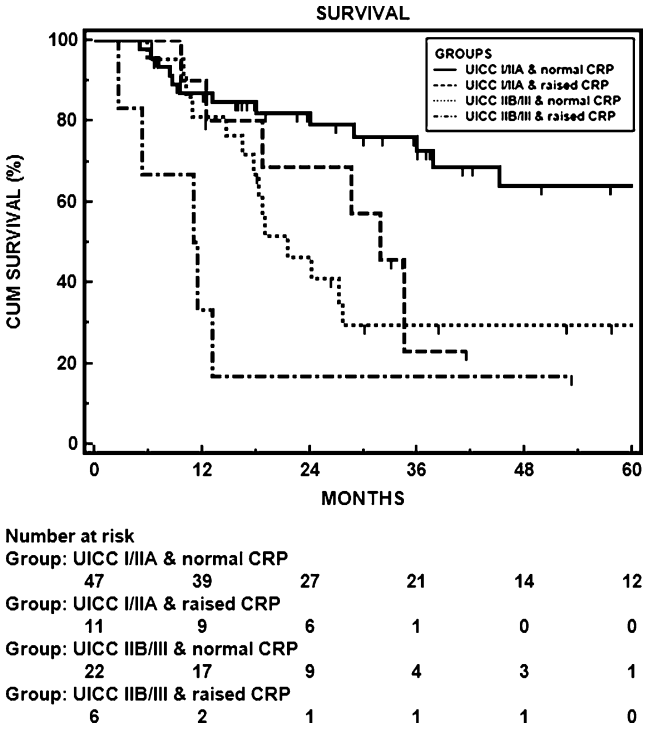

Figure 2 Kaplan–Meier curves stratified according UICC stage groups (I/IIA versus IIB/III) and CRP level (normal versus raised) in patients undergoing CRT.

indicator. In patients without neoadjuvant treatment, however, this selection of patients does not occur and the discriminative nature of the CRP is not apparent. This may also relate to the fact that patients proceeding straight to surgery generally have lower stage tumors than those having CRT. CRP levels were measured 2 weeks or less before surgery to avoid influence of acute inflammatory changes due to the CRP. To our knowledge, no data exist on changing levels of CRP over time following neoadjuvant therapy.

CRP levels were not measured before the initiation of CRT, and this must be acknowledged as an important limitation. However, these missing pretreatment CRP levels have not influenced our findings. Guillem et al.[12] measured CRP levels before neoadjuvant treatment in patients with esophageal cancer. They found that patients with raised CRP levels were more frequently non-responders, suggesting that CRP levels were associated with resistance to CRT and consequently with poorer survival. Another study analyzed the immunohistochemical CRP expression in SCC of the esophagus and found a significant poorer survival in tumors with CRP expression compared to tumors without CRP expression.[14] These studies strongly suggest an association of tumor biology and CRP levels.

One of the key elements after pretreatment is identifying which patients will actually benefit from surgery. Some authors have questioned the necessity of surgery after apparent complete pathological response.[15,16] Esophagectomy is still associated with substantial morbidity and mortality rates up to 60% and 14%, respectively.[17] Neoadjuvant treatment might also increase operative mortality in pretreated patients.[4] Patients with an apparent complete pathological response after neoadjuvant treatment have been shown to have a significant survival advantage.[18,19] However, an apparent complete pathological response alone is not reliable enough to measure efficacy of the neoadjuvant treatment, which can only be truly assessed after resection.[20]

The decision to perform surgery or not is also important from a quality of life point of view. Blazenby et al.[21] showed that only patients who survive for 2 years or longer return to their former quality of life. In our study, patients with a raised CRP level had a median survival of 13 months, and it is likely that many of these patients suffered from an impaired quality of life as a result of an operation, which was of no great benefit to them.

Table 5 Univariate and Multivariate Cox Regression Analysis for Outcome Survival for Patients who Have Undergone Neoadjuvant CRT

Univariate	Hazard ratio	95% CI	*p* value
Age (>65 versus ≤65 years)	0.66	0.34–1.29	0.225
Gender (male versus female)	0.57	0.28–1.17	0.129
UICC stage (IIB/III versus I/IIA)	2.83	1.49–5.37	0.002
Histology (SCC versus adenocarcinoma)	0.93	0.49–1.77	0.829
Radicality (R1 versus R0)	5.78	2.13–15.7	0.001
Major response (yes versus no)	0.49	0.22–1.11	0.087
Surgical technique (minimal invasive versus open)	1.77	0.69–4.51	0.236
CRP (raised versus normal)	2.17	1.08–4.39	0.031
Multivariate			
UICC stage (IIB/III versus I/IIA)	3.48	1.42–8.52	0.007
Radicality (R1 versus R0)	6.33	1.97–20.3	0.002
Major response (yes versus no)	1.39	0.48–4.10	0.544
CRP (raised versus normal)	5.07	1.92–13.43	0.001

Table 6 Subgroup Analysis of Patients Who Proceeded Directly to Surgery

Univariate	Hazard ratio	95% CI	*p* value
Age (>65 versus ≤65 years)	1.01	0.58–1.77	0.963
Gender (male versus female)	0.68	0.29–1.60	0.384
UICC stage (IIB/III versus I/IIA)	5.03	2.51–10.1	<0.001
Histology (SCC versus adenocarcinoma)	0.80	0.34–1.88	0.615
Radicality (R1 versus R0)	3.26	1.66–6.39	0.001
Surgical technique (minimal invasive versus open)	1.06	0.14–7.66	0.952
CRP (raised versus normal)	1.32	0.66–2.64	0.431
Multivariate			
UICC stage (IIB/III versus I/IIA)	4.53	2.22–9.23	<0.001
Radicality (R1 versus R0)	2.01	1.01–3.99	0.049
CRP (raised versus normal)	1.42	0.71–2.85	0.323

Today, the most commonly used re-staging examinations are endoscopy combined with EUS, CT and PET scan. The use of a PET scan, especially in combination with CT has improved re-staging and facilitates identification of patients who obtain a major response and may allow selection of those patients most likely to benefit from surgery.[22–24] However, to increase the accuracy of the restaging process, it would be helpful to have a non-imaging based tool such as a biomarker.

CRP has been shown to be a predictor of survival in many tumors, such as pancreatic, colorectal, and gastrointestinal cancers.[25] It is also a nonspecific marker of inflammatory reaction, and this might have influenced results. In our study, WCC were also analyzed and showed no difference between groups. Although CRP has been repeatedly shown to predict survival in cancer and is cheap and easy to measure, it is not widely used as a prognostic marker. Deans et al. included the CRP (≤5 versus >5 mg/l) in a risk score model for gastroesophageal cancer.[26] Patients with a raised CRP level over 5 mg/L had a 2.6 times higher probability of death from their disease.

Chemotherapy is well-known to cause a certain degree of hepatotoxicity.[27] This might have had an influence on CRP production in the liver and might therefore have influenced the results. The standard neoadjuvant treatment is based on 5-FU and cisplatin, and all patients in our study received this regimen. Whereas no data on liver impairment by cisplatin exists, 5-FU has been shown to impair liver function.[28] Therefore, we analyzed ALT and INR levels in our patients. These were largely normal, and the groups did not differ.

Apart from the CRP levels, UICC stage (IIB/III versus I/IIA) and completeness of the resection (R1 versus R0) were strong independent prognosticators for survival, consistent with the current literature.[29,30] The differentiation between UICC IIB/III and I/IIA relates to the presence of lymph node metastases, which is known to be one of the most important factors influencing survival.[31] After stratifying according to UICC stage groups and CRP levels, patients with raised CRP levels still had an unfavorable long-term survival compared with patients with normal levels. However, these differences did not reach statistical significance, most likely due to the low number in the stratified groups.

A major response to CRT, defined by a Mandard TRG of 1 or 2, was not significantly different between the groups.[13] In contrast to the current literature, we did not find the pathological response to neoadjuvant treatment to be an independent factor influencing survival.[32,33]

This study has several limitations. The study population is a selected group from an overall group of 474 patients. This may have introduced bias. The most important limitation of this study, however, is the relatively small number of patients, particularly in the raised CRP group. However, the difference in survival is so marked we think the difference is worth reporting. Given the ease and low cost of obtaining CRP levels, we think the marker is worthy of further prospective study with levels taken before and after neoadjuvant treatment.

Conclusion

We found preoperative CRP levels to be an independent prognostic marker for survival after neoadjuvant treatment in patients with esophageal cancer. Including preoperative serum CRP measurements in the re-staging process in patients who have undergone neoadjuvant treatment might prove an additional factor helping to select patients who are likely to benefit from surgery.

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ORIGINAL ARTICLE

An Antireflux Anastomosis Following Esophagectomy: A Randomized Controlled Trial

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Abstract
Background Reflux of duodeno-gastric fluid is a significant problem after esophagectomy with gastric conduit reconstruction. Symptoms may be severe and impact considerably upon the quality of life. Previous studies have suggested that a fundoplication type anastomosis may limit post-esophagectomy reflux.
Aim The purpose of this study was to determine whether a modified fundoplication at the gastro-esophageal anastomosis prevents reflux after esophagectomy.
Methods Prospective multicenter randomized controlled trial to compare a conventional end of esophagus to side of gastric conduit anastomosis with a modified fundoplication anastomosis in patients undergoing esophagectomy with intrathoracic anastomosis. Major outcomes were reflux symptoms, symptoms of dysphagia, and complications.
Results Fifty-six patients were enrolled. The fundoplication anastomosis was associated with significantly lower incidence of reflux (40% vs 70%), as well as a reduced incidence of severe reflux (8% vs 30%). Disturbance of sleep due to reflux was significantly reduced in the fundoplication group (18% vs 47%) as was the incidence of respiratory symptoms. The fundoplication anastomosis was not associated with an increase in dysphagia, and there was no difference in complications between the two groups.
Conclusions Fundoplication anastomosis during esophagectomy is effective in protecting patients from reflux symptoms after esophagectomy and improves quality of life, particularly with regard to sleep disturbance.

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Keywords Esophagectomy · Reflux · Esophageal cancer · Fundoplication · Anastomosis

Introduction

Esophagectomy is a major undertaking and can be associated with significant morbidity. When performed for cancer, many patients are not cured, and the quality of remaining life becomes paramount. Well-being after esophagectomy has been shown to correlate with the severity of physical symptoms,[1] and for this reason, refinement of surgical techniques to limit postoperative morbidity is important.

Esophagectomy utilizing a gastric conduit is frequently followed by reflux. Symptoms of reflux have been reported to

occur in up to 60–80% of such patients,[2] and objective tests for reflux suggest that all post-esophagectomy patients reflux when supine.[3] Severe heartburn (often manifested as cervical burning), troublesome belching, and regurgitation may not only be physically disturbing but also interfere with social function. It may be impossible for patients to lie flat, and sleep is often disrupted. Regurgitation of gastric content, particularly at night, introduces the risk of aspiration pneumonia. Furthermore, the pathological consequences of post-esophagectomy reflux are becoming apparent. Although the gastric conduit is vagotomized during esophagectomy, the denervated stomach has been shown to recover acid secretion capacity over 1 –3 years,[4] and bile reflux is also known to occur.[2] A well-conducted longitudinal study of post-esophagectomy patients found that the incidence of esophagitis at 36 months was 35%, and columnar metaplasia, including intestinal (Barrett's) metaplasia was found in 47%.[2]

A variety of strategies have been employed to limit reflux after esophagectomy, but none have been particularly successful.[5] In a previous retrospective study, we demonstrated that a modified fundoplication-type anastomosis is potentially effective,[6] suggesting that further evaluation of this strategy is appropriate. Hence, we conducted a prospective randomized controlled trial to evaluate the efficacy of the fundoplication anastomosis in controlling reflux after esophagectomy with gastric conduit reconstruction.

Methods

Study Design

A prospective muticenter randomized trial was undertaken.

From 2004 to 2007, patients were recruited into the trial from three sites in Australia (The Royal Adelaide Hospital, The Austin Hospital, and Flinders Medical Centre) and one site in the UK (The Royal Hallamshire Hospital). All surgeons involved in the study were familiar with the technique of fundoplication anastomosis as practiced by one of the authors (GGJ).[6] Patients planned to undergo radical esophagectomy with intrathoracic anastomosis were eligible for inclusion in the study. Patients were excluded from the study if an esophagectomy with cervical anastomosis was planned or if the stomach was not the planned conduit.

Power Calculations

Assuming a 50% survival at 12 months, it was estimated that 100 patients (50 in each group) would be required to demonstrate a 40% difference in reflux symptoms at 12 months.

Surgical Technique

A conventional esophagectomy was undertaken via an upper midline abdominal incision for gastric mobilization and conduit formation, and a right-sided thoracotomy for esophageal resection and anastomosis. The anastomosis was performed at or above the level of the azygos vein. The standard anastomosis was either a handsewn end (esophagus) to side (gastric) anastomosis completed in a single layer or circular-stapled (25 mm) end-side anastomosis as per surgeon preference. The technique of the fundoplication has been described in detail in a previous paper.[6] Briefly, the gastric tube is brought up behind the esophageal remnant. Sutures are placed as high as possible at the 3 and 9 o'clock positions of the esophageal remnant and secured to the greater curve of the gastric tube to hold the conduit in position and facilitate a 360° wrap around the esophagus and anastomosis, secured with non-absorbable sutures. If a complete wrap was deemed too difficult at the time of fashioning then a partial wrap was performed. A pyloric drainage procedure was performed in all cases.

Symptom Assessment and Follow-Up

The demographics, operative details, clinical outcome, and complications, in particular anastomotic complications, were recorded prospectively with follow-up at 3, 6, and 12 months after surgery. All patients were interviewed by a single interviewer at each institution. The interviewer was blinded to the type of anastomosis and used a standardized questionnaire.

The severity of postoperative reflux symptoms (heartburn and regurgitation) was scored with analogue scales (0–10), and the frequency of symptoms was recorded using standardized categorical scales. Sleep disturbance and modifications to sleeping arrangements were also recorded. The presence or absence of respiratory symptoms such as nocturnal cough, wheezing, or recurrent chest infections was recorded, as was sleep disturbance due to reflux. The presence of gas bloat, difficulty belching, and increased flatulence was recorded.

Dysphagia was assessed using a previously validated scoring system based on a nine-item graded food scale with no dysphagia scoring 0 and a maximum score of 45[7] as well as a 0–10 analog scale. The need for endoscopic dilation postoperatively was also recorded.

The impact of reflux upon the quality of life of patients was assessed using a simple categorical scale (nil, mild, moderate, severe) which asked patients to rate the disturbance reflux produces in their lives with sleep, eating, socializing, daily activities, and overall. The previously validated EORTC quality-of-life questionnaires (QLQ-C30, esophageal module OES18) for esophageal cancer patients

were also used to examine global as well as symptom specific quality of life.[8]

Ethics and Trial Registration

Ethics approval for the study was gained at each of the study centers by the governing Ethics Committee of the institution. This trial was registered with the Australian and New Zealand Clinical Trials Registry trial No. ACTRN 12605000587606.

Statistics

All data were analyzed on an intention to treat basis. Fisher's exact test was used to test for significance of proportions. Although results may be tabulated as percentages, calculations of Fisher's test were made on raw numbers. The homoscedastic Student's *t* test was used for parametric continuous data and the heteroscedastic Student's *t* test for data of unequal variance.

Means are expressed as mean ± SD.

All results were analyzed on an intention to treat basis.

Results

Demographics

Eighty eligible patients underwent esophagectomy at the three institutions during the recruitment period, and 56 were recruited to the study. The other 24 patients either declined participation (10), had a three-stage procedure (six), or were operated upon by surgeons not involved in the trial (eight). Of the 56 recruited to trial, 29 were randomized to undergo esophagectomy with fundoplication and 27 to undergo a standard anastomosis (without fundoplication). The majority of cases were performed at the Royal Adelaide Hospital (34) with the remainder being at the Royal Hallamshire, Austin Hospital, and Flinders Medical Centre. There was no significant difference between groups with respect to age, sex distribution, weight, smoking history, neoadjuvant and adjuvant therapy, preoperative comorbidty, tumor stage, and proportion of handsewn to stapled anastomoses (Table 1).

There was significant patient attrition through the follow-up phase of the study due either to death (12), disease recurrence (four), or patient choice/exhaustion (12). However, this was evenly distributed across both groups so comparable numbers remained at each follow-up time point (Fig. 1).

Surgical Details

The distribution of handsewn to stapled anastomoses is given in Table 1. Within the fundoplication group, only a partial (180°) fundoplication was possible in seven of the 29 patients.

Table 1 Randomization Outcome

Parameter	Wrap	Standard	Fisher's/*t* test*
Total	29	27	n.s.
Age	65	64	n.s.
Males	91%	74%	n.s.
Weight	75.5	71.6	n.s.
Smoking	10	12	n.s.
Comorbidity	70%	63%	n.s.
Neoadjuvant Rx	34%	33%	n.s.
Stage I/II/III	8:6:13	7:7:10	n.s.
Stapled/sewn	11:18	13:14	n.s.

*$p \leq 0.05$ considered significant

There were no significant differences in operative (30 days) mortality (one patient in each group) or morbidity (41% fundoplication group and 48% standard group). Anastomotic leak occurred in four patients in the fundoplication group (two clinical and two radiological) and in five of the standard group (four clinical and one radiological).

Efficacy of Reflux Control

The mean incidence of reflux symptoms in the fundoplication group and the standard anastomosis group across the 12-month follow-up period was 40% and 70%, respectively ($p = 0.04$ Fisher's exact test). The mean severity scores for heartburn symptoms were not different at 3 months follow-up, but at 6 and 12 months, scores were significantly lower in the fundoplication group (Fig. 2). In contrast, the mean severity scores for regurgitation were significantly different

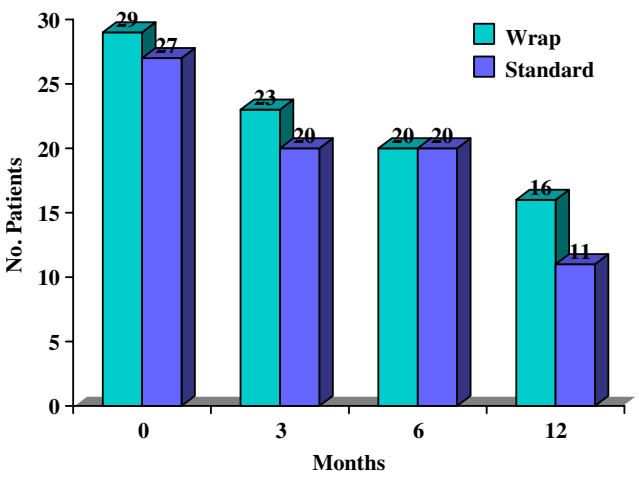

Figure 1 *Numbers* in each cohort at follow-up points; note that three patients who were assessable at 6 months were not assessable at 3 months due to postoperative complications.

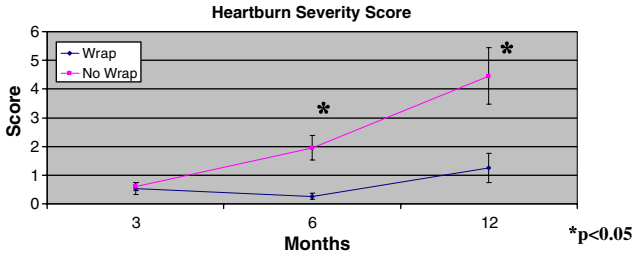

Figure 2 Postoperative mean heartburn severity scores.

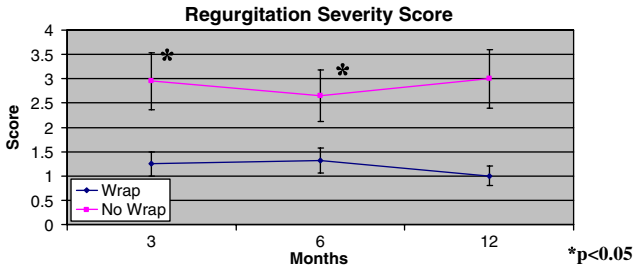

Figure 3 Postoperative mean regurgitation severity score.

at 3 and 6 months and approached significance (p=0.08) at 12 months follow-up (Fig. 3).

There was a trend toward lower heartburn (4.5±3.3 vs 0.2±0.6, p=0.07 at 12 months) and regurgitation (1.8±1.2 vs 0.8±0.3, p=0.2 at 12 months) scores if a total fundoplication had been performed compared to a partial fundoplication, but this did not reach statistical significance. If only total fundoplications are compared to standard anastomoses, with respect to heartburn and regurgitation scores the difference was of greater significance.

The incidence of severe reflux symptoms[1] was less in the fundoplication group compared to the standard group (mean incidence over 12 months 8% vs 30%). It was noticeable that the incidence of severe reflux increased over the 12 months in both groups, and this was due mainly to an increased incidence of severe heartburn type symptoms. However, in the fundoplication group, this was attributable to patients having a partial fundoplication. Only one patient with a total fundoplication had severe reflux at 12 months.

There was a significant reduction in the incidence of sleep disturbance due to reflux in the fundoplication group compared to the standard anastomosis group (Table 2). This impact was rated as moderate or severe in 40% of the standard anastomosis group but only 5% of the fundoplication group (p=0.01, Fisher's exact test).

Atypical symptoms of reflux were also less prevalent in the fundoplication group, in particular cough (25% vs 60%, $p \leq 0.05$, Fisher's exact test).

While there was an increased incidence in the use of proton pump inhibitor medication in the standard anastomosis group (34% vs 14%), this was not statistically significant (p=0.19).

Side Effects and Dysphagia

Dysphagia improved over the 12-month follow-up period in both groups. There was no significant difference in the incidence of dysphagia between the groups (Fig. 4), nor was there a difference in the severity of dysphagia between groups at each follow-up period (Table 3). There was no difference in the incidence of the need for dilation (20% wrap group vs 23% standard group).

Other side effects such as anorexia, bloat, excess flatus, diarrhea/dumping, and early satiety were not different between groups.

Quality of Life

The EORTC QLQ30 and OES 18 modules were used to assess postoperative quality of life. There was no significant difference in baseline quality of life scores preoperatively between the groups. Global and physical function scores were reduced in both groups compared to the preoperative state.

There was no significant difference in global quality of life and physical functioning scores between the two groups postoperatively except in relation to Insomnia score at 6 months follow-up which was lower in the fundoplication group (10±7 vs 42±12, p=0.04).

Similarly, esophageal symptom-specific scores were significantly lower with respect reflux (9.5±6.1 vs 36.1±13.2) and cough (13.3±6.0 vs 30.5±10) at 12 months but otherwise not significantly different.

Discussion

Our study has confirmed that with a standard end-side esophago-gastric anastomosis, classic reflux symptoms are very common (70%), and the fundoplication anastomosis protected many patients from reflux, with many fewer (40%) being symptomatic.

The severity of reflux was also reduced following fundoplication anastomosis. It is notable that at 3 months, heartburn severity was not different between the two study groups but was significantly lower in the fundoplication

Table 2 Incidence of Sleep Disturbance Due To Reflux

Months	Wrap	Standard	Fisher's exact test
3	17%	50%	0.02
6	15%	65%	0.01
12	25%	82%	0.005

[1] Severe reflux symptoms defined as heartburn or regurgitation analog severity score ≥5 and frequency weekly or greater.

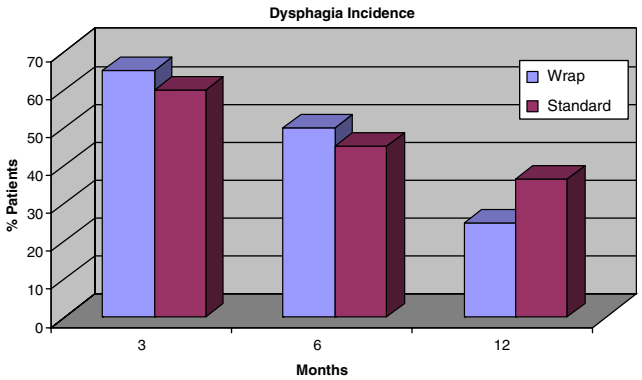

Figure 4 Incidence of postoperative dysphagia; there is no significant difference between groups.

group at 6 and 12 months. This might relate to an initial reduction in acid secretion due to vagotomy associated with resection of the esophagus but subsequent recovery of acid secretion thereafter.[4] In contrast, regurgitation scores remained relatively constant and significantly lower in the fundoplication group throughout the 12-month follow-up. This is probably because regurgitation is volume rather than acid dependent.

Of particular importance clinically is that the incidence of *severe reflux symptoms* was much reduced in the fundoplication group. In this regard, we noted that it seemed that only a total fundoplication was effective. Patients having a partial fundoplication did not seem to be protected against severe reflux particularly at 12 months. Technical issues probably drove the individual surgeon's choice to undertake a partial rather than a total fundoplication. Unfortunately, the study lacked sufficient numbers for subgroup analysis of partial and total fundoplication to determine if these patterns were statistically significant. In contrast to our observations, a small randomized trial of a partial fundoplication technique by Lundell et al. assessed reflux objectively by endoscopy and pH monitoring and found that the fundoplication anastomosis controlled reflux well.[9]

Atypical symptoms of reflux were also common. Nocturnal cough and sleep disturbance were notable, and again, the fundoplication anastomosis significantly reduced these symptoms. As a measure of the impact of reflux on quality of life, the disturbance of sleep was important. The impact of reflux symptoms on sleep was frequently rated as moderate or severe by patients with a standard anastomosis

Table 3 Dysphagia Severity Scores (Mean±SD)

Time (months)	Wrap	No wrap	*p* Value (*t* test)
3	2.9±2.7	2.8±3.1	0.85
6	1.5±2.4	1.5±2.3	0.94
12	0.4±0.8	1.6±3.1	0.19

but was uncommonly so in patients with a fundoplication anastomosis. This was mirrored in the EORTC quality of life questionnaire scores.

Importantly, the benefits seen in reflux control did not come at the expense of increased side effects. Standard fundoplication for reflux may induce side effects such as dysphagia, bloat, and early satiety. In the esophagectomy setting, however, such symptoms are frequent even with a standard anastomosis, and symptoms were not different in the fundoplication group.

The use of fundoplication in the esophagectomy setting has been sparsely reported previously and never in a randomized trial. Historical series by Butterfield[10] and Boyd et al.[11] describe the use of fundoplication after palliative resections of the lower esophagus, while more recently, Velanovich et al. describe a "split stomach" fundoplication after esophagectomy and demonstrate improved reflux control (20% vs 60%) with reduced anastomotic leak (0% vs 17%) compared to a nonrandomized cohort.[12] Although nonrandomized, these data support our findings that a fundoplication anastomosis can be effective in controlling post-esophagectomy reflux. Russell et al. also describe using a fundoplication type anastomosis reporting possible protection against anastomotic leak (0.4%).[13] We cannot comment on such a role from our study as the numbers are too small.

While the results of this study are encouraging, and the first of a randomized nature, there are some specific weaknesses. Unfortunately, the rate of recruitment for this study was overestimated in the original study timeline, and we were only able to recruit 56 patients, less than the initial target of 100. Nonetheless, with the numbers available, important clinical differences between the groups were found. The study documented subjective rather than objective measures of reflux control. We found that the majority of patients were reluctant to undergo objective reflux assessment (e.g., pH/bilitec) monitoring after esophagectomy, and we did not pursue this. However, it is the *symptoms* of reflux that are most relevant when considering quality of life in post-esophagectomy patients, and this study assessed clear endpoints in this regard; these outcomes provide important results that inform clinical practice.

It is important to consider other surgical factors that might contribute to postoperative reflux. The height at which the anastomosis is fashioned has long been considered important. An anastomosis below the level of the aortic arch was thought to be "refluxogenic" while one at the supra-aortic level less so.[14] The physiological argument for this is that with a lower anastomosis, more stomach is subject to positive intra-abdominal pressure thus promoting greater reflux.[15,16] In all our patients, anastomoses were at a supra-aortic level; yet, reflux was very common in patients with a standard anastomosis. McKeown[17] proposed that an anastomosis at the neck prevented reflux altogether.

However, reflux in patients with a cervical anastomosis has been documented by pH monitoring[18] and radionucleotide scans. The presence of a pyloric drainage procedure has an uncertain effect on post-esophagectomy reflux. On the one hand, it may facilitate gastric emptying and, thus, reduce gastro-esophageal reflux. On the other, it may promote duodenal reflux and in turn bile reflux into the esophagus. While many studies have addressed the role of pyloric drainage in terms of gastric conduit functioning,[19] few have specifically examined the effect on reflux, though Kobayashi et al.[20] noted a significant reduction in regurgitation and reflux symptoms when a drainage procedure is used. In our study, all patients had a pyloric drainage procedure. Thus, the reduction in reflux in the fundoplication group appears to be a direct effect of fundoplication.

A number of surgical techniques attempting to control reflux after esophagectomy have been reported[5] including intercostal muscle grafts to act as anti-reflux valves,[21] tunneling the esophagus through the muscular layer of the stomach[22,23] "inkwelling"[24,25] or creating a "globe" posteriorly invaginated anastomosis.[26] None have been subjected to a randomized controlled study and have historically been described in the setting of limited or palliative resections offering a substantial esophageal and or gastric remnant—something not available after modern radical resection for cancer.[27] The use of colonic interposition after esophageal resection eliminates gastro-duodenal secretions and, therefore, reflux. Its routine use has its proponents;[28] however, for most surgeons, a colonic conduit represents a major departure from their standard technique.

Conclusion

This study provides data from a randomized controlled trial which suggests that a fundoplication anastomosis offers good control of post-esophagectomy reflux. It has the advantage of being very simple to perform, requiring no major alteration to surgical technique. It may protect the patient from disabling symptoms and improve quality of life with respect to sleep disturbance from nocturnal reflux.

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ORIGINAL ARTICLE

Delay in Diagnostic Workup and Treatment of Esophageal Cancer

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Abstract
Introduction Esophageal cancer should preferably be detected and treated at an early stage, but this may be prohibited by late onset of symptoms and delays in referral, diagnostic workup, and treatment. The aim of this study was to investigate the impact of these delays on outcome in patients with esophageal cancer.
Methods For 491 patients undergoing esophagectomy for cancer between 1991 and 2007, patients' short- and long-term outcome were analyzed according to different time intervals between onset of symptoms, diagnosis, and surgical treatment.
Results Length of prehospital delay (from onset of symptoms until endoscopic diagnosis) did not affect patient's short- or long-term outcome. A shorter hospital delay between establishing the diagnosis of esophageal cancer on endoscopy and surgery was associated with lower overall morbidity and in-hospital mortality. Patients of ASA classes I and II experienced a shorter hospital delay than patients of ASA classes III and IV. Length of hospital delay between endoscopic diagnosis and surgery did not affect pathological tumor–node–metastasis stage or R0-resection rate. Longer hospital delay did not result in worse survival: Overall survival after esophagectomy for cancer was not significantly different between patients with hospital delay <5, 5–8, or >8 weeks (24.7%, 21.7%, and 32.3%, respectively; $p=0.12$).
Conclusion A longer hospital delay (between endoscopic diagnosis and surgery) resulted in worse patient's short-term outcome (higher overall morbidity and mortality rates) but not in a worse long-term outcome (overall survival). This may be explained by a more time-consuming diagnostic workup in patients with a poorer physical status and not by tumor progression.

Keywords Esophageal cancer · Diagnostic workup · Delay · Waiting list · Survival

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Introduction

The 5-year survival rate for esophageal cancer patients after esophagectomy with curative intent has improved up to 40%.[1–3] As further improvement in survival from a single modality approach, such as surgery, is unlikely, considerable interest has grown in other strategies that may improve patients' survival (neoadjuvant chemo- and/or radiotherapy in particular). In many types of cancer, the prognosis of patients with small, localized tumors is better than with locally advanced or metastatic disease. Similar to other malignancies such as colorectal and breast cancer, the outcome of esophageal cancer is related to the pathological tumor–node–metastasis (pTNM) stage of the disease.[2,4,5] Therefore, detection and treatment of esophageal cancer at an early stage could also improve long-term survival.

Early detection of esophageal cancer may be prohibited not only by the late onset of symptoms but also by delays in referral to an appropriate specialist, establishment of the diagnosis, further diagnostic workup, and start of treatment. However, the impact of these delays on both short- and long-term outcome for patients undergoing esophagectomy for cancer is unclear.

In patients with breast cancer, delays of 3–6 months between the onset of symptoms and start of treatment are associated with lower survival, caused by a more advanced tumor stage.[6] In two systematic reviews, no association was found between diagnostic and therapeutic delay and survival in colorectal cancer patients[7] nor between these delays and disease stage.[8] A few studies have investigated the impact of delays in diagnosis and treatment of esophageal cancer. Drawbacks of these studies are small numbers of patients included,[9] analyses that do not cover the complete track between onset of symptoms and surgical treatment,[10,11] combined patient groups with gastric and esophageal carcinoma,[12,13] and studies lacking survival analyses.[9,11–13]

We hypothesized that longer delays between onset of symptoms, endoscopic diagnosis, and surgical treatment are associated with a worse short-term outcome (morbidity, reoperation rate, and in-hospital mortality), worse tumor stage, and hence, worse long-term outcome (overall survival) following potentially curative esophagectomy in patients with esophageal cancer.

Patients and Methods

The Erasmus Medical Center in Rotterdam is a tertiary referral center for patients with esophageal cancer in The Netherlands. Most patients are referred to the Erasmus MC outpatient clinic for (surgical) treatment after the diagnosis of esophageal cancer has been established in a referring hospital (group A). The minority of patients is directly referred by the general practitioner (GP) to the Erasmus MC for clinical investigations of symptoms suggestive of cancer (group B). In all patients (groups A and B) upper gastrointestinal endoscopy with biopsy is (re)done in the Erasmus MC to confirm the diagnosis of esophageal cancer and to determine the exact location of the tumor. Staging is performed routinely with endoscopic ultrasonography, CT scanning of thorax and abdomen, and external ultrasound of the neck. Every patient is discussed in a weekly multidisciplinary oncology meeting in which a definitive treatment plan is designed. If eligible for surgery, patients are put on the waiting list for surgery. On the same day, the patient is referred to the Department of Anesthesiology for preoperative counseling. If needed, additional cardiac and/or pulmonary function tests are scheduled.

Between January 1991 and December 2007, 791 patients underwent esophagectomy for cancer of the esophagus or gastroesophageal junction in the Erasmus MC. To obtain a homogeneous cohort of patients in terms of treatment and to circumvent possible stage migration following chemo- and/or radiotherapy, patients receiving (neo)adjuvant therapy were excluded from this analysis. In our hospital, patients received neoadjuvant chemo(radio)therapy in the context of randomized controlled trials.[14,15] Induction chemo- and/or radiotherapy was given in patients with either a cT4 tumor without distant metastases or in patients with gross involvement of celiac trunk lymph nodes (M1a), who were not considered eligible for primary surgical therapy. There were 214 patients who were excluded because of chemo- and/or radiotherapy prior to surgery. In 44 patients, the hospital delay from endoscopic diagnosis to surgery could not be calculated, as the date of their first upper gastrointestinal endoscopy performed in the referring hospital was unknown. Another 42 patients were excluded, as they participated in a Barrett's esophagus surveillance program. Over recent years, multiple attempts for endoscopic treatment of early lesions delayed referral to the Department of Surgery in such way that this group was not representative for patients treated for (more advanced) esophageal cancer. Finally, data of 491 patients were analyzed in the present study. The vast majority of these patients underwent a transhiatal esophagectomy with locoregional lymphadenectomy only (N=477). In 14 patients, a transthoracic resection with extended lymphadenectomy was performed. The applied surgical techniques have been described previously.[3,16] Tumors were assigned pTNM stages according to the Union Internationale Contre le Cancer 2002 system.[17]

Data on patients' demographics, diagnostic tests, surgery, postoperative morbidity, in-hospital mortality, and survival have been collected prospectively and stored in a database by a data manager. From this database, the following time points were defined:

- Date of upper gastrointestinal endoscopy in the referring hospital, on which the diagnosis of esophageal cancer had been established by histology from biopsies (only applicable for group A)
- Date of first visit at the Erasmus MC outpatient clinic: Department of Surgery, Gastroenterology, or Medical Oncology
- Date of upper gastrointestinal endoscopy in the Erasmus MC, on which the diagnosis of esophageal cancer had been established by histology from biopsies
- Date of the multidisciplinary oncology meeting, after which the patient had been put on the operative waiting list if eligible for surgery
- Date of surgery.

To summarize all different time points that have been marked in the process between onset of symptoms and surgery, we divided this time span into two major time intervals that have been analyzed separately: pre-hospital and hospital delay (see Fig. 1). Subsequently, data were analyzed in three different ways:

- Impact of prehospital delay: time from onset of symptoms until diagnosis on first endoscopy (either in the referring hospital for group A or in Erasmus MC for group B)
- Impact of hospital-delay: time from diagnosis on patient's first endoscopy undertaken until surgery
- Impact of specific time intervals between diagnosis on first endoscopy and surgery. In order to examine the hospital-delay in more detail, the effect of specific time intervals between diagnosis in the referring hospital, first visit at the outpatient clinic in Erasmus MC, diagnosis on endoscopy in Erasmus MC, multidisciplinary oncology meeting, and surgery on short- and long-term outcome were analyzed. For this purpose, only data from patients in group A were used.

Statistics

Follow-up was recorded until December 2008 or until death if earlier and was complete for all patients. Statistical analysis for non-parametric data was used. Grouped data were compared using the chi-square, Mann–Whitney *U*, or Kruskall–Wallis *H* test. Patients who died due to complications following esophagectomy (in-hospital mortality) were not excluded from survival analysis. Overall survival was calculated from the date of operation until the date of last follow-up or death according to the Kaplan–Meier method. Disease-free survival was assessed from the date of operation until the date of disease recurrence in case of locoregional recurrence or distant metastases. Univariate analyses were performed with the log-rank test to identify prognostic variables associated with overall survival after esophagectomy. Data analyses were carried out with SPSS version 15.0 (SPSS, Chicago, IL, USA).

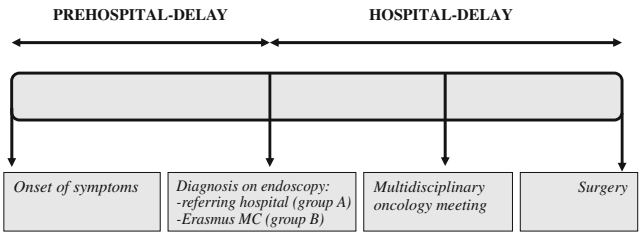


Figure 1 Analysis of prehospital and hospital delays encountered by patients who underwent surgical resection for esophageal cancer in Erasmus MC.

Table 1 Clinicopathological Characteristics of 491 Patients who Underwent Surgical Resection for Esophageal Cancer and Who Were Included in the Present Study

Age (in years)[a]	65 (28–89)
Gender	
Male	399 (81.3%)
Female	92 (18.7%)
ASA classification	
I	77 (15.7%)
II	316 (64.4%)
III	96 (19.6%)
IV	2 (0.4%)
Tumor location	
Proximal esophagus	8 (1.6%)
Mid esophagus	27 (5.5%)
Distal esophagus	196 (39.9%)
Gastroesophageal junction	260 (53.0%)
Histology	
Squamous cell carcinoma	73 (14.9%)
Adenocarcinoma	418 (85.1%)

ASA classification American Society of Anesthesiologists classification

[a] Age is given as median (range)

Results

Patients' characteristics are shown in Table 1. Three hundred sixty-five patients (74.3%), in whom the diagnosis esophageal cancer was established in another hospital, were referred to the Erasmus MC for further staging and treatment (group A). One hundred twenty-six patients (25.7%) were referred directly to the Erasmus MC by the general practitioner for investigation of symptoms suggestive of esophageal cancer (group B). Patients' first visit to the Erasmus MC was at the Department of Surgery (*N*=338, 68.8%), Department of Gastroenterology (*N*=147, 29.9%), or Department of Medical Oncology (*N*=6, 1.3%).

Impact of Prehospital Delay: Time from Onset of Symptoms Until First Endoscopy

The majority of patients underwent endoscopy for investigation of obstructive symptoms suggestive of cancer like dysphagia, odynophagia, and weight loss (*N*=462, 94.1%). Other indications for endoscopy encompassed investigation of hematemesis (*N*=12, 2.4%), anemia (*N*=9, 1.8%), or melena (*N*=8, 1.6%). Prehospital delay (from onset of symptoms until first endoscopy) lasted a median time period of 3.0 months (range, 0–36 months). Patient's short-term (morbidity, reoperation rate, and in-hospital mortality) and long-term outcome (overall 5-year survival) after esophagectomy were comparable for patients who experienced

Table 2 Impact of Prehospital Delay from Onset of Symptoms to First Endoscopy on Short- and Long-Term Outcome After Esophagectomy; Comparison of Prehospital Delay ≤3 Months (N=308) Versus >3 Months (N=183)

	Prehospital delay ≤3months, N=308	Prehospital delay >3months, N=183	p value
Morbidity	199 (64.6%)	104 (56.8%)	0.09
Reoperation	34 (11.0%)	16 (8.7%)	0.42
In-hospital mortality	18 (5.8%)	9 (4.9%)	0.66
Overall 5-year survival	24.0%	29.3%	0.10

symptoms for a period of 3 months or less versus more than 3 months until endoscopy was performed (Table 2).

Impact of Hospital Delay: Time from Endoscopic Diagnosis Until Surgery

The hospital delay from establishing the diagnosis of esophageal cancer on endoscopy (either in the referring hospital for group A or in Erasmus MC for group B) until surgery was 49 days (given as median, range of 5–175 days). This delay encompassed a median time period of 28 days (range, 0–147 days) from diagnosis on patient's first endoscopy until the multidisciplinary oncology meeting (staging delay), and a median time period of 15 days from this meeting until surgery (operative waiting list, range of 1–67 days). Median hospital delay between diagnosis and surgery increased during the study period (1991–2007): 3.9 weeks in 1991 toward 10.9 weeks in 2007 (Fig. 2). This increase in hospital delay should rather be ascribed to the 3.4 times increase in length of the operative waiting list (1.6 weeks in 1991 towards 5.6 weeks in 2007) than to the 1.5 times increase in staging delay (3.3 weeks in 1991 towards 4.9 weeks in 2007).

A shorter hospital delay between establishing the diagnosis of esophageal cancer on patient's first endoscopy and surgery was associated with significantly lower overall morbidity and mortality (Table 3). These associations

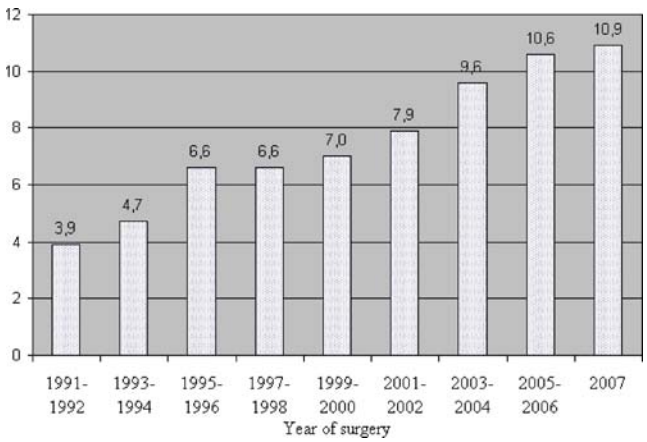


Figure 2 Median hospital delay (in weeks) between endoscopic diagnosis and surgery increased during the study period (1991–2007): 3.9 weeks in 1991 toward 10.9 weeks in 2007.

appeared to be linear: morbidity (p=0.001) and in-hospital mortality (p=0.01) increased with longer hospital delay. Patients of ASA classes I and II experienced a shorter hospital delay than patients of ASA classes III and IV (hospital delay <5 weeks, 28.8%; 5–8 weeks, 36.9%; and >8 weeks, 34.4% versus <5 weeks, 15.3%; 5–8 weeks, 41.8%; and > 8 weeks, 42.9%, respectively; p=0.02). Length of hospital delay did not affect pTNM stage or R0-resection rate (Table 3).

Longer hospital delay did not result in worse survival (Fig. 3): Overall 5-year survival was 24.7% in patients with a hospital delay less than 5 weeks, 21.7% in patients with a hospital delay between 5 and 8 weeks and 32.3% in patients in whom the hospital delay was more than 8 weeks. Although overall survival appeared to be longer in patients with a longer hospital delay, this difference was not statistically significant (p=0.12). Parameters found to be associated with overall survival in univariate analyses are shown in Table 4: age younger than 65 years, early pT stage (pT1 or pT2), no lymph node involvement (pN0), absence of distant metastatic disease (pM0), good differentiation grade of the tumor, R0 resection, and lymph node ratio smaller than 0.24 were favorable of improved overall survival. Survival analysis with regard to 5-year disease-free survival paralleled the overall 5-year survival curves (27.0%, 27.7%, and 38.3%, respectively; p=0.09).

Impact of Specific Time Intervals Between Endoscopic Diagnosis and Surgery (Group A)

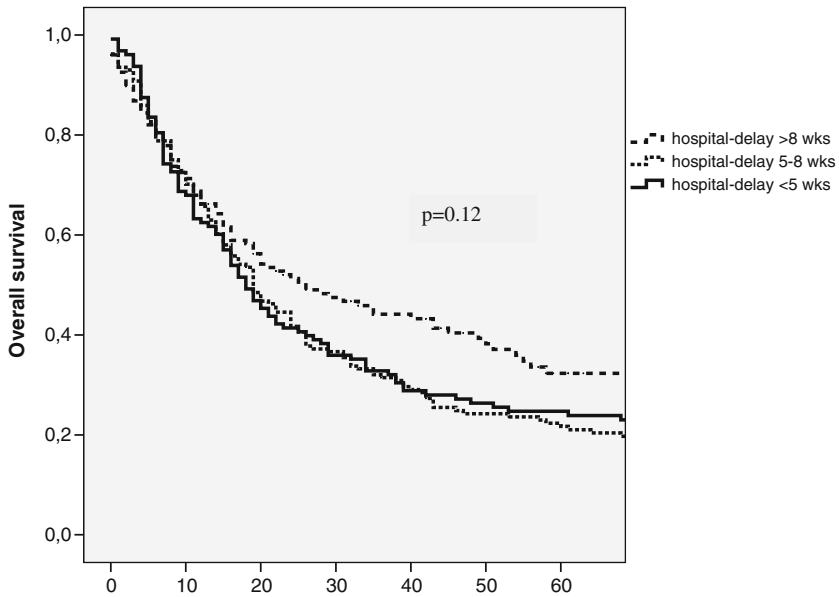
The median hospital delay was 53 days (range, 5–175 days) for patients in group A in whom the diagnosis esophageal cancer had been established in another hospital and who were referred to the Erasmus MC for surgical treatment (N=365). The breakdown of this delay is shown in Table 5, according to the different time intervals between diagnosis in the referring hospital, first visit to the outpatient clinic in Erasmus MC, diagnosis on endoscopy in Erasmus MC, multidisciplinary oncology meeting, and surgery.

When analyzing the impact of the separate time intervals, it appeared that the delay between the multidisciplinary oncology meeting and surgery (median, 15 days; reflecting the length of the operative waiting list) was the only time interval that influenced short-term outcome post-

Table 3 Impact of the Hospital Delay from Diagnosis on Patient's First Endoscopy Until Surgery: Hospital Delay <5 Weeks (N=128), 5–8 Weeks (N=186), and >8 Weeks (N=177)

	Delay <5weeks, N=128	Delay 5–8weeks, N=186	Delay >8weeks, N=177	p value
Morbidity	62 (48.4%)	122 (65.6%)	119 (67.2%)	<0.01
In-hospital mortality	2 (1.6%)	10 (5.4%)	15 (8.5%)	0.03
Reoperation	7 (5.5%)	20 (10.8%)	23 (13.0%)	0.10
pT stage				
pT1–pT2	30 (23.4%)	57 (30.6%)	54 (30.5%)	0.31
pT3–pT4	98 (76.6%)	129 (69.4%)	123 (69.5%)	
pN stage				
pN0	42 (32.8%)	66 (35.5%)	62 (35.0%)	0.88
pN1	86 (67.2%)	120 (64.5%)	115 (65.0%)	
pM stage				
pM0	103 (80.5%)	150 (80.6%)	131 (74.0%)	0.24
pM1a–M1b	25 (19.5%)	36 (19.4%)	46 (26.0%)	
Radicality of resection				
R0	86 (67.2%)	124 (66.7%)	130 (73.4%)	0.32
R1–R2	42 (32.8%)	62 (33.3%)	47 (26.6%)	

esophagectomy. Although in-hospital mortality was comparable between patients who had been on the waiting list for 15 days or shorter versus patients who were waiting for more than 15 days (p=0.14), length of the operative waiting list did influence morbidity (55.7% versus 67.1%, p=0.03), and a trend towards an increased reoperation rate could be noted (7.8% versus 13.9%, p=0.06). However, in contrast with the hospital delay between endoscopic diagnosis and surgery, none of the separate time intervals affected long-term survival.



No. at risk	0 months	12 months	24 months	36 months	48 months	60 months
> 8 weeks	177	107	68	48	38	24
5 – 8 weeks	186	122	78	54	39	34
< 5 weeks	128	80	53	42	32	30

Figure 3 Overall 5-year survival for esophageal cancer patients appeared longer for patients with a hospital delay between diagnosis on first endoscopy and surgery >8 weeks (N=177) versus patients with a hospital delay <5 weeks (N=128) or 5–8 weeks (N=186), although this difference did not reach statistical significance (p=0.12).

Table 4 Univariate Analyses of Potential Prognostic Variables Associated with Overall Survival After Esophagectomy for Cancer (N=491)

Variable	Five-year survival (%)	p value
Age		
≤65 years	30.2	0.001
>65 years	21.4	
Sex		
Male	25.4	0.84
Female	28.5	
ASA classification		
I–II	27.0	0.12
III–IV	22.2	
pT stage		
pT1–T2	53.3	<0.001
pT3–T4	15.0	
pN stage		
pN0	50.3	<0.001
pN1	12.2	
pM stage		
pM0	39.8	<0.001
pM1a–M1b	9.5	
Histology		
Squamous cell carcinoma	27.1	0.98
Adenocarcinoma	25.8	
Differentiation grade of tumor		
Good	69.1	
Moderate	29.5	<0.001
Poor	16.0	
Radicality of resection		
R0	35.5	<0.001
R1–R2	5.5	
Lymph node ratio		
≤0.24	36.0	<0.001
>0.24	12.0	
Referral		
By another hospital (group A)	25.9	0.65
By GP (group B)	26.2	
Prehospital delay		
≤3 months	24.0	0.10
>3 months	29.3	
Hospital delay		
<5 weeks	24.7	
5–8 weeks	21.7	0.12
>8 weeks	32.3	

ASA classification American Society of Anesthesiologists classification, *GP* general practitioner

Discussion

When initiating the current study, we hypothesized that longer delays between onset of symptoms, diagnosis, and surgical treatment are associated with worse short-term outcome (in terms of morbidity, reoperation rate, and mortality) and worse long-term outcome (overall survival) following esophagectomy for cancer. In the present series, it appeared that length of prehospital delay (from onset of symptoms until endoscopic diagnosis) did not influence patient's short-term outcome or overall 5-year survival. Onset of symptoms is a subjective measurement, and it may be that patients are not able to recall the exact moment that they first experienced discomfort. Furthermore, although little information is known about the tumor doubling time of esophageal cancer, the period of time in which a patient is symptomatic may be relatively short when compared to the total period between the first presence of malignant cells in the esophagus and the diagnosis of esophageal cancer. Unfortunately, we did not have information on delays caused by the GP (i.e., time between onset of symptoms and referral for endoscopy). Nevertheless, we do want to emphasize the importance of both patient and primary care education that will result in earlier notification of alarming symptoms such as dysphagia and weight loss.

A longer hospital delay from endoscopic diagnosis until surgery was associated with higher overall morbidity and mortality. This could be explained by a more thorough and time-consuming diagnostic workup in patients with a poorer physical status. Indeed, in the present study, patients of ASA classes I and II experienced a shorter hospital delay than patients of ASA classes III and IV. Alternatively, a longer delay prior to surgery may also have caused a worse physical status in esophageal cancer patients by means of malnutrition. However, this remains speculative, as our database did not provide detailed information with regard to patients' preoperative nutritional status (e.g., nutritional risk indices). When analyzing the impact of the separate time intervals between patient's first endoscopy and surgery, it appeared that the length of the operative waiting list was the time interval that influenced short-term outcome following esophagectomy the most. From the literature, it

Table 5 Delays Encountered by Esophageal Cancer Patients who have been Referred from an Other Hospital to the Erasmus MC for Surgical Treatment (group A, N=365)

Diagnosis on endoscopy elsewhere→ first visit outpatient clinic Erasmus MC	17	days (1–138)
First visit outpatient clinic Erasmus MC→ diagnosis on endoscopy Erasmus MC	6	days (0–36)
Diagnosis on endoscopy Erasmus MC→ multidisciplinary oncology meeting	7	days (0–95)
Multidisciplinary oncology meeting→surgery	15	days (1–67)
Total hospital delay		
Diagnosis on endoscopy elsewhere→surgery	53	days (5–175)

Lengths of delays are given as a median values with the corresponding range in brackets

is also known that the quality of life in newly diagnosed esophageal cancer patients who are waiting for surgery is seriously impaired.[18] Hence, it should be aimed for to keep this time interval to a minimum.

Our second hypothesis was that patients with longer delays would generally present with more advanced disease and that this relation between delay and stage would result in a poorer survival. However, pTNM stages were comparable in patients with a hospital delay <5, 5–8, or >8 weeks between endoscopy and surgery. Surprisingly, it appeared that overall survival was improved in patients with a longer hospital delay, although this difference was not statistically significant. This is in line with the results of Kötz et al.[10] who showed that a longer delay between diagnosis and surgical resection was associated with improved survival in esophageal cancer patients. However, the delay between diagnosis and surgery was not an independent prognostic variable on multivariate analysis in their study. Kötz et al.[10] noted that patients with a longer delay had a higher rate of complete tumor resection, suggesting that they were more appropriately selected for surgical treatment. In our series, we could not find evidence that patients were selected more appropriately, as both pTNM stage and R0-resection rate did not differ between patients with a shorter or longer hospital delay. However, hospital delay substantially increased especially over the last few years in our hospital (Fig. 2). This can probably explain the counter-intuitive correlation between longer hospital delay and improved long-term survival, which is rather reflecting state-of-the-art staging modalities, refined surgical techniques, and improved intensive care that have been introduced over the past years. Theoretically, it could also be possible that, in our hospital, patients did not undergo surgery anymore after a longer hospital delay in case the tumor progressed to a stage that was considered irresectable. However, in our patient group, the increased hospital delay can rather be ascribed to an increase in length of the operative waiting list than to an increased staging delay. As the decision on whether to operate or not has been made during the multidisciplinary oncology meeting, it is unlikely that a longer hospital delay led to a dropout of patients with irresectable tumors and, hence, a more selected patient group that underwent esophagectomy.

It is evident that efforts are taken to minimize delays experienced by patients with esophageal cancer between onset of symptoms, diagnosis, and surgical treatment. The National Health Service cancer plan was implemented in 2000 in the UK, indicating that all patients with relevant symptoms and suspected cancer should be able to see a specialist within 2 weeks of their GP referral. The introduction of these guidelines was associated with reductions in times to first outpatient visit, endoscopy, and diagnosis in patients with upper gastrointestinal cancer (esophageal or gastric).[19,20] However, the effectiveness of the NHS cancer plan is uncertain, as it can be questioned whether the slightly improved survival rates after 2000 can be ascribed to this plan.[21]

In our hospital, we recently introduced a new schedule of diagnostic services for patients with suspected esophageal cancer. It is attempted to see patients at the outpatient clinic of the Department of Surgery or Department of Gastroenterology within 1 week after referral. Furthermore, patients are offered all imaging modalities in 1 week, including upper gastrointestinal endoscopy, endoscopic ultrasonography, CT scanning of thorax and abdomen, and external ultrasound of the neck. The aim of this schedule is to minimize the delay between referral to our hospital and establishment of a definitive treatment plan for each individual patient.

In conclusion, length of prehospital delay (from onset of symptoms until diagnosis) did not affect patient's short- or long-term outcome. A longer hospital delay (between endoscopic diagnosis and surgery) resulted in worse patient's short-term outcome (higher overall morbidity and mortality rates) but not in worse long-term outcome (overall survival). This may be explained by a more time-consuming diagnostic workup in patients with a poorer physical status and not by tumor progression.

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ORIGINAL ARTICLE

Subclassification of Stage IV Gastric Cancer (IVa, IVb, and IVc) and Prognostic Significance of Substages

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Abstract
Background Although the prognosis of stage IV gastric cancer is poor, some patients with stage IV gastric cancer had a long-term survival after gastrectomy. The objective of this study was to subclassify stage IV gastric cancer according to survival differences, evaluate the prognosis by substage, and identify the factors associated with patient survival in each substage.
Methods The data from 1,176 patients who underwent gastric resection for stage IV gastric cancer between 1988 and 2007 at Tumor Hospital of Harbin Medical University were reviewed retrospectively. The patients were divided into three substages according to the survival differences: stage IVa (T1–2N3M0), stage IVb (T3N3M0 and T4N1–2M0), and stage IVc (T4N3M0 and TanyNanyM1). The clinicopathological characteristics as well as survival of the patients were evaluated retrospectively by substage.
Results There were no significant differences in survival among T3N3M0, T4N1M0, and T4N2M0 groups (p=0.884) and between T4N3M0 and TanyNanyM1 groups (p=0.192). The 5-year survival rates in stage IVa (T1–2N3M0), stage IVb (T3N3M0 and T4N1–2M0), and stage IVc (T4N3M0 and TanyNanyM1) were 22.7%, 9.9%, and 2.2%, respectively (p<0.001). Multivariate analysis showed the following independent prognostic factors for survival: subclassification, operation type, number of retrieved lymph nodes, curability, and chemotherapy for stage IV gastric cancer; curability, chemotherapy, and number of retrieved lymph nodes for stage IVa and IVb; chemotherapy and operation type for stage IVc. For 406 patients with curative resection in stage IVa and IVb, hematogenous recurrence (35.9%) was the dominant recurrence pattern in stage IVa, whereas the most common patterns of recurrence were peritoneal (40.8%) and locoregional recurrence (31.8%) in stage IVb.
Conclusions Subclassification of stage IV gastric cancer into IVa (T1–2N3M0), IVb (T3N3M0 and T4N1–2M0), and IVc (T4N3M0, TanyNanyM1) may be helpful to predict the outcome and determine the therapeutic strategies for patients with stage IV gastric cancer.

Keywords Stage IV · Gastric cancer · Subclassification

Introduction

Although results of treatment for gastric cancer have been improving with advance in diagnostic techniques and treatment methods, the prognosis of stage IV gastric cancer is still poor.[1,2] Stage IV gastric cancer includes seven groups: T1N3M0, T2N3M0, T3N3M0, T4N1M0, T4N2M0, T4N3M0, and T(any)N(any)M1 by the International Union Against Cancer classification.[3] The 5-year survival rate of stage IV gastric cancer is about 10%. However, the prognosis of stage IV gastric cancer can vary depending on the extent of disease and the potential for cure.[4,5] Some patients with stage IV tumors had a long-term survival after gastrectomy.[6,7] Therefore, a detailed classification of stage IV gastric cancer may be useful for more accurate evaluation of prognosis and more appropriate selection of therapeutic strategies.

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Some studies have demonstrated that subclassification of stage IV gastric cancer into stage IVa (T1–3N3M0) and stage IVb (T4N3M0 and M1) is an independent prognostic factor.[8,9] Other authors suggest that stage IV gastric cancer should be divided into three substages: stage IVA (T4N1–3M0), stage IVB (T1–3N3M0), and stage IVM (TanyNanyM1) for a more accurate prediction of prognosis and selection of appropriate treatment.[10] However, there may be survival differences between each group in the same substage, and the prognostic evaluation and therapeutic options may be inaccurate. Therefore, stage IV gastric cancer should be subclassified according to the survival differences among the seven groups.

In this study, we compared overall survival among each group in stage IV gastric cancer and separated stage IV gastric cancer into three substages according to the survival differences; we evaluated the prognosis of these substages and determined the factors associated with patient survival of each substage.

Patients and Methods

From January 1988 to December 2007, 4,875 patients with gastric cancer underwent gastrectomies in the Department of Gastroenterology at Tumor Hospital of Harbin Medical University. All patients were histologically confirmed gastric adenocarcinoma. Patients who had undergone previous gastric surgery or neoadjuvant chemotherapy were excluded. Among the 4,875 patients, 1,192 were diagnosed as stage IV gastric cancer, according to the sixth edition of the UICC TNM classification.[3] Of these, 16 patients who died of complications or other diseases within 30 days after surgery were excluded. Therefore, 1,176 patients with stage IV gastric cancer were enrolled in this study and separated into the following three substages according to the survival differences: stage IVa (T1–2N3M0), stage IVb (T3N3M0 and T4N1–2M0), stage IVc (T4N3M0 and TanyNanyM1).

The clinical and pathological features for each substage, including sex, age, tumor location, tumor size, type of operation, gross appearance, histological type, chemotherapy, the extent of lymph node dissection, number of retrieved and metastatic lymph nodes, recurrence, and survival, were analyzed on the basis of information in the medical records. All clinicopathologic variables were classified according to the Japanese Classification of Gastric Carcinoma.[11] The histology was grossly divided into the differentiated type (papillary and tubular adenocarcinoma) and the undifferentiated type (poorly differentiated adenocarcinoma, signet-ring cell carcinoma, mucinous carcinoma, and miscellaneous). Curative resection (R0) was defined as no tumor left macroscopically or microscopically after the operation. Tumor size was recorded by the maximum diameter. D2 lymph node dissection was defined as the removal of all perigastric lymph nodes and N2 group lymph nodes by location of primary tumor, whereas the lymph node dissection not satisfying D2 was defined as <D2 lymph node dissection. An extended gastrectomy included a resection of the adjacent organs such as the spleen, colon, pancreas, small bowel, liver, and kidney in addition to a subtotal or total gastrectomy. The patients with potentially curative resection routinely received a gastrectomy with D2 lymphadenectomy alone or with para-aortic nodal dissection. All chemotherapy for the enrolled patients was postoperative. The adjuvant chemotherapy was based on epirubicin, cisplatin, and fluorouracil.

Patients were followed routinely after surgery by serum carcinoembryonic antigen test at least every 3 months for the first year, every 6 months for the next 2 years, and every year for 5 years, and physical examinations, including abdominal ultrasonography, computed tomography scans, chest radiography, and endoscopy at least once each year. The outcome of all patients was collected by outpatient visits, telephone, mail, and death certificates. The follow-up period for the survivors ranged from 1 to 116 months, with a mean of 15.6 months and a median of 10.0 months. The recurrence pattern was classified as peritoneal, locoregional, hematogenous, distant lymph nodes, or unknown and was compared between the stage IVa and IVb patients with curative resection. The patients with the unknown recurrence pattern were also followed up, but they missed examinations. An endoscopic examination, abdominal computed tomography scans, cytological examination of peritoneal fluid, computed tomography of the chest, or bone scan was conducted to confirm recurrence. Locoregional recurrence included reappearance and progression of tumor in gastric bed, anastomotic site, or upper abdominal lymph nodes. Peritoneal recurrence was considered to be disease progression in peritoneal nodules, peritoneal wall thickening, or ascites with positive cytological findings. Patients with specific intra-abdominal or extra-abdominal organs involved, such as liver, lung, bone, brain, or adrenal glands, were considered to have hematogenous recurrence.[12] A distant lymph node recurrence was defined when the lymph nodes such as cervical lymph nodes were involved.

The categorical variables were compared using a chi-squared test. The continuous data are presented as mean (SD) and compared using the Mann–Whitney test. The overall survival and recurrence-free survival were evaluated by the Kaplan–Meier method. The log-rank test was used to determine univariate significance. Variables with an influence on the outcome on univariate analysis were included in the multivariate analysis. Multivariate analysis was performed by means of the Cox proportional hazards model, using the forward stepwise procedure for variable selection. Hazard ratios and 95% confidence intervals were

Table 1 Clinicopathological Features of Patients with Three Substages of Stage IV Gastric Cancer

Factors	IVa (n=59)	IVb (n=632)	IVc (n=485)	p value
Gender				
Male	38 (64.4)	411 (65.0)	339 (69.9)	0.209
Female	21 (35.6)	221 (35.0)	146 (30.1)	
Age, years				
Mean±SD	56.5±11.8	56.8±11.2	56.9±11.4	0.404
Range	28–74	24–83	26–88	
Tumor location				
Upper	12 (20.3)	126 (19.9)	106 (21.9)	0.422
Middle	18 (30.5)	202 (32.0)	170 (35.1)	
Lower	29 (49.2)	285 (45.1)	192 (39.6)	
Whole	0	19 (3.0)	17 (3.5)	
Operation type				
Subtotal gastrectomy	42 (71.2)	48 (7.6)	226 (46.6)	<0.001
Total gastrectomy	15 (25.4)	250 (39.6)	162 (33.4)	
Extended resection	2 (3.4)	334 (52.8)	97 (20.0)	
Tumor size, cm				
Mean±SD	6.7±3.7	7.3±3.8	8.3±4.0	<0.001
Range	2.5–15	3.5–18	3–20	
Borrmann type				
0	3 (5.1)	0	0	<0.001
1	7 (11.9)	3 (0.5)	18 (3.7)	
2	33 (55.9)	63 (10.0)	69 (14.2)	
3	10 (16.9)	408 (64.6)	292 (60.2)	
4	3 (5.1)	154 (24.4)	78 (16.1)	
5	3 (5.1)	4 (0.6)	28 (5.8)	
Histological type				
Differentiated	15 (25.4)	218 (34.5)	118 (24.3)	0.001
Undifferentiated	44 (74.6)	414 (65.5)	367 (75.5)	
Lauren classification				
Intestinal	17 (28.8)	254 (40.2)	147 (30.3)	0.006
Diffuse	39 (66.1)	347 (54.9)	318 (65.6)	
Mixed	3 (5.1)	31 (4.9)	20 (4.1)	
Lymphatic involvement				
Absent	13 (22.0)	227 (35.9)	206 (42.5)	0.003
Present	46 (78.0)	405 (64.1)	279 (57.5)	
Venous involvement				
Absent	21 (35.6)	107 (16.9)	70 (14.4)	<0.001
Present	38 (64.4)	525 (83.1)	415 (85.6)	
Neural involvement				
Absent	28 (47.5)	259 (41.0)	211 (43.5)	0.501
Present	31 (52.5)	373 (59.0)	274 (56.5)	
Depth of invasion				
T1	3 (5.1)	0	0	<0.001
T2	56 (94.9)	0	15 (3.1)	
T3	0	400 (63.3)	279 (57.5)	
T4	0	232 (36.7)	191 (39.4)	
Nodal status				
N0	0	0	13 (2.7)	<0.001
N1	0	87 (13.8)	149 (30.7)	
N2	0	145 (22.9)	199 (41.0)	
N3	59	400 (63.3)	124 (25.6)	

Table 1 (continued)

Factors	IVa (n=59)	IVb (n=632)	IVc (n=485)	p value
Curability				
R0	56 (94.9)	350 (55.4)	0	<0.001
R1, R2	3 (5.1)	282 (44.6)	485	
LN dissection				
≥D2	59	555 (87.8)	204 (42.1)	<0.001
<D2	0	77 (12.2)	281 (57.9)	
Chemotherapy				
Yes	43 (72.9)	389 (61.6)	329 (67.8)	0.038
No	16 (27.1)	243 (38.4)	156 (32.2)	
No. of retrieved LN (mean±SD)	43.5±13.2	33.2±16.9	21.9±3.2	<0.001
No. of metastatic LN (mean±SD)	23.9±6.9	19.4±11.8	10.3±7.9	<0.001

Numbers in parentheses are percentage. Substages are described in the "Method" section

LN lymph node, *SD* standard deviations

generated. A value of $p<0.05$ was considered to indicate statistical significance. All statistical analyses were conducted using SPSS statistical software, version 13.0 for windows (SPSS, Inc., Chicago, IL, USA).

Results

Clinicopathologic Characteristics

The clinicopathologic characteristics of the three substages of stage IV gastric cancer were shown in Table 1. There were no differences in the distributions of sex, age, tumor location, and neural involvement among these substages. The curative rate was significantly higher in stage IVa than that in stage IVb (94.9% vs 55.5%). The proportional frequency of extended gastrectomy procedure was higher in stage IVb (52.8%) compared with stage IVa (3.4%) and stage IVc (20.0%). All the patients in stage IVa underwent D2 or more extended lymph node dissection. The proportional frequency of D2 or more extended lymph node dissection was considerably higher in stage IVb than that in stage IVc (87.8% vs 42.1%). The distributions of tumor size, Borrmann type, histological type, Lauren classification, lymphatic involvement, venous involvement, chemotherapy, and number of retrieved and metastatic lymph nodes were significantly different among these substages.

Survival Analysis

The overall 1-, 3-, and 5-year survival rates of the patients with stage IV gastric cancer were 51.9%, 17.0%, and 7.4%, respectively. The median survival time was 13.5 months. The 5-year survival rates of patients in T1–2N3M0, T3N3M0, T4N1M0, T4N2M0, T4N3M0, and TanyNanyM1 groups were 22.7%, 9.2%, 11.8%, 9.8%, 2.7%, and 2.0%, respectively (Fig. 1). There were no differences in survival among T3N3M0, T4N1M0, and T4N2M0 groups (p=0.884) and between T4N3M0 and TanyNanyM1 groups (p=0.192). The 5-year survival rates in stage IVa (T1–2N3M0), stage IVb (T3N3M0 and T4N1–2M0), and stage IVc (T4N3M0 and TanyNanyM1) were 22.7%, 9.9%, and

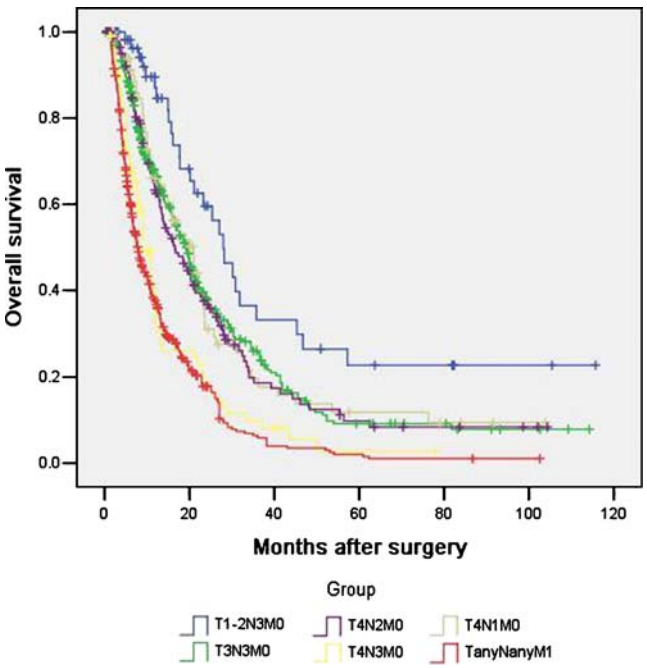

Figure 1 Survival among patients with T1–2N3M0, T3N3M0, T4N1M0, T4N2M0, T4N3M0, and TanyNanyM1. There were no survival differences among patients with T3N3M0, T4N1–2M0, and T4N2M0 (p=0.884) and between patients with T4N3M0 and TanyNanyM1 (p=0.192). The survival rate of patients with stage IVa (T1–2N3M0) was significantly higher than that of patients with stage IVb (T3N3M0 and T4N1–2M0; p=0.002). The survival rate of patients with stage IVb (T3N3M0 and T4N1–2M0) was higher than that of patients with stage IVc (T4N3M0 and TanyNanyM1; $p<0.001$).

Table 2 Univariate Analysis of Patients with Stage IV Gastric Cancer According to Three Substages

	IVa (n=59)		IVb (n=632)		IVc (n=485)	
	5YSR, % (median)	p value	5YSR, % (median)	p value	5YSR, % (median)	p value
Sex						
Male	21.1 (28.2)	0.876	10.1 (18.5)	0.454	2.3 (8.5)	0.149
Female	25.4 (28.0)		9.6 (20.3)		2.8 (7.5)	
Age, year						
<55	22.6 (30.8)	0.728	10.9 (21.2)	0.004	1.2 (9.2)	0.934
≥55	21.4 (27.8)		9.1 (14.1)		2.7 (8.0)	
Tumor location						
Upper	27.8 (27.0)	0.507	12.3 (19.1)	0.202	2.3 (8.2)	0.663
Middle	14.1 (28.0)		8.7 (18.4)		1.0 (7.5)	
Lower	25.0 (30.8)		9.3 (18.8)		1.8 (9.0)	
Whole	NA[a]		0 (19.5)		0 (5.4)	
Operation type						
Subtotal gastrectomy	29.2 (28.2)	0.181	9.5 (29.5)	0.084	5.0 (8.7)	0.001
Total gastrectomy	17.8 (30.8)		8.0 (27.7)		1.1 (8.0)	
Extended resection	0 (11.8)[b]		10.7 (34.1)		0 (7.5)	
Tumor size						
<8 cm	26.7 (27.2)	0.813	9.9 (20.0)	0.076	2.1 (9.1)	0.090
≥8 cm	17.5 (31.2)		10.0 (16.0)		2.8 (7.3)	
Borrmann type						
1	40.0 (57.2)	<0.001	0 (40.4)[b]	0.184	5.6 (14.2)	0.001
2	24.3 (25.3)		12.2 (20.6)		3.4 (13.0)	
3	0 (12.1)		12.1 (19.4)		1.8 (7.6)	
4	0 (6.2)[b]		6.4 (14.6)		1.5 (6.6)	
5	0 (16.1)[b]		0 (32.3)[b]		0 (8.1)	
Histological type						
Differentiated	37.5 (21.1)	0.565	11.9 (18.5)	0.235	3.2 (7.3)	0.477
Undifferentiated	16.8 (27.8)		7.5 (19.0)		1.5 (8.8)	
Lauren classification						
Intestinal	32.3 (35.7)	0.118	10.6 (19.9)	0.841	2.4 (8.8)	0.598
Diffuse	18.8 (28.0)		9.9 (17.6)		2.3 (8.1)	
Mixed	0 (14.9)[b]		0 (14.7)		0 (6.7)	
Lymphatic involvement						
Absent	28.6 (31.8)	0.410	11.3 (18.4)	0.309	1.2 (6.6)	0.750
Present	22.2 (26.7)		8.6 (19.4)		1.6 (9.2)	
Venous involvement						
Absent	25.5 (27.0)	0.790	16.7 (21.5)	0.137	4.8 (10.2)	0.073
Present	21.7 (30.1)		6.6 (14.6)		1.3 (8.0)	
Neural involvement						
Absent	25.7 (29.2)	0.882	10.6 (20.8)	0.452	2.4 (8.7)	0.841
Present	21.3 (25.6)		9.5 (17.6)		2.1 (7.6)	
No. of retrieved LN						
16–30	15.2 (11.8)	0.032	3.6 (13.3)	<0.001	0 (7.4)	0.059
31–50	20.2 (28.2)		14.7 (25.7)		0.9 (20.2)	
≥51	35.7 (46.7)		15.3 (29.6)		2.2 (13.3)	
Curability						
R0	24.8 (30.2)	0.024	19.9 (22.8)	0.001	NA[a]	NC[c]
R1, R2	0 (15.5)[b]		1.3 (15.1)		2.2 (8.2)	
LN dissection						
≥D2	22.7 (28.0)	NC[c]	10.5 (19.5)	0.008	1.9 (8.5)	0.699
<D2	NA [c]		5.7 (12.8)		2.6 (8.0)	

Table 2 (continued)

	IVa (n=59)		IVb (n=632)		IVc (n=485)	
	5YSR, % (median)	p value	5YSR, % (median)	p value	5YSR, % (median)	p value
Chemotherapy						
Yes	24.8 (30.8)	0.004	16.6 (23.4)	<0.001	2.7 (10.9)	<0.001
No	14.7 (15.7)		1.2 (13.4)		0.9 (5.9)	

Numbers in parentheses are percentage. Subgroups are described in the "Method" section

LN lymph node, *SD* standard deviations, *NA* not applicable, *NC* not calculated

[a] Because there was no patient, calculation of the 5-year survival rate and median survival time was not applicable

[b] Because of the small number of patients and short survival time, it was impossible to calculate the 5-year survival rate

[c] Because there were no patients in one of the two groups, the p value was not calculated

2.2%, respectively (p<0.001). The median survival times of the three substages were 28.0, 18.6, and 8.2 months, respectively.

Prognostic Factors

The multivariate analysis showed that subclassification, operation type, number of retrieved lymph nodes, curability, and chemotherapy were independent prognostic factors in patients with stage IV gastric cancer (Tables 3). For the five factors, subclassification had the highest relative hazard value (3.73). Table 2 shows the results of univariate analysis for the substages, and Table 3 summarizes the results of multivariate analysis. In stage IVa, the univariate analysis showed that Borrmann type, number of retrieved lymph nodes, curability, and chemotherapy were significant factors influencing survival. The multivariate analysis included three factors from the univariate analysis after excluding one (Borrmann type). In stage IVb, age, number of retrieved lymph nodes, extent of lymph node dissection, curability, and chemotherapy were associated with survival. Of the five factors, the multivariate analysis showed that curability, chemotherapy, and number of retrieved lymph nodes were independent prognostic factors. In stage IVc, operation type, Borrmann type, and chemotherapy had an effect on survival by univariate analysis. However, chemotherapy and operation type were determined to be independent predictive factors by multivariate analysis.

Recurrence-Free Survival and Recurrence Pattern of Stage IV Gastric Cancer with Curative Surgery

Figure 2 shows the recurrence-free survival curves for 406 patients with curative resection in stage IVa and IVb. There was significant difference of recurrence-free survival between stage IVa and IVb patients (p=0.012). The 1-, 3-, and 5-year recurrence-free survival rates were 64.6%, 24.8%, and 14.2% in stage IVa and 38.2%, 18.6%, and 11.7% in stage IVb, respectively. The median recurrence-free survival time was 16.9 months in stage IVa and 7.1 months in stage IVb. Table 4 presents the recurrence rates and patterns for the patients with curative resection. The recurrence rates were similar between the two substages (p=0.631). However, the recurrence patterns were significantly different between stage IVa and stage IVb (p<0.001). Hematogenous recurrence (35.9%) was the most common in stage IVa, followed by peritoneal (25.6%), distant lymph node (17.9%), and locoregional recurrence (12.8%).

In stage IVb, the most common patterns of recurrence were peritoneal (40.8%) and locoregional recurrence (31.8%).

Discussion

The prognosis of stage IV gastric cancer is still poor, although therapeutic outcome has improved because of early diagnosis and extensive radical surgery.[1,2] Many studies have attempted to determine the prognostic factors and select the appropriate therapeutic strategies for patients with stage IV gastric cancer.[4,13–15] However, there was no therapeutic standard accepted worldwide, and the treatment outcome is still unsatisfactory. Furthermore, stage IV gastric cancer includes: T1–3N3M0, T4N1–3M0, and TanyNanyM1 according to the sixth edition of the UICC TNM classification.[3] Therefore, it may be reasonable to subdivide stage IV gastric cancer according to the survival differences for the prognostic evaluation and the selection of therapeutic strategies. In this study, we found that there were no survival differences among patients with T3N3M0, T4N1M0, and T4N2M0 and between patients with T4N3M0 and TanyNanyM1. For patients with T1N3M0, it was impossible to calculate survival because of the small number of patients. Therefore, we divided patients with stage IV gastric cancer into three substages according to the survival differences: stage IVa (T1–2N3M0), stage IVb

Table 3 Multivariate Analysis of Factors Affecting the Survival of Stage IV Gastric Cancer and Three Substages

	HR	95% CI	*p* value
All cases (*n*=1,176)			
Subclassification			<0.001
IVa	1		
IVb	1.79	1.21–2.64	0.004
IVc	3.73	2.52–5.16	<0.001
Operation type			<0.001
Subtotal gastrectomy	1		
Total gastrectomy	1.19	0.95–1.48	0.127
Extended resection	1.87	1.45–2.42	<0.001
No. of retrieved LN			0.001
≥51	1		
31–50	1.26	0.97–1.64	0.086
16–30	1.66	1.26–2.18	<0.001
Curability			<0.001
R0	1		
R1, R2	2.03	1.68–2.47	
Chemotherapy			<0.001
Yes	1		
No	2.16	1.88–2.50	
IVa (*n*=59)			
No. of retrieved LN			0.024
≥51	1		
31–50	1.09	1.07–1.10	0.411
16–30	1.86	1.06–3.68	0.038
Curability			0.036
R0	1		
R1, R2	3.85	1.09–13.6	
Chemotherapy			0.006
Yes	1		
No	3.30	1.14–7.12	
IVb (*n*=632)			
No. of retrieved LN			0.044
≥51	1		
31–50	0.89	0.73–1.33	0.915
16–30	1.48	1.01–2.16	0.046
Curability			0.040
R0	1		
R1, R2	1.31	1.01–1.71	
Chemotherapy			<0.001
Yes	1		
No	2.04	1.51–2.75	
IVc (*n*=485)			
Operation type			0.002
Subtotal gastrectomy	1		
Total gastrectomy	1.14	0.91–1.44	0.248
Extended resection	1.61	1.24–2.01	<0.001
Chemotherapy			<0.001
Yes	1		
No	2.96	2.30–3.81	

Substages are described in the "Method" section

HR relative hazard, *CI* confidence interval, *LN* lymph node

(T3N3M0 and T4N1–2M0), and stage IVc (T4N3M0 and TanyNanyM1).

Many studies have already focused on defining prognostic factors for stage IV gastric cancer. These factors include surgical curability, distant metastasis, lymph node dissection, histological differentiation, lymphatic invasion, venous invasion, the number of metastatic lymph nodes, Borrmann type, and type of gastrectomy.[4,5,16] In the present study, we included the subclassified stage (IVa, IVb, and IVc) as a factor of the survival analysis for patients with stage IV gastric cancer and found that subclassification, operation type, number of retrieved lymph nodes, curability, and chemotherapy were independent predictive factors by multivariate analysis. Of the five factors, subclassification had the highest relative hazard value (stage IVc vs stage IVa). These findings suggest that subclassification of stage IV gastric cancer may be useful for the prognostic evaluation.

Stage IVa patients had the best survival outcome of the three substages. We found that curability, chemotherapy, and number of retrieved lymph nodes were independent prognostic factors for this substage. Curability was the strongest prognostic factors (hazard ratio, 3.85), and the curative rate was 94.9%, which accounted for good long-term survival in stage IVa. Number of lymph nodes retrieved had a prognostic impact on patients with stage IVa. Considering that the relative hazard ratio was 1.86 for 30 or fewer lymph nodes retrieved, the cutoff point for lymph node dissection should be more than 30. Tumors with lymphatic involvement

Table 4 Recurrence Pattern of Stage IVa and Stage IVb with Curative Resection

	IVa (*n*=56)	IVb (*n*=350)	*p* value
Recurrence			
Yes	39 (69.6)	255 (72.9)	0.631
No	17 (30.4)	95 (27.1)	
Recurrence pattern			
Peritoneal	10 (25.6)	104 (40.8)	<0.001
Locoregional	5 (12.8)	81 (31.8)	
Hematogenous	14 (35.9)	26 (10.2)	
Distant lymph nodes	7 (17.9)	17 (6.7)	
Unknown	3 (7.7)	27 (10.6)	

Numbers in parentheses are percentage. Stage IVa and stage IVb are described in the "Method" section

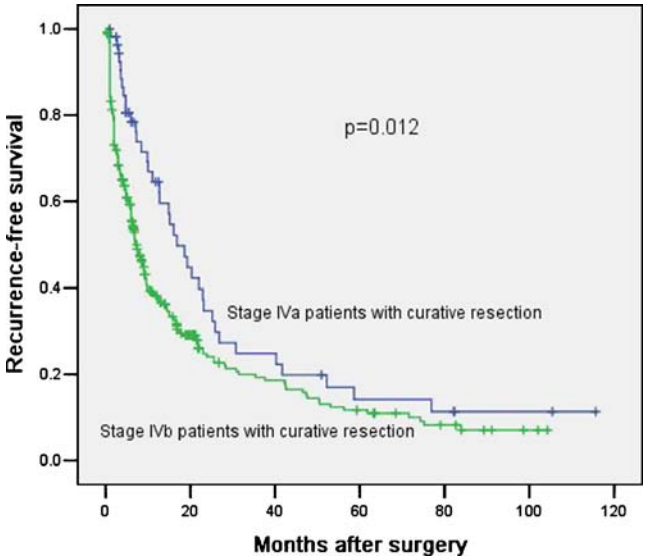

Figure 2 Recurrence-free survival of patients with curative resection in stage IVa (T1–2N3M0) and stage IVb (T3N3M0 and T4N1–2M0). The recurrence-free survival rate of patients with stage IVa was higher than that of patients with stage IVb (p=0.012).

were more common in stage IVa patients than that in stage IVb and IVc, which indicates that these tumors have a strong potential of lymphatic system invasion. Therefore, adequate number of lymph nodes retrieved was guarantee for curability. Chemotherapy had better outcomes than did no chemotherapy in stage IVa patients by multivariate analysis. This result underlines the role of chemotherapy after gastrectomy, consistent with a previous report,[17] and suggests that these tumors have already become a systemic disease rather than remaining a local lesion.

Stage IVb patients had significantly lower survival than did stage IVa patients. The patients were characterized by higher local grade of malignancy, compared with stage IVa patients. Curability, chemotherapy, and number of retrieved lymph nodes were independent prognostic factors of stage IVb patients. The multivariate analysis revealed that chemotherapy had the highest relative hazard value (2.04) in stage IVb, although the benefit of chemotherapy for patients with advanced gastric cancer is still controversial.[18] This result suggests that the role of chemotherapy has taken on added importance with the increase of local grade of malignancy in stage IV gastric cancer that is considered to be a systemic disease. An incomplete resection or positive margin status was associated with a less favorable prognosis in stage IVb (relative hazard, 1.31). D2 or more extended lymph node dissection for patients with stage IVb did not significantly prolong survival in the multivariate analysis in contrast to the univariate analysis. However, the relative hazard value of 30 or fewer lymph nodes retrieved was 1.48 by multivariate analysis. If the survival benefit of lymph node dissection for stage IV gastric cancer is attributed to the reduced absolute number of cancer cells in the body, as indicated by Yagi et al.,[4] then our findings are consistent with this result. The number of retrieved lymph nodes was a significant prognostic factor regardless of the extent of lymph node dissection in stage IVb.

The prognosis of stage IVc patients is the poorest with a 5-year survival rate of only 2.2%. In this substage, chemotherapy had the most important effect on survival. Some authors have demonstrated that a palliative gastrectomy is related to a survival benefit because of removal of gross disease; this procedure in these patients has been shown to achieve a better response to adjuvant therapy.[19] However, extended resection was an independent poor prognostic factor in stage IVc patients. This suggests that aggressive surgery for stage IVc patients does not provide any survival benefit.

In this study, we analyzed the recurrence-free survival of stage IVa and IVb patients with curative resection. However, the recurrence-free survival of stage IVc patients was not calculated because the patients in T4N3M0 group were not representative of the stage IVc patients, and the calculation of the patients in TanyNanyM1 group was not applicable. In stage IVa, the dominant recurrence pattern was hematogenous recurrence. This finding was probably caused by the low local grade of malignancy. We hypothesize that this tumor has special biological features that may be associated with a powerful potential of lymphatic and hematological system invasion. Thus, we suggest that the data from a large number of patients should be collected to comprehensively determine the biological characteristics. In stage IVb, the most common patterns of recurrence were peritoneal and locoregional recurrence. It may be associated with the high local grade of malignancy.

This study analyzed retrospectively the clinicopathologic features and prognosis of patients with stage IV gastric cancer, based on a 20-year experience. We are of the opinion that subclassification of stage IV gastric cancer might offer more useful and detailed information for predicting patient prognosis and determining therapeutic options. Ji et al.[10] suggested subclassification to stages IVA (T4N1–3M0), IVB (T1–3N3M0), and IVM (TanyNanyM1). These authors showed that the survival outcome of the T4N3M0 group was similar to that of the T4N1–2M0 group. However, we found that patients with T4N1–2M0 showed a much better survival than did patients with T4N3M0 and that the survival curve of the T4N3M0 and TanyNanyM1 groups showed no significant difference, consistent with the findings reported by Park et al.[8] Furthermore, we demonstrated that there was no significant difference among T4N1M0, T4N2M0, and T3N3M0 groups. Therefore, we suggest that patients with stage IV gastric cancer should be divided into three substages: stage IVa (T1–2N3M0), stage IVb (T3N3M0 and T4N1–2M0), stage IVc (T4N3M0 and TanyNanyM1).

Overall, stage IVa gastric cancer is a disease with extensive lymph node metastasis, a high possibility of curative resection and effective chemotherapy, the need for more radical lymph dissection, and a favorable survival. Stage IVa gastric cancer is prone to recurrence as hematogenous disease rather than peritoneal and locoregional disease. Stage IVb gastric cancer is representative of disease with extensive lymph node metastasis or adjacent organ invasion, requiring curative resection, adequate number of retrieved lymph nodes, and chemotherapy and was associated with an intermediate prognosis. The initial recurrence pattern is mainly peritoneal and locoregional recurrence. Stage IVc gastric cancer has the worst survival, and aggressive surgery does not offer any survival benefit, which shifts the therapeutic selection to chemotherapy. Our retrospective analysis shows the therapeutic value of surgical treatment and postoperative adjuvant chemotherapy in each substage, although it cannot offer the difference in adjuvant therapy among the three substages. Therefore, our results of this study support subclassification of stage IV gastric cancer into stage IVa (T1–2N3M0), stage IVb (T3N3M0 and T4N1–2M0), and stage IVc (TanyNanyM1).

In conclusion, there were no survival differences among patients with T3N3M0, T4N1M0, and T4N2M0 and between patients with T4N3M0 and TanyNanyM1. Therefore, subclassification of stage IV gastric cancer into stage IVa (T1–2N3M0), stage IVb (T3N3M0 and T4N1–2M0), and stage IVc (T any N any M1) may be useful for a more accurate prediction of patient survival and selection of therapeutic strategies. Subclassification of stage IV gastric cancer is an independent prognostic factor with highest relative hazard value. Multivariate survival analysis showed the following independent prognostic factors for substages: curability, chemotherapy, and number of retrieved lymph nodes for stage IVa and IVb and chemotherapy and operation type for stage IVc.

Potential and real conflict of interest No

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ORIGINAL ARTICLE

Surgery for Small Bowel Perforation in an Asian Population: Predictors of Morbidity and Mortality

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Abstract
Introduction Peritonitis from small bowel perforation is associated with prohibitive morbidity and mortality rates. The aims of our study were to review our institution's experience in the surgical management of small bowel perforation and to identify factors that could predict morbidity and mortality.
Methods A retrospective review of all patients who underwent operative intervention for peritonitis from small bowel perforation from January 2003 to May 2008 was performed. Patients were identified from the hospital's diagnostic index and operating records. The severity of abdominal sepsis for all patients was graded using the Mannheim peritonitis index (MPI). All the complications were graded according to the classification proposed by Clavien and group.
Results Forty-seven patients, of median age 68 years (18–95 years), formed the study group. Pneumoperitoneum on chest radiographs was seen in only 11 (23.4%) patients. Foreign body ingestion (17.0%), adhesions (14.9%), and malignancy (12.8%) accounted for majority of the pathologies. There was one patient who had several small bowel perforations from Degos disease. Small bowel resection was performed in the majority of the patients (74.5%). The mortality rate in our series was 19.1%, while another 57.4% patients had perioperative complications. On univariate analysis, American Society of Anesthesiologists score≥3, MPI>26, hypotension, stoma creation, abnormal electrolyte level, and renal impairment were related to worse outcome, while the three independent variables that were related to worse outcome after multivariate analysis were MPI>26, hypotension, and abnormal serum potassium level.
Conclusion Surgery for small bowel perforation is associated with significant morbidity and mortality rates. Patients with more severe peritonitis and physiological derangement were more likely to fare worse.

Keywords Intestinal perforation · Treatment outcome · Surgery

Introduction

Peritonitis from small bowel perforation is associated with prohibitive morbidity and mortality rates.[1,2] Despite advances in surgical technique, antimicrobial therapy, and perioperative intensive care support, the mortality rate has been quoted to be as high as 40%.[1,2] Prompt diagnosis is vital in ensuring the best possible outcome in these patients. Unfortunately, nonspecific clinical picture and the diverse etiologies with their own unique characteristics often delayed the diagnosis.[1–4]

Some of the common pathologies responsible for these perforations would include foreign body ingestion, infective causes, and Crohn's disease.[1–4] With the incidence of HIV infection rising worldwide, causations, such as tuberculosis, cytomegalovirus, and other rarer infective etiologies, are likely to become more prevalent.[5–7]

Primary small bowel anastomosis has always been considered safe,[8] with the necessity of stoma rarely discussed. Some of the risk factors associated with anastomotic dehiscence after primary anastomosis include hypoalbuminaemia, peritonitis, bowel obstruction, and hypotension.[9,10]

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In view of the numerous issues mentioned above, and the rarity of this topic being discussed in the literature, we undertook the study with the primary aim to review our institution's surgical experience in managing small bowel perforation. Our secondary aim was to identify factors that could predict perioperative complications.

Methods

Study Population

Tan Tock Seng Hospital is a 1,300-bed hospital, the second largest in Singapore, and provides secondary and tertiary medical care for about 1.5 million people. A retrospective review of all patients who underwent operative intervention for peritonitis from small bowel perforation from January 2003 to May 2008 was performed. Patients were identified from the hospital's diagnostic index and operating records. Patients who had small bowel perforation from peptic ulcer, postoperative anastomosis leakage, or abdominal trauma were excluded.

Decision for surgery was based on clinical assessment with the aid of plain radiographs or CT scans, which would be performed based on the surgeons' preference. Prior to the surgery, fluid resuscitation, and parenteral antibiotics would be administered to every patient. Nasogastric decompression would commence either pre- or intraoperatively depending on when the perforation was diagnosed. During the exploratory laparotomy, once the site of perforation was identified and the contamination controlled, the surgical procedure and the necessity of stoma were dependent on the surgeons' operative assessment. All gastrointestinal anastomoses were either hand-sewn or stapled. Prior to closure, copious lavage of the peritoneum would be performed. All patients would be transferred to the high dependency or surgical intensive care units postoperatively.

The data collected included age, gender, American Society of Anesthesiologists (ASA) score, comorbid conditions, presenting signs and symptoms, and clinical parameters. Laboratory values, including full blood count and renal panel, were also recorded. In addition, cause of perforation, operative findings and interventions, length of surgery, perioperative complications, mortality, and length of hospital stay were also documented.

The severity of abdominal sepsis for all patients was graded using the Mannheim peritonitis index (MPI)[11] (Table 1) with a score of >26 being defined as severe. The grades of complications (GOC) were in concordance to the classification proposed by Clavien and group[12,13] (Table 2).

Statistical analysis was performed using both univariate and multivariate analyses. The variables were analyzed to the various outcomes using the Fisher's exact test, and their odds ratio and 95% confidence interval were also reported. For the multivariate analysis, the logistic regression model was applied. All analyses were performed using the SPSS 16.0 statistical package (Chicago, IL), and all p values reported are two-sided, and p values of <0.05 were considered statistically significant.

Table 1 Mannheim Peritonitis Index[11]

Risk factor score		Score
Age>50 years old		5
Female sex		5
Organ failure[a]		7
Malignancy		4
Preoperative duration of peritonitis >24 h		4
Origin of sepsis not colonic		4
Diffuse generalized peritonitis		6
Exudate	Clear	0
	Cloudy, purulent	6
	Fecal	12

[a] Kidney failure = creatinine level>177 μmol/L, urea level> 167 mmol/L, or oliguria<20 ml/h; pulmonary insufficiency = PO_2< 50 mmHg or PCO_2>50 mmHg; intestinal obstruction/paralysis >24 h or complete mechanical ileus, shock hypodynamic, or hyperdynamic

Results

Study Group

Forty-seven patients formed the study group, with 55.3% of them older than 60 years old. Nearly half of study group had an ASA score of 3 (n=22, 46.8%). One third of the patients had at least two comorbid conditions, while nine (19.1%) were immunosuppressed. Though all patients had erect chest radiographs, pneumoperitoneum was seen in only 11 (23.4%) patients. Preoperative CT scan was performed in 32 (68.1%) patients, and some of the findings seen included pneumoperitoneum (n=21, 65.6%), abscess or inflammatory mass without extra-luminal gas (n=8, 25.0%), extravasation of oral contrast (n=1, 6.3%), and intestinal obstruction (n=2, 9.4%). Foreign bodies were also detected in several patients. Table 3 illustrates the various characteristics of this study group.

Clinical Parameters and Investigations

Eleven (23.4%) patients were hypotensive (systolic blood pressure <90 mmHg) on admission, with four of them requiring inotropic support in the emergency department. The majority of patients (n=36, 76.6%) had abnormal total white count, while anemia was present in about one third of

Table 2 Classification of Surgical Complications[12–13]

Grade of Complications (GOC)
Grade I: Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions
Grade II: Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included
Grade III: Requiring surgical, endoscopic or radiological intervention
Grade IV: Life-threatening complication(s) requiring ICU management (including organ dysfunction)
Grade V: Death of a patient

the study group. Preoperative electrolyte imbalances were also documented in about one third of the study group. Though serum albumin was only performed in 33 (70.2%) patients, it was abnormal in 28 (84.8%) of them (Table 4).

Operative Findings

There was a wide spectrum of pathologies responsible for the small bowel perforation in our study group. The three most common etiologies were foreign body ingestion (n=8, 17.0%), adhesions (n=7, 14.9%), and malignancy (n=6, 12.8%). Tuberculosis (n=5, 10.6%) and cytomegalovirus infection (n=1, 2.1%) accounted for the infective causes. Interestingly, one of our patients had numerous small bowel perforations from Degos disease. Nearly half of the study group (48.9%) had a MPI score of >26 (Table 5).

Small bowel resection was performed in the majority of the patients (n=35, 74.5%), while right hemicolectomy was performed in another six (12.8%). Three (6.4%) patients

Table 3 Characteristics of the 47 Patients who Underwent Surgery for Small Bowel Perforation

	Parameter (%)
Median age, range (years)	68 (18–95)
≤60	21 (44.7)
>60	26 (55.3)
Gender	
Male	30 (63.8)
Female	17 (36.2)
ASA status	
1	4 (8.5)
2	9 (19.1)
3	22 (46.8)
4	12 (25.5)
Premorbid condition	
Hypertension	20 (42.6)
Diabetes mellitus	8 (17.0)
Hyperlipidemia	11 (23.4)
Ischemic heart disease	9 (19.1)
History of cerebrovascular accident	6 (12.8)
Number of premorbid condition	
0–1	31 (66.0)
2–5	16 (34.0)
Immunosuppression	
No	38 (80.9)
Yes	9 (19.1)
3 patients has HIV infection	
2 patients on chemotherapy	
1 patient has SLE on corticosteroids	
3 patients has end-stage renal failure	

Table 4 Clinical Parameters and Laboratory Investigations of the Study Group

	Parameter (%)
Median systolic blood pressure (mmHg)	115 (64–172)
Hypotensive (<90 mmHg)	11 (23.4)
Not hypotensive	36 (76.6)
Median white blood cell count ($\times 10^9$/L)	12.0 (1.3–31.7)
<4.0 or >10.0	36 (76.6)
4.0 to 10.0	11 (23.4)
Median hematocrit (%)	39.1 (20.0–57.4)
<33.0	15 (31.9)
≥33.0	32 (68.1)
Median serum sodium level (mmol/L)	134 (110–146)
<135 or >144	21 (44.7)
135–144	26 (55.3)
Median serum potassium level (mmol/L)	4.0 (3.1–8.5)
<3.5 or >5.0	13 (27.7)
3.5–5.0	34 (72.3)
Median serum urea level (mmol/L)	6.2 (1.9–58.5)
≤9.3	30 (63.8)
>9.3	17 (36.2)
Median serum creatinine level (umol/L)	94 (25–1,020)
≤110	29 (61.7)
>110	18 (38.3)
Median serum albumin level (g/L)	22 (12–43)
<35	28 (59.6)
≥35	5 (10.6)
Not performed	14 (29.8)

Table 5 Surgical Observations and Perioperative Outcome of the Study Group

	Parameter (%)
Causes of perforation	
Foreign bodies	8 (17.0)
Adhesions	7 (14.9)
Idiopathic	7 (14.9)
Malignancy	6 (12.8)
Lymphoma	4
Leiomyosarcoma	1
Metastatic lung squamous cell carcinoma	1
Tuberculosis	5 (10.6)
Ischemic bowel	3 (6.4)
Meckel's diverticulum	3 (6.4)
Small bowel diverticuli	2 (4.3)
NSAID-induced ulcerations	2 (4.3)
CMV Gut	1 (2.1)
Crohn's disease	1 (2.1)
Degos disease	1 (2.1)
Incisional Hernia	1 (2.1)
Median Mannheim peritonitis index (MPI)	26 (6–43)
≤26	24 (51.1)
>26	23 (48.9)
Nature of anastomosis	
Handsewn	20 (42.6)
Stapled	13 (27.7)
No anastomosis as no bowel resection	4 (8.5)
Stoma	10 (21.3)
Grade of complications	
No complications	11 (23.4)
Grade I	3 (6.4)
Grade II	9 (19.1)
Grade III	2 (4.3)
Grade IV	13 (27.7)
Death or Grade V	9 (19.1)
Causes of death	
Septicemia	7 (14.9)
Bronchopneumonia	1 (2.1)
Cardiogenic shock	1 (2.1)

had wedge resection of the perforated Meckel's diverticulum, while one (2.1%) underwent en bloc small bowel resection and sigmoid colectomy for a small bowel malignancy that had invaded into the sigmoid colon. Primary closure of the perforation was performed in one (2.1%) patient. In another patient (2.1%), only drainage of the abscess during laparotomy was performed as the site of perforation was not uncovered. The foreign body, which was a fish bone, was identified in the abscess cavity.

Ten (21.3%) patients had stoma created. Hand-sewn and stapled anastomoses after bowel resection were performed in 20 (42.6%) and 13 (27.7%) patients, respectively. The majority of the patients (n=30, 63.8%) had surgery within 24 h of admission, and the median duration of the surgery was 135 min (50–315 min). *Escherichia coli* and *Klebsiella pneumoniae* were the two most common microorganisms cultured from the peritoneal fluid.

Outcome

The mortality rate in our series was 19.1% (n=9) with septicemia being the cause of death in the majority of them, while another 27 (57.4%) patients had associated perioperative morbidity. The median length of stay was 15 days (range, 4–150 days; Table 5).

There were five (11.8%) patients who developed wound dehiscence, while another patient (2.1%) had postoperative anastomotic leak that necessitated relook laparotomy. Two patients underwent tracheostomy for prolonged ventilation. One patient developed intra-abdominal abscess that failed percutaneous drainage and required laparotomy and drainage.

Analysis—Complications

Worse complications (GOC III to V) occurred more frequently in patients who had higher ASA scores (3–4), MPI>26, or were hypotensive on admission. Preoperative renal impairment, electrolyte imbalances, and creation of stoma were also associated with poorer outcome. Factors such as age, gender, type of anastomosis, and duration of surgery were not related. The three independent variables that were related to significant complications (GOC III to V) after multivariate analysis were MPI>26, hypotension on presentation, and an abnormal serum potassium level (Table 6).

Analysis—Stoma Creation

In our series, stoma was created in patients with higher ASA score (3–4) and MPI>26. Other risk factors included abnormal serum sodium and urea levels and hypotension on admission. After multivariate analysis, the independent variables were MPI>26, hypotension on presentation, and abnormal serum urea level (Table 7).

Discussion

Though our mortality rate was comparable to other series at 19.1%, it was still considerable. Apart from mortality, most of our patients had perioperative morbidity as only 11

Table 6 Analysis of the 47 Patients who had Worse Perioperative Outcome

Characteristics	GOC 0–II (n=23)	GOC III–V (n=24)	OR (95% CI)	P value
>60 years old	10 (43.5%)	16 (66.7%)	2.60 (0.80–8.49)	>0.05
Female gender	8 (34.8%)	9 (37.5%)	1.13 (0.34–3.70)	>0.05
ASA score 3–4	11 (47.8%)	23 (95.8%)	25.09 (2.89–218.28)	<0.001
≥2 premorbid conditions	6 (26.1%)	10 (41.7%)	2.02 (0.59–6.96)	>0.05
MPI>26	4 (17.4%)	19 (79.2%)	18.05 (4.19–77.76)	<0.001[a]
Hypotensive	1 (4.3%)	10 (41.7%)	16.15 (1.85–141.32)	0.004[a]
Abnormal WBC	18 (78.3%)	18 (75.0%)	0.83 (0.22–3.23)	>0.05
Hct (<33.0) (%)	6 (26.1%)	9 (37.5%)	1.60 (0.46–5.59)	>0.05
Abnormal serum sodium level	5 (21.7%)	16 (66.7%)	7.20 (1.95–26.54)	0.003
Abnormal serum potassium level	2 (8.7%)	11 (45.8%)	8.89 (1.69–46.63)	0.008[a]
Serum urea >9.3 (mmol/L)	3 (13.0%)	14 (58.3%)	9.33 (2.17–40.18)	0.002
Serum creatinine >110 (umol/L)	4 (17.4%)	14 (58.3%)	6.65 (1.73–25.64)	0.006
Serum albumin <35 (g/L)	10/13 (76.9%)	18/20 (90.0%)	2.70 (0.39–18.96)	>0.05
Operation after 24 h from admission	5 (21.7%)	9 (37.5%)	1.37 (0.38–4.89)	>0.05
Creation of stoma	1 (4.3%)	9 (37.5%)	13.20 (1.51–115.35)	0.010
Stapled anastomosis	8/19 (42.1%)	5/14 (35.7%)	0.76 (0.18–3.17)	>0.05
Duration of operation >2 h	10 (43.5%)	16 (66.7%)	2.60 (0.80–8.49)	>0.05

[a] Statistically significant on multivariate analysis

(23.4%) were discharged well without any perioperative complications. Some of the factors associated with poorer outcome in our series included worse peritoneal contamination and significant physiological derangement.

MPI has been recently adopted in our institution due to its ease of application and its ability to predict the outcome of patients according to the severity of the peritonitis.[14] This was affirmed in our series as patients with higher MPI scores were associated with worse perioperative outcome. Despite the advent of other scoring systems such as physiologic and operative severity score for the enumeration of mortality and morbidity and acute physiology and chronic health evaluation, the authors felt that MPI still has its roles in predicting surgical outcome in patients with peritonitis.

Besides MPI, those patients who were hypotensive or had deranged electrolyte levels were also more likely to

Table 7 Risk Factors Associated with Stoma Creation

Characteristics	No stoma (n=37)	Stoma created (n=10)	OR (95% CI)	P value
>60 years old	19 (51.4%)	7 (70.0%)	2.21 (0.49–9.89)	>0.05
Female gender	14 (37.8%)	3 (30.0%)	0.70 (0.16–3.18)	>0.05
ASA score 3–4	24 (64.9%)	10 (100.0%)	NA	0.043
≥2 premorbid conditions	13 (35.1%)	3 (30.0%)	0.79 (0.18–3.59)	>0.05
MPI>26	15 (40.5%)	8 (80.0%)	5.87 (1.09–31.56)	0.036[a]
Hypotensive	5 (13.5%)	6 (60.0%)	9.60 (1.98–46.50)	0.006[a]
Abnormal WBC	29 (78.4%)	7 (70.0%)	0.64 (0.16–3.07)	>0.05
Hct (<33.0) (%)	10 (27.0%)	5 (50.0%)	2.70 (0.64–11.36)	>0.05
Abnormal serum sodium level	16 (43.2%)	5 (50.0%)	1.31 (0.32–5.32)	0.003
Abnormal serum potassium level	9 (24.3%)	4 (40.0%)	2.07 (0.48–9.03)	>0.05
Serum urea >9.3 (mmol/L)	9 (24.3%)	8 (80.0%)	12.44 (2.22–69.63)	0.002[a]
Serum creatinine >110 (umol/L)	12 (32.4%)	6 (60.0%)	3.13 (0.74–13.19)	>0.05
Serum albumin <35 (g/L)	20/25 (80.0%)	8/8 (100.0%)	NA	>0.05
Operation after 24 h from admission	11 (29.7%)	6 (60.0%)	3.55 (0.83–15.09)	>0.05
GOC III to V	15 (40.5%)	9 (90.0%)	13.20 (1.51–115.35)	0.010
Duration of operation >2 h	18 (48.6%)	8 (80.0%)	4.22 (0.79–22.62)	>0.05

[a] Statistically significant on multivariate analysis

fare worse. The authors postulated that these factors would imply the depletion of any remaining physiological reserves, and these physiological derangements are often direct consequences of severe peritonitis.[15,16]

Also seen in our series and several others in the literature, the numerous pathologies responsible for the small bowel perforation made early preoperative diagnosis difficult. No specific clinical or laboratory finding has been shown to be specific enough.[2,3] Pneumoperitoneum on chest radiographs is often absent[2] and was seen in only 23.4% of our patients. These issues have resulted in the increased adoption of CT scans in the evaluation of patients presenting with acute abdomen in our institution and was performed in 68.1% of our patients. Some of the CT features suggestive of bowel perforation would include extraluminal air and oral contrast extravasation.[17] CT scan is also useful to differentiate bowel perforation from other acute abdominal conditions such as acute pancreatitis that could be managed non-operatively.

One of our most interesting cases must be the patient who had small bowel perforations from Degos disease. Degos disease causing bowel perforation is extremely rare with very few cases reported in the literature.[18] Degos disease is an occlusive arteriopathy involving small caliber vessels and is often progressive. It often leads to tissue infarction and its systemic variant involving the gastrointestinal tract is perhaps the most aggressive.[18] Intestinal perforation, like in our patient, is one of its most severe complications and accounts for majority of the mortalities in patients with systemic Degos disease. Our patient was discharged well but passed away few months later from other related complications.

Tuberculosis is the main infective etiology in our series. It typically affects the ileocecal area, and its management is often challenging.[19,20] Some of the complications that mandate surgical intervention would include perforation, bowel obstruction, and hemorrhage. The nutritional state of the patient, condition of the bowel, and length of diseased segments are just some of the factors to consider during surgery in these patients.[19,20]

Though seen in one patient, Crohn's disease is one of the more common pathologies responsible for small bowel perforation in the West.[1–4] The perforation may arise from active disease process, secondary to distal obstruction, or a consequence of steroid therapy.[3,4,21,22] While some authors advocated aggressive early surgical resection,[21] others have suggested nonsurgical treatment unless clinically indicated.[22] But when surgery is indicated, resection of the involved segment is the treatment of choice. Differentiation between Crohn's disease and tuberculosis is difficult as their clinical presentations, radiological features, operative findings, and even histological evaluation can be very similar.[21,22]

In our series, there were six (12.8%) patients who had perforation from small bowel malignancy. Perforation in malignant small bowel tumors could arise from tumor necrosis, bowel ischemia, or increased intraluminal pressure secondary to distal bowel obstruction.[23,24] The most common histological subtypes of primary small bowel cancers resulting in small bowel perforation include lymphoma, adenocarcinoma, and sarcoma, while metastatic lesions from various organs could also be responsible.[23,24]

Though the etiologies of small bowel perforation vary greatly, the surgical principles are perhaps less controversial. Early containment of the contamination, copious lavage, and resection of the diseased segment should be adopted. Even though suture plication of the perforation site was performed in one of our patients, this is no longer practiced in our institution. If possible, bowel resection and primary anastomosis is the treatment of choice. Apart from removing the diseased segments, resection also allows sufficient histological and/or microbiological evaluation of the specimen.[2–4] In cases of perforated Meckel's diverticulum, wedge resection of the diverticulum is acceptable.[25]

Primary small bowel anastomosis has generally been considered safe.[8] Some of the risk factors associated with anastomotic dehiscence would include hypoalbuminemia, hypotension, and peritonitis.[9,10] Fortunately, there was only one patient in our series with this adverse outcome. The authors postulated that our low rate of anastomotic dehiscence could be because stoma was created in a sizeable proportion of our patients (n=10, 21.3%). Even though those patients who had stoma created fared worse, the authors attributed this to the underlying factors that necessitated its creation rather than the procedure itself. Hence, the decision to exteriorize or primary anastomose after small bowel resection is perhaps dependent on the degree of physiological derangement, severity of peritoneal contamination, and the condition of the bowel.

Comparing our series to those in the literature,[1–4] the prevalence of the various etiologies appears to be geographically and economically related. While typhoid fever is the most common causation in developing countries, this is not the case in developed countries. And while Crohn's disease is a rare entity in Asians, tuberculosis is rarely seen in the West. The proportion of foreign body ingestion causing perforation is also likely to remain constant or rise in any graying population. The rise of HIV infection worldwide will likely bring about a new wave of infective causation, already evident by the number of tuberculosis and cytomegalovirus related perforations in our series.

Conclusion

Surgery for small bowel perforation is associated with significant morbidity and mortality rates. Patients with more severe peritonitis and physiological derangement were more likely to fare worse.

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ORIGINAL ARTICLE

Long-Term Outcome of Metachronous Rectal Cancer Following Ileorectal Anastomosis for Familial Adenomatous Polyposis

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Abstract
Background Total colectomy with ileorectal anastomosis (IRA) for familial adenomatous polyposis (FAP) carries a potential risk of metachronous cancer in the residual rectum. This study evaluated the risk of cancer development in the residual rectum.
Methods Ninety-six patients who underwent initial surgery for prevention and cure of FAP were studied, and a clinicopathologic comparison was conducted between 59 patients who underwent IRA and 24 who underwent total proctocolectomy.
Results The 5-year overall survival rates were 94% after IRA and 95% after total proctocolectomy with no significant difference. The incidence of dense-type rectal polyps (4/17, 24%) was significantly higher in patients who developed metachronous rectal cancer following IRA compared to that in patients who did not (1/39, 3%). Moreover, 60% of patients with dense-type colon polyps developed metachronous rectal cancer compared to 24% in patients without and 80% of those with dense type rectal polyps developed metachronous rectal cancer compared to 25% without. Endoscopic surveillance of the eight Tis or T1 patients was performed at intervals of 6 months to 1 year after IRA but was not performed in three T3 patients for more than 2 years.
Conclusions Effective IRA requires selection of patients without invasive rectal cancer and without dense rectal polyps in whom long-term postoperative follow-up of the residual rectum is possible.

Keywords Ileorectal anastomosis · Familial adenomatous polyposis · Metachronous rectal cancer

Introduction

The prevention of advanced colorectal cancer requires colectomy or proctocolectomy in patients with familial adenomatous polyposis (FAP) at a premalignant stage.[1] In Western countries, total proctocolectomy with ileal-pouch anal anastomosis (IPAA) is often indicated for preventive and curative resection, whereas total colectomy with ileorectal anastomosis (IRA) is more common in Japan. IPAA is an ideal strategy to reduce the risk of postoperative cancer in the residual rectum but often causes postoperative dyschezia[2,3] and deteriorated quality-of-life (QOL).[2–4] IRA provides superior postoperative bowel function compared to IPAA and is sometimes indicated in selected patients in Japan on this basis; however, the risk of cancer in the residual rectum is unavoidable after IRA, and prevention requires long-term endoscopic surveillance.

It has been suggested that IRA should be limited to patients with non-dense colorectal polyps;[5–8] patients with

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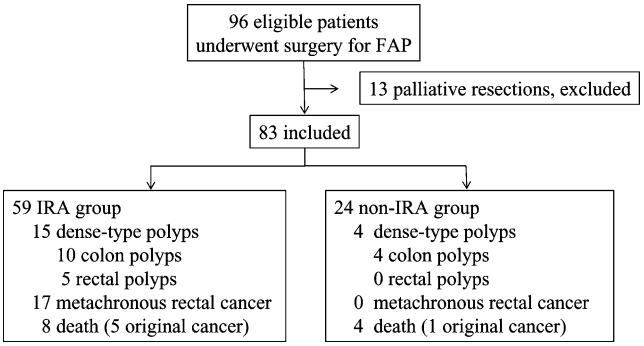

Figure 1 Study profile.

attenuated familial adenomatous polyposis;[9] young females who desire future pregnancy;[10] and patients for whom long-term follow-up can be conducted;[8] however, only a few studies have compared cancer recurrence and prognosis between IRA and IPAA. In this study, we examined these issues and identified risk factors for the development of metachronous cancer in the residual rectum following IRA.

Material and Methods

Ninety-six patients (male 62, female 34) who underwent initial surgery for prevention and cure of FAP at the National Cancer Center Hospital (Tokyo, Japan) between 1962 and 2007 were studied retrospectively. Patients who underwent palliative resections (partial colectomy, abdominoperineal resection, or ileostomy) were excluded. A clinicopathologic comparison was conducted between 59 patients who underwent IRA (IRA group) and 24 (non-IRA group) who underwent total proctocolectomy with ileoanal anastomosis (IAA) or IPAA, or total proctocolectomy with ileostomy. Age at the first operation, sex, surveillance period, density of polyps, presence of coexisting cancer, recurrence, overall survival, and relapse-free survival were examined in the two groups. More than 2,000 polyps in colon tissue samples were defined as dense-type polyposis and less than 2,000 polyps were defined as non-dense type. The number of polyps was counted roughly by the pathologist in charge. Rectal polyposis with more than 20 polyps on endoscopy was defined as a dense type, and less than 20 polyps were defined as a non-dense type. Clinicopathological factors were also compared between subgroups of patients who did and did not develop cancer in the residual rectum following IRA. Patients with intramucosal carcinoma were included in the subgroup who developed cancer in the residual rectum. Background and surgical data were obtained from a retrospective study of medical records. Since the study was a single-center observational design, approval by the institutional review board was not required in the present study.

Fisher's exact test and chi-square test were used for comparison between groups. Continuous nonparametric data were analyzed by the Mann–Whitney *U* test. Recurrence and survival rates were analyzed by the Kaplan–Meier method, and comparison of outcomes was conducted by log-rank test. A significant difference was assumed at $P<0.05$. Analyses were performed by using software (JMP, Version 7. SAS Institute Inc., Cary, NC).

Results

Figure 1 shows the study profile. Chronological changes in the operative procedure are shown in Table 1. IRA was performed in 42% patients (18/43) from 1962 to 1990 and in 77% (41/53) from 1991 to 2007.

The patient demographics are summarized in Table 2. Significantly more patients had coexisting rectal cancer in the non-IRA group; however, no significant differences were observed regarding the rate of patients with dense-type colorectal polyps between the two groups.

Prognosis was examined in all patients except for three Stage IV patients in the IRA group. The 5- and 10-year overall survival rates were 94% and 94%, respectively, in the IRA group, and 95% and 90%, respectively, in the non-IRA group, with no significant difference between groups (Fig. 2). There was also no significant difference in relapse-free survival rates between groups ($p=0.7111$; Fig. 3). There were eight deaths in the IRA group (five due to the original cancer and three of unknown cause) and four in the non-IRA group (one due to the original cancer, two due to other diseases, and one of unknown cause).

The patterns of cancer recurrence and metachronous cancer development in each group are shown in Table 3. Metachronous rectal cancer was detected in 17 patients in the IRA group.

A comparison of the 17 patients (30%) with metachronous cancer in the residual rectum and 39 patients (70%)

Table 1 Surgery for Familial Adenomatous Polyposis between 1962 and 2007

	1962–1990	1991–2007
IRA	18 (42)	41 (77)
IPAA	15 (35)	9 (17)
APR	5 (12)	1 (2)
Others	5 (12)	2 (4)
Total	43	53

Values in parentheses are percentages

IRA total colectomy with ileorectal anastomosis; *IPAA* total proctocolectomy with ileal-pouch anal anastomosis; *APR* abdominoperineal resection

Table 2 Patient Characteristics between IRA Group and Non-IRA Group

		IRA group (n=59)	Non-IRA group (n=24)	P value
Median age at operation (range)		30 (13–65)	31 (20–51)	0.9651
Sex	Male	35	19	0.1272
	Female	24	5	
Median follow-up (years)		8.9	16.1	0.1624
Colon polyps	Dense-type	10	4	1.0000
	Not dense-type	49	20	
Rectal polyps	Dense-type	5	0	0.3148
	Not dense-type	54	24	
Colon cancer	Present	30	7	0.0906
	Absent	29	17	
Rectal cancer	Present	5	7	0.0334
	Absent	54	17	
Pathological TNM stage for patients with cancer		31	11	
	0	12	2	0.5974
	I	5	2	
	IIA	1	0	
	IIB	0	0	
	IIIA	2	1	
	IIIB	5	4	
	IIIC	3	2	
	IV	3	0	

without cancer following IRA showed that cancer of the residual rectum occurred more frequently in patients with dense-type rectal polyps (p=0.0259; Table 4), and the incidence of dense-type rectal polyps (4/17, 24%) was significantly higher among those who developed metachronous rectal cancer following IRA compared to that in patients who did not (1/39, 3%). Moreover, 60% of patients with dense-type colon polyps developed metachronous rectal cancer compared to 24% in those without, and 80% of those with dense-type rectal polyps developed compared to 25% without.

Treatment after metachronous rectal cancer is demonstrated in Table 5. Initially, local therapy was performed for 10 of the 17 patients with cancer in the residual rectum, and surgery was performed on seven. Four of the 10 patients who initially received local treatment subsequently underwent radical surgery because of metachronous rectal cancer that could not be managed by endoscopic resection or pathological invasive cancer. Thus, surgery was required in 65% (11/17) of patients who developed cancer in the residual rectum. The surgery was performed at an average of 8.8 years after IRA (range 1.3−23.3 years).

Among the 11 patients who required radical surgery after IRA, eight with Tis-T1 invasion had undergone endoscopic surveillance at intervals of 6 months to 1 year after IRA, but the other three T3 patients did not undergo surveillance for more than 2 years before the second operation because of patient-related circumstances that had interrupted the

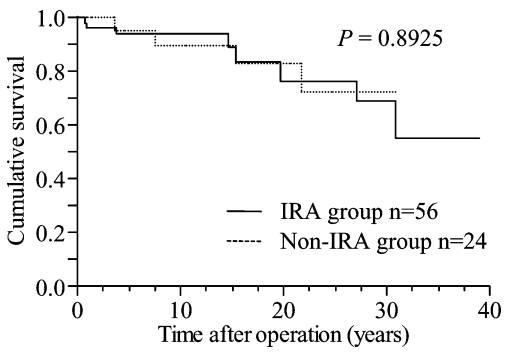

Figure 2 Overall survival rates in IRA and non-IRA groups.

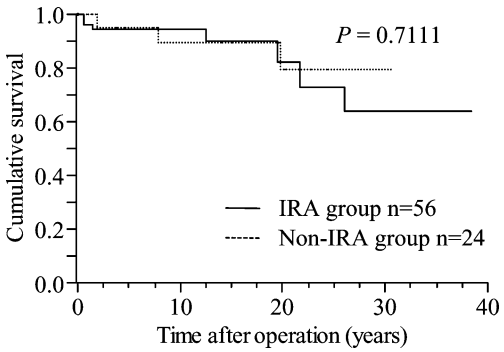

Figure 3 Relapse-free survival rates in IRA and non-IRA groups based on cancer recurrence.

Table 3 Pattern of Recurrence between IRA Group and Non-IRA Group

		IRA group (n=59)	Non-IRA group (n=24)
Metachronous cancer	Rectum	17	–
Recurrence	Liver	2	2
	Lung	0	0
	Small intestine	0	1
Total		19	3

Table 5 Treatment for Metachronous Rectal Cancer

Procedure	Initial treatment	Final treatment
Operation	7	11 (65)
Proctectomy with IAA or IPAA	5	9[a]
APR	2	2
Local resection	10	6 (35)
EMR	9	5
Trans-anal resection	1	1

Values in parentheses are percentages

IAA total proctocolectomy with ileoanal anastomosis; *IPAA* total proctocolectomy with ileal-pouch anal anastomosis; *APR* abdomino-perineal resection; *EMR* endoscopic mucosal resection

[a] One patient required total pelvic exenteration for the pelvic recurrence after IAA

postoperative surveillance. Regarding the surgical procedure of the 11 patients who required radical surgery after IRA, sphincter-preserving operations (IAA or IPAA) were performed in 88% (7/8) of patients with pathological Tis/T1 lesion, while 66.7% (2/3) of patients with pathological T3 lesion.

Discussion

A non-IRA procedure is ideal for preventive resection for patients with FAP to reduce the risk of metachronous cancer in the residual rectum, but IRA provides superior postoperative bowel function through alleviation of post-operative dyschezia and is sometimes indicated in Japan. Our findings demonstrate that IRA has no adverse effect on long-term prognosis, provided that appropriate surveillance is performed, despite the high risk of cancer development in the residual rectum. This suggests that IRA may be an option for selected patients who seem to be appropriately screened after IRA.

There have been several comparisons of IRA and non-IRA procedures, and IRA has been found to be superior to IPAA in that it provides a satisfactory level of postoperative defecation.[2,3] Some studies have reported improved QOL after IRA compared to IPAA,[2,3] but others have found no difference between these procedures based on findings from questionnaire surveys using the Short Form-36 Health Survey and the European Organization for Research and Treatment of Cancer Colorectal QoL Questionnaire.[4] Duijvendijk et al. had found no difference regarding QOL between the IRA and IPAA groups based on the responses to questionnaire surveys. Female fecundity has been found to deteriorate following IPAA compared with IRA,[10] which suggests that IRA might be superior in female patients who desire a future pregnancy, or IPAA should be performed after delivery. Meta-analysis by Aziz et al. of 12 reports published from 1991 to 2003 indicated that the development of adverse effects, such as bowel frequency, night defecation, and use of incontinence pads, was significantly lower after IRA than after IPAA, whereas fecal urgency was lower after IPAA.[11] Sexual dysfunction, dietary restriction, and postoperative complications did not differ between IRA and IPAA; however, the rate of reoperation within 30 days was significantly higher in patients who underwent IPAA than IRA (23.4% vs. 11.6%).[11]

The rate of cancer development in the residual rectum following IRA depends on the surveillance period and the

Table 4 Patients Characteristics with or without Metachronous Rectal Cancer after IRA

		Metachronous rectal cancer		P value
		Present (n=17)	Absent (n=39)	
Colon polyps	Dense-type	6 (60)	4 (40)	0.0520
	Not dense-type	11 (24)	35 (76)	
Rectal polyps	Dense-type	4 (80)	1 (20)	0.0259
	Not dense-type	13 (25)	38 (75)	
Colon cancer	Present	6 (22)	21 (78)	0.2520
	Absent	11 (38)	18 (62)	
Rectal cancer	Present	2 (50)	2 (50)	0.5770
	Absent	15 (29)	37 (71)	
Length of residual rectum	<11 cm	9 (35)	17 (65)	0.5702
	≥11 cm	8 (27)	22 (73)	

Values in parentheses are percentages

age of the patient.[12,13] Studies with follow-up periods of 5 years or longer have reported rates of 7–37%.[6,8,12–20] The risk rate of postoperative rectal cancer in the residual rectum in our study was 30% over a surveillance period of 8.9 years. This relatively high rate may have been due to the inclusion of Tis patients in the analysis. If the six patients with Tis tumors are excluded, the rectal cancer rate in this group would be only 20% (11/59). Although Tis lesions are regarded as adenomas in Western countries, it cannot be rejected that patients with Tis lesion in the residual rectum require resection of the lesion, and if local resection fails, radical surgery is indicated; therefore, patients with Tis lesions were included in the present study. However, most of the noninvasive lesions can be managed by endoscopic resection, thus, not requiring resection of the remnant rectum. Moreover, postoperative rectal cancer developed more often in patients with dense-type colorectal polyps (p=0.0259); therefore, we recommend that IRA is not indicated for patients with many colon polyps or those with 20 or more rectal polyps.

The correlation between the density of polyps and the rate of cancer development has been examined at a genetic level. Nieuwenhuis et al.[21] suggested that the severity of colonic polyposis may depend on the position of a mutation in the APC gene, with mutations between codons 1250 and 1464, and especially those at codon 1309, contributing to the severity of colonic polyposis. Other studies have proposed that mutations localized at the ends of the gene and in the alternatively spliced region of exon 9 cause a mild form of FAP, and it has been recommended that IRA should be limited to patients for whom a genetic diagnosis indicates a mild form of FAP.[6,9] Besides the density of polyps, development of a desmoid tumor should be considered in determining the indication for IRA, since a secondary proctectomy may be difficult to perform if cancer develops in the residual rectum in association with a desmoid tumor. Therefore, it has been proposed that IPAA should be selected for patients with a family history of desmoid tumor and those with a mutation located distal to codon 1444 in the APC gene.[1]

The stage at which cancer develops in the residual rectum clearly has a strong influence on prognosis. Vasen et al.[22] have reported that Dukes B, C, and D colon cancers account for 76% of cancers in the residual rectum, with most being detected at an advanced stage. In the present study, most patients were detected at an early stage, and Dukes B, C, and D colon cancers accounted for 29% (5/17). Detection at an early stage was arguably achieved by performing periodic endoscopic surveillance at intervals of 6 months to 1 year following surgery. Indeed, for the three T3 patients out of 11 patients who required further surgery, no endoscopic surveillance had been performed for 2 years or more before cancer was diagnosed; therefore, there may be no difference in prognosis after IRA and non-IRA procedures provided that appropriate surveillance is performed. Previous studies have shown that the main causes of death following IRA are cancer in the residual rectum, duodenal cancer,[23] and desmoid tumor;[24] however, duodenal and desmoid cancers may develop independently of the type of operative procedure. Therefore, IRA has no effect on overall survival when indicated appropriately in selected patients and with long-term periodic endoscopic surveillance to detect cancer in the residual rectum at an early stage. Vasen et al.[1] recommended intervals of 3 to 6 months for endoscopic follow-up of the rectum after IRA and suggested an indication for proctectomy in patients with multiple large (>5 mm) rectal adenomas with a high degree of dysplasia. Further acquisition of data is required to establish the appropriate interval for surveillance colonoscopy and to determine whether endoscopic resection is applicable following IRA.

Conclusion

Selection of an appropriate operative procedure for FAP requires consideration of a variety of factors, including the density of the colon or rectal polyps, whether future pregnancy is desired, the patient has a high risk of desmoid tumor, and the position of the mutation in an APC gene. Strict screening of patients will result in no difference in prognosis after IRA and non-IRA surgery, and we consider that the results demonstrated in the present study are essential in selecting suitable patients for IRA. Thus, IRA may be indicated for selected patients without invasive rectal cancer and without dense rectal polyps for whom frequent surveillance of the residual rectum can be performed over their lifetime.

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ORIGINAL ARTICLE

Single-Incision Laparoscopic Cholecystectomy: A Surgeon's Initial Experience with 56 Consecutive Cases and a Review of the Literature

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Abstract
Background We describe the results of a single surgeon's initial experience with single-incision laparoscopic cholecystectomy through his first 56 cases and provide a brief literature review on the development of this technique.
Methods Through a 2-cm vertical transumbilical incision, three 5-mm ports were placed using the Veress technique. One extracorporeal suture was utilized to provide cephalad retraction of the fundus, and a roticulating instrument grasping the infundibulum provided lateral retraction. The hilum was dissected, and the cystic duct and artery were clipped and divided. One 5-mm port was upgraded to a 10-mm port to allow the introduction of a retrieval bag, and the gallbladder was removed from the abdomen.
Results Of 56 patients, 54 successfully underwent a single-incision laparoscopic cholecystectomy. Two patients required conversion to either a conventional laparoscopic cholecystectomy or open cholecystectomy. The average age was 41 years (18–77) and the average BMI, 30.2 kg/m^2 (18.5–44.6). Mean operative time was 80 min (41–186). Length of stay was 0.3 days (0–2). The complication rate was 3/56 (5.4%).
Conclusions Our results suggest that single-incision laparoscopic cholecystectomy is a safe and effective alternative to four-port laparoscopic cholecystectomy that provides surgeons with an alternative minimally invasive surgical option and the ability to hide the surgical incision within the umbilicus.

Keywords Laparoscopy · Cholecystectomy · Surgical procedures · Minimally invasive

Introduction

The first laparoscopic cholecystectomy was performed by Erich Mühe in the County Hospital of Böblingen, Germany, on September 12th, 1985. Mühe describes designing and constructing his own laparoscope, called the Galloscope, and utilizing it before the era of video assistance. In fact, his technique, especially maintaining pneumoperitoneum proved to be so cumbersome that after performing the first six true laparoscopic cholecystectomies he abandoned the optically guided transumbilical approach under pneumoperitoneum for a single 3-cm subcostal incision approach where the gallbladder was removed under direct visualization.[1,2] The 23 years following Mühe have witnessed many competitive approaches to minimize the invasiveness of laparoscopic cholecystectomies with surgeons developing new instruments and techniques to decrease postoperative pain and improve cosmesis.[3,4] The most recent developments in laparoscopic surgery have been the combined advances in natural orifice transluminal endoscopic surgery and single-incision laparoscopic surgery (SILS). In deference to Mühe's rapid evolution from conventional laparoscopy to single-incision cholecystectomy, we report our experience with 56 cholecystectomies utilizing a single umbilical incision.

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Our technique employs a single 2-cm vertically oriented transumbilical incision to facilitate the placement of three 5-mm working ports (Fig. 1). We further utilized one extracorporeal stay suture to achieve the standard cephalad retraction of the gallbladder fundus. The lateral retraction of the infundibulum was accomplished with a roticulating instrument, allowing optimal exposure of the gallbladder hilum.

Methods

Patient Selection

Between November 2007 and August 2009, 56 patients underwent single-incision laparoscopic cholecystectomy at Yale-New Haven Hospital. All procedures were performed by the same surgeon. Patients who underwent this procedure either demonstrated symptomatic cholelithiasis, chronic biliary colic, acute cholecystitis, or gallstone pancreatitis. At the time of informed consent, patients were given the option to undergo either single-incision surgery or a traditional four-port procedure. Patients who were pregnant or whose American Society of Anesthesiologists (ASA) classification was 3 or 4 were excluded from consideration. Patient demographic data as well as height, weight, body mass index (BMI), length of operation, length of stay, perioperative complications, and surgical pathology were recorded to our database under an institutional-review-board-approved analysis.

Operative Technique

Patients were positioned on the operating table in a reverse Trendelenburg, right side up position. A 2-cm vertically oriented incision was made through the center of the umbilicus. A Veress needle (Versastep Veress system, Covidien, North Haven, CT, USA) was placed into the peritoneum and insufflated up to 15 mmHg with CO_2. Three 5-mm ports were placed through the same umbilical incision but through separate fascial incisions. For the last 18 cases, the SILS™ Port (Covidien, North Haven, CT, USA) was used instead. A 2-0 Polysorb suture on the Keith needle (Covidien, North Haven, CT, USA) was passed extracorporeally through the right upper quadrant close to the lowest rib and through the body of the gallbladder for cephalad retraction (Fig. 2). One roticulating grasper was used at the infundibulum for lateral retraction (Fig. 3). The gallbladder hilum was then dissected with a Maryland dissector to expose the cystic duct and cystic artery which

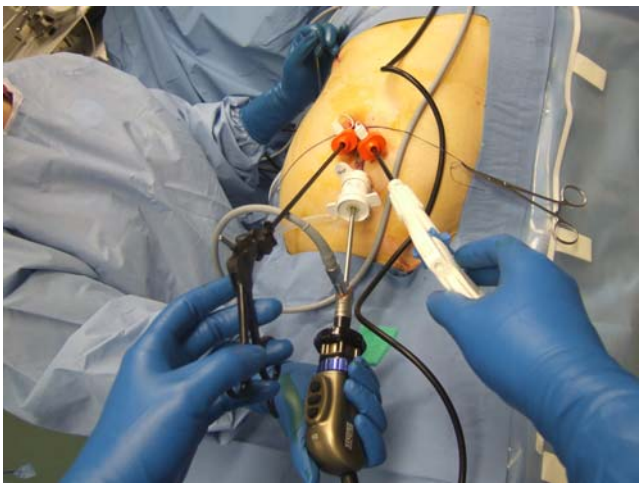

Figure 1 External positioning of three 5-mm ports through single vertical incision through the umbilicus.

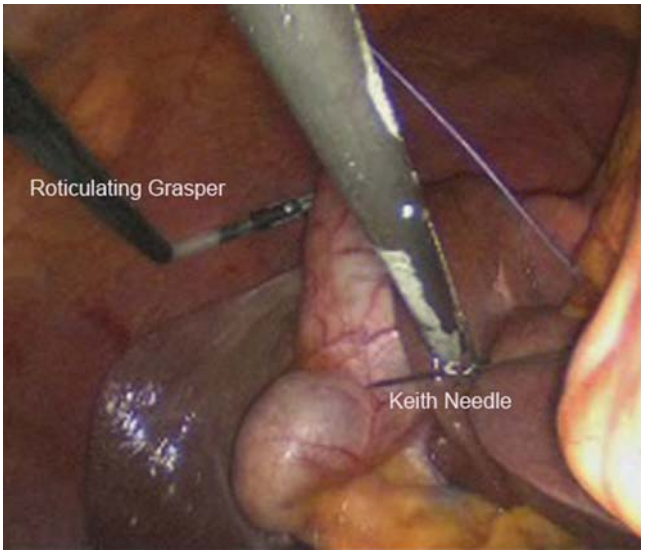

Figure 2 Placement of stay suture with Keith needle.

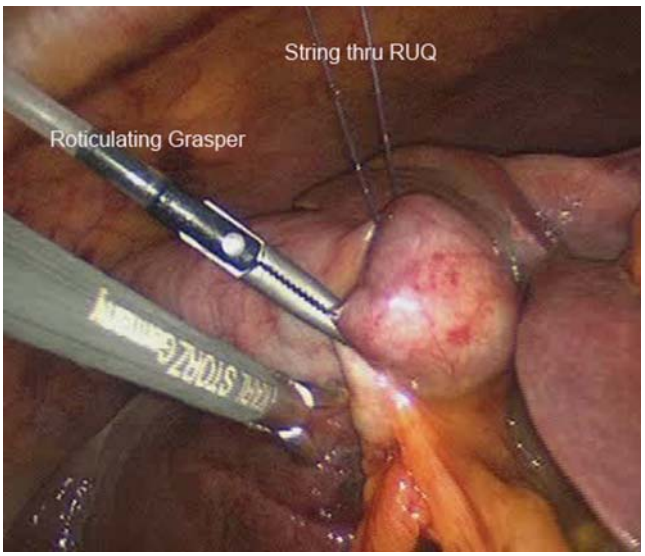

Figure 3 Lateral retraction of infundibulum with reticulating grasper and dissection of hilum.

were clipped with a 5-mm EndoClip™ (Covidien, North Haven, CT, USA) and then divided with scissors. The gallbladder was dissected from the gallbladder fossa with a hook electrocautery device. At this point, one 5-mm port was removed and another one was exchanged to an 11-mm port to facilitate the placement of an Endocatch bag™ (Covidien, North Haven, CT, USA). Then, the retrieval bag was placed beneath the gallbladder, and the retraction sutures were cut to allow removal of the gallbladder. The facial defect was then repaired with an 0 Ticron™ (Covidien, North Haven, CT, USA) suture in a figure of eight configuration, and the skin was reapproximated.

Results

Operative Results

Fifty-six patients were selected to undergo single-incision laparoscopic cholecystectomies between November 2007 and August 2009. Out of 56 patients, 54 (96.4%) successfully underwent a single-incision laparoscopic cholecystectomy. Of the remaining patients, one was converted to a standard four-port laparoscopic cholecystectomy for gangrenous cholecystitis due to failure to progress in a reasonable time. The second patient was converted to an open cholecystectomy due to dense adhesions and therefore difficult to identify anatomic landmarks. Moreover, a third patient underwent a concomitant resection of a Meckle's diverticulum which required both a longer operative time and length of stay. These patients were excluded from further analysis. Out of 53 patients, 47 (88.7%) were female; patients' average age was 41 years (range 18–77), and the average BMI was 30.2 kg/m^2 (range 18.5–44.6). Mean operative time was 80 min (range 41–186). Length of stay was 0.3 days (range 0–2).

Complications

Three complications were noted. The first complication involved a 39-year-old female who underwent an elective single-incision laparoscopic cholecystectomy for biliary colic. On postoperative day 7 (POD#7), she returned to the emergency department (ED) with complaints of nausea and vomiting and worsening right upper quadrant pain. Her white blood cell count at that time was found to be 24,000. Computed tomography demonstrated a 2.4×2.1-cm fluid collection within the gallbladder fossa; a follow-up hepatobiliary iminodiacetic acid (HIDA) demonstrated no bile leak. She was treated empirically with IV antibiotics and discharged home on hospital day 2 (HD#2) on oral antibiotics. The second complication was a morbidly obese 49-year-old female who underwent an elective cholecystectomy for biliary colic. Due to her multiple comorbidities, she was observed an additional day and discharged on POD#2. She returned to the ED on POD#4 with increasing right upper quadrant pain. Ultrasound demonstrated a 5.7× 2-cm fluid collection and a HIDA scan subsequently revealed a bile leak. The endoscopic retrograde cholangiopancreatography (ERCP) revealed a duct of Luschke leak, and a common bile duct stent was placed. The patient was discharged home on HD#3. Finally, the third complication was a 39-year-old female who underwent an elective cholecystectomy for biliary colic as well. She returned to the ED on POD#10 with complaints of right upper quadrant pain and bilious nausea and vomiting. An ultrasound was normal; however, magnetic resonance cholangiopancreatography showed a retained common bile duct stone; ERCP with stone retrieval and sphincterotomy were performed, and the patient was discharged home on HD#3.

Pathology

Pathology data were available for all 53 patients. Forty-three (81%) patients' pathology demonstrated cholelithiasis; 46 (87%) patients' pathology showed chronic cholecystitis; five (9%) patients had acute cholecystitis; three (5%) demonstrated cholesterolosis, and six (11%) patients demonstrated autolysis of the gallbladder.

Discussion

Single-incision transumbilical laparoscopic cholecystectomy was first described in the Italian literature in 1995.[5] In 1997, Navarra et al. published the first case series of 30 patients who underwent what they described as "one-wound laparoscopic surgery." Their method utilized three extracorporeal stay sutures and two 11-mm working ports, the incisions of which were connected at the end of the case to facilitate the removal of the gallbladder. The mean operative time was 123 min, and the mean postoperative stay was 1.8 days. One-wound complication was reported.[6]

In 1999, Piskun presented a series of ten patients (90% female). The authors reported no complications, and all patients were discharged within 24 h. Piskun used two extracorporeal stay sutures and utilized two 5-mm ports, which were combined to facilitate the removal of the gallbladder.[4]

In the last several years, there has been a resurgence of the popularity of SILS. Gumbs,[6] Cuesta,[7] and most recently Tacchino[8] have reported their experience with single-incision transumbilical laparoscopic cholecystectomies (Table 1). Gumbs describes his technique utilizing three 5-mm ports placed through a 2-cm transumbilical incision. An articulating grasper and a deflecting laparoscope are employed without the assistance of extracorporeal stay

Table 1 Comparison of Single-Incision Laparoscopic Cholecystectomies

Study	Year	Patients	BMI	Average length of stay	OR time	Ports	Stay sutures	Complications
Piskun[4]	1999	10	NR	<24 h	NR	2	2	
Cuesta[7]	2007	10	23	<24 h	70	2	1	
Gumbs[6]	2008	2	NR	<20 h	<60	3	0	
Tacchino[8]	2008	12	30	2.4 days	55	3	2	Perihepatic fluid collection Port site hematoma

sutures in two patients. Cuesta's method employs a horizontal transumbilical incision and two 5-mm ports. The gallbladder is retracted by a single extracorporeal Kirschner wire, which is manipulated within the abdomen by a proprietary device designed by the authors. The gallbladder is removed by connecting the skin bridge between the two ports. In this series, ten patients were described, and the mean operating time was 70 min. All patients were discharged within 24 h, and there were no perioperative or wound complications.

Most recently, Tacchino has published a technique making use of two crossed roticular instruments introduced through two 5-mm ports passed through a 12-mm intra-umbilical incision and two extracorporeal traction sutures. One port is upsized to 10 mm to facilitate the removal of the gallbladder. The authors describe their results in 12 patients with a mean operative time of 55 min. They report one persistent fluid collection and abdominal pain on postoperative day 2 and one periumbilical hematoma on postoperative day 7 (Table 1).

Our results are similar to those previously presented in the literature. Over the course of this series, our operative time improved from an average of 91 min for the first third of the cases to an average of 81 min for the second third of the cases and to just 64 min for the final third. While our technique described above most accurately resembles the last 43 cases, we experimented in the beginning with different techniques including no retraction suture in four cases and the use of two roticulating instruments in two cases. However, it quickly became obvious that the retraction suture and the use of only one roticulating instrument and a standard straight instrument do facilitate the ease of dissection and removal of the gallbladder. We have also adopted the use of an extracorporeal stay suture to assist in variable cephalad retraction. In our experience, there is minimal bile spillage from the placement of this stitch, and it has not torn through the gallbladder wall in any patient. Though this technique allows for appropriate visualization of the "critical view," a "top down" approach was never attempted and would likely be technically difficult to safely accomplish (Fig. 4).

Our case series possesses certain limitations. Our study was not based on an intention-to-treat analysis; rather it seeks to describe the characteristics of this new evolving technique. Therefore, three cases were excluded because they did not represent "routine" cholecystectomies. Further analysis is required to determine whether the conversion rate of this techniques differs from traditional four-port laparoscopic cholecystectomy. Moreover, ASA class 3 and 4 patients were intentionally excluded in this series. Though sicker patients may represent a large percentage of patients undergoing cholecystectomy, we are aware that a learning curve exists with this new procedure. We did not want to subject this population to potentially longer anesthesia times.

Intraoperative cholangiograms (IOC) are commonly performed during cholecystectomies. In this series, however, no IOCs were performed. Nonetheless, the single-incision technique does not prevent the use of standard cholangiography instruments at the surgeon's discretion. Though subjectively we believe the visualization of the gallbladder bed following removal of the gallbladder is comparable to that of a traditional four-port procedure, we experienced one duct of Luschke leak and one postoperative fluid collection in our series. A larger data set will be required to determine the true rate of these postoperative complications as well other theoretical risks including a potentially a higher incidence of

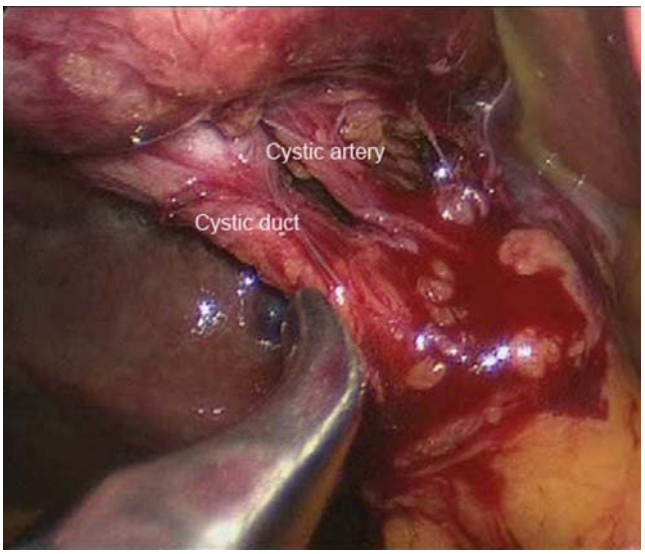

Figure 4 Completed dissection with critical view.

incisional/port site hernia formation, a potentially higher incidence of bile duct injury, and the possibility of increased rates of wound infection at the umbilicus. This new technique also raises other concerns such as increased cost of new ports and instruments as well as possible differences in postoperative pain and ability to return to work. These concerns will undoubtedly be the subjects of future studies.

In our practice, we give patients the choice of having their single-incision laparoscopic cholecystectomy or four-port laparoscopic cholecystectomy done as outpatients or with an overnight stay. All but one of our patients were discharged either the same day or on postoperative day 1. Our complication rate was 5.4%.

Despite potential limitations, a review of our initial data suggests that single-incision laparoscopic cholecystectomy is a safe and effective alternative to four-port laparoscopic cholecystectomy that provides surgeons with an alternative minimally invasive surgical option and the ability to hide the surgical incision within the umbilicus.

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ORIGINAL ARTICLE

Protective Effects of Early CD4$^+$ T Cell Reduction in Hepatic Ischemia/Reperfusion Injury

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Abstract
Aim CD4$^+$ T cells contribute to disturbances of liver microcirculation after warm ischemia/reperfusion (I/R). The aim of this study was to investigate a possible protective role of FTY720 (Sphingosine-1 phosphate receptor agonist) in this setting.
Material and Methods In an in vivo model (42 Wistar rats), ischemia of the left liver lobe was induced for 90 min under anesthesia with xylazine/ketanest. Sham-operated untreated ischemic and treatment group with FTY720 (1 mg/kg body weight intravenous) were investigated. The effect of FTY on I/R injury was assessed by in vivo microscopy 30–90 min after reperfusion (perfusion rate, vessel diameter, leukocyte–endothelial cell interactions, T cell infiltration), by measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), reverse transcription–polymerase chain reaction (RT–PCR) of interleukin (IL)-2, IL-6, IL-10, TNF-α, toll-like receptor 4 (TLR-4), and by histological investigation.
Results After 30 min of reperfusion, the number of T cells in sinusoids was increased four-fold. In the FTY group, the number of T cells was reduced to an half of the number of the ischemia group. Likewise, the number of adherent leukocytes in sinusoids (150.8±10.9% of s.o.) was reduced in the treatment group (117.3±12.2%; $p<0.05$ vs ischemia), leading to an improvement in perfusion rate in this group (85.0±4.6% of sham group) compared to nontreated animals (57.5±10.8%; $p<0.05$). According to improved microcirculation, AST/ALT values and histological tissue damage were reduced in the therapy group. RT–PCR revealed an increased expression of IL-2, IL-6, and TLR-4 in the nontreated ischemic group. This expression was clearly reduced in the treatment group.
Conclusion In conclusion, FTY720 ameliorates the microcirculatory, biochemical, and histological manifestations of hepatic I/R injury by preventing T cell infiltration. These results indicate that T cells are pivotal mediators in hepatic I/R and may have important implications early after liver transplantation and in warm ischemia.

Keywords Liver · T cells · Ischemia · Reperfusion

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Introduction

I/R injury is an important problem in clinical transplantation and is also implicated in a variety of nontransplant conditions, including myocardial ischemia, shock, and stroke. Clinical and experimental data have established that I/R injury has both immediate and long-term effects on the allograft, contributing both to acute rejection and chronic allograft dysfunction. I/R injury is a complex process that involves a variety of pathophysiologic mechanisms. Upregulation of adhesion molecule expression mediates increased adhesion of lymphocytes and neutrophilic granulocytes to organ endothe-

lium and their subsequent extravasation. These in turn release inflammatory cytokines and generate reactive oxygen species that mediate tissue damage. Total body or localized organ damage mediated by I/R injury is relevant in a variety of surgical fields such as transplantation medicine, cardiac surgery, and trauma surgery. Intervals of ischemia are also encountered during solid organ transplantation, myocardial revascularization, shock, and a variety of traumatic situations. The pathophysiology of liver I/R injury includes direct cellular damage as the result of the ischemic insult as well as delayed dysfunction and damage that results from activation of inflammatory pathways. Histopathologic changes include cellular swelling, vacuolization, endothelial cell disruption, neutrophil infiltration, and hepatocellular necrosis. The distal cascade of inflammatory responses that result in organ damage after I/R injury has been studied extensively. Activation of Kupffer cells with production of reactive oxygen species, upregulation of the inducible nitric oxide synthase and proinflammatory cytokines, and neutrophil accumulation contribute to inflammation-associated liver damage.[1–4]

One may therefore speculate whether a reduction in leukocyte adhesion and transmigration into the interstitium may reduce the incidence and severity of I/R-induced complications. FTY720 (2-amino-2-[4-octylphenyl]-1,3-propaneldiol hydrochloride), a synthetic structural analogon of sphingosine related to myriocin, is considered to be a possible drug targeting this problem.[5] FTY720 acts as a kind of super agonist and interacts with a G-protein-coupled sphingosine 1-phosphate receptor-1 (SIP1) located on thymocytes and lymphocytes. This causes an aberrant internalization of the receptor, which blocks the recirculation of T lymphocytes from the lymph node to peripheral blood. Additionally, to this T cell lymphopenia, a depletion of neutrophil granulocytes following FTY720 administration has also been postulated.[6] Preliminary results derived from this mode of action suggest a positive effect in I/R mediated by FTY720.[6,7]

The purpose of this study was to evaluate the effect of FTY720 administration on microcirculatory disturbances in an experimental model of warm liver I/R. Additionally, an analysis was performed of selected pathophysiological sequelae such as cytokine expression profiles or time course of transaminases in peripheral blood.

Materials and Methods

Animals, Operative Procedures

The investigation was performed in accordance with the German legislation on protection of animals, and the experiments were approved by the Committee on Animal Care (Regierungspräsidium Leipzig). Wistar rats (n=42, 250 g, inbred females) were fasted for 12 h and randomly assigned to three experimental groups: sham operation (n=14; 1 ml aqua dest.), ischemia without treatment (n=14; 1 ml aqua dest), and ischemia with FTY720 treatment (n=14; 1 mg/kg body weight (bw) intravenous, Novartis Pharmaceuticals Ltd., Basel, Switzerland). This dosage was chosen after preliminary tests of applications of 0.1, 1, and 10 mg/kg bw FTY720 and was given 12 h prior to operation.[8] Rats were anesthetized by intraperitoneal anesthesia with xylazine (2 mg/kg bw, Bayer, Leverkusen, Germany) and ketamine (40 mg/kg bw, Ratiopharm, Ulm, Germany). The animals were kept on their back on a heat dish (37°C), and microsurgery was conducted using a binocular microscope (×10–20 magnification, LEICA, Germany). Polyethylene catheters (PE 50, internal diameter 0.28 mm; Portex, Hythe, UK) were inserted into the right carotid artery and jugular vein. After a midline laparotomy, the blood supply to the left liver lobe was interrupted for 90 min by applying a microclamp to the vascular pedicle. The temperature of the ischemic liver was continuously controlled by a probe and kept at about 37°C. Sham-operated animals were subjected to the identical surgical procedure with a brief (2-s) interruption of blood flow to the left liver lobe.

Seven rats of each group were operated, and intravital microscopy was performed. The other seven rats of each group were used for biochemical and histological examinations in a 1-week follow-up.

Lymphocyte Separation

For intravital microscopic studies, $CD4^+$ cells were isolated from spleens of syngeneic rats using a magnetic cell sorting system (miniMACS, Miltenyi Biotec, Bergisch-Gladbach, Germany). Isolated $CD4^+$ cells were labeled with the fluorescent dye carboxyfluorescein diacetate succinimidyl ester (5 μmol/l, V-12883, Invitrogen, Karlsruhe, Germany). A total of 1×10^7 $CD4^+$ carboxyfluorescein-succinimidyl-ester-labeled cells was infused intra-arterially after 20-min reperfusion.

The purity of the T cell subsets was routinely greater than 95% as determined by fluorescence-activated cell sorting analysis. As stated in some other studies, the isolation procedure does not lead to T cell activation.[9,10]

Intravital Fluorescence Microscopy

The hepatic microcirculation was studied on the lower surface of the left liver lobe with the use of an intravital fluorescence microscope (Zeiss, Oberkochem Germany; eye pieces, ×10/20; objective, ×16/0.5 for water immersion; 100-W/2 HBO mercury lamp). The microscopic images were recorded by a CCD video camera (FK 6990-IQ; Cohu, Prospective Measurements, San Diego, CA, USA) and transferred to a video system (S-VHS Panasonic AG 7330; Matsushita Electric Ind., Tokyo,

Japan) for off-line evaluation. Quantitative assessment of the microcirculatory parameters was performed off-line by computer-assisted analysis of the videotaped images using CAPIMAGE (Dr. Zeintl, Heidelberg, Germany). The following parameters were analyzed: sinusoidal perfusion rate (perfused sinusoids/total number of sinusoids observed) and sinusoidal diameters (measured in 100 sinusoids per liver in the periportal zone, defined by dividing the sinusoid into three segments of equal length).[11]

T lymphocyte infiltration was visualized on the liver surface in sinusoids and postsinusoidal venules. Fluorescent T lymphocytes were infused intra-arterially within a time period of 30 min after reperfusion and ten randomly chosen areas of the liver surface were visualized using a special filter block (excitation 492–517 nm). Afterwards fluorescein-isothiocyanate-labeled dextran (0.1 ml, 5% MW 150,000; Sigma Aldrich) was administered to observe the sinusoids.

Leukocytes were labeled by an intravenous application of rhodamine 6G (0.1 ml, 0.05%, Sigma Aldrich, Deisenhofen, Germany) and visualized in postsinusoidal venules and sinusoids using an N2 filter block. Rolling leukocytes were defined as cells crossing an imaginary perpendicular through the vessel at a velocity significantly lower than the centerline velocity in the microvessel. Their numbers are given as cells per second per vessel cross section. Leukocytes firmly attached to the endothelium for more than 20 s were counted as permanently adherent cells and quantified as the number of cells per square millimeter of endothelial surface, calculated from the diameter and length of the vessel segment observed.[11] In sinusoids, the number of accumulated ("stagnant") leukocytes was counted in the scanned acini and is given in [1/acinus].

TaqMan Real-Time RT–PCR

Total RNA was isolated using the monophasic phenolguanidine isothiocyanate Trizol reagent (Invitrogen, Groningen, NL). To avoid degradation of RNA during the isolation procedure, 1 l/RNase inhibitor was added to 1 ml of Trizol reagent. cDNA was prepared from 1 g of total RNA by reverse transcription (RT) with SuperScript II RNase H-reverse transcription (Invitrogen, Karlsruhe, Germany) using random hexamer primers p(dN)6 (Roche Diagnostics GmbH, Mannheim, Germany). TaqMan primers and probes for β-actin, interleukin (IL)-2, IL-10, toll-like receptor (TLR)-4, and tumor necrosis factor (TNF)-α were designed from published rat mRNA.

The following primers were used:

IL-2 primer fwd: 5′-CCA TGA TGC TCA CGT TTA AAT TTT-3′; IL-2 primer rev: 5′- CAT TTT CCA GGC ACT GGA GAT G-3′, IL-2 probe: 5′-TTG CCC AAG CAG GCC ACA GAA TTG-3′ (NM 053836)

IL-6 primer: fwd5′- ATA TGT TCT CAG GGA GAT CCT GGA A-3′; IL-6 primer rev: 5′-CAG TGC ATC ATC GCT GTT CAT-3′; IL-6: probe: 5′- TGA GAA AAG AGT TGT GCA ATG GCA ATT CTG AT-3′ (NM 012589).

IL-10 primer fwd: 5′-AAG CTG AAG ACC CTC TGG ATA CAG-3′; IL-10 primer rev: 5′- TGC TCC ACT GCC TTG CTT TT-3′; IL-10 probe: 5′- ACG CTG TCA TCG ATT TCT CCC CTG TG-3′ (NM 012854).

TLR 4 primer fwd: 5′-TCT GAT CAT GGC ATT GTT CC-3′; TLR 4 primer rev: 5′-AGG GGG TTG AAG CTC AGA T-3′; TLR-4 probe: 5′-CTT GAA TCC CTG CAT AGA GGT ACT TCC T-3′ (NM 019178).

TNF-α primer fwd: 5′-CCA CGC TCT TCT GTC TAC TGA AC-3′; TNF primer rev: 5′- ACG GGC TTG TCA CTC GAG-3′; TNF probe: 5′-TCC CAA CAA GGA GGA GAA GTT CCC A-3′ (NM 012675).

β-actin primer fwd: 5′-CCC TGG CTC CTA GCA CCA T-3′; β-actin primer rev: 5′-GAG CCA CCA ATC CAC ACA GA-3′; β-actin probe: 5′-ATC AAG ATC ATT GCT CCT CCT GAG CGC A-3′ (NM 031144).

Primers and probes were selected to span two exons to prevent amplification of putative contaminations with genomic DNA. The probes were labeled with 6-carboxyfluorescein as a reporter dye at the 5′-end and 6-carboxytetramethylrhodamine as a quencher dye at the 3′-end. The TaqMan PCR was prepared in a final volume of 25 l containing 5 l of cDNA (diluted 1:10), 25 mM MgCl2, 2.5 l 1Amplitaq buffer A, 20M dNTP (each), 1.25 U Amplitaq Gold (PerkinElmer Applied Biosystems, Foster City, CA, USA), 200 nM fluorescently labeled oligonucleotide probe, and 900 nM of each oligonucleotide primer. The PCR was performed in an ABI PRISM 7700 Sequence Detector (PerkinElmer Applied Biosystems, Foster City, CA, USA) with continuous monitoring of fluorescence. The PCR conditions were 2 min at 50°C followed by an initial denaturation at 95°C for 10 min and 42-step cycles of 95°C for 15 s; 60°C for 1 min. A standard curve was constructed using a serial dilution of known copy numbers of cDNA fragments of the respective target genes cloned into the PCR-TOPO vector (Invitrogen, Inchinnan, UK). β-actin was used as an internal standard in each experiment.

Biochemistry

Preoperatively and again at 75 min, 2, and 24 h after reperfusion, 200 μl was drawn via jugular vein catheter for analysis of peripheral lymphocyte count, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. AST and ALT were determined at 37°C by standard enzymatic techniques (micromethod, Ektachem-Kodak).

Histology

Specimens were taken before liver manipulation and at 6 h, 2, and 7 days after reperfusion from the left lobe of the liver, fixed in 4% formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin–eosin. Histomorphologic alterations were semiquantitatively assessed by means of a scoring system from absent to severe. Impairment before organ manipulation was assessed by four parameters. Injury during warm ischemia, reperfusion, and follow-up was assessed by ten parameters as described elsewhere.[12] Score points were assigned according to the importance of each parameter for organ function. Score values were given in percent of maximal attainable score points.

Statistical Analysis

All data are presented as mean±SD. Statistical analysis was performed by one-way analysis of variance. If there were significant differences, the Student–Newman–Keuls test was used for direct comparison of the groups. Values of $p<0.05$ were considered significant.

Results

Hemodynamic Parameters

Sham operation did not affect mean arterial pressure or heart rate over time. Animals subjected to ischemia experienced transient systemic hypotension during ischemia with only incomplete recovery of systemic blood pressure after 90 min of reperfusion when compared to baseline ($p<0.05$). Heart rate remained quite stable during ischemia but was significantly reduced during reperfusion ($p<0.05$ vs baseline). However, FTY720 administration affected neither arterial pressure nor heart rate of the animals (data not shown).

Hepatic Microcirculation

Sinusoidal Diameters

The sham-operated group showed sinusoidal diameters of 9.4±2.9 μm. This value was set 100% and compared with the ischemic group. After ischemia, sinusoids were constricted to an average value of 74.4±6.2%. This constriction could be nearly reversed using FTY720 treatment. Prophylactic application of FTY720 resulted in diameters of 92.1±8.1% of the sham group value ($p<0.05$ vs ischemia).

Perfusion Rate

Sinusoidal perfusion was determined as an index of I/R-induced hepatic microvascular injury. Nearly 100% (96.2±1.8%) perfusion rate was found in the sham-operated animals. However, only 57.5±10.8% perfusion rate was measured in ischemic animals without treatment. After application of FTY720, a perfusion rate of 85.0±4.6% was measured ($p<0.05$ vs ischemia).

Leukocyte–Endothelial Cell Interactions

Within sinusoids, the number of stagnant leukocytes was rather low (2.0±0.4 per acini). This value was set as 100%. Ischemia significantly increased this number to 150.8±10.9% ($p<0.05$). The drug reduced the number of stagnant leukocytes to 117.3±12.2% of sham-operated animals ($p<0.05$ versus ischemia, Fig. 1, left side).

In postsinusoidal venules of sham-operated animals, only few rolling (1.7±0.2 mm/s) and adherent (39.5±8.0/mm^2) leukocytes were registered. In contrast, after 90 min of normothermic ischemia, the number of rolling (249.8±15.3%) and firmly adherent (319.5±18.1%) leukocytes was significantly increased. After FTY treatment, a significant decrease in rolling (161.1±14.5%) and adherent (167.3±14.8%) leukocytes was detected ($p<0.05$ versus ischemia).

T Cell Accumulation

Peripheral blood lymphocyte counts (sham 1,280±235 cells per microliter) were elevated in the ischemia group (1,853±563 cells per microliter) and significantly reduced by FTY720 pretreatment (653±132 cells per microliter) at 2 h after reperfusion ($p<0.05$, Fig. 1, right side).

The number of CD4$^+$ T cells accumulated in sinusoids was 0.7±0.2 per acinus in sham-operated animals. After 30 up to 90 min of reperfusion, the postischemic number of

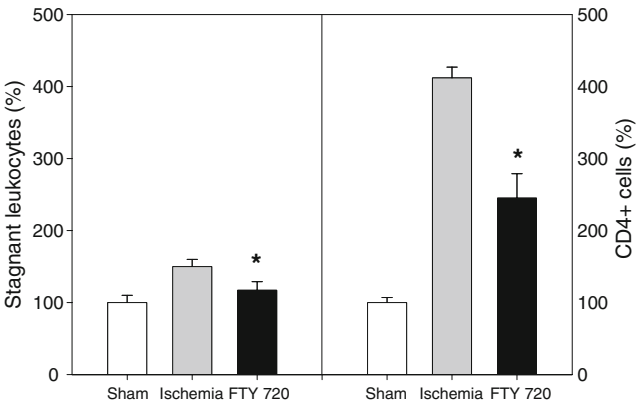

Figure 1 Numbers of stagnant leukocytes in sinusoids (*left side*) and accumulated CD4$^+$ cells in sinusoids (*right side*) in sham-operated, ischemia, and treatment groups after 90 min of ischemia and 20 min of reperfusion. *Asterisks*, significant difference between ischemia and therapy group ($p<0.05$).

$CD4^+$ T cells accumulated in sinusoids was increased in the nontreated ischemic group (2.9±0.2 per acinus; 408±38% of sham group), whereas in the FTY-pretreated animals (1.6±0.1/acinus; 238±24% of sham group) the number of accumulated cells remained low ($p<0.05$ versus ischemia, Fig. 1, right side).

After 30 min of reperfusion, 14±4% of intrasinusoidally accumulated $CD4^+$ T cells were found in the perivascular space, and this percentage was elevated to 25±3% at the end of in vivo microscopic observation. Moreover, the percentage of transmigrated T cells was reduced in FTY720-treated rats to 8±3% and 15±5% after 30 and 90 min of reperfusion, respectively.

Gene Expression of IL-2, IL-6, IL-10, TNF-α, and TLR-4 mRNA

Quantitative RT–PCR from hepatic tissue 1 h after reperfusion revealed a significant upregulation of TLR-4 (ischemia/therapy group 48.0±16.0-fold/29.3±12.9-fold, $p<0.05$), IL-2 (ischemia/therapy group 1.5±0.7-fold/0.6±0.3-fold, $p<0.05$), and IL-6 (ischemia/therapy group 31.2±18.7-fold/3.2±5.8-fold, $p<0.05$) in the ischemia group as compared to treated animals. TNF-α was significantly upregulated in both groups (ischemia/therapy group: 11.8±6.6-fold/7.7±4.0-fold, $p<0.05$), but the differences between the two groups did not reach statistical significance (Fig. 2). One day after reperfusion, TLR-4 and IL-2 furthermore were higher in the ischemia group. The IL-6 and TNF-α showed no differences (Fig. 3). Whereas IL-10 was equally high in both groups, after 1 day, the IL-10 showed a significant decrease in the FTY720 group but remained high in the untreated ischemic group.

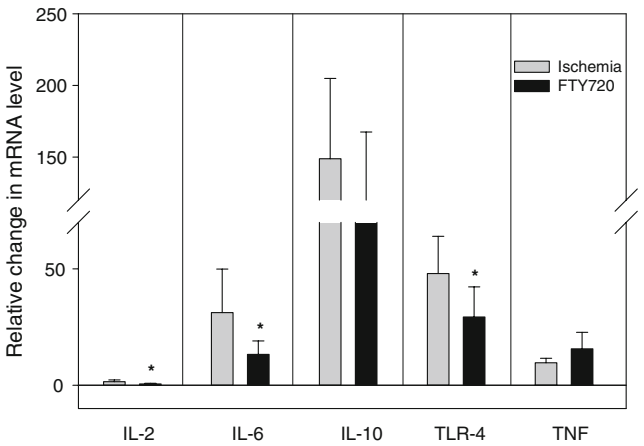

Figure 2 Intrahepatic mRNA expression of IL-2, IL-6, IL-10, TLR-4, and TNF-α 60 min after reperfusion given FTY720 (therapy group) or saline (ischemia group). *Asterisks*, significant difference between ischemia and therapy group ($p<0.05$).

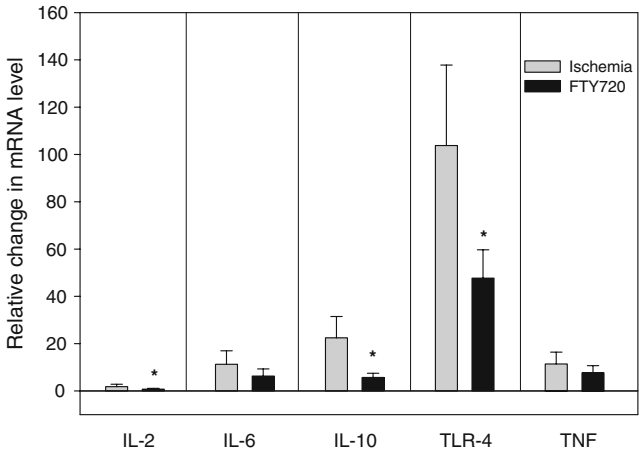

Figure 3 Intrahepatic mRNA expression of IL-2, IL-6, IL-10, TLR-4, and TNF-α 24 h after reperfusion given FTY720 (therapy group) or saline (ischemia group). *Asterisks*, significant difference between ischemia and therapy group ($p<0.05$).

Liver Enzyme Activities

In sham-operated animals, 2 h after reperfusion, AST (2.2±0.22 ukat/l) and ALT (1.7±0.3 ukat/l) values were not significantly increased compared with basal levels (AST 1.52±0.13 ukat/l; ALT 1.11±0.25 ukat/l). In the ischemia group, serum AST (9.2±1.0 ukat/l) and ALT (15.1±4.2 ukat/l) levels increased significantly ($p<0.05$) 2 h after reperfusion, reflecting the substantial loss of hepatocellular integrity.

In the treatment group, AST (7.15±1.2 ukat/l) and ALT (6.9±1.8 ukat/l; $p<0.05$) increase was reduced, indicating hepatoprotection by the drug (Figs. 4 and 5).

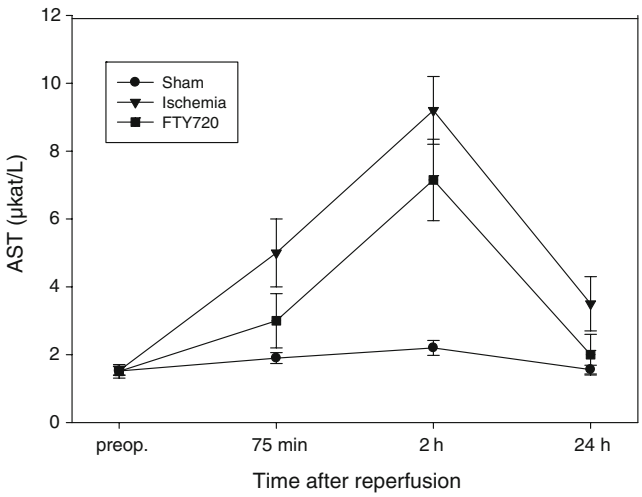

Figure 4 Serum levels of aspartate aminotransferase before ischemia, 75, 120 min, and 24 h after reperfusion. Values are given as mean± SD. *Asterisks*, significant difference between ischemia and therapy group ($p<0.05$).

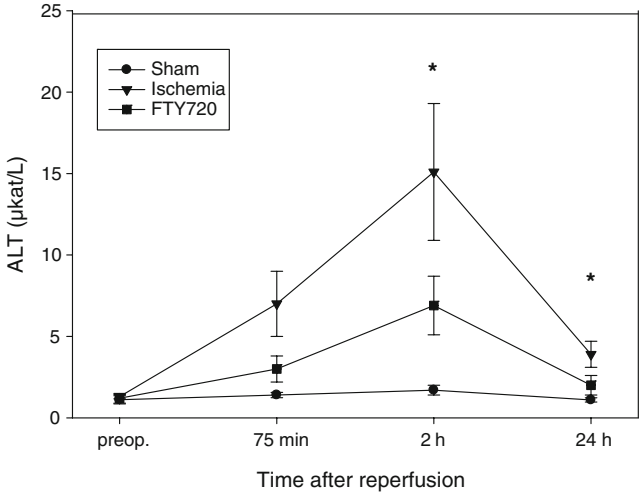

Figure 5 Serum levels of alanine aminotransferase before ischemia, 75, 120 min, and 24 h after reperfusion. Values are given as mean± SD. *Asterisks*, significant difference between ischemia and therapy group ($p<0.05$).

Histology

Before liver manipulation, there was no evidence of relevant morphologic damage in either group (score values: sham 0.28 ±0.03; ischemia 0.33±0.02; therapy 0.33±0.03). Nearly no changes were seen in the control group over time (score value 6 h after reperfusion 5.3±1.2). Six hours after reperfusion, histologic injury was found to be significantly lower in the therapy group ($p<0.05$). Slight increases in edematous injury (30%), reaction of the capsule of the liver (62%), and Kupffer cells (45%) were discovered in this group (Fig. 6). Histomorphologic alterations in the ischemia group included strongly developed interstitial and intracellular edema (58% and 87%, respectively), irregular trabecular disruption, hemorrhage, invasion of inflammatory cells, dilatation of the sinusoidal space, and sinusoidal congestion. Summarized injury in the ischemia group showed score values of 46.2± 8.3 versus 22.4±6.3 in therapy group ($p<0.05$). During follow-up (2 days postoperatively), an evident decrease in morphologic–pathologic alterations was observed in all groups in all investigated parameters. However, a significant difference in score values was still observed between the two groups (ischemia group, 25.5±4.8; therapy group, 14.3±3.8; $p<0.05$). After 7 days, no difference in score values could be found between the groups. All groups returned nearly to normal (Fig. 7).

Discussion

In the current study, we provide evidence that the selective blockade of T cell infiltration into the damaged tissue during reperfusion after warm ischemia leads to improved hepatic microvascular blood flow. This beneficial effect was characterized by a significant reduction in the percentage of nonperfused acini and sinusoids, resulting in a more homogeneous tissue perfusion. In addition, decreases in both leukocyte–endothelium interactions were observed, indicating improved microvascular perfusion.

After warm hepatic ischemia, various factors may contribute to localized disturbances observed in hepatic microvascular perfusion. As a consequence of an impaired transmembrane ion exchange during hypoxia, edema of hepatocytes and of sinusoidal endothelial cells with

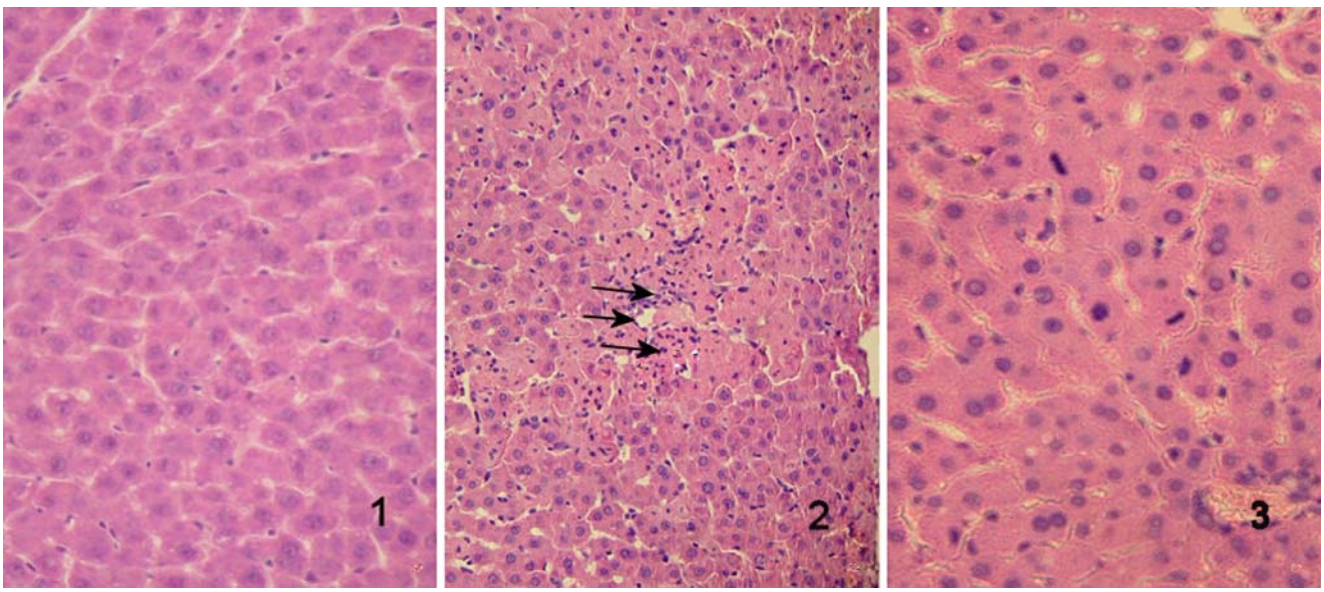

Figure 6 Histopathology of livers stained with hematoxylin–eosin (×400): (*1*) sham-operated control group; (*2*) I/R-injured rats (interstitial edema and infiltrated inflammatory cells indicated by arrows); (*3*) FTY720-treated group.

consecutive blood flow obstruction occurs and is paralleled by an increased intravascular hematocrit.[13] Additional endothelial cell damage occurs during reperfusion. Activation of Kupffer cells during this phase is followed by a secondary release of inflammatory mediators and oxygen radicals. In addition to the morphologic injury to parenchymal and nonparenchymal cells, functional alterations of presinusoidal and intrasinusoidal blood flow regulation contribute to the disturbances observed after reperfusion.

While previous studies have mainly focused on the participation of neutrophils as mediators of I/R injury,[14] recent investigations also demonstrated an important role for lymphocyte involvement.[1,7,15] Thus, modulation of lymphocyte function and lymphocyte concentration in peripheral blood might be beneficial for organ function and consecutive long-term survival. Therapeutic options, including the use of antibodies against specific lymphocyte antigens or lymphocyte-specific adhesion molecules, are still discussed.

In the present study, we investigated the influence of the synthetic drug FTY720, which inhibits the recirculation of T lymphocytes from secondary lymphoid tissues (lymph nodes, spleen, and Payer patches) to peripheral blood. FTY720 decreases total T lymphocyte concentration in peripheral blood and additionally upregulates several molecular markers that are related to intracellular homeostasis, including IL-10 and heat shock proteins.[5,6] Indeed, we found a protective effect of the drug in our treatment group shown by an improvement of the postischemic microcirculation protected by FTY720 treatment compared to controls.

Several mechanisms may account for the protective effect in I/R injury following FTY720 treatment. Matsuda et al. observed that systemic immunosuppression attenuated hepatocellular damage following I/R and described this to be due to an involvement of lymphocytes.[16] In this model, $CD4^+$ T lymphocytes adhered early in hepatic sinusoids, mediating consecutively a decrease of liver function. They also acted as cellular mediators for neutrophil recruitment.[17,18] In contrast, FTY720 may be protective owing to an upregulation of different cell protective molecules like heat shock proteins,[19,20] leading to stabilization of the endothelial layer or to a decreased sensitivity of the endothelial cells for inflammatory cytokines.[6,21] Concerning these activation processes and based on the current data available, an additional indirect protective effect of FTY720 could not be excluded.

Several investigations that have studied the involvement of T cell infiltration in hepatic IR injury corroborate our findings. Using a T-cell-deficient mouse (nu/nu) model, Zwacka et al.[22] observed the importance of T cells in mediating the subacute inflammatory injury caused by neutrophil infiltration in hepatic I/R injury. Adoptive transfer of T-cell-enriched splenocytes reconstituted this inflammatory response. They further showed that in vivo antibody depletion of CD4+, but not CD8+ T, cells abrogated indices of neutrophil-mediated inflammatory response. Others have shown that pretreatment with immunosuppressants, such as cyclosporine and FK506, has also been reported to reduce neutrophil accumulation and hepatic tissue injury following I/R,[23] possibly through inhibition of Kupffer cell cytokine-induced neutrophil chemoattractant release.[16]

The stimulation of extrahepatic lymphocytes may be a critical factor in regulating the inflammatory responses following IR injury in the liver. For example, it has been shown that rats splenectomized just prior to an ischemic insult to the liver demonstrated a reduction not only in neutrophil accumulation but also in biochemical and histological parameters of liver injury following 24 h of reperfusion.[24] Activation of lymphocytes at ectopic sites would be unaffected by FTY720 pretreatment, which could possibly account for the observed lack of effect on neutrophil recruitment. Additionally, the lymphocytosis observed in the untreated control group at 24 h of hepatic reperfusion may lend support to the notion that a systemic inflammatory response contributes to this process. Another possible explanation is that infiltrating lymphocytes could play a direct role in I/R-induced hepatocyte injury. Circulating lymphocytes in the rat are known to possess functions that are similar to those of granulocytes in humans, releasing toxic substances such as proteases and reactive oxygen species.[18]

Our analysis of hepatic cytokine gene expression patterns may lend further mechanistic insight into the inflammatory processes involved in hepatic I/R. Inhibition

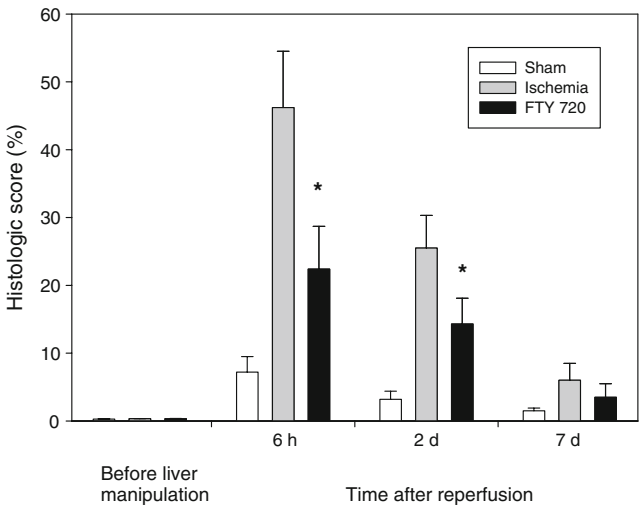

Figure 7 Semiquantitative histological score indices of the liver at different time points before manipulation and after reperfusion. *Asterisks*, significant difference between ischemia and therapy group ($p<0.05$).

of T cell infiltration afforded by FTY720 pretreatment was associated with a reduction in IL-2 expression, suggesting a Th-1-cytokine response may be an important mediator in this process, whereas the expression of the Th-2 cytokine, IL-10, was not significantly affected by FTY720 treatment. T cell activation involves antigen-dependent and antigen-independent pathways.[25] Antigen-independent T cell activation probably plays a pivotal role in I/R und can be upregulated by cytokines, including TNF-a, interferon c, IL-2, and IL-6. Therefore, inhibition of IL-2 upregulation in our model may influence T cell accumulation in the postischemic liver.

Another finding of this study was the marked inhibitory effect of FTY720 on the release of TLR-4. By recognizing bacterial/viral-specific pathogen-associated molecular patterns, TLR represents the host sentinel system responsive to infections.[26] Activation of TLRs triggers an inflammatory response that is mediated by macrophages, neutrophils, and complement. The induced chemokines/cytokines can mediate systemic responses and recruit leukocytes to sites of inflammation. In addition, antigen-presenting cells can be activated by TLR ligands, which may then initiate adaptive T cell responses. Relevant to the mechanism of IRI, endogenous ligands from damaged/stressed cells, including heat shock proteins, heparan sulfate, hyaluronan, and fibronectin, have the capacity to activate TLRs.[27] Indeed, endogenous TLR ligands representing the danger signal may initiate an immune response in the absence of infection. Therefore, a reduction in TLR-4 expression may be a maker for reduced I/R-induced inflammatory response of the liver.[28] Recent studies revealed that TLR activation also may trigger the activation of Kupffer cells during the inflammatory process of I/R.[29] Kupffer cells are known to release a host of inflammatory cytokines such as IL-1, MIP-2, and TNF-α in response to injury or stress.[30–32] Furthermore, a multitude of potentially toxic substances are released from activated Kupffer cells including reactive oxygen intermediates, proteases, and various eicosanoids, which may further induce hepatocellular damage.[33–35]

Several studies point out a remarkable impact of FTY720 to the plasma and tissue concentration of inflammatory and anti-inflammatory cytokines.[6,7,36] Anselmo et al. described an attenuated concentration of IL-1, IL-2, and IL-4 in liver tissue following I/R injury.[7] As far as the tissue mRNA IL-2 concentrations are concerned, their data are in line with our results. However, we were unable to point out a statistically significant difference between tissue concentrations of the other cytokines (TNF-α, IL-10) detected between our treatment group and the controls. The importance of expression of proinflammatory cytokines such as TNF-a and IL-6 was shown by Flach et al.[37] who analyzed the expression of TNF-α, transforming growth factor b, IL-6, IL-8, and IL-10 semiquantitatively in 40 patients undergoing liver transplantation and found that interindividual differences in the induction of TNF-a and IL-6 expression correlate with the clinical course. In several new studies, mainly in mouse models, IL-6 appears to be necessary in the early reperfusion period for liver cell regeneration.[38,39]

Conclusion

Our results indicate that the S1P receptor antagonist FTY720 has beneficial effects on the outcome after warm I/R injury of the rat liver by improving microcirculation and decreasing histologic damages. Furthermore, findings from the present study suggest that the diminishment of circulating T cells also may have anti-inflammatory potential through suppression of the mRNA expression of the genes of proinflammatory cytokines such as IL-6 and TLR-4. These findings may have implications for advanced liver resections with the need of hilus occlusion (Pringle maneuver). Further studies in models with cold ischemia are necessary to determine the importance of this treatment in liver transplantation.

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ORIGINAL ARTICLE

Surgical Complications Following Liver Transplantation in Patients with Portal Vein Thrombosis—A Single-Center Perspective

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Abstract
Introduction Portal vein thrombosis (PVT) was once considered a contraindication for liver transplantation (LTx) because of technical difficulties. Though no longer a contraindication, it remains a risk factor.
Aim A study of surgical complications following LTx in patients with and without PVT.
Patients and methods A retrospective review of 1,171 consecutive patients who underwent LTx between June 1995 and June 2007 was performed, and 78 recipients with PVT (study group) were compared with a stratified random sample of 78 contemporous recipients without PVT (control group) for postoperative complications. Both groups were comparable with respect to age, sex, race, and other confounding variables.
Results The rate of primary nonfunction (PNF) in the study and control groups was 9.0% and 1.3%, (p=0.063), while that of retransplantation was 17.9% and 7.7% (p=0.055), respectively. The mean donor risk index (DRI) among the patients with and without PNF in the study group was 2.58±0.44 and 2.08±0.42, respectively (p=0.014). A significantly higher number of packed red blood cells and fresh frozen plasma transfusions were observed in study group compared to controls (p=0.012, 0.007, respectively).
Conclusion A higher rate of PNF was related to the complexity of the surgical procedure and the use of donor livers with a high DRI. Higher rates of PNF eventually led to a higher rate of retransplant. A strategy of offering donor livers with a low DRI might be helpful in decreasing the rate of PNF. Further, a PV interposition graft in difficult cases instead of thrombectomy could lead to a lower rethrombosis rate.

Keywords Liver transplant · PVT · Infection · Biliary complications · Thrombotic complications

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Introduction

The incidence of portal vein thrombosis (PVT) in patients with end-stage liver disease varies from 5% to 15%.[1,2] When recognized in advance, PVT was once considered an absolute contraindication to liver transplantation,[2–4] until good results were obtained with thrombectomy or a venous interposition graft between the donor portal vein and splenomesenteric confluence.[5] With the evolution of new surgical techniques,[6] many of the technical difficulties were overcome, and encouraging results of liver transplantation (LTx) have been reported in patients with PVT.[7–9] Although

no longer considered a contraindication, PVT is still considered a risk factor for LTx.

The consequences of portal vein thrombosis are related to the extension of the thrombus. Upstream from the thrombus, there is little effect on the intestine as long as the mesenteric venous arches remain patent. Ischemia results from extension of the thrombus into the mesenteric veins and the mesenteric venous arches.[10] When ischemia is prolonged for several days, intestinal edema may follow, and translocation of intestinal bacteria may lead to sepsis. Downstream from the portal vein thrombus, the consequences for the liver are hardly discernible[11–13] due to the arterial "buffer" response, which consists of immediate vasodilatation of the hepatic arterial bed in response to a decreased portal vein flow[14] and a rapid development of collateral veins bypassing the thrombosed portion of the portal vein.[15] Portal pressure, however, is increased.[16] In other words, portal perfusion is maintained at the expense of portal hypertension.

Aim

The purpose of this paper is to study the surgical complications following LTx in patients with and without PVT at our center over a period of 12 years at our center.

Patients and Methods

Design

This is a retrospective cohort study.

Primary end-points:

- Rate of PV rethrombosis,
- Rate of primary non-function (PNF)
- Rate of retransplantation,
- Infectious complications,

A retrospective review of 1,171 consecutive patients who underwent LTx between June 1995 and June 2007 was performed, and 84 patients with PVT were identified with an incidence of 7.15%. The diagnosis was made on the basis of preoperative imaging and confirmed on the operative report. Six patients with live donor liver transplant were excluded from the study to minimize bias, so that 78 deceased donor liver transplant (DDLT) recipients (study group) with PVT were compared with a stratified random sample of 78 DDLT recipients without PVT (control group) for postoperative morbidity. The controls were chosen from all the remaining patients without PVT who underwent primary liver transplant during the same time period. One control was randomly selected for each patient from the remaining patients without PVT who underwent primary liver transplantation during the same study period. The baseline characteristics of the two groups were comparable with respect to age, sex, race, Model for End-Stage Liver Disease score (MELD), donor risk index (DRI; calculated, as described by Feng et al.[17]), cold ischemia time, warm ischemia time, and the primary indication for liver transplant (Table 1). Thus, at the outset, the two groups were equally susceptible to develop complications following LTx.

Data were collected by retrospective chart review on postoperative infectious complications (bacterial, viral, fungal, or mixed), portal vein rethrombosis, and primary nonfunction (PNF). We looked at all postoperative (30-day) infections including wound infection, infected hematoma, peritonitis, urinary catheter-related infections, and line sepsis and, based on culture reports, categorized them into bacterial, fungal, viral, or mixed infections. Rate of retransplant was also calculated. In addition, data were collected on etiology of liver failure, MELD score, DRI, preoperative investigations (CECT scan and/or MRI), intraoperative confirmation of PVT, and its extent. Data were also collected on number of blood products transfused (packed red blood cells (PRBC), fresh frozen plasma (FFP), and platelets) and length of hospital stay.

Statistical analyses were performed using SPSS Windows-based version 15.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). Median and range are used to describe FFP, Platelet and PRBC transfusions and length of hospital stay whereas normal variables are described using mean and standard deviation. All the categorical variables are described using frequency and percentage. The FFP, Platelet and PRBC transfusions were compared between the cases and controls using t-test after taking square root transformation. However, the length of stay was compared using Wilcoxon rank sum test. All the categorical variables were compared between the cases and controls using Pearson's Chi-square test and Fisher's exact test. A p value of <0.05 was considered significant.

Results

Classification of PVT

We classified PVT clinically into partial, if there was some preservation of portal flow, and complete, if there was complete occlusion of the lumen. Fifty-four (69.2%) patients in study group had partial PVT (pPVT), and 24 (30.8%) had complete PVT (cPVT).

PVT was further sub-classified anatomically into four types based on anatomic location of thrombus. This classification was based on preoperative imaging (CT or MRI scans) and intraoperative confirmation.

Type 1 was a pPVT in right or left PV branch (n=12, 15.4%), type 2 was a pPVT in main PV alone (n=34, 43.6%), type 3 was a pPVT in the main PV along with a

Table 1 Demographics

	Study (n=78)	Control (n=78)	p Value
Demographics (study period—June 1995 to June 2007)			
Sex			0.867
Males	51	50	
Females	27	28	
Race			1.000
Caucasian	68	68	1.000
Hispanic	4	4	1.000
Asian	3	3	1.000
African–American	2	2	1.000
Native American	1	1	1.000
Diagnosis			
Laennec's cirrhosis	22 (28.2%)	22 (28.2%)	1.000
Cryptogenic cirrhosis	17 (21.8%)	14 (17.9%)	0.547
HCV	15 (19.2%)	18 (23.1%)	0.556
NASH	9 (11.5%)	4 (5.1%)	0.148
Primary biliary cirrhosis	3 (3.8%)	4 (5.1%)	0.699
HBV	3 (3.8%)	5 (6.4%)	0.468
Autoimmune hepatitis	3 (3.8%)	3 (3.8%)	1.000
Hemochromatosis	2 (2.6%)	2 (2.6%)	1.000
Hepatocellular carcinoma	1 (1.3%)	2 (2.6%)	0.560
α-1-antitrypsin deficiency	2 (2.6%)	0	0.155
Hepatoblastoma	1 (1.3%)	0	0.316
Primary sclerosing cholangitis	0	3 (3.8%)	0.080
Drug toxicity	0	1 (1.3%)	0.316
Other variables			
Age (years) (mean ± SD)	56.76±11.32	57.67±9.33	0.581
Donor age (years) (mean ± SD)	50.21±18.95	51.81±17.75	0.590
Cold ischemia time (hours) (mean ± SD)	11.49±3.24	10.81±3.20	0.215
Warm ischemia time (minutes) (mean ± SD)	45±16	45±18	0.956
DRI[a] (mean ± SD)	2.11±0.44	2.08±0.48	0.675
MELD[b] (mean ± SD)	19.12±7.89	21.06±10.88	0.249

NASH nonalcoholic steatohepatitis, *HCV* hepatitis C viral infection, *HBV* hepatitis B viral infection, *LDLT* live donor liver transplant, *DDLT* deceased donor liver transplant, *DRI* donor risk index, *SD* standard deviation, *ns* not significant, *MELD* model for end stage liver disease

[a] DRI not available for 14 patients in the study group and five patients in the control group

[b] MELD not available for 14 patients operated before Feb 2002 in study group and 12 patients in the control group

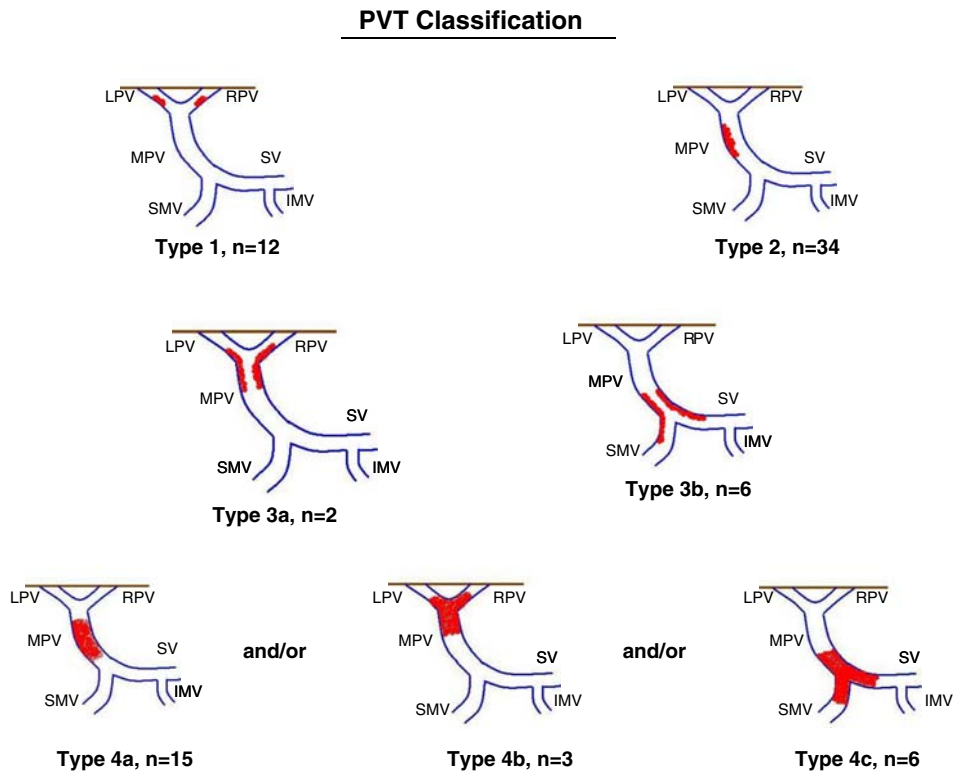
thrombus in right or left branch or both (type 3a: n=2, 2.6%) and/or a pPVT in main PV along with a thrombus in superior mesenteric vein (SMV) or splenic vein (SV) or both (type 3b: n=6, 7.7%; total type 3: n=8, 10.3%), and type 4 was a complete thrombus occluding the main PV alone (type 4a: n=15) with or without the right or left branch (type 4b: n=3), and SMV or SV or both (type 4c: n=6; total type 4, n=24, 30.8%, Fig. 1).

Surgical Management

The portal flow was established using portal vein thrombectomy alone in 58 (74.4%) patients, which includes 30 (38.5%) patients with type 2 PVT, seven (8.9%) patients with type 3 PVT, and 21 (27.0%) patients with type 4 PVT.

In eight (10.3%) patients, we had to use a jump/interposition graft because of extensive long-segment occlusion of PVT. This included four (5.1%) patients with type 2 PVT, one (1.3%) patient with type 3, and three (3.8%) patients with type 4a PVT. A venous jump graft to SMV was used in three (3.84%) patients, and a PV interposition graft was used in five (6.41%) patients. One of these patients was retransplanted twice, first for PNF (45 days after primary transplant) and then for HAT (29 days after second transplant), but finally died. In this group of patients where a jump graft was used, three other patients were retransplanted: one for PNF (10 days after primary transplant), one for portal vein rethrombosis, and one for intrahepatic abscess.

In 12 (15.4%) patients, no additional procedure was required since the thrombus was in the right or left PV branch close to the hilum and was removed along with recipient hepatectomy (Table 2). A higher rate of PV rethrombosis, though not statistically significant (p=1.0), was observed in patients who underwent a thrombectomy



Abbreviations: LPV: left portal vein, RPV: right portal vein, MPV: main portal vein, SMV: superior mesenteric vein, SV: splenic vein, IMV: inferior mesenteric vein.

Figure 1 Anatomical classification of portal vein thrombosis. *Type 1* Partial PVT in right or left PV branch. *Type 2* Partial PVT in main PV alone. *Type 3* Partial PVT in the main PV along with a thrombus in right or left branch or both (*type 3a*) and/or a pPVT in main PV along with a thrombus in superior mesenteric vein (*SMV*) or splenic vein (*SV*) or both (*type 3b*). *Type 4* Complete thrombus occluding the main PV alone (*type 4a*) with or without the right or left branch (*type 4b*), and SMV or SV or both (*type 4c*).

Table 2 Operative Findings

Surgical procedure used *n* (%)	Study (*n*=78)
No additional procedure	12 (15.4%)
Type 1	12 (15.4%)
Thrombectomy	58 (74.3%)
Type 2	30 (38.5%)
Type 3	7 (8.9%)
Type 4	21 (27.0%)
Interposition graft	8 (10.3%)
To superior mesenteric vein	3 (3.8%)
Type 2	2 (2.6%)
Type 4a	1 (1.3%)
To portal vein	5 (6.4%)
Type 2	2 (2.6%)
Type 3b	1 (1.3%)
Type 4a	2 (2.6%)

PVT portal vein thrombosis, *CT* computed tomography scan

alone (*n*=4, 6.89%) compared to those with PV interposition graft (*n*=0).

The median number of PRBC, FFP, and platelets transfused were 12, 10, and 10, respectively, in the study group and 9, 9.5, and 10, respectively, in the control group (p=0.012, 0.007, 0.139, respectively). The median length of hospital stay was 19 days in the study group and 15.5 days in the control group (p=0.039) (Table 3). On subgroup analysis of patients in the study group with partial or complete PVT, no significant difference was observed in the rate of biliary, infectious, or thrombotic complications, PNF, or rate of retransplantation in the two groups (Table 4).

PV Rethrombosis

The rate of PV rethrombosis following LTx in the study group was 6.4% (*n*=5). It was managed with rethrombectomy in two patients, retransplant in two, and by anticoagulation in one patient. In the control group, the rate was 2.6% (*n*=2). The difference in incidence in the two groups was not statistically significant (p=0.246, Table 3).

Table 3 Comparison Between Cases and Controls

	Study (n=78)	Controls (n=78)	p Value
Blood products transfused (median)			
PRBC	12 (range 2–81)	9 (range 1–60)	0.012
FFP	10 (range 2–91)	9.5 (range 2–64)	0.007
Platelets	10 (range 2–75)	10 (range 5–40)	0.139
Hospital stay (median, days)	19.0 (range 7–173)	15.5 (range 8–219)	0.039
Portal vein thrombosis, n (%)	5 (6.4%)	2 (2.6%)	0.246
Infectious complications, n (%)	43 (55.1%)	37 (47.4%)	0.360
Bacterial	35 (44.8%)	29 (37.2%)	0.570
Fungal	3 (3.8%)	0 (0.0%)	0.093
Viral	1 (1.3%)	2 (2.6%)	0.513
Mixed	4 (5.1%)	6 (7.7%)	0.432
Primary nonfunction, n (%)	7 (8.9%)	1 (1.3%)	0.063
Rate of retransplantation, n (%)	14 (17.9%)	6 (7.7%)	0.055

Infectious Complications

In the study group, the rate of infectious complications following LTx was 55.1% (n=43), of which 35 (44.9%) were bacterial, three (3.8%) were fungal, one (1.3%) was viral, and four (5.1%) were mixed infections. In the control group, the rate of infectious complications was 47.4% (n=37), of which 29 (37.2%) were bacterial, two (2.6%) were viral, and ix (7.7%) were mixed infections (Table 3). No fungal infections were observed in the control group. The difference in incidence of infectious complications between the two groups was not statistically significant (p=0.360).

Primary Non-Function and Retransplant

The incidence of PNF was 9.0% (n=7) in the study population, while only one (1.3%) patient in the control group had a PNF (p=0.063, Table 3). The mean DRI in patients with PNF was 2.58±0.44, while in patients in the study group who did not have PNF, DRI was 2.08±0.42 (p=0.014).

The rate of retransplantation in the study group was 17.9% (n=14) and 7.7% (n=6) in the control group (p=0.055). The most common cause of retransplant was PNF (n=6, 7.7%) in the study group and HAT (n=2, 2.6%), recurrent HCV (n=2, 2.6%) in the control group (Table 5). The most common cause of death in both groups was sepsis (n=19, 24.4% in study group, n=9, 11.5% in control group, p=0.095). The causes of retransplantation and death are summarized in Table 5.

Discussion

PVT is a well-recognized complication of end-stage liver disease and occurs in 5% to 15% of patients suffering from this condition.[1] An incidence of 7.15% was observed in our study. PVT has been reported traditionally as partial or complete.[18,19] We find this appropriate for comparison of clinical outcomes. Other authors have classified it into grades 1–4 depending upon the site of thrombus and percentage occlusion of the lumen,[20,21] which appears confusing and incomplete. We believe that an anatomic classification would be simple and more reasonable for reporting the findings and comparing results between institutions and have suggested one such classification (Fig. 1). However, for making clinical comparisons, classification of PVT into partial and complete appears appropriate.

When recognized in advance, PVT was once considered an absolute contraindication to liver transplantation[2–4] until

Table 4 Comparison Between Partial and Complete PVT

	pPVT (n=54)	cPVT (n=24)	p Value
Portal vein thrombosis, n (%)	3 (5.6%)	2 (8.3%)	0.644
Infectious complications, n (%)	32 (59.3%)	11 (45.8%)	0.271
Primary nonfunction, n (%)	5 (9.3%)	2 (8.3%)	0.895
Rate of retransplantation, n (%)	9 (16.7%)	5 (20.8%)	0.658

PVT portal vein thrombosis, *PRBC* packed red blood cells, *FFP* fresh frozen plasma, *DRI* donor risk index, *MELD* model for end-stage liver disease, *SD* standard deviation, *pPVT* partial portal vein thrombosis, *cPVT* complete portal vein thrombosis

Table 5 Causes of Retransplant and Death

Cause	Study (n=78)	Controls (n=78)
Retransplant *n* (%)		
Primary nonfunction	6 (7.7%)	0 (0.0%)
Hepatic artery thrombosis	2 (2.6%)	2 (2.6%)
Portal vein rethrombosis	2 (2.6%)	0 (0.0%)
Biliary cast syndrome	1 (1.3%)	1 (1.3%)
Recurrent HCV	1 (1.3%)	1 (1.3%)
Intrahepatic abscess	1 (1.3%)	0 (0.0%)
Recurrent HBV	1 (1.3%)	0 (0.0%)
Recurrent PSC	0 (0.0%)	1 (1.3%)
HBV	0 (0.0%)	1 (1.3%)
Death *n* (%)		
Sepsis	19 (24.4%)	9 (11.5%)
Unknown	3 (3.8%)	2 (2.6%)
Metastatic cancer	3 (3.8%)	4 (5.1%)
Cardiac arrest	3 (3.8%)	7 (9.0%)
Liver failure	1 (1.3%)	0 (0.0%)
Intracranial bleed	0 (0.0%)	1 (1.3%)

HCV hepatitis C viral infection, *PSC* primary sclerosing cholangitis, *HBV* hepatitis B viral infection

good results were obtained with thrombectomy or a venous interposition graft between the donor portal vein and splenomesenteric confluence.[5] Some of the recent studies have reported encouraging results of LTx in patients with PVT.[7–9] We did not exclude any patient, including those with complete thrombus extending to SMV or SV. The type of operative strategy depends on the extent of thrombosis. Thrombectomy and direct venous anastomosis is recommended in patients when the thrombosis is partial and involves portal vein with or without SMV.[6,20,22] In cases of complete PVT with patent SMV, venous jump graft to SMV is an alternative.[6,20,22] In cases where the portal vein is not amenable to thrombectomy, and the SMV is also thrombosed, the coronary vein can also be used for inflow.[22] For the patients with extensive and complete occlusion of the portal and proximal SMV as well as the distal SMV, cavoportal hemitransposition has been described;[23] however, the survival rate is significantly reduced when this technique is employed and the complication rate among survivors is significant.

In our study, during surgery, declotting of PV was done in all patients. If after declotting, good flow was established, nothing else was done; if not, then a jump graft to SMV or a PV interposition graft was used. Among 12 (15.4%) patients with type 1 PVT, no additional surgical procedure was needed since, being close to the liver hilum, this portion of portal vein was excised as a part of recipient hepatectomy. A good portal flow was achieved using thrombectomy alone in 58 (74.3%) patients (Table 2). In eight (10.3%) patients, we had to use a jump graft because of extensive long-segment occlusion of PVT. A jump graft to SMV was used in three and an interposition graft to PV in five patients.

Another major risk for liver transplant recipients with PVT is early rethrombosis. The incidence varies from 4.2% to 38.5%.[6,19,24–29] The use of therapeutic[28] or prophylactic[25] anticoagulation for 3 months to prevent thrombosis has been recommended. We routinely use anticoagulation after LTx to prevent rethrombosis. Partial rethrombosis might manifest with acute deteriorating liver function and complications secondary to portal hypertension, such as ascites or gastrointestinal bleeding. If detected early, it can be treated effectively with rethrombectomy. However, a delay in this diagnosis may lead to graft loss and retransplantation.[8] Frequent Doppler ultrasonography in the post-LTx period may prevent delay in the diagnosis of PVT and, therefore, the need for retransplantation and is recommended every 1 to 3 days during the first 2 weeks after LTx.[30,31]

The incidence of rethrombosis in our series was 6.4% (n=5). In two of these patients, there was a graft loss, and they had to be retransplanted. In one, it was managed with anticoagulation using heparin, and in another two patients, it required a re-exploration with rethrombectomy. One patient died following rethrombectomy, 1.4 months after re-exploration, the remaining four are alive and doing well. While in the control group, the observed incidence of portal vein thrombosis after LTx was 2.6% (n=2). The most important risk factor for rethrombosis is believed to be the extent of thrombus within the portal venous bed, and it has even been recommended to avoid retransplant in patients with complete PVT with extension throughout the portal venous bed.[8]

We found that four patients (6.9%) out of 58 who had undergone thrombectomy had a rethrombosis, while one patient out of three who received a jump graft to SMV had a rethrombosis and none of the five patients who received a PV interposition graft had rethrombosis. A probable explanation could be intimal injury following thrombectomy with resultant higher incidence of rethrombosis while patients with a PV interposition graft receive a healthy vessel with resultant no rethrombosis. The reason for rethrombosis in the patient who received a jump graft to SV was related to kinking of the vessel. A strategy of doing a PV interposition graft in difficult cases instead of thrombectomy could lead to a lower rethrombosis rate.

A significantly higher number of PRBC and FFP transfusion were recorded in study group as compared to the controls, highlighting the complexity of the surgical procedure with a difficult dissection and more intraoperative bleeding.

Interestingly, the incidence of PNF was 8.9% in the study population and 1.3% in the control group. This difference approached statistical significance. A possible explanation could be the fact that a difficult portal dissection in the presence of occluded portal vein and severe portal hypertension results in significant bleeding during hepatectomy. As a result, during the anhepatic phase, the patients develop severe acidosis and coagulopathy. The new liver graft is thus transplanted under less than optimal conditions in a compromised host, and the probabilities for failure are increased.[29] We postulate that this partly explains the high rate of PNF in the graft.

Out of the seven patients with PNF in the study group, six patients were retransplanted 3, 10, 29, 47, 8, and 5 days, respectively, after primary transplant. One died without intervention 45 days following LTx. The mean DRI in patients with PNF was significantly higher than patients in the study group who did not have PNF, which could also be a contributing factor for PNF.[17] The only patient with PNF in the control group died without intervention soon after primary transplant.

The rate of retransplantation in the study group was 17.9% (n=14), while in the control group, it was 7.7% (n=6). This difference could be accounted for by a higher incidence of PNF and portal vein rethrombosis in the study group compared to the controls.

Liver transplant recipients with PVT, especially the patients who have more than 50% of portal vein occlusion with or without SMV occlusion, are considered more prone to develop severe perioperative complications and a higher mortality rate.[8,20,32] Theoretically, one would assume a greater incidence of postoperative infectious complications in patients with complete and long-standing PVT as a result of mesenteric ischemia,[10] intestinal edema with consequent bacterial translocation, and sepsis. It is quite difficult, however, to attribute postoperative infection to PVT due to the involvement of several factors which can give rise to infection in the postoperative period. We looked at all postoperative (30-day) infections. We, however, did not find any statistically significant difference in postoperative infectious complications. However, there was a relatively higher incidence of fungal infections in the study group (n=3, 3.8%) compared to controls (n=0). This might suggest the need for prophylactic antifungal therapy in patients with PVT undergoing LTx, though the evidence in support for prophylactic antifungal therapy is not strong.

In conclusion, a higher rate of PNF was related to both the complexity of the surgical procedure and the use of donor livers with a high DRI. Higher rates of PNF eventually led to a higher rate of retransplant. A strategy of offering donor livers with a low DRI might be helpful in decreasing the rate of PNF in patients with PVT, though on the basis of this retrospective analysis alone, it is difficult to make a compelling argument. Further, a PV interposition graft in difficult cases instead of thrombectomy could lead to a lower rethrombosis rate. However, given the retrospective nature of the study, the evidence in support of these conclusions is not strong, and multicenter studies are needed to establish concrete recommendations.

Conflicts of Interest None

Prior Publication None

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ORIGINAL ARTICLE

Acute Hyperglycemia Worsens Hepatic Ischemia/Reperfusion Injury in Rats

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Abstract
Background/Aims Acute hyperglycemia is known to worsen ischemia/reperfusion (I/R) injury following myocardial infarction and stroke. We investigated whether acute hyperglycemia worsens injury and amplifies the inflammatory response evoked by hepatic I/R.
Methods Rats were pretreated with an intraperitoneal injection of 25% glucose or 0.9% sodium chloride (10 ml/kg BW). Subsequently, rats underwent partial (70%) hepatic ischemia for 45 min. After 4 h of reperfusion, hepatic injury, oxidative stress, inflammation, and heat shock protein expression were assessed.
Results Liver injury was increased in the hyperglycemic group with alanine aminotransferase (ALT) and aspartate aminotransferease (AST) serum concentrations of 7,832±3,374 and 10,677±4,110 U/L compared to 3,245±2,009 and 5,386±3,393 U/L ($p<0.05$ vs. control). Hyperglycemic I/R was associated with increased liver nitrotyrosine concentrations and increased neutrophil infiltration. I/R upregulated the protective heat shock proteins HSP32 and HSP70 in control animals, but this protective mechanism was inhibited by hyperglycemia: HSP32 expression decreased from 1.97±0.89 (control) to 0.46±0.13 (hyperglycemia), HSP70 expression decreased from 18.99±11.55 (control) to 3.22±0.56 (hyperglycemia), (expression normalized to sham, both $p<0.05$ vs. control I/R).
Conclusions Acute hyperglycemia worsens hepatic I/R injury by amplifying oxidative stress and the inflammatory response to I/R. The increase in injury is associated with a downregulation of the protective heat shock proteins HSP32 and HSP70.

Keywords Liver · Surgery · Inflammatory response · Neutrophils · Metabolism

Introduction

Acute hyperglycemia is frequently seen in hospitalized patients and induced by stressors such as acute illness and surgical trauma. Such transient increases in blood glucose concentrations may put patients at risk for adverse out-

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comes. Hyperglycemia independent of preexisting diabetes mellitus is an established risk factor for increased mortality and morbidity after cardiac surgery.[1] Patients without a history of diabetes who were hyperglycemic at admission to the hospital had higher mortality and lower functional outcomes than normoglycemic and even hyperglycemic diabetic patients.[2] Van den Berghe et al.[3] showed that intensive insulin therapy (IIT) reduces in-hospital mortality in surgical intensive care unit patients by 34% with subsequent investigations confirming that maintaining normoglycemia rather than glycemia-independent effects of insulin is responsible for the beneficial effects of IIT.[4,5] These findings emphasize the potential hazards of poor glucose control on patient outcome.

The detrimental effects of hyperglycemia do not require chronic exposure or preexisting diabetes. Animal models of acute hyperglycemia confirm the deleterious effects of even short episodes of hyperglycemia on cerebral[6] and renal ischemia/reperfusion (I/R) injury.[7] Proposed mechanisms for the detrimental effects of acute hyperglycemia are increased oxidative stress, an enhanced inflammatory response with cytokine activation[8,9] and impaired blood flow with reperfusion.[10]

Diabetic mice have been shown to be more susceptible to liver ischemia,[11,12] but so far, the effects of acute hyperglycemia on liver I/R injury have not been addressed. We therefore used a rat model of acute hyperglycemia to investigate its effects on hepatic I/R injury.

Material and Methods

Animal Model

All animal experiments were carried out with approval by the local committee on animal research. Animal care was in agreement with the National Institutes of Health guidelines for ethical research (NIH publication no. 80-123, revised 1985). Inbred male Lewis rats (Harlan, Indianapolis, IN, USA) were used for this study. Animals' weights on arrival at our facility were 250–300 g. Animals had access to standard laboratory diet and were maintained on a light–dark cycle. They were fasted 12 h prior to the start of the experiments. Prior to the study, animals spent several days in the animal care facility for acclimatization.

The rats were divided into hyperglycemic and control group. In the hyperglycemic group (HG, n=8), 2.5 g/kg glucose (25% solution) was injected intraperitoneally following the assessment of the baseline glucose serum concentration. The control group (CON, n=8) received 10 ml/kg 0.9% saline instead. Thirty minutes later, rats were anesthetized with isoflurane. Following liver exposure through a midline incision and collection of blood samples, hepatic ischemia was induced. Applying a 70% liver ischemia model, the liver was mobilized, and vascular structures to the left and median lobe were identified and clamped for 45 min using a bulldog clamp. The unoccluded right and caudate lobe allow outflow from the splanchnic circulation, thus avoiding venous congestion. For the duration of hepatic ischemia, the abdominal cavity was closed with clamps. Rectal temperature was continuously assessed using an electronic thermometer (RSP TM-200D, Respiratory Support Products Inc., Santa Ana, CA, USA using a Mallinckrodt probe, cat no. 502-0401, Mallinckrodt Inc., St. Louis, MO, USA) and held constant at 37°C using a heating lamp.

Following reperfusion, the animals received 5 ml of normal saline intraperitoneally, and the incision was closed in two layers. Animals were killed following a 4 h observation period. Blood and tissue were harvested. All tissue was immediately frozen in liquid nitrogen and stored at −80°C until further processing.

Sham experiments (Sham, n=5) served as reference for subsequent analysis. Sham experiments were identical to control I/R experiments except that hepatic vessels were not clamped. Hyperglycemic sham experiments (HG Sham, n=4) were added to the protocol to identify the effects of hyperglycemia alone.

Biochemical Markers of Liver Injury

Serum levels of aspartate aminotransferease (AST) and alanine aminotransferase (ALT) were determined at baseline and following 4 h of reperfusion. The analysis was done in the General Laboratory, San Francisco General Hospital, University of California, San Francisco.

Histology

Liver samples were fixed in 10% buffered formalin and processed for routine histology. Five-micron paraffin-embedded tissue sections were stained with hematoxylin and eosin and examined using standard light microscopy by a pathologist (R.R.) who was blinded to the experimental condition of the animals. Sections were scored from 0–4 for sinusoidal congestion, vacuolization of hepatocyte cytoplasm, and parenchymal necrosis as described by Suzuki et al. (Table 1).[13]

Intrahepatic Neutrophil Accumulation Assessment

Activity of myeloperoxidase (MPO), an enzyme stored in the azurophilic granules of neutrophils, was used to measure tissue neutrophil sequestration. We used a spectrophotometric method to assay tissue MPO activity. Frozen livers were thawed and extracted for MPO following

Table 1 Suzuki Score for the Assessment of Liver Damage Following Hepatic Ischemia/Reperfusion

Score	Congestion	Vacuolization	Necrosis
0	None	None	None
1	Minimal	Minimal	Single cell necrosis
2	Mild	Mild	−30%
3	Moderate	Moderate	−60%
4	Severe	Severe	>60%

homogenization and sonication. The assay is based on the oxidation of 3,3′,5,5′-tetramethyl benzydine by MPO in the presence of H_2O_2. Units of MPO activity were calculated using a standard curve derived from a MPO standard sample (Calbiochem, EMD Bioscience, La Jolla, CA, USA). MPO data are expressed as microunits per milligram of tissue per minute.

Protein Isolation and Western Blots

All steps for protein isolation were conducted at 4°C. Snap-frozen liver sections were homogenized in Tissue Protein Extraction Reagent (Pierce Biotechnology, Rockford, IL) containing 1 mm EDTA and 1:100 Protease Cocktail Inhibitor (Sigma, St. Louis, MO) and were centrifuged at 10,000×*g* for 5 min. The supernatant was aliquoted, snap-frozen, and stored at −80°C. Protein concentrations of liver homogenates were measured by the Pierce bicinchoninic acid protein assay with bovine serum albumin as the standard. Fifty micrograms of liver homogenates was separated on a Novex-NuPAGE 10% Bis-Tris sodium dodecyl sulfate polyacrylamide gel electrophoresis gel (Invitrogen) and transferred to nitrocellulose membrane using the XCell SureLock system (Invitrogen). A mouse anti-heat shock protein 70 (HSP70) monoclonal antibody (SC-24) and a goat anti-actin antibody (SC-1616) from Santa Cruz Biotechnology (Santa Cruz, CA) were used. In addition, a mouse anti-heat shock protein 32 (HSP32) monoclonal antibody from Stressgen (Ann Arbor, MI), a mouse anti-nitrotyrosine antibody from Abcam Inc. (Cambridge, MA), and a rabbit anti-cleaved caspase-3 monoclonal antibody (CST 9661) from Cell Signaling Technology (Danvers, MA) were used for the Western blots. The membranes were incubated with a 1:100 or 1:1,000 dilution of the primary antibody followed by a 1:10,000-fold dilution of a secondary anti-mouse or anti-goat immunoglobulin G from Santa Cruz Biotechnology. Immunoreactive proteins were developed using SuperSignal West Dura (Pierce Biotechnology) and visualized on the FluorChem 5500 Imaging system from Alpha Innotech (San Leandro, CA). Band intensities were quantified via spot densitometry.

Statistical Analysis

All data are presented as mean±SD. Comparison between study groups was performed using analysis of variance with post hoc Dunnett correction, with normoglycemic sham animals serving as controls. Comparison of the two ischemic groups alone was done using a two-tailed unpaired *t* test. p values<0.05 were considered as being statistically significant.

Results

Intraperitoneal injection and surgery alone resulted in an increase in serum glucose concentrations from 89±19 baseline to 182±32 mg/dL in the saline pre-treated group. In

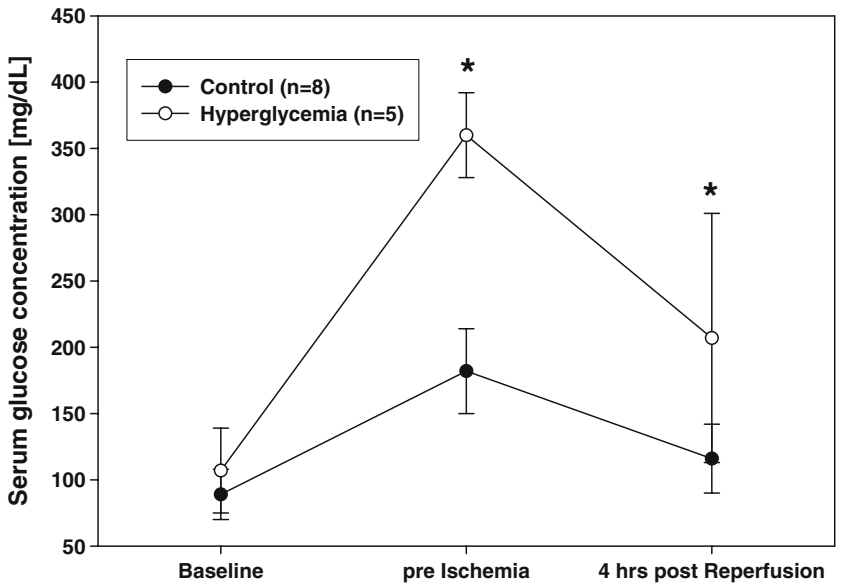

Figure 1 Intraperitoneal injection of saline and surgery alone increased serum glucose concentrations in control animals. Intraperitoneal injection of 2.5 g glucose/kg resulted in significantly higher serum glucose concentrations. After 4 h of reperfusion, glucose concentrations were still significantly higher in hyperglycemic animals. *$p<0.05$ vs. control.

the glucose-pretreated group, three animals were not considered for subsequent analysis due to an only moderate increase in serum glucose concentrations (<250 mg/dL, 30 min after treatment). In the five remaining animals, serum glucose concentrations before ischemia increased from 107±32 to 360±32 mg/dL. At the end of the 4-h reperfusion period, serum glucose concentrations remained higher in the glucose pretreated group (Fig. 1).

Serum Marker of Liver Injury Serum transaminase concentrations following I/R were higher in the glucose-pretreated animals: 7,832±3,374 vs. 3,245±2,009 U/L (ALT, $p<0.05$) and 10,677±4,119 vs. 5,385±3,393 U/L (AST, $p<0.05$). Transaminase concentrations after 4 h of reperfusion were correlated with glucose concentrations before ischemia of all animals that entered the study (Fig. 2).

Histology Both experimental groups showed liver damage including vacuolization and at least minimal congestion and single-cell necrosis (Fig. 3). Damage was graded using the Suzuki score. There was no statistical difference between hyperglycemic animals and control animals in Suzuki scores (6.0±2.2 vs. 6.1±1.8) or necrosis scores (2.0±0.8 vs. 2.0±0.8). Whether individual cell death after 4 h of reperfusion was attributable to necrosis or apoptosis could not be determined by histology. Using nuclear features to distinguish between types of cell death is not considered reliable,[14] and both experimental groups demonstrated zonal as well as spotty areas of dead hepatocytes (Fig. 3).

Apoptosis Cleaved caspase-3 expression was higher in control animals (2.12±0.47) vs. hyperglycemic animals (1.49±0.42) when compared to sham animals (1.00±0.10), indicating more apoptotic cells in livers from control animals Fig. (4)

Oxidative Stress Nitrotyrosine concentrations after 4 h of reperfusion were higher in hyperglycemic animals (1.63±0.54-fold when compared to control animals (1.00±0.30), $p<0.05$) indicating increased oxidative stress resulting in nitration of tyrosine residues of proteins by peroxynitrite.

Inflammation MPO activity in the liver after 4 h of reperfusion was higher in glucose-pretreated animals (5,383±2,512 vs. 2,219±2,086 mU/mg protein^{-1} min^{-1}, $p<0.05$), indicating increased neutrophil migration into the hepatic tissue of hyperglycemic animals (Fig. 5).

Heat Shock Protein activation I/R increased HSP32 expression in control but suppressed HSP32 expression in hyperglycemic animals (1.97±0.89-fold vs. 0.46±0.13-fold when normalized to sham animals, $p<0.05$; Fig. 6a). Hyperglycemia alone without I/R (hyperglycemic sham) did not affect HSP32 expression (0.92±0.20 vs. 1.00±0.16 when compared to control sham). I/R increased HSP70 expression in control animals more than in hyperglycemic animals (19.99±11.55-fold vs. 3.22±0.56-fold when normalized to sham, $p<0.05$; Fig. 6b). Again, hyperglycemia alone without I/R did not affect HSP70 expression (0.94±0.08 vs. 1.00±0.15) when compared to control sham.

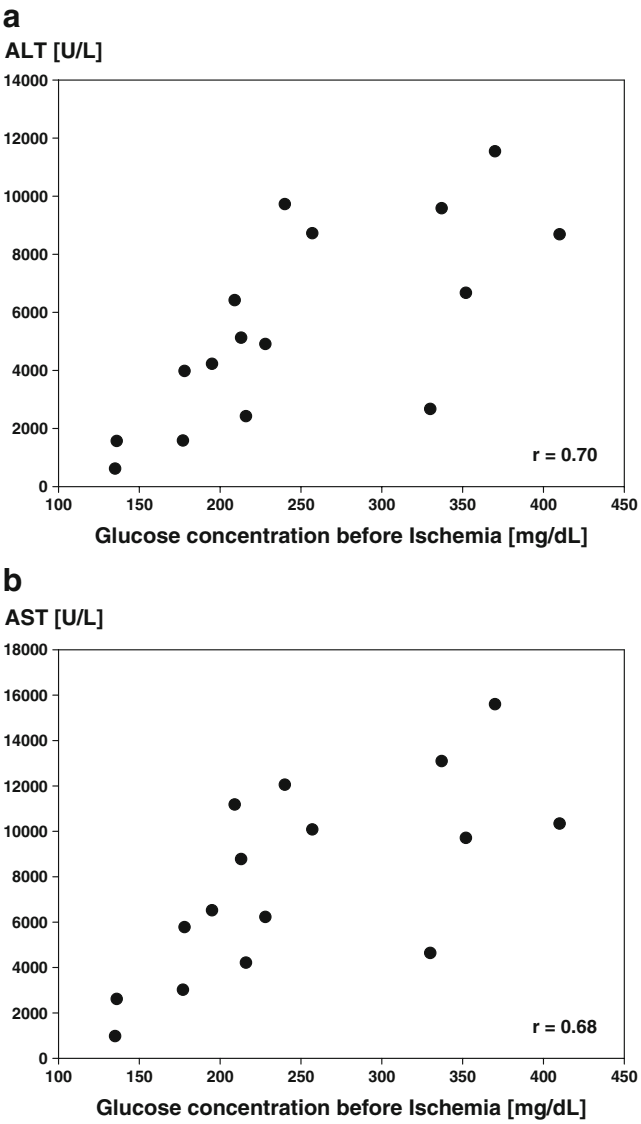

Figure 2 Correlation of serum glucose concentrations before the start of ischemia and serum concentrations of ALT (**a**) and AST (**b**) after 4 h of reperfusion. Liver injury, as assessed by transaminase concentrations and glucose concentrations, was correlated with correlation coefficients of $r=0.70$ (ALT) and $r=0.68$ (AST).

Discussion

Acute hyperglycemia during hepatic ischemia amplified the inflammatory response and resulted in elevated transami-

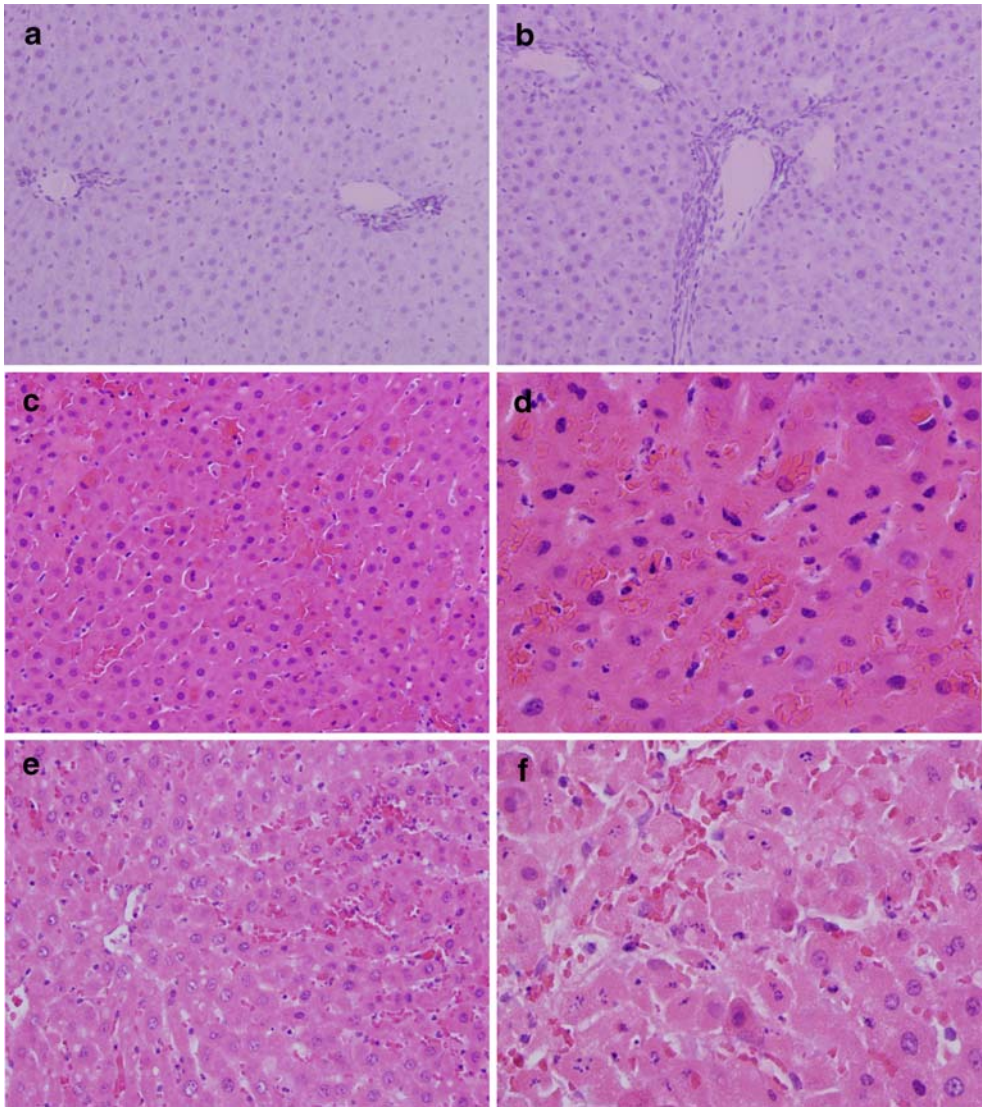

Figure 3 Sham-treated animals show no significant congestion at low power (**a** ×100). Vacuolization and cellular necrosis are not evident in periportal hepatocytes (**b** ×200) or in centrizonal areas. Hepatic architecture is unremarkable. In contrast, control animals following 45 min of ischemia and 4 h of reperfusion show diffuse, moderate sinusoidal congestion, with numerous sinusoidal channels distended by red blood cells in several areas of the liver section (**c** ×200). Two dying hepatocytes (likely evolving into Councilman bodies) are visible in the center of the field (**c**). Mild parenchymal vacuolization is visible in some hepatocytes, with several hepatocytes in this field showing irregular nuclear contours, chromatin condensation, pyknosis, and nuclear dust, histologic evidence of cell damage, and evolving cell death (**d** ×400). Moderate sinusoidal congestion also is seen in hyperglycemic animals treated with high levels of dextrose before ischemia (**e** ×200). This particular animal showed both zones of necrosis and patchy single-cell necrosis. Cell damage is seen in several adjacent hepatocytes containing vacuolated cytoplasm, pyknotic nuclei, and nuclear dust. These dying hepatocytes also show paler cytoplasm than their undamaged counterparts nearby (**f** ×400).

nase concentrations following I/R. The elevated serum glucose concentration at the start of ischemia seemed to be responsible for the increase in injury, as there was a strong correlation between serum glucose concentrations before ischemia and transaminase concentration after 4 h of reperfusion. We used a transient model of hyperglycemia starting only shortly before ischemia. Serum glucose concentrations were still higher in hyperglycemic animals at the end of the 4 h reperfusion period, albeit the graph (Fig. 1) clearly demonstrates a declining trend.

Hepatic I/R has been reported to result in hepatocyte death by two different pathways, necrosis and apoptosis. Whether apoptotic or necrotic cell death predominates following liver I/R has been the subject of debate. Based on terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, it was suggested that sinusoidal endothelial cells and then subsequently hepatocytes undergo apoptosis but rarely necrosis following 60 min of liver ischemia.[15] However, a later study applying a very similar ischemia model found only few apoptotic cells and

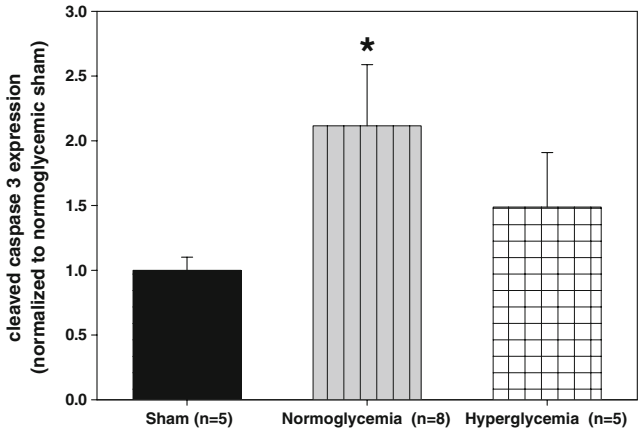

Figure 4 Cleaved caspase-3 expression was highest in control animals, suggesting preferential apoptotic cell death in animals that were not pretreated with glucose. Data are presented as mean±SD. *$p<0.05$ vs. sham.

predominantly necrosis following 60 min of ischemia when combining TUNEL with morphological criteria.[16] A subsequent review emphasized that apoptosis and necrosis share features and mechanisms that can make discrimination between both forms of cell death very challenging. In particular, the TUNEL assay is not suited to differentiate between necrosis and apoptosis, since DNA fragmentation was reported in apoptosis as well as necrosis.[17]

In the present study, histological assessment of the liver samples after 4 h of reperfusion could not reliably distinguish between apoptotic and necrotic cell death. A longer reperfusion could potentially facilitate histological analysis, but 4 h of reperfusion was chosen to enable the detection of inflammatory mediators. As a result, the histological scores were basically identical in both experimental groups, in spite of serologic evidence for increased

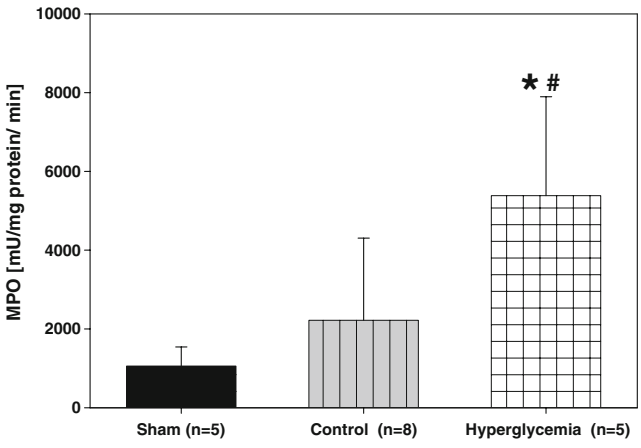

Figure 5 Myeloperoxidase activity was increased in liver homogenates of hyperglycemic animals when compared to control animals, indicating increased neutrophil accumulation after 4 h of reperfusion. Data are presented as mean±SD. *$p<0.05$ vs. sham, #$p<0.05$ vs. control I/R.

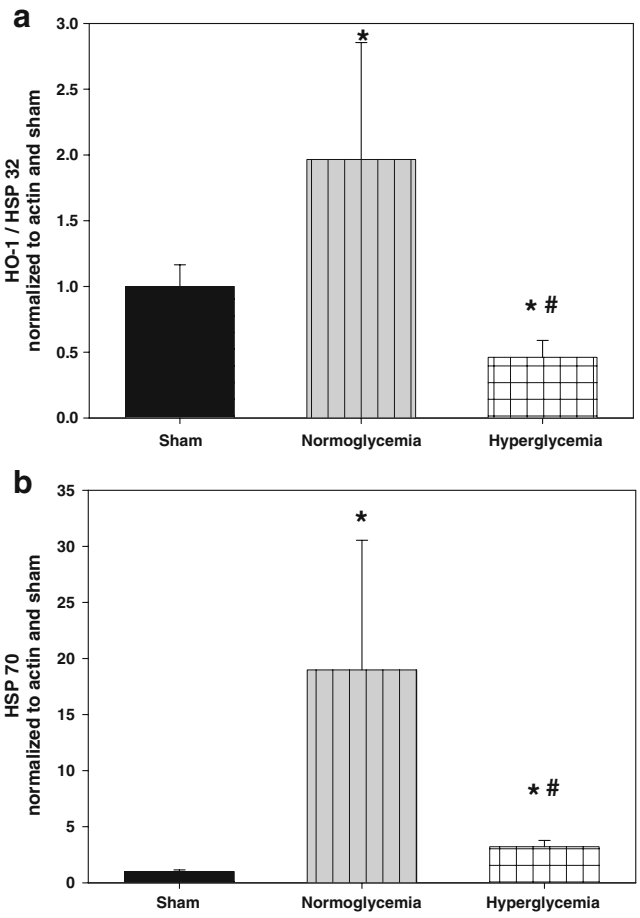

Figure 6 Heat shock protein expression as assessed by Western blot for HSP32 (hemeoxygenase-1) (**a**) and HSP70 (**b**). I/R resulted in a distinct activation of both HSP32 and HSP70 expression in control animals. However, hyperglycemia ameliorated the activation of HSP70 by I/R and suppressed HSP32 expression. Densitometric values were normalized to actin and are expressed as ratios of sham± SD. *$p<0.05$ vs. sham, #$p<0.05$ vs. control I/R.

necrotic cell death in hyperglycemic animals. The higher transaminase concentrations measured in the hyperglycemic group after 4 h of reperfusion reflect increased cellular breakdown due to necrosis with release of intracellular enzymes. Apoptosis maintains the barrier function of the cell membrane and would contribute only to a minor extent to elevated transaminase concentrations. Caspase-3 activation is considered the most reliable method for the detection of apoptosis.[14] We assessed caspase-3 activation to quantify the amount of apoptotic cell death and found higher apoptosis scores in the control group. The lower caspase-3 activation in the hyperglycemic group may be further evidence that necrosis, not apoptosis, is the preferential form of cell death in hyperglycemic conditions.

Hyperglycemia per se is known to increase oxidative stress and to cause a proinflammatory state.[8] Furthermore, hyperglycemia has been shown to amplify the inflammatory response caused by stressors such as LPS

administration.[18] Our results support the hypothesis that the mechanisms responsible for increased ischemic injury by hyperglycemia are the amplification of oxidative stress and of the inflammatory response normally seen with I/R. The increased concentration of nitrotyrosine containing protein is an established marker for severe oxidative stress. Reactive oxygen species, such as the superoxide radical, react with NO to form the more potent peroxynitrite species, which then subsequently nitrate tyrosine residues of proteins, leading to inactivation of key cellular proteins, DNA damage, and eventually cell death.[19] While Kupffer cell-induced oxidative stress is considered the first step in I/R injury,[20] it is followed by a profound inflammatory response that is largely responsible for the extent of I/R injury. This inflammatory response culminates in the hepatic accumulation of neutrophils, which directly damage hepatocytes by releasing oxidants and proteases. The MPO assay confirmed an increased neutrophil infiltration in the liver tissue of hyperglycemic animals. This neutrophil migration and infiltration is initiated by the production and release of cytokines such as tumor necrosis factor alpha and interleukin-6. Earlier studies demonstrated that hyperglycemia enhances cytokine production in response to stress.[21]

A surprising finding of the present investigation was the downregulation of HSP32 and HSP70 in hyperglycemic animals undergoing I/R. Hepatic I/R normally results in upregulation of HSPs, and the observed effects in livers from hyperglycemic animals differ distinctly from the situation in kidneys[7] and the brain,[22] where hyperglycemic I/R injury is associated with an increased activation of HSPs. We could demonstrate in sham experiments that hyperglycemia alone was not responsible for the downregulation of HSPs (data not shown) but that the combination of hyperglycemia and I/R is required to block or even suppress HSP activation. Since HSPs are one of the most potent protective mechanisms against I/R injury, it can be assumed that their suppression in hyperglycemic I/R contributes to the increased injury during acute hyperglycemia. Inhibition of HSP activation in response to ischemia has so far not been described in other organs and may represent a liver-specific (mal-)adaptation to hyperglycemia: It has been described before that diabetes does inhibit hepatic HSP70 activation by heat stress,[23] although subsequent studies did not confirm this finding.[24,25]

The mechanism responsible for the downregulation of HSPs remains to be defined. The expression of the heat shock genes encoding the different HSPs is regulated by heat shock transcription factors (HSFs), which are normally bound to HSPs within the cytosol. When cells are exposed to stress, HSFs are phosphorylated and form trimers that enter the nucleus and bind the heat shock elements located within the promoter of heat shock genes, thus initiating increased expression of HSPs.[26] It has been hypothesized that, in diabetes, the activation of HSF is inhibited in insulin-sensitive tissue.[27] In type 2 diabetic primates, livers had reduced HSP70 and HSP90 tissue concentrations that were related to 50% lower levels of the transcription factor heat shock factor 1.[28] But again, these results are challenged by a study that showed similar heat shock factor 1 content in livers from control and streptozotocin treated rats following heat stress.[24] Further interventional studies with activation of HSPs are planned to show whether the suppression of HSP activation is responsible for the worsened injury during hyperglycemia and whether activation of HSPs is capable of reversing such detrimental effects.

Conclusions

Acute hyperglycemia worsened liver injury as assessed by increased transaminase concentrations following hepatic I/R in rats. The effects of hyperglycemia on liver injury were associated with increased hepatic oxidative stress, an increased inflammatory response, and a suppression of HSP activation. These results, in spite of their descriptive nature, emphasize the need to better understand the role of hyperglycemia in organ injury, especially in clinical scenarios associated with a risk for ischemia. Glucose concentrations in this study were overall high, suggesting that glucose control may not need to be very aggressive to have beneficial effects. Preventing severe hyperglycemia alone may reduce I/R injury, thus avoiding the inherent risk of an IIT to cause undesired hypoglycemia.

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ORIGINAL ARTICLE

Solitary Necrotic Nodule of the Liver: Always Benign?

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Abstract
Objective Solitary necrotic nodule of the liver (SNNL) is a rare lesion and accepted as a benign entity. The aim of this study is to investigate the possible causes for the development of solitary necrotic nodules.
Methods Twenty-two retrospective solitary necrotic nodule specimens were examined to evaluate histologic features. The clinical records of these patients were reviewed, and clinical data were obtained for all patients.
Results Histologically, 17 of the 22 nodules were necrotic with surrounding fibrosis, and the remaining five nodules were completely fibrotic. Four of the 22 cases were found to have specific lesions within the nodules which may put light on the pathogenesis. Foci of metastatic carcinoma were identified in two of these four cases, and cuticle fragments of the hydatid cyst were identified in the other two cases. Clinical data showed that half of the cases with solitary necrotic nodule have an associated malignancy mainly involving the gastrointestinal system.
Conclusions SNNL is not always benign. The possible causes of this lesion include parasites and metastatic tumors. It is important to identify the minute foci of metastatic carcinoma for the appropriate management of this lesion.

Keywords Nodule · Necrotic · Liver · Parasites · Metastasis

Introduction

Solitary necrotic nodule of the liver (SNNL) is a very rare lesion. It was first described by Shepherd and Lee in 1983.[1] Slightly over 100 cases were reported in the English literature. The cases reported were clinically asymptomatic, and majority of the lesions were solitary. SNNL was described as the end stage of the natural history of the infections and degenerative causes.[2] Therefore, most of the cases were accepted as benign lesions. However, underlying causes of these lesions were identified in a small number of cases.[3]

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SNNL has not been adequately studied because of the scarcity of these lesions. The lack of sufficient data in the literature on SNNL encouraged us to conduct the present study. We report a case series of SNNL which put light on the etiopathogenesis of this rare lesion.

Methods

The files of the Pathology Department of Erciyes University Hospital were searched for liver lesions diagnosed as "solitary necrotic nodule" or "solitary fibrotic nodule" between January 1999 and May 2009. A total number of 22 patients who underwent surgical excision were identified. The clinical data including patient age, gender, clinical features, and medical history were obtained from the medical records. None of these patients received chemotherapy, radiation therapy, or hepatic ablative therapies prior to the surgery. Formalin-fixed paraffin-embedded tissue blocks were obtained from archives of the Department of Pathology. All blocks of the nodules were evaluated by performing three serial sections in 4-μm thickness. The

hematoxylin-and-eosin-stained slides were examined under light microscopy by two pathologists.

Results

Clinical Findings

Clinicopathologic features of the patients are summarized in Table 1. Of the 22 patients, 11 were female and 11 were male. Their ages ranged between 27 and 81 years with a mean of 60.7 years. Review of the patients' clinical data revealed that 13 patients (59%) have extrahepatic tumors, including five patients with colorectal carcinoma, five patients with gastric carcinoma, one patient with pancreatic carcinoma, one patient with cervix carcinoma, and one patient with ovarian dermoid cyst. Two of the remaining patients have hydatid cyst disease. None of the patients had associated chronic liver disorder.

The nodules were found during the surgery in 14 patients incidentally. In eight patients, nodules were detected by preoperative evaluation for surgery. Only four of the patients were suspected to have SNNL preoperatively. The presumptive diagnoses of hemangioma and cyst were made in the remaining four patients.

Computed tomography (CT) scans were performed in 13 patients; abdominal ultrasonography (USG) was performed in 15 patients, and both CT and USG were performed in five patients. Seven of the lesions were found by imaging studies while others were not displayed. CT scans demonstrated nodules in three patients, and USG demonstrated nodules in five patients. SNNL was described as hypodense nodules by CT and hypoechoic lesions with calcification by USG.

Seventeen nodules were accessible for the location in the liver, and ten nodules were located in the right liver lobe and seven nodules in the left liver lobe. Segmental distribution of the nodules was given in Fig. 1. All of these nodules were found to be located in the subcapsular region and treated with wedge resection or simple excision.

Pathologic Findings

Grossly nodules ranged from 4 to 14 mm in greatest diameter (mean 6.8 mm). All of the nodules were solitary. The center of the nodules was mainly composed of necrotic and eosinophilic granular material with fibrotic rim in 17

Table 1 Clinicopathologic Features of the 22 Patients with Solitary Necrotic Nodule of the Liver

Patient no.	Age/ gender	Tumor size	Associated disorder	Histology	Calcification
1	35/F	6	Hydatid cyst	Necrotic nodule with foci of hydatid cyst cuticles and foreign body reaction	+
2	69/F	6	Colon carcinoma	Fibrotic nodule with foci of adenocarcinoma	−
3	58/M	8	NA	Necrotic nodule	+
4	45/M	10	NA	Necrotic nodule	+
5	67/F	6	Colon carcinoma	Necrotic nodule with foci of adenocarcinoma	−
6	69/M	4	Cholelithiasis	Necrotic nodule	+
7	81/M	6	Gastric carcinoma	Necrotic nodule	+
8	50/F	4	NA	Necrotic nodule	+
9	61/F	5	Cholelithiasis	Necrotic nodule	+
10	74/M	5	Pancreatic carcinoma	Necrotic nodule	+
11	70/F	8	Chronic pancreatitis	Necrotic nodule	+
12	27/F	10	Ovarian Dermoid cyst	Necrotic nodule	−
13	65/F	7	Gastric carcinoma Hemangioma	Necrotic nodule	+
14	76/M	6	Gastric carcinoma	Fibrotic nodule	−
15	70/M	4	Gastric carcinoma	Necrotic nodule	+
16	45/M	10	Gastric carcinoma	Necrotic nodule	+
17	49/M	4	Colon carcinoma	Necrotic nodule	+
18	77/F	6	Rectal carcinoma	Necrotic nodule with foci of hydatid cyst cuticles	−
19	63/F	10	NA	Necrotic nodule	+
20	63/F	7	Cervix carcinoma	Fibrotic nodule	−
21	53/M	4	Rectal carcinoma	Fibrotic nodule	−
22	69/M	14	Hydatid cyst	Fibrotic nodule	−

NA not available

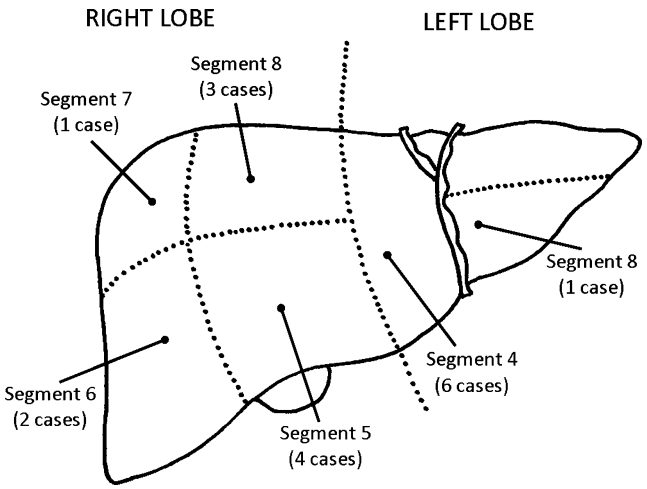

Figure 1 Localization of the 17 nodules within the liver.

cases. The remaining five nodules were entirely fibrotic without necrotic tissue. Of the 22 cases, 14 showed varying degrees of basophilic granular calcification. Ossification was not present in any of the cases. Four (18%) of the 22 cases displayed specific etiology for the development of SNNL. Small metastatic adenocarcinoma foci were identified in two nodules, one of them with centrally localized tumor (Fig. 2) and the other with peripherally localized tumor (Fig. 3). One of these nodules has a prominent necrosis accompanied by a small focus of tumor. These two cases of carcinoma-containing SNNL had synchronous colonic adenocarcinoma. Two of the nodules showed fragments of cuticular membrane (Fig. 4a). One case has a prominent foreign body reaction surrounding the cuticular membrane (Fig. 4b). Parasite was not identified. Chronic inflammation is also not associated with this disease. The adjacent liver parenchyma was noncirrhotic in all patients.

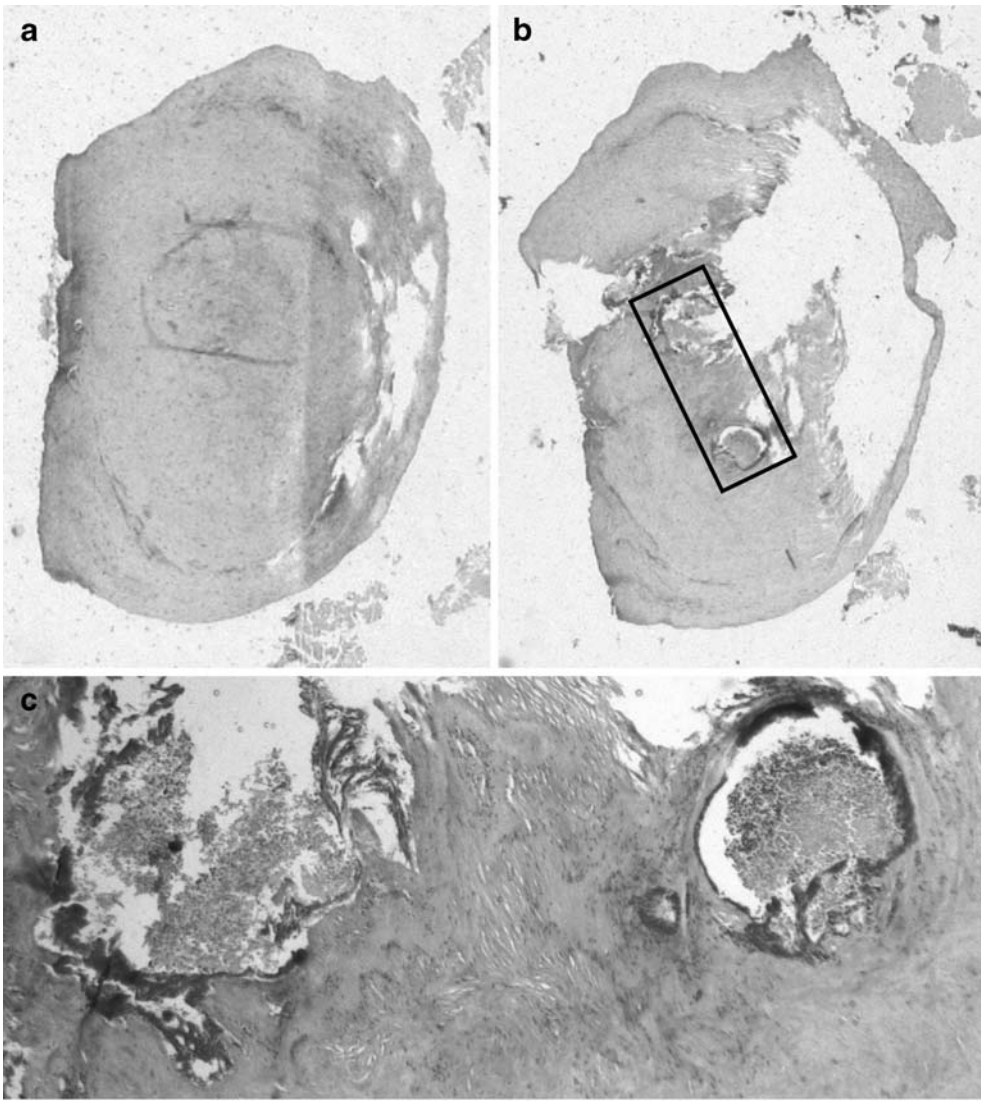

Figure 2 Liver lesion in case 5: initial section of the fibrotic nodule without tumor (**a**). Serial section of the nodule showing foci of metastatic adenocarcinoma (**b**), with centrally necrotic malignant glandular structures (**c**; hematoxylin and eosin).

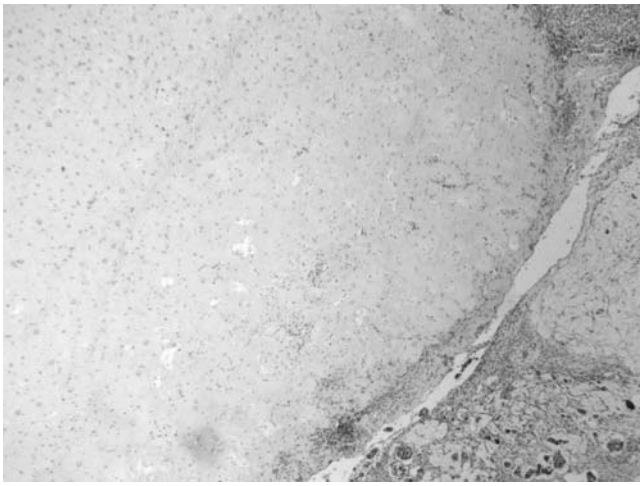

Figure 3 Metastatic mucinous adenocarcinoma at the periphery of the nodule in case 2 (hematoxylin and eosin).

Discussion

SNNL is described as solitary, small, subcapsular nodule. It is characterized by necrosis and fibrous tissue. Most of them are found incidentally by autopsy, surgery, and radiological examination.[4] They are small lesions. The mean diameter was 14 mm, and three fourths of these lesions were under 20 mm in a review by Zhou et al.[2] The mean diameter of the nodules in the current study was 6.8 mm, and the largest nodule was 14 mm in diameter. SNNL can be multiple, but the vast majority of the lesions were solitary.[5] It is reported that the lesion is more prevalent in older patients, and majority of the patients were in the seventh and eighth decades of life.[6] The study of Zhou et al. which reviewed the 68 previously reported SNNL cases pointed out a percentage of 57.4% with male predominancy which was slightly higher than our series (50%). They also showed mean age of 57.5 with a range of 27 to 85 years.[2] The current case series showed a similar age distribution ranging from27 to 81 years (mean 60.7).

Histologically, SNNL is characterized by necrotic core surrounded by heavily collagenized connective tissue. The proportion of the fibrotic and necrotic tissue mainly depends on the duration of the lesion. Sclerosis is a predominant feature of some of these lesions; therefore, the term fibrosing necrotic nodule was suggested by Tsui et al.[7] Most of our lesions have necrotic core surrounded by fibrosis (17 cases); only five cases were entirely composed of acellular hyaline eosinophilic collagen. More than half of our cases had calcification.

The etiology of this lesion is still uncertain.[8] Possible causes for this lesion included thrombi, hemangioma, trauma, and infections. In their original study, Shepherd et al. favored the traumatic or infectious etiology.[1] Parasitic infections are more likely to play an important role in certain cases.[7,9] Clouston et al. described fibrous nodules in a patient with filarial nematode.[9] Tsui et al. found *Clonorchis sinensis* in two of the seven patients with SNNL.[7] These two authors demonstrated parasites within the necrotic-calcified nodule.[7,9] Koea et al. showed necrotic parasitic remnants of *Capillaria hepatica* in an SNNL.[10] Sundaresan et al. reported the presence of the feeding vessels within the lesion, suggesting the hemangiomatous origin.[5] There have been reports of thrombosis associated with SNNL, and De Luca favored the ischemic hypothesis.[11] Previous reports described this entity as benign lesion, and most of them lack of specific etiology.[2,4,12] We showed that four of the 22 SNNL had focus of hydatid cyst and metastatic tumor which were not reported previously. This study shows that metastatic carcinomas can be identified within SNNL which were from the primary tumors of the gastrointestinal system. This study further supports that an infectious etiology is an important cause of SNNL depending on the geographic distribution of the infectious agent. This etiology may be liver flukes in East Asia and hydatid

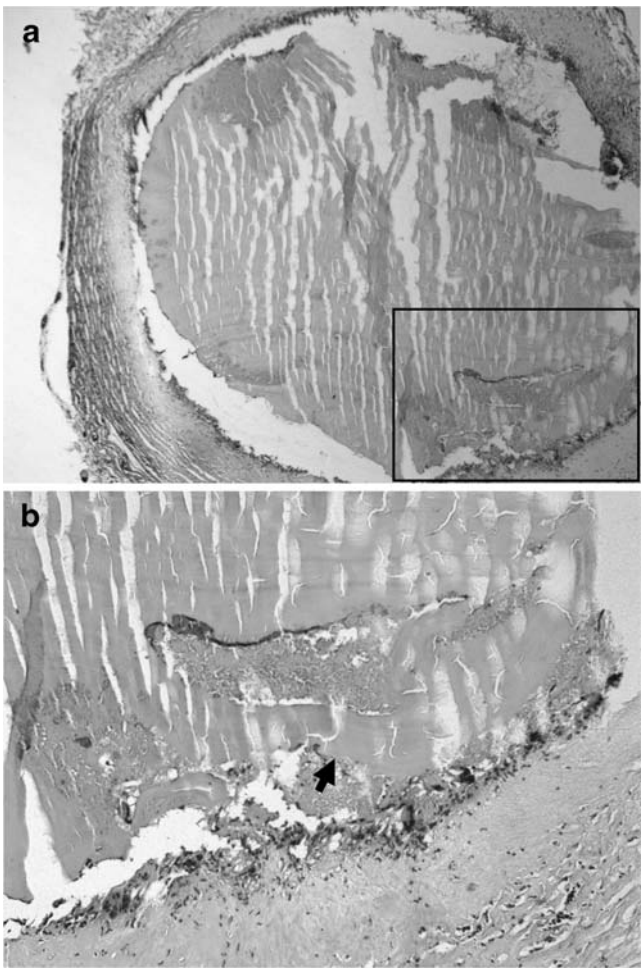

Figure 4 Necrotic nodule containing cuticles of hydatid cyst (*arrow*; **a** and **b**) and foreign body reaction in case 1 (hematoxylin and eosin).

cyst in Turkey, which are more prevalent than the other countries.

Even if the possible pathogenetic mechanisms have been described in several reports, we know little about the natural history of these lesions. Previous reports defined this entity as "burnt-out phase" of benign lesions.[11,13] We believe that SNNL is the end stage of the different lesions, either benign or malignant. It may be speculated that at least some of these lesions are "burnt-out" metastatic tumors because majority of the SNNLs were detected in patients with malignant tumors.[13] Our study showed that 55% (12 cases) of the cases associated with carcinomas in this series, confirming the previous reports. These carcinomas are mainly colorectal carcinomas.[13] Besides the metastatic disease, malignant tumors seem capable of affecting hepatic microvasculature, which may contribute to the development of SNNL.[5]

Most of the lesions were asymptomatic, and they were detected by preoperative evaluation for another cause or incidentally during surgery. Some of them were first detected incidentally at ultrasonography. The disease lacks characteristic clinical symptomatology.[4] All of our cases in this series were asymptomatic, and they were identified preoperatively/intraoperatively in accordance with the literature. SNNLs are small lesions, and they are difficult to detect on radiologic examination. In our series, we demonstrated nodules only in seven of the 22 patients. SNNLs are hypoechoic lesions on USG and round hypodense nodules on CT scans. Calcification, if present, helps to demonstrate the lesion even in smaller nodule (<1 cm). USG and CT findings are not specific for the disease. Radiologically, SNNL mimics primary and metastatic liver tumors. The imaging characteristics are very similar to the metastatic colorectal carcinoma, and the most important differential diagnosis of the SNNL is the solitary metastatic nodules. [10,14]

SNNL should not be assumed as almost always benign.[8] Exact diagnosis requires histological examination due to lack of characteristic radiological findings. Fine-needle aspiration and Tru-Cut biopsies have limited values for diagnosis of the lesion. Histological examination of the totally excised nodule is the best way to evaluate the lesion. Serial sections may be helpful to identify the focal lesions within the nodule. [15] To support this claim, we identified metastatic foci in two SNNLs. Our findings suggest the surgical resection of the nodule even in the absence of symptoms. Ablation therapy may be an alternative treatment in patients with metastatic disease. However, accurate diagnosis requires permanent histology and therefore resection of the nodule. Ablation therapy is not a treatment modality for infectious liver disease which may present as SNNL. Its high association with malignant tumors which has also shown to be high in this report and the presence of the small metastatic foci in the SNNL confirm resection as the treatment of choice.

In this study, we presented a series of 22 necrotic nodules, two of which show parasitic remnants and two metastatic malignancies. Therefore, the possibility of necrotic metastasis must be taken into consideration during assessment of the SNNL. We strongly recommend the surgical resection of these small, subcapsular, necrotic, calcified nodules and complete sampling through the histologic examination.

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ORIGINAL ARTICLE

Non-functional Neuroendocrine Carcinoma of the Pancreas: Incidence, Tumor Biology, and Outcomes in 2,158 Patients

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Abstract
Objective Pancreatic neuroendocrine cancer is a rare, indolent malignancy with no effective systemic therapy currently available. This population-based analysis evaluated the hypothesis that long-term survival benefit is greater with aggressive, rather than limited, surgical therapy.
Methods Non-functional pancreatic neuroendocrine carcinoma (NF-pNEC) cases diagnosed from 1973 to 2004 were retrieved from the SEER database.
Results A total of 2,158 patients with NF-pNEC were identified, representing 2% of all pancreatic malignancies. The annual incidence increased from 1.4 to 3.0 per million during the study period. On average, tumors measured 59±35 mm (median 50), and age at diagnosis was 59±15 years; 29% of patients were younger than 50. Nodal (44%) and systemic metastases (60%) were common. Overall the 5-, 10-, and 20-year survival rates were 33%, 17%, and 10%, respectively. Removal of the primary tumor significantly prolonged survival in the entire cohort (median 1.2 vs. 8.4 years; $p<0.001$) and among those with metastases (median 1.0 vs. 4.8 years; $p<0.001$). No survival difference was seen between enucleation and resection of the primary tumor (median 10.2 versus 9.2 years, $p=0.456$).
Conclusion This study suggests that surgical therapy improves survival among patients with localized, as well as metastatic, NF-pNEC. Enucleation may be oncologically equivalent to resection.

Keywords Pancreatic endocrine tumor · Islet cell carcinoma · Carcinoid · Incidence · Metastasectomy · Enucleation

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Introduction

The clinical manifestations and survival outcomes of neuroendocrine tumors vary significantly by their site of origin,[1–3] with pancreatic lesions being the most aggressive.[4] The heterogeneous morphology of neuroendocrine tumors, and the varying degrees of their clinical endocrine function, have prevented the adoption of a uniform pathologic classification. Although the 2000 World Health Organization (WHO) classification is recommended by most,[3,5] a prognostically superior staging and grading system was recently suggested by others.[6]

Lately, the use of the term *pancreatic endocrine tumor* has been recommended, whereas the use of older terms, such as *neuroendocrine* or *islet cell tumor* or *carcinoid*, have been discouraged.[5] The 2000 WHO classification provided a much needed framework for the integration of biologic behavior and histological features of pancreatic endocrine tumors.[3,5] In comparison, the use of the term *neuroendocrine carcinoma* is supported by the International Classification of

Diseases for Oncology[7] and is currently used in clinical practice. Therefore, for the purpose of this report, we adopted the term *neuroendocrine carcinoma* to describe pancreatic endocrine tumors with malignant and/or biologically unclear potential.

The natural history of pancreatic neuroendocrine tumors has been elucidated mostly by longitudinal studies on functional tumors,[8] however, there are multiple characteristics that differ between functional and non-functional tumors.[6,9–11] For example, insulinomas have approximately a 10% malignancy rate whereas non-functional tumors have a 92% malignancy rate.[12] A recent audit of 9,281 pancreatic neuroendocrine tumors, from the National Cancer Data Base, demonstrates that 85% were non-functional.[13] Most institutional studies[6,14] and database analyses[13,15] have combined functional and non-functional tumors. These data have contributed to the prognostic assessment of individual patients with pancreatic neuroendocrine carcinoma; however, their heterogeneity does not permit the establishment of good, evidence-based treatment algorithms.

A specific focus on pancreatic neuroendocrine tumors is warranted because (1) nationwide incidence data are not available, (2) characteristics differ depending on functional status[6,9–11] and site of origin,[1,2], and (3) surgical outcomes are associated with functional status.[16] In the current literature, there are only three institutional studies limited to non-functional pancreatic neuroendocrine tumors, which include at least 100 patients each.[14,17,18] In the absence of prospective trials, treatment effectiveness should be analyzed by large retrospective studies. Our objective was to evaluate the incidence of non-functional pancreatic neuroendocrine carcinomas (NF-pNEC) in the US population by collecting data from the Surveillance, Epidemiology and End Results (SEER) Program and to analyze outcome variables correlating with surgical treatment. We hypothesized that aggressive surgical intervention, including formal pancreatic resection and/or resection of metastases, is associated with improved survival compared to limited interventions, such as enucleation and/or no surgical treatment.

Methods

Identification of Pancreatic Neuroendocrine Carcinomas in the SEER Database

Diagnosis codes from the 3rd edition of the International Classification of Diseases for Oncology (ICD-O-3) are used to classify neuroendocrine tumors in the SEER database, which collects detailed information on the incidents of all malignant tumors within its respective populations.[7] The SEER registrars assign codes after review of the original pathology reports. Methods to differentiate between the benign, borderline, and malignant subtypes of neuroendocrine tumor are not fully validated and remain controversial.[3,5,10] Since it is recognized that over 85% of non-functional pancreatic neuroendocrine tumors have borderline or malignant biology,[10,12,14] the SEER program collects available data on clinical and pathological information for each case of pancreatic neuroendocrine tumors.[7]

A total of 2,531 pancreatic neuroendocrine tumors were identified, of which 2,158 (85%) were non-functional. Non-functional lesions included large cell neuroendocrine carcinoma (8013/3, n=7), islet cell carcinoma (8150/3, n=1,066), and neuroendocrine carcinoma (8246/3, n=1,085). All functional, atypical, and mixed tumors were excluded, as well as those designated carcinoid or enterochromaffin-like tumors (n=373). *Extent of disease* data was used to reconstruct the nodal and systemic metastatic status. Survival data is current as of November 2006.[7]

We analyzed the following outcome variables: year of diagnosis, patient gender and age at diagnosis, primary tumor size and grade, presence of lymph nodes and distant metastases, and type of surgical intervention. We did not include the Alaskan Native and Native Indian registries in our analysis of annual incidence because valid estimated annual censuses of these populations were not available.

Data Analysis

Values are expressed as mean±standard deviation (median). The 95% confidence intervals for annual incidence were calculated using the Poisson distribution. Categorical variables were analyzed with χ^2 test. Dichotomous outcomes were analyzed using multivariable logistic regression, and models were built with clinically significant variables identified in the SEER dataset. Continuous variables were compared using independent sample t test. Variance equality assumptions were validated using Bartlett's test. The Mantel–Haenszel trend test was used for evaluation of ordinal data. Kaplan–Meier estimates of survival were plotted, and survival differences were analyzed using the log-rank test. Proportional-hazards assumptions were tested using Schoenfeld's residuals. Multivariable survival analysis was performed using a stepwise forward inclusion algorithm of Cox proportional hazard model with inclusion and exclusion probabilities of 0.05 and 0.10, respectively. Statistical significance was assumed at $p \leq 0.05$.

Results

Demographics, Tumor Characteristics, and Incidence Rates

NF-pNEC accounted for 2% of 109,811 pancreatic malignancies registered between 1973 and 2004. The annual

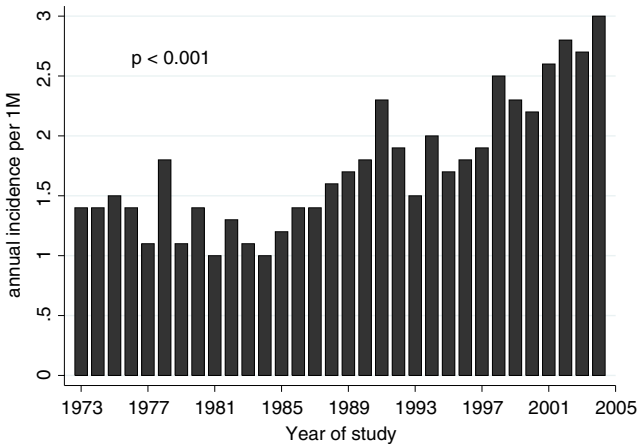

Figure 1 Annual incidence of non-functioning pancreatic endocrine carcinomas.

incidence increased from 1.4 per million in 1973 to 3.0 per million in 2004 (Mantel–Haenszel trend test χ^2 20.9, $p<$ 0.001, Fig. 1). The annual incidence over the first 5 years of the study was 1.34 cases per million (95% CI, 1.12–1.59). In the last 5 years of this study there were 1,087 cases in 415,088,938 person-years, resulting in an average incidence of 2.62 cases per million (95% CI, 2.47–2.78). Trend showed a significant change over the last 5 years of the study (Mantel–Haenszel trend test χ^2 4.2, p=0.040, Fig. 1).

The majority of patients were men (1,206/2,158; 55.9%). The mean age at diagnosis was 59±15 years (median 60 years) with 29% of cases younger than 50 years. Tumors measured 59±35 mm (median 50 mm) in diameter and were either located in the pancreatic head (42%), body (11%), tail (27%), or were diffuse (20%). There was no significant difference in tumor size between surgical and non-surgical treatments (58±36 mm vs. 59±34, p=0.394). Nodal metastases were present in 43.5% of patients (270 patients among 620 cases with known nodal status). Distant metastases were documented in 60% of patients (944 patients) with available data during their initial evaluation (n=1,573). Within the entire cohort, prior malignancy was reported in 15.1% of cases (326/2,158). Tumor grade was determined in 614 patients, with 34.2% grade I, 27.2% grade II, and 38.6% grade III and IV. Resection was performed in 46.2% of patients (735 out of 1,590 with available detailed information).

Is the Presence of Nodal and Systemic Metastases Predictable?

Using preoperative clinical variables only, we predicted the presence of nodal and distant metastases (Table 1). Interestingly, tumor size was predictive of nodal involvement, but not of systemic metastases. Conversely, age was not predictive of nodal involvement, but was predictive of systemic metastases. Discrimination ability of both models was poor (area under receiver operator curve 0.61 and 0.59, respectively), and thus they are of limited clinical utility.

Survival Analysis: Tumor and Patient Characteristics

At the censor date, 746 of 2,158 patients were alive. Of the 1,412 who died, 958 patients (67.8%) succumbed to NF-pNEC, and 454 died of other causes. Median survival was 2.2 years. Overall 5-, 10-, and 20-year survival rates were 33%, 17%, and 10%, respectively. Increasing age was associated with reduced survival. Patients with distant metastases at the time of diagnosis experienced significantly shorter overall survival than those without metastases (median 7.1 years vs. 1.4 years; p<0.001; Table 2 and Fig. 2). The presence of nodal metastases had no significant impact on the duration of survival in univariate analysis (median 6.0 years for node negative vs. 6.3 years for node positive; p=0.139). Higher tumor grade correlated with dismal overall survival (median 7 months for pooled grades III and IV) compared to low grade lesions (5 and 4.4 years for grades I and II, respectively; p<0.001; Fig. 3).

Survival Analysis: Effect of Surgical Treatment

Surgical removal of the primary tumor was performed in 46% of cases and was associated with prolonged survival (median 1.1 vs. 8.4 years; p<0.001). Analysis of survival between those who did and did not receive surgical resection after stratifying by distant metastases status demonstrated that, within both groups, patients treated with

Table 1 Multivariable Logistic Regression Models Predicting Lymph Node and Distant Metastatic Involvement from pNECs

	Lymph node metastasis			Distant metastasis		
	Odds ratio	p	95% confidence interval	Odds ratio	p	95% confidence interval
Gender (referent: male)	0.795	0.207	0.558–1.135	0.793	0.072	0.616–1.020
Age	1.000	0.961	0.988–1.012	1.018	0.001	1.009–1.027
Tumor size (mm)	0.986	0.001	0.980–0.992	1.002	0.245	0.998–1.005

Adjusted effects of preoperative variables (age, gender and tumor size) are indicated. Overall p values for both models is less than 0.001

Table 2 Proportions of Actual 5, 10, 15, and 20-Year Survivors

		Overall survival		Cancer-specific survival	
	n	M_0	M_1	M_0	M_1
5-year	1,573	0.58	0.22	0.70	0.39
10-year	228	0.43	0.08	0.55	0.20
15-year	65	0.30	0.06	0.39	0.17
20-year	8	0.21	0.06	0.39	0.17

Overall and pancreatic cancer specific survival rates are listed separately for cases initially presenting as metastatic and non-metastatic

surgical resection had a longer median survival. There was a significant increase in median survival for patients with resection without distant metastases (1.6 versus 11.3 years, $p<0.001$) and patients with distant metastases (1.0 versus 4.8 years, $p<0.001$). Enucleation compared to resection of the primary tumor was not a significant predictor of survival (median 10.2 versus 9.2 years, $p=0.456$) in the univariate analysis. Based on a multivariable Cox proportional hazard model, the most influential predictors of survival in the order of significance were resection of the primary tumor, low tumor grade, absence of distant metastases, and younger age (Table 3).

We also evaluated the survival benefit of surgical treatment for the subset of patients who presented with distant metastases ($n=614$). The likelihood of resection of the primary tumor was highly dependent on tumor grade: 79% of grade I and II primary tumors were resected compared to 25% of grade III and IV tumors ($p<0.001$). This strong association between tumor grade and surgical resection introduced substantial collinearity into the comprehensive Cox models for patients presenting with metastatic disease, thus a limited model using age and surgical therapy was used. Resection of either the primary tumor or distant metastatic site was associated with increased survival compared to no resection; the greatest survival benefit was seen in patients with the resection of both the primary tumor and metastases ($p<0.001$, Fig. 4, Table 4).

Discussion

Non-functional pancreatic neuroendocrine carcinomas represent about 2% of all pancreatic malignant tumors. In general, patients with pNECs manifest a prolonged survival;[14,16,19] however, there is a substantial variability in their clinical outcomes.[11,14] Despite a considerable amount of research, our understanding of natural history,[2,8,20] predictors of survival,[3,14,19] efficacy of multimodality therapy,[9,13,21,22] and prognosis[6,10,14,18] remains incomplete.

The SEER program is an excellent tool for population analysis of rare malignancies because of its data collection for over 30 years, extraordinary accuracy, and close approximation to the general US population.[7] Therefore, we conducted this study to elucidate some aspects of incidence trends, tumor characteristics, prognostic factors, and effectiveness of surgical therapy in patients with non-functional pNECs.

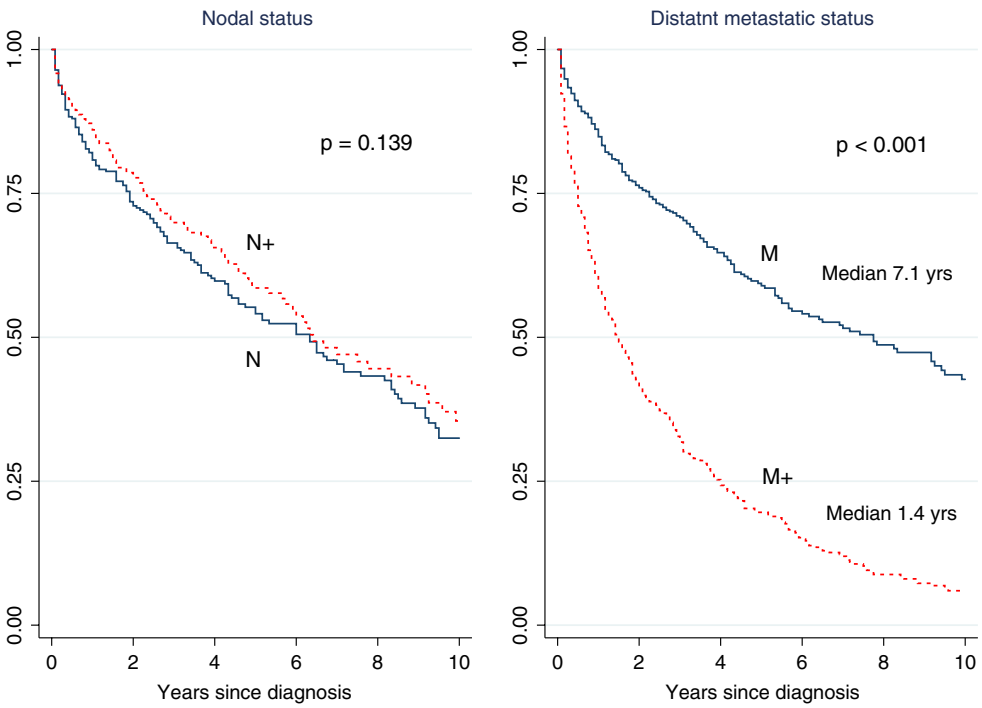

Figure 2 Survival estimates for patients by nodal status (*left panel*; median survival 6 and 6.3 years, $p=0.139$) and distant metastatic status at the time of diagnosis (*right panel*; $p<0.001$).

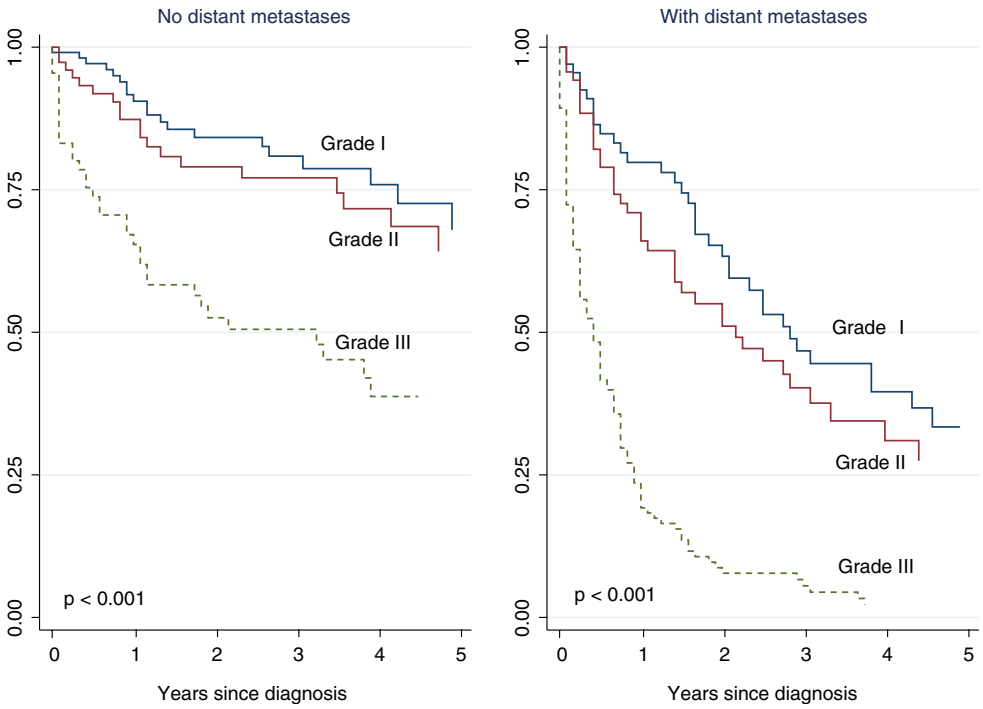

Figure 3 Survival estimates by tumor grade for patients without and with distant metastatic disease ($p<0.001$ for both).

In the SEER database, we identified 85% of pNECs as non-functional, which is similar to some previous findings.[13] An increasing incidence of all neuroendocrine tumors has been suggested over the last 50 years;[2] data from the Michigan registry[15] and Mayo clinic[11] demonstrate an increasing incidence of NF-pNEC. We also identified an increased incidence of clinically detectable NF-pNECs, with the annual incidence rate increasing from 1.4 to 3.0 new cases per million from 1973 to 2004.

There are substantial differences in the natural history and clinical behavior of neuroendocrine tumors arising in different anatomic sites.[2,3] Currently, pancreatic neuroendocrine tumors do not have a commonly accepted staging system, although a specific scheme was suggested.[3] While the American Joint Committee on Cancer staging excludes pNEC histology, it has good discrimination prognostic ability.[19] Tumor size was not predictive of survival in a large report from the MD Anderson Cancer Center,[18] but univariate analysis in two other large studies, suggested that small tumors (<2–3 cm) are associated with better survival.[14,17] Conversely, and in agreement with our data, tumor size and nodal status were not predictive of survival in the analysis of nearly 10,000 cases from the National Cancer Data Base.[13] Therefore, we, and others, believe that other factors, such as systemic metastases, local, vascular and lymphatic invasion, and grade,[5,10,18] are more powerful indicators of outcome. Additionally, in our study, tumor grade influence on survival was larger than the presence of distant metastases. Despite presumed variability in grading methodology among institutions, this variable retained its pivotal prognostic value.

A recent validation study of the WHO classification assessed 180 patients with non-functional pancreatic neuroendocrine tumors[14] and confirmed that distant metastatic spread and poor differentiation as negative prognostic markers. Conversely to our report, these authors identified nodal metastases as a negative predictor of survival among patients with malignant non-functional pancreatic neuroendocrine tumors. A proposed expert consensus-based TNM staging classification for pancreatic neuroendocrine tumors[3] utilizes tumor size and nodal metastases as predictors. On the contrary, we and others[6,16,18] found no survival predictive value of nodal metastases and tumor size.

Table 3 Multivariable Cox Regression Model for All Patients with NF-pNEC (n=2,158)

	HR	95% CI for HR		p
		Lower	Upper	
Age	1.022	0.999	1.043	0.051
T size (mm)	1.005	0.999	1.010	0.073
N status	1.382	0.809	2.361	0.236
M status	1.895	1.092	3.289	0.023
Grade				
I	1.000	Referent		
II	2.268	1.215	4.232	0.010
III	3.422	1.751	6.687	0.001
Resection of the primary site	0.237	0.132	0.424	0.001

Adjusted effect of age, primary tumor resection, nodal status, distant metastatic status, tumor grade, and size on survival. Overall model $p<0.001$

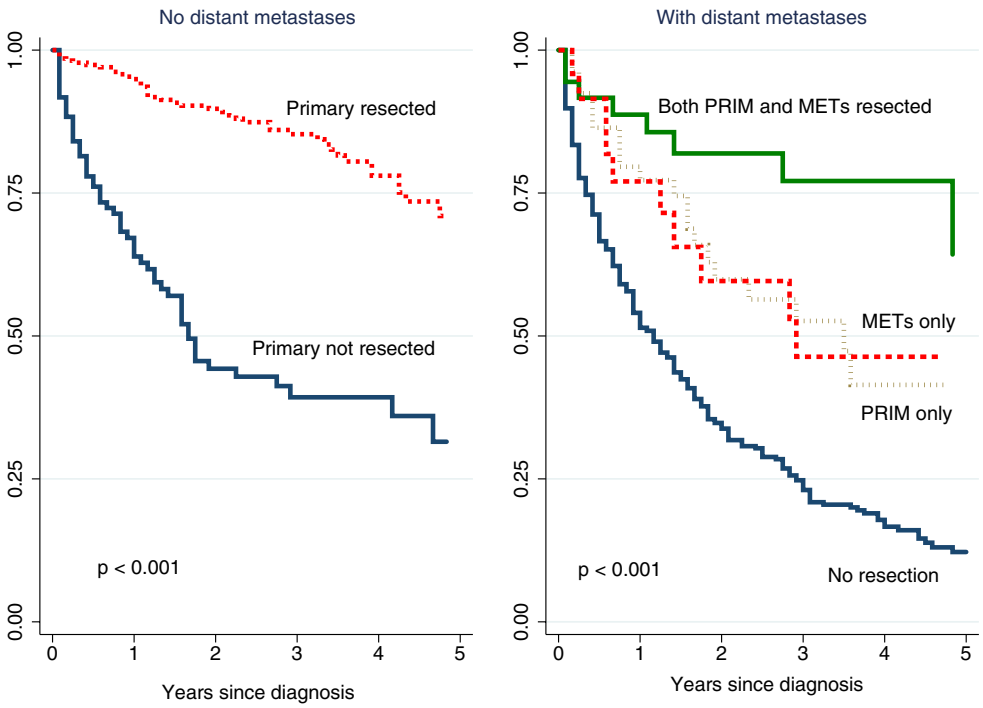

Figure 4 Survival estimates for patients according to metastatic status and resection of the primary tumor ($p<0.001$). Median survival times are listed in years.

Aggressive resection of both the primary tumor and metastasectomy is associated with improved survival in the present series. As expected, the largest benefit in this study was seen among patients undergoing the removal of both primary and metastatic sites. Patients with distant metastases undergoing resection of primary tumor only or metastases only, had similar survival rates of 3.5 and 2.9 years, respectively. Nevertheless, this was significantly longer than the median survival for those without any surgical treatment (1.0 year, $p<0.001$ for each). Other studies have specifically noted that a cytoreductive approach to hepatic metastatic disease[22–25] and nodal clearance[20] are associated with prolonged survival. Additionally, patients with liver metastases benefit from removal of primary neuroendocrine tumor alone.[26]

There are striking similarities between data presented here and those reported on 163 NF-pNEC treated at MD Anderson Cancer Center.[18] Both studies demonstrate a 60% distant metastatic involvement at presentation, beneficial effect of primary tumor resection, a lack of tumor size as a survival predictor, and similar overall survivals rates. It should be noted that despite the prolonged survival[9,18,21] this tumor can be fatal, and is cause of death in 67% of patients diagnosed with pancreatic neuroendocrine carcinoma.

We had hypothesized that enucleation is less effective in prolonging survival compared to formal pancreatic resection for treatment of pNEC, despite being associated with better functional outcomes.[27] Therefore, we evaluated enucleation versus formal resection for pNECs and found no survival difference between the two operations. It must be assumed that proper patient selection influenced this finding.

The present study is not prospective and all patients underwent individualized treatment, therefore, these results

Table 4 Multivariable Cox Regression Model for Patients with Metastatic NF-pNEC and Detailed Data on Resection of Primary and Distant Sites ($n=614$)

	HR	95% CI for HR		P
		Lower	Upper	
Age (per year)	1.030	1.023	1.038	<0.001
Resection of the primary site	0.457	0.306	0.683	<0.001
Resection of the metastatic site	0.404	0.245	0.668	<0.001

Adjusted effect of primary tumor resection and metastatic site resection on the survival are noted. Overall model $p<0.001$

cannot be viewed as a proof for the efficacy of surgical therapy. Nevertheless, we have demonstrated that surgical resection, including removal of metastases, is associated with improved survival. Multiple additional factors could influence these results including evolving terminology, changing registry protocols, and our inability to review histological material. Tumor grading for pNEC is in evolution and in the past has not been consistently reported. Determination of the malignant potential remains controversial in neuroendocrine tumors; however, most non-functional pancreatic neuroendocrine tumors are considered malignant.[10,12,14] Although these aspects may lower the reliability of our study, population characteristics remain important.

Conclusion

In summary, non-functional pancreatic NECs are uncommon, but their incidence is rising. Tumor size and nodal metastases do not predict survival, whereas grading and systemic metastases have a significant impact on survival. There is a clear association between survival and surgical therapy among select patients with both localized and metastatic disease. Moreover, resection and enucleation result in similar survival rates.

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ORIGINAL ARTICLE

Long-Term Outcome after 92 Duodenum-Preserving Pancreatic Head Resections for Chronic Pancreatitis: Comparison of Beger and Frey Procedures

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Abstract
Introduction Duodenum-preserving pancreatic head resection may be an alternative to pancreatoduodenectomy or drainage procedures for chronic pancreatitis. There are few studies directly comparing the long-term outcome after the operations described by Beger and Frey.
Methods One hundred thirteen patients underwent duodenum-preserving pancreatic head resection for complications of chronic pancreatitis. Follow-up was obtained in 92 patients (42 Beger, 50 Frey, median follow-up almost 5 years).
Results Overall/surgery-related perioperative morbidity was 30%/20% (Frey) and 40%/31% (Beger). In long-term follow-up (Frey vs Beger), 62% vs 50% were completely free of pain, but 6% vs 19% had pain at least once per week or daily, and 32% vs 31% experienced pain attacks at least once per year (n.s.). Diabetes mellitus occured in 60% vs 57% (de novo 34% vs 17%). Rates of exocrine insufficiency were 76% vs. 74% (de novo 34% vs. 33%). Median gain in body weight was 2.5 vs 1.5 kg (n.s.), respectively. Four patients had clinically relevant biliary complications during follow-up requiring reintervention.
Conclusions Our (nonrandomized) comparison of the long-term outcome after Frey and Beger procedures for chronic pancreatitis reveals a tendency for better pain control with the Frey operation. The functional outcomes were almost identical.

Keywords Chronic pancreatitis · Duodenum-preserving pancreatic head resection · Long-term outcome · Pancreatic pain · Pancreatic endocrine function · Pancreatic exocrine function

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Introduction

The management of patients with chronic pancreatitis does not only comprise an interdisciplinary approach by the gastroenterologist and the surgeon but also has led to the development of a wide armamentarium of surgical procedures competing for short- and long-term patient benefit. Pain in chronic pancreatitis has been described to be mediated through pancreatic duct obstruction, parenchymal hypertension, or sensory nerve injury[1–5] and remains the leading complaint of patients with chronic pancreatitis. Long-term success of an operative therapy, therefore, has to be judged on its ability to reduce pain in those patients. In the course of the disease, pancreatic exocrine and endocrine insufficiency[6] as well as local complications might occur, such as pseudocysts, extrahepatic cholestasis, gastric outlet or duodenal obstruction, aneurysmal hemorrhage, and portal venous hypertension due to stenosis or thrombosis.[7–9]

Operations, therefore, also have to be evaluated in their ability to retain acceptable quality of life for these patients.

The concept of the "inflammatory pancreatic head mass" as the pacemaker of both pain and progression of the disease has lead to the development of surgical techniques directed at resection of the pancreatic head, either by pancreatoduodenectomy with or without pylorus preservation or by a duodenum-preserving partial pancreatic head resection (DPPHR) with drainage of the pancreatic duct.[10–12]

While in recent years, the discussion on surgical strategy has focused on the question whether to perform a pancreatoduodenectomy or a duodenum-preserving operation, only one study has directly compared the long-term outcome of the two most widely used duodenum-preserving procedures described by Beger and Frey.[10,12,13]

The aim of this study was to compare the long-term outcome after Beger and Frey procedures for chronic pancreatitis in 92 patients with special focus on pain control and pancreatic exocrine and endocrine function. In a previous paper from our group,[14] we reported the long-term outcome after resection for chronic pancreatitis without subanalysis of the different types of DPPHR. After extended follow-up in these patients, we now present the outcomes depending on the type of DPPHR.

Patients and Methods

Since 1996, 113 patients underwent DPPHR for CP at our institution. Postoperative histological examination confirmed CP in all cases. Operative mortality in the entire cohort was 1/113 (0.9%). Prospective postoperative follow-up data of at least 6 months using standardized questionnaires were currently available and evaluated for this study in 92 patients operated between 1996 and 2007.

Preoperative Assessment

All patients had at least one cross-sectional imaging modality before surgery (CT or MRI). During the last years of the study period, MRI included MRCP and MR-angiography in the majority of patients. Until 2001, the majority of patients preoperatively had transfemoral arterial angiography to document vascular changes by CP. Angiography was abandoned afterwards due to better vessel detection in cross-sectional imaging. Seventy-two percent had an ERCP preoperatively, and 27% underwent preoperative biliary drainage.

Surgery and Perioperative Management

The operative and perioperative management of our patients undergoing pancreatic resection (for CP) has been described in detail.[7,14] During DPPHR according to Beger, the pancreatic duct was cannulated to exclude remaining pancreatic duct stones or relevant duct stenosis. The pancreatic anastomosis was also performed in an end-to-side technique using interrupted full-thickness polydioxanone sutures between the pancreatic stump and the draining jejunal loop. A bilioenteric anastomosis to the posterior wall of the jejunal loop was included in 24 (57%) of the 42 patients undergoing a Beger procedure. During the Frey procedure, reconstruction consisted in a side-to-side pancreatojejunostomy using running polydioxanone sutures. Perioperative octreotide was almost always applied for 5 to 7 days in the first years of this study period but abandoned during 2003. Before abdominal closure, flat silicon drains were placed at the pancreatic anastomosis and left in place for at least three postoperative days.

Definitions

Our standardized definition of pancreatic leakage was reported in detail before and consisted in increased amylase (>3 times serum amylase) in the drain output beyond the sixth postoperative day, the need of interventional drainage of abdominal fluid collections with a high amylase concentration or visible anastomotic insufficiency found during reoperation. Although we now use the definition of postoperative pancreatic fistula of the International Study Group of Pancreatic Surgery,[15] this definition could not be applied here because it had not yet been established during the early years of this study. Intraabdominal complications including gastrointestinal bleeding and wound infections were summarized as surgical complications. Diabetes mellitus was defined according to the criteria of the WHO classification. Patients in part underwent oral glucose tolerance tests or 24-h glucose profile determination. Exocrine insufficiency was defined as the presence of steatorrhea and/or the need for oral pancreatic enzyme supplementation. Other determinants of exocrine insufficiency (i.e., fecal elastase concentration) were not routinely obtained in our study.

Follow-Up Evaluations

Postoperative follow-up examinations were performed in several chronological steps. Questionnaires were mailed to the patients or handed out during outpatient consultations. They always included standardized items asking (among others) the presence of pain, pain intensity (including visual analog scales), pain frequency (none/daily/weekly/monthly/yearly), the presence of diabetes or steatorrhea, and the current specific medication (pancreatic enzymes, analgesics).[14] Furthermore, the need of specific treatment of CP was requested. In selected cases, patients and/or their home physicians were additionally contacted by phone. For the

analyses presented in this study, the results of the last follow-up evaluation per patient were considered.

Statistics

All perioperative and outcome data were prospectively entered into a computer-based database (SPSS software, SPSS Inc., Chicago, IL). For statistical comparisons, chi-squared test and Mann–Whitney *U* test were used.

Results

Demographic and Disease-Related Data

In both groups (Frey and Beger procedures), the vast majority of the patients had alcoholic CP and were male. At the time of surgery, the group of patients undergoing a Frey procedure had a slightly higher median age, a slightly longer preoperative duration of CP, and a higher rate of regional or generalized portal hypertension. As later outlined in the discussion, the presence of an advanced stage of portal hypertension frequently was a contraindication for trans-section of the pancreatic neck (i.e., during a Beger procedure or PD) and an indication for a Frey procedure.[7] However, only the occurrence of preoperative biliary drainage showed a statistical difference between the two groups (Beger 38% vs Frey 20%) and reflects the varying indications for these operations (Table 1).

Indications for Surgery

The indications for surgery of the 92 patients undergoing DPPHR are shown in Table 2. Patients with chronic pancreatitis requiring surgery in our series often presented with more than one indication or co-indication. In both groups, more than 90% of the patients had pain and/or recurrent pain episodes during attacks of CP as one leading indication for surgery. A relevant duodenal stenosis was present in 10% in each group. There was a significantly higher rate of patients with preoperative jaundice or with radiologically proven stenosis of the common bile duct in the group of patients undergoing a Beger procedure (Table 2).

Perioperative Outcome

Median duration of surgery was 55 min longer for Beger procedures ($p<0.01$; Table 3). Intraoperative requirement of blood transfusions (median 2 U) and postoperative length of stay (median 13 days) were similar in both groups. Overall postoperative complication rate was 30% (Frey) and 40% (Beger, n.s.), with surgical complications documented in 20% (Frey) and 31% (Beger, n.s.). There was a slightly, however, not significantly, lower rate of pancreatic leak, wound infection, and abdominal abscess in the Frey group. The rates of relaparotomy for complications were 6% and 7%, respectively (n.s.; Table 3).

Long-Term Outcome

Median postoperative follow-up was longer in the Beger than in the Frey group (62 months (range 6–137) vs 43 months (8–126); p = n.s.). This difference is explained in part by the fact that most of the Frey procedures were performed later during the study period and the Beger procedure was introduced earlier in our series of DPPHR.

Pain Assessment

At the last follow-up evaluation, 62% of the patients in the Frey group and 50% in the Beger group were completely free of pain ($p=0.25$; Table 4). There was a nonsignificant trend towards a lower overall incidence of pain and a lower frequency of pain episodes in the Frey group: Only three of 50 patients (6%) after the Frey procedure but eight of 42 patients (19%) after the Beger procedure reported pain to occur at least once per week (Table 4). Satisfactory pain control, defined here as absence of pain or the occurrence

Table 1 Preoperative Demographic and Disease-Related Characteritics of 92 Patients Undergoing a Frey or Beger Procedure for Chronic Pancreatitis (CP)

	Frey procedure $n=50$	Beger procedure $n=42$
Age (years, median, range)	45 (27–78)	41 (30–62)
Gender	78% male	86% male
BMI (median; range)	21.9 (15–35)	21.7 (16–30)
Preop. duration of CP (months, median, range)	59 (3–200)	48 (1–444)
Alcoholic CP	76%	79%
Calcifications	70%	76%
Pseudocysts	62%	62%
Diabetes	26%	40%
Portal hypertension	42%	26%
PBD	20%	38%

Differences between the groups: $p>0.1$ for all parameters except for preoperative biliary drainage ($p=0.05$)

PBD preoperative biliary drainage

Table 2 Indications for Surgery and Further CP-Related Characteristics in 92 Patients Undergoing a Frey or Beger Procedure for Chronic Pancreatitis

	Frey procedure n=50		Beger procedure n=42		*p* Value
	n	(%)	*n*	(%)	
Indication for surgery					
Pain	45	90	35	83	n.s.
Recurrent episodes	46	92	37	88	n.s.
Jaundice	7	14	13	31	0.05
Radiol. CBD-stenosis	14	28	28	67	<0.001
Duodenal stenosis	5	10	4	10	n.s.

More than one indication per patient possible

CBD common bile duct

of pain at most once per month, therefore, was found in 94% (Frey) and 81% (Beger), respectively. The same trend was documented for the use of analgesics. Application of narcotics was documented in 29% after a Beger procedure and in 16% after a Frey procedure (Table 4).

Exocrine Function, Diabetes, and Weight Difference

The rate of diabetes mellitus at the last follow-up evaluation was comparable in both groups (60% and 57%). Whereas the frequency of preoperative diabetes was higher in the Beger group, this difference was balanced by a higher rate of postoperative de novo diabetes in the Frey group (Table 5). The rates of preoperative, postoperative de novo, and overall exocrine insufficiency at the last follow-up were almost identical in both groups. Around half of the patients developed exocrine insufficiency postoperatively. At the last postoperative assessment, three of four patients in each group reported symptoms of exocrine pancreatic insufficiency (Table 5). At the last follow-up, a slight median gain in body weight was documented in both groups (1.5 vs 2.5 kg; p=0.5; Table 5).

Late Organ Complications

During the current follow-up period, four symptomatic biliary stenosis (two of those with impacted gallstones in the common bile duct) requiring intervention were documented (three after Frey and one after Beger procedure).

Discussion

It has been convincingly shown that surgical management is superior to endoscopic interventional therapy of pain in chronic pancreatitis.[16] Pain, however, is not the only indication that leads to surgery in chronic pancreatitis. Due to unknown factors that might reflect differences in pathophysiology, referral pattern, and geographic factors, it has been demonstrated that size of the pancreatic head tumor and concomitant local complications such as duodenal or biliary obstruction vary between German and American centers for pancreatic surgery.[17] Given the fact of varying indications for surgery in patients with chronic pancreatitis, different types of operative procedures have been performed with good short- and long-term results. Some authors have suggested that resectional procedures yield a better long-term outcome than drainage procedures due to resection of the "inflammatory pancreatic head mass."[1,18] In an effort to minimize organ resection, two popular DPPHR procedures were developed by Beger[10] and Frey.[12]

The duodenum preserving operations were introduced to limit resection of pancreatic tissue and alterations of the gastrointestinal passage in the treatment of a benign inflam-

Table 3 Perioperative Results in 92 Patients Undergoing DPPHR for CP

	Frey procedure *n*=50	Beger procedure *n*=42	*p* Value
Duration of surgery (min, median, range)	360 (195–600)	415 (235–740)	<0.01
Intraoperative blood transfusion (units, median, range)	2 (0–18)	2 (0–12)	0.93
Postoperative LOS (days, median, range)	13 (8–120)	13 (7–82)	0.84
Morbidity (*n*; %)			
Total	15 (30%)	17 (40%)	0.29
Surgical	10 (20%)	13 (31%)	0.23
Pancreatic leak	4 (8%)	5 (12%)	0.53
Wound infection	2 (4%)	5 (12%)	0.15
Abdominal abscess	1 (2%)	4 (10%)	0.11
Reoperation (*n*; %)	3 (6%)	3 (7%)	0.83

Table 4 Assessment of Pain, Pain Frequency, and Use of Analgesics at the Last Follow-up Evaluation after 92 DPPHR for Chronic Pancreatitis

	Frey procedure $n=50$		Beger procedure $n=42$		p Value
	n	(%)	n	(%)	
No pain	31	62	21	50	0.25
Pain (any frequency)	19	38	21	50	
Pain frequency					
No pain	31	62	21	50	0.26
Daily	0	0	3	7	
Weekly	3	6	5	12	
Monthly	10	20	7	17	
Yearly	6	12	6	14	
Use of analgesics					
None	37	74	25	60	0.29
Narcotics	8	16	12	29	
Other	5	10	5	12	

matory disease in the pancreatic head. Beger described a technique in which the pancreas is divided over the mesentericoportal axis and a subtotal resection of the pancreatic head was performed preserving the surrounding, nondiseased organs[19] (Fig. 1b). The Frey operation, in contrast, combines a limited local excision of the pancreatic head with a longitudinal pancreaticojejunostomy[12] (Fig. 1a).

To our knowledge, there is only one prospective randomized study[13,20,21] aiming at comparing these two procedures directly in short- and long-term follow-up. In a prospective randomized trial comparing the Beger and the Frey procedure, Izbicki and coauthors[20] demonstrate a significant reduction in pain scores in 95% resp. 94% of the patients after 1.5 years after the operation. In their long-term follow-up,[13] the authors report their result of 74 patients equally and randomly assigned to the Frey and the Beger operation. These patients were reassessed after a meaningful interval of over 8 years. The authors reported no differences between the two procedures concerning pain control or the occurrence of endocrine or exocrine insufficiency. The important additional information that was drawn from this study was that both the functional scale and the symptom scale demonstrated a significant improvement in the quality of life as well as in pain control independent of the type of operation performed. To our knowledge, this is the only long-term observation comparing both techniques. Beger et al.[22] demonstrated in a long-term nonrandomized observation in 303 patients receiving a Beger operation that even though surgery can provide a good long-term pain control, the progression of the disease leads to endocrine (61%) or exocrine insufficiency (71%) on the long run. Other authors as well have underlined the fact that pancreatic insufficiency develops independently of the surgical therapy applied.[23]

In our current manuscript, we report the perioperative and long-term outcome of the two most commonly performed types of DPPHR today, the procedures described by Beger and Frey. Our patient collective is characterized by a preoperatively long-standing (4 to 5 years) chronic pancreatitis with a high rate of local complications and a high rate of inflammatory pancreatic head masses. Due to the variety of symptoms the operative procedure was not chosen at random, as the Beger procedure was used in cases of bile duct stenosis, because of the possibility of sufficient bile duct decompression with this method. Likewise, the two treatment groups differ in the rate of extrahepatic cholestasis and preoperative endoscopic drainage, while being comparable concerning other preoperative parameters and indications for surgery. It is of note that the Frey

Table 5 Exocine and Endocrine Pancreatic Function and Weight Gain at the Last Follow-up Evaluation after 92 DPPHR for Chronic Pancreatitis

	Frey procedure $n=50$		Beger procedure $n=42$		p Value
	n	(%)	n	(%)	
Preoperative diabetes	13	26	17	40	0.14
Postoperative de novo diabetes	17	34	7	17	0.06
Diabetes (total)	30	60	24	57	0.78
Preoperative exocrine insufficiency	21	42	17	41	0.88
Postoperative de novo exocrine insufficiency	17	34	14	33	0.95
Exocrine insufficiency (total)	38	76	31	74	0.81
Weight gain (kg; median; range)	1.5	(−12–14)	2.5	(−13–37)	0.5

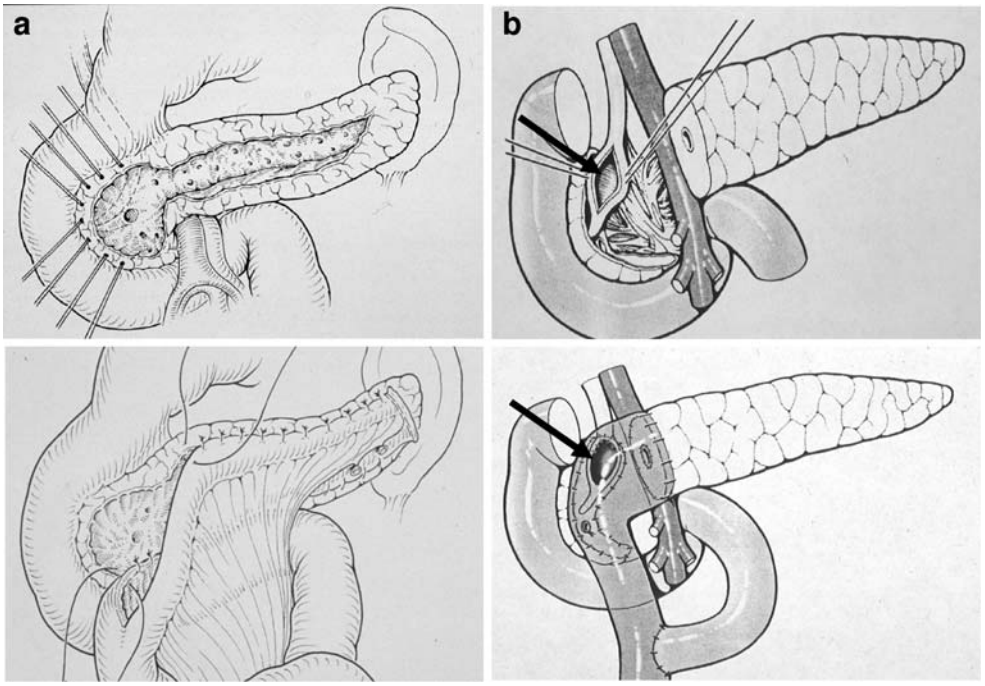

Figure 1 **a**, **b** Illustrations demonstrating the status after the resectional part (*upper images*) and after/during pancreatic anastomosis (*lower images*) during the Frey (**a**) and the Beger procedure (**b**). Note the incised common bile duct and the subsequent inclusion of a bilioenteric anastomosis after a separate incision in the posterior wall of the jejunal loop (*arrows* in **b**).

procedure was introduced after the Beger procedure in our series. The Frey procedure was individually chosen in a few cases with CP and severe generalized extrahepatic portal hypertension where a potentially indicated resection (i.e., PD or Beger procedure) were contraindicated because of peripancreatic venous collaterals and the inability to interventionally recanalize the mesentericoportal axis.[7] Both of these limitations, the decision for an operation according to the underlying complications and the successive introduction of the Beger and the Frey operation to our armamentarium for chronic pancreatitis, yield some danger of bias in our retrospective study. We do, however, judge this danger as very important for the occurrence of perioperative morbidity and as limited when we are focusing on the long-term results of these surgical techniques. One might, however, argue that portal vein thrombosis or biliary obstruction might reflect a more advanced stage of disease which might lead to a higher occurrence of diabetes or exocrine insufficiency. Here, we see a potential bias of our observations.

As far as the blood loss, we found no differences which might be due to the fact that we had a quite large percentage of patients with portal hypertension in both groups. We do think as well that none of the procedures is necessarily more likely to require blood transfusion compared to the other.

The primary indication for surgery in chronic pancreatitis remains pain. In our collective of 92 patients after a median follow-up of more than 4 years, we show that pain, which is present in almost every patient preoperation, is controlled sufficiently in a high percentage of patients after the operation; 60% resp. 74% of the patients upon follow-up did not require any pain medication at all. Among those who had to stay on pain medication after the operation, the majority was dependent on narcotics, which per se might be difficult to withdraw even after the operation. The pain control rate was not significantly different in the Beger and the Frey group. Both groups had many patients with signs of advanced CP, reflected by a higher rate of local complications due to tumor size such as biliary obstruction or portal vein thrombosis.

Even though both procedures were very effective in controlling the main symptom (pain), we found a high rate (around 75%) of exocrine and a somewhat lower rate of endocrine pancreatic insufficiency (around 60%) in our patients. The incidence of exocrine pancreatic insufficiency was almost identical after Beger and Frey procedures and reflects the progressive damage to function of the gland that cannot be sufficiently be altered by the operation. It has been suggested that neither medical nor surgical therapy can change the decline in pancreatic exocrine and endocrine function in CP in the long run, and our results can be seen as a support to this hypothesis. It, therefore, has to be emphasized again that apart from the management of complications, pain control is the primary goal of surgical therapy of CP. Interestingly enough, however, we found an almost significant trend ($p=0.06$) to a higher rate of new onset diabetes (17% vs. 34%) in the groups of patients that received the Frey operation. This might reflect a loss of more islet cells due to an extension of the procedure and resection to the pancreatic tail which anatomically has a higher yield of islet cells. This observation, however, only reflects a trend and has to be seen under the limitations of a

retrospective observation, the nonrandomization of the groups, and the varying co-indications for surgery in both groups, as mentioned previously in our manuscript.

In conclusion, this study compared the Beger and Frey procedures for the surgical management of CP. Using a differentiated and individualized approach to CP, with the Beger procedure performed in cases with extrahepatic cholestasis, the long-term pain control as well as the long-term exocrine and endocrine pancreatic function, is comparable after the Beger and Frey procedures. Based on the currently available data, we, therefore, propose the following individualized management of patients with chronic pancreatitis.

As for pain control, the Beger and Frey procedures are comparable; we suggest the Frey procedure as the primary operation of choice. The Frey procedure is technically less demanding as a surgical liberation of the mesenterico-portal axis is not necessary, which can be challenging for the surgeon in the context of severe local inflammation.[24] In complicated and advanced cases which have been reported to be far more common in a German collective[17] and who present with a biliary, portovenous, or duodenal obstruction, the Beger operation offers the possibility to reconstitute biliary flow and allows for portovenous and duodenal decompression. There are of course studies that demonstrate a higher incidence of pancreatic cancer in patients with chronic pancreatitis[25] but more alerting as well in patients who had previous pancreatic drainage.[26–29] In our series, we could document the development and lethal outcome of pancreatic cancer in only one of the 92 patients after DPPHR, but follow-up regarding long-term risk of pancreatic cancer is still limited. Patients with recurrent chronic pancreatitis of unknown etiology have to be evaluated by surgeons. In cases with the suspicion of malignancy, the oncologic resection by pylorus preserving pancreatic head resection or the classical Whipple procedures remain the treatments of choice.

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CASE REPORT

Emergence of Imatinib Resistance Associated with Downregulation of C-Kit Expression in Recurrent Gastrointestinal Stromal Tumor (GIST): Optimal Timing of Resection

Vikas Dudeja · Leonard H. Armstrong · Pankaj Gupta · Howard Ansel · Sabeen Askari · Waddah B. Al-Refaie

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Abstract
Introduction Gastrointestinal stromal tumors (GISTs) are the most common gastrointestinal mesenchymal tumors. The activating mutation in the KIT (c-kit; CD117) proto-oncogene with subsequent tyrosine kinase activation plays a central role in the pathogenesis of GIST. Tyrosine kinase inhibitors are an integral part of GIST therapy. Initial response to neoadjuvant imatinib can be expected in up to 70% of the patients, thus offering an opportunity to surgically treat those with locally advanced primary or recurrent GIST. This favorable response to imatinib, however, is plagued with development of secondary resistance during the course of therapy.
Case description We herein report a case of recurrent locally advanced GIST in an elderly man, with excellent performance status, successfully managed with the integration of neoadjuvant targeted therapy and surgery.
Discussion Continued monitoring by a multidisciplinary team, including a surgeon, is vital for the success of neoadjuvant imatinib therapy for unresectable primary or recurrent GIST in the context of emergence of secondary resistance. As such, surgeons should participate in managing imatinib-treated GIST, as resection may become a viable curative option. This case also highlights that major oncologic resections can be safely performed in older persons when their performance status and comorbidities are carefully considered.

Keywords C-kit · Elderly · Gastrointestinal stromal tumor · Imatinib mesylate · Neoplasm · Drug resistance · Surgery

Case Description

We report the case of an 80-year old male, performance status Eastern Cooperative Oncology Group (ECOG)/ Zubrod score 0, who underwent pre-referral resection of gastrointestinal stromal tumors (GISTs) of the lesser curvature of the stomach in 2006. The histopathology revealed an intensely c-kit positive, 7-cm GIST with low mitotic count (less than five mitoses per 50 high-power fields). The tumor demonstrated a predominantly spindled (focally epithelioid) morphology with focal areas of necrosis and muscularis propria invasion. Overlying mucosa was not involved. Given the pathologic features (tumor size and mitotic count), the tumor was classified as "intermediate risk" based on guidelines proposed by National Institute of Health GIST Workshop 2001 for defining risk of aggressive behavior[1] (Fig. 1). He remained disease free for 1 year, when he was found to have asymptomatic recurrence occupying the entire lesser sac and involving the root of the mesentery on surveillance cross-sectional imaging (Fig. 2a). The recurrence also demonstrated avid fluorodeoxyglucose uptake on positron emission tomography (PET). Because of his excellent performance status and in an attempt to render the locally advanced GIST resectable, a multidisciplinary treatment

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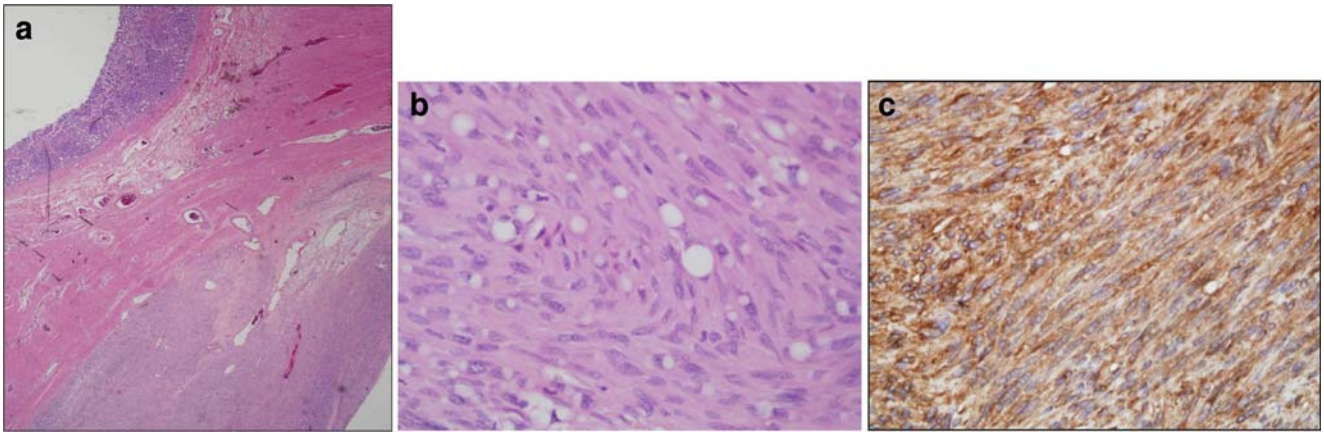

Figure 1 Histopathologic features of GIST prior to imatinib therapy. **a** GIST within gastric wall. High-power view (**b**) showing a cellular, cytologically bland, spindle cell tumor with prominent perinuclear vacuoles. **c** Tumor cells strongly mark for CD117 immunohistochemical stain.

decision was made to initiate imatinib therapy at 400 mg orally once daily. He demonstrated early (Fig. 2b) and continued (Fig. 2c) radiographic response to the imatinib therapy on cross-sectional imaging and PET scan for 8 months. During the course of treatment, the imatinib dose was decreased to 300 mg/day due to imatinib-related edema and fatigue. At 10 months (Fig. 2d), the radiographic response had plateaued, but the recurrent disease had regressed and was resectable with potential multiorgan resection. To avoid losing the window of opportunity for resection, a multidisciplinary decision was made to resect the recurrent GIST. He underwent a distal gastrectomy with Roux-en-Y gastro-jejunostomy and omentectomy with achievement of microscopically negative surgical margins (R0 resection). The final pathology showed a 6.5-cm, c-kit negative, cystic GIST with a low mitotic count (less than one mitosis per 50 high-power fields) and negative resection margins. Therapy-related regressive changes with hemorrhage and necrosis were noted (Fig. 3). A separate 2.8-cm omental GIST nodule was also identified. Postoperatively, adjuvant sunitinb therapy was initiated. The patient currently remains asymptomatic and without evidence of clinical or radiographic disease recurrence for 14 months.

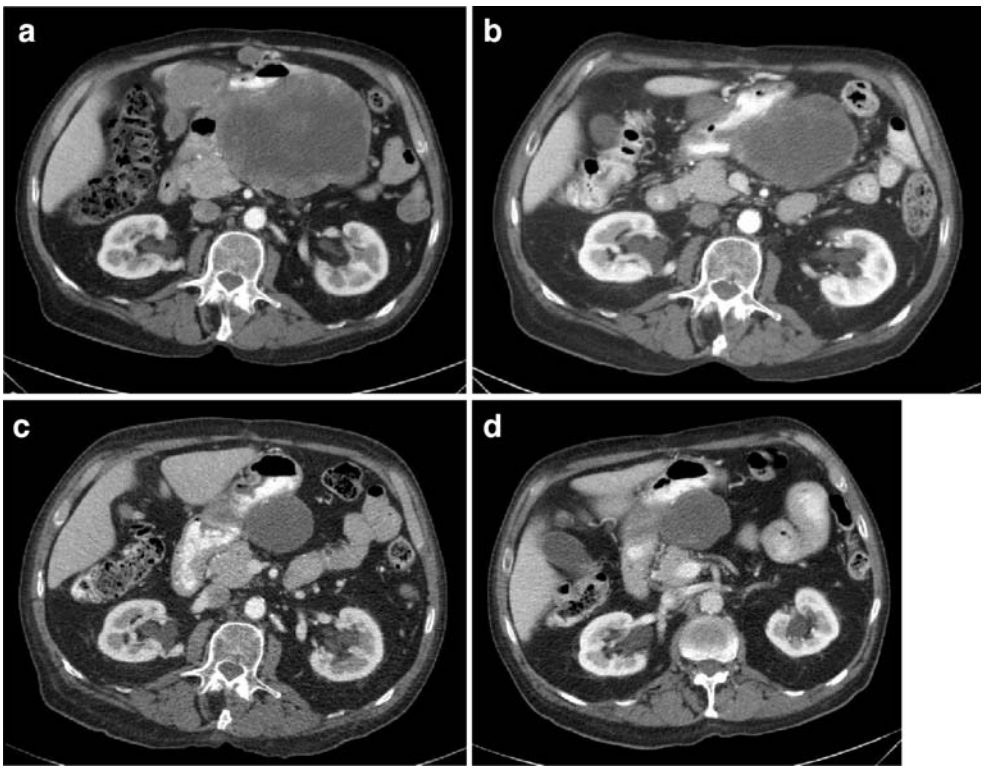

Figure 2 Cross-sectional imaging during the course of imatinib therapy. **a** CT scan at presentation, **b** after 2 months of imatinib therapy, **c** after 8 months of imatinib therapy, and **d** after 10 months of imatinib therapy. The response plateaued between 8 and 10 months.

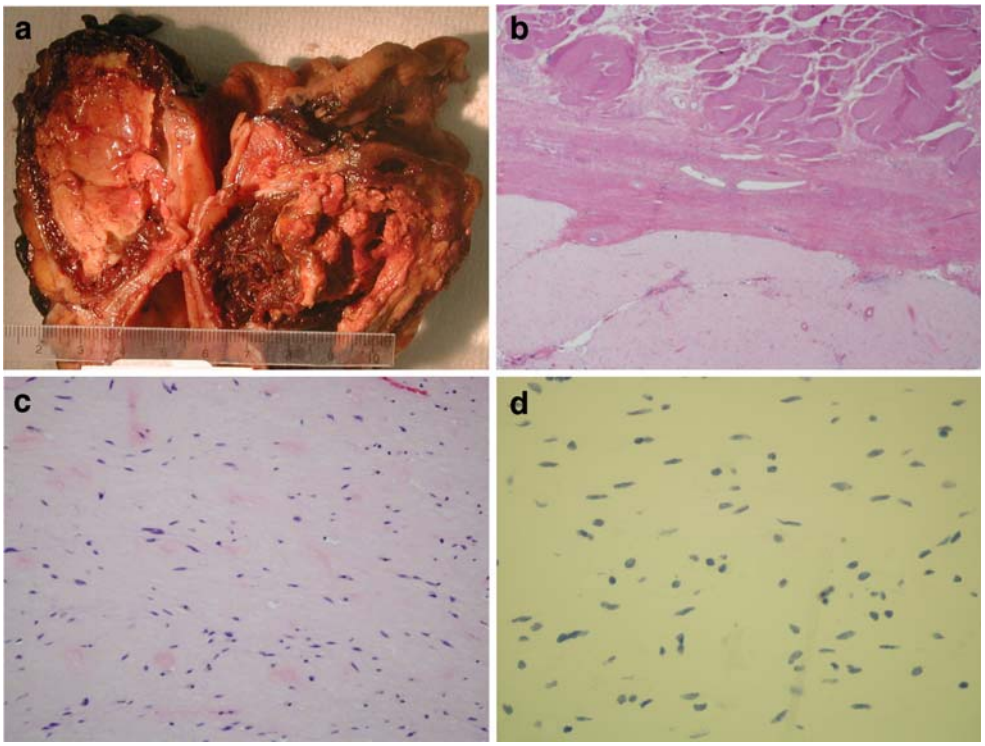

Figure 3 Pathology images showing altered tumor morphology following therapy. **a** Gross image of distal gastrectomy specimen showing an extramural tumor mass with cystic degeneration, hemorrhage, and necrosis. Medium- (**b**) and high-power images (**c**) demonstrate microscopic findings of a hypocellular tumor adjacent to gastric wall. Continued treatment with imatinib led to resistance, and the resected recurrent GIST lacked any detectable c-kit expression (**d**).

Discussion

We report a case of secondary imatinib-related resistance in an older patient with recurrent locally advanced unresectable GIST who underwent R0 resection after demonstrating early response to neoadjuvant imatinib therapy. Imatinib-related resistance is an evolving phenomenon that surgeons should be aware of when caring for patients with GIST on imatinib therapy for locally advanced, recurrent, or metastatic GIST. This case also highlights the important role that surgeons should play in managing GIST beyond those with primary resectable disease.

Patients with GIST who are being treated with neoadjuvant imatinib to downstage their disease are typically followed up for several reasons. Evaluation of response to imatinib therapy early in the course of treatment will help clinicians assess and predict treatment response by categorizing their patients into three sub-groups: (1) early responders, (2) those with stable disease, and (3) those with progressive disease while on targeted therapy. The current literature suggests that patients who show early radiographic response to imatinib therapy have a greater probability of undergoing R0 resection and of prolonged disease-free survival, as it is with our case.[2] Early responders are typically continued on imatinib (or other targeted) therapy to enhance their rates of R0 resection in the context of organ-sparing surgery. It also important to expect that in some instances, the disease may stabilize or progress on imatinib therapy.[3] Up to 15% of patients with GIST harbor primary resistance to imatinib.[4] The options for such patients are limited to escalation of imatinib dose or switching to alternative tyrosine kinase inhibitors such as sunitinib. Because of its potential effects in prolonging survival, sunitinib has been approved for use in imatinib-resistant GIST. The efficacy of sunitinib, however, is limited to partial response in 13% and stable disease in 40% patients over a period of 24 weeks.[5] Sunitinib-related side effects such as fatigue, diarrhea, hand-foot syndrome, hypertension, and myelosuppression are observed in up to 20% of patients.[6]

The present case also highlights that early response to imatinib should not instill a false sense of security. Continued close monitoring is important to detect secondary resistance. Close follow-up with cross-sectional imaging is important since response to imatinib therapy, in terms of maximal achievable response and time to progression, is variable; thus the duration of therapy needs to be individualized.[2, 7] Given the varied patterns of response to targeted therapy, patients on neoadjuvant imatinib need to be followed up by a multidisciplinary team with input from experienced surgeons to predict resectability. Once maximal response to imatinib therapy is achieved and the disease is considered resectable, further watchful surveillance can be counter-productive due to emergence of secondary resistant mutants and progression of disease with continued therapy. In our case, the close follow-up of the

patient with serial cross-sectional imaging provided us with a window of opportunity at which time though the tumor response to imatinib had plateaued, the tumor had regressed to a size where an organ-sparing resection was possible. A diminishing response should suggest that the maximal response has been achieved, and the surgical resection should be considered if the disease is resectable in patients with reasonable performance status. This is important since there is evidence in the literature of missed opportunities due to delay in surgical intervention, with poor outcomes.[2]

Emergence of secondary resistance to imatinib is a peculiar phenomenon. It is believed that exposure to imatinib drives selection of imatinib-resistant clones and ultimate failure of imatinib therapy and disease progression. In the current case report, the patient demonstrated early response. However, with continued imatinib treatment, the tumor response plateaued, signaling development of secondary resistance. Remarkably, even though the primary GIST tumor was c-kit positive, the recurrent tumor resected after neoadjuvant imatinib therapy was c-kit negative. Though emergence of imatinib-resistant clones with secondary c-kit mutations is well described,[8] this phenomenon of emergence of clones which are c-kit negative on immunostaining (suggesting downregulation of c-kit expression by the tumor cells) has not been described before. This observation further indicates that in patients who have received imatinib (for GIST or even other indications such as chronic myeloid leukemia), the lack of c-kit staining should not detract from a possible diagnosis of GIST.

Perhaps one of the other interesting aspects of this case is the decision to perform major oncologic resection in the elderly. Our patient highlights the importance of carefully evaluating performance status and the presence or absence of comorbidities, rather than making age-based treatment decisions. Performance status and comorbidities in older persons vary widely, as they may not have the same endurance for major surgical procedures. The surgical literature also supports the view that outcomes in patients undergoing major oncologic resections, e.g. pancreatectomies, are affected by performance status in addition to chronologic age.[9] Studies have now shown that older patients with good performance status can undergo major oncologic surgeries safely.[10–12] Most recently, a large multihospital risk-adjusted study of the American College of Surgeons National Surgical Quality Improvement Program has demonstrated that older age is associated with worse short-term outcomes after major oncologic resections.[13] However, the effect of age was not prohibitively worse and is comparable to that of other preoperative factors, thus supporting risk-based treatment decision-making on the part of both surgeons and their patients.[13] A detailed analysis of preoperative performance status and comorbidities is therefore important to ensure favorable short- and long-term operative outcomes.

Summary

Neoadjuvant imatinib therapy has made surgical resection feasible for patients presenting with recurrent unresectable GIST. While on neoadjuvant therapy, patients should be continually evaluated by surgical team for resectability since patterns of response and progression are variable. Once maximal response is achieved and the tumor is deemed resectable, R0 surgical resection should be considered in patients with good performance status. Continued therapy in such patients entails risk of emergence of secondary resistance with progression of the disease, which may preclude the benefit of surgical resection. Imatinib therapy can lead to selection of c-kit negative imatinib-resistant clones of cancer cells.

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HOW I DO IT

Completion Mucosectomy for Retained Rectal Mucosa Following Restorative Proctocolectomy with Double-Stapled Ileal Pouch–Anal Anastomosis

Maria E. Litzendorf · Arthur F. Stucchi · Susana Wishnia · Amy Lightner · James M. Becker

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Abstract
Introduction Colectomy with ileal pouch–anal anastomosis has become widely accepted and is now considered the procedure of choice for patients with ulcerative colitis (UC) as well as familial adenomatous polyposis (FAP).
Discussion The clear patient advantage of functional continence has pushed this procedure to the forefront in treating both UC and FAP. As a result, the procedure continues to evolve with recent debate centering on the question of whether to perform a double-stapled technique without rectal mucosectomy or a handsewn anastomosis following transanal mucosectomy. Although continence and complication rates continue to be hotly debated, it is understood that performing the stapled procedure does leave a rectal cuff, which carries with it the possibility of disease persistence or recurrence. As such, if the rectal cuff becomes symptomatic or dysplastic, it must be removed. This is accomplished by performing a transanal completion mucosectomy and reconstructing the ileal pouch–anal anastomosis.

Keywords Completion mucosectomy · Restorative proctocolectomy · IPAA · Stapled anastomosis · Handsewn anastomosis · Ileoanal anastomosis

Introduction

Ulcerative colitis (UC), a chronic inflammatory bowel disease that affects the mucosa of the colon and rectum, has been recognized as a distinct disease entity for over 150 years. The last century has seen a myriad of surgical treatments aimed at removing the diseased colon and rectum from patients refractory to medical therapy.[1] Until about 30 years ago, the only surgical option for patients with UC or familial adenomatous polyposis (FAP) was a total proctocolectomy with a Brooke ileostomy.[2] Although this operation removes all the diseased colon and rectum as well as any subsequent risk of malignant transformation, it was never well received by patients or their physicians due to the significant problems associated with a permanent, incontinent abdominal ileostomy. Thus, surgeons sought alternatives to total proctocolectomy and ileostomy that could provide the patient with continence and acceptable function.

Although there were early attempts at continence-sparing operations, such as the continent ileostomy or Kock pouch,[3] these operations were fraught with technical complications and poor functional results. With the resurgence of restorative proctocolectomy with anal sphincter preservation, first proposed by Ravitch and Sabiston in the late 1940s,[4] there was new hope for patients wishing to avoid a permanent abdominal stoma and remain continent following a proctocolectomy. Since its reintroduction 30 years ago,[5,6] the procedure has undergone numerous technical advances including the addition of an ileal pouch that significantly improved functional outcome.[7,8] As surgeons became more familiar with the technical aspects

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of the procedure and the management of early and late complications, the use of restorative proctocolectomy became more prevalent. Today, most surgeons agree that restorative proctocolectomy with ileal pouch–anal anastomosis (IPAA) is the definitive operation for the surgical treatment of patients with UC, FAP,[9–11] and, more recently, hereditary nonpolyposis colorectal cancer.[12]

The advent of the modern IPAA in the early 1980s brought with it operative advances that focused on several issues including the functional utility of the various pouch configurations (S, W, or J) and staging of the procedure with a temporary loop ileostomy. For the most part, the debate surrounding these controversies has been settled by prospective trials with most major centers now utilizing a J pouch configuration with a two-stage procedure. The first operation consists of a total proctocolectomy, endorectal IPAA, and diverting loop ileostomy while the second stage involves the closure of the ileostomy approximately 8–12 weeks later. Despite a consensus on these technical points, preservation of the surgical anal canal, most notably the anal transition zone (ATZ), has long been a significant source of controversy among experts and still overshadows the overall benefit of this remarkable surgical achievement. To create the pouch–anal or ileoanal anastomosis, the surgeon can choose to either handsew or staple the ileal pouch to the anal canal. This is an important operative decision, with significant long-term health implications. If the decision is made to use a stapling device, a cuff of rectum containing intact anorectal mucosa must remain. In sharp contrast, all the anorectal mucosa is removed transanally prior to the handsewn procedure. Thus, when the stapled technique is utilized, the ATZ is preserved; alternatively, with the handsewn procedure, a mucosectomy is performed at the level of the dentate line which completely eliminates the mucosa from the ATZ and the proximal cuff of rectal epithelium.[13] Hence, these techniques differ dramatically in the amount of rectal mucosa that remains following surgery.

Mucosectomy with Handsewn Versus Double-Stapled Anastomosis

When restorative proctocolectomy was originally envisioned by Ravitch and Sabiston in the late 1940s,[4] it was based on the premise that UC is mucosal disease and following proctocolectomy, a mucosal proctectomy or mucosectomy could be performed that selectively dissects away all the disease-bearing columnar mucosa above the dentate line and prevent recurrence of the disease. This technique would ostensibly preserve continence by sparing the rectal muscular cuff and the anal sphincter apparatus. Then, in place of the much maligned permanent ileostomy, the continuity of the intestinal tract could be reestablished by extending the terminal ileum into the pelvis endorectally and circumferentially suturing it to the anus in an end-to-end fashion.[4]

The controversy began in the late 1980s when the newly developed circular end-to-end anastomosis (EEA) stapler was first utilized to complete the ileoanal anastomosis as an option to the more technically demanding and tedious mucosal proctectomy and handsewn technique.[14] In order to better understand the underlying reasons for the debate, key anatomical landmarks of the anal surgical canal from an operative perspective must be clearly delineated (Fig. 1). Perhaps the most relevant surgical landmark of significance to this controversy is the dentate line, proximal to which lies rectal mucosa and distally the anoderm. Since UC is a mucosal disease, often originating in the rectum and progressing proximally, the rectal mucosa must always be

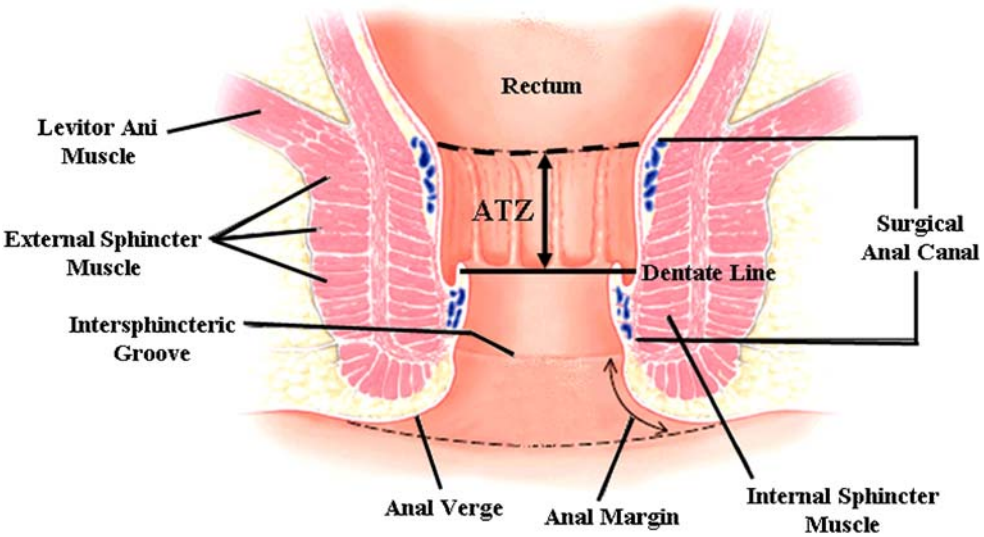

Figure 1 Anatomy of the surgical anal canal showing key anatomical landmarks. Most notable is the dentate line above which lies the rectal mucosa. Adapted with permission from Beck.[15]

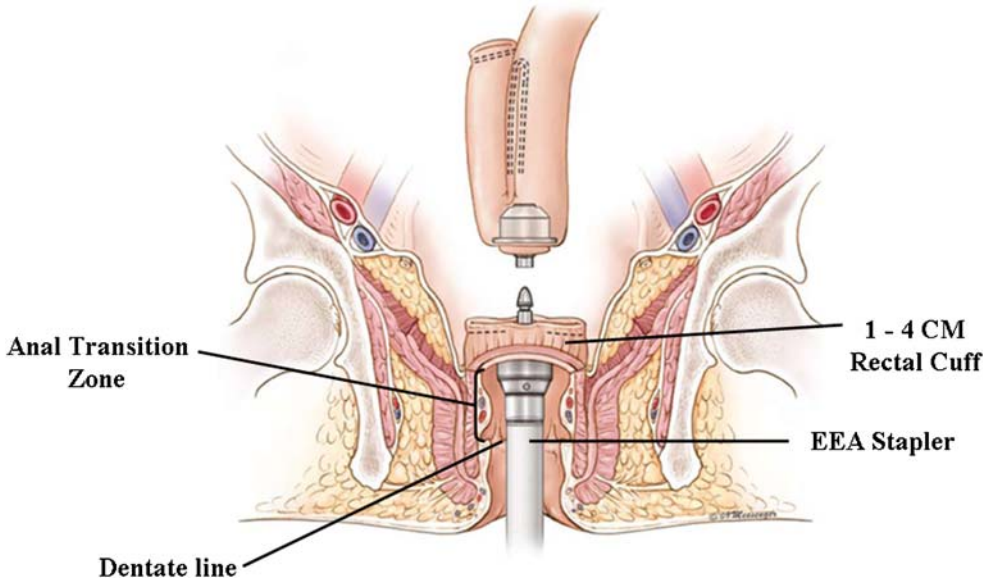

Figure 2 Double-stapled ileal pouch–anal anastomosis technique requires the use of end-to-end anastomosis or EEA stapler. Retained rectal mucosa remains distal to the anastomosis. The anvil is affixed to the apex of the newly created ileal pouch with a purse-string suture. After the head of the EEA stapler is advanced into the anal canal, the connection pin is located next to the staple line at the site where the rectum was previously divided at the floor of the pelvis. The anvil of the stapler within the pouch is then brought down into the pelvis and carefully aligned and fit onto the prefixed pin of the stapler. The stapler is then fired to secure the pouch to the rectal cuff. Copyright 2008 Lori A. Messenger, CMI, with permission.

considered a disease-bearing tissue and, if left behind after surgery, is at significant risk for disease persistence or recurrence and malignant degeneration. The surgical anal canal including the ATZ is surrounded by the internal anal sphincter. This muscle, responsible for the maintenance of resting anal tone, is innervated by the autonomic nervous system and is under involuntary control. Proponents of the stapled anastomosis argue that mucosal proctectomy weakens the internal sphincter, thereby potentially reducing postoperative resting tone and compromising continence. The external sphincter which surrounds the internal sphincter and is innervated by somatic nerves generates the voluntary anal squeeze and is not thought to be affected during mucosal proctectomy.

In order to accommodate the stapled anastomosis, the rectum is dissected down to the level of the pelvic floor and divided several centimeters above the dentate line (Fig. 2). Although this positioning ensures that the final pouch–anal anastomosis is within the surgical anal canal, the diseased rectal mucosa within the ATZ remains intact. Herein lies the crux of this controversy and the fundamental flaw of the stapled IPAA surgical technique. The retained rectal mucosa in the ATZ proximal to the dentate line is disease-bearing tissue that is highly susceptible to symptomatic

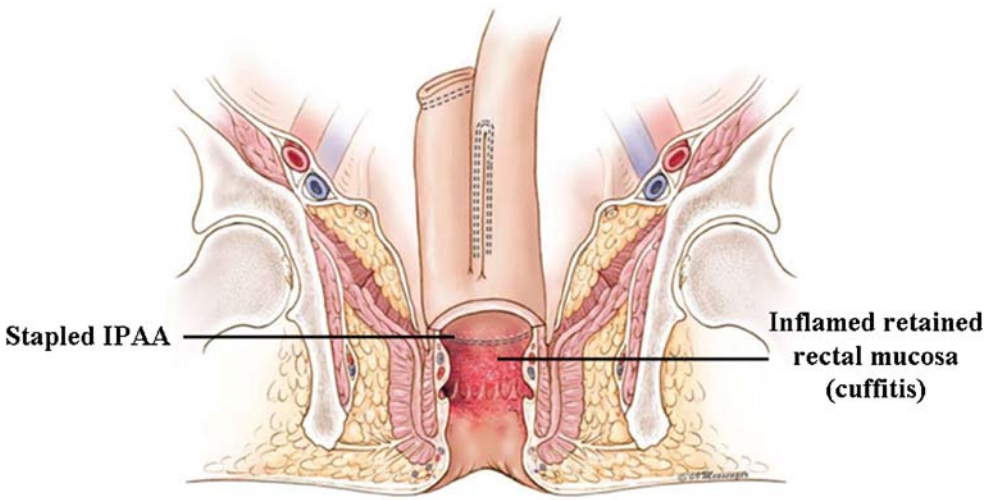

Figure 3 Active inflammation ("cuffitis") in the retained rectal mucosa. Copyright 2008 Lori A. Messenger, CMI, with permission.

Table 1 Comparison of Handsewn vs. Stapled Ileal Pouch–Anal Anastomosis

Handsewn	Stapled
Advantages	
Excellent long-term function	Excellent long-term function
No disease recurrence	Higher resting sphincter pressure
No or very low cancer risk	Improved nocturnal continence
No annual surveillance required	Easier to learn, low failure rate
	Less manipulation of anal canal
Disadvantages	
Risk of damage to anal sphincter	Possibility of disease recurrence
Technically demanding	Risk of dysplasia or cancer
	Annual surveillance required
	Chronic inflammation in ATZ

From Lovegrove et al.[18] Reprinted with permission from Wolters Kluwer Health

disease recurrence, chronic inflammation, or cuffitis (Fig. 3) and is at significant risk of dysplasia and cancer.[13] For this reason, some surgeons advocate a more individualized approach to the anastomotic technique based on the presence of dysplasia or cancer in the preoperative endoscopic evaluation.

Although preservation of the ATZ purportedly improves functional results,[16–19] shortens operative times,[20] and reduces septic complications,[21,22] considerable controversy still exists regarding long-term outcomes as a result of recurrent or persistent disease and the risk of malignant degeneration in the ATZ. In sharp contrast, surgeons who advocate mucosal proctectomy, as we do at our center, emphasize that the complete removal of all rectal mucosa not only confers the highest likelihood of a complete surgical cure but more importantly removes all future risk of malignant transformation.[10] However, as mentioned above, opponents argue that because a mucosectomy involves removal of the highly innervated region of cuboidal transitional epithelium that divides columnar and squamous epithelia within the surgical anal canal, the sphincter complex could be easily damaged and functionally compromised.[13,23]

Each technique has advantages and disadvantages in long-term functional outcomes, operative and postoperative complications, and risk of neoplasia (Table 1).[18] Many younger surgeons favor the double-stapled technique because this is the simpler operation, easier to learn, and it may have a lower risk of failure.[24] As mentioned, although the mucosectomy requires greater manipulation of the anal canal with potential risk of damage to the sphincter mechanism, the stapled IPAA leaves diseased rectal mucosa within the ATZ which can lead to disease persistence or recurrence and inflammation at any time. There are a number of symptoms associated with chronic inflammation of the ATZ including a pouchitis-like syndrome called cuffitis, increased urgency and frequency, sinus tracts, strictures, and chronic pelvic pain, as well as dysplasia and cancer (Table 2). However, it is the risk of malignant transformation of retained rectal mucosa with the double-stapled technique that requires vigilant lifelong surveillance.[25,26] In those cases where the symptoms of retained rectal mucosa become overbearing or refractory to medical therapy, it becomes necessary to perform a completion mucosectomy which involves the removal of the retained rectal mucosa from the ATZ and reconstruction of the ileal pouch–anal anastomosis as described below. Given the paucity of data in this realm, the long-term fate of the ATZ in the surgical management of UC has yet to be determined.

Completion Mucosectomy: Operative Technique

Patients who are experiencing symptoms associated with retained rectal mucosa after IPAA undergo pouchoscopy with biopsies, as well as anorectal manometry to establish baseline sphincter function prior to surgery. Patients are seen preoperatively by our stoma nurses for ileostomy marking. A bowel prep is given the day before surgery.

After administration of general anesthesia, the patient is placed in the lithotomy position, and the anal canal is irrigated with dilute betadine solution. The patient is prepped, draped, and positioned with sterile stirrups, and a Foley catheter is placed using sterile technique. The table is

Table 2 Symptoms and Presentation of Chronic Inflammation of the Anal Transition Zone

Recurrence of original ulcerative colitis
Pouchitis-like symptoms including urgency, frequency (>10 BM/day) and bloody diarrhea
Incontinence or leakage
Stricture
Cuffitis or chronic inflammation of the ATZ
Chronic pelvic pain
Dysplasia
Rectal cancer

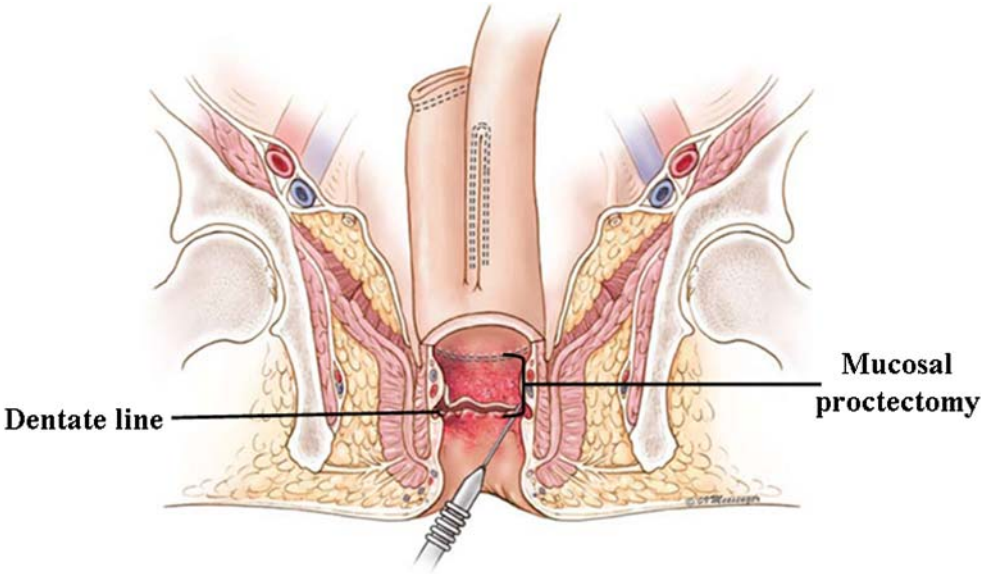

Figure 4 Transanal mucosectomy. The mucosal proctectomy is begun at the level of the dentate line in order to remove all disease-bearing retained rectal mucosa. Needle tip electrocautery on the cut setting allows the tissue planes to be more easily identified, and once a circumferential incision is made with the cautery, the mucosa is then carefully stripped away from the surrounding rectal cuff and underlying sphincter mechanism. Copyright 2008 Lori A. Messenger, CMI, with permission.

placed in the Trendelenburg position, and a Lonestar retractor is then used for exposure of the anal canal. The dentate line is identified, and 20 ml of dilute epinephrine is circumferentially infiltrated into the submucosa. Using needle tip electrocautery on the cut setting a circumferential incision is made at the level of the dentate line. The retained rectal mucosa is then dissected away from the anal sphincter (Fig. 4). The dissection is primarily blunt, taking care not to damage the sphincter mechanism. Electrocautery is used for hemostasis, again exercising caution to preserve all muscle fibers. The mucosal dissection continues cephalad until the ileal pouch is identified. The mucosa is then transected, making certain that the proximal segment contains circumferential ileal pouch (Fig. 5). After transection of the remnant rectal mucosa, the pouch may require additional mobilization to facilitate construction of the new ileal pouch–anal anastomosis. Allis-Adair clamps are placed on the distal pouch circumferentially. The clamps serve as a handle to facilitate the reconstruction of the ileal pouch–anal anastomosis assuring that the ileal pouch can be mobilized and advanced such that there is no tension on the pouch prior to completing the anastomosis. Interrupted 3–0 polyglycolic acid sutures are used to anastomose the apex of the ileal pouch to the dentate line. Dissection continues laterally along the pouch, again to ensure that the sphincter mechanism and muscular rectal cuff remain intact. If the pouch requires further mobilization, a laparotomy is indicated. If this is the case, we find that the pouch is seldom

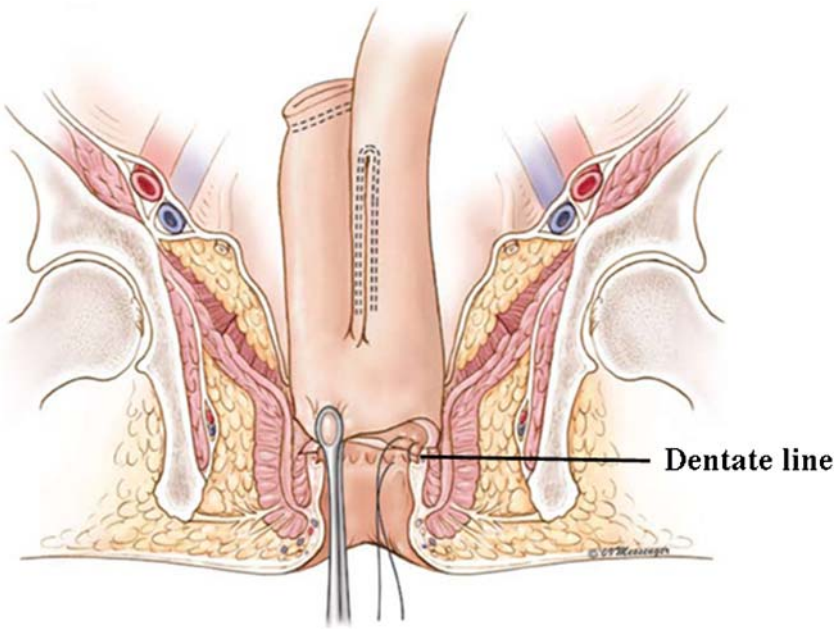

Figure 5 Construction of a new ileal pouch–anal anastomosis. Once the retained mucosa has been dissected away from the rectal cuff and transected, the ileal pouch–anal anastomosis is recreated. Allis clamps help maintain the pouch orientation as full-thickness pouch is re-approximated to the dentate line assuring that there is no tension on the pouch prior to completing the anastomosis. Copyright 2008 Lori A. Messenger, CMI, with permission.

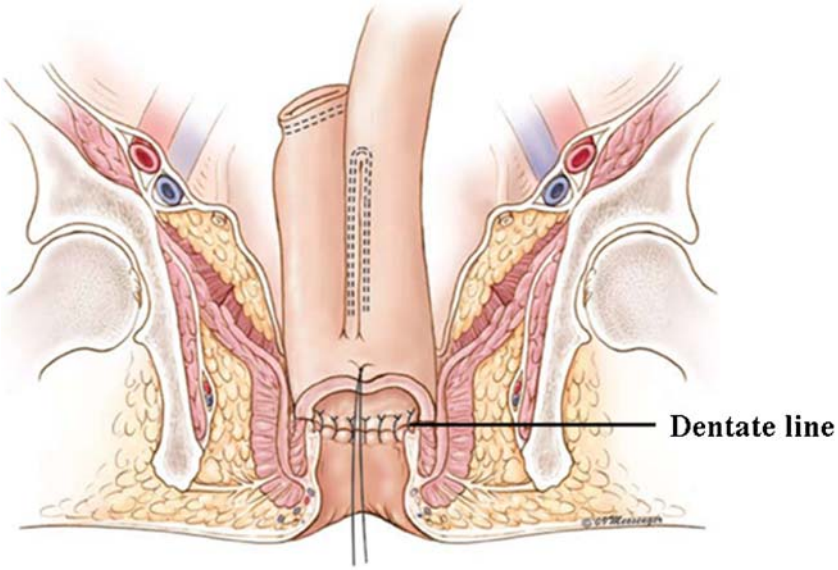

Figure 6 Final configuration of ileal pouch–anal anastomosis. The retained rectal mucosa has been removed, and a new handsewn anastomosis created between full-thickness ileal pouch and the anoderm. Copyright 2008 Lori A. Messenger, CMI, with permission.

salvageable and routinely perform a laparotomy to resect the pouch after it is mobilized out of the pelvis before constructing a standard 15-cm J pouch as has been previously described.[9] The ileal mesentery is mobilized to the exit of the superior mesenteric artery from the pancreas. Relaxing incisions in the mesentery may be required. In either case, the pouch orientation is maintained carefully so as not to compromise the ileal blood supply. The apex of the J pouch is then advanced into the pelvis in an endorectal position. The distal pouch is secured to the anal sphincter in four quadrants with interrupted 2–0 polyglycolic acid sutures. The purse string in the apex of the pouch is then cut to allow the enterotomy to open. The full-thickness pouch is then sutured to the dentate line circumferentially with 3–0 polyglycolic sutures, thus completing a side-to-end ileal pouch–anal anastomosis (Fig. 6).

The patient is then taken out of the lithotomy position. The surgical team changes gowns and gloves and a new surgical instrument table is used for creation of the loop ileostomy as has been previously described.[9] After returning to the abdomen, the pouch is identified as it enters the pelvis. A site approximately 40 cm proximal to the pouch is chosen for creation of the loop ileostomy. The loop of ileum is brought out through the previously marked ostomy site. Prior to closing the midline incision, we routinely place an anti-adhesive barrier in the pelvis and under the midline incision. Once the fascia and skin are closed, the loop of ileum is opened transversely and matured over a rod with 4–0 polyglycolic acid sutures. Sagittal views of the ileal pouch with a stapled anastomosis and inflamed retained rectal mucosa are shown in Fig. 7a, and the postoperative view following the completion mucosectomy and ileoanal anastomosis is shown in Fig. 7b.

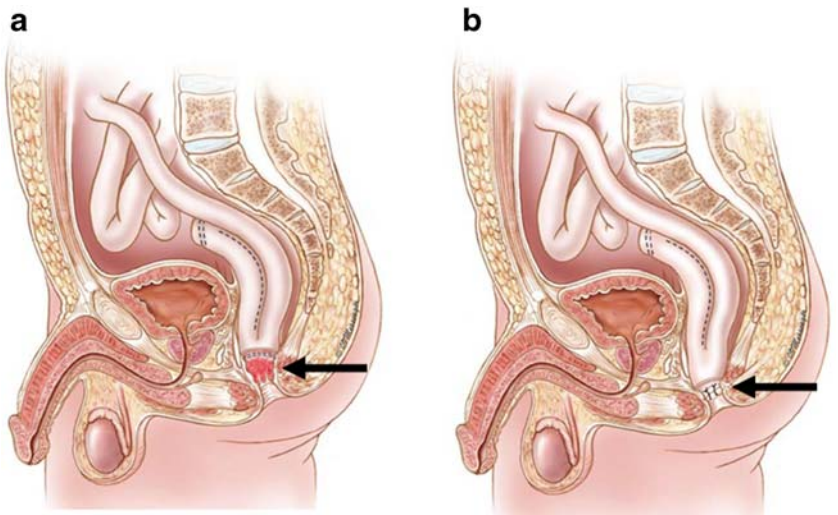

Figure 7 **a** Sagittal view showing active inflammation (*arrow*) in retained rectal mucosa; **b** sagittal view showing new handsewn ileal pouch–anal anastomosis (*arrow*) after removal of retained rectal mucosa. Copyright 2008 Lori A. Messenger, CMI, with permission.

Postoperative Care

Most of our patients are hospitalized for an average of 5 days and are discharged home on a low fiber diet. Approximately 4 weeks after the operation, patients undergo a barium radiographic study to assess the integrity of the ileal pouch and the ileoanal anastomosis. Anal manometry is repeated to ensure the anal sphincter mechanism is intact. If results are satisfactory, patients undergo closure of their ileostomy approximately 8 weeks after the initial surgery. Following closure of the ileostomy, patients are followed at regular intervals with anal manometry at 1 year and ileal pouchoscopy with surveillance biopsies every 5 years.

Outcomes

Although a recent meta-analysis of 21 studies between 1988 and 2003 comparing 2,699 handsewn with 1,484 stapled IPAA patients showed no significant differences in the incidence of postoperative complications or early postoperative outcomes between either anastomotic technique, it did show that patients who underwent a stapled IPAA had higher anorectal physiologic measurements which was reflected in significantly improved nocturnal continence.[18] Interestingly, the study also demonstrated that the stapled IPAA group showed a higher incidence of dysplasia in the ATZ, and while it did not reach statistical significance (P=0.080), the relevance of this finding in the context of the procedure cannot be overlooked. Although the relative risk of long-term neoplastic transformation in the retained rectal mucosa could not be quantified by this study, the statistical strength of this trend warrants closer scrutiny by pundits of preserving the ATZ. This fact is highlighted in more recent studies clearly demonstrating the presence of chronic inflammation in the ATZ in nearly 90% of IPAA patients with retained mucosa.[19,27] Although chronic inflammation appears to have limited clinical impact, the mere presence of ATZ inflammation in such a high number of patients clearly warrants life-long surveillance with biopsies. The authors of the present communication feel strongly that chronic inflammation of the ATZ cannot persist for years without a significant clinical impact, not to mention an increased risk for malignant degeneration over time. This was apparent in our own series of patients with complications related to retained rectal mucosa in which we assessed the outcomes after completion mucosectomy.

In our series, 27 patients who underwent completion mucosectomy for retained rectal mucosa failed protracted medical therapy before the diagnosis was confirmed by biopsies. Over two thirds presented with cuffitis or chronic inflammation in the ATZ which was resolved by a completion mucosectomy performed by a single surgeon (JMB). Bowel movements (≥10 per day) and the incidence of pouchitis-like symptoms (≥80%) were significantly reduced at 3 and 12 months postoperatively. Day or night incontinence (≥60%) was also significantly reduced by over 70% at 3 and 12 months. Over 90% of patients reported being moderately or very satisfied with their surgical outcome.

Summary

Completion mucosectomy is a low-risk and effective surgical option for which patients experiencing unremitting complications associated with chronic inflammation in the ATZ can expect a good outcome. Although IPAA with either anastomotic technique is safe and results in a rapid and profound improvement in quality of life, the long-term risk for dysplasia in patients who received a stapled IPAA is only recently coming to light,[18] undoubtedly due to the high number of stapled-IPAA patients with chronic inflammatory changes in the ATZ.[19] For these patients, life-long surveillance has become necessary.

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REVIEW ARTICLE

Management of Acute Post-operative Portal Venous Thrombosis

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Abstract
Background Portal vein thrombosis can be a devastating, but often overlooked, complication of hepatobiliary procedures. Symptoms of acute portal vein thrombosis range from nondescript abdominal pain to septic shock secondary to mesenteric ischemia.
Discussion The surgeon must be cognizant of these symptoms and the potential for portal vein thrombosis after any hepatobiliary procedures as an expedient diagnosis and treatment is necessary in order to prevent thrombus propagation, bowel ischemia, and death. This report outlines the symptoms, diagnosis, and a review of the literature on the treatment of acute portal vein thrombosis after hepatobiliary surgery with a special note made regarding a case of portal vein thrombosis after pancreatectomy and autologous islet cell transplantation.

Keywords Portal vein thrombosis · Islet cell transplantation · Chronic pancreatitis · Portal vein thrombectomy

Introduction

Portal vein thrombosis refers to any thrombosis developing in the portal vein, its branches, or with extension into the splenic, superior mesenteric, or inferior mesenteric veins. As with any venous thrombotic condition, the etiology of acute portal vein thrombosis (PVT) can be categorized based on Virchow's triad of venous stasis, hypercoaguable state, and endothelial injury. These etiologies are not independent of each other and often times several factors may coexist.[1] Venous stasis may occur with conditions in which intrahepatic blood flow is impeded, such as with the Budd–Chiari syndrome or portal hypertension associated with cirrhosis.[2,3] Hypercoaguable states may be divided into inherited and acquired disorders with inherited disorders encompassing such disease states as antithrombin III deficiency, protein C/S deficiency, and factor V Leiden mutation. Acquired states of hypercoaguability include malignancy, myeloproliferative disorders, oral contraceptive medications, and pregnancy. Finally, states of endothelial injury include intra-abdominal infections/inflammatory processes such as pancreatitis, cholecystitis, or diverticulitis/colitis and also involve direct injury or manipulation of the portal vein which may occur with splenectomy, surgical shunts, liver transplantation, or abdominal surgeries.[2,4] It is this last group of etiologies, specifically acute PVT after hepatobiliary surgery, which is the focus of this review.

Most literature regarding PVT after hepatobiliary surgery refers to liver transplantation with a reported incidence of 2–6%.[5,6] Smoot et al. reported a 5% acute (less than 30 days post-operative) PVT rate in patients who underwent portal vein reconstruction during pancreaticoduodenectomy (PD).[7] Although there was a difference in rates based on reconstruction with polytetrafluoroethylene interposition graft versus lateral venorrhaphy and primary end-to-end reconstruction, the difference was not significant (33%

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versus 12%, respectively, $p=0.16$). The low incidence of acute PVT may be secondary to a lack of detection until chronic changes have occurred. These chronic manifestations of PVT most often present with esophageal varices and subsequent rupture as Witte et al. demonstrated 60% of their cohort with chronic PVT presented with hematemesis.[8,9] In addition, splenomegaly is a common finding secondary to increased resistance to splenic outflow and is reportedly found in 75–100% of patients.[4,8,10] Histologically, increased reticulin deposition has been demonstrated around the hepatic portal triads reminiscent of non-cirrhotic portal fibrosis.[4] The utility of liver biopsy in the setting of PVT is limited, but this finding does demonstrate that hepatic architectural changes do take place and perhaps potentiate portal hypertension. Although the formation of varices, variceal hemorrhage, and portal hypertension is not seen in acute PVT, as it is in chronic PVT, the most feared complication is propagation of the thrombus into the superior mesenteric vein resulting in bowel ischemia, sepsis, and death. Certainly, mortality rates are higher in cases with associated mesenteric ischemia. The ability to diagnose and, therefore, treat PVT is of paramount importance in order to prevent the catastrophic case of mesenteric ischemia resulting from this complication.

Signs and Symptoms

The symptoms of acute PVT are usually non-specific but may involve vague abdominal pain, nausea, and potentially fevers. Klempnauer et al. reported that 71% of the patients in their series had a presenting symptom of acute abdominal pain, 13% presented with abdominal colic, and 6.5% of patients in their series presented with bloody stool.[11] Ascites is usually a rare presenting sign, but if present, is usually transient because collateral circulation has not yet developed. Otherwise, the presence of ascites denotes chronic liver dysfunction.[8] Laboratory values are usually nondescript as liver function tests are usually normal, although mild elevations in transaminases, alkaline phosphatase, and bilirubin can be seen.[4,8] Sharp increases in liver function tests should raise the suspicion of the clinician of the potential for PVT, especially when taken in the context of other signs and symptoms. Decreased white blood cell and platelet counts may also be present when associated with hypersplenism, but an increased white blood cell count in the presence of metabolic acidosis, increased abdominal pain, and hemodynamic instability should warrant further diagnostic imaging as the potential for bowel ischemia is great.[4,8] Hemodynamic instability outside of that associated with septic shock does not typically occur with acute PVT. It has been suggested that with portal vein occlusion, hepatic arterial flow increases but a hyperkinetic state soon develops. A significant decrease in systemic vascular resistance with a concomitant increase in cardiac output has been noted.[10] A high index of suspicion must, therefore, be present in patients with the above signs and symptoms when they are out of the ordinary for what should be expected after typical hepatobiliary procedures.

Diagnosis

Acute PVT after hepatobiliary surgery may not be suspected because of a lack of symptoms and relative paucity of cases reported. However, several modalities exist in order to secure the diagnosis of portal vein thrombosis. The choice of imaging in order to visualize the location and extent of portal thrombus depends on each individual institution's ability to mobilize the proper resources in order to provide an expeditious diagnosis and ultimately, treatment (Table 1). Color Doppler has been used to visualize portal thrombus but is extremely user-dependent, may be limited secondary to body habitus or overlying bowel gas, often cannot visualize acute thrombus secondary to its non-echogenic nature, and often cannot be acquired late at night. However, the fact that it is non-invasive and inexpensive makes it a valuable screening tool. The sensitivity and specificity for color Doppler to detect portal thrombosis vary and range from 89% to 93% and 92% to 99%, respectively.[12,13] Compared to color Doppler evaluation, computed tomography (CT) of the abdomen, especially when coupled with thin cuts through the porta hepatis, yields results similar to that seen with Doppler. The advantages of abdominal CT include a high sensitivity (90%) and specificity (99%) to diagnose PVT as well as more accurate delineation of the portal vein anatomy that contains thrombus.[13] However, cost may preclude its use in some instances. Magnetic resonance angiography (MRA), although costly and time-consuming, can provide exquisite detail of the portal anatomy including flow direction and disturbances. In regards to acute PVT, MRA usually is not required but is instead more useful in the chronic state of thrombosis seen in patients with liver failure who may be considered for liver transplantation. Historically, the gold standard for the diagnosis of PVT is portal venography. Not only does this allow diagnosis but also treatment of the thrombosed vessels, although it is more invasive with associated complications. In one small series, portal venography was correlated with the surgical presence of PVT and had a sensitivity of 100% and specificity of 90%.[12] The surgeon, therefore, can utilize a multimodality approach as it relates to the workup of this potentially lethal complication.

Treatment Options

Anticoagulation

Treatment of portal vein thrombosis is dictated by the acuity of the thrombus and associated complications. Serial abdominal exams as well as serial lactic acid levels, liver function tests, and factor V levels should be measured to assess for the progression of potential bowel ischemia and liver dysfunction. After securing the diagnosis of PVT, therapeutic anticoagulation with heparin should be instituted as soon as possible in order to prevent propagation of thrombus with its associated repercussions. It has been demonstrated that expeditious anticoagulation results in a greater likelihood of portal vein recanalization. Turnes et al. retrospectively evaluated 38 patients who had the diagnosis of acute portal vein thrombosis either alone or in combination with other associated veins (splenic, mesenteric). Anticoagulation was instituted in 27 patients within 30 days of the onset of their symptoms. Twelve patients demonstrated recanalization with 50% demonstrating complete recanalization of the thrombosed portal vein versus the 11 patients who did not receive anticoagulation in which no evidence of recanalization was demonstrated. Of interest is the fact that in this group of 12 patients who demonstrated recanalization, 83% (ten of 12) were started on anticoagulation within 1 week of symptom onset versus the remaining two patients who were started on anticoagulation greater than 1 week after diagnosis.[14] This study was confirmed in a prospective analysis of patients with acute PVT in which 38% of patients with associated ascites had recanalization versus 65% of patients without ascites at 1 year. The presence of ascites was an independent predictor of failure of anticoagulation to produce recanalization of the portal vein.[15] This is likely secondary to the fact that ascites dictates a more chronic process and this further supports the necessity of the expeditious institution of anticoagulation in patients with acute PVT. Thrombus burden also has an effect on response to anticoagulation therapy and should be taken into account when selecting patients for anticoagulation alone in the treatment of acute PVT. In a retrospective study performed by Condat et al., patients presenting between 1983 and 1999 with acute portal vein thrombosis, as defined by (a) the onset of recent

Table 1 Comparison of Portal and Mesenteric Vein Thrombosis Etiology and Diagnosis in Selected Series

Author	*N*	Etiology (%)					Diagnosis (%)			Location (%)		
		Hypercoag	Malignancy	Infxn/Infl	Operative	Idiopathic	U/S	CT	MRA	PV	SMV	Comb
Janssen[1]	172	27	24	17	23	16	–	–	–	89	0	11
Demertzis[42]	1	0	0	0	0	100	–	100	–	100	0	0
Klempnauer[11]	31	6.5	29	0	32.3	0	39	16	0	26	61	13
Zyromski[26]	1	0	0	0	100	0	100	–	–	0	0	100
Dutta[43]	20	25	5	0	10	50	–	–	–	20	5	75
Amitrano[44]	121	69.4	–	10	19.2	0	–	–	–	33.9	17.3	48.8
Condat[16]	33	54.5	3	36.3	3	24.2	84.8	66.6	0	–	–	–
Henao[45]	1	0	0	0	100	0	0	100	0	0	0	100
Malkowski[21]	33	66.7	0	0	0	33.3	–	–	–	75.8	0	24.2
Ozkan[46]	1	0	0	0	100	0	100	100	0	0	0	100
Stambo[34]	1	0	0	0	100	0	0	100	0	100	0	0
Kaplan[47]	1	0	0	100	0	0	100	0	0	0	0	100
Turnes[14]	38	57.9	0	18.4[a]	18.4[a]	21.1	81.6	84.2	23.7	26	0	43
Hollingshead[24]	20	–	–	–	–	–	30	60	10	15	10	75
Thomas[b]	1	0	0	0	100	0	100	100	0	100	0	0

In some instances multiple etiologies were identified for a single patient and are reported together. In addition, more than one diagnostic imaging may have been used for a single patient and are likewise reported together. Etiologies include hypercoaguable state which includes acquired/hereditary, oral contraceptives, or myeloproliferative disorder (*hypercoag*), malignancy, infectious/inflammatory (*infxn/infl*), operative, or idiopathic. Diagnostic imaging involved the use of Doppler ultrasonography (*U/S*), computed tomography (*CT*), or magnetic resonance angiography (*MRA*). Location of thrombus was identified as either portal vein (*PV*), superior mesenteric vein (*SMV*), or a combination of portal vein and superior mesenteric vein involvement (*Comb*)

[a] Infectious, inflammatory, and operative etiologies were not discriminated in this study and are thus listed together

[b] The current series of PVT after AICT is listed

abdominal pain, (b) no evidence of chronic portal hypertension, and (c) the absence of porto-portal collaterals on imaging studies, were evaluated for recanalization of the portal vein after anticoagulation but without operative thrombectomy or lytic therapy. A total of 27 patients with acute thrombus who were anticoagulated had follow-up imaging either by color Doppler ultrasound or CT scan at a mean of 4.9 months from their initial imaging which demonstrated an acute PVT. The group demonstrated that complete recanalization was achieved more frequently in cases where thrombosis involved only the portal vein or superior mesenteric vein (eight of 11, 73%) versus more extensive involvement of the portal venous system (two of 16, 13%). Of note, two patients did not receive any anticoagulation treatment, and there was no recanalization noted in either of these cases on follow-up imaging.[16] Finally, Sheen et al. reported their series of nine patients diagnosed with acute PVT of which five (55.5%) resolved with anticoagulation alone at a median of 197 days after diagnosis.[17] Immediate anticoagulation is, therefore, a viable option for patients with acute PVT in order to restore portal vein flow, albeit a slower option. Systemic anticoagulation, however, may be contraindicated in the immediate post-operative period, necessitating invasive modalities in order to restore portal flow.

Thrombolytic Therapy

Evidence exists that only approximately 50% of patients will have complete recanalization of the portal vein with anticoagulation as the sole modality of treatment.[16–20] Condat et al. reported only 37% of patients in their study having complete recanalization with 55.5% and 7.4% of patients demonstrating incomplete or no recanalization with only anticoagulation as treatment, respectively.[16] In cases where anticoagulation may be contraindicated, site-directed thrombolytic therapy may be an appropriate alternative. Techniques of thrombolytic therapy differ in that thrombolytics can be infused via a catheter positioned in the superior mesenteric artery (SMA) to achieve indirect lysis of PV thrombus or in the portal vein itself. Like the use of anticoagulation, expedient institution of venous thrombolytic therapy demonstrated a higher rate of thrombus resolution compared to delayed treatment. Malkowski et al. showed that 36% of patients had "excellent" recanalization results when symptoms did not exceed 14 days compared to 0% recanalization in those who had symptoms that persisted greater than 30 days.[21] Numerous series have demonstrated the excellent response rate of site-directed venous thrombolysis with rates ranging from 75% to 100% partial or complete recanalization (Table 2). An alternative

Table 2 Comparison of Portal and Mesenteric Vein Thrombosis Treatment Patterns and Response Rates in Selected Series

Author	*N*	Treatment (%)			Treatment response (%)			Median time to Tx (days)
		Surg	Lytics	Anti-Coag	Surg	Lytics	Anti-Coag	
Janssen[1]	172	0.6	0	27	NR	–	NR	28
Demertzis[42]	1	100	100	0	100	100	–	0
Klempnauer[11]	31	35.5	16	13	91	100	0	7
Zyromski[26]	1	100	0	0	100	–	–	0
Amitrano[44]	121	0	3.3	33.9	–	100	24.4	–
Condat[16]	33	0	0	94	–	–	80.6	14
Henao[45]	1	0	100	0	–	100	–	–
Malkowski[21]	33	0	85	0	–	82	–	–
Ozkan[46]	1	0	100	0	–	100	–	21
Stambo[34]	1	100	100	0	100	100	–	5
Kaplan[47]	1	0	100	0	–	100	–	5
Turnes[14]	38	0	0	71	–	–	44.4	6
Hollingshead[24]	20	10	100	0	100	75	–	12.5
Thomas[a]	1	100	100	0	100	100	–	1

Treatment (percent) refers to the percentage of patients who underwent the specified modality of treatment for acute PVT. Many series involved the use of anticoagulation early in the treatment algorithm but this was not the primary mode of treatment for the PVT. Where indicated, anticoagulation is designated as the sole method of treatment of the PVT with corresponding treatment response rates. Some patients received more than one treatment, such as combined thrombolytic and mechanical thrombectomy

Surg surgical thrombectomy, *Lytics* pharmacologic thrombolytic therapy, *Anti-coag* anti-coagulation therapy, *NR* not reported

[a] The current series of PVT after AICT is listed

strategy of thrombolytic delivery is via the superior mesenteric artery. Proponents of SMA-directed thrombolytics argue that arterial infusion allows resolution of small venous thrombi that cannot be treated with PV thrombolytic infusion.[22,23] Hollingshead et al. utilized either the PV or SMA route for thrombolysis of PV or mesenteric venous thrombosis. A comparison of the PV versus SMA routes demonstrates a similar time from symptom presentation to thrombolysis (11.3 versus 15 days, respectively), but increased duration of thrombolysis (29.4 versus 42.3 h, respectively) and better recanalization with the PV route compared to the SMA route (83% partial recanalization versus 50%, respectively). Arterial infusion has, therefore, been shown to result in longer infusion times, delayed time to resolution of thrombus, and inefficient thrombus resolution when compared to portal venous thrombolysis.[24] With either modality, there is a reduction in thrombus burden with restoration of portal flow that is more expeditious than anticoagulation therapy alone and helps to avoid the complications of inadequate recanalization seen with anticoagulation alone (Table 2).

Operative and Mechanical Thrombectomy

Operative thrombectomy for SMV thrombosis was first reported by Mergenthaler and Harris in 1968 after a PD for duodenal neoplasm.[25] Since then, many advances have been made in the diagnosis and treatment of PV/SMV thrombosis after hepatobiliary surgery. Patients who, after hepatobiliary surgery, develop signs of ischemic bowel secondary to porto-mesenteric vein thrombosis should be anticoagulated and proceed directly to laparotomy for bowel resection. It is at that time that some authors argue that portal vein thrombectomy should be carried out in order to immediately reduce thrombus burden. This is especially true in the case where venous reconstructions

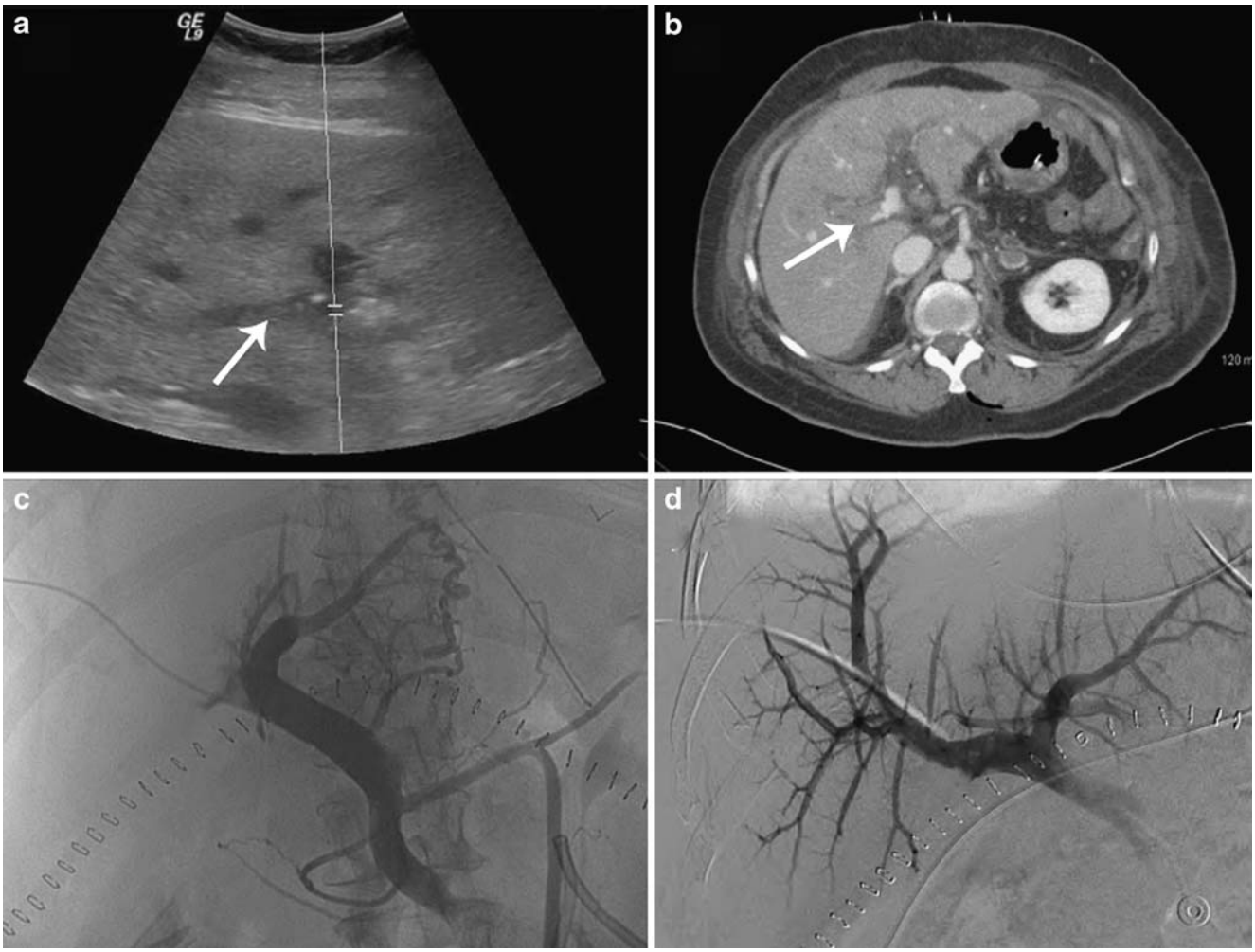

Figure 1 Diagnostic imaging of acute portal vein thrombosis pre- and post-thrombolytic treatment. **a** Color duplex ultrasonography demonstrates PVT in the right branch of the portal vein and was confirmed by CT (**b**). **c** Initial portal venography confirms the findings of ultrasonography and CT and confirms a patent superior mesenteric vein. **d** After mechanical thrombolysis and 33.5 h of r-TPA therapy, there was almost complete resolution of the PVT. *Arrows* indicate large thrombus burden in the right portal vein.

have been performed involving the portal vein, which may be the case during PD secondary to tumor involvement of the PV/SMV confluence or during liver transplantation.

In these situations, operative thrombectomy affords the surgeon the opportunity to inspect any vascular anastomosis and revise if needed.[26] This is especially important in cases of liver transplantation when graft survival is dependent on hepatopetal flow. At one time, portal vein thrombosis was considered a contraindication to liver transplantation, but even complete PVT is no longer considered a contraindication to surgery.[27] An evaluation of the efficacy of operative portal vein thrombectomy is best accomplished through an analysis of the liver transplantation literature. Although cases of portal vein thrombectomy at the time of liver transplantation relate to episodes of chronic PVT, this management gives insight into re-thrombosis rates that may be applicable to thrombectomy for acute PVT. Portal vein thrombectomy alone at the time of transplantation has been demonstrated to have a re-thrombosis rate of 4.2–38.5%.[28–31] In many of the cases of re-thrombosis, patients had to undergo repeat liver transplantation or underwent observation. In one series, surgical thrombectomy was carried out in six patients with acute PVT after liver transplantation, with a success rate of 83%, demonstrating its efficacy.[32] Additionally, Klempnauer et al. reported one case of re-thrombosis (n=11, 9%) after initial thrombectomy for porto-mesenteric thrombosis or various etiologies.[11] In their series, five patients received thrombolytic therapy via a mesenteric vein and none of these patients developed re-thrombosis. The trend with operative thrombectomy is thus to infuse thrombolytics concurrently as complete thrombectomy is extremely difficult as small adherent thrombi often are still attached to the vessel wall serving as a nidus for thrombus propagation. The use of thrombolytics, therefore, treats these undetected foci of thrombus. Adani et al. demonstrated this in their series of three patients who developed PVT after a liver transplantation, liver resection, and a splenectomy. Systemic heparinization at the time of diagnosis followed by mechanical thrombectomy and lytic treatment resulted in a 0% re-thrombosis rate in these patients.[33] In addition, the use of newer mechanical thrombectomy devices such as the AngioJet reholytic mechanical thrombectomy system (Possis Medical) has demonstrated promising results with a complete resolution of a PVT that occurred as a result of a pancreatic biopsy in one report.[34] Operative thrombectomy is, therefore, an alternative treatment of acute PVT but proper patient selection must be implemented. When performed by itself, high rates of re-thrombosis have been reported so follow-up imaging and a high index of suspicion must be present in order to detect potential re-accumulation of thrombus. Operative/mechanical thrombectomy performed concomitantly with thrombolytics has been demonstrated to provide at least equivalent results to site-directed thrombolytics but with a more expedient resolution of the thrombus and should be the procedure of choice except when absolute contraindications to thrombolytic therapy are present.

Special Circumstances

The process of portal vein thrombosis usually occurs in the presence of endothelial injury, hypercoaguable state, malignancy, sepsis, or portal hypertension. Although rare, portal vein thrombosis can occur in conjunction with autologous islet cell transplantation (AICT) and can be a devastating complication. In our experience at the University of Cincinnati, having performed 107 AICT cases to date, we have encountered one case of PVT after purified AICT. The case involved a 61-year-old female with a history of recurrent acute on chronic pancreatitis who underwent AICT and was diagnosed with PVT by color

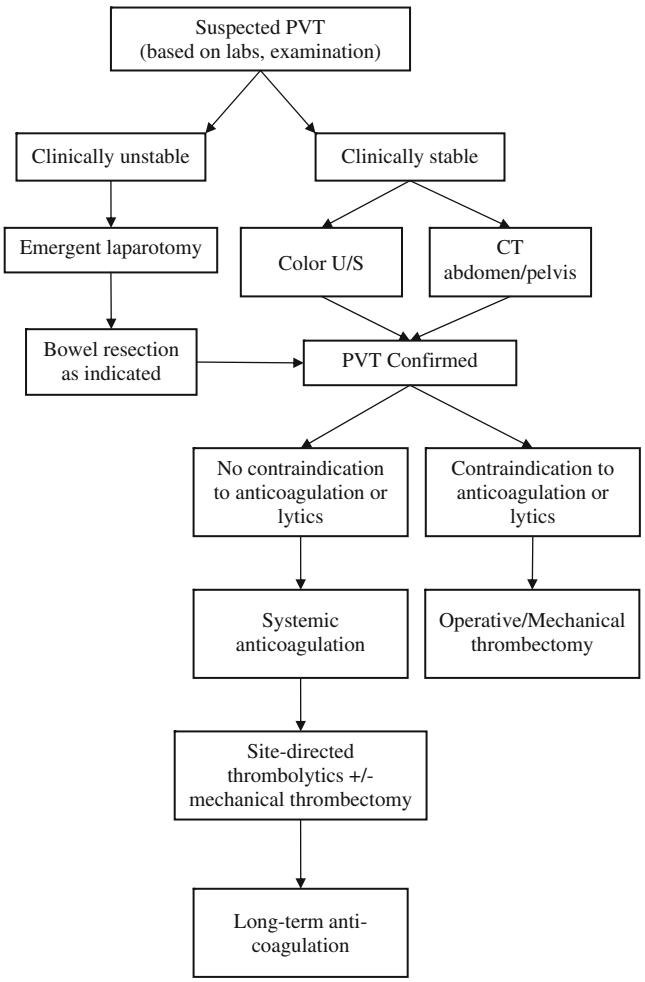

Figure 2 Diagnostic and treatment algorithm for acute portal vein thrombosis after hepatobiliary surgery.

Doppler and CT angiogram when her post-operative liver function tests became elevated (Fig. 1a, b). The patient was immediately anticoagulated with heparin and taken to the interventional radiology suite where portal venography was performed demonstrating right PV occlusion, thrombosis of multiple left portal veins, but a widely patent main PV (Fig. 1c). Utilizing a combination of mechanical thrombectomy and thrombolytics, patency of the portal vein was restored by post-operative day 3 after less than 35 h of thrombolytics (Fig. 1d). Institutions that perform autologous islet transplantation appear to have a lesser risk of portal/mesenteric venous thrombosis compared to cadaveric islet transplantation. Wahoff et al. presented 48 cases of AICT without an incidence of portal vein thrombosis.[35] In 2001, the Leicester group reported their experience over 54 months of 24 patients who underwent AICT. In this series, one patient (4.2%) developed a partial portal vein thrombosis who was treated with anticoagulation for 6 months.[36] Finally, Argo et al. from Alabama reported in June 2008 of 26 patients who underwent AICT in which none of them developed PVT.[37] Prior to new techniques of islet preparation and purification, portal vein thrombosis after AICT had a higher incidence likely due to the larger volume of islets required for transplantation as well as an increased thrombogenicity of the crude preparation thought to be due to elevated thromboplastin activity.[38] The preparation of islet cells has been modified throughout the years since first being described by Mirkovitch and Campiche and modified by Horaguchi and Merrell.[39,40] The most common methods are variations of the method described by Ricordi et al. which is currently employed at our institution.[41] With the advent of this preparation method, the incidence of PVT after AICT has significantly decreased such that it is a rare complication of autologous islet cell transplantation.

Conclusion

Portal vein thrombosis after hepatobiliary surgery is a rare yet important complication. Diagnosis can be made by the cost-effective color Doppler ultrasound or the higher resolution CT scan to delineate portal vein obstruction. Sensitivity and specificity for these two tests are similar, but each has its own distinct set of positive and negative attributes. Once portal vein thrombosis is diagnosed, treatment is determined by the clinical situation. In most cases, PVT is treated with immediate anticoagulation in order to limit the propagation of thrombus. As indicated previously, only 50% of patients will have complete resolution of their PVT and may need further intervention in order to prevent the complications of chronic PVT thrombosis such as portal hypertension. With the advent of more sophisticated devices and improved interventional techniques, we recommend site-directed thrombolytics to the area of thrombus as this results in excellent recanalization in a relatively short period of time with low re-thrombosis rates (Fig. 2). At the same time, this algorithm avoids laparotomy in the face of recent hepatobiliary procedures which could prove difficult or be fraught with iatrogenic injuries. That being said, in patients with evidence of bowel ischemia secondary to thrombus propagation, laparotomy must be employed in order to eradicate the source of septic shock in a patient. In these situations, site-directed thrombolytics should still be employed either alone or coupled with operative/mechanical thrombectomy in experienced hands. With this management algorithm and a high index of suspicion, the complications of acute portal vein thrombosis, whether associated with hepatobiliary procedures or not, can be limited, and restoration of normal portal venous flow can be attained.

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GI IMAGE

Inverted Meckel's Diverticulum with Ectopic Pancreatic Tissue as a Source of Severe Gastrointestinal Bleeding

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Abstract
Introduction Meckel's diverticulum is the most common congenital anomaly of the gastrointestinal tract. Authors present a 67-year-old woman treated for iron deficiency anemia for the past 5 years. Suddenly, her disease was presented with painless severe gastrointestinal bleeding (fresh melena). Inverted Meckel's diverticulum with ectopic pancreatic tissue as a source of severe gastrointestinal bleeding was diagnosed by intraoperative enteroscopy.
Conclusion A combination of inversion of Meckel's diverticulum with ectopic pancreatic tissue is extremely rare, particularly in elderly patient. Capsule endoscopy, double balloon enteroscopy, and ultimately intraoperative enteroscopy may be helpful in timely diagnosis.

Keywords Meckel's diverticulum · Intussusception · Intraoperative enteroscopy · Ectopic pancreas

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Introduction

Meckel's diverticulum is the most common congenital anomaly of the gastrointestinal tract (1–3% of the population in autopsy studies, twice as more frequently found in males). It derives from incomplete obliteration of the yolk stalk (omphalo-mesenteric duct). Meckel's diverticulum is a true diverticulum with all layers of the intestinal wall present. It arises from the antimesenterial border, located usually 100 cm proximal to the ileo-cecal valve, has its own mesentery and blood supply from a terminal branch of the superior mesenteric artery. Diverticula that do not contain normal ileal mucosa may harbor ectopic glandular tissue: gastric (~50%), duodenal Brunner's glands, pancreatic acinar tissue, colonic mucosa, endometrium, hepatobiliary tissue, or their combination. Meckel's diverticulum is usually asymptomatic, only about 2% develop a complication over the course of their life. Sixty percent of patients having complications are younger than 2 years, painless bleeding (from peptic ulceration in ectopic gastric mucosa) is the most common. *Helicobacter pylori* may colonize the gastric mucosa of Meckel's diverticulum, but it likely plays no role in bleeding diverticula. Other complications of Meckel's diverticulum comprise diverticulitis, iron deficiency anemia, intestinal obstruction, and perforation (from foreign bodies, diverticulitis, peptic ulceration, or blunt

abdominal trauma). A longer diverticulum (length>2 cm) is associated with a higher risk of complications. Bacterial overgrowth, intussusception, volvulus, strangulation, Littre's herniation, phytobezoars, formation of enteroliths, and malignant transformation (carcinoid, adenocarcinoma, or leiomyosarcoma) are all very unusual.[1–3]

We report an unusual case of severe gastrointestinal bleeding from inverted Meckel's diverticulum with ectopic pancreatic tissue in an elderly patient.

Clinical History and Histological Findings

A 67-year-old woman was treated for iron deficiency anemia for the past 5 years. Suddenly, her disease was presented with painless severe gastrointestinal bleeding (fresh melena) elsewhere. Bleeding required 6 U of blood within 24 h. The source of the bleeding was not identified either by gastroscopy or colonoscopy, and the patient was referred to our department as a case of acute obscure overt bleeding. Enteroclysis revealed a large polyp (8 cm in length) 80 cm proximal to the ileo-cecal valve and nearly obstructing the entire intestinal lumen (Fig. 1). Intraoperative enteroscopy was carried out as the next step because of supposed multiple small bowel polyps. Surprisingly, an inverted Meckel's diverticulum was found with three ulcers; one of them with adhering blood clot (Fig. 2). An area of whitish tissue was discovered at the tip of the intussusceptum after its disimpaction (Fig. 3). The surgeon decided to resect the diverticulum together with 10 cm of the adjacent ileum (Fig. 4). Histology confirmed Meckel's diverticulum with ulcers (Fig. 5). A pancreatic tissue was identified in the whitish tissue by histology. Heterotopic pancreatic tissue was fully formed with acinar tissue, islets, and draining duct (Fig. 6). There was no gastric mucosa in the diverticulum. The postoperative course was uneventful, and the patient was released from the hospital 10 days later.

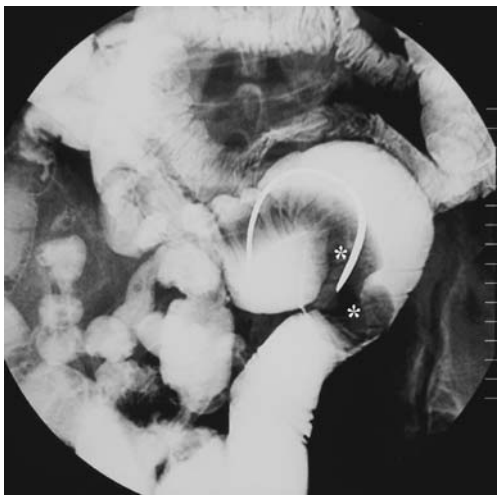

Figure 1 Enteroclysis. Smoothly marginated intraluminal mass in the ileum simulated an intraluminal polyp (*asterisk*).

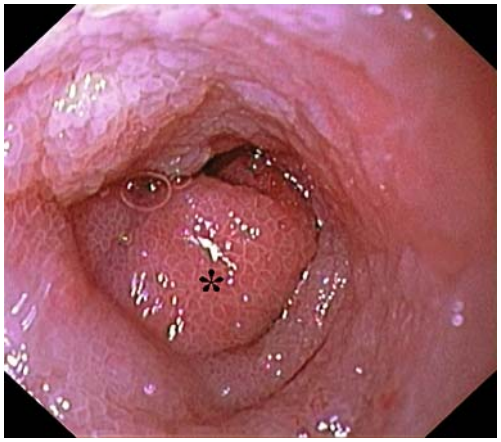

Figure 2 Intraoperative enteroscopy. Obstruction of the ileum caused by inverted Meckel's diverticulum. Swollen mucosa of the diverticulum is nicely visible (*asterisk*).

Discussion

Meckel's diverticulum is the most common congenital anomaly of the gastrointestinal tract (1–3% of the population in autopsy studies, twice as more frequently found in males). Sixty percent of patients having complications are younger than 2 years; painless bleeding (from peptic ulceration in ectopic gastric mucosa) is the most common.[1]

We present a remarkable case of inverted Meckel's diverticulum as a quite rare cause of painless severe acute gastrointestinal bleeding. We have found only 15 reports of inverted diverticulum[4,5] and only a few cases similar to ours,[6–8] but none of them was described in elderly patients. Other cases presented with iron deficiency anemia, diarrhea and vomiting, strangulated intestinal obstruction, intussusception,

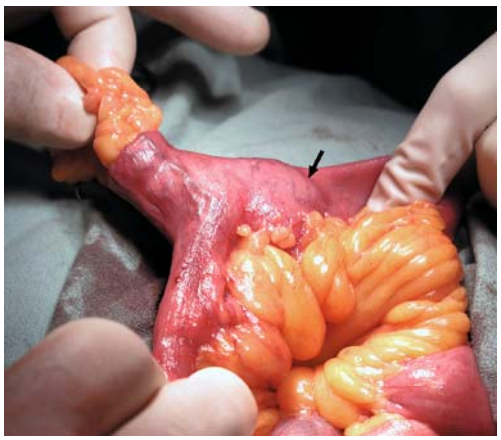

Figure 3 Surgical field—view of open abdominal cavity. Extraction of the Meckel's diverticulum; large part of it is still inverted (*arrow*).

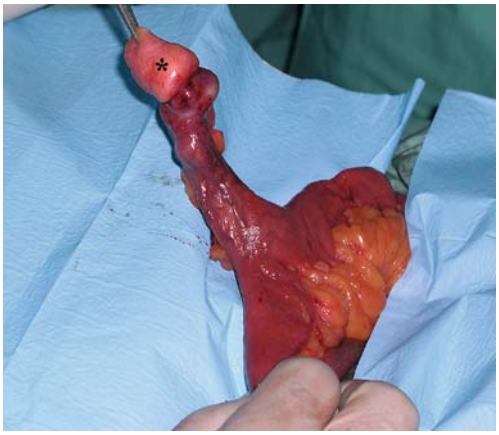

Figure 4 Surgical field—view of open abdominal cavity. Meckel's diverticulum after extraction is seen. White tissue on the tip (*asterisk*) is the heterotopic pancreas.

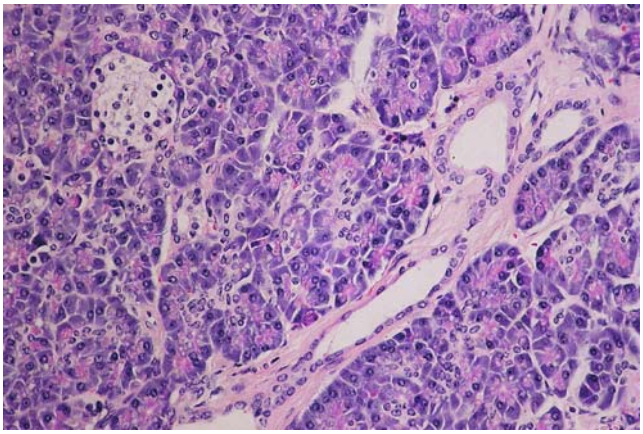

Figure 6 Ectopic pancreatic tissue. Hematoxylin–eosin.

mimicking Crohn's disease or tumor.[5] Once inverted, the diverticulum may serve as the site of intestinal obstruction or lead point for an ileo-ileal or ileo-colic intussusception.[6,8] Ectopic pancreatic tissue is a rare condition. Heterotopic pancreas is a rare cause of intussusception. It is supposed that this lesion is of vitellointestinal tract origin, conceptually similar to a Meckel's diverticulum but without a diverticulum as such. Heterotopic pancreatic tissue occurring alone is more common in the proximal small intestine, duodenum, and stomach than in the ileum, and it is often asymptomatic.[6]

More than 2,800 papers were published on Meckel's diverticulum over the past five decades (according to a PubMed search). However, most publications have been either small series or case reports. The largest series was published by Park et al. as the Mayo Clinic experience with 1,476 patients (collected from 1950 to 2002).[3] Only 16% were symptomatic. Among 180 adult patients, bleeding (69 out of 180; 38%), obstruction (61 out of 180; 34%), and diverticulitis (50 out of 180; 28%) were the most common complications. The authors do not mention any case of inverted Meckel's diverticulum.[3]

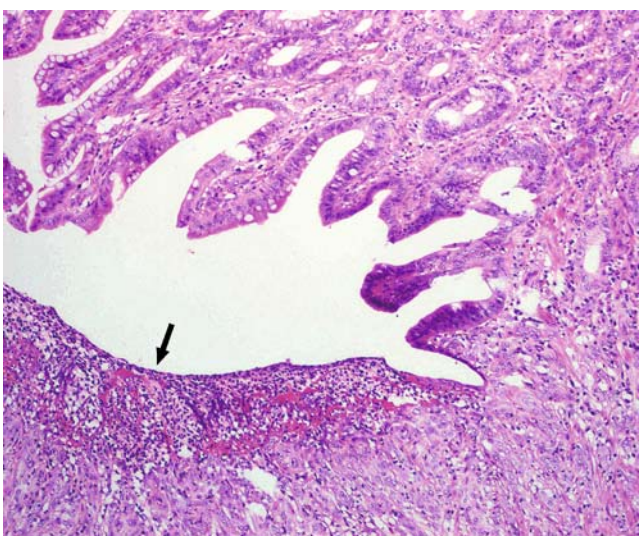

Figure 5 Ulcer of the Meckel's diverticulum (*arrow*). Hematoxylin–eosin.

A ^{99m}technetium pertechnate scintigraphy is a principal investigation, it detects ectopic gastric mucosa in Meckel's diverticulum, pretreatment with pentagastrin or H_2-receptor antagonists reduces false negative results. Pentagastrin accelerates Tc uptake, and an H_2-receptor antagonist decreases Tc release by the gastric mucosa.[1] However, only one half of Meckel's diverticula harbor gastric mucosa. Other diagnostic tools comprise [computed tomography (CT)/magnetic resonance] enteroclysis, intraoperative enteroscopy,[9] Doppler ultrasonography,[10] angiography, and recently wireless capsule endoscopy[11,12] and double balloon enteroscopy.[5,13]

Abdominal radiographic findings are most often nonspecific in these cases unless the patients have intestinal obstruction or intussusception. Enteroclysis shows an elongated, smoothly marginated intraluminal mass that parallels the long axis of the intestine and frequently has a bulbous or club-like tip. It may also appear as a pedunculated intraluminal polyp.[4] CT characteristically shows the inverted diverticulum as a central core of fat attenuation surrounded by a collar of soft-tissue attenuation. At sonography, the inverted diverticulum has a target-like appearance with central hyperechogenicity from the core of mesenteric fat or a double target appearance when the entire section of the small intestine containing the inverted diverticulum is visualized. Doppler sonography may reveal anomalous vessels.[10] The differential diagnosis for an elongated tubular filling defect produced by an inverted Meckel's diverticulum on barium images of the small intestine includes elongated pedunculated polyps such as Peutz-Jeghers syndrome.[9] The principal differential diagnosis for an inverted Meckel's diverticulum on CT scans is a lipoma. Intestinal lipomas have fat attenuation at CT, but they lack the collar of soft-tissue attenuation that is seen in inverted Meckel's

diverticulum.[14] When the vitalline artery is seen in the ilea lumen on angiography, inverted Meckel's diverticulum should be considered.

In our particular case, inverted Meckel's diverticulum also mimicked elongated pedunculated polyp on enteroclysis. It was not until intraoperative enteroscopy that the correct diagnosis was determined. Surgical resection in the same anesthesia provided a final solution.

Conclusions

Meckel's diverticulum is the most common anomaly of the gastrointestinal tract. However, most of them are asymptomatic lifelong. Clinical symptoms arise from complications of the diverticulum which are very rare in elderly people. Preoperative diagnosis of a complicated Meckel's diverticulum may be challenging because clinical and imaging features overlap with those of other causes of acute abdomen. In case of severe painless acute obscure overt bleeding, Meckel's diverticulum should be considered even in elderly patients. A combination of inversion of Meckel's diverticulum with ectopic pancreatic tissue is extremely rare. Capsule endoscopy, double balloon enteroscopy, and ultimately intraoperative enteroscopy may be helpful in timely diagnosis.

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LETTER TO THE EDITOR

Should We Trust the Results of Multivariate Analysis?

Alain Braillon

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In 66 patients, Shiba et al. studied 11 variables for early recurrence of HCC, ten for disease-free survival and ten for overall survival.[1]

Too few patients, too many variables for the guidelines on subject to variable ratio for multivariate statistical analysis.[2]

Moreover, using cut-points to derive subgroups is not appropriate when there is a continuous distribution of the values with no obvious modal values.

Lastly, *p* values must be adjusted for multiple testing, in this case three.

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LETTER TO THE EDITOR

Reply to Should We Trust the Results of Multivariate Analysis?

Hiroaki Shiba · Shigeki Wakiyama · Katsuhiko Yanaga

Received: 4 November 2009 / Accepted: 30 November 2009 / Published online: 5 January 2010

Keywords Transfusion · Hepatectomy · Hepatocellular carcinoma · Multivariate analysis

Reply

We thank the editors for the opportunity to respond to the letter by Dr. Braillon regarding our article.[1]

In his letter, Dr. Braillon pointed out small sample size, low subject to variable ratio, inappropriate use of cut-points to derive subgroup and necessity of *p* value adjustment for multiple testing.

In our study,[1] the sample size was 66, and the number of subjects evaluated was 26 for disease-free survival and ten for overall survival. For multivariate analysis of disease-free survival (Table 2), we used five variables that were significant by the Logrank test (Table 1): gender, $ICGR_{15}$, model for end-stage liver disease score, tumor factor, and blood products transfused. Therefore, the subject to variable ratio of disease-free survival was 5.2:1 in multivariate analysis. Also, for multivariate analysis of overall survival (Table 3), we used four variables that were significant by the Logrank test (Table 3): gender, $ICGR_{15}$, tumor factor, and blood products transfused. Therefore, the subject to variable ratio of overall survival was 2.5:1 in multivariate analysis. In statistical analyses, relative importance of the sample size *versus* the subject to variable ratio remains controversial.[2] The recommended minimal sample size varies from 50 to 400,[3, 4] while the lowest subject to variable ratio recommended ranges from 2:1 to 10:1.[5] Therefore, the sample size and the subject to variable ratio in our study seem to be within the recommendation limits. A description that we used only variables selected by the Logrank test for multivariate analyses in the *Results* section would have avoided misunderstanding.

In multivariate analysis, we used gender as a nominal scale qualitative data, tumor factor as an ordinal scale qualitative data, and $ICGR_{15}$, model for end-stage liver disease score, and dose of blood products transfused as a quantitative data. The tumor factor was classified into two groups, T1 or T2, and T3 or T4, which we thought was appropriate. Therefore, we did not use any cut-points in the multivariate analysis.

Finally, we agree that *p* value adjustment is necessary to avoid accidental significant difference in univariatel analysis using excessive variables. However, in our study, we used univariate analyses to select variables to be used for multivariate analyses. Therefore, *p* value adjustment does not seem necessary.

We thank Dr. Braillon for raising these important issues in the statistical analysis of our article.

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Table 1 Univariate Analysis of Disease-free and Overall Survival After Hepatic Resection

Factor	Parameters N	Disease-free survival		Overall survival	
		Median (year)	*p* Value	Median (year)	*p* Value
Age (years)					
<60	27	2.51	0.061	3.27	0.388
≥60	39	2.15		2.93	
Gender					
Male	56	2.37	0.001	3.26	<0.001
Female	10	1.40		2.65	
ICG_{R15} (%)					
<15	44	2.38	0.003	3.50	0.020
≥15	22	1.52		2.61	
Child classification					
A	60	2.37	0.119	3.26	0.062
B or C	6	1.78		2.02	
MELD score					
<10	61	2.36	0.034	3.07	0.118
≥10	5	1.42		3.02	
T factor					
T1 or T2	46	2.60	0.013	3.26	0.032
T3 or T4	20	1.48		2.86	
Type of resection					
Anatomical	22	2.28	0.679	3.82	0.747
Partial	44	2.36		2.98	
Duration of operation (min)					
<300	38	2.60	0.528	3.55	0.825
≥300	28	2.23		2.37	
Blood loss (g)					
<1000	44	2.36	0.670	2.98	0.215
≥1000	22	2.23		3.36	
Hepatitis virus					
HBV	26	2.44	0.182	3.29	0.767
HCV	28	2.10		3.00	
No	12	2.31		3.10	
Blood products transfused					
With	22	1.51	0.038	2.59	0.001
Without	44	2.40		3.16	

MELD score, model for end-stage liver disease score; *T factor*, tumor factor; *HBV*, hepatitis B virus; *HCV*, hepatitis C virus

Table 2 Multivariate Analysis of Disease-free Survival After Hepatic Resection

Factor	Odds Ratio (95%CI)	*p* Value (multivariate)
Gender (female)	2.773 (1.044-7.367)	0.041
ICG_{R15} (%)	1.013 (0.978-1.050)	0.464
MELD score	1.027 (0.855-1.233)	0.777
T factor (T1 or T2)	2.975 (1.332-6.644)	0.008
Total blood products transfused (units)	1.017 (1.006-1.028)	0.002

MELD score, model for end-stage liver disease score; *T factor*, tumor factor

Table 3 Multivariate Analysis of Overall Survival After Hepatic Resection

Factor	Odds Ratio (95%CI)	*p* Value (multivariate)
Gender (female)	11.595 (2.771-48.515)	<0.001
ICG_{R15} (%)	1.022 (0.973-1.073)	0.387
T factor (T1 or T2)	7.653 (1.701-34.433)	0.008
Total blood products transfused (units)	1.027 (1.014-1.040)	<0.001

T factor, tumor factor

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