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Elective Resection of Colon Cancer by High-Volume Surgeons Is Associated with Decreased Morbidity and Mortality

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Abstract
Background The purpose of this study was to determine whether morbidity and mortality in patients undergoing elective resection of colon cancer are associated with surgeon or hospital volume.
Methods Using the Nationwide Inpatient Sample database, we identified all adult patients who underwent elective resection for colon cancer as their primary procedure between 2003 and 2007. Cases were divided into three groups according to the mean number of resections performed annually by each surgeon: low volume (≤4/year), intermediate volume (5–9/year), or high volume (≥10/year). Annual hospital case-load was also categorized as low volume (≤30/year), intermediate volume (31–60/year), and high volume (≥61/year). Multiple logistic regression models were used to identify differences in morbidity and mortality.
Results A total of 54,000 patients underwent resection of colon cancer by 7,313 surgeons in 1,398 hospitals. After adjusting for important covariates including hospital volume, colon cancer resection by high-volume surgeons was an independent predictor of decreased morbidity (odds ratio [OR], 0.91; 95% CI, 0.85–0.97) and mortality (OR, 0.75; 95% CI, 0.65–0.86). Mortality was lowest among patients operated on by high-volume surgeons in high-volume hospitals (2.2% vs. 3.9%; OR, 0.56; 95% CI, 0.46–0.68).
Conclusions In patients undergoing elective resection of colon cancer, procedures done by high-volume surgeons are associated with decreased morbidity and mortality.

Description of the study Retrospective database analyses of the impact of surgeon and hospital volume on outcomes in elective colon cancer resection.

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Introduction

In 2009, there were over 106,100 cases of colon cancer and 40,800 cases of rectal cancer diagnosed in the USA, making it the third most common cancer in both genders.[1] Surgical treatment is the only chance for cure in these two malignancies. Postoperative mortality for colon cancer resection has been reported to range between 1% and 6% in different series.[2] Patient and procedure-related characteristics have been used in different scoring systems to predict the risk of postoperative complications and mortality associated with surgical resection. However, other factors related to the surgeon and the hospital may affect the risk of postoperative complications or death.[3,4]

Higher surgeon and hospital volume have been associated with a reduction in postoperative morbidity and mortality following various surgical procedures.[3,4] The impact of hospital volume on the outcome of rectal cancer resection has been evaluated in different studies.[5–8] The effect of surgeon volume has also been evaluated in series including both colon and rectal resection.[9–11] However, few studies have examined the influence of these volume-associated variables in colon cancer resection only.[12] These studies included patients undergoing nonelective resections, which are associated with an increased risk of postoperative mortality. Findings from these previous studies may be linked to this increased mortality/morbidity in nonelective setting. However, these results may not hold true when considering only elective procedures. A recent study has shown that hospitals with favorable outcomes for elective resections do not necessarily achieve the same results for nonelective resections.[13] Patients requiring emergent surgery for colon cancer are less likely to have the opportunity to choose their hospital or their surgeon. However, patients with colon cancer requiring elective surgery may be interested in the impact of hospital- and surgeon-related characteristics on the outcome of the planned resection.

In order to address these issues, we used a population-based nationwide US database to evaluate the impact of hospital and surgeon volume on the outcomes of elective resection for colon cancer.

Methods

Data Source

Data were extracted from the Healthcare Cost and Utilization Project (HCUP) Nationwide Inpatient Sample (NIS) database for the years 2003 to 2007.[14] The NIS is the largest all-payer database of national hospital discharges (~8 million per annum) maintained by the Agency for Healthcare Research and Quality. It represents a 20% stratified random sample of non-federal acute-care hospitals in the USA including community, general, and academic centers, but not long-term care facilities. Stratified random sampling ensures that the database is representative of the US population and that it accounts for ~90% of all hospitalizations. Each data entry includes a patient identifier, demographic data, hospital transfer status, admission type (emergency, urgent, or elective), primary and secondary diagnoses (up to 15), procedures (up to 15), insurance status, hospital charges, length of stay (LOS), and hospital characteristics. NIS data compares favorably with the National Hospital Discharge Survey, supporting the validity of this database.[15]

Study Population

We used the International Classification of Diseases 9th Version, Clinical Modification (ICD-9-CM) procedure codes to identify 54,000 adult patients (≥18 years) who underwent elective partial (ICD-9-CM, 45.7) or total abdominal colectomy (45.8) for colon cancer (153) between 2003 and 2007.[16] Patients undergoing emergent or urgent resection were not included.

Classification of Cases by Surgeon and Hospital Volume

Based on the mean annual number of resections, surgeons and hospitals were categorized into three volume groups using tertiles allowing distribution of surgical cases evenly between groups. In the NIS, each surgeon has a standardized identifier which allowed us to link different records of the same surgeons across the study period. NIS hospital sampling varies across years; thus, each surgeon is not necessarily surveyed every year. For surgeons sampled in different years, an estimated average volume was calculated based on the total number of procedures performed over the study period. This also allowed to make sure that the same surgeon sampled multiple times was not considered as multiple different ones. To estimate the magnitude of procedures and the weighted sample design of NIS database, we used SUDAAN to calculate the corresponding estimated procedures across the USA. Surgeon case volume was divided in tertiles to form three even groups corresponding to low- (≤4/year), intermediate- (5–9/year), or high- (≥10/year) volume surgeon group. Annual hospital case-load was also averaged and divided with the same method to form the following categories: low volume (≤30/year), intermediate volume (31–60/year), and high volume (≥61/year).

Covariates

We examined the following patient-related variables to adjust for different case-mix between hospitals: age, race, gender, type of health insurance (private vs. non-private), cancer stage (local involvement, nodal involvement, or organ metastasis [see below]), type of resection performed (partial colectomy, ICD-9, 45.7; total abdominal colectomy, ICD-9, 45.8), presence of local perforation (ICD-9, 569.83) or mechanical obstruction (ICD-9, 569.83), malnutrition (ICD-9, 262, 263, 269.8, 783.3, 799.4, and 783.21), and other comorbidities. We used the Elixhauser algorithm (cancer excluded) to categorize the number of comorbidities for each patient (0, 1, 2, and ≥3 comorbidities).[17] As previously described, the cancer stage was determined using ICD-9 codes that identify lymph node involvement (196–196.9) or the presence of organ metastasis (197–

198.89).[10] If a patient had neither of these codes, it was assumed that the cancer was local.

Outcomes

In addition to in-hospital mortality and morbidity, we assessed length of hospital stay (LOS) and total hospital charges adjusted for inflation to 2007 dollars using the US Consumer Price Index for medical care.[18] Finally, we identified the occurrence of postoperative complications in the following categories: wound-related, infectious, cardiovascular, renal, pulmonary, gastrointestinal, and vascular. We also looked at the requirement for a second procedure related to postoperative complications during the index hospitalization. The specific ICD-9-CM codes used to define these complications have been outlined previously.[19]

Statistical Analysis

The distribution of case mix variables in each of the volume groups was compared using Fisher's exact, chi-square, and *t* tests as appropriate. Based on theses analyses, logistic regression analysis was then used to model the associations between the volume groups and outcomes, adjusting for significant variations in case mix. Multiple linear regression was used to model the continuous outcomes of LOS and hospital charges. Due to the skewed distribution of LOS, a natural log transformation was performed to achieve a more normal distribution for the analysis. All models employed generalized estimating equations to account for the hierarchical nature of the data (i.e., clustering of discharges within hospitals) and complex sampling design of the NIS database. Discharge-level weights published by HCUP were used to produce 95% confidence intervals (CI) for point estimates and to reflect nationwide data during the study period.[20]

To assess the interaction of surgeon and hospital volume, the groups were further subdivided into nine groups according to both surgeon and hospital volume, and the same regression analysis was performed. All statistical analyses were performed using SAS-callable SUDAAN (version 9.0.1; Research Triangle Institute, Durham, NC, USA); differences were considered statistically significant at a p value less than or equal to 0.05.

Results

Between 2003 and 2007, 54,000 patients underwent an elective colon resection for cancer by 7,313 surgeons at 1,398 hospitals. Table 1 shows the distribution of cases in each volume group and the corresponding distribution of surgeons and hospitals. The majority of surgeons (68%) and hospitals (73%) were in the low-volume groups. In the low-volume surgeon group, 4,952 surgeons (68%) performed 16,379 cases (30%), at a mean rate of 2.1 cases per year. In the medium-volume surgeon group, 1,676 surgeons (23%) performed 20,533 (38%) procedures, for a mean of 6.4 cases per year. Only 9% of surgeons performed ten or more elective colon cancer resections annually. These surgeons operated on 17,088 patients (32%), for a mean of 15.2 cases per year. According to hospital volume groups, 17,962 cases (33%) were performed at 1,021 low-volume hospitals (73%), at a mean rate of 10.7 cases per year. In the medium-volume hospital group, 18,406 (34%) cases were performed in 261 (19%) hospitals, at a mean rate of 43 cases per year. For the high-volume hospital group, 17,632 cases (33%) were performed at 116 hospitals (8%), at a mean rate of 90.2 cases per year. High-volume surgeons were operating mostly in high-volume hospital (50.7%) but also in medium- (33.6%) and low-volume hospital (15.7%), whereas low-volume surgeons were distributed mostly in low-volume

Table 1 Summary of volume groups in colon surgery

	Total	Low	Medium	High
Surgeons				
Definition of group (cases per year)	–	≤4	5–9	≥10
No. of surgeons	7,313	4,952 (68%)	1,676 (23%)	685 (9%)
No. of cases	54,000	16,379 (30%)	20,533 (38%)	17,088 (32%)
Mean annual case volume	–	2.1	6.4	15.2
Hospitals				
Definition of group (cases per year)	–	≤30	31–60	≥61
No. of hospitals	1,398	1,021 (73%)	261 (19%)	116 (8%)
No. of cases	54,000	17,962 (33%)	18,406 (34%)	17,632 (33%)
Mean annual case volume	–	10.7	43.0	90.2

hospital (45.7%) but also in medium- (31.5%) and high-volume hospital (22.8%).

Characteristics of Surgeon and Hospital Volume Groups

The mean age of the study population was 71.2 years (Table 2). Patients were equally distributed by gender (48% male, 52% female). Patients were predominantly white (65%) and mostly Medicare recipients (62.9%). Only 17.3% of patients had no comorbidities, while 33.2% had three or more comorbid conditions. The majority of patients (62.2%) had only local involvement; 23% had nodal involvement and 14.9% had organ metastases. Although all patients underwent elective resection, 23.3% had diagnosis codes indicative of mechanical obstruction and 2.4% had local perforation. Most procedures were partial colectomies (98.2%); 1.8% involved a total colectomy. Laparoscopic procedures were performed in 3.8% of patients.

Table 2 summarizes the population case mix by surgeon and hospital volume groups. There were significant differences in baseline characteristics between surgeon volume groups in all case mix variables except for gender and the number of comorbidities. High-volume surgeons operated on slightly older patients, more white patients, more privately insured patients, and more with localized disease. High-volume surgeons operated on fewer patients with obstruction or perforation and were more likely to perform total colectomy and operate laparoscopically.

Similar differences in baseline characteristics were identified between the hospital volume groups in all case mix variables except gender, cancer stage, and obstruction. High-volume hospitals admitted slightly younger patients, more with private insurance, patients with less comorbidities, and fewer patients with perforation. Laparoscopic procedures and total colectomy were also performed more often in high-volume hospitals.

Surgeon Procedure Volume

Mortality rates for high-volume and low-volume surgeons were 2.6% and 3.9%, respectively. Increased surgeon volume was associated with lower mortality and complication rates (Table 3). After adjusting for case mix and

Table 2 Summary of patient characteristics by volume groups

	Total	Surgeon volume groups			*p* value	Hospital volume groups			*p* value
		Low	Medium	High		Low	Medium	High	
Age, years	71.2	70.9	71.2	71.3	<0.0001	71.6	71.1	70.4	<0.0001
Female gender	52.3	52.7	52.1	52.2	0.55	52.5	51.5	53.0	0.0209
Health insurance									
Private	29.5	28.5	29.6	30.3		24.9	30.1	33.6	
Medicare	62.9	61.6	63.4	63.6	<0.0001	65.7	62.7	60.3	<0.0001
Other	7.6	10.0	7.0	6.1		9.5	7.3	6.1	
White race	65.2	60.6	65.2	69.6	<0.0001	64.3	67.1	64.1	0.0373
Elixhauser comorbidity index									
0	17.3	17.2	16.9	17.9		16.8	16.5	18.6	
1	24.9	24.5	24.8	25.4	0.27	24.1	24.9	25.7	0.0012
2	24.6	24.9	24.6	24.5		24.5	24.7	24.7	
≥3	33.2	33.5	33.8	32.3		34.6	34.0	31.0	
Malnutrition	4.8	5.7	4.9	3.9	<0.0001	5.5	5.0	3.9	0.0001
Transfusion	21.4	24.2	21.1	19.1	<0.0001	24.8	19.6	19.8	<0.0001
Primary procedure									
Partial colectomy	98.2	98.5	98.4	97.7	0.0002	98.6	98.3	97.7	<0.0001
Total colectomy	1.8	1.5	1.7	2.3		1.4	1.7	2.4	
Laparoscopic procedure	3.8	2.7	3.5	5.2	<0.0001	2.6	3.8	5.0	<0.0001
Perforation	2.4	3.4	2.1	1.6	<0.0001	2.8	2.3	2.0	<0.0001
Obstruction	23.3	25.4	23.4	21.3	<0.0001	22.6	24.3	23.1	0.08
TNM									
Local	62.2	59.8	62.1	64.4		63.2	61.6	61.7	
Nodal involvement	23.0	23.9	22.9	22.1	<0.0001	22.1	23.6	23.2	0.06
Metastasis	14.9	16.3	15.0	13.4		14.7	14.8	15.1	

Table 3 Summary of the crude and case mix-adjusted outcomes by volume groups

Characteristics	Total	Surgeon volume group[a]			Hospital volume group[b]		
		Low	Medium	High	Low	Medium	High
Mortality							
Crude % (95%CI)	3.1 (3.0–3.3)	3.9 (3.6–4.3)	3.0 (2.7–3.2)	2.6* (2.3–2.8)	3.6 (3.3–3.9)	3.2 (2.9–3.5)	2.6* (2.4–2.9)
Adjusted odds ratio (95% CI)		1.00 Ref	0.81*** (0.73–0.90)	0.75*** (0.65–0.86)	1.00 Ref	1.01 (0.87–1.16)	0.87 (0.76–1.00)
Any complication							
Crude % (95% CI)	33.6 (32.9–34.2)	36.1 (35.2–37.0)	33.1 (32.2–34.0)	31.7* (30.6–32.9)	33.1 (32.1–34.0)	34.6 (33.5–35.7)	33.0** (31.7–34.3)
Adjusted odds ratio (95% CI)		1.00 Ref	0.91*** (0.85–0.96)	0.91*** (0.85–0.97)	1.00 Ref	1.11*** (1.04–1.18)	1.09 (1.09–1.18)
Length of stay							
Crude (days) (95% CI)	7.0 (4.9–11.0)	7.7 (5.3–12.1)	6.9 (5.0–10.9)	6.5* (4.5–10.0)	7.3 (5.2–11.2)	7.0 (4.9–11.2)	6.6* (4.6–10.5)
Adjusted % change (95% CI)		Ref	−4.9%*** (−6.8, −3.0)	−3.0% (−6.8, 1.0)	Ref	3.0% (1.0, 6.2)	3.0% (−1.0, 6.2)
Charges							
Crude ($)	38,116	40,904	36,835	37,379*	36,576	39,145	38,471*
Adjusted % change (95% CI)		Ref	−8.6%*** (−10.4, −5.8)	−7.7%*** (−10.4, −3.9)	Ref	9.4% (1.0, 17.4)	10.5%*** (2.0, 19.7)

*$p<0.0001$; **$p=0.08$; ***statistically significant

[a] Adjusted for hospital volume

[b] Adjusted for surgeon volume

hospital volume, the adjusted odd ratios (aOR) for mortality among patients operated on by medium-volume and high-volume surgeons were 0.81 (95% CI, 0.73–0.90) and 0.75 (0.65–0.86), respectively. Complications were reduced in both the medium- and high-volume surgeon groups by almost 10%. Hospital charges were also significantly reduced in medium- and high-volume surgeons, with an adjusted percentage decrease of 8.6% and 7.7%, respectively, compared with low-volume surgeons. This corresponds to an estimated mean reduction of $3,200 and $2,900 dollars, respectively, for each case. Compared to patients operated on by low-volume surgeons, the medium-volume surgeon group was associated with a slight reduction in LOS (−4.9%; 95% CI, −6.8, −3.0), while the reduction in the high-volume surgeon group did not reach statistical significance. Comparisons between medium-volume and high-volume surgeon groups did not reveal significant differences in mortality or complications.

Hospital Procedure Volume

High-volume hospitals were associated with a 2.6% mortality rate, while this rate increased to 3.6% in low-volume hospitals. After adjusting for case-mix and surgeon volume, hospital volume was not significantly associated with postoperative mortality; however, high-volume hospitals had a trend toward reduced mortality compared to low-volume hospitals (aOR, 0.87; 95% CI, 0.76–1.00). On the other hand, mortality was similar between medium- and high-volume hospitals (aOR, 1.01; 95% CI, 0.87–1.16). Interestingly, complications were significantly increased in the medium-volume hospital group (aOR, 1.11; 1.04–1.18) compared to low-volume hospitals. High-volume hospitals had a trend toward more complications (aOR, 1.09; 1.00–1.18). LOS was not significantly associated with hospital volume. However, hospital charges were increased in high-volume hospitals (adjusted change, 10.5%; 95% CI, 2.0–19.7%), while the difference in medium-volume hospitals did not reach statistical significance (adjusted change, 9.4%; 95% CI, 1.0–17). Comparisons between medium-volume and high-volume hospital groups did not reveal significant differences in any of the outcome measures.

Surgeon vs. Hospital Volume

When analyzed individually after adjusting for each other, only surgeon volume was shown to influence patient mortality. We also compare the interaction between these two variables when considered simultaneously. Figure 1 shows the effect of surgeon volume in the three different hospital setting. Increased surgeon volume was associated with a trend toward a reduced risk of in-hospital mortality in each hospital volume. This is particularly significant in medium- and high-volume hospitals. Even if hospital volume is not an independent predictor of mortality, it seems to confer additional risk reduction for patient operated on by medium- or high-volume surgeons. Low-volume surgeons had a similar risk of mortality no matter the volume of the hospital where they work. Interestingly, in high-volume hospitals, medium-volume surgeons achieved the same reduction in mortality risk as high-volume surgeons (aOR, 0.68).

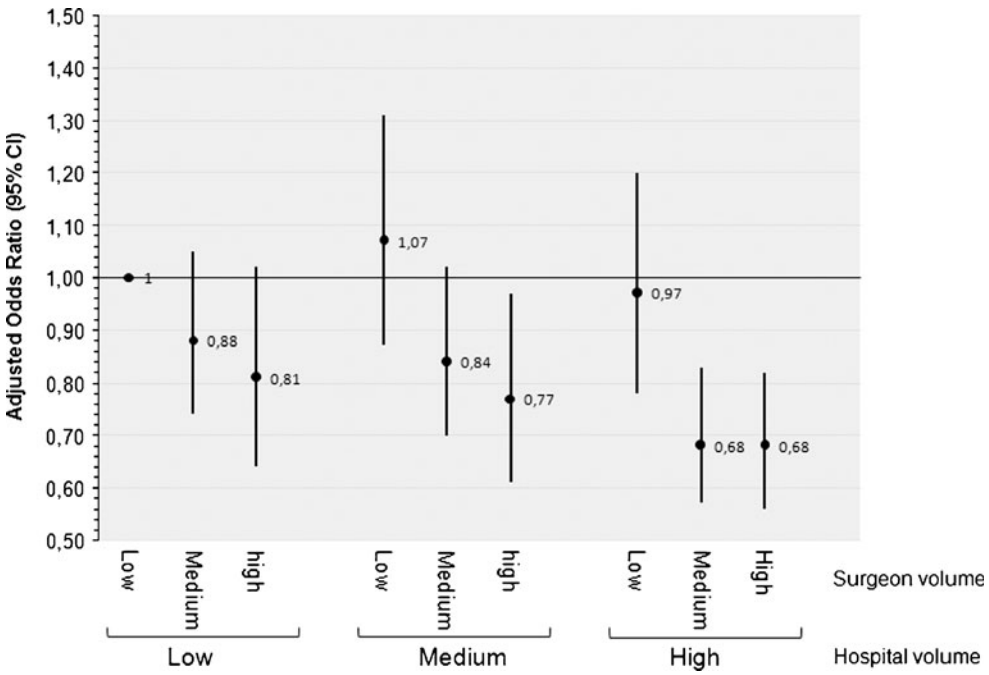

Fig. 1 Effect of surgeon volume on the risk of patient mortality in different hospital settings. Low-volume hospital/low-volume surgeon group used as the reference group. Adjusted odds ratio (95% CI) for risk of postoperative mortality

Other Independent Predictors of Mortality

Other factors besides surgeon and hospital volume were associated with an increased risk of mortality. After adjustment for hospital and surgeon volume, a higher rate of in-hospital mortality was independently associated with increased age, malnutrition, more comorbidities, the presence of local perforation, obstruction or organ metastases, and in patients undergoing a total colectomy. Female gender, private insurance, and laparoscopic procedures were associated with a lower risk of mortality. Regression model results for non-volume variables are included in Table 4.

Discussion

Surgeon Volume

This analysis of 54,000 patients who underwent elective resection for colon cancer reveals that patients operated on by medium- or high-volume surgeons have a reduced risk of in-hospital mortality compared to those operated on by low-volume surgeons. Specifically, patients operated on by surgeons performing at least ten resections for colon cancer annually had a 25% lower odds of death compared to patients having their resection by surgeons doing four or fewer procedures per year. Patients operated on by medium-volume surgeons also experienced a reduced risk of in-hospital mortality compared to patients resected by low-volume surgeons (aOR, 0.81).

Table 4 Regression model results for non-volume related variables predicting postoperative mortality

	Adjusted odds ratio[a]	95% CI
Age		
<60 years	1.00	Ref
60–80 years	1.81*	1.43–2.28
≥80 years	4.15*	3.27–5.27
Female gender	0.79*	0.72–0.87
Private insurance	0.56*	0.46–0.69
White race	0.96	0.85–1.09
Malnutrition	2.17*	1.88–2.51
Laparoscopic	0.52*	0.36–0.76
Primary procedure		
Partial colectomy	0.60*	0.45–0.81
Total colectomy	1.00	Ref
Elixhauser comorbidities		
0	1.00	Ref
1	1.21	0.99–1.47
2	1.27*	1.03–1.58
≥3	1.72*	1.39–2.12
Perforation	4.94*	4.06–6.00
Mechanical obstruction	1.16*	1.03–1.32
TNM		
Local	1.00	Ref
Nodal involvement	0.96	0.85–1.09
Metastasis	1.95*	1.76–2.17

*Statistically significant

[a] After adjustment for surgeon and hospital volume

Our results confirm those of other authors who have used different administrative databases. Karanicolas et al. used a nationwide Canadian database to study the impact of surgeon volume on in-hospital mortality in patients undergoing colorectal resection for malignant or benign diseases and found a survival advantage among patients treated by high-volume surgeons.[11] Harmon et al. compared the outcome of colorectal resections for cancer and found that high-volume surgeons achieve a lower mortality rate in three different hospital volume settings.[10] The adjusted risk of in-patient death was reduced by 36% among high-volume surgeons compared to low-volume surgeons.[10] Roger et al. also described a reduction in 30-day mortality and overall long-term mortality in patients undergoing colorectal resection for cancer by high-volume surgeons.[9] Schrag et al. found similar results in a cohort of patients limited to colon cancer with a crude mortality reduction of 2% when operated by surgeons with a high volume of procedures.[21] Ko et al. examined a cohort of patients from NIS hospitalized in 1996 and found a 17% reduction in the risk of in-hospital mortality for patients operated on by surgeons performing more than eight resections for colon cancer yearly.[22]

Larson et al. used data from the COST trial to study the effect of surgeon volume on disease-free and 5-year survival for colon cancer resection.[23] They did not find a correlation between survival and surgeon volume. However, they used the number of cases included in the COST trial by each surgeon yearly to define surgeon volume. Surgeons participating in this study would likely have done additional cases that were not included in the study. Thus, this stratification may not represent the real case volume of these surgeons. Also, the requirement to participate in this study (>19 previous laparoscopic colonic resections) likely limited the inclusion to surgeons that would be considered high-volume surgeons in other studies, and therefore, these results are probably not generalizable to an unselected population of surgeons.

A potential explanation for the discrepancy between these studies relates to differences in the definitions of surgeon volume across reports. These definitions seem to vary from one study to another because the authors use different periods of time as denominator (e.g., 1, 3, or 5 years). However, when comparing these different case-

volume categories using a common 1-year basis denominator, most of the authors consider surgeons doing more than ten procedures yearly as high-volume surgeons.[9,10,23] We used tertiles to divide patients according to surgeon and hospital volume. Other methods such as the utilization of lowess curve to determine cutoff points may have been used to increase the chance of finding differences between volume groups. As a post hoc analysis, we performed a lowess curve to see if this would revealed any specific "breakpoints" that would represent better cutoff points, but none were identified. This is in line with the findings of Karanicolas et al. who evaluated the effect of surgeon volume as a continuous measure instead of using quartile and found a linear correlation between in-hospital mortality and surgeon volume.[11] The optimal number of resections per year to achieve the best outcome is open to debate; however, it seems clear that surgeon volume significantly influences postoperative mortality.

Hospital Volume

Hospital volume was not associated with a reduction in hospital mortality in our cohort.

Previous studies evaluating the effect of hospital volume on perioperative mortality have found divergent results. For example, Rogers et al. described a correlation between hospital volume and 30-day mortality when comparing patients undergoing colorectal resection for cancer.[9] In a similar setting, Harmon et al. did not find a statistically significant association.[10] Similarly, Karanicolas et al. did not observe an improvement in outcome in patients who underwent colorectal resection for various indications in high-volume hospitals.[11] For long-term results in a US veteran's population, Rabeneck et al. reported 7% and 11% increases in 5-year survival for colon and rectal cancer, respectively, in patients who underwent surgical resection in high- vs. low-volume hospitals.[24] Others authors have described a reduction of mortality among high-volume hospitals for the resection of colon cancer.[12,25,26]

The relation between hospital volume and in-hospital mortality seems to be more complex than the effect of surgeon volume and imply different factors. One could assume that the relation between surgeon volume and mortality may be explained by a reduction in technical errors, improved pre- and postoperative care, better patient selection, and/or improvement in clinical judgment acquired with the experience and the volume of cases. On the other hand, the factors implied in the relation between hospital volume and mortality is less evident at first sight. Billingsley et al. investigated this relationship and found that the availability of *sophisticated clinical services* accounted for the majority of the improvement in postoperative mortality.[26] Other factors such as the nurse-to-patient ratio and the percentage of registered nurses have also been found to influence the hospital-related mortality in medical and surgical patients.[27] Hospital volume may not correlate closely with these factors, and this may explain in part the variability in the association with postoperative mortality.

Although hospital volume was not associated with better outcome in the overall cohort, further analysis revealed an association with a lower risk of mortality when compared according to surgeon volume subgroups (Table 4). This suggests that the surgeon volume has the largest influence on in-hospital mortality. However, hospital volume seems to confer additional reduction in the risk of mortality inside medium- and high-volume surgeon groups. Few studies have evaluated the impact of both surgeon and hospital volume in colon cancer. Schrag et al. compared the effect of surgeon and hospital volume in a cohort of colon cancer resections and concluded that both factors predict outcome; however, hospital volume had a stronger effect.[21] They based their conclusion on the findings of a "somewhat more favorable" outcome for patients undergoing surgery at high-volume hospitals by low-volume surgeons than for patients operated on at low-volume hospitals by high-volume surgeons. Harmon et al. in a study that also included rectal cancer concluded that high-volume surgeons have better outcome in all different hospital settings. They also concluded that medium-volume surgeons had outcomes more closely related to hospital volume, with a performance equal to high-volume surgeons in high-volume hospitals and results comparable to low-volume surgeons in low-volume hospitals.[10] We did not find this type of association in our study, with medium-volume surgeons having better outcome than low-volume surgeons in all hospital settings. In a colorectal cancer resection population, Rogers et al. found that both hospital volume and surgeon volume were independent predictors of outcome after adjusting for each other in multivariate analysis.[9] These comparisons are always difficult since most of high-volume surgeons are usually operating in high-volume center and low-volume surgeons in low-volume center; it may be hard to differentiate what is due to surgeon or hospital volume. However, in our study, after adjusting for hospital volume, surgeon volume was still an independent predictor of mortality.

Although some may consider these results as an indication to concentrate colon cancer resection in some selected centers to achieve high-volume dedicated surgeons, this is unlikely to be realizable according to the burden of colon cancer throughout the USA. Others have argued in favor of directed distribution of selected operations within each hospital to certain surgeons.[3] This may allow individuals with a special interest to become high-volume surgeons in regional centers. However, a

detrimental effect of this selection may occur by further reducing the experience of low-volume surgeons and increasing their mortality rate for inevitable urgent or emergent resection that they must face.[11] Hillner and others have emphasized that identification of the mechanisms underlying variation in outcomes should facilitate initiatives tailored to address specific shortcomings.[28] This may results in specific changes in surgical training, surgical techniques, or in the organization of care.

Our study has several unique strengths. First, we used the largest database of national hospital discharges in the USA. Data are collected from all payer agencies and include data from community, general, and academic centers of all states. This may reduce the influence of regional differences in treatments or outcomes. Unlike the SEER database, the NIS is not limited to patients over 65 years of age and to Medicare beneficiaries. Most of the previous studies in this field used data from early 1990s and, therefore, may not reflect recent advances in surgical techniques and perioperative care. Our study includes more recent data (2003–2007) and may be a better reflection of current trends. We limited our analysis to colon cancer resection as rectal cancer is associated with more challenging surgical techniques and is influenced by other factors such as neoadjuvant chemoradiation. Unlike previous studies, we excluded urgent resections which are commonly associated with worse outcomes. Patients presenting acutely are usually not in a position to choose between different institutions or surgeons. Our intent was to define the effect of hospital and surgeon volume on surgical outcomes when limited to a cohort of patients presenting for an elective colon cancer resection.

All studies using administrative database rely on the validity of the data included; coding errors are inherent to any database. However, since we used a large sample size, these are unlikely to have influenced the results of our study. Another limitation of our study is the lack of information on specific characteristics of surgeons (e.g., training or certification) and hospitals (e.g., nurse-to-patient ratio or access to specialized services) that may contribute to the volume-outcome relationship. The utilization of in-hospital mortality also limits the evaluation of post-discharge outcomes. One may argue that 30-day mortality would be a better measure of quality. However, this is associated with a less reliable capture of events and is not available in this particular database. Previous studies have reported a good correlation between in-hospital mortality and 30-days mortality.[29] Inpatient mortality is also associated with length of stay as this defines the period of observation. High-volume surgeon group was associated with slightly shorter LOS (7.7 vs. 6.5 days, $p<0.001$), and this may have influenced the capture of the main outcome. In-hospital mortality may also not correlate with long-term survival rate in a cancer population. However, the evaluation of long-term survival would be influenced by several other factors such as type of adjuvant treatment and compliance with follow-up.

In summary, we found a significant reduction of postoperative mortality for patients undergoing elective colon cancer resection by medium- and high-volume surgeons. Hospital volume was not independently associated with a reduction in postoperative mortality. Although hospital volume seems to confer a small benefit when compared according to surgeon volume groups, the latter remains the most important predictor of outcome. Further research is needed to identify specific factors that mediate these differences in patient outcomes.

Conflicts of interest No conflicts of interest exist.

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2010 SSAT QUICK SHOT PRESENTATION

Medically Managed Hypercholesterolemia and Insulin-Dependent Diabetes Mellitus Preoperatively Predicts Poor Survival after Surgery for Pancreatic Cancer

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Abstract
Introduction Although patients with pancreatic ductal adenocarcinoma (PDAC) frequently require medications to treat pre-existing conditions, the impact of these treatments on outcomes post-resection is unknown. The purpose of this study was to determine the impact of preoperative medications on overall survival after pancreatic resection.
Methods Multi-institutional data on preoperative medications and outcomes in patients undergoing resection for PDAC were analyzed. Univariate and multivariate analyses were performed to determine which medications were predictive of early mortality.
Results Of the 518 patients resected for PDAC, 13.3% were being treated preoperatively with insulin, 14.8% were on a statin, 1.7% were on steroids, and 7.6% were on thyroxin. On univariate analysis, patients taking preoperative insulin had a higher 90-day mortality rate relative to those not on insulin (13.0% vs. 4.8%, $p=0.024$), and those on a statin had a higher 90-day mortality than those who were not (10.8% vs. 4.6%, $p=0.035$). Preoperative steroids and thyroxin were not associated with 90-day mortality ($p=0.409$ and $p=0.474$, respectively). Insulin and statin use was a stronger predictor of 90-day mortality than history of diabetes ($p=0.101$), BMI≥30 ($p=0.166$), cardiac disease ($p=0.168$), pulmonary disease ($p=1.000$), or renal dysfunction ($p=1.000$). Older patients also had a higher risk of early postoperative death ($p=0.011$). On multivariate analysis, only preoperative insulin usage and statin treatment independently predicted early mortality (odds ratio (OR)=3.043; 95% confidence interval (CI), 1.256–7.372; $p=0.014$, and OR=2.529; 95% CI, 1.048–6.104; $p=0.039$, respectively). Based on the beta coefficients, a simple scoring system was devised to predict survival after resection from preoperative medication use. Zero points were assigned to patients who were on neither insulin nor a statin, one point to those who were on one or the other, and two points to those who were on both insulin and a statin. The score correlated with early postoperative survival (90-day mortality rates of 3.4%, 11.5%, and 13.3% for 0, 1, and 2 points, respectively, $p=0.004$). Increasing score was also associated with poorer long-term outcomes, with a median overall survival of 19.6, 15.6, and 11.2 months for 0, 1, and 2 points, respectively ($p=0.002$, median follow-up 14.4 months).

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Conclusions Patients with PDAC being treated for pre-existing diabetes or hypercholesterolemia with either insulin or statin-based therapy have an increased risk of early postoperative mortality. A simple scoring system based on preoperative medications can be used to predict early and overall survival following resection.

Keywords Pancreatic cancer · Diabetes · Insulin · Statin

Introduction

The relationship of pancreatic ductal adenocarcinoma (PDAC) with diabetes mellitus (DM) and its associated comorbid conditions, such as obesity, is complex. The increased prevalence of DM among patients with PDAC relative to the general US population (40% vs. 7%) has generated a multitude of data from prospective, as well as population-based and case–control studies implicating DM or impaired glucose tolerance as a risk factor for, or alternatively as a consequence of, PDAC.[1–4] Pre-existing DM, moreover, has been shown to be an adverse prognostic indicator in a variety of cancer patients, notably those with malignancies of the breast, endometrium, and colorectum.[5] Others have suggested that specifically hyperinsulinemia, whether disease or drug-induced, may be responsible for these observations, whereas DM treated with certain other anti-hyperglycemics, such as metformin, may be protective.[6]

Related comorbidities, such as obesity, have also been attributed as risk factors for PDAC independently of DM.[7] Statin-based therapies for hypercholesterolemia that are often used as part of the treatment regime for these patients, meanwhile, have been variably implicated in either promoting or attenuating tumor growth for a variety of cancers.[8] The biological mechanisms for these seemingly disparate clinical observations, and whether they are primarily a consequence of a particular disease profile or the medications used in their treatment, remain to be completely elucidated. In this study, we sought to determine if preoperative insulin and statin use, independent of underlying DM or obesity, is associated with post-resection outcomes in patients diagnosed with PDAC.

Materials and Methods

An IRB-approved, multi-institutional database of patients who underwent surgical resection for PDAC from January 1, 2000 to January 1, 2009, was queried. Those diagnosed with other pancreatic or periampullary neoplasms were excluded. Data were collected prospectively from members of the Central Pancreas Consortium (University of Louisville, Louisville, KY; University of Cincinnati, Cincinnati, OH; University of Wisconsin, Madison, WI; University of North Carolina, Chapel Hill, NC; Emory University, Atlanta, GA) on a variety of preoperative clinicopathologic factors including comorbidities and medication use, as well as on outcomes following surgical resection. These data were prospectively entered into each institution's pancreatic cancer database and then subsequently brought together in a menu-driven central database. Diabetes was defined by history and/or outpatient fasting blood glucose >126 mg/dl, random plasma glucose ≥200 mg/dl, or inpatient plasma glucose ≥200 mg/dl prior to 7 AM in 2 days.[9] Univariate analyses were performed to determine which clinicopathologic factors were associated with 90-day postoperative mortality. Multiple logistic regression analyses were also used to determine whether preoperative insulin or statin use could independently predict early mortality using direct entry of covariates which achieved significance on univariate analysis. Beta coefficients from the multivariate analysis were then used to construct a simple scoring system to predict early mortality and overall survival (OS) based on preoperative insulin and statin use. Kaplan–Meier analysis was performed to determine if this score was associated with overall survival and a multivariate Cox proportional hazards model was developed to determine if the score could predict survival independent of tumor size, nodal ratio, and margin status. All analyses were performed with PASW version 18.0 (IBM/SPSS Chicago, IL, released July 2009).

Results

There were 518 patients in this study, with a median age of 67 years (range, 36–93) and a median follow-up of 14.4 months. The 90-day mortality rate was 5.6%. Clinicopathologic characteristics for this cohort are presented in Table 1. On univariate analysis, patients who died within the 90-day postoperative period were more likely to be older (p=0.011) and have been treated with either preoperative insulin or a statin (p=0.024 and p=0.035, respectively). Preoperative steroid or thyroxin use, tumor size >2 cm, nodal ratio, margin status, gender, or the presence of other medical comorbidities, including pre-existing diabetes or BMI≥30 were not associated with early mortality.

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Table 1 Univariate analysis of preoperative medications and clinicopathologic factors associated with early mortality

Preoperative factor	<90-day survival (n=29), n (%)	>90-day survival (n=486), n (%)	p value
Age			0.011
Insulin	9 (36.0)	60 (16.0)	0.024
Statin	9 (36.0)	74 (15.2)	0.035
Thyroxin	3 (10.3)	36 (7.4)	0.474
BMI≥30	3 (10.3)	106 (21.8)	0.166
DM	13 (44.8)	146 (30.0)	0.101
Steroids	1 (3.4)	8 (1.6)	0.409
Male gender	16 (55.2)	234 (48.1)	0.567
Respiratory history	2 (6.9)	47 (9.7)	1.000
Renal failure	1 (3.4)	17 (3.5)	1.000
Cardiac history	10 (34.5)	107 (22.0)	0.168
Tumor size >2 cm	25 (86.2)	392 (80.8)	0.703
Nodal ratio			0.484
Positive margins	9 (31.0)	126 (25.9)	0.734

The overall perioperative complication rate for this cohort was 56.8% (Table 2). Of all the factors examined for association with early mortality, only preoperative steroid use was associated with increased complication rate within 30 days of resection (p=0.006 by Fischer's exact test).

To determine which factors were independent predictors of early mortality, a multivariate analysis was performed using direct entry of those factors which were significant on univariate analysis (Table 3). Both preoperative insulin use (odds ratio (OR)=3.043; 95% confidence interval (CI), 1.256–7.372; p=0.014) as well as statin use (OR=2.529; 95% CI, 1.048–6.104; p=0.039) were independently associated with 90-day mortality. Age >70 also retained significance in this model, which demonstrated both adequate fit and discrimination (Ĉ, p=0.836, AUROC=0.730).

A simple scoring system was then constructed based on the beta-coefficients from the multivariate analysis. Zero points were assigned to those patients who were taking neither insulin nor a statin preoperatively. One point was assigned to those taking either one of these medications, and two points were assigned to individuals being treated with both preoperative insulin and a statin. This score was found to correlate with early postoperative mortality (Fischer exact test, p=0.04, Table 4), as well as worse OS with a median survival of 19.6 months, 15.6 months, and 11.2 months, for 0, 1, and 2 points, respectively (log-rank test, p=0.002, Fig. 1 and Table 4).

In considering whether patients taking either preoperative insulin or a statin were afflicted by more aggressive tumor biology or more frequently underwent incomplete resections, a Cox regression analysis was performed to determine if our preoperative medication score was independent of tumor size >2 cm, nodal ratio, and margin status. As indicated in Table 5, this score remains an independent predictor of overall survival in this model (p=0.009). It should be noted that while there is a significant difference between score 1 and 0, and score 2 and 0, the

Table 2 Complication rates encountered for entire cohort within 30 days of PDAC resection

Complication	Rate, n (%)
Any	294 (56.8)
Delayed gastric emptying	99 (19.1)
Wound infection	58 (11.2)
Intra-abdominal abscess	41 (7.9)
Respiratory complication	39 (7.5)
Sepsis	30 (5.8)
Pancreatic fistula	25 (4.8)
Cardiac complication	20 (3.9)
GI bleed	20 (3.9)
Anastomotic leak	12 (2.3)
UTI	12 (2.3)
DVT/PE	11 (2.1)
ARF	8 (1.5)
Other	37 (7.1)

GI gastrointestinal, *UTI* urinary tract infection, *DVT/PE* deep vein thrombosis/pulmonary embolism, *ARF* acute renal failure

Table 3 Multivariate logistic regression of preoperative factors predicting early mortality

Preoperative factor	Odds ratio	95% CI	p value
Age>70	2.732	1.131–6.598	0.026
Insulin	3.043	1.256–7.372	0.014
Statin	2.529	1.048–6.104	0.039

Table 4 Correlation of preoperative medication score with early mortality and overall survival

Points	*N* (%)	90-day survival (%)	Median OS (months)
0	365 (70.9)	96.6	19.6
1	108 (21.0)	88.5	15.6
2	13 (2.5)	86.7	11.2

difference between score 1 and 2 is smaller, and does not reach statistical significance, although there is a trend. Overall, however, the score remains predictive of survival. Tumor size, increasing nodal ratio, and positive resection margins were also independent predictors of overall survival (p=0.005, p<0.0005, and p<0.0005, respectively).

Discussion

Numerous prospective and observational studies have suggested a link between DM and PDAC. This relationship is complex, with DM being reported as either a risk factor for, or a consequence of, pancreatic cancer.[1–4] Although a number of studies implicate DM and perioperative hyperglycemia as risk factors for increased postoperative morbidity and mortality after major surgery, the impact of this disease and associated comorbid conditions, such as hypercholesterolemia on outcomes post-resection for PDAC is an area requiring further study.[10–12] Specifically, whether the preoperative use of insulin or statins are markers of postoperative survival for patients diagnosed with PDAC, independent of underlying DM or obesity, has yet to be evaluated.

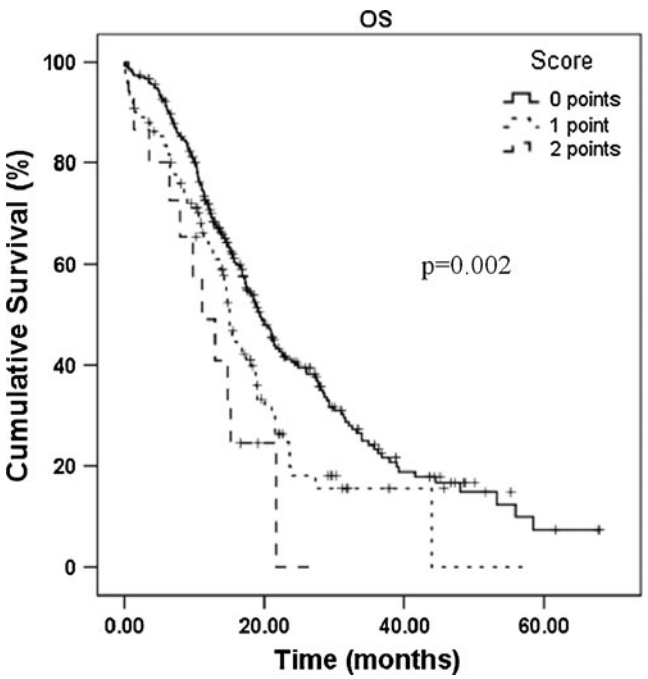

Fig. 1 Kaplan–Meier curves for overall survival comparing increasing preoperative medication score

Table 5 Cox regression analysis of factors influencing overall survival

Factor	Odds ratio	95% CI	*p* value
Preop med score			0.009
0 vs. 1	0.755	0.581–0.980	0.035
2 vs. 1	1.610	0.851–3.044	0.143
Margins			<0.0005
R1 vs. R0	1.481	1.158–1.894	0.002
R2 vs. R0	4.177	1.827–9.548	0.001
Tumor size >2 cm	1.531	1.133–2.067	0.005
Nodal ratio>0.2	1.594	1.268–2.005	<0.0005

In this study, we found that preoperative insulin use is an independent predictor of both 90-day mortality as well as overall survival. DM itself however, was not found to be predictive of postoperative mortality on univariate analysis. In other studies, DM and poor glycemic control has been shown to be a poor prognostic factor in a number of cancers. A recent meta-analysis including 48 studies with a minimum 3 months follow-up, demonstrated broadly defined DM to be associated with increased all-cause mortality in patients with cancer, particularly of the breast, endometrium, and colorectum, but interestingly, not of the pancreas.[5] Although a large observational study found that DM was associated with increased mortality from pancreatic cancer, diabetes in this study was defined by history only and there was no control for method of treatment.[13] Another prospective cohort study, in an attempt to more clearly define a subset of patients with impaired glucose tolerance, found increasing post-load plasma glucose levels to be associated with mortality from pancreatic cancer, independent of age, race, smoking history, and BMI, although no treatment variables were included.[14] While we also attempted to more clearly define DM, by both history and more objective biochemical criteria, no association was found between this disease and 90-day postoperative mortality. Findings of a recent retrospective study from Chu et al. demonstrated that DM was an independent risk factor for certain postoperative complications such as pancreatic fistula and acute renal failure, but as in our study, DM was not associated with early postoperative mortality (60-day mortality 4.3 vs. 3.0%, p>0.05).[9] In a related study using a similar patient population, although assessing long-term outcomes, this group did find that new-onset DM (<24 months before the diagnosis of PDAC) was predictive of poorer OS, independent of tumor characteristics and

related comorbidities, such as BMI.[15] Interestingly, preoperative DM, regardless of time of onset, was also independently associated with increased tumor size.

Our novel finding that preoperative insulin use is an independent predictor of both early postoperative mortality and OS suggests that hyperinsulinemia itself may be responsible for some of the findings described above. Insulin, by increasing the bioavailability of insulin-like growth factor-1 (IGF-1) through mutual competition for binding proteins, has potential mitogenic effects in vivo.[16–18] The IGF-1 receptor in turn has also been shown to be overexpressed in pancreatic cancer cell lines, and signaling through this receptor has been associated with inhibition of apoptosis as well as promotion of both angiogenesis and cell proliferation.[19–22] Conversely, metformin, by decreasing hepatic gluconeogenesis and acting as a peripheral insulin sensitizer, may attenuate the proliferative actions of insulin on pancreatic cell lines.[23, 24] In a hamster model of pancreatic cancer, treatment of animals with metformin reduced hyperinsulinemia and abrogated the development of PDAC by inhibition of pancreatic ductal cell proliferation.[25] An alternative mechanism by which metformin may be protective against PDAC is through activation of an AMP-activated protein kinase pathway, with downstream inhibition of cell proliferation via mammalian target of rapamycin as well as promotion of cell cycle arrest and apoptosis through p53 and p27.[26–29] These biochemical mechanisms may represent an explanation for recent observational studies demonstrating increased cancer-related mortality in diabetic patients taking either exogenous insulin or sulfonylurea monotherapy, relative to those patients exposed to metformin.[30–32] A case–control study conducted at M.D. Anderson found that patients taking metformin specifically had a decreased risk of developing pancreatic cancer, whereas those patients taking either exogenous insulin or an insulin secretagogue had an increased risk of pancreatic cancer relative to those not taking these medications.[6] Similarly, a retrospective cohort study from the UK found that concomitant use of metformin abrogated the increased risk of pancreatic and colon cancer that was found in diabetic patients taking insulin or insulin secretagogues.[33] Thus, the simple presence of diabetes, defined either by patient history or even with more rigorous biochemical criteria based on blood glucose, does not account for various treatment regimes that may include exogenous insulin, insulin secretagogues, or metformin, leaving open the possibility of grouping together patients who are taking agents that have opposing effects on circulating insulin levels and potentially tumor progression. This might explain some of the disparate findings in the literature with respect to DM and mortality from PDAC, and suggests that hyperinsulinemia, whether drug or disease-induced, could be used as a surrogate for DM in these types of studies. While it should be noted that we have not been able to control for the severity of diabetes within the population of patients who are on insulin, our main interest was to understand the predictive value of the medications themselves. The presence of other potential confounding factors which may have been unaccounted for, such as smoking history, should however, be acknowledged. Future prospective series evaluating survival in patients with specific preoperative diabetic drug regimens may strengthen our understanding of the role of preoperative insulin as a poor prognostic indicator and the potential protective function of preoperative metformin.

Other comorbid conditions associated with DM, such as obesity, have also been found to be linked with pancreatic cancer. While we did not find that mortality is associated with certain comorbidities, such as cardiac history or renal failure, which may in part be due to a relatively small sample size, similar to our findings, a large prospective cohort analysis using data from the Whitehall Study, did not find BMI or hypercholesterolemia to be risk factors for mortality from pancreatic cancer.[34] A recent retrospective case–control study, however, found obesity (BMI≥30) to be associated with reduced overall survival in pancreatic cancer independent of DM.[7] Of note, this study did not control for statin use which may have been correlated with obesity. The role of statin use on the risk and progression of pancreatic cancer is controversial. Statins have been shown to both decrease pancreatic cell invasion and metastasis, and conversely to also impair anti-tumor immune responses in vitro.[8, 35] While some observational studies suggest that statin use is associated with a decrease in overall cancer incidence, this finding is inconsistent and not corroborated by recent meta-analyses.[36–39] Indeed, clinical trial data have shown significantly increased cancer rates in elderly patient populations with prolonged statin use.[40, 41] A meta-analysis from 2008, including a total of 12 RCTs, cohort and case–control studies, also demonstrated no reduction in risk of pancreatic cancer from low-dose statin use.[42] While there remains incongruent evidence on the role of statins in pancreatic cancer incidence, our finding that preoperative statin use is an independent predictor of both early postoperative mortality and poorer OS, suggests that for patients diagnosed with PDAC, treatment with these medications is a negative prognostic marker when present.

Conclusion

Preoperative insulin and statin use are independent predictors of outcome after PDAC resection. This novel finding allowed for the creation of a simple scoring system which correlated with both 90-day mortality and overall survival. We found that this preoperative medication score predicted survival independent of traditional clinicopathologic variables such as

tumor size, nodal ratio, and margin status. Although this is a multi-institutional study, widespread use of the score will require prospective validation in other series.

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ORIGINAL ARTICLE

Prognostic Factors for Post-recurrence Survival in Esophageal Squamous Cell Carcinoma Patients with Recurrence after Resection

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Abstract
Objective The survival of recurrent esophageal cancer is poor. But reports regarding prognostic factors for post-recurrence survival are limited. We analyzed the recurrence pattern and the prognostic factors for post-recurrence survival in esophageal squamous cell carcinoma with recurrence after resection.
Methods Two hundred sixty-eight patients were included. Tumor recurrence occurred in 115 (42.9%) patients. Recurrence pattern was classified as locoregional, distant, and combined recurrence. The post-recurrence survival was defined as the interval between initial recurrence and either death or the last follow-up.
Results Mediastinum lymphadenopathy was the most common site for locoregional recurrence, whereas lung, liver, and bone were the most common sites for distant recurrence. The overall 1- and 2-year post-recurrence survival rates were 32.6% and 12.6% with a median survival after recurrence of 6.0 months. The independent prognostic factors included liver recurrence (HR=2.255, 95%CI=1.073–4.741, p=0.032), time to recurrence ≤10 months (HR=2.657, 95%CI=1.438–4.911, p=0.002), and no treatment for recurrences (HR=2.745, 95%CI=1.635–4.608, p<0.001).
Conclusions We identify liver recurrence, early recurrence, and no treatment for recurrence as risk factors for dismal post-recurrence survival.

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Keywords Esophageal cancer · Recurrence · Squamous cell carcinoma · Surgery

Introduction

Esophageal cancer is one of the deadliest cancers with rapidly rising incidence.[1] Even after resection with curative intent, the 5-year survival is rarely >25%.[2] Furthermore, a large number of patients would suffer from tumor recurrences and more than 50% of tumor recurrences occur within 1 year after operation.[3–9] As for the patients with recurrent esophageal cancer after resection, only limited reports addressed the outcome after recurrence, and most indicated extremely poor prognosis. The median survival after recurrence is usually <1 year.[5–10] Many factors, such as tumor location, invasion depth, lymph node metastasis, degree of cell differentiation, vascular invasion, and lymphatic invasion, have been reported to affect survival

and recurrence in esophageal cancer patients.[4–6,8–11] In contrast, very little information regarding the predictors for post-recurrence survival in recurrent esophageal cancer after resection could be found in the literature. In this report, we retrospectively reviewed 268 patients who underwent esophagectomy for esophageal squamous cell carcinoma (ESCC). We aim to analyze the pattern of recurrences and investigate the prognostic factors for post-recurrence survival in patients with recurrence after esophagectomy and lymphadenectomy for ESCC.

Patients and Methods

Study population

A consecutive series of 381 patients who underwent esophagectomy for cancer at Taipei-Veterans General Hospital between 2000 and 2008 was studied. Preoperative staging included physical examination, laboratory tests, esophagogastroduodenoscopy, flexible bronchoscopy (for upper third and middle third tumors), barium esophagography, computed tomography (CT) scans from neck to upper abdomen, ultrasound of the abdomen, and radionuclide bone scans. The positron emission tomography/CT scan became a routine preoperative staging examination for esophageal cancer since 2007. The presence of lymph node enlargement was not a contradiction as long as the nodes are included in the resection. The exclusion criteria included: (1) patients with non-squamous cell carcinoma (n=37); (2) patients who received neoadjuvant chemoradiation (n=33), since the "T" and "N" status may be affected; (3) patients who did not received transthoracic esophagectomy (n=26) and thus the extent of intrathoracic lymphadenectomy may not be adequate; (4) patients with incidental findings of M1 stage during operation (n=9, lung (three), liver (two), omentum (three), and pleural seeding (one)); (5) patients with microscopic or macroscopic residual tumor cells at cut end (n=20); and (6) patients with in-hospital mortality (n=22, 5.8%). The Institutional Review Board of Taipei-Veterans General Hospital approved this study design.

Treatments

The surgical methods included transthoracic esophagectomy and left-sided thoracoabdominal approach. In the transthoracic esophagectomy, esophagectomy and systematic mediastinum lymph node dissection were performed in the thoracic stage. Esophageal substitute mobilization and dissection of paracardial nodes and enlarged celiac axis nodes were performed in the abdominal stage. Then, the gastric tube was pulled to the cervical incision for anastomosis. Cervical lymph node sampling was also completed in the cervical stage. In the left-sided thoracoabdominal approach, the incision extends from below the scapula, across the costal margin, and obliquely toward the umbilicus. The left side pleural cavity and abdominal cavity were exposed simultaneously. The principle of dissection in left-sided thoracoabdominal approach was similar to that of the transthoracic esophagectomy method. Determination of the pathological stage was according to the 7th edition AJCC TNM staging system.[12] Adjuvant therapy was offered to all patients with pT3/T4 stage and positive lymph node metastases.

Follow-up

All patients were followed-up at our outpatient department with an interval of 3 months for the first 2 years, 6 months for 2–5 years, and then annually. Routine follow-up exams include serum tumor marker, chest radiography, and CT scan from the neck to the upper abdomen. Endoscopy and radionuclide bone scans were obtained as clinically indicated. Diagnosis of recurrence was based on histological, cytological, or radiological evidences. Tumor recurrences were classified as locoregional recurrence, distant recurrence, and combined recurrence. Recurrences at the anastomotic site or within the area of previous resection and nodal clearance in the mediastinum or upper abdomen were classified as locoregional recurrence. Distant recurrence was defined as hematogenous metastasis to solid organs or recurrence in the pleura or peritoneal cavity. Simultaneous locoregional and distant recurrences were classified as combined recurrence. The interval between first treatment and detection of recurrence was defined as time to recurrence. The post-recurrence survival was defined as the interval between the detection of initial recurrence and either death or the last follow-up. The principle of treatment for recurrence followed the National Comprehensive Cancer Network (NCCN) guideline.[13] Recurrences were managed with best supportive care or palliative therapy depending on the patient's performance and the surgeon's decision. The palliative treatments included surgery, chemotherapy, radiotherapy, and combined chemoradiation. The best supportive care purposed to relieve patient's symptoms and supported the quality of life regardless of the stage of the disease and the need for further treatment.

Statistics

A chi-square test was used to compare categorical variables and ANOVA for the comparison of continuous variables.

Calculation of post-recurrence survival and construction of survival curve were performed by the Kaplan–Meier method. To assess the prognostic factors for post-recurrence survival, univariate and multivariate analyses by means of Cox regression model were used. To avoid overfitting and selection bias, the full model approach incorporating all candidate variables was used in the multivariate analysis.[14] All calculations were performed using SPSS 17.0 software, and a p value of <0.05 was considered significant.

Results

Patient Demographics and Recurrence Pattern

Two hundred sixty-eight patients met the criteria. The patient characteristics are summarized in Table 1. Patients with more advanced stage of disease tended to have a higher rate of recurrence. Adjuvant therapy was offered to all patients with T3/T4 stage or positive lymph node

Table 1 Characteristics of patients with and without recurrence

Variables	Recurrence					Total (n=268)
	Without (n=153)	Local (n=41)	Distant (n=50)	Combined (n=24)	p value	
Age, mean (±SD)	61.0 (12.1)	61.9 (11.2)	60.3 (10.7)	62.2 (12.1)	0.888	61.1 (11.7)
Sex					0.320	
Male	135	40	46	22		243
Female	18	1	4	2		25
Surgical approach					0.343	
Thoracotomy, three-hole	145	39	45	24		253
Left thoracoabdominal	8	2	5	0		15
T					0.024*	
1	37	9	4	1		51
2	35	9	11	2		57
3	69	22	32	17		140
4	12	1	3	4		20
N					0.002*	
0	75	21	11	10		117
1	46	12	14	8		80
2	15	7	17	5		44
3	17	1	8	1		27
Stage					0.001*	
I	26	9	1	1		37
II	69	16	13	9		107
III	58	16	36	14		124
Grade					0.172	
Well-differentiated (G1)	22	3	6	4		35
Moderately differentiated (G2)	121	34	36	15		206
Poorly differentiated (G3)	10	4	8	5		27
Location					0.551	
Upper third	16	4	4	3		27
Middle third	102	26	27	16		171
Lower third	35	11	19	5		70
Tumor length (cm)	4.4 (2.3)	4.3 (1.9)	5.1 (2.0)	5.9 (2.6)	0.006*	4.7 (2.3)
Chemoradiation status					0.008*	
None	104	30	22	17		173
Adjuvant chemoradiation	49	11	28	7		95

A chi-square test was used to compare categorical variables and ANOVA for comparison of continuous variables

SD standard deviation

*p<0.05

Table 2 Pattern of recurrence in 115 patients

	No. of patients (%)
Locoregional recurrence	41 (35.7)
Distant recurrence	50 (43.5)
Combined recurrence	24 (20.9)
Locoregional recurrence	65
Mediastinum lymphadenopathy	39 (60.0)
Cervical lymphadenopathy	13 (20.0)
Anastomosis	9 (13.8)
Celiac lymphadenopathy	7 (10.8)
Distant metastasis	74
Lung	35 (47.3)
Liver	23 (31.1)
Bone	18 (24.3)
Pleural effusion	10 (13.5)
Brain	5 (6.8)
Other abdominal organ	6 (8.1)

metastases; however, only 95 patients completed the whole course of therapy.

During a mean follow-up of 27 months, tumor recurrence after resection developed in 115 patients (115/268, 42.9%). The median time to recurrence was 10 months. More than half (75/115, 66.2%) recurrences happened within 1 year after operation. The patterns of recurrences included locoregional only in 41 (41/115, 35.7%) patients, distant only in 50 (43.5%) patients, and combined recurrences in 24 (20.9%) patients (Table 2). For locoregional recurrences, 39 (39/65, 60.0%) patients presented with mediastinum lymphadenopathy, 13 (20.0%) had cervical lymphadenopathy, 7 (10.8%) had celiac lymphadenopathy, and 9 (13.8%) patients had locoregional recurrence at the anastomostic site. For distant recurrences, 35 (35/74, 47.3%) patients presented with lung, 23 (31.1%) with liver, 18 (24.3%) with bone, 10 (13.5%) with malignant pleural effusion, 5 (6.8%) with brain, and 6 (8.1%) with other intra-abdominal organs metastases. Treatments for recurrences included surgery for 1 patients, chemotherapy for 33 patients, radiotherapy for 12 patients, and combined chemoradiation for 28 patients. Forty patients received best supportive care due to poor performance status.

Factors Predicting Post-recurrence Survival

The overall 1- and 2-year post-recurrence survival rates were 32.6% and 12.6% (Fig. 1). Median survival after recurrence was 6.0 months (95% CI=5.6–8.4 months). Univariate analysis (Table 3) identified tumor invasion depth, tumor length, combined type recurrence, liver metastasis, time to recurrence, and treatment for recurrences as prognostic factors for post-recurrence survival. In the multivariate analysis (Table 4), liver metastasis, time to recurrence, and treatment for recurrences remained independent prognostic factors for post-recurrence survival.

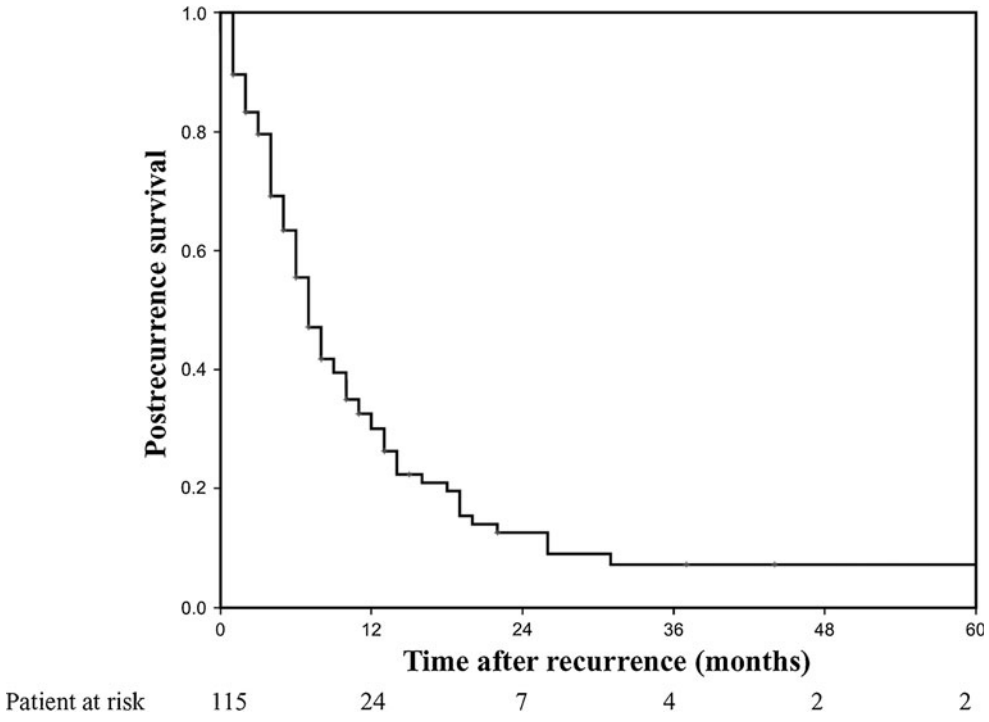


Fig. 1 Post-recurrence survival in 115 patients with recurrent esophageal cancer after esophagectomy. Survival curves were plotted by the Kaplan–Meier method

Table 3 Univariate analysis for post-recurrence survival in 115 patients with recurrent esophageal cancer after esophagectomy

Variables	HR	95% CI	*p* value
Age	1.001	0.981–1.021	0.931
Sex			
Male	1	–	–
Female	1.101	0.402–3.015	0.852
Surgical approach			
Thoracotomy, three-hole	1	–	–
Left thoracoabdominal	0.994	0.735–1.346	0.971
T			
1	1	–	–
2	1.203	0.529–2.736	0.659
3	1.842	0.900–3.770	0.095
4	3.552	1.295–9.743	0.014*
N			
0	1	–	–
1	1.611	0.959–2.704	0.071
2	1.277	0.719–2.270	0.404
3	1.606	0.752–3.430	0.221
Stage			
I	1	–	–
II	1.141	0.499–2.606	0.755
III	1.589	0.718–3.516	0.253
Grade			
Well differentiated (G1)	1	–	–
Moderately differentiated (G2)	0.610	0.320–1.163	0.133
Poorly differentiated (G3)	0.469	0.201–1.091	0.079
Location			
Upper third	1	–	–
Middle third	1.084	0.531–2.213	0.826
Lower third	1.195	0.511–2.343	0.816
Tumor length (cm)	1.124	1.024–1.234	0.014*
Chemoradiation status			
None	1	–	–
Adjuvant chemoradiation	1.106	0.889–1.378	0.366
Recurrence type			
Local only	1	–	–
Distant only	1.548	0.953–2.516	0.078
Combined	2.264	1.269–4.037	0.006*
Local recurrence site			
Mediastinum (with vs without)	0.895	0.570–1.405	0.630
Cervical (with vs without)	0.696	0.369–1.314	0.264
Celiac (with vs without)	1.568	0.693–3.281	0.300
Anastomosis (with vs. without)	1.220	0.562–2.650	0.615
Distant metastasis site			
Lung (with vs. without)	1.245	0.780–1.986	0.359
Liver (with vs. without)	2.506	1.447–4.339	0.001*
Bone (with vs. without)	1.592	0.891–2.845	0.116
Brain (with vs. without)	1.061	0.386–2.920	0.909
Pleural effusion (with vs. without)	1.583	0.758–3.306	0.222
Abdominal organ (with vs. without)	1.315	0.530–3.260	0.555
Time to recurrence			
>10	1	–	–
≤10	2.231	1.440–3.456	<0.001*
Treatment for recurrence			
Yes	1	–	–
No	1.952	1.272−2.997	0.002*

Analysis was performed using the Cox regression model

HR hazard ratio, *CI* confidence interval

*$p<0.05$

Liver is the only distant metastasis site that predicted worse survival. When stratified by median time to recurrence (10 months), patients with early recurrence (≤10 months) had worse survival. In contrast, palliative treatments, instead of supportive care only, for recurrence was a favorable factor for post-recurrence survival. Patients with more risk factors (liver recurrence, early recurrence, and no treatment for recurrence) would suffer from poorer post-recurrence survival (Fig. 2). The median survival in patients with zero, one, two, and three risk factors were 14 (95% CI=8.7–19.3), 7 (95% CI=5.2–8.8), 4 (95% CI=1.6–6.4), and 2 (95% CI=1.1–2.9), respectively ($p<0.001$). One year post-recurrence survival rate was 61.75%, 30.7%, and 4.3% for patients with zero, one, and two risk factors, respectively, whereas none with three risk factors survived more than 1 year.

Discussion

Pattern of tumor recurrence differs among types of cancers. In some types of cancers, recurrence may arise soon after

Table 4 Significant variables in multivariate analysis for post-recurrence survival in 115 patients with recurrent esophageal cancer after esophagectomy

Variables	HR	95% CI	*p* value
Liver (with vs. without)	2.255	1.073–4.741	0.032
Time to recurrence (≤10 vs. >10 months)	2.657	1.438–4.911	0.002
Treatment for recurrence (no vs. yes)	2.745	1.635–4.608	<0.001

Analysis was performed using the Cox regression model. The full model approach incorporating all candidate variables was used. Only significant variables were listed

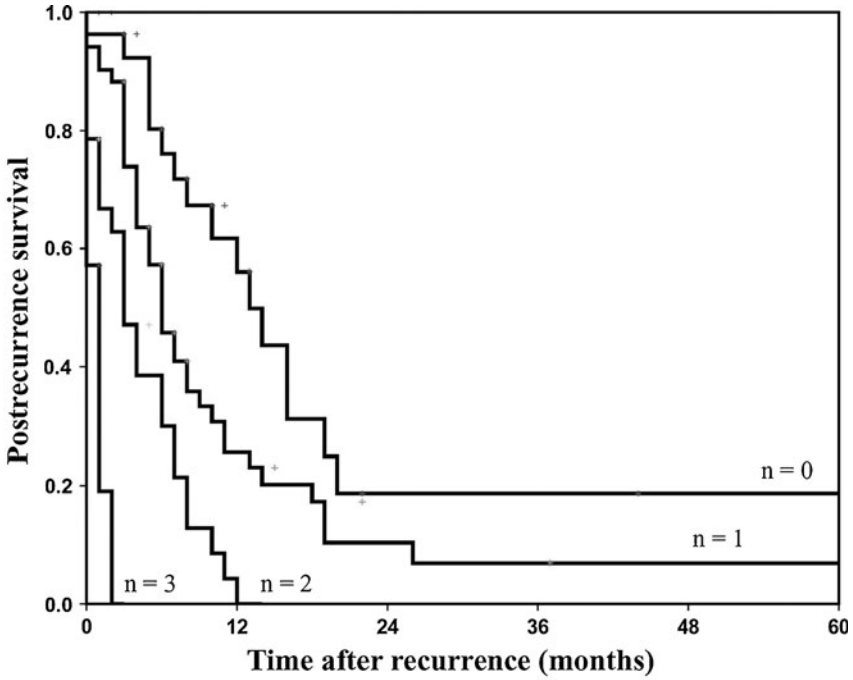
HR hazard ratio, C*I* confidence interval

*$p<0.05$

primary treatment, whereas in others it occurs years after. The disseminated cancer cells may even survive in distant organ microenvironment in a dormant state until they meet all requirements for metastatic outgrowth.[15] The concept of "tumor self-seeding" by circulating cancer cells also explains the early development of locoregioanl recurrence after tumor resection in some cancers.[16] An awareness of recurrent pattern in each type of cancer is essential to patient follow-up protocol.[6] Understanding the predictive factors for post-recurrence survival helps identify high-risk patients, select appropriate treatments, and improve patient outcome after recurrence.

The reported recurrence rate after curative resection for esophageal cancer ranges from 36.8% to 59.2%.[7,17] Locoregional recurrences may occur along the entire "esophageal bed" from the cervical lymph node, anastomostic site, and the mediastinum to intra-abdominal lymph nodes with different frequencies according to the primary localization of tumor.[3,17,18] As for the distant recurrence, the most common involved organs were the lung, liver, and bone in most reports.[3–5,8,17,19] In some reports, soft tissue and skin were also frequent sites for distant recurrence.[3,5,17] In a study by Smit et al.,[17] 40.3% of distant recurrences were noted in the skin or soft tissue, which was the most frequent site for distant recurrences. In our study, the most common sites for distant recurrences were the lung, liver, and bone, followed by the malignant pleural effusion, brain, and other intra-abdominal organs including the spleen, kidney, pancreas, and adrenal glands.

The prognosis of recurrent esophageal cancer is extremely poor. The median post-recurrence survival ranges between 2.7 and 10.0 months in the literature.[5–10] Dresner and Griffin[5] reported the recurrence pattern following radical esophagectomy with two-field lymph node dissection in 176 patients. Among 85 patients with proven recurrences, the median post-recurrence survival was only 2.7 months. They also showed a relative survival advantage in patients with cervical recurrence, but there was no survival difference in patients with interventional therapy compared with those with symptomatic treatment alone. In contrast with Dresner and Griffin's observation, Kunisaki et al.[10] studied 166 patients who underwent curative esophagectomy. Seventy-two developed recurrence, and they identified that each treatment, including chemotherapy, radiotherapy, and chemoradiation, significantly affected survival after recurrence. Abate et al.[6] also showed that



Patient at risk

Risk factor number (n)						
0	29	11	2	2	1	1
1	51	12	5	2	1	1
2	28	1				
3	7					

Fig. 2 Three prognostic factors including liver recurrence, early recurrence, and no treatment for recurrence were identified as risk factors for poor post-recurrence survival. The median survival in patients with zero, one, two, and three risk factors were 14 (95% CI=8.7–19.3), 7 (95% CI=5.2–8.8), 4 (95% CI=1.6–6.4), and 2 (95% CI=1.1–2.9), respectively ($p<0.001$). Patients with more risk factors would suffer from poorer post-recurrence survival. Survival curves were plotted by the Kaplan–Meier method

median post-recurrence survival was significantly longer in patients treated for recurrence (9 vs. 3 months, $p=0.001$).

With regard to the type of recurrence, Mariette et al.[8] reported a significantly longer median post-recurrence survival for regional dissemination than for distant recurrence. They also demonstrated that patients with cervical recurrence had significantly longer survival than those with recurrence at other sites, which was similar to the observation in reports by Dresner and Griffin and Kato et al.[5,20] However, Bhansali et al.[9] reported no difference in different types of recurrences. The median post-recurrence survival was 7 and 9 months in patients with locoregional and distant recurrence, respectively, after radical esophagectomy for ESCC in their study.

Another reported prognostic factor for post-recurrence survival is the interval from esophagectomy to detection of recurrence. Osugi et al.[21] indicted that the time to recurrence correlated with survival after recurrence in ESCC patients who underwent esophagectomy and extended lymphadenectomy. Shimada et al.[7] also showed that patients with time of recurrence <1 year had worse 1-year survival after treatment for recurrence. In accordance with previous literature, we also showed treatment for recurrence and time to recurrence as prognostic factors for post-recurrence survival. Whereas chemotherapy or radiation for recurrence was a favorable factor for post-recurrence survival, patients with early recurrence (time to recurrence ≤10 months) suffered worse prognosis. In addition, we identified liver metastasis as an independent prognostic factor for post-recurrence survival. Liver recurrence was noted in 23 of 115 patients with recurrent esophageal cancer in the current study. The 1-year post-recurrence survival rate in patients with recurrence other than liver was 36.3%, whereas none with live recurrence survived more than 1 year. The post-recurrence survival was significantly shorter in the presence of liver metastasis. In contrast, distant recurrence at other organs had no prognostic value on survival after recurrence.

The current study presented the results of a "surgical series." Since multidisciplinary approaches which highlight the importance of neoadjuvant chemoradiation have shown the survival benefits, we have changed our policy and followed the NCCN guideline using induction chemoradiation.[13,22] Further comparison on the post-recurrence survival difference between patients with and without neoadjuvant chemoradiation is needed. In summary, 115 of 268 ESCC patients developed recurrences after esophagectomy and lymphadenectomy. The survival after recurrence is very poor, with 1- and 2-year post-recurrence survival of 32.6% and 12.6%. We identify T3/T4 stage, liver recurrence, early recurrence, and no treatment for recurrence as risk factors for poor post-recurrence survival. Patients with more risk factors would suffer from poorer post-recurrence survival. Our results may provide a guide to identify high-risk patients, select appropriate treatments, and improve patient outcome after recurrence.

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ORIGINAL ARTICLE

Therapeutic Options for Management of Pharyngoesophageal Corrosive Strictures

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Abstract
Introduction Pharyngoesophageal strictures due to corrosive injury raise difficult therapeutic problems due to the site of stricture, the possible association with laryngeal injury and the presence of downstream esophageal strictures. We present here our approach to management of 51 consecutive patients with pharyngoesophageal strictures seen over a 30-year period.
Methods Patients (51) with PES were managed by one of several options depending on the individual case, viz. dilatation alone, dilatation followed by esophagocoloplasty, dilatation after cervical esophagostomy with or without an esophagocoloplasty, pectoralis major or sternocleidomastoid myocutaneous flap inlays with or without esophagocoloplasty, pharyngocoloplasty with tracheostomy, and neck exploration followed by esophagocoloplasty if a lumen was found in the cervical esophagus.
Results The overall results were excellent with satisfactory swallowing restored in 45 out 51 patients (88.2%). There was one death and three incidences of complications, two patients with temporary cervical salivary fistula, and one patient in whom swallowing could not be restored because of lack of suitable conduit. The mean dysphagia score was improved from a pre-operative value of 3.6 to 1.5 post-operatively.
Conclusion In conclusion, pharyngoesophageal strictures require considerable expertise in management, and one should be aware of various options for this purpose. The choice of procedure depends on site of stricture, time of presentation after the corrosive injury, relationship of the stricture to the laryngeal inlet, status of the larynx and the airway, length of the stricture, presence or absence of a lumen distal to the stricture in the cervical esophagus, and presence or absence of strictures further downstream. With proper treatment, mortality is negligible and morbidity minimal and is usually restricted to temporary salivary fistula.

Keywords Corrosive injury · Pharyngoesophageal stricture · Benign esophageal stricture · Surgery for corrosive stricture

Introduction

Proximal esophageal and pharyngoesophageal strictures (PES) as a consequence of corrosive injuries are not uncommon and pose special problems in management because of the site of involvement and possible associated laryngeal injury.[1] In one study, the incidence of PES was reported to be 24.1% of all corrosive injuries.[2] PES may be the sole area of narrowing with a relatively normal distal esophagus, or they may be associated with dense stricturing of the thoracic esophagus. A short length of the cervical esophagus beyond the PES is often spared. PES may also be associated with corrosive burns of the larynx with varying degrees of narrowing of the glottic inlet. Many of these patients with PES are either resistant to dilation or require frequent dilatations, thus significantly compromising the patient's quality of life. There are reports of pharyngo-colo-gastrostomy or jejunostomy proximal to the PES with relatively few complications and satisfactory

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functional results.[3] However, such results are in general rare in patients with PES. Surgical bypass to the uninvolved pharynx proximal to the cricopharyngeal junction with or without excision of the esophagus is often associated with severe difficulty in learning to swallow in many patients because of frequent episodes of aspiration. The surgical outcome is worse than that in patients with an esophageal substitute anastomosed to a healthy cervical esophagus.[1]

As a therapeutic principle, we have found that it is advisable to widen the pharyngoesophageal junction and perform the bypass to the cervical esophagus. This paper describes the experience with 51 pharyngoesophageal strictures treated in a single unit over a 30-year period. Various therapeutic options are described and an algorithm presented to manage this difficult problem.

Patients and Methods

A total of 51 patients were seen by the unit for dysphagia due to high esophageal or PES over a 30-year period from 1977 to 2006.

The PES was due to accidental injury in 11 patients and suicidal attempt in 40 patients. The agent involved was acid in 44 out of 51 patients, usually goldsmiths' solvent or aqua regia (a 3:1 mixture of hydrochloric acid and nitric acid) and bathroom cleaning acid (concentrated hydrochloric acid). Both preparations are easily available commercially without any regulations for purchase. Three patients had alkaline injury due to caustic substances, and the agent was unknown in four patients.

Evaluation of the esophagus was carried out by endoscopy after preliminary dilatation, thin oral contrast studies in patients with some residual lumen or by a CT scan with air contrast.

For this study, dysphagia was graded as follows;

Grade I: Normal swallowing
Grade II: Occasional difficulty with solid food bolus
Grade III: Difficulty with both solids and liquids present at all times
Grade IV: Total inability to swallow with dribbling of saliva

Post-operative swallowing was graded 4 weeks after surgery, at 6 months and on every follow-up visit thereafter. The current dysphagia score was evolved since it was easy to administer, easy to grade, reproducible, and was simple for patients (many of whom were illiterate) to respond in postal follow-up. The follow-up protocol consisted of personal examination at 4 weeks, 3, 6, and 12 months and thereafter annually for 5 years. Further follow-up was by postal contact.

The patients were managed in one of the following ways depending on the findings. Informed consent was obtained prior to intervention in every patient after discussing in detail the various therapeutic options and their expected outcome. The time frame between injury and surgical intervention varied from 6 months to 2 years. Delayed intervention was in those patients who were referred to the unit from other centers after varying durations.

Group I (n=9)

All patients who had a residual lumen capable of admitting a guide wire were first offered dilatation as the first therapeutic modality. They were subjected to surgical bypass only if they required frequent dilatations or were rapidly restenosing or if they had a non-dilatable stricture further downstream in the esophagus.

Group II (n=5)

These were patients with no demonstrable lumen capable of allowing passage of a guide wire or patients in whom the extreme proximity of the stricture to the laryngeal inlet led to respiratory embarrassment caused by the proximal end of the inflated balloon occluding the glottic inlet (Fig. 1a, b). They were first subjected to a neck exploration along the anterior border of the right sternocleidomastoid. The cervical esophagus was identified and opened. Retrograde dilatation established a passage to the hypopharynx. The opening in the esophagus was converted into an esophagostomy. A nasogastric tube was brought out through the esophagostomy (Fig. 2). This tract was used for repeated

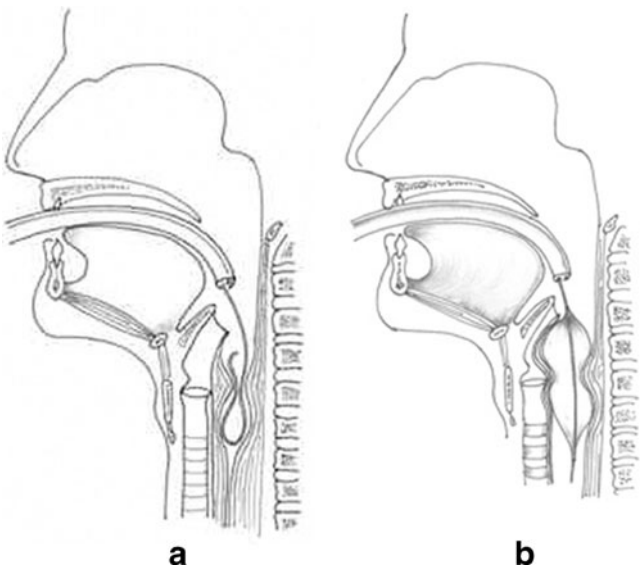

Fig. 1 Problems with dilatation of pharyngoesophageal strictures, **a** over the wire dilators and **b** balloon dilators

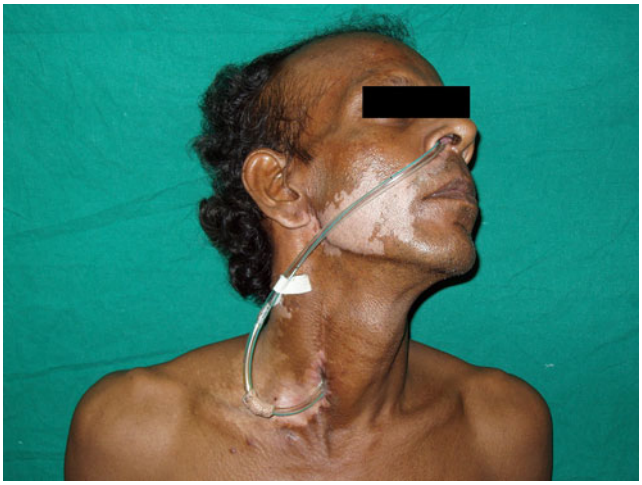

Fig. 2 Patient with cervical esophagostomy with a nasogastric tube brought out through the esophagostomy

dilation of the pharyngoesophageal stricture by passing a guide wire and then over the wire Savary–Gilliard dilators. After each session of dilatation, the nasogastric tube was re-inserted and brought out through the esophagostomy and kept in situ till the next dilatation to keep the tract open. Dilatations were done at weekly intervals. Once the lumen was established an esophagocoloplasty was done distal to the now dilated stricture through a left neck approach. The procedure for esophagocoloplasty described by the unit has been published earlier.[4] The esophagostomy on the right side usually closes spontaneously. In patients in whom spontaneous closure takes a long time, it is a simple procedure under anesthesia to close the esophagostomy with interrupted sutures after separation from the skin. The anastomosis of the colon to the cervical esophagus was a wide 5×5 cm side to side anastomosis to the cervical esophagus beyond the pharyngoesophageal junction. The blind end of the colonic conduit was closed with interrupted non-absorbable sutures. The native esophagus beyond the anastomosis was left in situ and not removed. The larynx was not excised in any patient. The conduit was placed substernally.

Group III (n=3)

Patients with short segment pharyngoesophageal obstruction due to granulation tissue or synechiae were managed by excision of granulation and adhesiolysis under vision with the help of a direct laryngoscope. Repeated sessions were necessary to obtain a stable lumen.

Group IV (n=11)

Patients who had a dense non-dilatable stricture of the pharyngoesophageal junction with a normal distal esophagus of at least 5 cm length distal to the pharyngoesophageal stricture fell into groups IV and V.

In group IV, the PES stricture was repaired using an island pectoralis major myocutaneous flap inlay through a right neck approach. The details of the technique have been described elsewhere.[5] In brief, the steps of the procedure include exposure of the cervical esophagus below the PES through an incision along the anterior border of the right sternocleidomastoid muscle and laying open the PES, thus exposing healthy pharynx above and healthy esophagus below. The defect thus created is closed in one layer using a paddle of skin as an island over the pectoralis major muscle (PMMC flap). This paddle of skin is about 4–5 cm wide and of sufficient length to extend at least 1 cm beyond the strictured segment with the skin facing the lumen of the laid open pharyngoesophageal segment. The donor area was primarily closed. All 11 patients had in addition strictures of the thoracic esophagus. Eight could be managed by dilatation (group IVA) and three required esophagocoloplasty through a left neck approach 4–6 weeks later since dilatation was not possible (group IVB).

Group V (n=15)

Since the PMMC flap left an unsightly scar on the chest wall the PMMC inlay was replaced by a sternomastoid muscle myocutaneous inlay flap in this group. The indications were the same as for group V .The details of the procedure have been published earlier.[6] The procedure for the SMMC flap inlay is similar to the PMMC inlay flap except that it is based on the sternocleidomastoid muscle. Two patients had only a PES with no obstruction distally and were relieved after an SMMC flap without the need for further intervention. This constituted group VA. Twelve patients after an SMMC flap required treatment of strictures in the thoracic esophagus. Six of these could be managed by dilatation (group VB) and six required esophagocoloplasty through an approach along the anterior border of the left sternomastoid (group VC). One patient after an SMMC flap for a PES underwent an attempt at coloplasty for the thoracic esophageal stricture. However, the attempt was unsuccessful since the colonic vascular anatomy was unsuitable (group VD).

Group VI (n=2)

Patients with total destruction of the larynx and a permanent tracheostomy were managed by a mid colon pharyngocoloplasty with the pharyngocolic anastomosis done above the PES. The anastomosis was done in a side to side fashion in two interrupted layers. The blind cut end

of the colon beyond the anastomosis was closed by interrupted non-absorbable sutures in two layers.

Group VII ($n=6$)

In this group of patients, no lumen was detected in the cervical esophageal segment beyond the PES by contrast study or CT scan. A neck exploration was done in them to identify the cervical esophagus for retrograde dilatation if feasible. No lumen was demonstrable below the hypopharynx in four patients up to the clavicle. These patients underwent a feeding gastrostomy or jejunostomy only since it was considered that a pharyngocolic bypass would result in a very high rate of aspiration as the laryngeal inlet was wide open, the epiglottis partly destroyed leaving the glottis exposed, and the upward movement of the larynx on deglutition compromised due to fibrosis (group VIIA). In the other two patients, a lumen was identified in the cervical esophagus. An esophagostomy was done on the right side distal to the PES for dilatation as described in group II. Once the lumen was stabilized which took 3 to 4 months, an esophagocoloplasty was performed through the left neck approach (group VIIB).

Results

The 51 patients ranged in age from 18 to 43 years (mean 25.6 years). There were 8 men and 43 women. All patients had a stricture involving the pharyngoesophageal junction. Two patients in addition had extensive laryngeal involvement with complete closure of the glottic opening which required a permanent tracheostomy.

The procedures performed for these 51 patients and the outcome of these procedures is shown in Table 1. Out of the 51 patients, 36 (71%), in addition to a pharyngoesophageal stricture, also had strictures involving the esophagus further downstream.

The patients were followed up at 4 weeks, 3,6, and 12 months post-intervention and thereafter annually for up to 5 years. Further follow-up was postal except when they had some complications to report when they were seen personally.

Group I

Dilatation was possible in nine patients who had a narrow residual lumen permitting passage of a guide wire. The mean pre-procedure dysphagia score in this group was 3.2.

Table 1 Therapeutic procedures and results

Group and subgroup		Procedure	Number	Associated distal stricture	Pre-operative mean dysphagia score	Post-operative mean dysphagia score	Complications	Mortality
I		Dilatation	9	Nil	3.2	1.6	–	–
II		Dilatation+coloplasty	5	5	3.8	1.0[a]	–	1
III		Excision of granulation+ adhesiolysis	3	Nil	3.0	1.7	–	–
IV	A	PMMC+dilatation	8	8	3.3	1.3	–	–
	B	PMMC+coloplasty	3	3	3.7	1.0	2[b]	–
V	A	SMMC alone	2	Nil	4.0	1.0		–
	B	SMMC+dilatation	6	6	4.0	1.2	–	–
	C	SMMC+coloplasty	6	6	4.0	1.0	–	–
	D	SMMC+failed coloplasty	1	1	4.0	4.0	1[c]	
VI		Tracheostomy plus pharyngocolic bypass	2	2	3.5	1.0	–	–
VII	A	Neck exploration alone	4	4	4.0	4.0[d]	–	–
	B	Neck exploration—Lumen found[e]	2	2	4.0	1.5		
Total			51	36			3	1

PMMC pectoralis major myocutaneous inlay flap, *SMMC* sternomastoid myocutaneous inlay flap

[a] Excludes patient who expired

[b] Temporary cervical salivary fistula

[c] Coloplasty could not be done after successful SMMC reconstruction since the vascular anatomy of the colon was unsuitable

[d] Only feeding gastrostomy/jejunostomy done

[e] Treated like group II

The lumen could be stabilized to admit a size 15 Fr dilator by repeated bougienage at 2-week intervals over a period of few months. Since there was no distal stricture, the patients were relieved of their dysphagia and were able to take solid food (Fig. 3a, b). The mean post-operative dysphagia score was 1.6 (Table 1). Four patients could eat normally, and five patients had occasional difficulty with solid food. They were advised six monthly follow-up. During the follow-up period, one patient required re-dilatation and was subsequently asymptomatic.

Group II

There were five patients whose PES could be stabilized by dilatation after establishment of a right cervical esophagostomy to facilitate passage of a guide wire. The period taken to obtain stable esophageal lumen varied from 6 to 12 months. Dense or non-dilatable thoracic esophageal stricture downstream in the esophagus in these patients mandated esophagocoloplasty by the previously described procedure.[4] One patient died in the immediate post-operative period following esophagocoloplasty because of massive aspiration. The remaining four patients were able to eat solid food after the coloplasty and had an uneventful recovery. The pre- and post-procedure dysphagia scores were 3.8 and 1.0, respectively (Table 1). The patient who expired has been excluded from the post-operative dysphagia score.

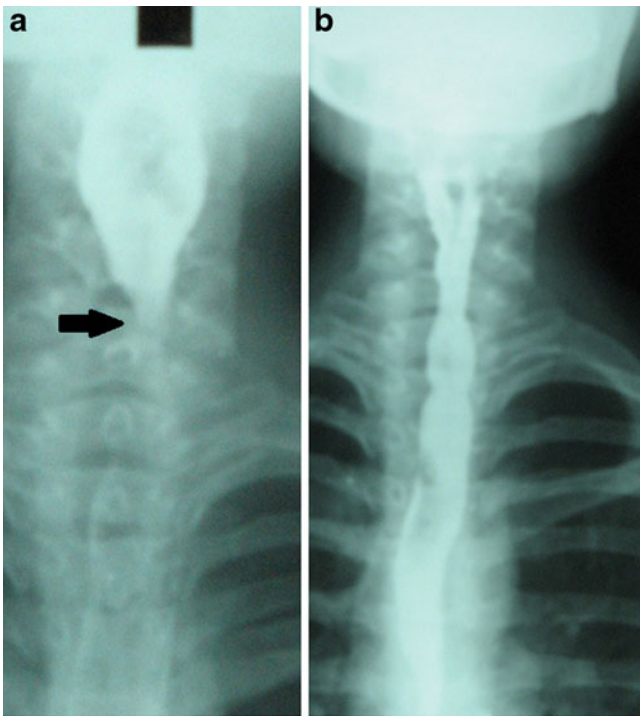

Fig. 3 Showing the lumen **a** before dilatation and **b** post-dilatation

Group III

There were three patients who were seen early (within 12 weeks) after the corrosive injury with grade IV dysphagia. The cause of dysphagia was extensive granulation tissue and adhesions between the epiglottis and arytenoids and the posterior and lateral pharyngeal walls as shown by endoscopy. Multiple sessions of cauterization of granulation tissue and lysis of adhesions were carried out till patients were able to take soft diet. They are on follow-up with periodic dilatation, if required. Post-operative swallowing was satisfactory with change of the mean pre-operative dysphagia score of 3.0 to a post-operative value of 1.7 (Table 1). Two of three patients have occasional difficulty with solid food and one has normal swallowing.

Group IV

This group consisted of 11 patients. As a first step, the pharyngoesophageal stricture was corrected using an island PMMC flap (Fig. 4a–c). Eight of these patients who had a dilatable esophageal stricture of the middle and lower one third were put on a dilatation schedule (group IVA). Lumen was stabilized over a few months. Six patients were able to eat normally, and two have occasional difficulty with solids. The mean pre- and post-procedure dysphagia scores in this group were 3.3 and 1.3, respectively. In three patients, the thoracic esophageal stricture could not be dilated. These patients were subjected to esophagocoloplasty through a left neck approach (group IVB). There was an uneventful post-operative course, restoration of normal swallowing, and reduction of dysphagia score to a mean of 1.0 from 3.7 (Table 1). Two patients with a PMMC flap had a transient cervical salivary fistula which closed spontaneously.

Group V

It consisted of 15 patients in total, all with grade 4 dysphagia. In two patients, PES was the sole involvement (group VA). After an SMMC inlay, normal swallowing was restored in both patients. In 12 patients, about 5 cm of the cervical esophagus beyond the PES was spared with dense stricture of the thoracic esophagus. As a first stage, correction of the PES was obtained by a SMMC inlay flap through a right neck approach (Fig. 5). There was uneventful healing in 6 to 8 weeks. Six of these twelve patients with a non-dilatable stricture downstream had an esophagocoloplasty (group VC). Normal swallowing was restored in all patients. Six others with dilatable strictures were placed on a dilatation program (group VB). In this group, post-dilatation swallowing was normal in five, and one patient had occasional difficulty with solids. In one

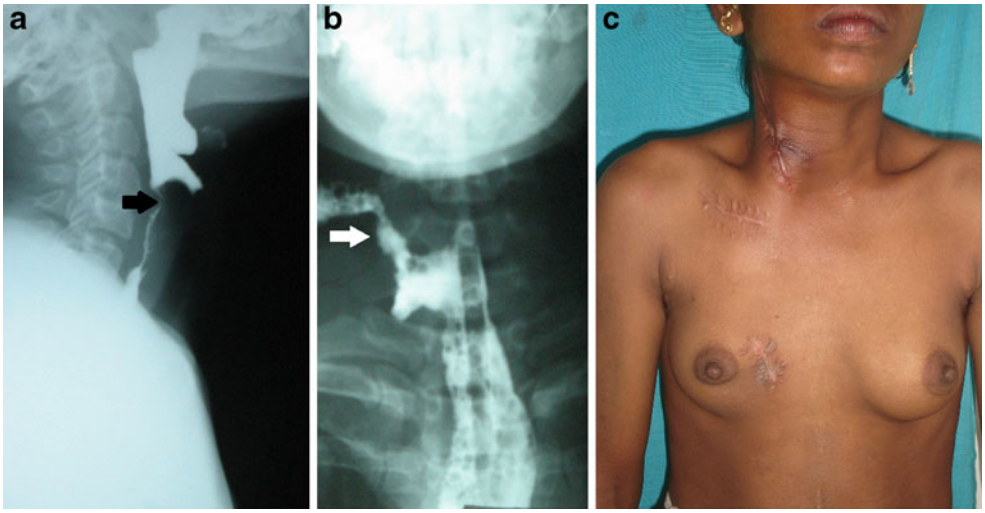

Fig. 4 **a** Pre-PMMC after failed dilatation and **b** post-PMMC with the now dilated segment (a salivary fistula is also shown), and **c** post-PMMC clinical photograph

patient after an SMMC flap, since there was a dense downstream stricture of the esophagus, laparotomy was carried with esophagocoloplasty in mind. However, the stomach was thickened and contracted, and the colonic arterial anatomy precluded a colonic conduit since the marginal artery was thin and interrupted. The patient was left with a feeding jejunostomy only (group VD). The mean pre- and post-procedure dysphagia score in group V is shown in Table 1.

Group VI

Two patients who had extensive laryngeal involvement with permanent tracheostomy had an esophagocoloplasty with the proximal side to side anastomosis being made between the conduit and the hypopharynx (Fig. 6). Both patients recovered uneventfully with reduction of mean pre-operative dysphagia score of 3.5 to 1.0 post-operatively (Table 1).

Group VII

Six patients had no demonstrable cricopharyngeal opening and no demonstrable esophageal lumen by CT scan. As a last resort, the neck was explored through a right-sided approach. No lumen was found on a right neck exploration, and only a feeding jejunostomy was done in three patients and a feeding gastrostomy in one. Since the larynx was intact, they were not considered for a pharyngocoloplasty (group VIIA). In the other two, a lumen could be demonstrated. A cervical esophagostomy was performed for dilatation as described in group II. Following stabilization of the PES, the two patients underwent an esophagocoloplasty with uneventful recovery. One patient had restoration of normal swallowing, and the other had occasional difficulty with solids only (group VIIB).

The outcome, complications, pre- and post-procedure dysphagia scores in all the groups and subgroups are shown

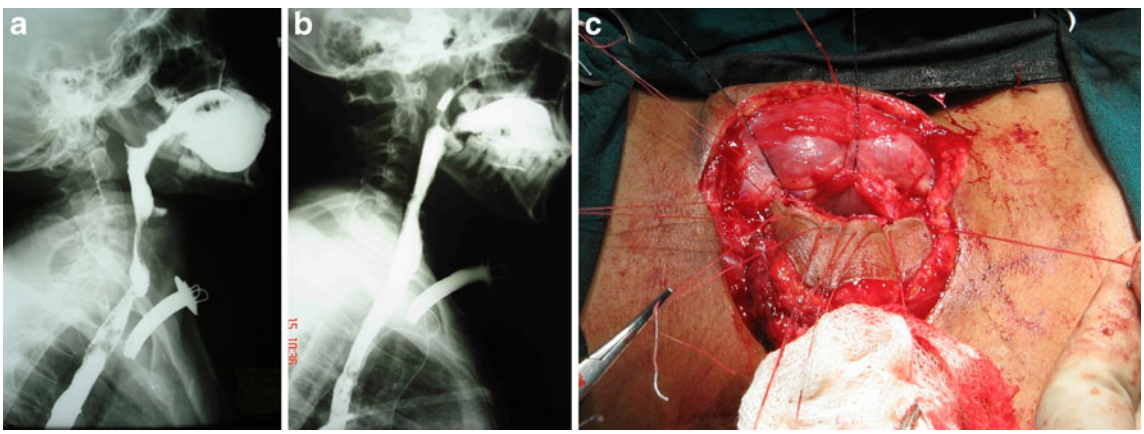

Fig. 5 **a** Pre-SMMC after failed dilatation, **b** post-SMMC showing the reconstituted esophagus, and **c** intra-operative photograph showing the SMMC flap ready to be inlaid

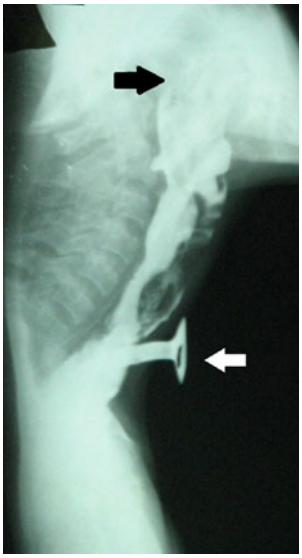

Fig. 6 Pharyngo-coloplasty with permanent tracheostomy

in Table 1. It was found that the swallowing ability seen at 3 months post intervention was stable with no deterioration in those in whom normal swallowing was restored. One patient in group I required a single additional dilatation after 18 months and was asymptomatic thereafter. Three patients in group III require occasional endoscopic dilatations but have not requested yet for a bypass since they are satisfied with the results.

There were no readmissions for aspiration-related complications in the follow-up period.

Discussion

Corrosive strictures which involve the hypopharynx, the pharyngoesophageal junction, or the proximal esophagus raise important therapeutic considerations in addition to the problems seen in those that affect the mid- or distal esophagus. Patients with high esophageal and pharyngoesophageal strictures constitute more than a third of patients with esophageal stricture following corrosive ingestion. The patients may or may not have associated strictures of the esophagus further downstream. In this study, 36 out of 51 patients with PES also had mid- or distal esophageal stricture.

In patients with PES, swallowing mechanisms are often compromised by fibrosis of the soft palate or pharynx which frequently extends to the piriform sinus or the glossoepiglottic folds.[7] Any attempt to treat the esophageal obstruction by anastomosing the conduit to the pharynx with or without resection of the esophagus is associated with significant problems with swallowing, and frequent episodes of aspiration in a large percentage of patients. This happens even in patients in whom considerable time is spent in re-learning how to swallow.

Reconstruction of a segment of the esophagus distal to the pharyngoesophageal junction and performance of anastomosis at this site is met with better results.[1] Since strictures are most common and most severe at sites of anatomical narrowing of the esophagus, it is found in many patients that there is a pharyngoesophageal stricture, a relatively spared segment of cervical esophagus and dense stricture at the site of crossing of the aortic arch extending down to the gastroesophageal junction. Demonstration of the existence of this patent cervical esophageal segment is important for therapeutic reasons. Endoscopy and radiocontrast studies are not useful since the stricture is very tight. We have found a CT scan after swallowing air to be very useful investigation for this purpose.[8] It almost always shows the lumen of the cervical esophagus, if present.

Dilatation still remains the first choice therapeutic modality although the failure rate of dilatation is higher in caustic stenosis compared to anastomotic stenosis and peptic strictures.[9,10] This is probably related to the long, narrow, rigid nature of the stricture and also the multiplicity of strictures following corrosive induced injury. Dilatation is also likely to be successful when instituted early.[11] However, there are two problems with dilatation viz. (a) frequently, there is no lumen to enable passage of a guide wire for use of over-the-wire dilators and (b) when a balloon dilator is used, the extreme proximal nature of the stricture results in the proximal part of the inflated balloon occluding the larynx. This causes acute respiratory embarrassment and necessitates abandoning of the procedure.

If a patent segment of the esophagus can be demonstrated below the pharyngoesophageal stricture, an esophagostomy can be established through a right neck approach along the anterior border of the sternocleidomastoid distal to the PES. After a week, a guide wire can be passed transorally across the stricture exiting through the esophagostomy, and this can be used for Savary–Gilliard dilatation. The stricture can be kept open between dilatations by leaving a nasogastric tube to exit out through the esophagostomy. Once the lumen of the pharyngoesophageal stricture is stabilized, an esophagocoloplasty can be done through a left-sided neck approach. This was done in five patients. One of the five patients died in the immediate post-operative period due to massive aspiration during sleep. Others were relieved of their dysphagia. If the pharyngoesophageal stricture is the only segment of the esophagus to be narrowed, stabilization of the stricture by dilatation through an esophagostomy can be followed by asking the patient to progressively swallow liquids, semisolids and solids. Once normal swallowing is established, the esophagostomy can be closed if it does not close spontaneously as it usually does.

Patients who come early after the corrosive ingestion may have only granulation tissue or synechiae between the

arytenoids and the posterior pharyngeal wall as the cause of their dysphagia. Repeated excision of the granulation tissue, cauterization, and adhesiolysis under anesthesia can result in improvement of dysphagia without the need for any major procedure.

In some patients, the pharyngoesophageal stricture is longer and extends up to several centimeters into the cervical esophagus. In these patients, other options are called for as mere dilatation is unlikely to stabilize an optimal lumen. The procedure carried out should not only widen the stricture but also result in a capacious cervical esophagus lined by epithelium without a tendency to re-stricture. For this purpose, an island myocutaneous flap is optimal. The length of the flap should be about a centimeter longer at either end than the length of the stricture and its width should be about 5 cm. We have used both the PMMC and the SMMC flap for this purpose in 11 and 15 patients, respectively. Both flaps are optimal and have a fairly good blood supply. In those with additional distal non-dilatable strictures, the myocutaneous flap inlay which is done by an approach through the right side of the neck can be followed by a second stage esophagocoloplasty through the left side of the neck .For others without a distal stricture, this procedure itself is curative.

If there is a distal dilatable stricture, it can be treated by dilatation after the myocutaneous flap inlay. Both procedures, the SMMC and the PMMC flaps for corrosive strictures have been described in detail by this unit.[5,6] Of the two myocutaneous flaps, the SMMC has several advantages. It is in close proximity to the stricture; hence, dissection over the chest wall with consequent scarring, which is particularly unacceptable to female patients, is not required. The PMMC flap also has to be tunneled over a long distance across the chest wall and the clavicle which occasionally leaves an unsightly bulge. The blood supply of the PMMC is more robust than the SMMC particularly in patients who have a thin atrophic sternomastoid muscle. The PMMC flap can also support a wider and longer island of skin. The island SMMC flap can be based either superiorly or inferiorly as required depending on the convenience of positioning of the flap.

Patients who have extensive laryngeal scarring requiring a permanent tracheostomy are easier to manage. Since the risk of aspiration is eliminated they can be treated by a colonic bypass with the proximal anastomosis being made to the lateral wall of the pharynx. Two patients treated thus made an uneventful recovery.

There were six patients in whom no lumen was demonstrable in the cervical esophagus beyond the pharyngoesophageal stricture by CT scan. The patients had normal larynx and were considered unsuitable for the pharyngocolic anastomosis for fear of aspiration. Neck exploration was carried out with the hope of finding a lumen in the esophagus which could be widened by a myocutaneous flap. A lumen could be demonstrated in two of the six patients in the cervical esophagus. The two patients were managed by an esophagostomy, serial dilatation, and an esophagocoloplasty after stabilization of the lumen as described earlier. The important issue here is that neck exploration may be the only certain measure to identify the esophageal lumen when all pre-operative investigations have not been useful. CT scan may also fail for this purpose if the pharyngoesophageal connection is totally interrupted. Dense fibrosis had completely obliterated the lumen in the remaining four patients, and the procedure had to be abandoned. Therefore, there would

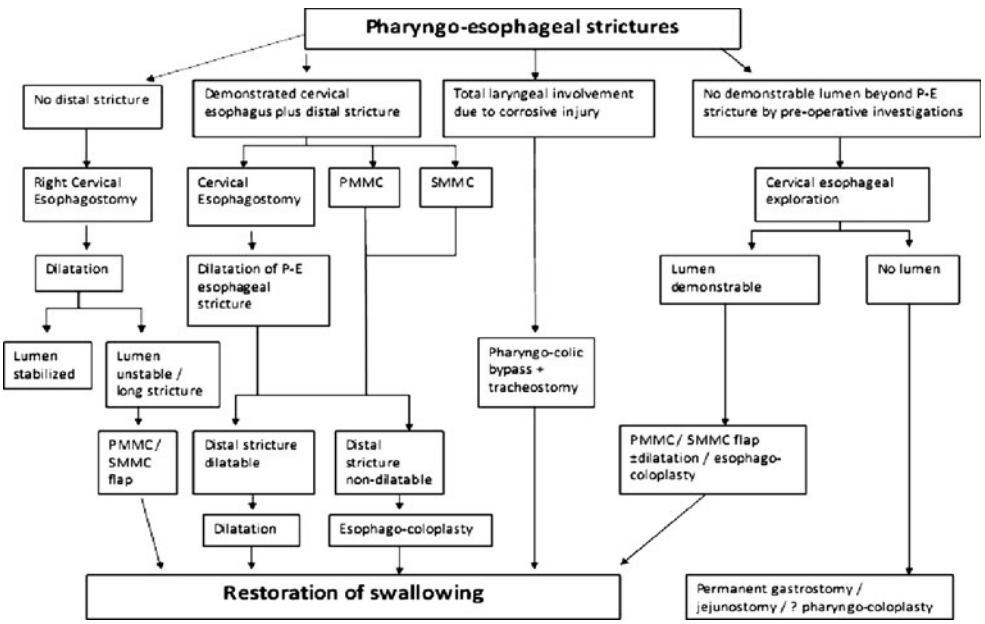

Fig. 7 Algorithm for management of pharyngoesophageal strictures

remain a proportion of patients in whom it will not be possible to do an esophagocoloplasty. They may be considered for a permanent feeding jejunostomy or gastrostomy or counseled and taken up for a pharyngocolic anastomosis with post-operative rehabilitation to enable swallowing.

We had only one mortality, and the morbidity was minimal and predominantly due to temporary cervical salivary fistula. Other series had a much higher complication rate of up to 25%, although cervical anastomotic leak still remained the most common.[12,13] Published large studies of colon interpositions have reported higher rates of mortality, higher fistula rates up to 24%, and a significant rate of anastomotic stenosis of 8.5%.[1,14,15]

Other procedures such as free jejunal grafts were not tried by us.[16] These require considerable technical expertise, are useful only for short segments, and are associated with a high rate of early post-operative morbidity.[17,18] We have also not used the platysma myocutaneous flap described by Zhou et al.[15] or the gastric tube interposition of Matsuki et al.[19]

In conclusion, pharyngoesophageal strictures require considerable expertise in management, and one should be aware of various options for this purpose. The need for a flexibility of approach in the management of PES has been emphasized earlier.[20] The choice of procedure depends on site of stricture, time of presentation after the corrosive injury, relationship of the stricture to the laryngeal inlet, status of the larynx and the airway, length of the stricture, presence or absence of a lumen distal to the stricture in the cervical esophagus, and presence or absence of strictures further downstream.

The algorithm used in our unit for PES is shown in Fig. 7. We have found this to be extremely useful in decision-making. Mortality is negligible and morbidity is minimal and is usually restricted to temporary salivary fistula. It must be emphasized that the approach is not empiric. The options select themselves based on certain parameters.

a. Flimsy adhesions with overgrowth of granulation tissue are best managed by endoscopic adhesiolysis and fulguration. Patients with this clinical situation are, however, rare.
b. For short PES strictures with a passable lumen, the treatment of choice is dilatation. Other options are considered only if dilatation fails, there is an esophageal perforation or the stricture rapidly restenoses.
c. Longer PES, more than 1 or 2 cm in length, are best managed by a myocutaneous inlay flap since they usually re-stenose after dilatation. The choice between a PMMC and an SMMC is not arbitrary. PMMC has a better and more reliable vascular supply. It can be broader and longer. However, the flap leaves an unsightly scar over the chest wall and an unseemly bulge over the clavicle. It is used in patients in whom the sternomastoid muscle is unsuitable, is very thin, has an inadequate blood supply, or the length and breath required are more than can be offered by an SMMC flap.
d. Pharyngo-colic bypass is used by us only when the laryngeal inlet is irretrievable scarred and patients have a permanent tracheostomy.
e. When all pre-operative investigations fail to show a cervical esophageal lumen beyond the pharyngoesophageal junction, neck exploration may still be warranted and may demonstrate a salvageable lumen in a small proportion of patients.
f. How to deal with associated distal strictures beyond the thoracic inlet depends on whether the strictures are dilatable or not. Patients with dilatable strictures are first offered dilatation and those who fail to respond or are non-dilatable are offered a colonic conduit.

Awareness of multiple treatment options makes the choice for both the patient and the surgeon easier.

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ORIGINAL ARTICLE

Intestinal Cancer Risk in Crohn's Disease: A Meta-Analysis

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Abstract
Aim of the study To clarify the intestinal cancer risk in Crohn's disease (CD).
Methods 20 clinical studies (1965–2008) with a total of 40,547 patients with Crohn's disease-associated cancer (CDAC) were included in the meta-analysis ("inverse variance weighted" method).
Results The incidence of CDAC in any CD patient was 0.8/1,000 person years duration (pyd) (CI, 0.6–1.0). The incidences of different carcinomas were: colorectal cancer 0.5/1,000 pyd (CI, 0.3–0.6), small bowel carcinoma 0.3/1,000 pyd (CI, 0.1–0.5), and cancers arising from CD-associated fistulae 0.2/1,000 pyd (CI, 0.0–0.4). Compared to the incidence in an age-matched standard population, the risk of colorectal cancer was increased by factor 2–3 and of small bowel cancer by factor 18.75, respectively. Mean patient age at diagnosis of CD-associated colorectal cancer was 51.5 years, thus 20 years earlier than in a standard population. The mean duration of CD until diagnosis of CDAC was 18.3 years. Duration of CD, age at diagnosis of CD, and anatomical area of CD involvement had no significant influence on cancer incidence.
Conclusions CD is a risk factor for colorectal cancer, small bowel cancer, and fistula cancer; however, compared to ulcerative colitis, cancer risk is moderate.

Keywords Crohn's disease · Carcinoma · Colorectal cancer · Small bowel cancer · Meta-analysis

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Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are two distinct yet similar diseases that are both characterized by chronic and relapsing inflammation of the gastrointestinal tract. The initiating event occurs well before the patients are symptomatic. Studies have shown that these two forms of inflammatory bowel disease (IBD) are influenced by a variety of factors, including genetic,[1,2] environmental,[3,4] and immunologic factors,[5] that lead to the chronic nature of IBD and the relapsing course common in these two diseases.

UC is a well-defined risk factor for colorectal carcinoma,[6–15] and duration and extent of the disease have been identified as important risk factors. However, there have been relatively few studies dealing with malignancies in CD. Since the first case of an adenocarcinoma was described in a patient suffering from CD in the 1950s, an increased rate of small[16–22] and large bowel cancer has been assigned to CD in various studies,[23,24] whereas other studies could not confirm these findings.[25–28]

The aim of this study was to determine the incidence and prevalence of CD-associated carcinoma (CDAC) by meta-

analytical techniques. We analyzed the influence of disease duration, patient age, localization of Crohn's disease, and further variables which are candidate risk factors for CDAC.

Our analysis includes 20 studies with 40,547 patients fulfilling stringent inclusion criteria for a meta-analysis of cancer risk in CD.

Patients and Methods

Identification of Original Studies

All published studies on CD and cancer risk were identified by conducting a literature search on Medline and Cochrane Library using the following keywords: "Crohn's disease", "cancer", and "carcinoma". A comprehensive search of reference lists of all review articles and of the retrieved original studies was performed to find studies not identified by the Medline search. We identified 244 independent publications (1965–1st August 2008).

Inclusion and Exclusion Criteria

English and German language articles were included if they contained data on patient cohorts affected by CD and if they reported CDAC. The studies had to provide a cancer incidence, or the calculation of cancer incidence had to be possible from the data provided. For definition as CDAC, a malignancy had to arise from an intestinal segment affected by CD.

Review articles, studies on cancer mortality statistics (not cancer incidence), duplicate publications, and case reports were excluded. We excluded all publications in which diagnosis of carcinoma was made before the diagnosis of CD. In addition, we excluded case series in which all patients developed a carcinoma; these studies were regarded as case reports with larger patient numbers.

Data Extraction

The included studies were read by two authors (MGL and MH) who extracted study and patient characteristics using a special search mask containing 113 specific parameters. Differences in data extraction were resolved by consensus.

Statistical Analysis

Statistical analysis was performed using the SPSS Software for Windows, version 15.0.1 (Nov. 2006; Chicago, USA). The meta-analysis was conducted according to Lipsey and Wilson[29] using macros for SPSS written by David B. Wilson.[30] Overall estimation of effect size was computed using the "inverse variance weighted" method and a stratified analysis was performed using the "inverse variance weighted one-way ANOVA" and a "random effects model", since the included studies differed concerning their variables and a uniform study design was not present. The heterogeneity in our study was tested by Q-statistics. The significance level was determined as $p < 0.05$.

Incidence values are given per 1,000 person years duration (pyd) with a confidence interval of 95%.

Results

A total of 244 papers were identified. In detail, 15 articles were written neither in English nor in German. Twenty seven articles were identified as reviews and excluded. Six clinical studies contained patient cohorts which were published in duplicate. Fifty six publications did not correspond to the diagnostic criteria of CDAC (see "Patients and Methods"). Seven studies provided no sufficient statistical description. Ninety one case reports were excluded. Twenty two studies were excluded, in which all reported patients developed CDAC (case series).

Twenty clinical studies reporting 40,547 patients finally met the inclusion criteria for the meta-analysis (Table 1).[6,26,31–48] Twelve studies were conducted in the USA and Canada, and eight studies were conducted in Europe.

Overall CDAC Incidence and Prevalence

The meta-analysis calculated an overall CDAC incidence in any CD patient of 0.8/1,000 pyd (95% CI, 0.6/1,000–1.0/1,000), meaning that during a 1-year observation period 0.8 CD patients out of 1,000 developed CDAC. There was a significant variance between the studies ($p < 0.0001$). The overall prevalence of CDAC in any CD patient was 1.01% (95% CI, 0.71–1.31). Again, there was a significant variance between the studies ($p < 0.0001$).

Twelve studies conducted in the USA and Canada, and eight studies conducted in Europe provided the same pooled incidence of 0.8/1,000 pyd (USA/Canada, 95% CI, 0.6/1,000–1.1/1,000; Europe, 95% CI, 0.4/1,000–1.2/1,000; $p > 0.05$).

For analysis of a potential trend in incidence over time, publications were stratified into three groups by year of publication: 1965–1983 (four studies), 1984–1994 (11 studies), and 1995–2008 (five studies). Overall incidences were 0.7/1,000 pyd (95% CI, 0.1/1,000–1.3/1,000), 0.8/1,000 pyd (95% CI, 0.5/1,000–1.1/1,000), and 1.1/1,000 pyd (95% CI, 0.5/1,000–1.6/1,000), respectively. There was no significant difference between

Table 1 Details of included studies

Author	Country	Publication year	Study duration (years)	Study design	Follow-up	Number of CD patients	CDAC Prevalence (%) ± SEM
Atwell et al.[31]	UK	1965	28	Referral center cohort	Retrospective	212	1.41 ± 0.81
Choi et al.[32]	USA	1994	34	Referral center cohort	Retrospective	3,124	0.89 ± 0.17
Connell et al.[33]	UK	1994	29	Referral center cohort	Retrospective	450	1.33 ± 0.17
Connell et al.[34]	UK	1994	53	Referral center cohort	Retrospective	2,500	0.60 ± 0.15
Ekbom et al.[35]	Sweden	1990	18	Population based	Prospective	1,655	0.60 ± 0.19
Freeman et al.[36]	Canada	2001	20	Referral center cohort	Retrospective	877	0.68 ± 0.28
Gollop et al.[26]	USA	1988	39	Population based	Retrospective	103	1.94 ± 1.36
Greenstein et al.[37]	USA	1980	16	Referral center cohort	Retrospective	579	1.90 ± 0.57
Kersting et al.[6]	Germany	2007	15	Referral center cohort	Retrospective	306	2.61 ± 0.91
Korelitz et al.[38]	USA	1983	N.A.	Population based	Retrospective	16,469	0.52 ± 0.06
Ky et al.[39]	USA	1998	14	Referral center cohort	Retrospective	1,000	0.70 ± 0.26
Kyle et al.[40]	UK	1992	34	Population based	Prospective	1,008	1.29 ± 0.36
Lashner et al.[41]	USA	1992	9	Referral center cohort	Retrospective	1,500	0.47 ± 0.18
Lindgren et al.[42]	Sweden	1996	10	Population based	Prospective	312	0.96 ± 0.55
Otto et al.[43]	Germany	1980	11	Referral center cohort	Retrospective	161	2.48 ± 1.23
Petras et al.[44]	USA	1987	9	Referral center cohort	Retrospective	3,500	0.31 ± 0.09
Ribeiro et al.[45]	USA	1991	29	Referral center cohort	Retrospective	1,701	2.59 ± 0.38
Sigel et al.[46]	USA	1999	20	Referral center cohort	Retrospective	2,883	1.04 ± 0.19
Slater et al.[47]	USA	1984	21	Referral center cohort	Retrospective	1,227	0.24 ± 0.14
Yamazaki et al.[48]	USA	1991	26	Referral center cohort	Retrospective	980	1.63 ± 0.40

Pooled CDAC prevalence = 1.01% (95% CI, 0.71–1.31)

CD Crohn's disease, *CDAC* Crohn's disease-associated cancer, *N.A.* not available, *SEM* standard error of the mean

the groups ($p > 0.05$), meaning that there was no significant trend in CDAC incidence over time.

Incidence and Prevalence of Crohn's Disease-Associated Colorectal Cancer

All included studies provided information about the localization of the observed CDACs. This allowed the calculation of pooled incidences for the different localizations.

CD-associated colorectal carcinoma (as reported by 20 studies) had a pooled incidence of 0.5/1,000 pyd (95% CI, 0.3/1,000–0.6/1,000). The prevalence was 0.24% (95% CI, 0.19–0.28).

Incidence and Prevalence of Crohn's Disease-Associated Small Bowel Carcinoma, and Other Cancers

The pooled incidence of CD-associated small bowel carcinoma (as reported by 12 studies) was 0.3/1,000 pyd (95% CI, 0.1/1,000–0.5/1,000), the corresponding prevalence was 0.16% (95% CI, 0.12–0.21).

Carcinomas arising from CD-associated fistulae were reported in nine publications. The pooled incidence of carcinomas in fistulae was 0.2/1,000 pyd (95% CI, 0.0/1,000–0.4/1,000). The pooled prevalence, calculated from two studies, was 0.55% (95% CI, 0.15–0.95).

Eight studies reported cases of other cancer localizations. There were four cases of anal carcinoma, one gastric cancer, and four cancers with lacking information about the exact localization of the primary tumor. The pooled prevalence of cancers at these various localizations was 0.11% (95% CI, 0.04–0.19).

For the different cancer localizations (colorectal, small bowel, fistulae, other), there was a significant variance in prevalence between the groups ($p < 0.01$) and within the groups ($p < 0.0001$).

Age of Patients at Diagnosis of CD and at Cancer Diagnosis, Mean Age of Study Population

The mean patient age at diagnosis of CD was 33.3 years (95% CI, 31.5–35.1). There was a significant heterogeneity between the different studies ($p < 0.0001$). The mean age at diagnosis of CDAC was 50.7 years (95% CI, 48.6–52.9). For colorectal cancer, mean age at cancer diagnosis was 51.5 years (95% CI, 51.4–51.6). The mean duration of CD until diagnosis of CDAC was 18.3 years (95% CI, 17.0–19.6).

The mean age of the study population (pooled from all 20 publications) was 44.49 years (95% CI, 44.48–44.50). The mean age of the population developing a colorectal cancer during the observation period was 44.50 years (95% CI, 44.49–44.52).

Comparison of CD-Associated and Sporadic Cancers

For interpretation of the calculated pooled incidence of colorectal carcinoma, corresponding data of standard populations [*USA*—data of the Surveillance, Epidemiology and End Results (SEER) Program[49]; *UK*—data of the Office of National Statistics[50]] are summarized in Table 2. There was no difference in overall incidence of colorectal carcinoma as incidences in both standard populations (0.58/1,000 pyd) were within the confidence interval of the study population (0.5/1,000 pyd; 95% CI, 0.3/1,000–0.6/1,000). However, the study population suffering from CD was rather young, as reflected by a mean age of 44.5 years. Age-matched standard populations (40–44 years, 45–49 years) had incidences of colorectal carcinoma as low as around 0.2/1,000 pyd (0.11 to 0.26/1,000 pyd). As these values are outside the 95% confidence interval of the study population (0.3/1,000–0.6/1,000 pyd), this means that the incidence of colorectal carcinoma is significantly increased in CD by factor 2–3. Importantly, the mean age at diagnosis was 51.5 years compared to a mean age at diagnosis of 71.0 years in a standard population.[49]

In the general US population, small bowel carcinoma is a rare event with an incidence of 0.016/1,000 pyd (95% CI, 0.015/1,000–0.017/1,000 pyd).[49] In the study population, the pooled incidence of CD-associated small bowel carcinoma was significantly increased by factor 18.75 [0.3/1,000 pyd (95% CI, 0.1/1,000–0.5/1,000)].

Influence of CD Duration on CDAC Incidence

To analyze the influence of CD duration on CDAC risk, patients were stratified into three groups of disease duration: ≤10 years, >10–20 years, and >20 years. The incidences of CDAC (all localizations) in the three groups were 0.4/1,000 pyd (95% CI, 0.1/1,000–0.8/1,000), 0.8/1,000 pyd (95% CI, 0.7/1,000–1.0/1,000), and 1.2/1,000 pyd (95% CI, 1.0/1,000–1.5/1,000), respectively. Although indicating a trend towards a threefold increase of incidence over time, there was no significant difference between the three groups ($p > 0.05$), but a significant variance within the groups ($p < 0.0001$).

Influence of CD Extent on CDAC Incidence

In patients with involvement of both small bowel and colon, the pooled incidences were 0.4/1,000 pyd (95% CI,

Table 2 Incidence and age distribution of colorectal carcinoma

	Study population (Crohn's disease)		General population	
			USA	UK
Mean age	44.5 (44.49–44.52) years			
Incidence	0.5/1,000 (0.3/1,000–0.6/1,000) pyd		0.58/1,000 pyd	0.58/1,000 pyd
		Age 40–44:	0.14/1,000 pyd	0.11/1,000 pyd
		Age 45–49:	0.26/1,000 pyd	0.23/1,000 pyd
Mean age at diagnosis	51.5 (51.4–51.6) years		71.0 years	

The overall incidence of colorectal carcinoma is similar in the study population (Crohn's disease) and in the standard populations (USA and UK). However, the mean age of the study population is 44.5 years, and a comparison to age-matched groups of the standard populations (40–44 years, 45–49 years) reveals a twofold to threefold increased incidence in the study population. Furthermore, in Crohn's disease colorectal carcinoma is diagnosed about 20 years earlier (51 vs. 71 years) than in sporadic cases [USA—age-adjusted SEER incidence rate, 1992[49]; age-specific (crude) SEER incidence rates, 1992–2007[49]; UK—Office for National Statistics: cancer statistics—registrations 1992[50]]

0.3/1,000–0.6/1,000) for colorectal cancer, 0.3/1,000 pyd (95% CI, 0.1/1,000–0.6/1,000) for small bowel cancer, and 0.1/1,000 pyd (95% CI, 0.05/1,000–0.5/1,000) for cancers in fistulae.

In patients with isolated involvement of the colon, the pooled incidence of colorectal cancer was 0.4/1,000 pyd (95% CI, 0.3/1,000–0.5/1,000).

In patients with isolated involvement of the small bowel, small bowel carcinoma had an incidence of 0.2/1,000 pyd (95% CI, 0.1/1,000–0.4/1,000).

An influence of disease extent on CDAC risk could not be shown as there was no significant difference between the groups ($p > 0.05$).

Influence of Age at Diagnosis of CD on CDAC Incidence

Patients were stratified into three groups depending on their age at diagnosis of CD: ≤30 years, 31–40 years, and >40 years. The incidences of CDAC in the three groups were 0.4/1,000 pyd (95% CI, 0.1/1,000–0.7/1,000), 0.5/1,000 pyd (95% CI, 0.2/1,000–0.8/1,000), and 0.3/1,000 pyd (95% CI, 0.1/1,000–1.1/1,000). There were no significant differences between the groups, indicating that the age at diagnosis of CD had no influence on CDAC incidence.

Discussion

This meta-analysis including more than 40,000 CD patients demonstrates that CD is a risk factor for intestinal cancer. The incidence of CD-associated cancers was 0.8/1,000 pyd and the corresponding prevalence was 1.01%.

For colorectal cancer, the incidence was 0.5/1,000 pyd (95% CI, 0.3/1,000–0.6/1,000). Although this value is comparable to the incidence in standard populations in the USA and the UK (0.58/1,000 pyd),[49] one has to take in account that CD patients were rather young in the studies included, as reflected by a mean age of 44.50 years (95% CI, 44.49–44.52). Compared to an age-matched standard population (incidence 0.11–0.26/1,000 pyd),[49] patients with CD have a twofold to threefold increased risk of colorectal carcinoma. Importantly, the mean age of colorectal cancer development was 51.5 years in this meta-analysis, thus the onset of colorectal cancers was about 20 years earlier than in a standard population.[49]

Compared to a standard population,[49] CD increases the risk of small bowel cancer 18.75-fold. However, as small bowel cancer is a rare event, the absolute incidence in CD is slightly lower than for colorectal carcinoma, 0.3/1,000 pyd (95% CI, 0.1/1,000–0.5/1,000).

To our knowledge, this is the first study reporting the incidence of cancers arising from CD-associated fistulae, 0.2/1,000 pyd (95% CI, 0.0/1,000–0.4/1,000). Although this value is lower than for colorectal and small bowel cancer, this incidence means a relevant risk as fistulae cancers are not present in non-IBD-affected individuals.

Although the risk of colorectal cancer is significantly increased in CD, it remains far lower than in long-standing UC. The risk of colorectal carcinoma in UC is as high as 2/1,000 pyd after 10 years of disease duration, 7/1,000 pyd after 20 years, and 12/1,000 pyd after 30 years, leading to a cumulative probability of colorectal cancer of 18% after 30 years.[9] This means that in long-standing UC the risk of colorectal cancer is more than 20-fold higher than in CD (0.5/1,000 pyd).

Although CD has been regarded as potential risk factor for intestinal carcinoma due to the chronic intestinal inflammation and a dysplasia–carcinoma sequence,[51,52] single studies were not able to clearly demonstrate this correlation. While several studies claimed an increased cancer risk,[16–24] other studies did not find any correlation.[25–28] This is easily explained by the only moderate increase in cancer risk compared to UC. Meta-analytical techniques applied in the present study, however, allow the detection of the moderately increased cancer risk.

The results of the present meta-analysis are in line with other published meta-analyses. Three meta-analyses on intestinal cancer risk in CD have been published to date. They included population-based studies that provided a ratio of observed to expected cases of cancers or that provided a relative risk value.[53–55] This allowed the calculation of pooled relative risks. In CD, these studies calculated a relative risk of 1.9–2.5 for colorectal carcinoma and of 27.1–28.4 for small bowel carcinoma. These meta-analyses included a rather small number of population-based studies (6–14), as studies that reported incidences, but lacked relative risk estimations, were not considered for analysis.

We extended the available data pool by also including studies that reported crude incidences, without providing relative risks. This allowed to double the number of studies included ($n = 20$), thus providing a larger basis for meta-analytical calculation. However, several of the population-based studies had to be excluded in our meta-analysis as it could not be clarified if all reported cancers arose from CD-affected bowel segments (defining CD-associated cancer).

This meta-analytical technique, as already employed by Eaden for the analysis of colorectal cancer risk in UC,[9] generates pooled crude incidences. For interpretation, one has to compare these incidences to those of an age-matched standard population. In our study, this leads to a twofold to threefold increased risk of colorectal cancer and to a 18.75-fold increase of small bowel cancer. These values, based on a completely different pool of studies, are within the confidence intervals of the abovementioned pooled relative risks and therefore confirm the findings of previous analyses.[53–55]

The definition of an appropriate control population is an inevitable limitation of our study. As most of the studies

were not population based, exact data on the background populations do not exist. By using standard population data on cancer incidences in the USA and in the UK in the 1990s (when most studies were performed), we achieved a reasonable estimation of the control population.

Using similar techniques like in our meta-analysis, von Roon has calculated a pooled incidence of CD-associated colorectal carcinoma of 0.68/1,000 pyd (0.49/1,000–0.87/1,000), based on 22 studies.[55] This value is comparable to our pooled incidence of 0.5/1,000. Importantly, due to different selection criteria, only four studies were included both in our and in von Roon's meta-analysis. This underlines that the effect is robust to different study selection and therefore is a reliable indicator of cancer risk in CD.

Unlike previous studies, we performed a meta-analysis of patient age at CD diagnosis, at cancer diagnosis, and calculated the mean patient age during the observation period. The latter allowed the comparison of the incidences to an age-matched standard population. Furthermore, we report an onset of colorectal carcinoma in CD patients that is about 20 years earlier than in a standard population.

While duration of disease is a very important risk factor for colorectal cancer development in UC, it seems not to play that important role in CD. Our meta-analysis did not show significant changes in CDAC incidence with longer disease duration despite a trend toward higher incidences with long-standing disease. This allows two conclusions: on the one hand, risk does not increase significantly over time; on the other hand, cancer risk is present even in patients with newly diagnosed CD. However, the mean disease duration until diagnosis of a CDAC was 18.3 years.

Two population-based studies[17,28] indicate that there is a further increased colorectal cancer risk with early onset of CD (<25 years) (pooled relative risk 21.46).[55] However, our meta-analysis did not confirm an influence of patient age at CD diagnosis on cancer risk. Probably the risk was overestimated in the abovementioned studies due to the small number of patients included (n = 562).

Finally, our data did not show a significant influence of the anatomic segments involved by CD on cancer risk. In particular, ileocolic and isolated colonic involvement had comparable risks of colorectal cancer.

It has to be considered that besides chronic intestinal inflammation several confounders could increase the cancer risk in CD patients, e.g., the use of immunosuppressives, or smoking; these confounders are not controlled in the studies included. Concerning immunosuppressives, it has to be mentioned that during the observation period of the included studies only very few patients received potent novel biologicals (anti-TNF-alpha antibodies). The effect of this new therapy on cancer risk has to be analyzed in the future as both an increase in cancer risk due to immunosuppression and a reduction due to better control of inflammation seem possible.

Our study has several important clinical implications. First of all, CD definitely is a risk factor for colorectal cancer, small bowel cancer, and cancer arising from fistulae. However, compared to UC, where cancer development is one of the main problems with long-standing disease, the risk is only moderately elevated. Despite the only moderate risk of cancer development, CD-associated intestinal cancer is a relevant problem. Our meta-analysis shows that CD patients develop intestinal cancers about 20 years earlier than healthy individuals, so we are confronted with malignancies in younger patients. Furthermore, CD-associated carcinomas frequently present in advanced stages, are poorly differentiated, and have a poor prognosis.[6,56,57] Unfortunately, many CD-associated carcinomas are not diagnosed until they are found intraoperatively.[6]

Like in UC, we advocate colonoscopy screening in CD patients to allow early detection of colorectal dysplasia or carcinoma. Based on the finding that the mean age of cancer development is around 50 years and that the mean duration of CD until cancer diagnosis is around 18 years, we recommend to start screening colonoscopy at the age of 40 years, or if duration of CD is longer than 10 years.

Early detection of small bowel carcinoma remains an unsolved problem. Routine (MR) enteroclysma or capsule endoscopy could potentially detect these malignancies at an early stage.[58] However, differentiation between inflammatory stenosis and malignancy will remain difficult. Furthermore, the high costs of these procedures prevent their routine use for the screening of asymptomatic individuals. We propose to use these procedures in every patient who has a change of symptoms, as this might be an indicator of a CDAC. In case of doubt about the dignity of a bowel stenosis, we advocate a surgical approach for definitive diagnosis and therapy.

In CD, situations may arise in which parts of the colon are not available to endoscopic surveillance, usually due to stenosis of a distal colonic segment. In these cases, we propose to dilate the stenosis to perform a complete colonoscopy. If this is not possible, we recommend to resect the colon as a prophylactic measure as it is at relevant risk for the development of CD-associated cancer.

Finally, any fistulae that do not respond to pharmacological and surgical treatment must raise suspicion of a fistula-associated cancer.

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ORIGINAL ARTICLE

Inflammatory Myofibroblastic Tumor of the Small Bowel Mesentery: An Unusual Cause of Abdominal Pain and Uveitis

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Abstract
Introduction An inflammatory myofibroblastic tumor (IMT) of the small bowel mesentery with diffuse immunohistochemical staining for the anaplastic lymphoma kinase 1 gene is reported in a patient who presented with abdominal pain and uveitis.
Discussion To our knowledge, only seven cases of IMT affecting the small bowel mesentery have previously been reported in the English literature. The association of IMT and uveitis is a rare phenomenon, previously reported in patients with IMT affecting the head and neck. Surgical resection of the IMT resulted in rapid resolution of the uveitis.
Conclusion IMT should be considered in the differential diagnosis for a mesenteric mass.

Introduction

Inflammatory myofibroblastic tumor (IMT) is an uncommon neoplasm first described in the lung[1] and originally thought to occur predominately in pediatric patients. Extrapulmonary IMTs have also been described in the liver,[2] spleen,[3] mesentery,[4,5] head and neck,[6,7] heart,[8] and retroperitoneum.[9] IMTs have also been reported in patients of all ages ranging from neonates to the elderly. Although IMT was originally felt to be reactive in origin, growing evidence suggests that it is a true neoplasm with the potential for invasion of surrounding structures, local recurrence, and distant metastases.[10] Up to half of patients with IMTs have a chromosomal abnormality in the anaplastic lymphoma kinase (ALK) 1 gene on chromosome 2p23 resulting in the activation of a tyrosine kinase receptor that is normally expressed only in neural tissue.[11] Recent studies have attempted to correlate the presence of ALK 1 gene expression with local recurrence, distant metastases, and overall prognosis in patients with IMT.

Patients with IMTs most commonly present with pain. They may also present with a syndrome of fatigue, anemia, and weight loss.[10] The pathophysiology of IMT remains unclear, although inflammatory, infectious, and neoplastic mechanisms have been suggested as potential etiologic factors. Similar to retroperitoneal fibrosis, IMT has been reported in conjunction with other autoimmune diseases and/or symptoms.[12] Herein, we report the eighth case of a mesenteric IMT in the English literature[4,5,13–17] in a patient presenting with abdominal pain, fatigue, weight loss, uveitis, and anemia.

Case Report

A 34-year-old Latino man presented with a 2-month history of intermittent, crampy periumbilical and left-sided abdominal pain and a 1-year history of intermittent low back pain. In addition, he developed photophobia and his eyes became red and painful. A complete review of systems was significant for fatigue, diaphoresis, weight loss, and

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palpitations. He denied associated anorexia, nausea, vomiting, change in bowel habits, melena, or hematochezia.

His past medical history was significant for two seizures at the age of 18 and a transient ischemic attack at age 29. His physical exam revealed marked conjunctival injection bilaterally. He had a nondistended abdomen that was soft and nontender with no palpable masses.

Laboratory studies were notable for a normochromic, normocytic anemia with a hematocrit of 36.1%. Abdominal computed tomography revealed a 4.1×5.1×4.5-cm isodense mesenteric mass just caudal to the transverse duodenum and anterior to the inferior vena cava (Fig. 1) with surrounding inflammatory changes, adjacent lymphadenopathy, and enlarged right iliac lymph nodes. There was no associated bowel or liver lesions.

An exploratory laparotomy revealed a solid mass in the small bowel mesentery just beyond the takeoff of the main trunk of the superior mesenteric artery with inflammatory changes extending into the mesentery of the right colon and adjacent enlarged mesenteric lymph nodes. The small and large bowel were normal in appearance and palpation. There were no liver or peritoneal metastases.

The patient underwent a right hemicolectomy and resection of 15 cm of terminal ileum. A mesenteric mass was identified. Gross examination demonstrated a circumscribed and yellow-tan nodular tumor measuring 6.5×6×4.2 cm with focal necrosis (Fig. 2). Microscopic examination revealed a neoplasm composed of spindle cells with no cytological atypia, arranged loosely in a myxoid background, with admixed lymphocytes, plasma cells, and eosinophils (Fig. 3a and b). No mitotic figures were identified. Immunohistochemical stain for ALK1 was diffusely positive in tumor cells (Fig. 3c); focal positivity for smooth muscle actin (SMA) was seen (Fig. 3d). Tumor cells lacked desmin expression. Special stains (Gomori methenamine silver and Ziehl–Neelsen) were negative for microorganisms. The excised lymph nodes were negative for malignancy.

On postoperative day 1, the patient had a low-grade fever, tachycardia, and a witnessed seizure. A neurological evaluation and computed tomography of the head were negative. The rest of his postoperative course was unremarkable. He was discharged on postoperative day number five and was seen in follow-up 1 week later with resolution of his eye pain and photophobia and marked improvement in his conjunctival injection. A diagnosis of anterior uveitis was made on a postoperative ophthalmologic exam and he was started on steroid eye drops. The patient returned for ophthalmology follow-up 1 month after surgery and his uveitis had resolved and his steroid eye drops were discontinued.

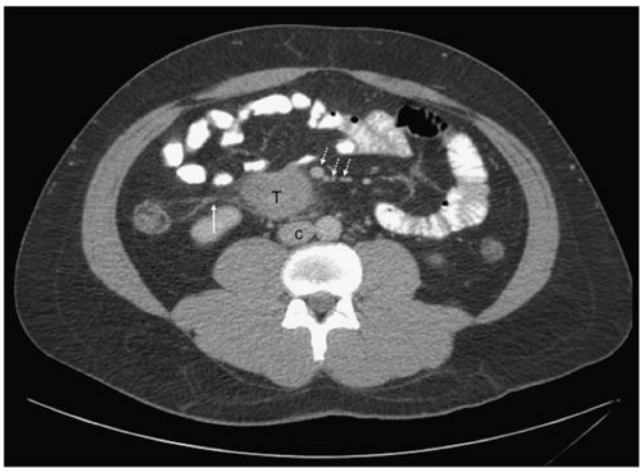

Fig. 1 Abdominal CT scan showing the mesenteric mass (*T*) anterior to the inferior vena cava (*c*) with adjacent lymphadenopathy (*white dotted arrows*) and inflammatory changes extending into the mesentery of the right colon (*white solid arrow*)

Discussion

IMT is classified by the World Health Organization as a neoplasm of intermediate biologic potential because of its potential for invasion of surrounding structures and development of local recurrence and distant metastases.[18,19] IMT has also been variously referred to as inflammatory pseudotumor, plasma cell granuloma, pseudosarcomatous myofibroblastic lesion, and inflammatory myofibrohistiocytic lesion. IMT is characterized by spindle cell proliferation in a variable background of inflammatory cells.[18] A benign IMT cannot be differentiated from a malignant tumor based on radiographic appearance alone. In a mesenteric location, the differential diagnosis includes lymphoma, sarcoma, desmoid tumor, carcinoid tumor, and a gastrointestinal stromal tumor.[4]

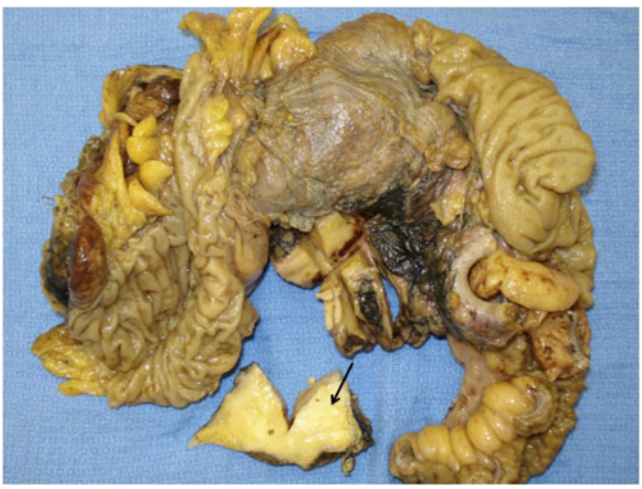

Fig. 2 Right hemicolectomy specimen with transected mesenteric mass (*arrow*)

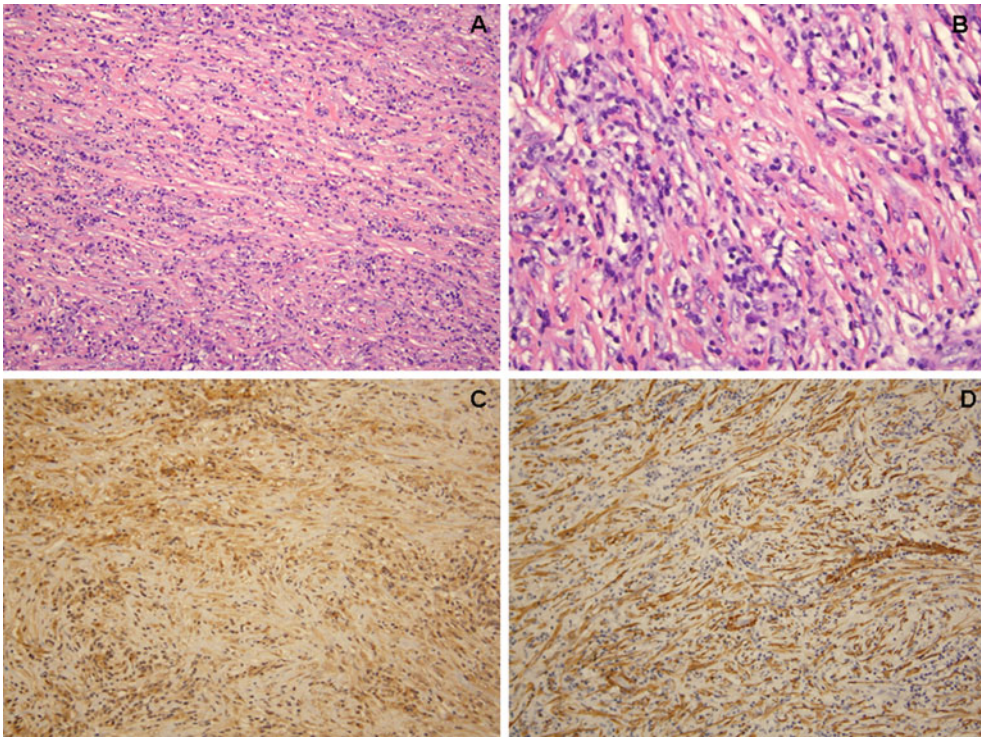

Fig. 3 Inflammatory myofibroblastic tumor. **a** Spindle and plump cells in diffuse inflammatory background (hematoxylin–eosin ×100). **b** Inflammatory infiltrate with lymphocytes and eosinophils (hematoxylin–eosin ×200). **c** ALK1 is expressed in tumor cells (×200). **d** SMA stain (×200)

The characteristic pathologic and molecular features of IMT help distinguish it from other mesenteric tumors.[10] IMTs are characterized by uniform-appearing spindle cells which on ultrastructural examination are predominately myofibroblasts and, to a lesser extent, fibroblasts. IMTs are positive for smooth muscle actin in 80–90% of cases, express desmin in 60–70% of cases, and have focal keratin reactivity in one third of cases.[10] The spindle cells are present within a background of inflammatory cells, mainly plasma cells, lymphocytes, and eosinophils. Approximately 50% of IMTs are positive for ALK 1 on immunohistochemistry whereas most tumors considered in the differential diagnosis of an IMT in the small bowel mesentery are ALK 1-negative.[10]

The etiology of IMT is unknown. IMTs have been reported in association with infectious agents,[20,21] trauma, surgery,[22] autoimmune disease, and most recently with chromosomal abnormalities in the ALK 1 gene. IMT has been reported in patients with a variety of autoimmune diseases, including rheumatoid arthritis,[2,23] systemic lupus erythematosus,[24] Sjögren's syndrome,[25] and adult-onset Still's Disease.[26] The associated fever, weight loss, anemia, and fatigue that were seen in our patient with IMT and reported by others seem to be related to the effects of proinflammatory cytokines, similar to other chronic inflammatory and autoimmune disorders.[2] The potential for immune-mediated phenomena is particularly relevant in our patient who had concomitant uveitis. Reports of IMT with associated uveitis are rare, and the handful of cases in the literature typically involved a primary IMT of the head and neck region.[6,7] In those cases, the proximity of the IMT to the eye was thought to account for the development of uveitis. However, uveitis associated with a primary mesenteric IMT suggests the presence of circulating systemic inflammatory cytokines or autoantibodies.

Although intraocular aspiration was not used to definitively establish the etiology of the uveitis in our patient, we suspect that the uveitis was a systemic manifestation of the mesenteric IMT. The onset of ocular symptoms occurred in association with the onset of the abdominal pain. Additionally, the patient experienced marked improvement in his scleral injection and resolution of his photophobia and eye pain less than 2 weeks following resection of the IMT. One week after hospital discharge, he was examined by an ophthalmologist and was diagnosed with uveitis, similar to what is seen with other autoimmune disorders and was treated with ocular steroids. It is conceivable that the uveitis may have resolved completely after tumor excision without any other intervention.

Advances in molecular pathology have demonstrated that up to one half of all IMTs have mutations in the ALK1 gene,[27] a gene that was first identified in association with anaplastic large cell lymphoma. The presence of chromosomal abnormalities, local recurrence, and distant metastases[4,28,29] establish that IMT is a true neoplasm. Researchers are currently investigating whether ALK 1 tumor positivity is predictive of tumor behavior and prognosis. In a series of 59 cases of IMT selected for histologic atypia or tumor

aggressiveness, Coffin et al. reported that 56% were ALK 1-positive. ALK 1-positive tumors were more common in younger patients and patients with local recurrence. Metastatic disease was limited to ALK 1-negative tumors.[27] As a result, we have chosen to follow our patient with a yearly computed tomogram of the abdomen to evaluate for recurrence. Although other groups have reported that ALK 1-positive tumors are associated with improved prognosis,[28,30] the relationship between ALK 1 positivity and IMT prognosis has not been definitively established.

A complete en bloc resection of the tumor is the mainstay of therapy. Chemotherapy with or without radiotherapy has been used for patients with incomplete tumor resection, positive margins, tumors not amenable to resection, and metastatic disease despite lack of definitive data establishing the efficacy of adjuvant therapy. Adjuvant chemotherapy regimens that have been reported include vincristine or vinorelbine with methotrexate,[31,32] ifosfamide regimens with carboplatin or doxorubicin[32,33] and imatinib.[33] Corticosteroids have been used as a primary form of treatment for IMT with demonstrated tumor regression[34,35] and also as an adjunct to reduce inflammation and swelling of surrounding tissue in patients with IMTs involving the central nervous system.[32] Radiotherapy has been demonstrated to have some benefit, mainly in pulmonary IMT.[36–38] There is currently no evidence that chemotherapy or radiotherapy have a role for patients following complete resection. Despite the frequently reported use of adjuvant therapy for IMT in the literature, there is no definitive data establishing that it is efficacious and there are no standardized treatment protocols.[32]

Butrynski and colleagues have recently reported the use of crizotinib, an ALK tyrosine kinase inhibitor, for the treatment of IMT. One patient with an IMT positive for ALK rearrangement demonstrated sustained partial response after surgical resection and crizotinib therapy, but another patient with ALK-negative IMT did not respond to crizotinib after tumor debulking.[33] For the subset of IMT patients with ALK rearrangements, ALK tyrosine kinase inhibitors represent a potential promising modality for targeted neoadjuvant or adjuvant therapy for locally invasive, incompletely resected, locally recurrent, and unresectable tumors.

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ORIGINAL ARTICLE

Ischemic Preconditioning Attenuates Lactate Release by the Liver During Hepatectomies Under Vascular Control: A Case–Control Study

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Abstract
Background We have previously demonstrated lactate release by the liver itself in hepatectomies performed under selective hepatic vascular exclusion. We hypothesized that ischemic preconditioning applied in this setting might lead to a reduction of hepatic lactate production.
Methods Twenty-one patients underwent hepatectomy under inflow and outflow occlusion combined with ischemic preconditioning (IP group, n=21). These patients were matched 1:1 with patients subjected to the same technique of hepatectomy under vascular occlusion without ischemic preconditioning (control group, n=21). The transhepatic lactate gradient (hepatic vein–portal vein) was calculated before liver dissection and 60 min post-reperfusion.
Results In the control group, the transhepatic lactate gradient before liver resection was negative indicating consumption by the liver. After 60 min post-reperfusion, this gradient became positive, indicating net lactate production by the liver (0.2±0.3 vs. −0.3±0.2 mmol/L, P<0.001). In the IP group, the liver consumed lactate both before resection and 60 min post-reperfusion (gradients −0.2±1.1 and −0.1±0.6 mmol/L, respectively). The magnitude of lactate release by the liver correlated with systemic hyperlactatemia post-reperfusion and 24 h postoperatively (r^2=0.54, P<0.001 and r^2=0.67, P<0.001, respectively). Significant correlations between the transhepatic lactate gradient post-reperfusion and peak postoperative AST as well as the apoptotic response of the liver remnant were also demonstrated (r^2=0.72, P<0.001 and r^2=0.66, P<0.001, respectively).
Conclusion The microcirculatory derangement and cellular aerobic metabolism breakdown elicited by ischemia–reperfusion insults can be prevented with hepatoprotective measures such as ischemic preconditioning. The transhepatic lactate gradient could act as a monitoring and prognostic tool of the efficacy of ischemic preconditioning.

Keywords Reperfusion injury · Lactate kinetics · Anaerobic metabolism · Apoptosis · Hepatoprotection

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Introduction

Liver resections, especially when quite extensive, can be complicated by major hemorrhage, which adversely affects short- and long-term patient outcome.[1] Therefore, techniques involving some type of vascular control have been devised to ensure a less hemorrhagic surgical field.[2,3] Selective hepatic vascular exclusion (SHVE), which entails simultaneous clamping of the portal triad and of the major hepatic veins along with caval flow preservation, is one of the vascular occlusion techniques that allow the safe performance of major liver resections.[3,4] However, the aforementioned technique, like all procedures involving some type of vascular exclusion, is invariably associated with ischemia/reperfusion (I/R) injury, which can diminish the capacity of the liver remnant to maintain adequate postoperative function.[5,6]

Research on I/R has focused on the mechanisms underlying the complex microcirculatory derangement occurring in this type of injury.[5–7] We have previously demonstrated lactate release by the liver during hepatic resections performed under SHVE, a fact implying that the functional impairment accompanying I/R injury might involve hepatic metabolism breakdown with diversion to anaerobic pathways.[8] Pharmacological and surgical interventions have been developed aiming at attenuating the problems inherent in I/R injury.[9–11] Ischemic preconditioning (IP)—a short period of ischemia followed by a period of reperfusion prior to the prolonged ischemia—seems to confer protection against the longer ischemic period and subsequent reperfusion insult.[12] Extensive experimental studies and a few clinical series have shown that IP is beneficial not only for the liver but for a variety of other tissues subjected to I/R.[12–16]

Since the complex biomolecular cascade of I/R insult and the exact hepatoprotective mechanism of IP have not been fully elucidated yet, we designed a matched case–control protocol aiming to investigate regional lactate kinetics in the hepatosplanchnic area when IP is applied. Based on our previous work in which we showed that the liver produces lactate during hepatectomies performed under SHVE,[8] we formulated the hypothesis that IP applied in the same setting of hepatectomies under SHVE might lead to a reduction of lactate production by the liver as an indication of aerobic metabolism preservation in the cellular level. Secondly, we sought to investigate whether IP used intraoperatively attenuates systemic hyperlactatemia post-hepatectomy.

Materials and Methods

Study Design and Setting

The study protocol was approved by the Hospital's Ethics Committee and written informed consent was obtained from all patients. In our institution, since September 2007, our surgical team employs the preconditioning technique in all liver surgery procedures performed under vascular control. Twenty-one consecutive patients, in whom intraoperative biopsies and regional lactate kinetics calculations were available, were pooled from this patient population. Patients with severe comorbidity, preoperative underlying diffuse liver pathology (e.g., cirrhosis), history of diabetes, previous liver resection, or any liver-related intervention (e.g., chemoembolization or radiofrequency ablation) were not considered. The selected patients were matched with control subjects extracted from a cohort of historical controls subjected to the same technique of hepatectomy without IP and operated between the years 2004 and 2006. Matching criteria were age, gender, and the performance of the procedure by the same surgical team. The same exclusion criteria applied for the selection of a control patient whereas the presence of intraoperative biopsies and lactate measurements were a prerequisite for the match pairing.

Operative Data

Preoperative workup of the liver lesions included computerized tomography, ultrasonography, and magnetic resonance imaging. Intraoperative ultrasonography was also carried out in all patients to ensure resectability and delineate the extent of the resection. Anesthetic management was accomplished by general anesthesia using a standardized drug regimen. Intraoperative transfusion requirements were set so as to keep hemoglobin ≥9 g/dL.

Access to the liver was obtained by a bilateral subcostal incision. Abdominal exploration was then carried out and the liver was fully mobilized from its ligaments and completely disconnected from the retrohepatic inferior vena cava (IVC). The extrahepatic trunks of the major hepatic veins were then dissected free and encircled with vessel slings. Afterwards, liver inflow and outflow were occluded by simultaneous clamping of the portal triad including any aberrant vessels with a Satinsky clamp and the hepatic veins with curved Bulldog clamps. By this technique, the liver was completely deprived from any blood perfusion while the flow in the IVC remained undisturbed during parenchymal transection. In the IP group, 10 min of inflow occlusion were followed by 15 min of reperfusion before the longer period of inflow and outflow occlusion were applied while in the control group the same technique of vascular isolation was employed without IP. After completion of the liver resection, the liver remnant was reperfused by releasing the vascular clamps on the hepatic veins followed by those on the hepatoduodenal ligament. Hemorrhage and bile leak from the traumatic liver surface were treated meticulously by suturing of all visible vessels and

ducts on the cut surface with 3-0 and 4-0 polypropylene. In all patients, a drain was left in place close to the liver surface.

Lactate Measurements

Blood samples were obtained from the radial artery, portal vein, and from the hepatic vein of the non-tumor-bearing liver lobe, simultaneously during the dissection phase of the operative procedure immediately before applying any vessel clamps (T_0). The needle used to sample portal and hepatic vein blood was 25G in size. The second set of samples was obtained from the same vessels 60 min post-reperfusion (T_1).

Blood gases were analyzed using a blood gas analyzer (Radiometer ABL-700 Series; Copenhagen, Denmark). Blood lactate levels were measured using an analyzer that employs a technique based on membrane-bound enzyme electrode methodology (YSI 2300 Stat Plus; Yellow Springs Instrument Co, Yellow Springs, OH, USA). The precision of this enzymatic method of lactate measurement has been previously validated.[17]

To measure lactate consumption or production by the liver both before and after liver resection, the transhepatic gradient was calculated as hepatic venous lactate minus portal venous lactate in millimoles per liter. Care was taken to ensure that samples were not diluted with excess heparin or contaminated with saline and were all analyzed within 5 min of drawing.

Data Collection and Postoperative Measurements

Wedge-shaped liver biopsies were obtained prior to inflow and outflow occlusion of the liver and 60 min after reperfusion, just before abdomen closure. The specimens were fixed in 10% formaldehyde solution and embedded in paraffin, after which sections 4 μm thick were cut and stained with the TUNEL assay. This assay is based on labeling the free 3′-OH ends of DNA strand breaks that are produced after DNA fragmentation during apoptosis, with fluorescent nucleotides in an enzymatic labeling with terminal deoxynucleotidyl transferase. We used the commercial kit Apoptosis-1, 5 (YLEM, Rome, Italy) for the TUNEL assay. Ten random fields were analyzed for each TUNEL-stained tissue sample. All slides were examined by the same pathologist who was unaware to the patient group. Data were expressed as mean ± SD percentage of nuclei containing apoptotic bodies per high-power field.

Postoperatively, arterial lactate levels and serum aspartate aminotransferase (AST) were assessed daily and complications requiring specific treatment or prolonging hospital stay were recorded.

Statistics

The primary endpoint of the study was the impact of IP on hepatic lactate production post-reperfusion. Secondary outcome measures were peak postoperative AST values, peak postoperative arterial lactate concentrations, and TUNEL scores in biopsies. Prior to the study, sample size estimation indicated that approximately 21 patients should be included in each group in order to detect a clinically relevant difference of 30% in hepatic vein lactate concentrations between the two groups with a power of 0.80 and an alpha error of 0.05. The standard deviation of hepatic vein lactate concentration post-reperfusion was estimated to be 0.97 mmol L^{-1} from our previous study.[8]

Variables were tested for normality of distributions with the Kolmogorov–Smirnov test. Comparisons of quantitative data between the two groups were performed with the unpaired *t* test or the Mann–Whitney test, depending on whether the variables followed a normal or non-normal distribution. The chi-square test was used for comparisons of qualitative data. Comparisons before and after the liver resection within the same group were carried out with the paired *t* test or the Wilcoxon sign rank test. Correlation between data was tested by using the Pearson product moment correlation coefficient test. Results are expressed as mean ± SD or as median (range) depending on normality of distributions. A value of $P < 0.05$ was considered as statistically significant. Statistical analysis was performed by the use of SPSS for Windows v.16.0 statistical software (SPSS Inc., Chicago IL, USA).

Results

Demographic and operative data of the two groups of patients as well as their postoperative course are presented in Table 1. The two groups were comparable with respect to patient characteristics, type of hepatectomy, type of hepatic lesion, total operative time, warm ischemia time, blood loss, and requirement for blood transfusion. No significant difference was found between the two groups regarding the occurrence of postoperative complications whereas the duration of ICU stay or hospital stay was also similar.

Lactate measurements are presented in Table 2. In the control group, arterial lactate levels increased significantly 60 min after reperfusion in comparison to preresection levels (3.4±0.7 vs. 2.1±0.4 mmol/L, $P<0.001$). In the IP group, however, arterial lactate levels 60 min after reperfusion did not differ from baseline values (2.4±0.4 vs. 2.2±0.3 mmol/L, $P=0.206$). In the control group, before liver inflow and outflow occlusion the hepatic vein–portal vein (transhepatic) lactate gradient was suggestive of lactate consumption within the liver (−0.3±0.2 mmol/L). After

Table 1 Characteristics of patients undergoing liver surgery with ischemic preconditioning (IP group) and without ischemic preconditioning (control group) and their postoperative course

	Control group (n=21)	IP group (n=21)
Age, years [median (range)]	63 (27–76)	65 (25–76)
Sex, male/female	14/7	14/7
Hepatic lesion, malignant/benign	17/4	19/2
Number of segments resected >2	11/10	12/9
Operative time, min (mean ± SD)	197.1±23.2	191.1±31.1
Ischemic time, min (mean ± SD)	44.0±8.7	43.3±9.1
Blood loss, mL [median (range)]	450 (260–1,800)	450 (270–2,000)
Blood transfusion, units [median (range)]	1 (0–5)	0 (0–7)
Chest infection	2	3
Pleural effusion	4	4
Bile leak	2	1
Wound infection	1	0
ICU stay, days [median (range)]	1 (0–7)	1 (0–8)
Hospital stay, days [median (range)]	8 (5–19)	9 (5–20)
Mortality	0	0

No statistically significant differences were observed

ICU intensive care unit

reperfusion, the transhepatic lactate gradient changed significantly in comparison to baseline values indicating that the liver had become a net producer of lactate (0.2±0.3 vs. −0.3±0.2 mmol/L, P<0.001). On the contrary, in the IP group there was indication of lactate consumption within the liver both before resection and 60 min after reperfusion (transhepatic lactate gradient −0.2±1.1 and −0.1±0.6 mmol/L, respectively). In the control group, the transhepatic PO_2 gradient (portal vein PO_2−hepatic vein PO_2) decreased significantly after reperfusion as compared to preresection values (19.0±11.2 vs. 25.4±7.6 mmHg, P=0.01). In the IP group, however, the transhepatic PO_2 gradient after reperfusion was no different from baseline (25.8±8.1 vs. 26.4±7.7 mmHg, P=0.623). The transhepatic lactate gradient 60 min after reperfusion in the 42 patients correlated with systemic arterial lactate levels at the same timepoint (r^2=0.54, P<0.001) (Fig. 1).

Hepatocellular injury was attenuated in the IP group in comparison to the control group as this was expressed by peak postoperative serum AST levels (336±248 vs. 510±291 IU, P=0.043). Similarly, arterial lactate levels 24 h postoperatively were lower in the IP group as compared to the control group (1.9±1.2 vs. 2.7±1.0 mmol/L, P=0.041). The magnitude of hepatic lactate release post-reperfusion was found to correlate strongly with both arterial lactate 24 h postoperatively and peak postoperative AST values (r^2=0.67, P<0.001 and r^2=0.72, P<0.001, respectively) (Figs. 2 and 3).

Regarding apoptotic activity, the IP group showed significantly fewer hepatocytes positive to TUNEL staining

Table 2 Systemic and regional lactate kinetics in the two groups of patients before hepatic resection (T_0) and 60 min after reperfusion (T_1)

	Control group (n=21)	IP group (n=21)
Arterial lactate (mmol/L)		
Before resection (T_0)	2.1±0.4	2.2±0.3
60 min after reperfusion (T_1)	3.4±0.7*	2.4±0.4**
Portal vein lactate (mmol/L)		
Before resection (T_0)	2.6±0.9	2.3±0.9
60 min after reperfusion (T_1)	2.8±0.8	2.4±0.4
Hepatic vein lactate (mmol/L)		
Before resection (T_0)	2.2±0.9	2.1±0.9
60 min after reperfusion (T_1)	3.0±0.9*	2.3±0.4**
Transhepatic lactate gradient (mmol/L)[a]		
Before resection (T_0)	−0.3±0.2	−0.2±1.1
60 min after reperfusion (T_1)	0.2±0.3*	−0.1±0.6**
Transhepatic PO_2 gradient (mmHg)[b]		
Before resection (T_0)	25.4±7.6	26.4±7.7
60 min after reperfusion (T_1)	19.0±11.2*	25.8±8.1**

Results are presented as mean ± SD

*p<0.05 in comparison to T_0, **p<0.05 vs. the other group at the same timepoint

[a] Transhepatic lactate gradient: hepatic vein lactate − portal vein lactate

[b] Transhepatic PO_2 gradient: portal vein PO_2−hepatic vein PO_2

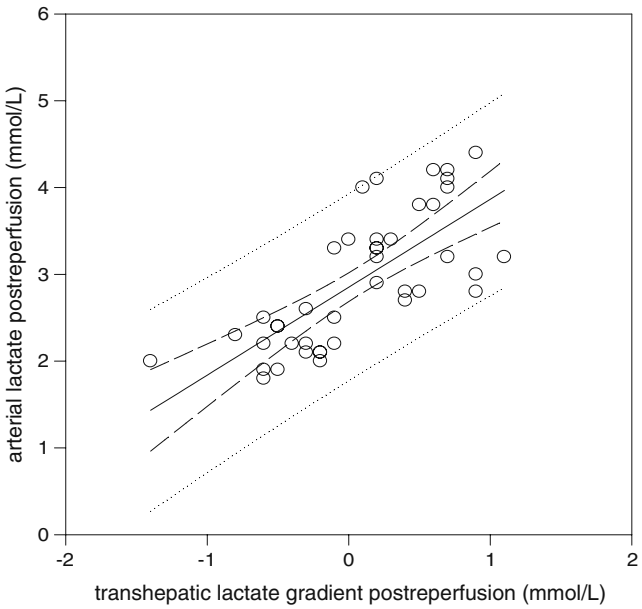

Fig. 1 Transhepatic lactate gradient 60 min post-reperfusion versus systemic arterial lactate levels at the same timepoint in the 42 matched patients. A transhepatic lactate gradient <0 denotes consumption; a gradient >0 denotes production. Systemic arterial lactate 1 h post-reperfusion is directly related to hepatic lactate release post-reperfusion (r^2=0.54, P<0.05)

in comparison to the control group 60 min after reperfusion (26.6±8.8% vs. 36.3±9.4% percentage of nuclei containing apoptotic bodies per high-power field, P=0.001) (Fig. 4). The percentage of positively staining nuclei was found to

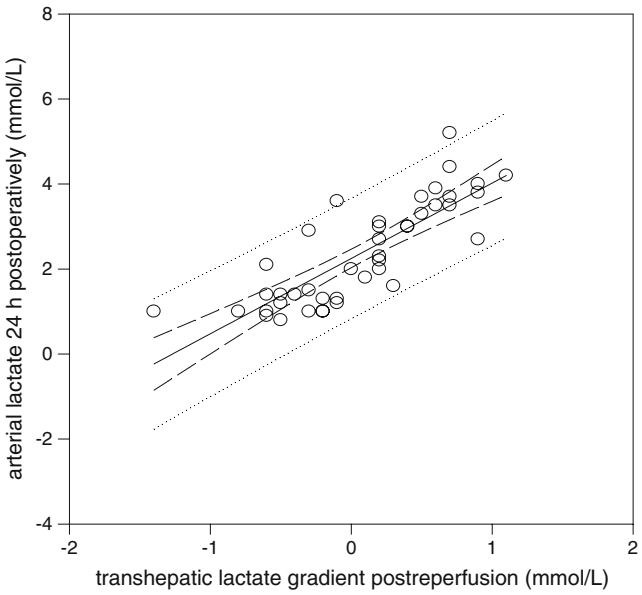

Fig. 2 Transhepatic lactate gradient 60 min post-reperfusion versus systemic arterial lactate levels 24 h postoperatively in the 42 matched patients. A transhepatic lactate gradient <0 denotes consumption; a gradient >0 denotes production. Systemic arterial lactate 24 h postoperatively are strongly correlated to hepatic lactate production post-reperfusion (r^2=0.67, P<0.05)

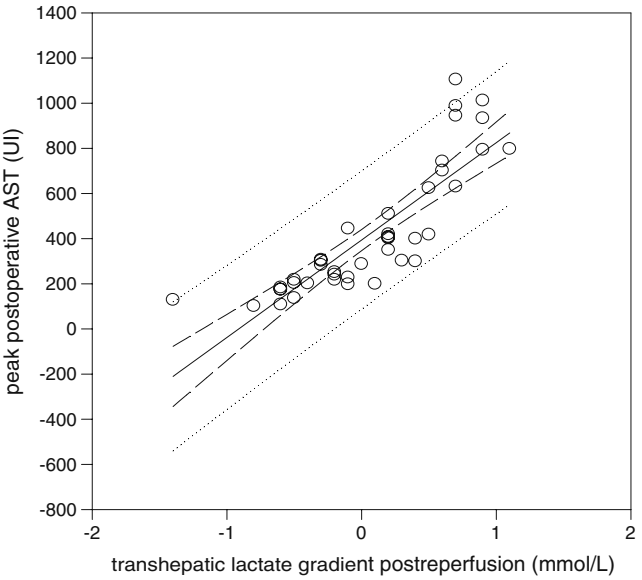

Fig. 3 Transhepatic lactate gradient 60 min post-reperfusion versus peak postoperative aspartate aminotransferase (AST) in the 42 matched patients. A transhepatic lactate gradient <0 denotes consumption; a gradient >0 denotes production. Peak AST values demonstrate a strong correlation to hepatic lactate release post-reperfusion (r^2=0.72, P<0.05)

correlate significantly with hepatic lactate production post-reperfusion (r^2=0.66, P<0.001) (Fig. 5).

There was no correlation between the transhepatic lactate gradient post-reperfusion and the length of warm ischemic time. Finally, no correlation between hepatic lactate release post-reperfusion and clinical outcomes (length of hospitalization, ICU stay, or morbidity) was demonstrated.

Discussion

The main findings of our study were the reduction of hepatic lactate release when IP was applied in hepatectomies under inflow and outflow exclusion of the liver and the attenuation of systemic hyperlactatemia post-hepatectomy in this setting. We also demonstrated a positive correlation between the transhepatic lactate gradient post-reperfusion and peak postoperative AST as well as the apoptotic response of the liver remnant.

Liver resections under some type of vascular control have been favored by many surgeons since they can ensure a less hemorrhagic surgical plane by taking advantage of liver tolerance to normothermic warm ischemia.[2,3] These vascular exclusion techniques, however, are invariably associated with some degree of I/R injury, which has led to the conception and development of various methods to prevent or alleviate the harmful process triggered by reperfused ischemic tissues.[9,10] One of these techniques is IP—a short spell of ischemia followed by a period of

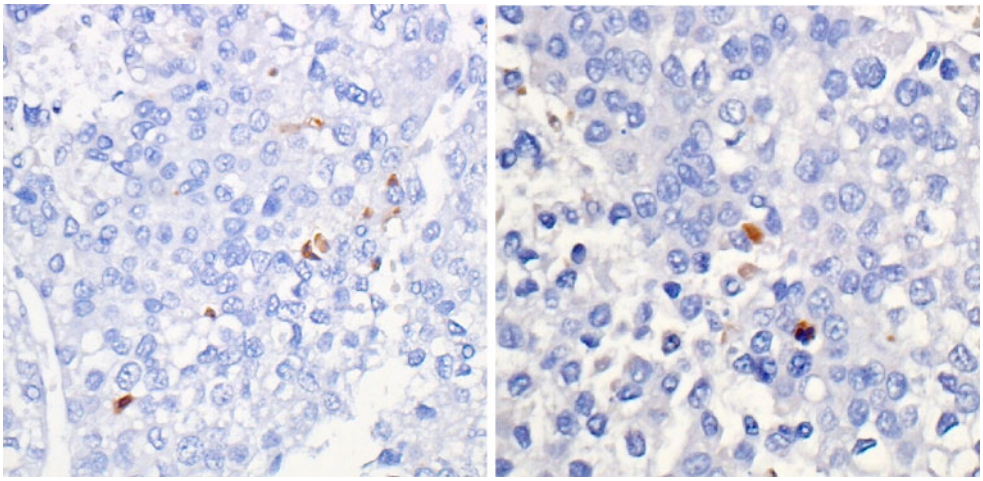

Fig. 4 Apoptosis recorded as the percentage of positively staining nuclei per high-power field was higher in the control group (*left*) as compared to the ischemic preconditioning group (*right*). (TUNEL staining ×200, apoptotic bodies appear *brown* in color)

reperfusion before prolonged ischemia. IP seems to enhance the readiness of liver cells to activate crucial mechanisms in order to withstand the subsequent major ischemic insult.[12,13,18]

The exact pathophysiologic processes initiating the complex biomolecular cascade of I/R injury or the mechanisms by which IP confers hepatoprotection have not been fully elucidated yet.[6,19,20] In a previous study from our institution, we demonstrated lactate release by the liver in hepatectomies performed under combined inflow and outflow vascular exclusion.[8] That investigation was the first to directly assess hepatic lactate production in the clinical context of liver resections. In fact, assessment of hepatic lactate production during liver surgery has been scantily reported and investigated because of the technical difficulty in ensuring access to the portal vein. Hepatic vein catheterization provides information reflecting the status of the entire hepatosplanchnic circulation and cannot differentiate between the liver and the intestine as the source of increased lactate production. The technique used by our team entails selective inflow and outflow exclusion of the liver, thus allowing for the transhepatic lactate gradient to be calculated by providing direct access to the portal and major hepatic veins. The timing of the second sampling was set at 60 min post-reperfusion to allow for the full evolution of any underlying I/R injury. This waiting period did not prolong the course of the operation unnecessarily since, in our experience, approximately 1 h post-reperfusion is the time required to meticulously control hemorrhage and bile leak before starting wound closure.

The present study, in accordance to our previous report, implicated the liver as the source of lactate release after reperfusion when no hepatoprotective measures were applied. In contrast, when preconditioning was applied the liver remained a consumer of lactate as before reperfusion.

The reason I/R insults can result in sustained impairment in lactate metabolism even 1 h after liver perfusion has been restored is not clear. It has been speculated that ischemia followed by reperfusion leads to sinusoidal vasoconstriction and complex interactions between Kupffer cells, leukocytes, and endothelial cells, which in turn might result in subsequent activation of inflammatory cytokines and vasoactive substances.[20–23] Cytokine-mediated acceleration of glucose uptake and utilization predominantly by Kupffer cells could be followed by increased synthesis of pyruvate and lactate as a high turnover mass effect.[24] Actually, injury to the endothelial cells has been shown to result in inhibition of pyruvate dehydrogenase in vitro, a mechanism leading to pyruvate accumulation in the cell because its production rate exceeds cellular capacity to metabolize it via the citric acid cycle.[25,26] Thus, the majority of the pyruvate produced from glycolysis might be diverted away from oxidative metabolism within the Krebs cycle and

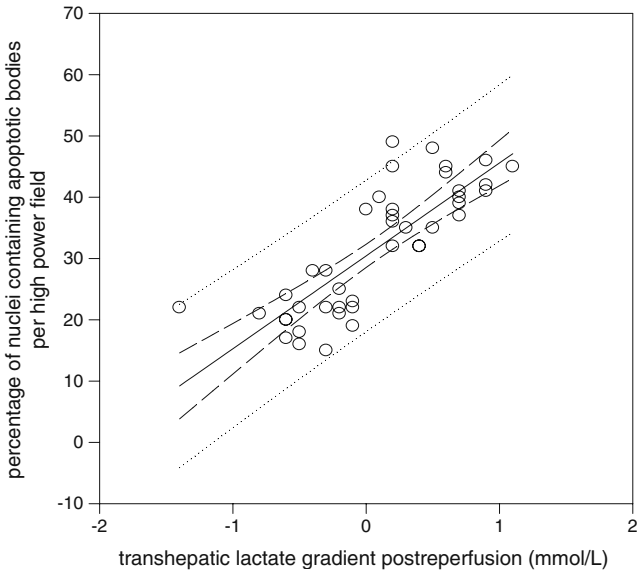

Fig. 5 Transhepatic lactate gradient 60 min post-reperfusion versus percentage of nuclei containing apoptotic bodies per high-power field in the 42 matched patients. A transhepatic lactate gradient <0 denotes consumption; a gradient >0 denotes production. Apoptosis increases significantly as hepatic lactate production increases (r^2=0.66, P<0.05)

consequently released into the circulation as lactate.[27] Alternatively, the presence of microcirculatory shunting might be implicated in local tissue derangement accompanying I/R injury. Decreased oxygen extraction suggesting shunting at the level of the sinusoidal vasculature has been demonstrated in fulminant hepatic failure by Bihari et al.[28] In our study, limitation of maximal oxygen extraction manifested as a decreased transhepatic PO_2 gradient post-reperfusion was demonstrated in the control group. The notion that a decreased oxygen supply always underlies hyperlactatemia has been questioned recently. In fact, in animal models of sepsis, ample intracellular oxygen has been shown despite the generalized septic state.[29] In human studies, serum lactate levels have been found to correlate poorly with systemic oxygen delivery, further lending support to the fact that the inability of the hepatic parenchyma to utilize oxygen as an energy substrate rather that an underlying oxygen deficit is one of the pathophysiologic mechanisms involved in lactic acidosis.[30]

In contrast, as shown by our results, it seems that the microcirculatory impairment and cellular aerobic metabolism breakdown elicited by I/R can be prevented by IP. The beneficial effect on remnant liver function evident by the lower peak AST levels as well as the antiapoptotic effect of IP shown by our investigation is in accordance with previous studies.[13,16,31] The mechanism by which IP attenuates liver apoptotic response seems to involve down-regulation of apoptotic mediators like caspase-3, activation of the antiapoptotic Akt-kinase pathway, or modulation of the expression of apoptotic genes.[13,32–34] To the best of our knowledge, our study is the first to examine the effect of IP on regional lactate kinetics and specifically on hepatic lactate production when combined inflow and outflow vascular exclusion of the liver is applied. In the IP group, the transhepatic lactate gradient post-reperfusion remained negative, indicating continued hepatic consumption of lactate rather than production. Additionally, the transhepatic PO_2 gradient post-reperfusion was not different from baseline suggesting satisfactory oxygen utilization by the liver parenchyma. The mechanism underlying the reduction of lactate release by the liver when preconditioning is applied remains open to speculation. It is postulated that IP suppresses cytokine release, enhances the production of hepatoprotective adenosine, and increases ATP availability by slowing the rate of ATP depletion.[18,19,35,36] This complex array of events possibly interferes with all the aforementioned pathogenetic mechanisms involved in lactate production by the liver. In fact, the favorable effects of adenosine on lactate kinetics have already been documented in a variety of other tissues. In experimental work by Sayeed et al., lung slices from guinea pigs given endotoxin showed a decrease in lung adenosine triphosphate along with lactate accumulation.[37] In isolated guinea pig hearts, adenosine attenuated anaerobic glycolysis and lactate accumulation in hypoperfused and ischemic myocardium.[38] Similarly, in dogs subjected to 60 min of regional myocardial ischemia, preconditioning decreased the rate of lactate release after the subsequent sustained ischemic insult.[39] Studies on the effect of adenosine on liver lactate production are lacking. Adenosine and cyclic adenosine 5-monophosphate have been demonstrated to convey liver protection by inhibiting leukocyte entrapment, down-regulating Kupffer cell activation, and improving hepatic tissue blood flow.[40] Therefore, the favorable role of adenosine on liver lactate metabolism is possibly mediated through Kupffer cell suppression along with the aforementioned inhibition of cytokine release, mechanisms which, in turn, might decrease cytokine-mediated glucose uptake and lactate synthesis by non-parenchymal liver cells. Secondly, the enhancement of regional hemodynamic status attributed to adenosine might prevent local dysoxic responses, which are often expressed as increased lactate release. It should be noted that in our study we opted for hemodynamic stability intraoperatively and we kept hemoglobin levels ≥9 g/dL. This way, we could be almost certain that the observed regional alterations in lactate kinetics and on the transhepatic PO_2 gradient as well as the favorable effect of IP on both is solely attributable to the surgical manipulations we employed (vascular exclusion with or without IP) and not to systemic hemodynamic compromise or to inadequate systemic oxygen delivery brought about by low hemoglobin.

According to our results, the transhepatic lactate gradient was found to correlate with systemic hyperlactatemia 1 h after reperfusion. It does not seem reasonable to assume that any organs other than the liver might be the source of the raised systemic lactate levels. In specific, intestinal lactate production does not seem to contribute to increased lactate levels since portal vein lactate after reperfusion was not found to be significantly different from baseline. Although there is a temporary ischemic insult imposed upon the gastrointestinal track during hepatic dissection due to portal vein occlusion, it seems to be short-lived and rapidly reversed after reperfusion. So the etiology of systemic hyperlactatemia post-reperfusion seems to mainly involve lactate production and release into the circulation by the liver itself. Moreover, the positive correlation between the transhepatic lactate gradient and arterial lactate levels measured 24 h postoperatively indicates a significant contribution of hepatic lactate release to the extent of postoperative lactic acidosis. The transhepatic lactate gradient was also found to correlate with the extent of postoperative hepatocellular injury, as expressed by peak AST values and the magnitude of apoptotic response. This indicates the possible prognostic significance of lactate release by the liver in hepatic resections under blood flow deprivation. The lack of correlation with clinical outcomes

in our study was probably due to the short warm ischemic times and the exclusion of patients with abnormal liver parenchyma. Furthermore, our study was not powered to the detection of a specific clinical outcome measure and certainly firmer conclusions can be reached with larger randomized studies sufficiently powered to detect differences in outcome. Perhaps the clinical utility of IP would have been more evident if ischemic time had been longer or if there had been more heterogeneity in the patient population by including patients with abnormalities in the liver parenchyma (i.e., steatotic or cirrhotic) or patients subjected to hepatectomies leaving behind very marginal liver remnants.

Conclusion

Ischemia-inflicted energy deficiency, further exacerbated by the noxious series of events accompanying restoration of blood flow, seems to play a crucial role in lactate generation in liver I/R injury. On the other hand, preconditioning, via up-regulation of the process of cellular ATP production, reduces the energy imbalance that occurs during subsequent liver ischemia and thereby diminishes the rate of hepatic production of lactate. A negative transhepatic lactate gradient post-reperfusion, indicating a reduction of lactate release by the liver, could act as a prognostic tool of the efficacy of hepatoprotective measures such as IP, well before other biochemical markers become evident.

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ORIGINAL ARTICLE

Prognostic Factors and 10-Year Survival in Patients with Hepatocellular Carcinoma After Curative Hepatectomy

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Abstract
Purpose There were contrary results about the effects of hepatitis B e antigen (HBeAg) positivity on the long-term survival in patients with hepatocellular carcinoma (HCC) after curative resection.
Patients and Methods Medical records of 170 HCC patients who underwent curative liver resections were retrospectively reviewed. The 10-year survival rate and correlations among clinical, laboratory, and pathological data, especially HBeAg, were analyzed.
Results Fifty-two patients survived more than 10 years. The 10-year actual overall survival (OS) rate was 30.6%, and the actual disease-free survival (DFS) rate was 24.1%. The median OS and DFS were 76 and 35 months, respectively. In multivariate analysis, HBeAg positivity (P=0.032; hazard ratio [HR], 3.041), presence of a satellite nodule (P=0.007; HR, 4.166), and elevated ICG R15 (P=0.003; HR, 4.915) had a significant negative correlation with the 10-year DFS rate. In addition, HBeAg positivity (P=0.044; HR, 3.725) and recurrence (recur within 1 year, P<0.001; HR, 41.296; recur after 1 year, P=0.03; HR, 4.848) were found as independent factors which were negatively correlated to the 10-year OS.
Conclusions The presence of HBeAg was significantly correlated to DFS and OS after curative resection for HCC. Active treatment of B viral hepatitis before and after surgery should be provided to prolong survival in patients with 5–10-cm HCC.

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Keywords HBeAg · Tumor size · Hepatocellular carcinoma · 10-Year survival

Introduction

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer worldwide.[1] In Korea, the incidence of HCC is highest in patients with liver cirrhosis and chronic hepatitis caused by the vertical transmission of hepatitis B virus (HBV).[2]It is the second most common cause of mortality in Korean adults.[3]

The early detection rate of HCC has recently increased with the development of tumor markers, such as alpha-fetoprotein (AFP) and protein induced by vitamin K antagonist-II, and screening programs using imaging such as abdominal ultrasonography. In addition, for HCCs smaller than 3 cm, the treatment outcomes of nonsurgical methods—for example, percutaneous ethanol injection (PEI) therapy, percutaneous radiofrequency ablation

(RFA), and microwave coagulation therapy—are comparable to those of hepatectomy.[4–7]However, in a nationwide survey conducted in Japan with 7,000 patients, hepatectomy was associated with a 2-year recurrence rate of 35.5%, which was lower than 55.4% in RFA group and 73.3% in PEI group.[8]In the United States population survey conducted with 5,000 patients, hepatectomy was associated with a 5-year actuarial survival of 35%, which was higher than 20% in ablation group.[9]Although several surgical and nonsurgical treatment methods for HCC have been proposed and used, liver transplantation remains as the ideal method because it treats both HCC and an underlying liver disease.[10,11]However, liver transplantation for HCC remains uncommon because of the shortage of donors, strict criteria, and high recipient dropout rates.[12,13]

With the decrease in mortality after hepatectomy (from 20% in 1970s[14] to <5% in 1990s[15–17]), the indications for this surgery have expanded.[18,19] Nonetheless, because of poor liver function and delayed diagnosis, the percentage of HCC patients eligible for hepatectomy is not high, ranging from 10% to 50%.[10,20] In addition, due to a high recurrence rate and deterioration of liver function, long-term survival after hepatectomy remains low.

The 10-year overall survival (OS) rate after curative hepatectomy has ranged from 10.5% to 20% and the 5-year OS rate from 29% to 58%.[21–26] However, the 10-year actual survival rates are rarely reported and vary from 0.9% to 21.8%.[23,27,28] The actual survival rate differs from the OS rate, probably because only the former is more affected (i.e., reduced) by various clinical factors.[29] These negative factors reportedly include, among others, the presence of satellite nodules, absence of tumor capsule, advanced tumor stage according to the tumor–node–metastasis classification, vascular invasion, and presence of microscopic tumor in the resection margin. In this study, we determined prognostic factors and assessed the 10-year actual survival rate in HCC patients who had undergone curative hepatectomy in our hospital and been followed for more than 10 years.

Patients and Methods

Patients

We examined the records of 172 patients who underwent curative hepatectomy for HCC between January 1995 and December 1999 at Severance Hospital, Yonsei University Health System. Two patients were excluded from the analysis because they were lost to follow-up, leaving 170 patients in the study. This study was approved by the internal review board of Yonsei University Severance Hospital. Informed consent was not required because of the retrospective nature of the study.

Laboratory Tests

Serum liver function tests and tumor markers alpha-fetoprotein (AFP) and indocyanine green retention rate at 15 min (ICG R15) were preoperatively assessed. In the analyses, AFP was divided into three categories (<10, 10–400, and >400 IU/mL), as was ICG R15 (<10%, 10–20%, and >20%).

Imaging Studies and Staging

Diagnostic imaging tests included ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and hepatic artery angiogram. Tumor was assessed according to the Barcelona Clinic Liver Cancer (BCLC) system and the 7th American Joint Committee on Cancer (AJCC) system.

Operation and Histologic Assessment

Hepatectomy was categorized as anatomical resection and nonanatomical resection. A curative hepatectomy was defined as a case without macroscopic evidence of residual tumor.[30] Vascular invasion was classified based on the pathological examination as not present, microscopic, or macroscopic. The width of the resection margin from the tumor was classified as positive (i.e., containing tumor cells), ≤1 cm, and >1 cm. The number of tumors was classified as either single or multiple. The size of tumor was classified by its maximal diameter as <5, 5–10, and >10 cm.

Follow-up and Treatment Modalities for Recurrent HCC

All patients were seen in outpatient clinics every 3 months after surgery, and AFP and CT were regularly assessed for tumor recurrence. Recurrence was defined by the presence of hypervascular and early washout tumors on dynamic CT, MRI, and angiography or by a diagnosis of HCC by a radiologist. Treatment modalities for recurrent HCC were transcatheteric arterial chemoembolization (TACE)/transcatheteric arterial chemoinfusion (TACI), surgical resection, and others (including radiofrequency ablation, percutaneous holmium injection, percutaneous ethanol injection, chemotherapy, and radiotherapy).

Outcomes

The associations of clinical manifestations, laboratory test results, and histopathological characteristics with the 10-year OS rate and the disease-free survival (DFS) were analyzed.

Statistical Analysis

Statistical analysis was performed with SPSS version 15.0 software (SPSS Inc., Chicago, Illinois, USA). All continuous

results are presented as the median (range). All categorical results are presented as the number (percentage). The OS rate and DFS rate were calculated by the Kaplan–Meier method. Cox's proportional hazard model was used for univariate and multivariate analyses of prognostic factors. Statistical significance was defined by a P value <0.05.

Results

Baseline Characteristics

Demographic and clinical characteristics of 170 patients are presented in Table 1. Male gender predominated (n=138, 81.2%), and the median age was 51 years (range, 17–73). HBV was the most common underlying cause of HCC (n=129, 75.8%). Ninety (52.9%) patients had a history of HCC treatment before surgical resection. TACE and TACI were the most common forms of preoperative treatment (n=57, 63.3%) and were administered median one time (1–8 times). Three patients had previously undergone hepatectomy, so their surgeries in this study were repeated procedures.

Pathological Characteristics and Tumor Stage

Anatomical resection predominated (n=157, 92.4%), 68 of them (40%) underwent major resection exceeding a hemihepatectomy, and 3 of them underwent extended hemihepatectomy. The pathological results indicated that 11 patients (7.4%) had microscopic resection margin invasion, and 7 (4.3%) had macroscopic vascular invasion (Table 2).

Table 1 Baseline characteristics (N=170)

Variable	Median (range)	No. of patients (%)
Sex		
M/F		138:32
Age (years)	51 (17–73)	
Hepatitis		
None		31 (18.2)
HBV		129 (75.8)
HCV		4 (2.4)
HBV+HCV		1 (0.6)
Alcohol		2 (1.2)
Unknown		3 (1.8)
Preoperative AFP (IU/ml)	44.8 (0.5–50,000)	
<10		60 (37.0)
10–400		51 (31.5)
>400		51 (31.5)
Child–Pugh class		
A		150 (93.2)
B		11 (6.8)
ICG R15 (%)	10 (1–68.3)	
<10		78 (51.7)
10–20		58 (38.4)
>20		15 (9.9)
Preoperative treatment		
No		80 (47.1)
Yes		90 (52.9)
Method of preoperative treatment		
TACE/TACI		57 (63.3)
Holmium injection		7 (18.9)
Systemic chemotherapy		2 (2.2)
Resection		3 (3.3)
Intraarterial injection		4 (4.4)
Combined treatment		17 (18.9)

HBV hepatitis B virus, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *ICG R15* indocyanine green retention rate at 15 min, *TACE* transarterial chemoembolization, *TACI* transarterial chemoinfusion

Overall Survival and Disease-Free Survival

The 10-year actual OS rate and actual DFS rate were 30.6% and 24.1%, respectively. The 1-year OS rate was 85.3%, 3-year 64.9%, 5-year 54.3%, and 10-year 43.5% (Fig. 1a). The DFS rate was 70.4% for 1 year, 48.4% for 3 years, 42.2% for 5 years, and 35.5% for 10 years (Fig. 1b). The median OS and DFS were 76 and 35 months, respectively.

The Univariate and Multivariate Analysis to Identify Prognostic Factors Affecting OS

The univariate analysis showed that the OS rate was significantly and negatively correlated with the presence of satellite nodules, resection margin invasion, vascular invasion, and recurrence, regardless of recurrence time. The 10-year OS rate of hepatitis B e antigen (HBeAg)-negative patients was 28.3%, which was higher than the 23.8% of HBeAg-positive patients, but the difference was not significant (P=0.238; hazard ratio [HR], 1.466; 95% confidence interval [CI], 0.777–2.765; Table 3).

The variables that were significantly and negatively correlated with the OS rate were HBeAg positivity and recurrence in multivariate analysis. Recurrence within 1 year after hepatectomy (P<0.001; HR, 41.296) is more negatively correlated with OS than recurrence after 1 year (P=0.03; HR, 4.848; Table 3).

Table 2 Pathologic characteristics and tumor stage

Variable	Median (range)	No. of patients (%)
Size (cm)	4.5 (0.9–6)	
<5		88 (52.7)
5–10		60 (35.9)
>10		19 (11.4)
Satellite nodule		
Absence		137 (80.6)
Presence		33 (19.4)
Number		
Single		122 (71.8)
Multiple[a]		48 (28.2)
Surgical margin (cm)	1 (0–10)	
0[b]		11 (7.4)
≤1		48 (32.2)
>1		90 (60.4)
Vascular invasion		
No		89 (55.3)
Microscopic		65 (40.4)
Macroscopic		7 (4.3)
BCLC stage		
0		10 (6.0)
A		139 (83.2)
B		12 (7.2)
C		6 (3.6)
7th AJCC T stage		
T1		76 (47.2)
T2		73 (45.3)
T3a		5 (3.1)
T3b		7 (4.4)

BCLC Barcelona Clinic Liver Cancer, *AJCC* American Joint Committee on Cancer

[a] The number of tumors including microscopic satellite nodules

[b] Abutting the tumor at resection margin

The Univariate and Multivariate Analyses to Identify Prognostic Factors Affecting DFS

The univariate analysis showed that the overall DFS rate significantly and negatively correlated with male, larger tumor size, the presence of satellite nodules, resection margin invasion, vascular invasion, higher AFP, and higher ICG R15 (Table 4).

The multivariate analysis showed that overall DFS rates were significantly and negatively correlated with HBeAg positivity, the presence of satellite nodules, vascular invasion, and higher ICG R15 (Table 4).

The Analysis for Interaction Between Tumor Size and Prognostic Factors

According to tumor size, the role of HBeAg was contrarily reported. The interactions of tumor size and significant factors were assessed in multivariate analysis of OS. The results showed that in patients with a tumor <5 cm, recurrence within 1 year is the only significant factor. In patients with a tumor between 5 cm and 10 cm, HBeAg is the most significant factor for OS (Table 5).

Treatment Modalities and Outcomes of Recurrent Disease

Of 170 patients, 52 (30.6%) did not have any evidence of local recurrence or distant metastases. Of the other 118 patients who did, 50 (29.4%) had recurrence within 1 year after surgery, and 51 (30%) had recurrences after 1 year. In the remaining 17 patients, the exact recurrence time could not be assessed. TACE/TACI was the most common treatment for recurrence and was administered to 52 patients (44.1%). A repeat resection was performed in only six patients (5.1%) and three of them underwent resection involving other organs. For 16 patients (13.6%), unspecified palliative treatment was provided (data not shown). Among the patients who had recurrences, median survival after the initial curative hepatectomy was significantly longer in those who underwent TACE/TACI (42 months;

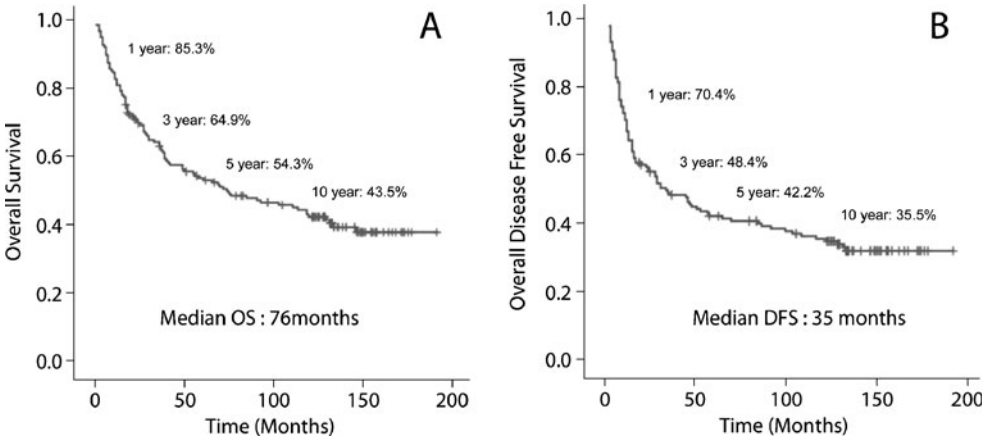

Fig. 1 The overall survival rate (**a**) and disease-free survival rate (**b**) for hepatocellular carcinoma after curative resection

P=0.008) or repeat resection (70 months; P=0.012) than among those who underwent only palliative treatment (9 months; Fig. 2a). The median survival after the treatment for the recurrence was again significantly longer with TACE/TACI (26 months; P<0.001), repeat resection (25 months; P=0.006), and other treatment methods (12 months; P=0.004) than it was following initiation of palliative treatment (6 months; Fig. 2b).

Table 3 Factors affecting overall survival

	Univariate			Multivariate		
Variable	HR	95% CI	*p*	HR	95% CI	*p*
Age (years)						
≥65 vs. <65	1.385	0.739–2.597	0.31			
Sex						
Male vs. female	1.474	0.849–2.558	0.168			
HBeAg						
Positive vs. negative	1.466	0.777–2.765	0.238	3.725	1.037–13.385	0.044
Tumor size (cm)						
5–10 vs. <5	0.988	0.635–1.536	0.957	1.263	0.545–2.927	0.586
>10 vs. <5	1.84	1.014–3.339	0.045	0.93	0.237–3.648	0.917
Satellite nodule						
Presence vs. absence	2.169	1.387–3.389	0.001	1.84	0.658–5.145	0.245
Resection margin						
Positive vs. >1 cm	2.847	1.471–5.51	0.002	0.379	0.102–1.407	0.147
≤1 cm vs. >1 cm	0.818	0.504–1.328	0.417	0.944	0.248–3.598	0.933
Vascular invasion						
Positive vs. negative	1.603	1.06–2.423	0.025	0.214	0.037–1.237	0.085
AFP (IU/mL)						
10–400 vs. <10	1.426	0.872–2.331	0.157			
>400 vs. <10	1.376	0.842–2.247	0.202			
ICG R15 (%)						
10–20 vs. <10	1.187	0.753–1.872	0.461	0.572	0.215–1.52	0.262
>20 vs. <10	1.918	0.955–3.85	0.067	2.501	0.365–17.144	0.351
Time to recurrence						
>1 year vs. no recurrence	3.179	1.776–5.69	<0.001	4.848	1.169–20.1	0.03
≤1 year vs. no recurrence	12.091	6.578–22.225	<0.001	41.296	7.536–226.298	<0.001
7th AJCC T stage						
T2 vs. T1	0.927	0.327–2.628	0.887	2.406	0.284–20.371	0.421
T3a vs. T1	1.429	0.513–3.977	0.495	12.298	0.867–174.384	0.064
T3b vs. T1	1.936	0.432–8.678	0.388	25.153	0.589–1074.876	0.092

HR hazard ratio, *CI* confidence interval, *HBeAg* hepatitis B 'e' antigen, *AFP* alpha-fetoprotein, *ICG R15* indocyanine green retention rate at 15 min, *AJCC* American Joint Committee on Cancer

Table 4 Factors affecting disease-free survival

	Univariate			Multivariate		
Variable	HR	95% CI	*p*	HR	95% CI	*p*
Age (years)						
≥65 vs. <65	0.798	0.415–1.536	0.5			
Sex						
Male vs. female	1.848	1.031–3.314	0.039	5.216	0.957–28.424	0.056
HBeAg						
Positive vs. negative	1.106	0.579–2.115	0.76	3.041	1.103–8.383	0.032
Tumor size (cm)						
5–10 vs. <5	0.917	0.594–1.416	0.696	0.955	0.351–2.597	0.928
>10 vs. <5	0.045	1.013–3.322	0.045	2.361	0.476–11.706	0.293
Satellite nodule						
Presence vs. absence	1.693	1.068–2.684	0.025	4.166	1.467–11.834	0.007
Resection margin						
Positive vs. >1 cm	2.898	1.465–5.735	0.002	0.542	0.201–1.459	0.225
≤1 cm vs. >1 cm	0.796	0.5–1.267	0.337	0.435	0.106–1.781	0.247
Vascular invasion						
Positive vs. negative	1.74	1.16–2.609	0.007	8.854	1.476–53.115	0.017
AFP (IU/mL)						
10–400 vs. <10	1.752	1.08–2.843	0.023	1.393	0.439–4.418	0.574
>400 vs. <10	1.517	0.921–2.499	0.101	0.867	0.335–2.245	0.769
ICG R15 (%)						
10–20 vs. <10	1.591	1.029–2.46	0.037	4.915	1.729–13.971	0.003
>0 vs. <10	2.213	1.031–4.752	0.042	3.762	0.646–21.911	0.141
7th AJCC T stage						
T2 vs. T1	0.743	0.29–1.909	0.538	3.766	0.64–22.164	0.143
T3a vs. T1	1.176	0.468–2.959	0.73	1.524	0.122–19.063	0.744
T3b vs. T1	1.326	0.316–5.561	0.7	0.259	0.01–6.958	0.421

HR hazard ratio, *CI* confidence interval, *HBeAg* hepatitis B 'e' antigen, *AFP* alpha-fetoprotein, *ICG R15* indocyanine green retention rate at 15 min, *AJCC* American Joint Committee on Cancer

Discussion

As surgical techniques improved over the last several decades, hepatic resection in patients with HCC is considered a safe procedure and the gold standard of treatment with a curative intention. Nevertheless, surgical resection is often contraindicated due to deterioration of hepatic function and excessive tumor burden, both of which may result from delayed diagnosis. Fortunately, early diagnosis of HCC has recently increased because of regular examination of high-risk patients and advancement of radiological diagnostic techniques. Despite the decrease in surgery-related mortality and the increase in possible surgical approaches, long-term outcome of surgical resection remains unsatisfactory in many cases due to early recurrence. After hepatectomy, the 5-year OS rate has been reported as 29% to 58%, and the actual survival rate has been reported as 30% to 50%. Similarly, the 10-year OS rate has been reported as 10.5% to 20%, and the 10-year actual survival rate at 0.9% to 21.8%.[23,27,28] However, there were a few reports about the 10-year OS rate. Reported prognostic factors have included the status of HBV infection, AFP levels, Child–Pugh classification, ICG R15 value, perioperative transfusion, size and number of tumors, presence or absence of satellite nodules, and presence of vascular invasion.

In our study, 52 patients survived for longer than 10 years, and 41 of these patients had DFS for longer than 10 years. The OS rate in our study was 43.5%, and the actual survival rate was 30.6%, both of which are higher than previously reported ones. In our study, HBeAg positivity, the presence of satellite nodules, vascular invasion, and higher ICG R15 value were negative prognostic factors in terms of 10-year overall DFS rate. It is well known that intrahepatic metastasis, vascular invasion, and resection margin invasion are high risk factors for recurrence.[30,31] In accordance with this, we found that the presence of satellite nodules and vascular invasion were significant factors with a negative correlation with the 10-year overall DFS rate. However, we could not find a correlation resection margin invasion with the 10-year overall DFS rate. The ICG R15 value reportedly reflects

Table 5 Factors affecting overall survival according to the tumor size

Tumor size						
	<5 cm			5–10 cm		
Variable	HR	95% CI	*p*	HR	95% CI	*p*
HBeAg						
Positive vs. negative	3.41	0.134–85.595	0.457	12,725.318	12.382–10,527,714.496	0.006
Satellite nodule						
Presence vs. absence	2.736	0.243–30.776	0.415	14.801	1.12–195.668	0.041
Resection margin						
Positive vs. >1 cm	0.201	0.016–2.453	0.209	0.002	0–0.706	0.038
≤1 cm. vs. >1 cm	0.361	0.007–19.036	0.615	0.126	0.005–3.136	0.207
Vascular invasion						
Positive vs. negative	0.263	0.008–8.279	0.448	0.012	0–0.792	0.012
ICG R15 (%)						
10–20 vs. <10	0.468	0.125–1.748	0.258	7.849	0.317–194.469	0.208
>20 vs. <10	2.718	0.114–64.61	0.536	76.175	0.945–6,142.65	0.053
Time to recurrence						
>1 year vs. no recurrence	5.215	0.217–125.565	0.309	0.268	0.01–7.02	0.429
≤1 year vs. no recurrence	45.293	1.524–1,345.991	0.028	0.143	0.003–7.666	0.339
7th AJCC T stage						
T2 vs. T1	2.206	0.184–22.32	0.564	0.029	0–3.73	0.153
T3a vs. T1	16.091	0.231–1,119.232	0.199	2.532	0.12–53.623	0.551

HR hazard ratio, *CI* confidence interval, *HBeAg* hepatitis B 'e' antigen, *ICG R15* indocyanine green retention rate at 15 min, *AJCC* American Joint Committee on Cancer

residual liver function and is a significant prognostic factor for the DFS rate.[32] Some surgeons recommended >1 cm resection margin for HCC because long-term survival is achieved more likely with a wide resection margin.[33–35] However, we found that the median survival was 35 months in cases with negative margins smaller than 1 cm and 36 months in cases with margins larger than 1 cm (P=0.337), and the distance was not associated with the DFS rate. Our findings regarding the 10-year OS rate were similar to those of studies of the 5-year OS rates; that is,

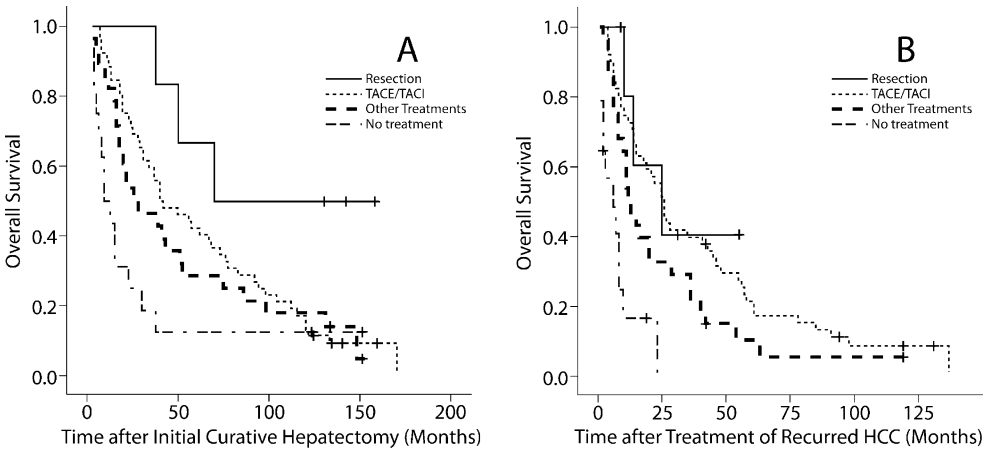

Fig. 2 The overall survival (**a**) and disease-free survival rate after recurrence (**b**) according to treatment for hepatocellular carcinoma recurrence after curative resection. The resection group (**a**) shows longest median survival (70 months) after initial curative hepatectomy. This median survival is statistically significant to compare with no-treatment group, but is not to compare with TACE/TACI or other treatment group. The resection (25 months), TACE/TACI (26 months), and other treatment (12 months) groups (**b**) show statistically longer median survival than no-treatment group (6 months), but they don't show statistical significance between each other

residual liver function and the progression status of the tumor were significant prognostic factors. In addition, factors pertinent to surgery, such as surgical methods and transfusion, were not associated with the 10-year overall DFS rate.

Age, liver cirrhosis status, surgical method, ICG R15 value, the presence or absence of satellite nodules and vascular invasion, and the number of tumors have all been reported as significant factors correlating with the 10-year OS rate.[27,28,32,36] In our study, the status of tumor progression and factors pertinent to recurrence were significant. In the multivariate analysis, the state of HBV infection and the time to recurrence were significant factors. In other studies, the status of HBV infection state and liver function were more important than tumor characteristics in correlation with 10-year survival.[32]

In our univariate analysis, the 10-year survival rate of HBeAg-negative patients was 28.3%, and that of HBeAg-positive patients was 23.8%. However, the difference was insignificant (P=0.238). The multivariate analysis showed that HBeAg positivity was significantly correlated with the 10-year survival rate (P=0.044, HR=3.725). Adachi et al.[37] reported that active hepatitis viral infection in the parenchyma is independently associated with intrahepatic recurrence after hepatectomy, particularly multicentric recurrence. Several studies reported the influence of hepatitis C virus (HCV) on long-term survival of patients with HCC. HCV–Ab positivity is a risk factor for recurrence of HCC and lower survival.[28] However, in cases with a low level of HCV RNA, even with HCV–Ab positivity, indicating low HCV activity, liver function was well maintained even after 10 years. In these patients, the recurrence rate of HCC was low. In addition, interferon treatment has been associated with longer survival in some patients with HCC associated with HCV.[38] In our study, only 5 of 170 patients with HCC had HCV infection, so the effects of the latter were difficult to assess. Our multivariate analysis showed that HBeAg positivity was a significant factor correlating with DFS (P=0.032, HR=3.041) and OS (P=0.044, OR=3.725). Patients with HBeAg positivity generally have a high level of viral activity. Thus, HBeAg positivity has been considered a greater risk for deterioration of liver function and intrahepatic recurrence. However, some published studies that have evaluated the association of HBV's activity with DFS and OS reported opposite results. Some studies of small HCCs have indicated that HBeAg positivity correlated with recurrence within 1 year, but not with long-term survival rates.[39,40] Similarly, in another study of HBV-DNA, an indicator of viral activity, it revealed that HBV-DNA was a major significant risk factor for delayed recurrence independent of tumor size, but did not significantly correlate with survival rates.[41] In our study, we analyzed the interaction of tumor size and HBeAg positivity. In HCC with 5–10 cm size, HBeAg positivity was significantly correlated with OS rates. In HCC with >10 cm size, HBe positivity could not be evaluated because of small sample size.

Until now, early recurrence has been shown to be the factor exerting the worst effect on prognosis.[30,42] In our study, we defined early recurrence as recurrence occurring within 1 year, and 50 patients (42.4%) suffered from this. The 10-year survival rate in these patients was 2%, while that of those with recurrences after 1 year was 23.5%. In addition, early recurrence showed a stronger negative risk than did HBeAg positivity (HR, 41.296 [P<0.001] vs. 3.725 [P=0.044]).

Numerous studies have been conducted on adjuvant therapies after hepatectomy, but none demonstrated that these therapies effectively increased the DFS or OS rates. In some studies, transarterial irradiation (^{131}I) embolism increased the DFS and OS rates; however, this was based on limited data, thus, more studies need to be conducted.[37] Ultimately, early detection of recurrence and aggressive treatment are important for improving long-term survival of patients with HCC after curative hepatectomy. The most effective and widely accepted therapy for recurrent HCC in terms of increasing OS rate is re-resection. Although no randomized prospective studies on re-resection in this setting have been published, to our knowledge, retrospective studies have shown that re-resection is associated with higher survival rates than other treatments of recurrence.[26,43]

In our study, resectable and localized recurrences were associated with the longest survival (Fig. 2a), regardless of the site of recurrence. The median survival (after treatment of the recurrence) was 26 months in TACE group and 25 months in resection group. Nevertheless, a limitation to our study was that we did not control the disease status. The effect of treatment methods for recurrent disease will be reevaluated for a difference in a subsequent large prospective study.

Conclusion

Factors with significant correlations to the 10-year OS rate were HBeAg positivity and recurrence. Factors with significant correlations to DFS were HBeAg positivity, ICG R15 values, and tumor characteristics. Therefore, after hepatectomy, aggressive prevention and/or treatment of HBV infection and therapy designed to maintain liver function may improve DFS and OS. In particular, an active HBV infection has a significant negative correlation with DFS and OS rates and with the liver function. Thus, in patients with 5–10-cm HCC, controlling such viral infection may be important in improving long-term survival. Therefore, trials of anti-B viral therapy in patients with 5–10-cm HCC are needed.

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ORIGINAL ARTICLE

Prognostic Value of Cirrhosis for Intrahepatic Cholangiocarcinoma After Surgical Treatment

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Abstract
Background The surgical outcome and prognostic factors of intrahepatic cholangiocarcinoma are not fully understood. This study aimed to establish the clinical significance of cirrhosis for prognosis in patients with intrahepatic cholangiocarcinoma after surgery.
Methods One hundred fifteen patients with intrahepatic cholangiocarcinoma who underwent surgical resection between December 2001 and January 2008 were retrospectively analyzed. The prognostic significance of clinicopathologic factors including cirrhosis was assessed by univariate and multivariate analyses.
Results Thirty-two of the 115 patients (28%) had liver cirrhosis. Complete tumor removal (R0 resection) was performed in 42 patients (75%). Overall median survival time was 21 months, with 1-, 3-, and 5-year actuarial survival rates of 68%, 27%, and 17%, respectively. There was a significant difference in survival between patients with cirrhosis and those without cirrhosis (P=0.027). Univariate analysis showed that cirrhosis, vascular invasion, hepatic duct invasion, lymph node metastasis, positive surgical margin (R1), and TNM stage were significantly associated with poor survival. Multivariate analysis showed that cirrhosis, positive surgical margin, and lymph node metastases were related to survival, with hazard ratios of 2.49, 3.53, and 4.16, respectively.
Conclusions Cirrhosis is an independent factor for poor prognosis in intrahepatic cholangiocarcinoma after surgery.

Keywords Intrahepatic cholangiocarcinoma · Surgery · Prognostic factors · Cirrhosis

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a rare primary liver cancer with a global increasing trend in recent years.[1,2] The incidence of ICC varies greatly among different areas of the world, and this variation is related to the distribution of risk factors.[3,4] More than 80% of these cases occurred in developing countries located in Asia and Sub-Saharan Africa, with 55% from China alone.[4] This circumstance is likely due to the high prevalence of hepatolithiasis and hepatitis B viral (HBV) infection in China,[5–7] and they are also the main known risk factors in East Asia.[3,8–11] To date, surgical resection is still the primary and most effective means to treat ICC. However, the prognosis following surgery for ICC has been poor, and prognostic factors that influence survival after surgical resection have not been well defined.[12–15] Cirrhosis commonly results from HBV infection and hepatolithiasis, and it has been to shown to be one of the factors related to poor prognosis after surgical treatment of hepatic cell carcinoma (HCC).[16–18] The present study examined the

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Table 1 ICC patient and tumor characteristics in the present study

Characteristics	*N* (total=115)
Mean age (year)	64
Sex (male/female)	71/44
Associated with hepatolithiasis	82
Associated with Hbv	24
Associated with cirrhosis	32
Elevated serum CA19-9	89
Elevated serum CEA	58
Macroscopic type	
Mass-forming type	87
Periductal infiltrating type	25
Intraductal growth type	3
Mean size (cm)	5.6
Positive lymph node metastases	46
TNM stage	
I–II	40
III–IV	75

clinicopathologic features and outcome of intrahepatic cholangiocarcinoma to determine whether the present of cirrhosis may be a significant prognostic factor after surgical resection.

Patients and Methods

Between December 2001 and January 2008, a total of 115 patients with ICC were submitted to surgery at our hospitals. ICC was defined as adenocarcinoma arising from the second order or more distal branches of intrahepatic bile ducts. Tumor stage was defined according to the pathologic tumor node metastasis classification proposed by the International Union Against Cancer.[19] Hilar cholangiocarcinoma (Bismuth type I, II, III, or IV) and combined hepatocellular and cholangiocarcinoma were excluded from this study. This study was approved by local committee on medical research ethics.

Preoperative imaging workup was performed to evaluate the extent of the disease. This workup included computed tomography (CT), magnetic resonance imaging (MRI), and abdominal ultrasonography. For selected patients, percutaneous transhepatic cholangiography, endoscopic retrograde cholangiography, or magnetic resonance cholangiopancreatography (MRCP) was performed. Liver cirrhosis and HCC were confirmed histologically in each case. Preoperative liver function status was assessed by Child's grading, liver biochemistry status, and indocyanine green (ICG) clearance test.[20] An ICG retention at 15 min (ICG R-15) of less than 14% was considered safe for major resection.[17] Criteria to exclude patients from resection were the presence of distant metastases, peritoneal carcinomatosis, extensive vascular involvement, multiple intrahepatic metastases, and Child's C cirrhosis. The extent of resection was defined according to the Brisbane classification.[21] Surgical mortality was defined as death occurring within 1 month after surgery. Surgical morbidity was defined according to the classification proposed by Dindo et al.[22] Curative resection was defined a negative resection margin at histopathological definitive examination.

After surgery, a regular follow-up with clinical examinations, blood workup, and ultrasonography evaluation was performed after 1 and 3 months, and then every 6 months. Suspected recurrences were confirmed with CT or MRI with MRCP. Chest CT or bone scanning was performed in cases of recurrence or clinical suspicion of distant metastases. The treatment of recurrent disease included surgery, systemic chemotherapy, interventional radiological therapy, and supportive therapy.

Table 2 Operative procedures for intrahepatic cholangiocarcinoma

Surgical procedure	Total (*n*=113)	Cirrhotic patients (*n*=31)	Non-cirrhotic patients (*n*=82)
Type of hepatectomy			
Left hemihepatectomy	33	9	24
Right hemihepatectomy	8	2	6
Left trisectionectomy	13	3	10
Right trisectionectomy	6	2	4
Central trisegmentectomy	8	2	6
Right trisegmentectomy	5	1	4
Bisegmentectomy	22	7	15
Segmentectomy	18	5	13
Combined resection			
Extrahepatic bile duct	29	8	21
Caudate lobe	32	8	24
Portal vein	6	2	4
Hepatic artery resection	2	0	2

Table 3 Clinicopathologic and surgical characteristics of patients

Variables	With cirrhosis	Without cirrhosis	*P*
Mean age (years)	63	64	0.829
Gender (M/F)	20/11	49/33	0.643
Child classification			0.041
A	25	82	
B	6	0	
Tumor size (cm)	4.9±1.3	5.4±1.5	0.343
Tumor-free margin (cm)	1.0±0.2	1.2±0.2	0.472
TNM stage			
I	1	2	0.518
II	13	23	
III	8	24	
IV-A	9	33	
Positive lymph node metastasis	32%	26%	0.238
R0 resectability	68%	73%	0.567

Statistical Analysis

Data were collected and analyzed with SPSS statistical software (version 13.0; SPSS, Chicago, IL, USA). Statistical analysis was performed using the chi-square test, Fisher exact test, or Spearman rank–correlation test as appropriate. Survival of the patients was compared according the Kaplan–Meier method, and differences between the survival curves were tested using the log-rank test. We considered the treatment day as time zero, and patients that survived to the end of follow-up were considered censored. Univariate and multivariate survival analysis were performed using the Cox proportional hazards regression model. For the multivariate model, we used 0.20 as the cutoff *P* value to select the analyzed factors from the univariate analysis data. Backward stepwise multivariate analysis was also used to find independent prognostic factors. A value of *P* less than 0.05 was considered statistically significant.

Results

One hundred fifteen ICC patients, including 71 men and 44 women with a median age of 64 years (range 33–81 years), received a median follow-up period of 23 months (range 5–78 months). The characteristics of the patients with intrahepatic cholangiocarcinoma are shown in Table 1. Thirty-two of the 115 patients (28%) had liver cirrhosis. Complete tumor

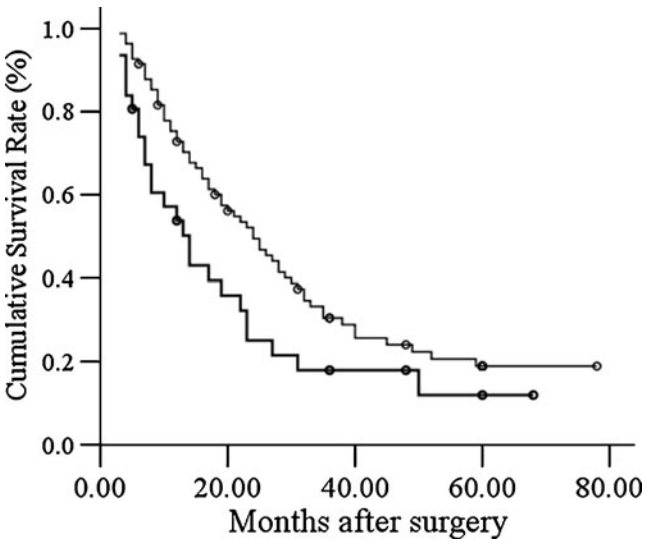

Fig. 1 Comparison of overall survival rates in patients with or without cirrhosis. *Thin line* noncirrhotic patients, (*n*=82); *thick line* cirrhotic patients (*n*=31). *Empty circle* patients who were alive at the last follow-up

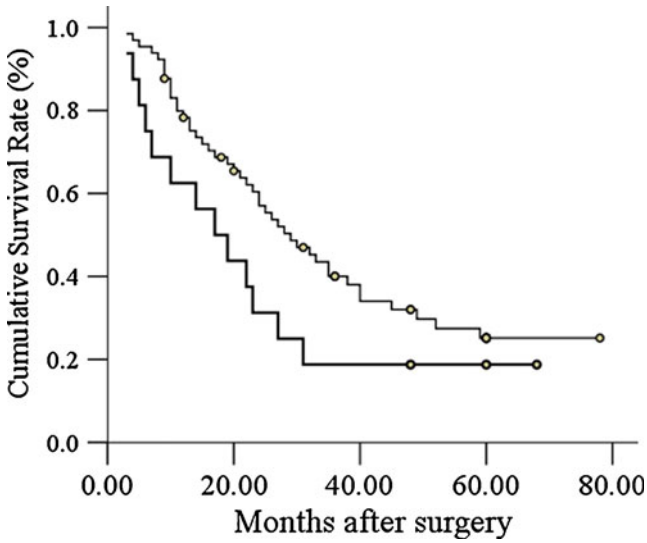

Fig. 2 Comparison of overall survival rates in R0 resection patients with or without cirrhosis. *Thin line* noncirrhotic patients (*n*=82); *thick line* cirrhotic patients (*n*=31). *Empty circle* patients who survived to the last follow-up

Table 4 Univariate analysis with respect to outcome in patients undergoing hepatic resection for intrahepatic cholangiocarcinoma

Factors	No. of patients	Survival (%)		*P* value
		3 years	5 years	
Age (year)				
<65	58	24	15	0.63
≥65	55	30	19	
Gender				
Male	69	25	13	0.179
Female	44	30	23	
Cirrhosis				
Absent	82	30	19	0.033
Present	31	17	11	
Serum CA19-9 (U/ml)				
<37	26	36	23	0.089
≥37	87	24	15	
Serum CEA (ng/ml)				
<5	56	31	21	0.374
≥5	57	23	13	
Tumor size (cm)				
<5	53	34	23	0.182
≥5	60	21	13	
Macroscopic type				
MS	85	29	19	0.21
PI/IG	28	21	12	
Vascular invasion				
Absent	88	33	22	<0.0001
Present	25	6	0	
Intrahepatic metastasis				
Negative	81	31	19	0.11
Positive	32	17	12	
Lymph node metastasis				
Negative	82	53	22	0.012
Positive	31	12	4	
Margin status				
R0	81	34	24	0.006
R1	32	9	0	
Histologic grading				
G1–G2	72	31	20	0.23
G3–G4	41	20	12	
TNM stage				
I–II	39	42	25	0.031
III–IV	74	19	13	

removal (R0 resection) was performed in 81 patients (71%). Extrahepatic bile duct resection was performed in 39 (34%) patients in whom the tumor was judged to have invaded the biliary confluence. Portal vein resection and reconstruction was necessary in three patients (3%). There was a postoperative morbidity rate of 35% (Dindo class II in 16%, IIIa in 11%, IIIb in 5%, and IVa in 3%), and there were two (2%) in-hospital deaths (class V according to the Dindo classification) after surgical resection. One of these patients died on postoperative day 9 because of pulmonary infarction, and the other patient died of hepatic failure 28 days after the operation. The most frequent complications observed were pneumonia (10%), liver dysfunction (7%), biliary fistula (7%), abdominal abscesses (4%), pleural effusions (3%), and cholangitis (2%), leakage of hepaticojejunostomy (1%), and pulmonary infarction (1%).

Two patients died of complications that were thought to be unrelated to the primary disease. We therefore analyzed survival in 113 patients after excluding the two in-hospital deaths. Patients with intrahepatic cholangiocarcinoma were treated by left hepatectomy (n=33), right hepatectomy (n=8), left trisectionectomy (n=13), right trisectionectomy (n=6), central trisegmentectomy (n=8), right trisegmentectomy (n=5), bisegmentectomy (n=22), or segmentectomy (n=13). In addition, the following procedures were performed: extrahepatic bile duct resection (n=29), portal vein resection with end-to-end anastomosis (n=6), and hepatic artery resection with end-to-end anastomosis (n=3). The operative procedures among cirrhotic and non-cirrhotic patients are summarized in Table 2. A comparison of the clinicopathologic and surgical factors in the patients with or without cirrhosis is shown in Table 3. With the exception of preoperative liver function status, the difference between the two groups was not statistically significant.

The overall median survival time was 21 months, with 1-, 3-, and 5-year actuarial survival rates of 68%, 27%, and 17%, respectively. There was significant difference in the survival rates between cirrhotic (1 year, 52%; 3 years, 17%; 5 years, 11%; median survival time, 14 months) and non-cirrhotic (1 year, 73%; 3 years, 30%; 5 years, 19%; median survival time, 25 months) patients (P=0.033; Fig. 1). A further analysis in the subgroup patients who underwent R0 resection showed there were significant difference in the survival rates between cirrhotic and non-cirrhotic patients (median survival time,17 vs. 29 months; P=0.044; Fig. 2).

Table 5 Multivariate analysis of factors related to survival

Factors	Hazard ratio	95% Confidence limits	*P* Value
Cirrhosis (present vs. absent)	2.49	1.17–5.30	0.035
Lymph node metastases (present vs. absent)	3.53	1.23–10.16	0.028
Margin status (R1 vs. R0)	4.16	1.87–9.26	<0.0001

The univariate analysis of prognostic factors of ICC is summarized in Table 4. Cirrhosis, vascular invasion, lymph node metastasis, positive surgical margin, and TNM stage were statistically significant risk factors affecting the outcome of the patients with ICC. Cox's regression multivariate model identified cirrhosis, positive surgical margin and lymph node metastases as being significantly related to survival with hazard ratios of 2.49, 3.53, and 4.16, respectively (Table 5).

Discussion

Although surgical resection offers the patient with ICC the best chance for prolonged survival, the long-term survival of these patients is still low.[14,15,23,24] Thus, there is an urgent need for understanding the prognostic factors. The present study used univariate and multivariate analysis to calculate overall survival (OS) rates of 113 ICC patients after surgical treatment. In univariate analysis, cirrhosis, vascular invasion, lymph node metastasis, positive surgical margin, and TNM stage were associated with unfavorable OS rate for ICC patients. Applying the multivariate Cox's proportional hazard model to data from this investigation revealed that aggregated overall survival with respect to cirrhosis, positive surgical margin, and lymph node metastases.

Concerning the surgical treatment for intrahepatic cholangiocarcinoma, improved survival results after curative R0 resection have been reported in recent years.[14,15,23–27] In our series, margin-negative R0 resection was significantly associated with a favorable outcome in univariate analysis. The 5-year survival rates for patients with R0 resection and R1 resection were 24% and 0%, respectively. These studies suggested that better survival results could be achieved by margin-negative R0 resection, including vascular resection for intrahepatic cholangiocarcinoma.

The prevalence of lymph node metastases varies from 27% to 73%. The long-term prognosis in patients with positive lymph nodes is poor, with a 5-year survival rate of zero to 17%.[28–33] In accordance with the literature, the presence of lymph node metastases in our series was related to shorter survival, with a 5-year survival rates of 4% for *N*+ patients and 4% for N0 patients (P=0.012).

The incidence of cirrhosis in this study (28%) was obviously higher than in Western countries.[8,34] It is associated with high prevalence of HBV infection and hepatolithiasis in southern China.[5,6,35,36] In our series, ICC patients with cirrhosis had higher lymph node metastasis and lower R0 resectability rate than those without cirrhosis, but the difference was not statistically significant. Cirrhosis was significantly related to poor survival of the patients with ICC after surgery in our analysis. We also showed that cirrhosis is an independent factor of poor prognosis in these patients. Subgroup analysis revealed cirrhotic patients had worse survival than non-cirrhotic ones even in R0 resection group. So far, no other reports have shown this association as clear as in the current study.

One possible explanation for the relationship between cirrhosis and poor prognosis in ICC patients is that cirrhosis may interfere with early diagnosis of ICC, and thus delay detection and treatment of the ICC. Another possibility is that death due to deterioration of liver function is much faster than tumor progression in some patients. Resultant fatal complications include liver failure, esophageal variceal bleeding, and encephalopathy.

Conclusion

In conclusion, we report that cirrhosis is an independent factor of poor prognosis for patients with ICC, and is thus a useful indicator to predict the outcome of patients with ICC who undergo surgical resection of the tumor. ICC patients with cirrhosis should be followed-up carefully. Further studies are needed to evaluate the efficacy of surgical treatment for intrahepatic cholangiocarcinoma with cirrhosis.

Conflict of interest There is no conflict of interest in the study.

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ORIGINAL ARTICLE

Evaluating Systemic Stress Response in Single Port vs. Multi-Port Laparoscopic Cholecystectomy

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Abstract
Background and Aims Acute-phase proteins and inflammatory cytokines mediate measurable responses to surgical trauma, which are proportional to the extent of tissue injury and correlate with post-operative outcome. By comparing systemic stress following multi-port (LC) and single-incision laparoscopic cholecystectomy (SILC), we aim to determine whether reduced incision size induces a reduced stress response.
Methods Thirty-five consecutive patients were included, 11 underwent SILC (mean ± SEM; age 44.8±3.88 year; BMI 27±1.44 kg/m^2) and 24 underwent LC (56.17±2.80 year; 31.72±1.07 kg/m^2, $p<0.05$). Primary endpoint measures included levels of interleukin-6 and C-reactive protein measured pre- and post-operatively. Length-of-stay (LOS) and postoperative morbidity were secondary endpoints.
Results No statistically significant differences were found between SILC and LC for interleukin-6 and C-reactive protein levels, LOS and duration of surgery. There was also no correlation between systemic stress response and operative parameters. There were no intra-operative complications.
Conclusion SILC appears to be a safe, feasible technique with potential advantages of cosmesis, reduced incisional pain, and well-being recommending its use. These data indicate no difference in systemic stress and morbidity between SILC and LC. A larger, multi-centred, randomised prospective trial is warranted to further investigate and confirm this finding.

Keywords Cytokines · Acute phase response · Single incision laparoscopic surgery (SILS) · Cholecystectomy

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Introduction

Over recent decades, the evolution of laparoscopic techniques has transformed much of traditional surgery. Compared to an open approach, minimally invasive techniques have proven effective in reducing surgical trauma, thereby improving patient recovery and length of hospital stay.[1,2] Benefits of improved postoperative pain and cosmesis are now well established for many operations.[3–6] The benefits displayed by conventional laparoscopic cholecystectomy (LC) have established it as the gold standard for gallbladder removal;[7] however, surgeons have since sought to further reduce the size and number of incisions[5,8,9] with single-incision laparoscopic surgery (SILS) being one of the latest innovations.

SILS is a technique which offers theoretical advantages of reduced pain and complications by limiting the number of incisions to one.

In general surgery, SILS involves the introduction of laparoscopic instruments via the same access point in the abdominal wall; typically the umbilicus, which can result in an almost invisible scar.[10] Issues to overcome with SILS include in-line instrument clashes, and loss of triangulation, with articulating instruments and extra long laparoscopes being developed as a result.[11]

SILS demonstrates feasibility and reproducibility, with surgical safety and outcomes remaining uncompromised in published series of benign disease.[10,12] Although the cosmetic benefit[12,13] and reduced incisional pain are described,[13] the literature has not reported any other significant differences between SILS and conventional laparoscopy. Systemic stress response, postoperative morbidity, and patient satisfaction are yet to be defined.

The extent of surgical trauma has been evaluated by assessing the systemic stress response.[14] Total white cell count (WCC) and acute phase reactant C-reactive protein (CRP) are known indicators of tissue injury.[4,14] In addition, the cytokine response to surgical injury has been well documented.[2,15] An acute-phase response is triggered following surgical injury which can be detected in peripheral blood.[2] The cytokine interleukin-6 (IL-6) is a major mediator of this response. Following acute injury, IL-6 produced by virtually all cells peaks in the circulation 4–6 h post injury.[15] IL-6 levels have been shown to be proportional to the extent of injury;[15] however, this exact mechanism remains unknown. IL-6 can regulate the synthesis of hepatic acute-phase proteins such as CRP. Increases in CRP plasma levels following surgery are positively correlated with the increase seen in IL-6.[16]

Several authors have examined cytokine profiles following LC, mini-open, and open cholecystectomy (OC).[17] Grande et al.[16] observed that postoperatively, patients undergoing OC had significantly greater increases in serum levels of IL-6 and CRP compared to LC. These findings are also supported by other investigators.[2,4,18,19] Authors have demonstrated a significant difference in the systemic stress profiles following different surgical approaches despite the technique of cholecystectomy remaining the same; supporting that surgical trauma produces a measurable response, the magnitude of which being proportional to the extent of tissue injury.[14,15,19]

With regard to postoperative morbidity, exaggerated elevations in IL-6 have been shown to be linked with the onset of major clinical complications.[20] It has also been reported that postoperative plasma levels of IL-6 are early indicators of postoperative wound infections.[21]

A comparison of inflammatory mediators following SILC and LC provides a model to investigate the extent to which the systemic response is influenced by surgical access. Since the technique of cholecystectomy is the same for both approaches, we hypothesise that any difference in systemic response can be attributed to the difference in the size and number of incisions. We hypothesise the reduction in total incision size seen in SILC will result in a reduced systemic stress response with a potential decrease in postoperative morbidity.

Patients and Methods

Study Design and Subjects

This single centre, non-randomised study received ethical approval by the St. Mary's Hospital Research Ethics Committee (REC ref:08/H0712/146). Data were collected at St Mary's Hospital from February through May 2010. All patients undergoing SILC or LC were considered for inclusion. Patients were excluded if they had co-morbidities resulting in raised inflammatory markers (such as autoimmune disease, malignancy or infection). Informed consent was obtained after verbal and written information were given. Performing the SILC technique was at the discretion of the surgeon.

There is no data in the literature to base a power calculation on; however, based on previous studies comparing cytokine variations in LC and open cholecystectomy,[16,18] we aimed to reach a sample size of at least 11 SILC and 11 LC subjects.

Operative Techniques

All operations were performed by one of five attending-led operative teams. A standardised anaesthetic protocol was followed for all patients.

SILS Cholecystectomy

The technique used at our institution has been previously described in the literature by our institution and the technique used for this study was the same.[22] In short, SILC involved the introduction of laparoscopic instruments and a 5 mm diameter 30° laparoscope into the umbilicus via a 12 mm bladed, but disarmed port and a 5 mm Dexide port (Covidien, Mansfield, MA, USA).[1] With two suspension sutures (0 silk) placed in the right upper quadrant and through the fundus and infundibulum, respectively, traction of the gallbladder was maintained. The principle of cholecystectomy was then carried out in the traditional

[1] Covidien, Mansfield, MA, USA

fashion adhering to principles of safety during dissection, demonstration of the critical view and wide posterior window and clipping of the cystic duct and artery, with gallbladder retrieval into an endo bag through the umbilical incision.

Multi-port Laparoscopic Cholecystectomy

This technique was performed using three 5-mm ports and one 10-mm port. Ports were positioned in the standard fashion at the mid-epigastrium, right lateral, right subcostal and umbilical positions, respectively. Unlike the sutures used in SILC, the assistant surgeon maintained traction of the gallbladder. The principle of cholecystectomy was then carried out as previously described. Conversion to either LC or OC in SILC and LC, respectively, was performed when the surgeon felt it necessary. Reasons for conversion were reported in the operative notes.

We have considered that the larger single incision (12 mm) and multiple fascial incisions used in SILC will be more traumatic than the typical umbilical incision in LC (10 mm); however, we believe it will overall result in a decreased systemic stress response. There are still no robust long-term data on incisional hernia rates following this larger incision however there is a hypothetical increased risk of port-site herniation.

Data Collection

Perioperative data was recorded for all patients as illustrated in Table 1. Primary endpoint measures included plasma levels of IL-6 and CRP. LOS and postoperative morbidity were secondary endpoints.

Patient demographics, indication for surgery and co-morbidities were recorded. Operative time was measured from the first incision to the closure of the final wound. As operative time can be affected by unforeseen delays such as faulty equipment and/or the experience of the surgeon, the grade of the surgeon and delays were recorded. Operative parameters were documented from the operative notes and personal observation. LOS was measured from the incision time to the patient's discharge time. Any readmissions were added to the patient's original LOS.

Sample Method and Times

Peripheral venous samples were taken preoperatively as baseline. Postoperative samples were subsequently collected at 6±24 h from the incision time. For cytokine analysis, 6 ml blood samples were taken into EDTA blood collection tubes. Within 30 min of collection, samples were centrifuged for 15 min at 1,000×*g*, before the supernatant was separated, aliquoted and samples were stored at −80°C for subsequent analysis.

Table 1 Assessed parameters

Patient demographics	Sex
	Age
	Height, weight and BMI
	Presenting condition
	Co-morbidities—ASA grade
	Past surgical history
Operative parameters	Incision time
	Closure time
	Length of surgery
	Number of incision(s)
	Size of incision(s)
	Total size of incision
	Grade of surgeon
	Bile spillage
	Intra-operative complications/notes
	CO_2 insufflation
	Drain
	Conversion[a]
Outcome	Length of hospital stay (LOS)
	Follow up questionnaires[b]

[a] Conversion is defined as the addition of one or more trocars to the SILS technique and conversation to open in the LC technique

[b] Carried out at 2 weeks and 2 months

Follow Up

Patients were followed up at 2 weeks and 2 months postoperatively. Data collected using a standardised questionnaire included wound healing, postoperative pain, gastrointestinal symptoms and any post-operative medical consultations.

Cytokine Assays

IL-6 levels were measured using a commercially available high-sensitivity enzyme-linked immunosorbent assay (ELISA; Quantikine HS [High Sensitivity] Human IL-6, R&D Systems Europe)[2] according to manufacturer's instructions. All readings taken from the ELISA plates were standardised by assessing positive control values that were assayed in duplicate on each plate. All samples were assayed in duplicate and the standard curve ran from 10 to 0.156 pg/ml.

[2] ELISA; Quantikine HS [High Sensitivity] Human IL-6, R&D Systems Europe

Statistical Analysis

All analyses were carried out using statistical software within GraphPad Prism (Version 5.03, San Diego, CA, USA).[3] Fisher's exact test was used to evaluate significant differences of categorical variables when the sample size was <10. Mann–Whitney *U* test was used in the analysis of non-parametric variables with a larger sample size. Spearman's rank was used for correlations of continuous variables. Statistical significance was set at $p<0.05$.

Results

Patient inclusion and participation is detailed in Fig. 1. The total number of patients available for analysis was 35. The demographics of all 35 patients are shown in Table 2. SILC subjects (2 M:9 F) were typically younger and had a lower BMI compared to LC subjects (7 M:17 F; $p<0.05$). No other significant difference was found in the demographics of patient groups.

Operative Outcomes

Mean duration of surgery was longer for SILC (86.91±8.97 vs. 79.08±4.24 min) however this was not statistically significant. Attendings were more likely to perform SILC (82% vs. 42%; $p=0.04$). No significant difference was seen in the cases of bile spillage and total CO_2 insufflation. Of the LC group, three patients were converted from SILC due to either poor visibility or unclear anatomy. Intra-operatively there were no complications (Table 3) aside from the higher proportion of drains inserted in the LC group (7 vs. 0).

Systemic Stress Response

The systemic stress response of the patient was measured as a change from baseline and compared at 6 and 24 h postoperatively. No significant variations between the baseline values of each group were noted, making them comparable.

IL-6

IL-6 levels significantly increased from baseline to 6 h post operation in the LC group (2.28±0.40–8.65±1.83 pg/ml; $P<0.0001$, Fig. 2a) as well as in the SILC group (1.57±0.28–5.1±1.20; $P=0.0006$), with a greater percentage increase in the LC group, although this was not statistically significant between the groups. At 6 h, IL-6 levels were higher in the LC group compared to the SILC group, but again, this was not statistically significant ($p=0.0673$; Fig. 2a). In the LC group there was a decrease in IL-6 concentration between 6 and 24 h (Table 3). There was no correlation between operative time and systemic stress response ($p=0.94$). There was no significant difference in plasma levels of TNF-α and WCC between the two groups at any time point (data not shown).

CRP

Plasma CRP levels (Fig. 2b) postoperatively increased in both groups at 6 h; this was not statistically significant. There was a remarkable increase in CRP from 6 to 24 h postoperatively in the LC group ($p<0.05$), demonstrating that CRP may peak at 24 h.

As previously described, there is a learning curve associated with any laparoscopic technique and the complexity of the SILS technique, makes it particularly challenging.[3] Although there was a difference in the grade of surgeon performing the SILC and LC techniques, analysis comparing the grade of surgeon vs. the systemic stress response (Fig. 3a) found no statistically significant difference; incidentally, the attendings generated a slightly higher IL-6 response in both study groups compared to residents but this was not significant. Duration of surgery (Fig. 3b) also showed no significant difference between residents and attendings. Bile spillage between the groups was not shown to be significant.

Postoperative Outcomes

Mean LOS was slightly shorter in the LC group compared to the SILC group (0.97±0.35 vs. 0.86±0.11 days; $p=0.42$). There was one readmission within the SILC group due to erythema and pain at the umbilicus. This readmission was included in the patient's LOS and may have skewed the SILC group's mean LOS.

Follow up revealed that the LC group visited a doctor on more occasions than the SILC group (14% vs. 0%). With regard to the wound(s); within the LC group, 38% stated that at least one wound had not healed compared to 29% of the SILC group. The LC group also had a higher proportion of minor wound site bleeding (9% vs. 0%) and infection (5% vs. 0%; Table 3).

Discussion

The surgical trauma induced inflammatory response is well defined within the literature.[2,15] Primarily, the physiological response to surgical trauma is equal to that of infection or injury i.e. the induction of the acute-phase response;

[3] GraphPad Prism (Version 5.03, San Diego, CA, USA)

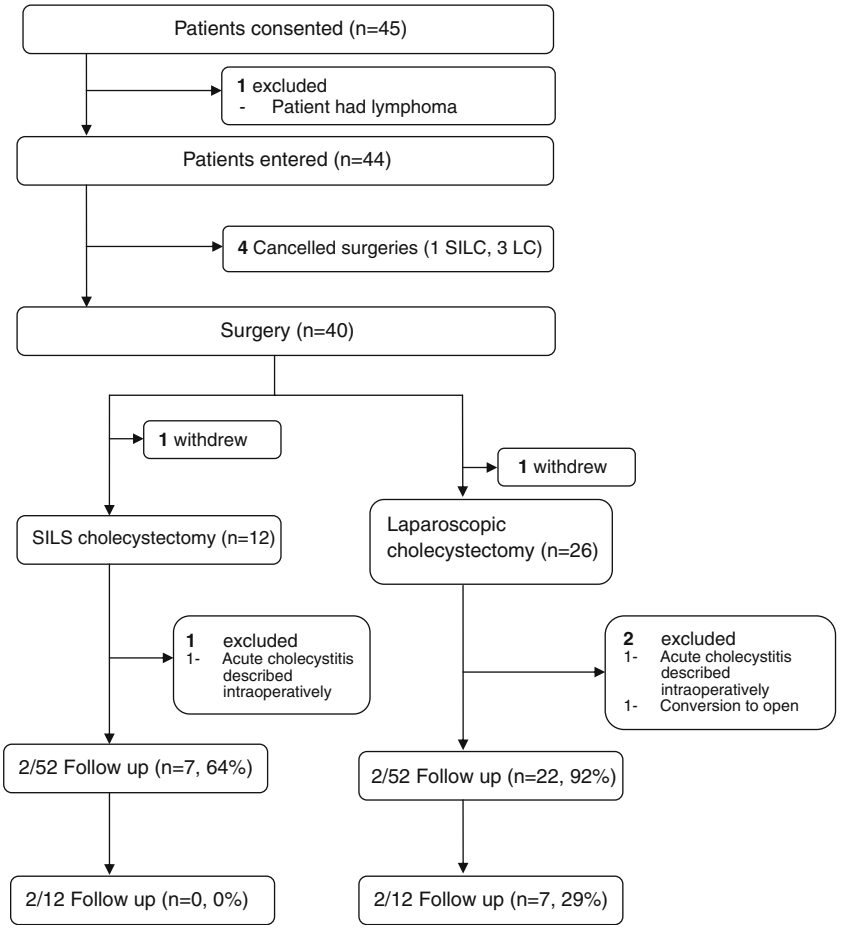

Fig. 1 Flow of subjects (follow up at 11/05/2010)

Table 2 Patient demographics

Characteristics			Patients with SILC (n=11) (%)	Patients with LC (n=24) (%)	p Value
Sex	Male		2 (18%)	7 (29%)	0.6855
	Female		9 (82%)	17 (71%)	
Mean	Age (years)		44.82 [3.88]	56.17 [2.80]	0.0218
	Weight (kg)		75.21 [6.36]	86.35 [2.33]	0.0190
	Height (m)		1.658 [0.04]	1.657 [0.02]	0.9574
	BMI (kg/m^2)		27 [1.44]	31.72 [1.07]	0.0219
Co morbidities	ASA grades	1	5 (45)	9 (38)	
		2	6 (55)	13 (54)	
		3	0 (0)	1 (4)	
		4	0 (0)	1 (4)	
Indications for surgery	Biliary colic		2 (18)	5 (21)	
	Symptomatic gallstones		6 (55)	16 (76)	
	Abdominal pain		0 (0)	1 (4)	
	Previous gallstone pancreatitis/ cholangitis/cholecystitis		2 (18)	2 (8)	
Past surgical history	Nil		3 (27)	8 (33)	
	Upper GI surgery		0	2 (8)	
	Lower GI surgery		2 (18)	4 (17)	
	Non GI		6 (55)	10 (42)	

[] Standard error of the mean (SEM)

Table 3 Operative, post operative outcomes and systemic stress markers

Characteristics				Patients with SILC (n=11)	Patients with LC (n=24)	p Value
Operative parameters	Operative time (mins)			86.91 [8.97]	79.08 [4.24]	0.3108
	Total incision size (mm)			13.64 [1.26]	33 [1.29]	<0.0001
	Grade of surgeon	Attending		9	10	0.0354
		Resident		2	14	
	Bile spillage	Yes		4	6	0.6889
		No		7	18	
	Conversion			3	0	
	Total CO_2 insufflation (litres)			240.3 [81.12]	118.5 [32.23]	0.1996
	Complications			nil	nil	
Intra-operative findings	Acute cholecystitis			0	0	
	Mucocele			0	3	
	Inflamed gallbladder			3	8	
	Stone impeded in Hartmanns pouch			2	3	
	Gallbladder Adhesions			0	6	
	Gallstone/sludge spillage			0	1	
	Abnormal anatomy			1	2	
	Umbilical hernia			1	1	
	Drain			0	7	
	Liver pathology			1	2	
	Distended gallbladder			2	4	
Inflammatory markers	IL-6 (pg/ml)	Time points	n (SILC, LC)			
		t=0	(11, 22)	1.571 [0.28]	2.278 [0.40]	0.2146
		t=6	(11, 24)	5.100 [1.20]	8.648 [1.83]	0.0673
		t=24	(0, 3)	–	7.422 [0]	–
	CRP (mg/l)	t=0	(11, 22)	3.227 [0.53]	4.682 [0.75]	0.2403
		t=6	(11, 24)	3.609 [0.73]	5.292 [0.79]	0.3251
		t=24	(0, 3)	–	46.000 [25.63]	–
Post operative	LOS (days)			0.97 [0.35]	0.86 [0.11]	0.4238
	Follow up (2/52)			n=7	n=22	
		No complications		4 (57%)	5 (23%)	
		Wound—infection		0 (0%)	1 (5%)	
		Wound—bleeding		0 (0%)	2 (9%)	
		Wound—scarring		1 (14%)	1 (5%)	
		At least one wound not healed		2 (29%)	11 (50%)	
		GI Symptoms		4 (57%)	12 (55%)	
		Non GI Symptoms		3 (43%)	8 (36%)	

[] SEM

LOS Length of stay

The 24 h sample was omitted for SILC cases due to the majority being day cases

reflected in cytokine function and cellular messenger systems.[2] The magnitude of these changes is reflected proportionally to the extent of the surgical trauma.[4]

Many studies support LC as the gold standard over the traditional open approach based on results demonstrated by cytokine response profiles.[4,16,23] To date, there has been no study comparing the systemic stress response of SILC vs. LC. The results of this trial reject the hypothesis that a single incision results in a decreased systemic stress response.

In this trial, postoperative IL-6 levels significantly increased over baseline values in both SILC and LC groups; supporting the described acute-phase response following surgery.[2,15] However, no significant differences

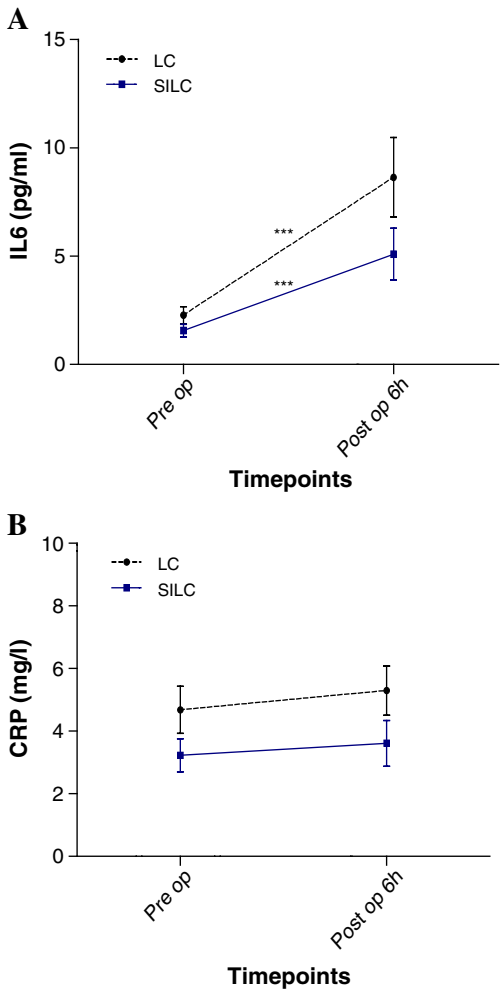

Fig. 2 Plasma concentration of IL-6 and CRP preoperatively (t=0 h) and postoperatively (t=6 h) A: IL-6, a significant difference is seen between t=0 and t=6 in both SILC group (p=0.0006) and LC group (p<0.0001). *Error bars* denote standard error of the mean (SEM). ***p<0.001; **0.001–0.01 (Mann–Whitney U test)

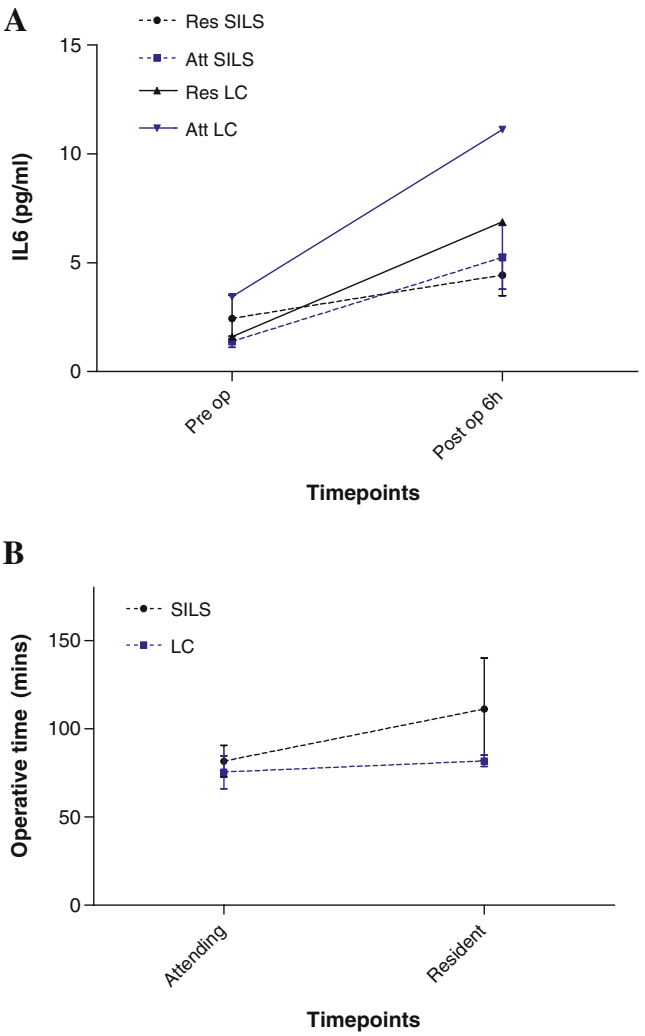

Fig. 3 **a** Grade of surgeon vs. the systemic stress response (IL-6) in SILC and LC. No significant difference found between residents or attendings (Mann–Whitney U Test).

	p value IL-6
Res SILC 6h vs. Att SILC 6h	**0.9091**
Res LC 6h vs. Att LC 6h	**0.2081**

b Grade of surgeon vs. operative time. No significant difference (p= 0.372; Kruskal-Wallis test). *Error bars* denote SEM

were found between the groups although there was a trend for the SILC group to have lower IL-6 plasma levels at 6 h post surgery.

There was no significant difference in the CRP levels postoperatively between SILC and LC. Authors[16] have stated that in the acute-phase response, CRP production is proportional to the increase in IL-6. Our findings conversely showed no significant correlation between IL-6 and CRP (r=−0.23, p=0.29)

The majority of SILC cases were day cases and so the 24 h sample was often omitted. This therefore resulted in a lack of data for SILC at 24 h. As CRP peaks at 24 h,[2] the absence of these time points in the SILC group did not give a representative comparison of CRP in SILC vs. LC. Routine analysis of CRP was performed with staff blind to the two patient groups therefore ruling out potential detection bias.

Total CO_2 insufflation was higher in the SILC group (240.3 L vs. 118.5, p = ns). Recent studies suggest that CO_2 pneumoperitoneum may influence systemic stress.[2] It is thought that the production of cytokines, namely TNF-α and IL-1, in peritoneal macrophages is suppressed due to the acidic environment of CO_2. These findings are not wholly reliable as in SILC, a portion of the total CO_2 "insufflated" is unaccounted for by gas leakage at the port site.[3]

Early studies comparing multi-port LC to OC reported an increased incidence in bile duct injuries.[24,25] However, recent studies have shown that the reported intraoperative complication rate of SILC is comparable to LC;[26–28] in our

study, there were no incidences of complications in either the SILC or LC cohorts. In this trial, we found that there was no difference in the LOS and overall, the SILC group had a better postoperative recovery with fewer cases of consulting a doctor and wound complications. The sample sizes in this study were similar to those of previous studies that demonstrated significance with a comparable methodology.[16,18] Although not suggested by the data, a type 2 error always needs to be considered, especially when dealing with relatively small sample sizes. The ethical issues associated with randomisation meant that we conducted this trial without randomisation. This led to the trial being open to selection bias, as demonstrated in the significant differences in age, weight and BMI. Although this heterogeneity was unavoidable, other characteristics, baseline values and operative parameters were comparable between the groups. The learning curve of the surgeon may have also introduced procedural bias. Nevertheless, our results showed that the grade of surgeon had no effect on systemic stress.

In conclusion, this trial did not demonstrate a significant difference in systemic stress or postoperative morbidity between SILC and LC, identifying SILC to be quite comparable to LC. To overcome the limitations of this study a larger, multi-centred, randomised prospective trial is warranted; to further investigate and confirm our findings. However, based on the results of this trial, we suggest that SILC is a safe and feasible technique, which has at least equivalent peri-operative outcomes to LC with obvious advantages of cosmesis and theoretical advantages of reduced analgesic requirements and well-being in this patient group.

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ORIGINAL ARTICLE

Anatomic Variations of Intrahepatic Bile Ducts in a European Series and Meta-analysis of the Literature

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Abstract
Background Accurate knowledge of biliary anatomy and its variants is essential to ensure successful hepatic surgery; however, data from European countries are lacking.
Methods Two hundred cholangiograms obtained from patients submitted to whole liver transplantation were reviewed; donors' characteristics were related to the prevalence of typical biliary anatomy and its variants. A comprehensive literature search was performed with MEDLINE and EMBASE from 1980 to 2010 to investigate whether geographical origin could be related to biliary abnormalities.
Results Typical biliary anatomy was observed in 64.5% of cases, but female donors more frequently presented an anatomic variation; typical anatomy was present in 55.0% of females and in 74.0% of males (P=0.005). Twenty-two reports were identified by the literature search with a total of 7,559 cases, including the present series; heterogeneity was low (Q=14.60; I2<5.0%) after exclusion of three outlier reports. Prevalence of typical biliary anatomy was similar in Europeans and Americans (~60%); a slightly higher prevalence was observed in Asiatics (~65%).
Conclusions Anatomic variants seem to be more frequent in females, probably as a consequence of different embryologic development. Available data suggest that typical biliary anatomy can be more frequent in Asiatics, but an accurate means of classification is essential to making comparison realistic.

Keywords Bile ducts · Intrahepatic · Abnormalities · Anatomy · Surgery

Introduction

An accurate knowledge of normal intrahepatic bile duct ramifications and their variations, as well as the variations in the confluence of the hepatic ducts and their first order ramie, is of crucial importance for liver and biliary tract surgeons. Lack of knowledge in this surgical area can lead to definitive and often lethal injuries in both conventional hepatectomies and hepatectomies for living donor liver transplantation, where both recipient and donor must conserve an adequate functional liver. The right hepatic duct drains the segments of the right liver lobe (V-VIII) and has two major branches: the right posterior duct, draining the posterior segments, VI and VII, and the right anterior duct, draining the anterior segments, V and VIII. The right posterior duct usually runs posterior to the right anterior duct and fuses it from a left (medial) approach to form the right hepatic duct. The left hepatic duct is formed by segmental tributaries, draining segments II-IV. The common hepatic duct is formed by fusion of the right hepatic duct, which is usually short, and the left hepatic duct. The bile duct draining the caudate lobe usually joins the origin of the left or right hepatic duct. The largest published series, originating from operative cholangiograms, report this normal biliary anatomy to range between about 58% and

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68% of cases.[1,2] The most common anatomic variants described in the branching of the biliary tree involve the right posterior duct and its fusion with the right anterior or left hepatic duct. As mentioned earlier, the right posterior duct normally passes posteriorly to the right anterior duct and joins it from the left to form the right hepatic duct, which then forms a junction with the left hepatic duct to form the common hepatic duct. Drainage of the right posterior duct into the left hepatic duct or at its confluence with the right anterior duct is the most common anatomic variant of the biliary system and is reported in about 30% of cases.[1,2]

While the epidemiology of extrahepatic biliary abnormalities is well described in the literature, especially as regards pancreaticobiliary duct maljunction,[3,4] few data are available regarding the epidemiology of intrahepatic biliary abnormalities. In fact, in opposition to what has been observed for extrahepatic biliary anatomy, no data are reported about sex prevalence, regional or ethnical disparities, or correlation with other demographical characteristics. The first aim of the present study was, therefore, to analyze the prevalence of intrahepatic biliary abnormalities in relationship with these general characteristics. The second aim of the present study was to estimate how and if intrahepatic biliary abnormalities could be different in different geographical areas of interest; data regarding intrahepatic abnormalities have mostly involved American or Asiatic patients and no comparison has ever been performed between these populations and Europeans. A comprehensive literature search was therefore performed and a meta-analysis on the prevalence of typical biliary anatomy was carried out.

Materials and Methods

Data regarding biliary anatomy were obtained from cholangiograms of patients who underwent whole liver transplantation. From January 2006 until May 2010, 366 adult liver transplantations from deceased donors were performed in 334 patients at the Department of Surgery and Transplantation of the University of Bologna. Since the aim of the present study was to investigate the intrahepatic anatomy of the biliary system, only cases with adequate intra- or postoperative routine cholangiography were included in the analysis. Biliary reconstruction depended on the morphology of the bile ducts of both liver graft and recipient and, in the majority of cases, a simple duct-to-duct reconstruction was performed placing a t-tube across the anastomosis. Of the 366 transplants, 166 cases were excluded for the following reasons: 62 cases with a choledoco-jejunal anastomosis or a duct-to-duct reconstruction without t-tube; 97 cases with unavailable or unsatisfactory imaging of the intrahepatic biliary anatomy, six split liver transplantations, and one liver originating from a non-Italian donor (South Africa). The final study population consisted of 200 transplant procedures, using whole liver grafts originating from deceased Italian donors, with a duct-to-duct reconstruction, performed with a t-tube and with adequate intra- or postoperative cholangiography (Table 1).

The following donor data were collected for each transplant: sex, age, cause of death, place of birth, and blood type. Cholangiograms were reviewed by two independent investigators (E.P. and C.Z.), and in the event of disagreement on biliary anatomy definition a third investigator (G.E.) was consulted. Typical biliary anatomy (type 1) was defined when the right posterior duct drained into the right hepatic duct, and both the right and left hepatic ducts converged into the common hepatic duct (Fig. 1). Trifurcation (type 2) was defined when the right posterior duct drained into the junction of the right anterior duct and the left main duct. Abnormal right configuration included an anomalous drainage of the right posterior duct, draining into the left main hepatic duct (type 3a) or into the common hepatic duct (type 3b). Other rare variants were also investigated and are reported in the present analysis .[1,2]

In order to compare data originating from the present study and different series published from different geographical areas, a comprehensive literature search of the

Table 1 Clinical and demographical characteristics of the study population

Variable	Study population (no.=200)
Sex	
Male	100 (50.0%)
Female	100 (50.0%)
Age (mean; S.D./median; range)	57.8±18.2/61 (11–88)
Cause of death	
Cerebrovascular	136 (68.0%)
Trauma	40 (20.0%)
Other	24 (12.0%)
Birth place	
North Italy	129 (64.5%)
Central south Italy	71 (35.5%)
Blood type	
O	86 (43.0%)
A	79 (39.5%)
B	21 (10.5%)
AB	14 (7.0%)
Intrahepatic biliary anatomy	
Typical	129 (64.5%)
Atypical	71 (35.5%)

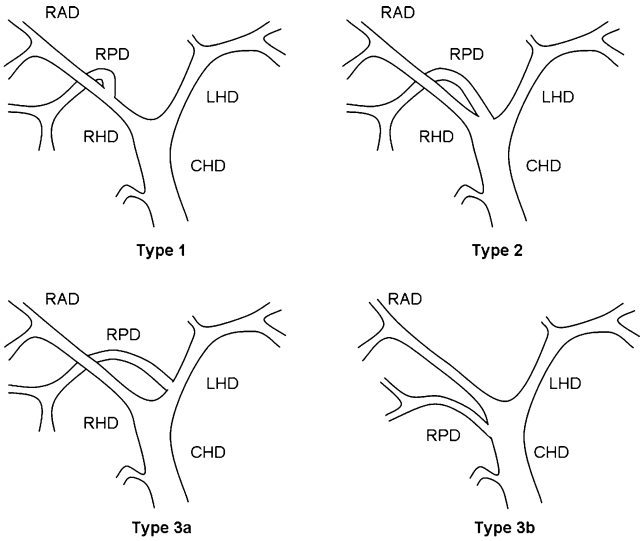

Fig. 1 Schematic representation of intrahepatic bile duct anatomy. Type I is conventional, type 2 is a trifurcation pattern with a common confluence of the right posterior and the right anterior segmental ducts, type 3 is an abnormal right duct configuration that includes type 3a in which the right posterior duct drains into the left hepatic duct, and type 3b in which the right posterior ducts drains directly into the common hepatic duct. *RAD*, right anterior duct; *RPD*, right posterior duct; *RHD*, right hepatic duct; *LHD*, left hepatic duct; *CHD*, common hepatic duct

MEDLINE and EMBASE databases was performed, covering the period from January 1980 to January 2010, and conducted independently by two investigators (M.Z. and P.G). Since the target of the present review included only observational studies, the Meta-analysis of Observational Studies in Epidemiology group recommendations were used to conduct and report the findings.[5] The following medical subject heading terms were used for the bibliographic search: "bile ducts," "bile ducts, intrahepatic," "bile ducts, abnormalities," "bile ducts, anatomy," "bile ducts, radiology," "bile ducts, surgery." Inclusion or exclusion of studies was performed hierarchically based on the title of the report first, followed by the abstract, and then by the full text. If the initial study was followed by a more complete study or studies that included the original dataset, the most recent and complete report was chosen. Such linked studies were identified on the grounds of authorship, institutions, design, length of follow-up, and study populations. We finally reviewed the reference lists of all selected studies for additional citations; disagreement on study selection was resolved by consulting a third investigator (M.C.). In studies that classified biliary anatomy by both magnetic resonance cholangiography and conventional intraoperative cholangiography, prevalence of typical anatomy, and anatomic variants were related to conventional cholangiography. Chosen reports were divided on the basis of the geographical region of provenance; in particular, five reports were from Europe, six were from North or South America, and 11 from Asian countries.[1,2,6–25]

Statistical Analysis

Categorical variables were defined in a number of cases and percentages and compared with the chi-square test as appropriate. A P value less than 0.05 was considered statistically significant. Publication bias was examined through the use of funnel plot.[26] The overall variation in typical biliary anatomy prevalence that was attributable to between-study heterogeneity was assessed with the Cochran Q test; the I2 index was also defined in order to better interpret the heterogeneity magnitude, percentages around 25%, 50%, and 75% were interpreted as low, medium, and high heterogeneity, respectively.[27,28] Studies potentially influencing heterogeneity were therefore removed from the analysis and results compared. For each published report, the 95% confidence interval (C.I.) for prevalence of typical biliary anatomy was properly calculated using the binomial distribution. In consideration of the relatively small number of studies, a P value of less than 0.10 was used to indicate heterogeneity rather than the conventional cut-point of P=0.05; in addition, pooled prevalence of typical biliary anatomy was calculated assuming a fixed or a random effect model on the basis of the heterogeneity test.[29] Statistical analysis was performed with SPSS (SPSS 13.0, SPSS Inc., Chicago, IL, USA) and Excel 2003 (Microsoft Corporation, USA).

Results

The prevalence of different biliary anatomic variants and their relationship with donor characteristics are reported in Table 2. Typical biliary anatomy (type 1) was observed in 129 cases (64.5%), trifurcation (type 2) was present in 28 cases (14.0%), right posterior duct draining into the left main hepatic duct (type 3a) was observed in 24 cases (12.0%) and right posterior duct draining into the common hepatic duct (type 3b) was observed in 16 (8.0%). Other complex biliary variations accounted for three cases (1.5%). Females presented a variation of the typical biliary anatomy more frequently. In fact, a normal biliary anatomy was present in 55.0% of cases, significantly lower than that observed in males in whom a normal anatomy was present in 74.0% of cases (P=0.005). Regarding the specific atypical pattern of the biliary anatomy, trifurcation (type 2) was 3.7 times more frequent in females with respect to males and a trend toward a higher prevalence of the right posterior duct draining into the common hepatic duct (type 3b) was also observed, with a female to male ratio of 1.7; type 3a and

Table 2 Demographical charact eristics in relationship with the intrahepatic biliary anatomy observed

Variable	Intrahepatic biliary anatomy				
	Type 1	Type 2	Type 3a	Type 3b	Other
All cases (no.=200)	129 (64.5%)	28 (14.0%)	24 (12.0%)	16 (8.0%)	3 (1.5%)
Sex					
Male (no.=100)	74 (74.0%)*	6 (6.0%)	13 (13.0%)	6 (6.0%)	1 (1.0%)
Female (no.=100)	55 (55.0%)*	22 (22.0%)	11 (11.0%)	10 (10.0%)	2 (2.0%)
Cause of death					
Cerebrovascular (no.=136)	93 (68.4%)	18 (13.2%)	16 (11.8%)	7 (5.1%)	2 (1.5%)
Non-cerebrovascular (no.=64)	36 (56.3%)	10 (15.6%)	8 (12.5%)	9 (14.1%)	1 (1.6%)
Birth place					
North Italy (no.=153)	98 (64.1%)	19 (12.4%)	19 (12.4%)	14 (9.2%)	3 (2.0%)
Central south Italy (no.=47)	31 (66.0%)	9 (19.1%)	5 (10.6%)	2 (4.3%)	0 (0.0%)
Blood type					
O (no.=86)	52 (60.5%)	13 (15.1%)	10 (11.6%)	8 (9.3%)	3 (3.5%)
A (no.=79)	50 (63.3%)	13 (16.5%)	10 (12.7%)	6 (7.6%)	0 (0.0%)
B (no.=21)	15 (71.4%)	1 (4.8%)	3 (14.3%)	2 (9.5%)	0 (0.0%)
AB (no.=14)	12 (85.7%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)

*P=0.005, type 1 versus all other types

other complex biliary variations were found to be similar in the two groups (Table 2).

No differences were observed in biliary anatomy in relationship with cause of death (P=0.218) and place of birth; in this latter case, donors originating from north Italian regions showed a typical biliary anatomy in 64.1% of cases and donors originating from central and south Italian regions in 66.0% of cases (P=0.811). Donors of blood type O had a typical biliary anatomy in 60.5% of cases, similar to those of blood type A in whom a typical anatomy was observed in 63.3%; conversely, a slight increase was observed in donors of blood type B and AB. Donors of blood type B had a typical anatomy in 71.4% of cases and those of blood type AB in 85.7%. This difference was not significant (P=0.278), but it should be noted that donor sex was not uniformly distributed among blood type groups; females accounted for 55.8% of blood type O donors, for 50.6% of blood type A donors, for 28.6% of blood type B donors, and for 42.9% of blood type AB donors.

Table 3 reports the distributions of normal biliary anatomy and its variants in a series published in the last three decades originating from the literature search. Twenty reports were identified by the literature search, including the present series, a total of 7,559 cases have a classification of intrahepatic biliary anatomy. Including the present study, reports from Europe accounted for 794 cases (10.5%), from America for 4,146 cases (54.8%), and from Asia for 2,619 cases (34.6%). The total number of typical anatomy cases reported was 4,598 (60.8%) and total biliary anatomic variants were 2,961 (39.2%); in particular, trifurcation was described in 904 cases (11.9%) and an abnormal right configuration in 1,324 cases (17.5%).

Considering typical biliary anatomy prevalence, the Q test showed a medium heterogeneity between the 21 studies involved (Q=34.32; P=0.046) as confirmed by an I2 of 35.9% (Fig. 2); the funnel plot showed that outliers were represented by the report by Yoshida (1996, Asia), Puente (1983, America), and Sharma (2008, Asia). The removal of these reports from the analysis led to a very low heterogeneity (Q=14.60; P=0.747) as confirmed by an I2 less than 5.0%. Thus, the pooled prevalence of normal biliary anatomy of the remaining reports was 63.0% (95% C.I.=59.9–66.2%) (Fig. 3). Studies were grouped on the basis of region of origin to verify whether a different prevalence could effectively exist between Europeans, Americans, and Asiatics (Fig. 4). The pooled prevalence of typical anatomy from European reports (Q=7.36; P=0.195; I2=32.1%) was 59.7% (95% C.I.=54.3–64.9%). Since it is possible that American and Asiatic outliers contained meaningful information regarding the ethnic variability of biliary anatomy, prevalence was calculated both without and with them. The prevalence of typical anatomy from American reports (Q=2.01; P=0.736; I2< 5.0%) was 63.2% (95% C.I.=54.4–72.0%), but after the re-inclusion of the report by Puente (Q=3.38; P=0.641; I2= 5.0%) prevalence dropped to 58.1% (95% C.I.=55.8–60.3%). In Asiatic reports (Q=2.64; P=0.954; I2<5.0%), the pooled prevalence of typical anatomy was 65.2% (95% C.I.=60.9–69.6%); the re-inclusion of the reports by Yoshida and Sharma (Q=11.03; P=0.355, I2=9.4%) led to a slightly lower prevalence of typical anatomy of 64.8% (95% C.I.=61.8–67.8%).

Table 3 Review of the literature reporting biliary anatomic variants

Author	Year	Region	No. of cases	Imaging technique	Intrahepatic biliary anatomy		
					Type 1	Type 2	Type 3a/3b
Present study	2010	Italy	200	Cholangiography	129 (64.5%)	28 (14.0%)	40 (20.0%)
Kashyap [25]	2008	USA	36	MRC	22 (61.1%)	4 (11.1%)	8 (22.2%)
De Filippo [24]	2008	Italy	350	MRC	202 (57.7%)	27 (7.9%)	35 (10.0%)
Sharma [23]	2008	India	253	Cholangiography	134 (52.9%)	29 (11.5%)	64 (25.3%)
Kim [22]	2008	Korea	33	MRC	25 (75.8%)	1 (3.0%)	3 (9.1%)
Karakas [21]	2008	Turkey	112	MRC	61 (54.5%)	16 (14.3%)	35 (31.2%)
Song [20]	2007	Korea	111	MRC	67 (60.4%)	9 (8.1%)	30 (27.0%)
Sirvanci [19]	2007	Turkey	62	Cholangiography	43 (69.3%)	6 (9.7%)	11 (17.7%)
Cho [18]	2007	Japan	60	CTC	38 (63.3%)	14 (23.3%)	8 (13.3%)
Vidal [17]	2007	France	45	MRC	36 (80.0%)	2 (4.4%)	4 (8.9%)
Kitami [16]	2006	Japan	158	CTC	115 (72.8%)	8 (5.1%)	25 (15.8%)
Macdonald [15]	2005	USA	39	Cholangiography	24 (61.5%)	3 (7.7%)	8 (20.5%)
Chen [14]	2005	USA	56	MRC	33 (58.9%)	7 (12.5%)	15 (26.8%)
Wang [13]	2005	USA	62	CTC	35 (56.0%)	7 (11.0%)	19 (30.6%)
Ayuso [12]	2004	Spain	25	MRC	10 (40.0%)	1 (4.0%)	8 (32.0%)
Lee [11]	2004	USA	108	MRC	78 (72.2%)	6 (5.6%)	20 (18.5%)
Okubo [10]	2004	Japan	110	Cholangiography	71 (64.5%)	6 (5.4%)	21 (19.1%)
Choi [9]	2003	Korea	300	Cholangiography	188 (62.7%)	29 (9.7%)	53 (17.7%)
Kitagawa [8]	2003	Taiwan	170	Cholangiography	113 (66.5%)	36 (21.2%)	31 (18.2%)
Nakamura [7]	2002	Japan	120	Cholangiography	78 (65.0%)	11 (9.2%)	29 (24.2%)
Cheng [6]	1997	Taiwan	210	Cholangiography	138 (65.7%)	35 (16.7%)	31 (14.7%)
Yoshida [2]	1996	Japan	1,094	Cholangiography	741 (67.7%)	193 (17.7%)	153 (13.9%)
Puente [1]	1983	Chile	3,845	Cholangiography	2,217 (57.6%)	426 (11.1%)	673 (17.5%)

MRC magnetic resonance cholangiography, *CTC* computed tomography cholangiography

Discussion

Accurate preoperative assessment of hepatic biliary anatomy is essential to ensure safe and successful hepatic surgery, and epidemiological knowledge of intrahepatic biliary abnormalities could thus be of importance. The first interesting finding of the present analysis is that anatomic variants of the biliary tree are more frequently observed in females rather than in males, with a female to male ratio that ranges from 1.7 for an abnormal right configuration of the posterior right duct to 3.7 for biliary trifurcation. Despite large data series reported from American and Asian countries, this specific feature has never been investigated until now.[1,2,6–25] Even if this finding may seem strange, an accurate knowledge of the embryology of the biliary tract could help in understanding. The origin of both intra- and extrahepatic biliary structures is the embryonic diverticulum that arises from the ventral surface of the foregut.[30] Extrahepatic biliary abnormalities are reported to be more frequently observed in females than in males.[3,4] In particular, the congenital maljunction of the pancreaticobiliary tract, defined as a union of the pancreatic and biliary duct that is located outside the duodenal wall, is the anomaly that has been most studied in the literature.[3,4] This abnormality had an unexplained female/male preponderance, commonly reported as 3:1. Other biliary abnormalities are also more frequently observed in females rather than in males; choledochal cysts are frequently observed in the presence of a pancreaticobiliary maljunction, but 20–50% of these did not present such an association, supporting the hypothesis that choledochal cysts are purely congenital in nature. This extrahepatic biliary abnormality is reported to be three to four times more frequent in females than in males.[31] These observations well support the finding of the present study of a higher prevalence of anatomic biliary variants in females because such abnormalities can follow the same ductal plate pathway. Apart from this embryologic explanation of this peculiar result, the knowledge that females can more frequently present an abnormal biliary duct configuration not only adds some epidemiological knowledge to the anatomy of the bile ducts but can also help surgeons in planning potential living donor evaluation or split procedures of deceased liver donors.

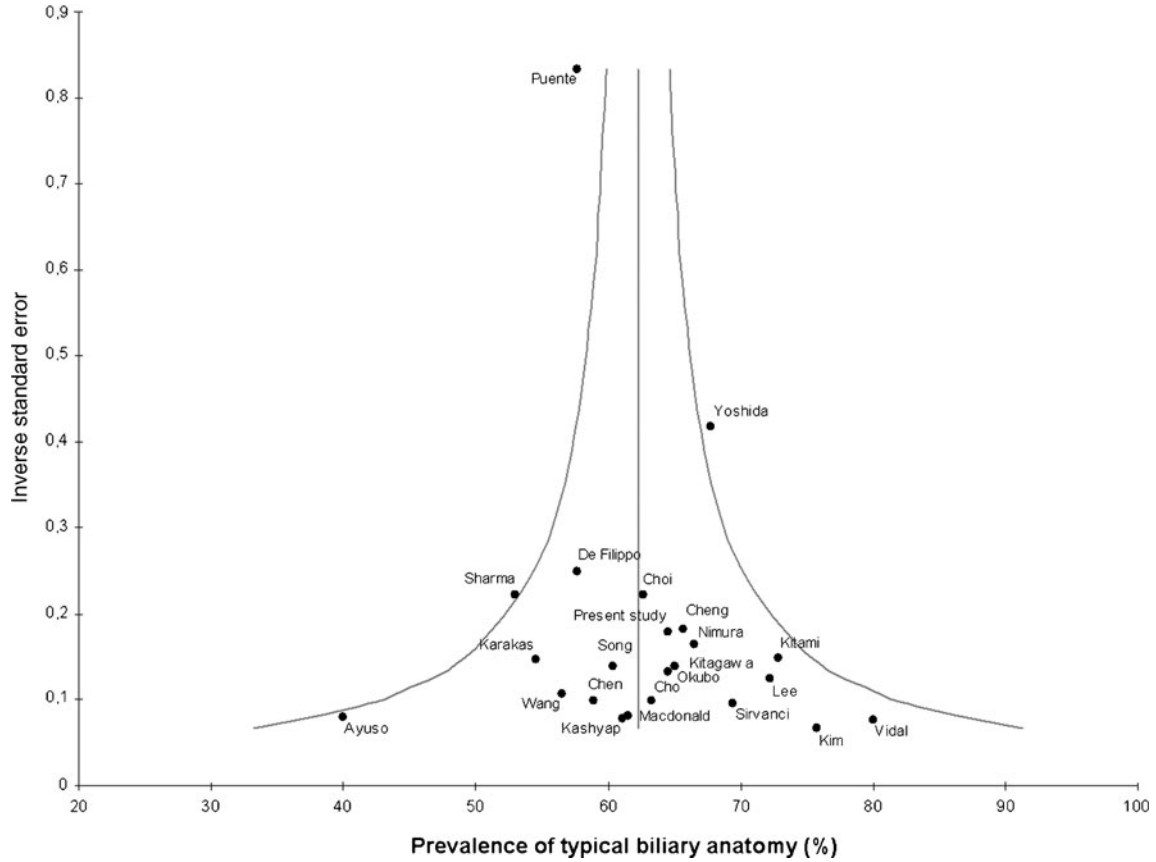

Fig. 2 Funnel plot of studies reporting prevalence of typical biliary anatomy. Outliers are represented by the reports by Yoshida, 1996 (Asia); Puente, 1983 (America); and Sharma, 2008 (Asia)

As already mentioned, most of the data concerning biliary anatomic variants are from American and Asian countries, whereas fewer data from European series are available. To the best of our knowledge, this is one of the largest European series that describes biliary anatomy in this specific population of interest, and the first one to assess biliary anatomy by means of cholangiography in this population.[12,17,19,21,24] This study is also the very first meta-analysis of observational studies regarding biliary anatomy and could thus be helpful in summarizing the large case series reported in the literature into a single useful review. The literature review showed that Europeans and Americans share similar prevalence rates of typical biliary anatomy and, in contrast, of anatomic variants even with the re-inclusion of the outlier report of Puente.[1] From an ethnicity point of view, this finding is not surprising since both populations can be considered Caucasian. On the contrary, Asiatics seem to have a slightly higher prevalence of typical anatomy, even if the re-inclusion of the outlier reports of Yoshida and Sharma tone down this observed difference.[2,23] These differences, as well as those observed in trifurcation and abnormal right configuration prevalence rates, should be taken into account with caution. In particular, it is often difficult to distinguish a biliary trifurcation from an abnormal insertion of the right posterior duct on the origin of the main left hepatic duct. Other biliary anatomic variants can be present in concomitance with these abnormalities, leading to a different classification of anatomy. Such difficulties can lead to interpret a trifurcation as a type 3a, or a different anatomic variant as normal, a trifurcation or a type 3 variant and vice versa. In addition, the possible absence of different spatial projections in radiological imaging can lead to a misunderstanding of these often very subtle differences. These observations are well supported by the literature that reports wide ranges for both biliary trifurcation (4.0–23.3%) and abnormal right duct insertion (8.9–30.6%). On the basis of data currently available in the literature, only a slightly higher prevalence of typical biliary anatomy in Asiatics could be supposed, but this observation is far from being conclusive because of difficulties in obtaining a uniform classification.

Conclusion

Typical intrahepatic biliary anatomy is present in about 60% of Europeans, but anatomic variants seem to be more frequent in females rather than males, probably as a

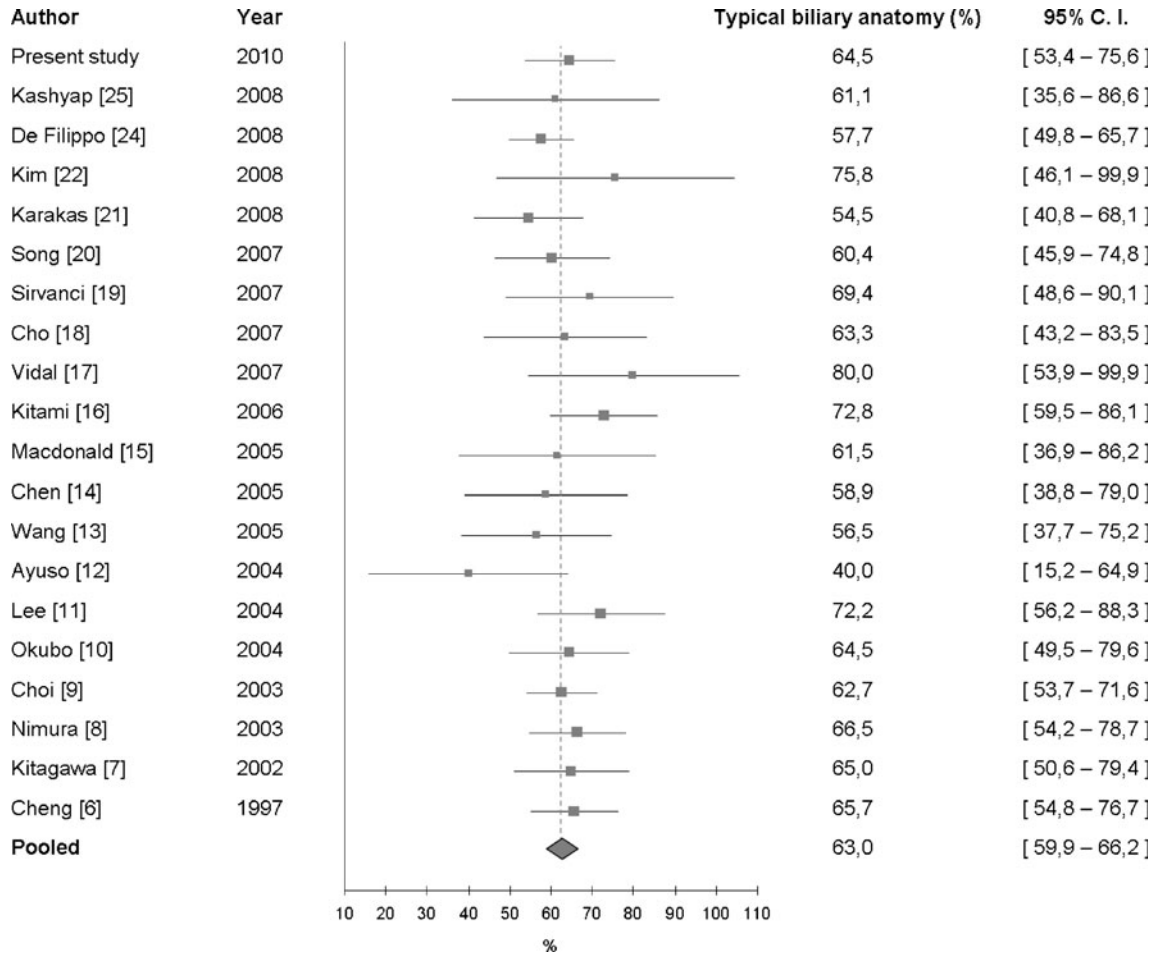

Fig. 3 Forest plot of studies reporting prevalence of typical biliary anatomy after exclusion of outliers identified by funnel plot (Q=14.09; P= 0.660; I2<5.0%). Outliers are represented by the reports by Yoshida, 1996 (Asia); Puente, 1983 (America); and Sharma, 2008 (Asia)

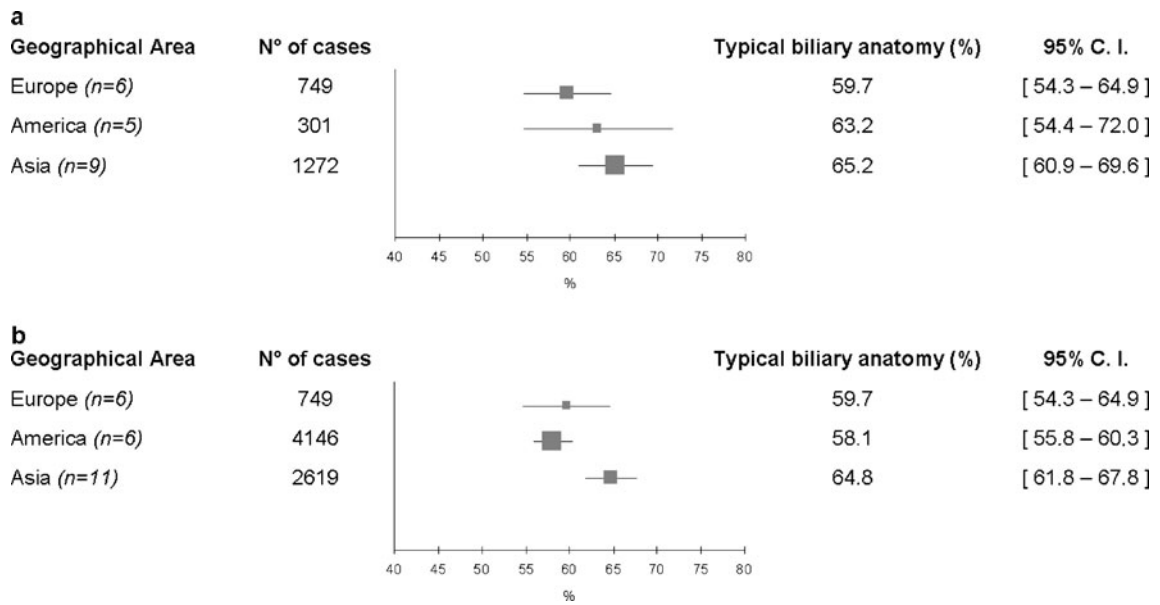

Fig. 4 Forest plot of studies reporting prevalence of typical biliary anatomy after **a** exclusion of outliers and **b** re-inclusion of outliers. Outliers are represented by the reports by Yoshida, 1996 (Asia); Puente, 1983 (America); and Sharma, 2008 (Asia)

consequence of different embryologic development, as already observed for extrahepatic biliary abnormalities. Currently available data suggest that typical biliary anatomy can be more frequently observed in Asiatics, but an accurate and standardized method of classification is essential in making comparison realistic. Further analyses of published data regarding the gender issue raised by the present analysis are warranted.

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ORIGINAL ARTICLE

Pancreatic Heterotopia of the Duodenum: Anatomic Anomaly or Clinical Challenge?

Marius Distler · Felix Rückert · Daniela Aust · Hans-Detlev Saeger · Robert Grützmann

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Abstract
Introduction Pancreatic heterotopia (PH) is a common congenital anomaly and can occur anywhere in the gastrointestinal tract (GIT). In most cases, these heterotopias are asymptomatic and are only incidentally detected upon pathohistological examination or autopsy. We analyzed our cases of duodenal PH with respect to their clinical relevance and impact.
Materials and Methods Our prospectively collected pancreatic database was retrospectively analyzed. Thirty-four cases of duodenal PH were found. Specimens were reviewed by a GI pathologist. Classification was performed according to Heinrich (Type I acini, ducts, and islet cells; Type II acini and ducts; Type III only ducts).
Results From January 2000 to June 2009, we performed 534 pancreatic head resections. Thirty-two patients (6.0%) were found to have duodenal PH. Indications for pancreatic resections (pylorus-preserving pancreaticoduodenectomy, $n=26$; Whipple, $n=6$) were as follows: chronic pancreatitis, $n=16$; malignancies, $n=9$; cystic neoplasms, $n=5$; and neuroendocrine neoplasms, $n=2$. PH was also detected after two partial duodenal resections. In total, two cases of duodenal PH were found to be symptomatic. According to Heinrich, the following types were found: Type I, $n=12$; Type II, $n=17$; and Type III, $n=5$ (total $n=34$).
Conclusions PH is rare and in most cases detected incidentally during pathohistological examination. However, in two of our patients, surgery was performed due to symptoms. Therefore, in patients with unclear pancreatoduodenal lesions, PH should be considered as a possible diagnosis. Resection is indicated for symptomatic cases.

Keywords Pancreatic heterotopia · Pancreatic resection · Heinrich's classification

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Abbreviations

GIT	Gastrointestinal tract
PH	Pancreatic heterotopia
PPPD	Pylorus-preserving pancreaticoduodenectomy

Introduction

Pancreatic heterotopia (PH) is defined as pancreatic tissue located outside of the pancreas without any anatomical or vascular connection to the pancreas. Ectopic pancreatic tissue may occur anywhere in the gastrointestinal tract (GIT), but it is mostly found in the upper GIT.[1,2] The exact origin of PH is unknown, but a popular theory is that the ectopic tissue separates itself from the pancreas during embryonic rotation and fusion of the dorsal and ventral pancreatic buds.[3,4] Following this theory, it is believed that

ectopic pancreatic tissue in the stomach and duodenum is a derivative of the dorsal segment, while tissue in the jejunum and ileum originates from the ventral segment.[1,4,5]

The incidence of heterotopic pancreatic tissue is low (0.5–15%),[2] and until now, no specific symptoms have been described. In most cases, PH is asymptomatic and is only incidentally detected by pathohistological examination of the specimen.[2,6] However, all diseases that occur in the genuine pancreas can arise in heterotopic tissue.[2,4,7,8]

According to the literature, symptomatic cases have only been described in several case reports, and series of symptomatic PH are very rare.[2,9–11] Therefore, we analyzed our cases of duodenal PH with respect to their clinical relevance and impact.

Materials and Methods

Using our prospective pancreatic database, we retrospectively analyzed cases for PH of the duodenum during the period between January 2000 and June 2009. We found a total of 34 cases (♀=8, ♂=26). The mean patient age was 54 years (range 24–75). Clinical symptoms and pathological findings as well as the surgical procedures performed were recorded; sizing of the duodenal lesion was not possible for all cases (Table 2).

Samples were stained with hematoxylin and eosin, and each specimen was reviewed by a senior GI pathologist (D.A.) to determine whether the components of pancreatic tissue, including acini, ducts, and islets of Langerhans, were present. Classification was performed according to the system described by Heinrich[12] (Table 1).

Results

During the above-mentioned period, a total of 534 pancreatic head resections were performed in our department. PH of the duodenum was found in 32 patients (6.0%) during postoperative pathohistological examinations. The pancreatic head resections (pylorus-preserving pancreaticoduodenectomy (PPPD) n=26; Whipple, n=6) were conducted for the following indications: chronic pancreatitis, n=16; malignancies, n=9; cystic neoplasms, n=5; and neuroendocrine tumors, n=2 (Table 2). Additionally, duodenal pancreatic tissue was detected after a left resection with partial duodenal resection in a case of

Table 1 Heinrich's classification

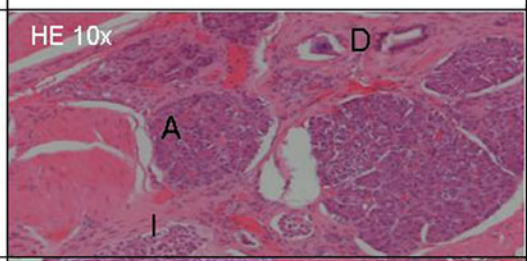
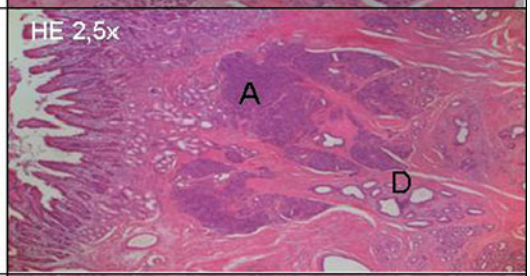
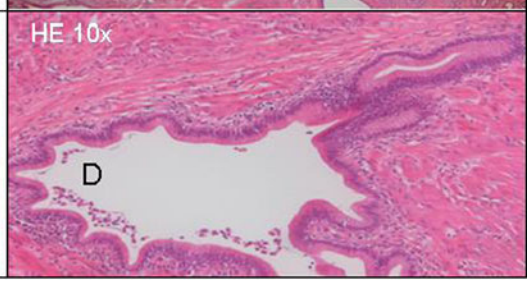
	Structure:	Histology:
Type I	All components of pancreatic tissue: ***Acini [A], Ducts [D] and endocrine Islet cells [I]***	
Type II	Exocrine components of pancreatic tissue: ***Acini [A] and ducts [D] but no Islet cells***	
Type III	Mainly contains ***pancreatic ducts (possibly cystic) [D], no acini, no Islet cells***	

Table 2 Patient Cohort

No.	Age (y) Sex	Clinical Manifestation	Operation	Heinrich's Type of PH	Histology
1	47 ♂	Obstructive Jaundice/Tumor Pancreatic Head	Whipple	Type II	Cholangio carcinoma
2	34 ♂	Obstructive Jaundice/Papillary Tumor	PPPD	Type II	Papillary carcinoma
3	64 ♂	Obstructive Jaundice, Duodenal Stenosis/Papillary Tumor	PPPD	Type III	Papillary carcinoma
4	49 ♀	Obstructive Jaundice/Papillary Tumor	PPPD	Type II	Papillary carcinoma
5	72 ♂	Obstructive Jaundice, Pancreatic Stenosis/Tumor Pancreatic Head	PPPD	Type I	Papillary carcinoma
6	60 ♂	Recurrent Pancreatitis/Epigastric Pain	PPPD	Type I	NET
7	58 ♀	Diarrhea/Cystic Tumor Pancreatic Head	PPPD	Type II	NET
8	60 ♂	Recurrent Pancreatitis, Stenosis Pancreatic Duct by Cystic Tumor	PPPD	Type II	IPMN
9	66 ♀	Epigastric Pain/Cystic Tumor Pancreatic Head	PPPD	Type I	IPMN
10	71 ♂	Cystic Tumor Pancreatic Head/Stenosis Pancreatic Duct	PPPD	Type II	IPMN
11	70 ♀	No symptoms/Suspicious Tumor Pancreatic Head	Whipple	Type I	Cyst adenoma
12	75 ♀	Epigastric Pain/Cystic Tumor Pancreatic Head	PPPD	Type III	Cystic neoplasia
13	57 ♀	Obstructive Jaundice, Epigastric Pain/Tumor Pancreatic Head	PPPD	Type I	PDAC
14	56 ♂	Recurrent Pancreatitis, Pseudocyst Pancreatic Head/ Epigastric Pain	PPPD	Type II	PDAC
15	67 ♂	Obstructive Jaundice/Tumor Pancreatic Head	PPPD	Type I	PDAC
16	70 ♂	Obstructive Jaundice/Tumor Pancreatic Head	PPPD	Type I	PDAC
17	46 ♂	Recurrent Pancreatitis/Epigastric Pain	Whipple	Type II	C.P.
18	62 ♂	Recurrent Pancreatitis/Pancreatic Fistula to Duodenum	Left resection with partial duodenal resection	Type II	C.P.
19	35 ♂	Recurrent Pancreatitis, Epigastric Pain/Duodenal Stenosis	Whipple	Type II	C.P.
20	54 ♂	Recurrent Pancreatitis, Epigastric Pain	PPPD	Type II	C.P.
21	63 ♂	Recurrent Epigastric Pain/Tumor Pancreatic Head	PPPD	Type I	C.P.
22	54 ♂	Recurrent Pancreatitis/Cystic Tumor Pancreatic Head, Pain	PPPD	Type III	C.P.
23	29 ♀	Recurrent Pancreatitis, Pseudocyst Pancreatic Head/ Epigastric Pain	PPPD	Type III	C.P.
24	49 ♀	Pseudocyst Pancreatic Head, Stenosis Pancreatic Duct/Pain	PPPD	Type II	C.P.
25	52 ♂	Recurrent Pancreatitis, Tumor Pancreatic Head/Stenosis Pancreatic Duct	PPPD	Type I	C.P.
26	24 ♂	Recurrent Pancreatitis, Pseudocyst Pancreatic Head/ Epigastric Pain	PPPD	Type II	C.P.
27	56 ♂	Recurrent Pancreatitis/Stenosis Pancreatic Duct, Pain	PPPD	Type II	C.P.
28	46 ♂	Recurrent Pancreatitis/Tumor Pancreatic Head	PPPD	Type I	C.P.
29	39 ♂	Abdominal Pain/Tumor Pancreatic Head	PPPD	Type II	C.P.
30	46 ♂	Recurrent Pancreatitis, Duodenal Obstruction, Obstructive Jaundice, Pain	Whipple	Type II	C.P.
31	47 ♂	Recurrent Pancreatitis, Abdominal Pain, Pseudocysts	PPPD	Type I	C.P.
32	47 ♂	Recurrent Pancreatitis/Epigastric Pain	PPPD	Type III	C.P.
Symptomatic cases of PH					
33	60 ♂	Epigastric pain, Nausea, Vomiting, Melena/Suspicious Duodenal Tumor, Duodenal Stenosis	Whipple	Type I	PH
34	56 ♂	Abdominal Pain, Nausea, Vomiting/Duodenal Stenosis, Suspicious Duodenal Tumor	Partial Duodenal Resection	Type II	PH

C.P. chronic pancreatitis, *PDAC* pancreatic ductal adeno carcinoma, *NET* neuro endocrine tumor, *PH* pancreatic heterotopia

chronic pancreatitis and after a partial duodenal resection due to a suspicious duodenal tumor (total cases, n=34; Table 2). Overall, according to Heinrich's classification, the following types of heterotopia were found: Type I, n=12; Type II, n=17; and Type III, n=5 (total n=34).

In total, a direct relationship between symptomatic heterotopia of the duodenum and the indication for an operation was observed in two cases. In one patient with duodenal stenosis, a Whipple procedure was performed. This patient presented with symptoms of epigastric pain, nausea, vomiting, and melena. Endoscopy showed a stenotic submucosal tumor of the duodenal bulb. Preoperative biopsy revealed signs of inflammation. The patient had no report of alcohol abuse or chronic pancreatitis, and serum pancreatic enzymes (amylase/lipase) levels were not elevated. MRI showed a tumor in the bulbus duodeni and the descending duodenum with a possible infiltration of the pancreas (Fig. 1). Malignancy could not be excluded by imaging even though the CA 19-9 levels were not elevated. Therefore, the patient underwent a pancreaticoduodenectomy (Whipple procedure). Pathohistological examination revealed a nearly 4-cm duodenal tumor arising from the pancreatic side of the duodenal wall. No signs of chronic pancreatitis or malignancy were found. The tumor was graded as a Type I heterotopy according to the Heinrich classification (patient 33, Table 2).

The other symptomatic patient presented with nausea, vomiting, and unspecific abdominal pain, most likely due to duodenal obstruction. Endoscopy revealed a polypoid duodenal tumor with incipient stenosis. Because of the unknown character of the lesion, a partial duodenal resection was performed. Frozen section could not confirm a malignancy; therefore, we refrained from a full oncological resection. Postoperative examination resulted in the diagnosis of a pancreatic heterotopy with a diameter of nearly 3 cm (Type II according to Heinrich's classification; patient 34, Table 2).

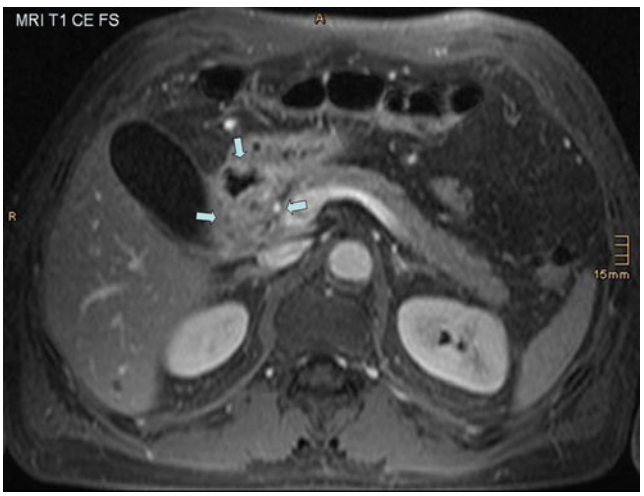


Fig. 1 MRI of a duodenal tumor, histologically proven to be heterotopic pancreatic tissue. *Arrows* mark the boundaries of the mass

The other patients (n=32) presented with symptoms of pancreatic malignancy or chronic pancreatitis including obstructive jaundice, recurrent pain, or pseudocysts. In most of these cases, a pancreatic tumor was detected during the preoperative examinations. Indications for surgery typically included clinical symptoms or diagnostic findings (patients 1–32, Table 2). Specific symptoms related to the occurrence of duodenal PH could not be detected in this analysis.

In four patients with histologically proven chronic pancreatitis (Whipple, n=1; PPPD, n=3), pancreatic heterotopia of the duodenal wall was assumed to be responsible for pancreatitis during the postoperative pathohistological examination. Interventions in these patients were performed due to abdominal pain, duodenal obstruction, obstructive jaundice, and pseudocysts. Furthermore, all patients had a history of alcohol abuse and chronic pancreatitis (patients 29–32, Table 2). In summary, in 2/34 cases (5.9%), nonspecific abdominal symptoms related to a duodenal PH directly led to an operation.

Discussion

PH is rare, and a clinical diagnosis prior to surgery appears to be difficult because typical clinical symptoms have not been reported.[2,4,13] Common locations for ectopic pancreatic tissue include the stomach (25–36%), the duodenum (17–36%), and the jejunum (15–22%). Rare locations include the esophagus, the gallbladder, or the common bile duct.[11,14,15] Recently, Heller et al. reported ectopic pancreatic tissue in the brain.[16] In our study, we focused on the duodenum; and after duodenectomy, heterotopic pancreatic tissue was found in 6% of cases.

Depending on its location and diameter, heterotopic pancreatic tissue can lead to unspecific symptoms.[14,17] This is especially true for tumors in the duodenum due to the anatomic character of this region of the digestive tract and because PH is most commonly found in this localization.

In our analysis, two patients exhibited typical signs of duodenal obstruction and abdominal pain due to a duodenal tumor of 3–4 cm in diameter. The most common symptom of pancreatic heterotopia described in the literature is abdominal pain.[18] Hemorrhage due to mucosal erosion, ulcer formation, and perforation, especially in the small bowel, have also been described.[11,14]

However, only these two patients (5.9%) showed symptoms that were directly related to PH. As the diagnosis was reached after onset of obstructive symptoms, it is reasonable that PH may have grown over a longer period

until it became symptomatic. In regards to the other analyzed cases, the presenting symptoms were related to the prevailing diagnosis and not due to the existence of PH.

Because of the unspecific set of symptoms, the clinical diagnosis of PH is challenging. This challenge is demonstrated by our study where none of our patients were diagnosed preoperatively. Although endoscopy was performed and the tumoral lesion could be shown, the biopsies were futile because the lesion was not represented in the specimen. The reasons are that the ectopic tissue is typically located in the submucosa (76%), in the muscular layer (17%), or in the subserosa (10%).[19] Therefore, endoscopy often shows a submucosal swelling covered by normal mucosa, making an endoscopic biopsy diagnosis difficult.[17]

Endoscopic ultrasound may help to identify a submucosal lesion, especially when combined with fine needle aspiration, and a sensitivity of up to 100% is possible.[20] By CT, ectopic pancreatic tissue enhances similar to normal pancreatic tissue; however, the findings are usually nonspecific and the diagnosis of submucosal lesions is difficult.[21] In our analysis, endoscopic ultrasound was not routinely used and preoperative CT/MRI scans also could not differentiate the lesions. Furthermore, especially in the duodenum, it was difficult to distinguish the tumor from the original pancreas. A clinical discrimination between gastrointestinal stromal tumors, lymphomas, adenomatous polyps, peptic ulcer disease, or malignancies from heterotopic pancreatic tissue is often impossible.[11,14,17] Therefore, histopathological examination is essential to diagnose PH.

According to the literature, any disease of the ordinary pancreas can also arise in the heterotopic tissue. Cases of acute pancreatitis and chronic pancreatitis, as well as the occurrence of pseudocystic changes, have been reported.[4,11,22,23] Only a few cases of malignant transformation to adenocarcinoma or acinar cell carcinoma in PH are reported in the literature.[2,8,24] Nevertheless, malignancy must be included in the differential diagnosis and must therefore be excluded. Furthermore, there have been reports about neuroendocrine tumors arising from pancreatic heterotopias.[11]

In four of our patients, it was speculated that pancreatic heterotopia might be the cause of chronic pancreatitis. However, all patients had a history of alcohol abuse and, thus, the development of chronic pancreatitis was much more likely. Generally, a relationship between clinical symptoms and the occurrence of pancreatic heterotopia must be definitively proven, especially other diagnoses must first be excluded. Thus, in this analysis, only two cases (5.9%) had clinical symptoms clearly attributed to duodenal pancreatic heterotopia.

If possible, local surgical excision seems to be the most adequate procedure for patients with symptomatic PH.[11] Supporting this belief, we performed a partial duodenal resection in one of our cases due to a symptomatic and suspicious duodenal tumor after the exclusion of malignancy. The patient was well thereafter. Histologically proven PH without any complications or symptoms can be treated conservatively. If symptoms develop, complete removal is indicated; even extended surgical resections seem to be justified, especially if malignancy is assumed.

In summary, PH of the duodenum is a rare diagnosis. Most of the diagnoses are incidental, especially because specific symptoms are not observable. The diagnosis of ectopic duodenal pancreatic tissue remains challenging, but it should be considered in the differential diagnosis. If serious symptoms or complications associated with the lesion occur, surgical excision is the treatment of choice.

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ORIGINAL ARTICLE

Urinary and Sexual Disorders After Laparoscopic TME for Rectal Cancer in Males

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Abstract
Background Urinary and sexual dysfunctions are frequent after surgery for rectal cancer. Total mesorectal excision (TME) improves local recurrence and survival rates, and does not hamper recognition and sparing of hypogastric and pelvic splanchnic nerves. It is not known how laparoscopic rectal resection could change functional complication rates.
Materials and Methods From a global series of 1,216 laparoscopic interventions for colorectal diseases, 35 cases of males less than 70 years old, undergoing rectal resection and TME for a T1-3M0 medium and low rectal cancer were selected. Urinary and sexual functions after the operations were retrospectively recorded by means of specific tools (International Prostate Symptom Score (IPSS) and IIEF questionnaires, respectively).
Results None of the patients necessitated permanent or intermittent catheterization. More than half the patients had no complaints about urinary functions; about one third had nocturia; 72% of the patients had an IPSS less that 10, and no case of IPSS worse that 31 was recorded. Sexual desire was reduced and spontaneous erectile function was impaired in almost half the cases, while induced erections were possible in about 90% of cases; about 70% of patients still had the possibility of penetration and a normal ejaculation and orgasm after the intervention.
Discussion and Conclusions The present series confirms previous data and contribute to the creation of a benchmark specifically related to the laparoscopic approach to which surgeons should face when informing the patients before the operation. While severe urinary dysfunction is rare, sexual impairment remains a serious concern after rectal resection with TME.

Keywords Rectal cancer · TME · Laparoscopy · Urinary · Sexual · Functional outcome

Introduction

The injury of hypogastric and pelvic splanchnic nerves during rectal cancer resection are well-known, being the reported incidence of urinary dysfunction 10–30% and erectile dysfunction 40–60%. Urinary and sexual functions are controlled by the sympathetic nerves that originate from the superior hypogastric plexus and by the parasympathetic nerves (erigentes nerves, pelvic plexus, and its branches). These nerves can be accidentally damaged during the dissection of mesorectum, when the plan of separation between the visceral pelvic fascia and the parietal pelvic fascia (the so-called "Heald's holy plane") is not strictly recognized and respected. The damage to the sympathetic nerves causes, in particular, unstable bladder and ejaculation disorders while the damage to the parasympathetic system leads to a lack of detrusor contraction and erectile problems.[1] The sympathetic component is recognized and respected in more than 90% of patients, while there is considerable variability in rates of recognition of the parasympathetic component (53–96%), which is located deeper in the pelvis.[1]

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Rectal tumors can be managed laparoscopically with total mesorectal excision (TME); in experienced hands the complication rate and short-term oncological results are comparable to those of open surgery.[2,3] However, little is known about the incidence of urinary and sexual disorders after rectal resection with laparoscopic TME. From a theoretical point of view, a magnified view of the pelvis can help the identification of autonomic nerves.

The present study examines the incidence of genitourinary dysfunctions in a consecutive series of patients who underwent laparoscopic anterior resection of the rectum with TME.

Materials and Methods

From September 1994 to December 2007, 1,216 laparoscopic interventions for colorectal diseases were performed in our department. Out of 237 resections for rectal cancer, only patients operated from 1 January 2001 to 1 January 2008 were taken into consideration. After the exclusion of 67 female patients, 36 patients aged over 70 years, 79 patients with proximal cancer (more than 12 cm from the anal margin), 27 patients undergoing Miles intervention, 26 patients with locally advanced cancer (T4) and/or metastatic disease, five patients lost to follow-up and 14 died patients, 35 young males who underwent laparoscopic anterior resection with TME for medium and low, T1-T3 rectal cancer were available for the analysis of urinary and sexual postoperative complications and represent the object of the present study.

Preoperative workup included visit with anamnesis, colonoscopy, thoraco-abdominal CT scan, CEA and CA 19.9. All patients were operated by the same team with the already described, strictly standardized, surgical technique,[4] having as basic principles the total mesorectal excision (TME), with adequate radial margins of resection, the preservation of autonomic nerves, the ligature of inferior mesenteric vessels at their origin, and minimal intraoperative manipulation of the cancer. The TME includes the complete removal of the mesorectum containing the inferior mesenteric artery and vein, fat, and lymph nodes through a precise dissection along the avascular plane comprised of the lateral and the visceral pelvic fascia. The preservation of autonomic nerves is done by identifying and saving the preaortic superior hypogastric plexus and hypogastric nerves, which join the parasympathetic nerves (nerves erigentes) to form the inferior hypogastric plexus, which is anterior and lateral in the pelvis, on both sides. From ganglion cells of the inferior hypogastric plexus, postganglionic fibers originate from the subperitoneal rectum (these fibers are cut up anyway) and to the genitourinary organs (these fibers should be respected).

The pre- and postoperative management has been carefully standardized; all the patients underwent mechanical bowel preparation (which is actually abandoned), together with a diet free of slag, antibiotic prophylaxis with cefazolin 2 g IV or piperacillin+tazobactam 4.5 g IV at anesthesia induction, venous thrombosis prophylaxis with low molecular weight heparin, and postoperative pain therapy with NSAIDs and tramadol (opioids were avoided). All patients underwent bladder catheterization before surgery; the catheter was removed on average at postoperative day 2 (range, 1–5 days). Feeding was allowed meanly on postoperative day 3. Drains were regularly positioned and removed on postoperative day 6.

All patients included in the study were personally interviewed (phone interview was avoided) by two male doctors between February and April 2009, when the mean follow-up was 27 months (range, 13–104 months), using two specific tools. Urinary function was studied by the administration of a questionnaire with seven questions—frequency, nocturia, weak urinary stream, hesitancy, intermittence, incomplete emptying, and urgency—based on the International Prostate Symptom Score (IPSS).[5] Two patients were suffering from prostatic hypertrophy before undergoing intervention and therefore were excluded from the study of urinary function. The score for each question ranged from 0 to 5. The IPSS is calculated by summing the scores assigned to each question. Sexual impairment was analyzed by means of questions about sexual function before and after surgery, investigating the sexual desire, the erectile function (presence of spontaneous and induced erections), the rigidity of erection and the possibility of penetration, the orgasmic function (orgasm and/or ejaculation), based on the modified International Index of Erectile Function.[6] One patient had already impotence before surgery, so he was excluded from the analysis.

Results

The 35 patients analyzed had a mean age at the time of the intervention of 56 years (range, 35–69 years). The tumor was located in the lower rectum (0–6 cm from the anal verge) in 21 patients and medium rectum (7–12 cm from the anal verge) in 14 patients. A neoadjuvant chemoradiotherapy was performed in 25 patients (71.4%). The average duration of the intervention was 235 min (range, 160–330 min) for resections with mechanical anastomosis and 290 min (range, 300–440 min) for those with manual anastomosis, and in no case was the initial approach converted to laparotomy. The length of the removed piece was 31.2 cm on average (range, 24–45 cm), the mean number of examined nodes was 16.5, and the percentage of complete mesorectum removal (as confirmed by the

histology) was 85.7%. At final histological examination, two patients had a tumor stage Tis, 12 T1, 7 T2, 14 T3, while seven patients had lymph node metastases. There were no intraoperative complications, and postoperative complications have been reported in nine cases, three of which required a second operation. There were no deaths (Table 1).

After the intervention, in no case was the catheter replaced after removing it; none of the patients necessitated permanent or intermittent catheterization. Table 2 shows the results of telephone interviews about the urinary function. More than half the patients had no complaints; about one third had nocturia, which is a symptom that is frequent in the general population; 72% of the patients had an IPSS less that 10, and no case of IPSS worse that 31 was recorded.

Table 3 reports the results of the analysis of sexual disorders. Among the 34 patients sexually active before surgery, sexual desire was reduced and spontaneous erectile function was impaired in almost half the cases, while induced erections were possible in about 90% of cases; about 70% of patients still had the possibility of penetration and a normal ejaculation and orgasm after the intervention.

Discussion

Before the introduction of TME, the incidence of genito-urinary complications after resection of rectal cancer was very high (30% for urinary dysfunctions and 40–50% for sexual dysfunctions),[7] due to lesions of the autonomic nerves in the pelvis and/or along the distal aorta. These rates were linked to the type of performed surgery, i.e., to the distance from the aorta of the inferior mesenteric artery section and to the level of attention and then nerve preservation.[8] In fact, when classifying the kind of nerve sparing, in cases of complete destruction of the pelvic autonomic nerves, 78% of patients do not regain bladder sensation and are discharged with an indwelling catheter and 58% of them definitively lost bladder sensation.[9] When a lateral node dissection is added, rate of severe urinary and sexual dysfunctions may also be significant, up to 27% and

Table 1 Clinical and surgical features of 35 males with T1-T3NxM0, medium, or low rectal cancer patients undergoing laparoscopic rectal resection and TME

Mean age (range)	56 (35–69)	
Neoadjuvant therapy	25 (71.4%)	
Tumor location	Medium	14 (40.0%)
	Low	21 (60.0%)
Performed anastomosis	Knight-Griffen	30 (85.7%)
	Colo-anal hand-sewn	5 (14.3%)
Temporary diverting ileal stoma	33 (94.2%)	
Mean retrieved nodes (range)	16.5 (7–57)	
Mesorectal excision	Complete	30 (85.7%)
	Partial	5 (14.3%)
R1-R2	0	
Operating time	Knight-Griffen	235 min (160–330)
	Colo-anal hand-sewn	290 min (300–440)
Conversion in laparotomy	0	
30-day mortality	0	
Major morbility, surgical morbility	9 (25.7%), 7 (20.0%)	
Anastomotic leak	4 (11.4%)	
Abdominal collection	2 (5.5%)	
Occlusion	1 (2.8%)	
Reinterventions	3 (8.5%)	

R1 microscopic residual, *R2* macroscopic residual

Table 2 Urinary symptoms after laparoscopic rectal resection with TME for rectal cancer (IPSS) in 33 patients that complained no urinary symptoms before the intervention

	Frequency (%)	Urgency (%)	Incomplete emptyng (%)	Weak stream (%)	Hesitancy (%)	Nocturia (%)	Intermittence (%)
Not at all	48.5	63.6	57.5	66.6	69.6	36.3	54.5
Less than 1 time in 5	15.1	21.2	18.1	9.1	9.1	30.3	12.1
Less than 1/2 the time	15.1	15.1	15.1	15.1	15.1	15.1	9.1
About 1/2 the time	15.1	0	3.0	3.0	6.1	15.1	9.1
More than 1/2 the time	3.0	0	0	3.0	0	3.0	6.1
Almost always	3.0	0	6.1	3.0	0	0	9.1
IPSS							
0–10	24 pts			72.7%			
11–20	7 pts			21.2%			
21–30	2 pts			6.0%			
31–35	0 pts			0%			

pts patients

44–90%, respectively,[10,11] even if specific attention is paid to nerve sparing. The concept of TME has its roots in the finding that tumors of the rectum spread from the mesorectal tissue contained in the visceral pelvic fascia. From an oncological perspective, TME is a surgical procedure associated with a lower risk of local recurrence.[12,13] TME is also associated with less urinary and sexual dysfunction, if the parietal fascia covering the sacrum is spared.[14] Recent studies—having, as objective, a specific analysis of urinary and sexual dysfunction after TME—have confirmed a reduced incidence of severe postoperative impairment, when specific attention is paid to nerve preservation; in such cases, the actual reported rate is respectively 4–10% and 5–30%.[10,11,15,16]

We should confirm a low percentage of severe urinary dysfunction, as in only one patient (3%), IPSS score was more than 20 (severe dysfunction), moreover this patient did not need intermittent or permanent catheterization. Serious urinary dysfunction such as neurogenic bladder was not encountered. This result is in agreement with recent studies. Sterck, for instance, reported that in about 90% of the patients, postoperative bladder function became normal and only 10% suffered from bladder denervation after 6 months.[15] Recording urinary dysfunctions generally do not suffer from a methodological point of view based on the interpretation of symptoms by the patient. This is why we did not try to obtain an instrumental evaluation that was not influenced by the psychological factors. Post-void residual urine and

Table 3 Sexual habits after laparoscopic rectal resection with TME for rectal cancer in 34 patients formerly normal (IIEF)

Sexual desire	Unchanged	18	52.9%
	Reduced	16	47.1%
Erectile function, spontaneous	Yes both before and after intervention	17	50%
	Yes before and not yet after the intervention	14	41.1%
	No before nor after the intervention	3	8.8%
Erectile function, induced	Yes both before and after intervention	30	88.2%
	Yes before and not yet after the intervention	4	11.8%
	No before nor after the intervention	0	0%
Stiffness and possibility of penetration	Normal	10	29.4%
	Stiffness lowered but penetration possible	14	41.1%
	Stiffness lowered and penetration impossible	8	23.5%
	Penetration impossible both before and after the intervention	2	5.8%
Ejaculation/orgasm	Both normal	23	67.6%
	Organs without ejaculation	9	26.4%
	Both absent	2	5.8%

IIEF International Index of Erectile Function

uroflowmetry are not strictly related to symptoms. For example, Kim reported a significant change in mean maximal urinary flow rate and voided volume, but not in residual volume.[17] What is really important is the symptoms score; from this point of view, rectal resection for cancer with TME usually does not impair significantly the quality of life of the patients. The only author reporting a significant increase in the total IPSS was Kim. In this series, IPSS increased after surgery from 6.2 to 9.8 (<0.05).[17] Mean postoperative IPSS score of our series was 8.9, and we cannot compare it with pre-surgery data, as a reliable scoring is impossible on the basis of a general anamnesis. The majority of patients finally did not complain significant symptoms about urinary functions after surgery.

On the other hand, sexual dysfunction after TME remains a serious problem. Previous experiences report only 31% of recovered erectile function and only 19% of recovered normal ejaculatory function in the first postoperative year in patients aged less than 60 even after a nerve sparing surgery.[9] Other series reported better data, with about 30% of preoperatively potent men having sexual dysfunction postoperatively,[15] as it was also in the Nesbakken experience (30% of patients reported some reduction in erectile function, 10% reported retrograde ejaculation, and 4% became impotent).[18] Masui reports that in 87.7% and 66.9% of patients erectile and ejaculatory potencies were maintained, and these rates were also higher when complete preserving operations were done instead of hemilateral autonomic nerve preservation (92.9% and 82.5%, respectively).[19] Pocard reports that in nine men that were sexually potent in the preoperative period, sexual activity and potency were unchanged, while retrograde ejaculation was reported in one who previously had had normal antegrade ejaculation. After 3 months, four patients reported a reduced rigidity of erection, returning to normal by 1 year.[20]

The reasons for such a wide range of complications have multiple explanations. First, frequently the preoperative genitourinary function was not evaluated; when questionnaires are submitted in a retrospective manner, the patients do analyze the problems they actually have also taking into account some psychological factors, and frequently they remember their previous sexual habits quite differently from what they really were. Second, patient groups that are analyzed are not always consistent because some series included patients undergoing rectal resection with sphincter preservation together with patients undergoing Miles intervention, that are affected by a very high incidence of sexual complications; some other studies do mix patients undergoing TME together with patients with proximal cancers, undergoing partial mesorectal excision. Furthermore, in some studies males and females were analyzed together. Third, the time at which the analysis is done is crucial, as the more frequent the postoperative follow-up the greater the chance of recovery. Significant improvements were detected also 2 years after the intervention; patients who did well from an oncological perspective should be psychologically better towards chronic functional impairment. Eventually, fecal diversion closure also plays a role in the physical integrity feeling and in the sexual performances.

In our study, starting from a large database, including more than 250 rectal cancers operated on, we did focus on a homogenous population, for which the knowledge of the real risk of having chronic postoperative sequelae is worth knowing. So, males aged less than 70, with cancers from less than 12 cm from the anal verge, undergoing restorative proctocolectomy for T1-T3 M0 tumors were analyzed. Females less frequently have functional consequences than males; the same is true for proximal cancers, in which the mesorectal excision should be done two thirds of the length of the mesorectum (the so-called partial mesorectal excision), to spare the pelvic plexus while the contrary is attended for Miles operation and bulky tumors. Finally, older patients do have frequently preoperative impairment of both urinary and sexual functions. Moreover, in this series the eventual psychological effect of persisting ileostomy was eliminated by performing the questionnaire at a long distance time (mean, 27 months; range, 13–104 months), when all but one patient had their ileostomy closed. Our series showed a rate of postoperative impotence (understood as erection not sufficient for sexual intercourse) of 23.5% and the absence of ejaculation in 11% of patients, which is in line with more recent studies. The International Index of Erectile Function domains that were more likely to be impaired were sexual desire (reduced in 47.1% of patients) and stiffness (reduced in 64.6% o f patients).

Some effort to improve nerve identification and sparing were published, for example, cavernous nerve identification and integrity before and after pelvic dissection were assessed intraoperatively, both visually by an experienced surgeon and by using the CaverMap nerve stimulator,[21] but results were similar to those offered by the simple vision. The laparoscopic approach has some advantages compared to the open. The magnification of the image helps the operator to identify the connective space interposed between the two fascia. The 30° optics can be considered as a "third eye" of the surgeon, allowing to reach the narrow lower portion of the pelvis and to perform under direct vision some maneuvers that in open surgery are under the exclusive control of touch; all this is to improve the 'identification and respect for the pelvic autonomic nerves. However, despite these obvious advantages of laparoscopy approach, some studies reported a high rate of nerve injury after laparoscopic TME. In 2002, Quah reported a higher incidence of postoperative impotence

after laparoscopy than after open approach.[22] A significant difference was noted in males, with seven of 15 sexually active men in the laparoscopic group reporting impotence or impaired ejaculation, compared with only one out of 22 patients having an open operation (P=0.004). According to the author, a possible explanation would be the highest percentage of bulky and distal tumors, requiring, more frequently, Miles intervention, and the highest rate of complete mesorectal excisions that were done in laparoscopic interventions, which would lead to an increased risk of nerve damage. A greater radicalism would result in increased nerve damage. A subsequent study, analyzing data from the UK Medical Research Council Conventional versus Laparoscopic-Assisted Surgery In Colorectal Cancer trial,[23] confirmed that overall sexual function and erectile function tended to be worse following laparoscopic TME than after open rectal surgery in men (overall function; difference, −11.18 (95% CI, −22.99 to 0.63), P=0.063; erectile function, difference, −5.84 (95% CI. −10.94 to −0.74), P=0.068). In this study too, total mesorectal excision (TME) was more commonly performed in the laparoscopic rectal group than in the open rectal group, and it was an independent predictor of postoperative male sexual dysfunction (OR, 6.38; P=0.054). In contrast, a recent study hypothesizes a significant advantage in laparoscopic approach, attributed to improvement in visibility by the magnification feature of laparoscopic surgery, reporting a rate of postoperative impotence of 5% versus 29% of the patients treated by laparotomy, while no differences were detected in urinary dysfunctions.[24] However, the number of patients in this study was low (17 versus 18). Two further series of patients undergoing laparoscopic TME with autonomic nerve preservation that were retrospectively analyzed without a control group are available. The first, taking into account the quality of surgery (it was determined that the learning curve for this surgical technique necessitated that colorectal surgeons carry out at least 20 such procedures) and the preoperative functions of the patients (only 60/98 patients were finally analyzed), reported in 32 male patients that ejaculation was good in 56.3% of the cases, fair in 18.7%, and poor in 25%. The potency was good in 62.5% of the patients, fair in 15.6%, and poor in 21.9%.[25] In the second series, including 50 patients aged less than 75, sexual desire was maintained by 55.6%, ability to engage in intercourse by 57.8%, and ability to achieve orgasm and ejaculation by 37.8% of the patients.[26] Such results are similar to those reported in our series, and confirm that laparoscopic approach does not worsen the results obtained in open experience. Probably, although there are not currently published studies on this topic, robotic surgery can achieve better results by being able to give a better view of the anatomical detail.

The high variability of results in the published studies clearly depict the extreme complexity of an analysis of clinical data that suffer the specificity of these problems, it being difficult to be collected from the patients and to be compared. We should only provide a picture of the impact of urinary and sexual problems after surgery, while a critical advice about the means to prevent such problems is no longer reliable. Meanwhile, the data we reported, confirming the occurrence of complications in the sexual function in a high percentage of cases, should certainly be clarified and formalized in the patient informed consent.

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ORIGINAL ARTICLE

Analysis of Factors Associated with Prognosis After Colorectal Cancer Resection in 174 Chinese Elderly Patients

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Abstract
Purpose The purpose of the present study was to investigate risk factors associated with prognosis in elderly patients with colorectal cancer (CRC) and to determine treatment and follow-up strategies.
Materials and Methods CRC patients (age ≥70) who were treated with curative operation were studied. We compared 57 patients whose survival time was less than 2 years with 117 patients with survival time exceeding 5 years, based on the clinical, pathologic, and preoperative clinical laboratory analysis findings. A risk scoring system on basis of factors determined by multiple logistic regression analysis was explored and validated by both receiver operating characteristic and survival analysis.
Results Neuroticism, rural residence, deep layer invasion, lymphovascular invasion, and high serum CEA levels were found to be associated with adverse prognosis in the multivariate logistic regression model. Risk scoring system based on these factors showed that the patients with total score exceeding 2.5 had a significantly poorer prognosis ($P<0.05$), which was validated by survival analysis.
Conclusions Patients with neuroticism, rural residence, deep layer invasion, lymphovascular invasion, and high serum CEA level should be regarded as a high-risk group; a simple scoring system based on these factors could be used to evaluate the risk and facilitate treatment of CRC for elderly patients.

Keywords Colorectal cancer · Elderly patients · Prognosis

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Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors in the world. Its incidence rises with increasing age.[1,2] Increasing incidence of CRC with higher age and extended life expectancy have resulted in a rapidly growing population of elderly patients with CRC coming for treatment. Currently, although people aged 70 and over account for nearly 50% of all cancer patients, less than 10% of patients over 70 years are included in clinical trials.[3–5] Therefore, the treatment efficacy and prognosis in elderly populations remain unclear.

The clinical and pathological characteristics and treatment patterns of elderly patients with CRC in the developed countries have been documented.[6–10] However, the information on tumor features and prognosis for older patients from the developing countries is relatively scarce. In

addition, the clinicopathological attributes of this disease in Asian patients are different from the rest of the world.[11] In the present study, we collected the clinical, biological, and pathological characteristics of patients over 70 years with CRC at a large institution in recent two decades. The aim of this study was to investigate the potential risk factors for elder patients by the comparison of tumor characteristics between the two groups, survival time less than 2 years and exceeding 5 years, and to determine the factors influencing prognosis and treatment decisions.

Materials and Methods

Patients

We retrospectively identified 57 patients with survival time less than 2 years and 117 patients with survival time exceeding 5 years who underwent curative operation in the Department of Colorectal Surgery of Cancer Hospital of Fudan University (Shanghai, China) between January 1988 and December 2004. The patients' age is all over 70 years, and in the present study, all consecutive cases were occurring longitudinally. All specimens examined were taken from vital cores of histopathologically confirmed cancers at primary surgery. To eliminate treatment bias, the patients who underwent chemotherapy or radiotherapy both at pre- or post-operation were excluded. Tumor samples were reviewed by at least two experienced pathologists, and tumor staging was made based on the system of the International Union Against Cancer. For survival analysis, overall survival was defined as the time from the date of the initial diagnosis to the date of patient death or last follow-up. The research protocol was approved by the Ethics Committee at Cancer Hospital of Fudan University, and all patients gave consents for the study. Patients were excluded if they had undergone surgery for colorectal disease. Characteristics of patients were shown in Table 1.

Eysenck Personality Questionnaire

We used an abbreviated version of the Eysenck Personality Questionnaire (EPQ-RSC), which is a Chinese edition of the EPQ-RS.[12] There are 48 items in the questionnaire. EPQ-RSC allows measurement of two dimensions of personality: extraversion and neuroticism. Individual scores was assessed with T value $T = 50 + 10\times$ (subjects scores − mean scores)/SD, where male/female, mean=6.89/7.28, SD=3.08/3.48; extraversion was assessed with $T \geq 56.7$, neuroticism with $T \leq 43.3$. On both scales, higher scores indicate a greater tendency to have the personality trait. Extraversion represents liveliness and sociability, whereas neuroticism represents emotional instability and anxiousness.[13]

We examined 19 factors based on clinical, pathologic, and preoperative clinical laboratory analysis findings. The clinical factors included gender, age, irregular eating habit, residence, personality, comorbid illness, cigarette smoking, and alcohol intake. The pathologic factors included location, largest tumor diameter, histopathology, differentiation, and so on. The preoperative clinical laboratory analysis factors included P53 and serum carcinoembryonic antigen (CEA) level on admission. Operation was regarded as curative when no residual cancer cells (R0) were considered to be left behind.

Statistical Analysis

The variables were compared between survival time less than 2 years and that exceeding 5 years using the chi-squared tests. Univariate and multivariate logistic regression models were applied to evaluate the effects of variables on risk of colorectal cancer. The univariate influence of binary variables on the outcome was assessed by contingence tables, Fisher's two-tailed exact test, and risk calculation (odds ratio and its 95% confidence interval). Factors found to be significant in the univariate analysis were then in a stepwise LR analysis, and the logistic function was constructed with the calculated values of the β coefficient for the LR model and for each independent variable. Factors identified to be significant in the multivariate analysis were used to establish a scoring system to predict colorectal cancer of the elderly patients. The performance of the established scoring system was evaluated by the receiver operating characteristic (ROC) curve analysis and calculation of the prediction accuracy and was further validated using subsequent survival analysis. Survival curves were generated by the Kaplan–Meier method, and univariate survival distributions were compared with the use of the log–rank test. A P value of <0.05 was considered statistically significant. All statistical analyses were conducted using the statistical program, SPSS version 13.0 (SPSS Inc., Chicago, IL).

Results

Univariate Predictors

A total of 169 patients from 70 to 88 years old were included in this study. There were 26 males and 31 females with the average age of 74.25±6.77 years in survival time less than the 2-year group, and 61 males and 51 females with the average age of 73.90±4.42 years in survival time

Table 1 Patients' and tumors' characteristics stratified by survival time of the elderly colorectal cancer patients

Characteristics	Categories	≤2 years (n=57)	≥5 years (n=112)	P value[a]
Age (years)	70–80	52 (91%)	103 (92%)	0.840
	≥80	5 (9%)	9 (8%)	
Sex	Male	26 (46%)	61 (55%)	0.330
	Female	31 (54%)	51 (45%)	
Largest tumor diameter	<5cm	29 (51%)	41 (37%)	0.100
	≥5cm	28 (49%)	71 (63%)	
Histopathology	Adenocarcinoma	48 (84%)	93 (83%)	0.730
	Mucinous adenocarcinoma	7 (12%)	12 (11%)	
	Signet ring cancer	2 (4%)	7 (6%)	
Differentiation	Well-moderate	43 (75%)	94 (84%)	0.210
	Poor	14 (25%)	18 (16%)	
pT stage	T1	13 (23%)	15 (13%)	0.016*
	T2	10 (18%)	29 (26%)	
	T3	13 (23%)	45 (40%)	
	T4	21 (37%)	23 (21%)	
pN stage	N0	17 (30%)	91 (81%)	0.000*
	N1	14 (25%)	14 (13%)	
	N2	26 (45%)	7 (6%)	
Retrieved lymph node	<12	35 (61%)	51 (46%)	0.070
	≥12	22 (39%)	61 (54%)	
Perineural invasion	Yes	11 (19%)	32 (29%)	0.260
	No	46 (81%)	80 (71%)	
Lymphovascular invasion	Yes	11 (19%)	44 (39%)	0.036*
	No	46 (81%)	68 (61%)	
Localization	Right hemicolon	9 (16%)	19 (17%)	0.840
	Transversum	3 (5%)	8 (7%)	
	Left hemicolon	4 (7%)	4 (4%)	
	Colon sigmoideum	8 (14%)	19 (17%)	
	Rectum	33 (58%)	62 (55%)	
Irregular eating habit	Absent	31 (54%)	59 (53%)	0.871
	Present	26 (46%)	53 (47%)	
Residence	Urban	11 (19%)	44 (30%)	0.025*
	Rural	46 (81%)	78 (70%)	
Personality	Neuroticism	43 (75%)	57 (51%)	0.003*
	Extroversion	14 (25%)	55 (49%)	
Alcohol intake	Absent	33 (58%)	63 (56%)	0.871
	Present	24 (42%)	49 (44%)	
Cigarette smoking	Absent	37 (65%)	71 (63%)	0.867
	Present	20 (35%)	41 (37%)	
Comorbid illness	Hypertension	13 (23%)	28 (25%)	0.123
	Diabetes	7 (12%)	13 (12%)	
	Cardiovascular disease	9 (16%)	34 (30%)	
	No comorbidity	28 (49%)	37 (33%)	
CEA	≤5mg/L	16 (28%)	63 (56%)	0.001*
	>5mg/L	41 (72%)	49 (44%)	
P53	−−−	36 (63%)	79 (71%)	0.453
	+−−	11 (19%)	11 (10%)	
	++−	4 (7%)	9 (8%)	
	+++	2 (4%)	7 (6%)	
	Not available	4 (7%)	6 (5%)	

[a] P value are obtained from χ^2 test

*P<0.05, statistically significant

exceeding the 5-year group. The median follow-up time was 52.55 months (range 4.20–181.30 months).

The univariate analysis (Table 2) demonstrated that factors significantly associated with prognosis were histopathology ($P<0.001$), pT stage ($P=0.010$), pN stage ($P=0.030$), lymphovascular invasion ($P=0.011$), residence ($P=0.013$), personality ($P<0.001$), and serum CEA level ($P=0.012$). Having tumors with signet ring cancer or with poor T/N stage or with lymphovascular invasion or with elevated serum CEA level and so on were indicators of poor prognosis of CRC. The histopathology was categorized into three subsets (adenocarcinoma, mucinous adenocarcinoma, and signet ring cancer), and the univariate binary logistic regression analysis demonstrated that the proportion of patients with poor prognosis differed significantly between any two subsets. There were no significant differences in gender, age, or differentiation ($P>0.05$).

Multivariate Predictors

Further multivariate analysis (Table 2) showed that five variables (pT stage, lymphovascular invasion, residence, personality, and serum CEA level) were significantly correlated with prognosis while histopathology and pN stage were not independently risk factors ($P>0.05$).

Risk Scoring System

To evaluate the risk factors identified to be significant in the multivariate analysis, we explored a scoring system to predict the prognosis of the elderly CRC patients. These five variables were incorporated into a risk scoring system by assigning points to various features according to their odds ratios (OR) values in the multivariate analysis (Table 2), as listed in Table 3. A score of 0 was assigned to features with OR value less than 3, score 1 for those with OR value exceeding 3, and score 2 for those with OR value exceeding 10. Points of these five variables were then totaled to yield an overall risk score. The mean total score was 2.2±0.1 points for patients with survival time less than 2 years and 0.7±0.1 points for those with survival time exceeding 5 years, which had a significantly lower total score than the former ($P<0.05$, t test). The probability of having poor prognosis of CRC increased stepwise with the total score (Table 3). The performance of the established value of scores was evaluated by the ROC analysis and calculation of the prediction accuracy. The ROC curve is the graphic representation of this reciprocal relationship between sensitivity and specificity, calculated for all possible threshold values, each operating point on the ROC curve represents the combination of sensitivity and

Table 2 Uni- and multivariate analysis of prognosis factors for elderly colorectal cancer patients

Selected variable	OR	95% CI	*P* value
Univariate analysis			
Sex	0.625	0.346–1.133	0.122
Age group	0.691	0.271–1.756	0.620
Largest tumor diameter	0.814	0.482–1.384	0.410
Histopathology	1.432	0.755–2.602	<0.001*
Differentiation	1.494	0.814–2.755	0.780
pT stage	0.513	0.243–1.082	0.010*
pN stage	1.121	0.803–1.575	0.030*
Perineural invasion	0.923	0.463–1.792	0.600
Lymphovascular invasion	0.382	0.181–0.798	0.011*
Residence	1.893	1.198–3.515	0.013*
Irregular eating habit	1.078	0.663–1.745	0.746
Personality	8.745	3.688–18.034	<0.001*
Alcohol intake	0.916	0.671–1.251	0.581
Cigarette smoking	1.245	0.864–1.543	0.316
Histological type	0.865	0.683–1.276	0.596
CEA	4.343	1.786–8.953	0.012*
Multivariate analysis			
pT stage (T3–4 vs. T1–2)	3.106	1.179–8.135	0.014*
Lymphovascular invasion (Yes vs. No)	2.764	1.132–6.752	0.032*
Residence (rural vs. urban)	3.483	1.633–7.417	<0.001*
CEA (>5 vs. ≤5 mg/L)	3.532	1.543–9.432	0.006*
Personality (neuroticism vs. extroversion)	12.796	5.943–27.687	0.019*

*$P<0.05$, statistically significant

Table 3 Distribution of groups in various total scores

Total points	0 [no. (%)]	1 [no. (%)]	2 [no. (%)]	3 [no. (%)]	4 [no. (%)]
≤2 years	4 (5.6)	5 (25.0)	26 (53.1)	19 (76.0)	3 (75.0)
≥5 years	67 (94.4)	15 (75.0)	23 (46.9)	6 (24.0)	1 (25.0)

specificity at a given threshold value.[14] In this study, ROC analysis showed that the best cutoff value to distinguish was 2.5. The area under ROC (AUC) which is a measure for the diagnostic accuracy was 0.751 (95% confidence interval (CI) 0.659–0.843, $P<0.05$) (Fig. 1). When the cutoff value was set at 2.5, the Youden's index (sensitivity+specificity−1) reached the largest value (0.415). The sensitivity was 56.0%; the specificity was 85.5%; the prediction accuracy was 69.9%.

Patients' Survival

Survival curves were generated by the Kaplan–Meier method, and univariate survival distributions were compared with the use of the log–rank test. Firstly, the elderly patients with CRC were categorized into five subgroups in accordance with overall risk score (0, 1, 2, 3, 4). Kaplan–Meier curves showed significantly survival difference among five subgroups ($P<0.05$) (Fig. 2). The prognosis of elderly patients with CRC worsened with the risk score increasing. The 5-year survival rates in score 0–4 were 72.7%, 66.9%, 55.2%, 15.6%, and 20%, respectively ($P<0.05$). The median survival times of the five subgroups were 85.4, 82.7, 62.0, 40.4, and 22.2 months, respectively. Subsequently, according to cutoff value of 2.5, we divided the patients into two subgroups (>2.5 and <2.5) to perform a survival analysis. As shown in Fig. 3, there are significant differences on overall survival between two subgroups ($P<0.05$). The median survival times of the two subgroups were 65.4 and 39.7 months, respectively.

Discussion

Colorectal cancer is one of the most common cancers in the elderly,[15] and its incidence rises with increasing age.[1,2] Currently, the majority of colon and rectum tumors arise in patients aged 70 and over; however, data showed that these patients received insufficient treatment for CRC.[16] Although many factors may account for the reluctance to receive standard cancer treatment in elderly CRC patients, such as elderly people have a limited life expectancy, comorbid problems may complicate or even preclude operation and chemotherapy, elderly patients may be more susceptible to side-effects of operation and chemotherapy which may decrease their quality of life,[17] and socioeconomic condition and studies on the efficacy and toxicity of chemotherapy in elderly group are limited,[18,19] recent study has shown that colorectal surgery appears to be safe in most elderly patients.[9] The aim of the present study

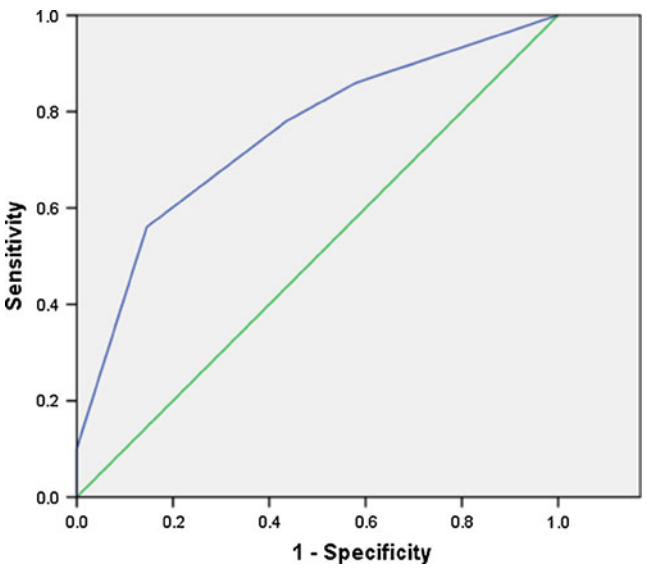

Fig. 1 ROC curve of total scores of clinicopathologic parameters to evaluate calculation of the prediction accuracy. AUC=0.751 (95% CI= 0.659–0.843); $P<0.05$

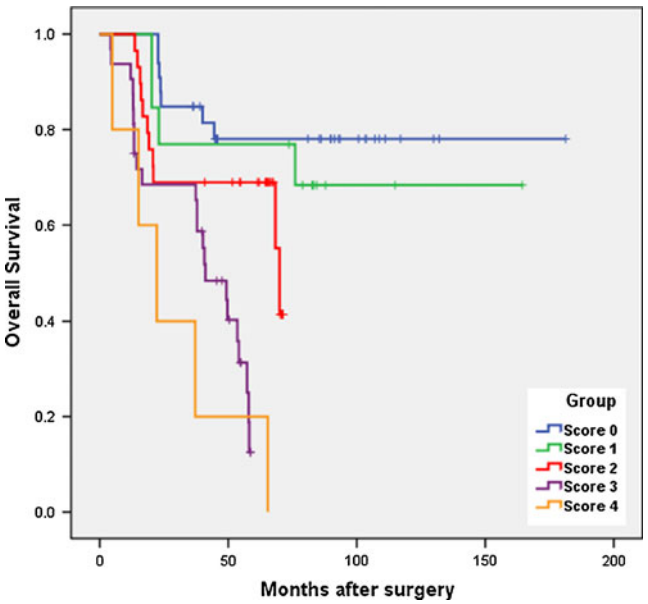

Fig. 2 Kaplan–Meier curves showed significantly survival difference among five subgroups in accordance with overall risk score (0, 1, 2, 3, 4) ($P<0.05$)

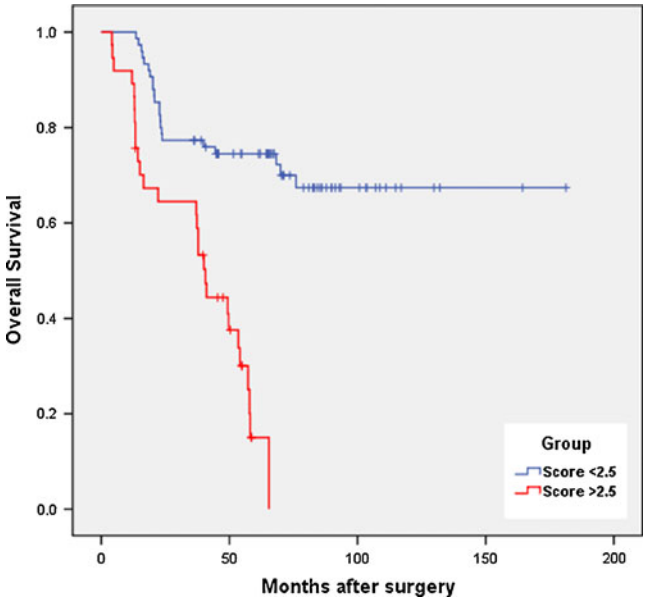

Fig. 3 Kaplan–Meier curves showed significantly survival difference between two subgroups in accordance with cutoff value 2.5 ($P<0.05$)

was to identify the risk factors of the elderly patients (≥70 years) by extreme analysis and establish a simple scoring system based on these factors to predict prognosis and guide therapy for elderly patients.

The present study documented that neuroticism, rural residence, deep layer invasion, lymphovascular invasion, and high serum CEA level were positively related to adverse prognosis for elderly patients. It has been claimed that neuroticism is thought to be more prone to chronic stress. Chronic psychosocial stress is thought to affect lifestyle and the immune system[20,21] and may thus contribute to the development of cancer. The colorectum is known to be an organ sensitive to stress.[22,23] Kune et al.[24] found an increased risk of colorectal cancer associated with a personality profile, characterized by repression, denial, non-expression of anger, social desirability, conflict avoidance, and the suppression of reactions that may offend others. As known, the elderly patients were a special cohort with a high morbidity of mental illness. Two processes are thought to mediate the link between psychosocial factors of the elderly patients and the development of CRC: one was that the aged people susceptible to chronic stress are associated with unfavorable lifestyle factors, such as smoking, alcohol consumption, lack of physical activity, obesity, and unhealthy eating.[25–27] The other possibility was that chronic stress may have an impact on the neuro-endocrinoimmunological network that may lead to the development of cancer via immunosuppression.[21]

Like many cancers, CRC might involve a complex interplay of risk factors, including biologic and social conditions. Evidence has been mounting to indicate that area-based or neighborhood socioeconomic characteristics are associated with cancer outcomes.[28] Studies for several cancer sites have shown that individuals living in poor areas are more likely not to utilize cancer screening services and present at a late stage compared with individuals living in affluent areas.[29] In addition, several studies have detected geographic disparities in stage at diagnosis for breast, prostate, and CRC. The geographic areas noted in these studies as having a high incidence of late stage disease were predominately lower-income areas.[30] In the rural areas of China, the individual's income and education level is relatively lower compared to those in urban areas.[31] In this study, it was shown that the elderly patients living in rural areas have poorer survival. Several surveys of rural residents have identified numerous barriers to obtaining preventative health care services including lack of health insurance, long travel distances, low health literacy, education levels, and language barriers, and these impact an individual's ability to navigate the medical system, understand screening options and recommendations, and communicate with healthcare professionals.[32]

Depth of invasion is one of the most important prognostic factors of CRC.[33] When we focused on prognosis of the elderly patients with CRC, depth of invasion was also identified as a risk factor. This result is consistent with previous work documenting the inverse relationship between depth of invasion and survival.[34] Lymphovascular invasion (LVI) has been widely acknowledged as a useful independent pathological indicator for predicting prognosis in colorectal cancer. Patients with LVI usually have a higher chance of disease progression and poorer prognosis.[35] In the present study, we also found that the presence of lymphovascular invasion was a negative factor, signifying that poor pathologic features are closely related to poor consequence.

We identified preoperative high serum CEA level (>5μg/L) as a risk factor for prognosis of the elderly patients with CRC. The majority of previous studies evaluating CEA levels preoperatively revealed it to be an important and independent prognostic indicator of survival[36] with patients having an elevated preoperative level having decreased 5-year survival. Although the use in the management of patients with colorectal cancer remains controversial, preoperative CEA elevation was a highly significant prognostic factor for survival, and it was confirmed by multivariate analysis in the present study. Additionally, Carriquiry and Piñeyro reported that a preoperative elevated CEA level was associated with a 3.74 relative risk of recurrence,[37] suggesting that CEA may play an important biological role in the development of cancer.

Comorbid disease and exercise tolerance are reported to be important factors for survival in most colorectal cancer studies; however, conflicting finding was observed in the

present study. The reasons for the discrepancy may partly include the following: (a) selection bias that could have had an effect. Elder patients with severe comorbid illness were excluded in our study because they are less likely to be given operation than younger patients; and (b) relatively small sample sizes may also influence the results. To achieve more accurate insight into the quality of care received by older patients, it would be useful to further evaluate the impact of comorbidity in a large scale study.

In the present study, we identified several prognostic factors of CRC for the elderly patients and evaluated the validity of them by both ROC curve and survival analysis. We anticipate that these findings may have implications for the management of the elderly patients with CRC. First, to select out high-risk CRC patients and maximize the benefits of adjuvant therapy, these independent prognostic markers could potentially be helpful in identifying aggressive phenotypes with CRC. Second, more intensive strategies of follow-up may be explored in patients harboring these factors, especially for those living in rural areas.

Conclusion

In conclusion, clinicopathologic features including neuroticism, rural residence, deep layer invasion, lymphovascular invasion, and high serum CEA level were inversely related to prognosis for elderly patients. Based on these factors, a simple scoring system could be used to evaluate the risk and facilitate treatment of CRC for elderly patients.

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Conflict of Interest None.

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ORIGINAL ARTICLE

The Potential of Osteopontin as a Therapeutic Target for Human Colorectal Cancer

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Abstract
Objective Osteopontin (OPN), a phosphorylated glycoprotein, is involved in tumor progression and metastasis. Previously, we have reported that high OPN mRNA expression level possessed clinicopathological or prognostic significance in human colorectal cancer (CRC). The aim of this study is to investigate whether OPN can serve as a novel molecular target for CRC therapy.
Material and Methods Western Blot assay was performed to detect the expression of OPN protein in 18 CRC and corresponding nontumor colon tissue samples. RNA interference (RNAi) was employed to knockdown endogenous OPN expression in CRC cell line (LoVo). MTT, colony formation, and tumorigenicity assays were performed to analyze the effect of OPN downregulation on the in vitro and in vivo proliferation of CRC cells. Wound healing and Matrigel invasion assays were performed to analyze the effect of OPN downregulation on migration and invasion of CRC cells. A clonogenic cell survival assay after radiation was performed to analyze the effect of OPN downregulation on the radiosensitivity of CRC cells.
Results The relative level of OPN protein expression in CRC tissues was significantly higher than that in corresponding nontumor colon tissues ($P<0.05$). We found that RNAi-mediated OPN downregulation could inhibit not only in vitro proliferation but also in vivo tumorigenicity of CRC cells. In addition, OPN downregulation could suppress in vitro invasion capacity and enhance in vitro radiosensitivity of CRC cells, which might be associated with decreased levels of MMP-2 and -9 expression.
Conclusion RNAi-targeting OPN could inhibit proliferation, invasion and enhance radiosensitivity of human CRC cells. Therefore, OPN could serve as a novel molecular target for gene therapy of CRC.

Keywords Osteopontin · Colorectal cancer · RNA interference · Invasion · Radiosensitivity

Introduction

Colorectal cancer (CRC) is the third most common malignancy around the world. Annually, over 945,000 people develop CRC around the world, and around 492,000 patients die from the disease. [1] The number of deaths from CRC has decreased as a result of improved tests that allow early detection of the cancer, when it can be more easily treated. Current treatment of CRC mainly includes surgery and chemotherapy along with radiotherapy. [2] Despite those advances in clinical treatment, the prognosis of CRC patients especially with metastasis still remains very poor. Therefore, there is an urgent need to develop new therapeutic targets and strategies, both of which can be realized through an increased understanding of the molecular mechanisms governing CRC progression.

OPN is a secreted multifunctional glyco-phosphoprotein, which plays important roles in many biological processes, such as inflammation, angiogenesis, tissue remodeling, tumor progression, and metastasis. [3] The overexpression of OPN has been found in a variety of human cancers such

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as lung cancer, breast carcinoma, esophageal cancer, endometrial cancer, gastric cancer, and malignant pleural mesothelioma. [4–9] Previously, we reported that the level of OPN mRNA expression in CRC cells or tissues was significantly higher than that in normal intestinal epithelial cell line or corresponding nontumor colon tissues. Additionally, we also showed that the expression level of OPN mRNA was significantly associated with lymph node metastasis, lymphatic invasion, venous invasion, and TNM stage. [10] However, whether OPN can serve as a novel therapeutic target for CRC has not been fully investigated. In the present study, RNA interference (RNAi) strategy was employed to downregulate the expression of OPN gene in CRC cells and investigate the effects of OPN downregulation on proliferation, invasion, and radiosensitivity of CRC cells.

Materials and Methods

Tissue Samples

A total of 18 CRC and corresponding nontumor colon tissue samples were obtained from patients who underwent surgical operation at the Department of Surgery in the Affiliated hospital of Nanjing Medical University. None of the patients received preoperative treatment such as radiation or chemotherapy. Using the American Joint Committee on Cancer TNM classification, tumors were classified as stage I in 11 specimens (61.1%), stage II in four (22.2%), and stage III in three (16.7%). All samples were obtained from patients who gave informed consent to use excess pathological specimens for research purposes only. Specimens were frozen and stored in liquid nitrogen until use. The Nanjing Medical University Medical Center Institutional Review Board granted permission for the study.

Plasmid Construction

The cDNA sequence of OPN was obtained from GenBank (J04765.1). The potential target sequences for RNA interference were scanned with the siRNA Target Finder and Design Tool available at the Ambion, Inc. website. OPN–shRNA (corresponding to osteopontin 1,186–1,203 bp) and control–shRNA were designed and synthesized as follows: shRNA/OPN (sense, 5′-*GATCCGAAACTCCCTGTAAACTAATTCAA-GAGATTAGTTTACAGGGAGTTTCTTTTTTGTC-GACA*-3′), and shRNA/control (sense, 5′-*GATCC AATCAATGACCTCATCGAATTCAAGAGATT-CGATGAGGTCATTGATTTTTTTTGTCGACA*-3′). All of above sequences were inserted into the BamHI and HindIII sites of pSilencer4.1-CMVneo plasmid (Ambion, USA). The successfully constructed plasmid vectors were named pSilencer-shRNA/OPN and pSilencer–shRNA/control, respectively.

Transfection

LoVo cells (at a density of 2.0×10^5 cells/well) were plated in 6-well culture plates and then were transfected with pSilencer–shRNA/OPN or pSilencer–shRNA/control vector using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's instructions. Stable transfectants were selected with G418 (800 mg/ml) for 14 days. The two stable transfectants were named LoVo–shRNA/OPN and LoVo–shRNA/control, respectively.

Reverse Transcription-Polymerase Chain Reaction

Total RNA was extracted from cells or tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) and was reverse transcribed using reverse transcription-polymerase chain reaction (RT-PCR) kits (Applied Biosystems, Foster City, CA, USA). The primers of OPN, MMP-2 and -9, were designed as follows: sense 5′-*AGTTCTGAGGAAAAG-CAGC*-3′; reverse 5′-*CCCCTACCGGAACATACG*-3′; MMP-2, sense 5′-*ACCTGGATGCCGTCG-TGGAC*-3′; reverse 5′-*TGTGGCAG-CACCAGGGCAGC*-3′; MMP-9, sense 5′-*CTTTGTAGGGTC-GGTTCTG*-3′; reverse 5′-*CCTGTGAGTGGGTTGGATT*-3′. The primers of β-actin (an internal control) were designed as follows: sense, 5′-*TGACGGGGTCACCCACACTGTGCC-CATC*-3′; reverse, 5′-*CTAGAAGCATTTGCGGTGGACG*-3′. PCR conditions were 30 cycles consisted of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. Each PCR product was separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Western Blot Assay

Protein was extracted and SDS-PAGE performed as previously described. [11] OPN, MMP-2 and -9, and β-actin antibodies were from Santa Cruz Biotechnology (CA, USA). Western blot quantification was performed using ImageJ software (Image Processing and Analysis in Java). All Western blot experiments were performed in triplicate.

MTT Assay

The mock or stably transfected LoVo cells were cultured in 96-well plates and were harvested for a standard tetrazolium bromide (MTT) assay as previously described. [12] All assays were performed in octuplicate and repeated at least three times.

Colony Formation Assay

Approximately 1.0×10^3 mock or stably transfected LoVo cells were plated in 10-cm culture dishes. After 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were manually counted. Visible colonies were manually counted.

Tumorigenicity Assay

For tumorigenicity assays, suspensions of 6.0×10^6 cells in a volume of 0.1 ml of PBS were injected s.c. into the second right mammary fat pad of 7–8-week-old female athymic nude mice which were maintained under pathogen-free conditions. The inoculations were done in five mice for each group. Tumor diameter was measured in two dimensions with a caliper twice a week. The mean tumor volume was measured and calculated according to the formula: $V = 0.4 \times D \times d^2$ (V volume, D longitudinal diameter, d latitudinal diameter).

Wound-Healing Assay

When mock or stably transfected LoVo cells were seeded and grown to confluence, a scratch was set with a pipette tip running though the dish and cultured under standard conditions for 72 h. Plates were washed twice with fresh medium to remove non-adherent cells and then photographed.

In vitro Matrigel Invasion Assay

Invasion assay was performed in a 24-well transwell chamber (Costar, Bodenheim, Germany). The 8-lm pore inserts were coated with Matrigel (Becton Dickinson Labware, Bedford, MA, USA) and used to perform matrigel invasion assay as previously described. [13]

Clonogenic Survival Assay

The mock or stably transfected LoVo cells were seeded in triplicate at limiting dilutions in 6-well plates for about 24 h in DMEM medium supplemented with 10% FBS until attached. 24 h later, the cells were using to performed to clonogenic survival assay as previously described. [14]

Statistical Analysis

Statistical analysis was carried out using SPSS 13.0 software. Values were presented as means±SE. We used ANOVA to compare mean values. A probability level of 0.05 was chosen for statistical significance.

Results

Detection of OPN Protein Expression in CRC and Corresponding Nontumor Colon Tissues

Western Blot assay was performed to detect the expression of OPN protein in 18 cases of CRC and corresponding nontumor colon tissue samples. As shown in Fig. 1, the average level of OPN protein in CRC tissues (1.14±0.08) was significantly higher than that in corresponding non-tumor colon tissues (0.37±0.12; $P<0.05$).

The Effect of OPN Downregulation on In vitro Proliferation of CRC Cells

To detect the effect of pSilencer–shRNA/OPN on OPN gene expression, we established stable transfectants by G418 selection. Compared with mock LoVo cells, LoVo–shRNA/OPN cells showed a decreased level in OPN

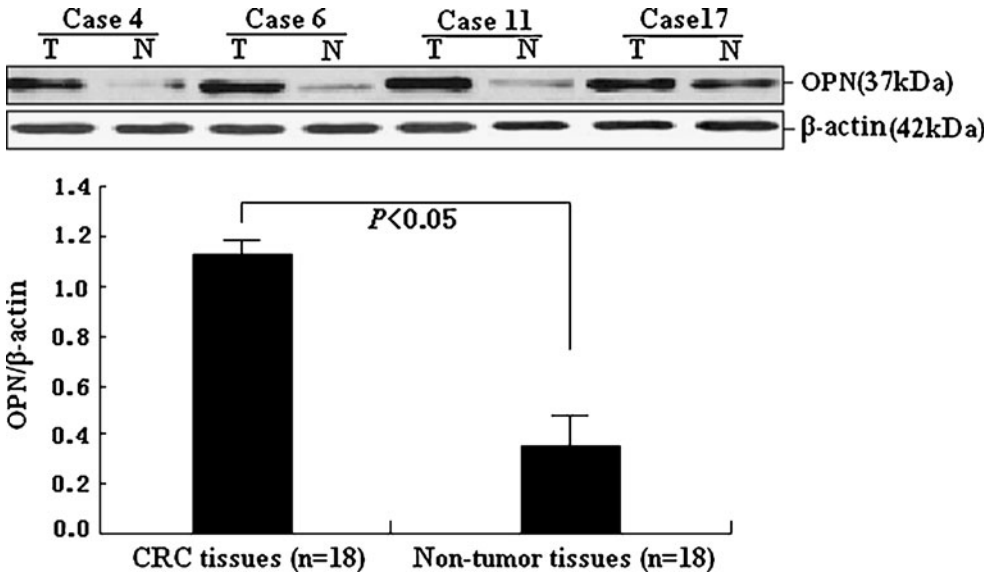

Fig. 1 Western blot analysis of OPN protein expression in 18 CRC and corresponding nontumor colon tissue samples. The average level of OPN protein expression in CRC tissues was significantly higher than that in corresponding nontumor colon tissues ($P<0.05$). β-actin was used as an internal control. *T* tumor tissues, *N* nontumor colon tissues

mRNA and protein expression levels (Fig. 2a). To analyze the effect of OPN knockdown on in vitro proliferation of CRC cells, MTT and colony formation assays were performed. As shown in Fig. 2b, RNAi-mediated OPN downregulation could significantly decrease the growth rate of tumor cells in a time-dependent manner ($P<0.05$). Meanwhile, the number of colonies developed from LoVo–shRNA/OPN cells was significantly lower than that developed from LoVo–shRNA/control or mock LoVo cells ($P<0.05$; Fig. 2c). These results showed that RNAi-mediated OPN downregulation could significantly inhibit in vitro proliferation of CRC cells.

The Effect of OPN Downregulation on In vivo Tumorigenicity of CRC Cells

Then, we explored the effect of OPN downregulation on the tumorigenicity of LoVo cells in nude mice. Subcutaneous injection of mock or stably transfected LoVo cells into athymic nude mice was performed. To confirm OPN knockdown by siRNA, tumor homogenates were subjected to immunohistochemistry of OPN protein. The staining of OPN protein expression in tumors developed from LoVo–shRNA/OPN cells was significantly weaker than that in tumors developed from control cells (Fig. 3a). Compared with that of tumors from the control cell groups, the average volume of tumors from LoVo–shRNA/OPN group was markedly reduced ($P<0.05$; Fig. 3b). At day 28, the average weight of the tumor masses from LoVo–shRNA/OPN group was lower than that from control cell groups ($P<0.05$; Fig. 3c). These suggested that RNAi-mediated OPN downregulation could significantly inhibit in vivo proliferation of CRC cells.

The Effect of OPN Downregulation on In vitro Invasion and Migration of CRC Cells

In Matrigel transwell assay (Fig. 4), a representative experiment demonstrated a marked reduction in the invasion of LoVo–shRNA/OPN cells compared with control cells (mock LoVo and LoVo–shRNA/control). The number of LoVo–shRNA/OPN cell that invaded through Matrigel-coated membrane was significantly lower than that of control cells ($P<0.05$). In cell scratch wound healing assay, the speed of LoVo–shRNA/OPN cells migrated to the scratched area was significantly smaller than that of mock LoVo or LoVo–shRNA/control cells (Fig. 5). OPN downregulation could significantly inhibit in vitro invasion

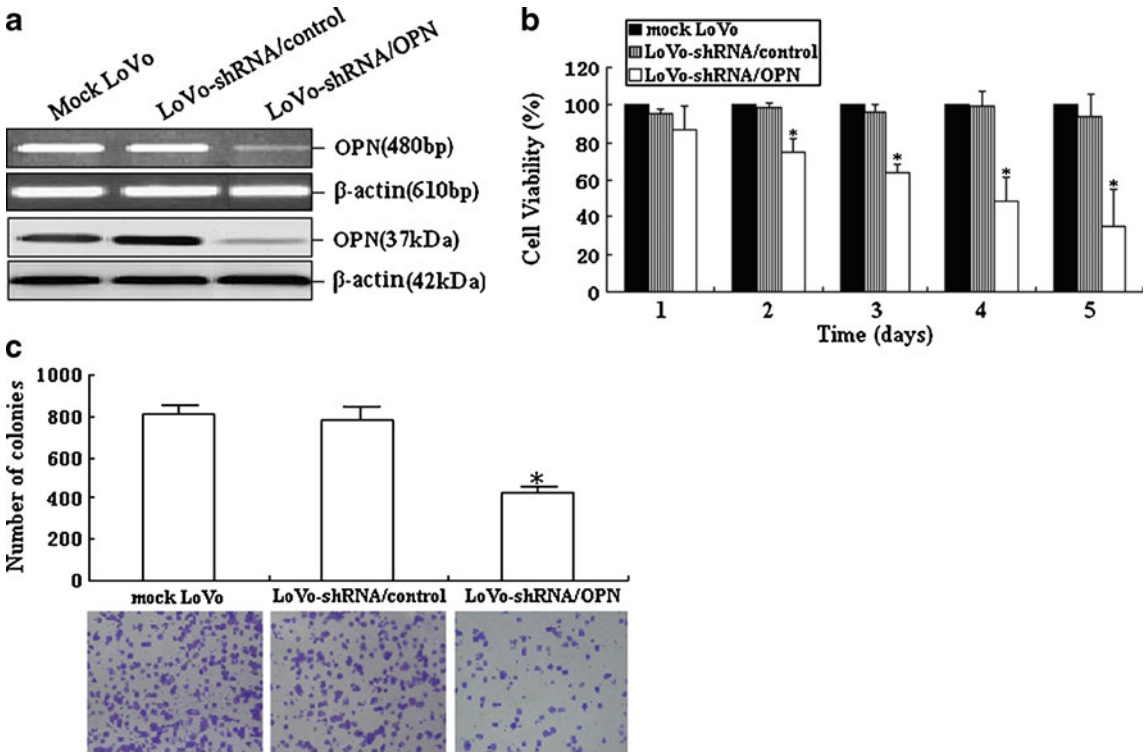

Fig. 2 siRNA-mediated OPN inhibition on in vitro proliferation and colony formation of LoVo cells. **a** RT-PCR and Western blot analysis of OPN mRNA and protein expression in mock (LoVo) or stably transfected LoVo cells (LoVo–shRNA/control and LoVo–shRNA/OPN). **b** MTT analysis of the cell viability of mock LoVo, LoVo–shRNA/control or LoVo–shRNA/OPN cells. **c** The results of colony formation assay. The LoVo–shRNA/OPN cells showed much less colonies than mock LoVo or LoVo–shRNA/control cells. All experiments were performed in triplicate, *$P<0.05$

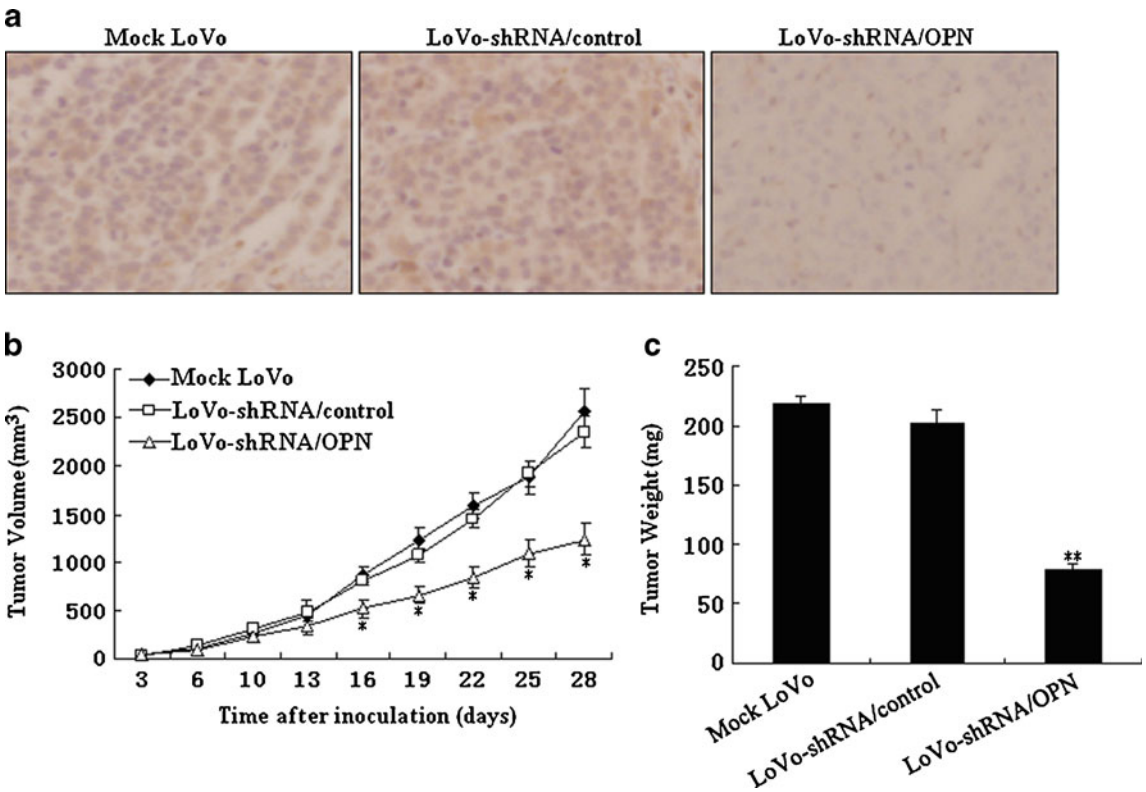

Fig. 3 Determining in vivo tumorigenicity of LoVo cells. **a** Immunostaining of OPN protein in tumors developed from mock LoVo, LoVo–shRNA/control or LoVo–shRNA/OPN cells. **b** Proliferation of tumors in the mice injected with mock LoVo, LoVo–shRNA/control or LoVo–shRNA/OPN cells. **c** Average tumor weight at day 28 after the inoculation of mock LoVo, LoVo–shRNA/control or LoVo–shRNA/OPN cells. *$P<0.05$, **$P<0.01$

and migration of LoVo cells. Liu, et al.[15] have reported that OPN could induce MMP-2 and -9 expressions via NF-κB-mediated signaling pathways in prostate cancer. Meanwhile, the expression of MMP-2 and -9 has been found to be correlated with angiogenesis and metastasis of colorectal cancer. [16] Herein, we investigated the effects of OPN downregulation on the level of MMP expression in LoVo cells. Compared with control cells, LoVo–shRNA/OPN

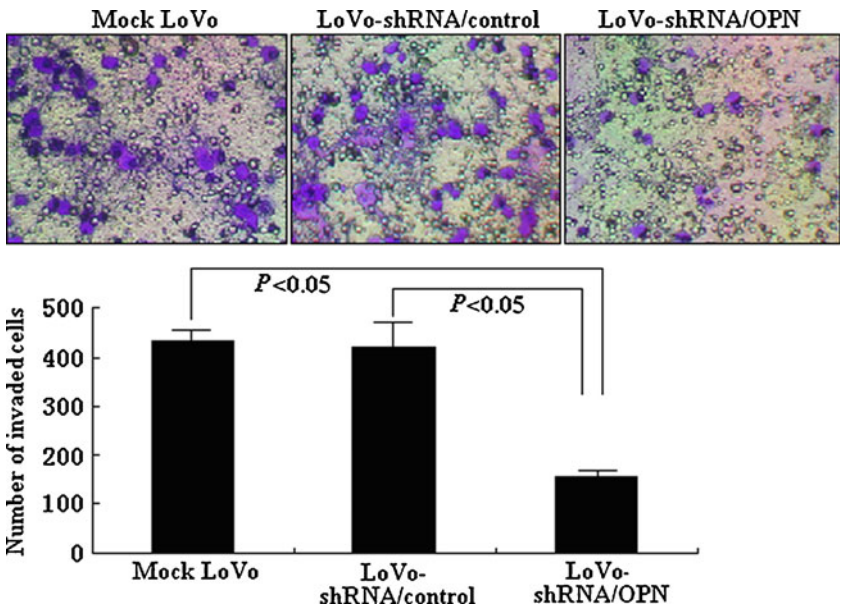

Fig. 4 Analysis of OPN inhibition on transwell invasion of LoVo cells. The invasive LoVo cells were stained and counted under microscope. Quantitative results for the transmembrane ability of each group of cells. *$P<0.05$, statistically significant difference between LoVo–shRNA/OPN cells and mock LoVo or LoVo–shRNA/control cells

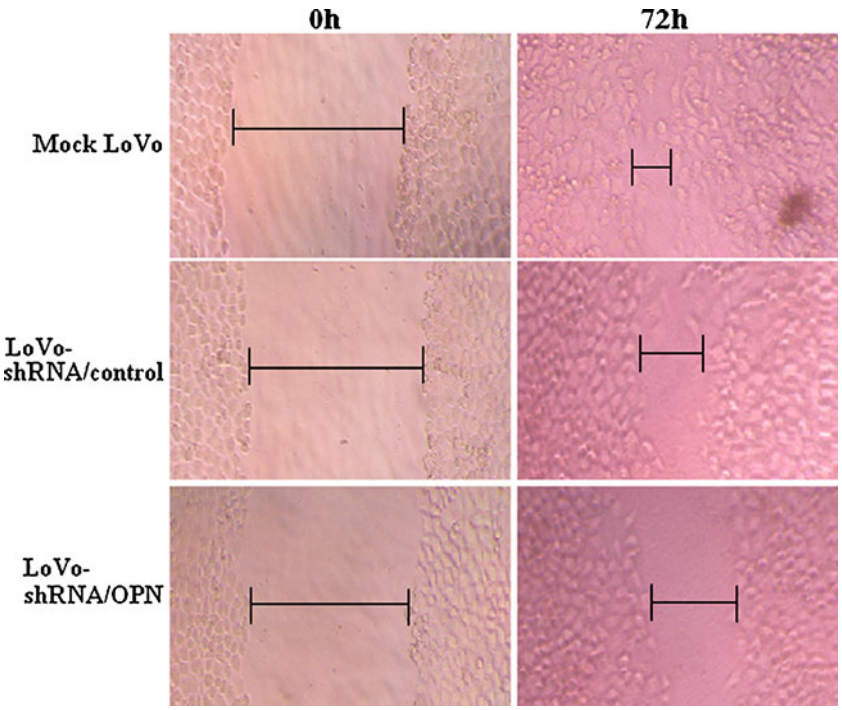

Fig. 5 Analysis of OPN inhibition on in vitro migration of LoVo cell; 72 h after wounding, cells with extended membrane protrusion moved into the wounded areas

cells showed a decreased level in MMP-2 or -9 mRNA and protein expression ($P<0.05$; Fig. 6). Thus, OPN downregulation could inhibit the MMP (MMP-2 and -9) expression in CRC cells, which might lead to decreased invasion capacity of CRC cells.

The Effect of OPN Downregulation on In vitro Radiosensitivity of CRC Cells

To detect the effect of OPN downregulation on in vitro radiosensitivity of LoVo cells, clonogenic survival assay was performed. Then, the survival rates of the mock or stably transfected LoVo cells after X-ray irradiation (radiation dose range: 0–20 Gy) were determined. The 50% effective dose (ED_{50}) of the mock LoVo, LoVo–shRNA/control, and LoVo–shRNA/OPN cells was 16.8, 15.2, and 9.82 Gy, respectively. The radiosensitivity of the LoVo–shRNA/OPN cells significantly improved compared to the radiosensitivity of mock LoVo or LoVo–shRNA/control cells ($P<0.05$; Fig. 7). OPN downregulation could induce radiosensitivity enhancement in CRC cells.

Discussion

Evidence has shown that the high levels of OPN expression can promote tumor progression and cell survival. [17] Multiple and complex mechanisms have been reported to be involved in the role of osteopontin in cancer, including

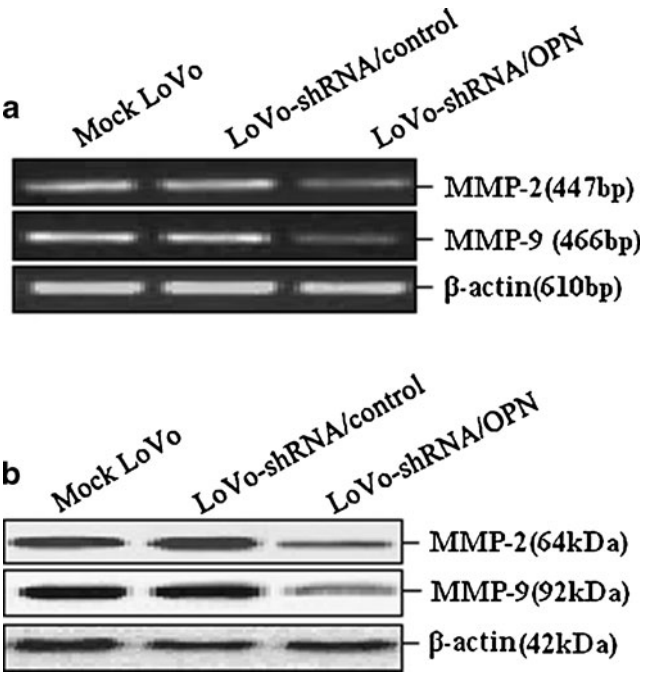

Fig. 6 Analysis of MMPs expression in LoVo cells. RT-PCR (**a**) and Western blot (**b**) analysis of MMP-2 and -9 mRNA and protein expression in mock (LoVo) or stably transfected LoVo cells (LoVo–shRNA/control and LoVo–shRNA/OPN). β-actin was used as an internal control. All experiments were performed in triplicate, $*P<0.05$

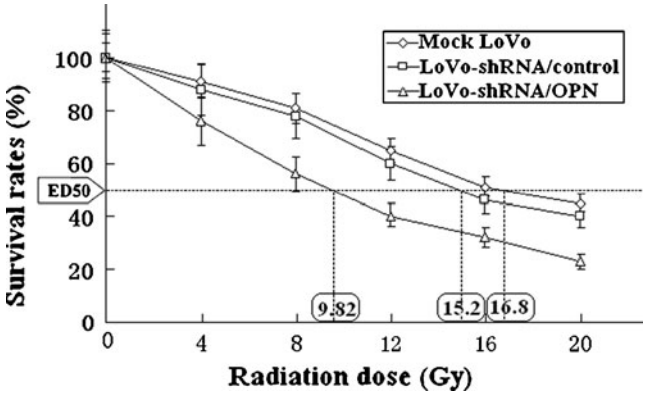

Fig. 7 The effect of OPN inhibition on in vitro radiosensitivity of LoVo cells. The ED_{50} of mock LoVo, LoVo–shRNA/control or LoVo–shRNA/OPN cells was 16.8, 15.2, or 9.82 Gy, respectively. All experiments were performed in triplicate

interactions with cell surface receptors, growth factor/receptor pathways, and proteases. [18] The overexpression of OPN gene has been found in many human cancers including CRC. Previously, we have reported that the status of OPN mRNA expression in CRC potentiates important clinicopathogical or prognostic significance. Multivariate analysis also showed that high OPN mRNA expression might be a prognostic marker for CRC patients. Meanwhile, other groups reported that OPN could be identified as lead marker of colon cancer progression by using pooled sample expression profiling.[19] Experimental data from a screen of 12,000 human genes have recently uncovered OPN as a lead marker of colon cancer progression and established a significant association between the degree of OPN expression and advancing Astler Collier stage.[20] Moreover, a gradient of increasing osteopontin expression was also observed when a precursor polyp (with low osteopontin expression) was directly compared with its adjacent, associated invasive cancer (high osteopontin expression). Recently, Wild et al.[21] found that OPN combined with other serum makers could be used for the early detection of colorectal cancer. Imano et al.[22] showed that OPN in the central area might have induced high microvascular density, which led to liver metastasis. The significantly elevated OPN protein levels during normal epithelium to carcinoma progression may contribute to the increased fibroblast–myofibroblast transition determining stem cell niche in colorectal cancer. [22] OPN deregulation is an early event in intestinal tumorigenesis which may promote tumor development by altering either proliferation or apoptosis to increase tumor cell numbers, but Martinez, et al.[23] found that OPN expression in the intestine is dependent on c-myc binding sites in the promoter. All these experimental data suggest that the overexpression of OPN is associated with colon tumorigenesis and plays critical roles in the progression of CRC.

Considering the important roles of OPN in tumor development, whether OPN could serve as a molecular target for cancer therapy is drawing more and more attention. Matsuura et al.[24] reported that simvastatin or RNA interference could suppressed ECM invasion by decreasing OPN expression. Gong et al.[25] showed that OPN siRNA might offer a new potential gene therapy approach for human gastric cancer. On the basis of this "proof-of-concept" study, Mi et al.[26] concluded that RNA aptamer targeting of OPN has biologically relevance for modifying growth and metastasis of breast cancer cells. Meanwhile, Hahnel et al.[27] showed that an OPN knockdown could improve radiobiological effects of breast cancer cells, suggesting that OPN seems to be an attractive target to improve the effectiveness of cancer radiotherapy. Wai et al.[28] showed that osteopontin silencing by small interfering RNA could suppress in vitro and in vivo CT26 murine colon adenocarcinoma metastasis, but the therapeutic significance of OPN in human CRC needs to be further elucidated.

To further explore the roles of OPN in the tumorigenic properties of CRC cells and whether OPN could be a molecular target for CRC therapy, plasmid vector expressing shRNA/OPN was constructed, and the effects of siRNA-mediated OPN inhibition were evaluated in vitro. We here first evaluated the effects of siRNA treatment against OPN in human CRC cells. In the present study, we demonstrated that the siRNA-mediated OPN inhibition in LoVo cells led to an inhibition of cell proliferation and colony formation, similar to the previous study demonstrated in other human cancers. [29, 30] Furthermore, we also observed an effective suppression of CRC tumorigenicity through knockdown of OPN gene expression in vivo. We significantly suppressed the tumorigenicity of LoVo cells by pSilencer–shRNA/OPN treatment 4 weeks after injection. This further suggests the therapeutic value of OPN inhibition for CRC treatment. To our knowledge, this is also the first time that the therapeutic value of OPN inhibition in human CRC has been demonstrated in vivo. Since invasion is also native feature of carcinoma, we furthermore explored the ability of mobility and invasion of siRNA vector-transfected LoVo cells. In vitro transwell indicated that the number of LoVo–shRNA/OPN cells was fewer than that of mock and blank vector-treated ones. Additionally, the cell scratch wound healing assay also showed that the speed of LoVo–shRNA/OPN cells migrated to the scratched area was significantly smaller than that of control cells. It is interesting as the experiments shown that siRNA-mediated OPN inhibition inhibited not only the proliferation but also the capability of mobility and invasion of CRC cells. Radioresistance of tumors are major obstacles that often lead to the failure of clinical cancer therapy. An important strategy for improving cancer

therapy is the development of approaches that are more selective and mechanistic for overcoming tumor resistance. To date, little is known regarding the association between OPN overexpression and radiosensitivity of CRC cells. In this study, for the first time, we were able to demonstrate that siRNA-mediated OPN knockdown could significantly increase the ED_{50} of LoVo cells to radiation, but the exact molecular mechanism needs to be further elucidated in future.

Conclusion

Taken together, our study showed that RNAi-mediated OPN downregulation could significantly inhibit proliferation, reduce invasion and enhance radiosensitivity of human CRC cells. Therefore, OPN may be a potential molecular target for gene therapy of CRC and siRNA-targeting OPN is of potential value to be used as a new method for the radiosensitization of human CRC in the future.

Acknowledgments This work was supported by the Project Sponsored by the Scientific Research Foundation for the Beijing Returned Overseas.

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ORIGINAL ARTICLE

Electroacupuncture in Reduction of Discomfort Associated with Barostat-Induced Rectal Distension—A Randomized Controlled Study

Wing-Wa Leung · Alice Y. M. Jones · Simon S. M. Ng · Cherry Y. N. Wong · Janet F. Y. Lee

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Abstract
Background This pilot study aims to explore the effectiveness of electroacupuncture in reduction of colorectal discomfort caused by Barostat-induced rectal distension.
Method Subjects scheduled for a colonoscopy screening procedure were recruited and randomized to receive either electroacupuncture (EA) or sham acupuncture (SA) (short stud instead of needle) for 45 min to acupuncture points Hegu (LI4), Neiguan (PC6), and Zusanli (ST36). A balloon catheter attached to the Dual Drive Barostat machine was then inserted into the subjects' rectal region. Colorectal discomfort after each incremental pressure (4 mmHg) rise was assessed by visual analog and a four-point subjective discomfort scale. Blood beta-endorphin level was measured before, immediately after acupuncture, at 24 mmHg, and at maximal tolerable inflation pressure.
Results Forty subjects completed the study. Rectal discomfort was reported at a higher inflation pressure in the EA group compared to the SA group ($p<0.05$). Twelve subjects in the EA group were able to tolerate the maximal inflation pressure (48 mmHg) compared to only four in the SA group. Beta-endorphin levels increased significantly in the EA group but not in the SA group.
Conclusion Electroacupuncture appeared to be effective in reduction of colorectal discomfort during Barostat-induced rectal distension. The role of electroacupuncture during colonoscopy warrants further investigation.

Keywords Colonoscopy · Rectal discomfort · Barostat · Electroacupuncture

Introduction

Early detection of pre-malignant colonic pathology can reduce the risk of cancerous development, and colonoscopy is a common procedure for examination and early screening for pathological changes of the large bowel. While minimally invasive, this procedure is often associated with abdominal pain and discomfort, especially when the colonoscope is passed through a colonic angulation.[1] The discomfort felt by the patient during colonoscopy may diminish the patient's tolerance to the procedure and jeopardize screening accuracy. A combination of narcotic analgesia and benzodiazepines has been conventionally used to minimize abdominal pain and discomfort during colonoscopy.[2,3] However, these medications have significant side effects such as nausea, vomiting, and may even induce respiratory arrest.[4,5] These unwanted effects may prolong recovery time and increase

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post-intervention risk and nursing care demands in the outpatient setting.[6] The undesirable effects of pharmacologically induced sedation have promoted the search for alternative management strategies to minimize patient discomfort during colonoscopy.

Acupuncture is used worldwide in the management of musculoskeletal pain and neurological disorders.[7,8] However, the role of acupuncture in providing relief of gastrointestinal discomfort during endoscopic procedures has not been studied. Only one clinical study reports the use of acupuncture to relieve gastrointestinal discomfort in patients with irritable bowel syndrome.[9] The mechanism by which acupuncture induces pain relief is believed to be associated with the release of endogenous opioids.[10] Endorphin levels in relation to gastrointestinal discomfort during colonoscopy have not been reported.

This study aimed to investigate the role of acupuncture in reducing colorectal discomfort during colonoscopy. A dual drive Barostat, a device commonly used to investigate visceral pain sensation by mimicking air inflation during colonoscopy,[11] was used to generate a standardized pattern of colorectal discomfort. Our hypothesis was that acupuncture would raise the threshold for colorectal discomfort, possibly by inducing increased plasma beta-endorphin levels.

Materials and Methods

Approval was granted by the Ethics Review Committees of the involved university and hospital prior to data collection (Approval number: HSEARS20080416003; CRE-2008.089-T). Written informed consent was obtained from each subject prior to commencement of the study.

Subjects

Subjects with ages ranging from 18 to 65 years registered for a colonoscopy examination were invited to participate in the study. Exclusion criteria included known cardiovascular dysfunction (American Society of Anaesthesiologists grade III or above), irritable bowel syndrome (Rome II classification),[12] renal impairment, previous abdominal surgery or experience of colonoscopy, gastrointestinal complaints, pregnancy, or previous experience with acupuncture. Subjects meeting the inclusion criteria were randomized to either the electroacupuncture (EA) or sham acupuncture (SA) groups. The respective intervention code was generated randomly by a computer program (Random Allocation Software, version 1.0, Isfahan University of Medical Sciences, Iran); the group allocation number was then placed in a sealed opaque envelope which was opened by the investigator responsible for implementation of EA or SA at the time of intervention.

Bowel Preparation

Patients were screened for impaired renal function prior to bowel preparation with “Fleet phosphosoda”. While this solution is no longer in common usage in the United Sates, it is still the standard solution for colonic preparation in hospitals in Hong Kong. All subjects were instructed to engage in a low fiber diet 3 days before the study. On the day before the measurement procedure, each subject drank 90 mL of Fleet® sodium phosphate solution (CB Fleet Co., Inc., Lynchburg, USA) to induce bowel movement and prepare the colon for examination. Participants were asked to empty their bowel at home before coming to the hospital for examination.

Equipment

A dual-lumen polyethylene catheter (Model CR3-0005, Mui Scientific, Mississauga, Ontario, Canada) was attached to a 10-cm long and 600-mL capacity polyethylene bag (Model CT-BP600R, Mui Scientific) using a sterile surgical silk suture (Ethicon, Mersilk Soie, Somerville, New Jersey, USA), with the surgical knots sealed by latex glue. One lumen of the catheter allowed passage of compressed air for bag inflation and the other was attached to a transducer for monitoring the pressure inside the bag. The catheter was attached to the dual drive Barostat (Distender Series II; G &J Electronics, Inc., Toronto, Canada) which controlled the rate of inflation and deflation of the bag electronically. To ensure that the bag attachment was airtight, the bag was inflated to 60 mmHg for 5 min and placed in sterile warm water to ensure that there was no leakage of air from the system (Fig. 1).

Acupuncture Protocols

Acupuncture points Hegu (LI4), Neiguan (PC6), and Zusanli (ST36) were identified. LI4 is located on the

Fig. 1 The Barostat balloon system

dorsum of the hand, between the first and second metacarpal bones; PC6 is located on the palmar aspect of the forearm, 2 cun above the transverse crease of the wrist between the flexor carpi radialis and palmaris longus tendons; ST36 is located on the anterior aspect of the leg, 3 cun below the knee cap and one fingerbreadth from the anterior crest of the tibia. One cun is the distance between the interphalangeal creases of the subject's middle finger.[13] These points were chosen because LI4 and PC6 are reportedly used to reduce abdominal pain, [14,15] and ST36 regulates colorectal muscle contractility in conscious rats.[16] These acupuncture points were swabbed with alcohol following a standard antiseptic process.

A sterile acupuncture needle, 25 mm in length and 0.22 mm in diameter (Hwato®, Suzhou Medical Appliance Factory, China), was inserted, via a sterile plastic tube stabilized by a foam block, into the identified acupuncture points of the subject (Fig. 2). The foam block acted to blind both the assessor and the subject to the applied intervention. The acupuncture needle was inserted to a depth of 15 mm and connected to a six-channel programmable electrical stimulator (ES-160, ITO Company Limited, Tokyo, Japan) in the EA group. Forty-five minutes of alternate current stimulation was administered at 1–10 Hz, with an intensity just sufficient to provoke muscle contraction.

Blunt-tip acupuncture needles (Hwato®, Suzhou Medical Appliance Factory, China), 10 mm in length and 0.22 mm in diameter, were prepared and inserted through the foam block without penetrating the skin. The programmable electrical stimulator was connected for 45 min to the needle without electrical output, despite an activated output light.

Experimental Procedures

All subjects were scheduled for their colonoscopy screening procedure in the afternoon. At 9 a.m. on the same day, subjects were invited to attend the laboratory to conduct the study. They were instructed to rest in the supine position for 30 min and randomized to receive 45 min of either EA or SA in the supine position. The subject then adopted a left side-lying position with both knees flexed to 90°. The folded polyethylene bag, attached to the Barostat, was then slowly inserted into subject's rectum to a distance 10 cm from the anal verge. The tube was taped to the buttock, and the subject was asked to assume a supine position and rest for 15 min. Complete deflation of the polyethylene bag was first ensured, followed by 60 s of bag inflation and then 60 s of complete bag deflation, with a 4-mmHg stepwise incremental increase in rectal pressure to either a maximum of 48 mmHg or when the subject indicated that he/she could not tolerate further discomfort. The EA or SA stimulation continued throughout the Barostat procedure. At the end of the Barostat procedure or if a maximum tolerable inflation pressure was reached, the needles were

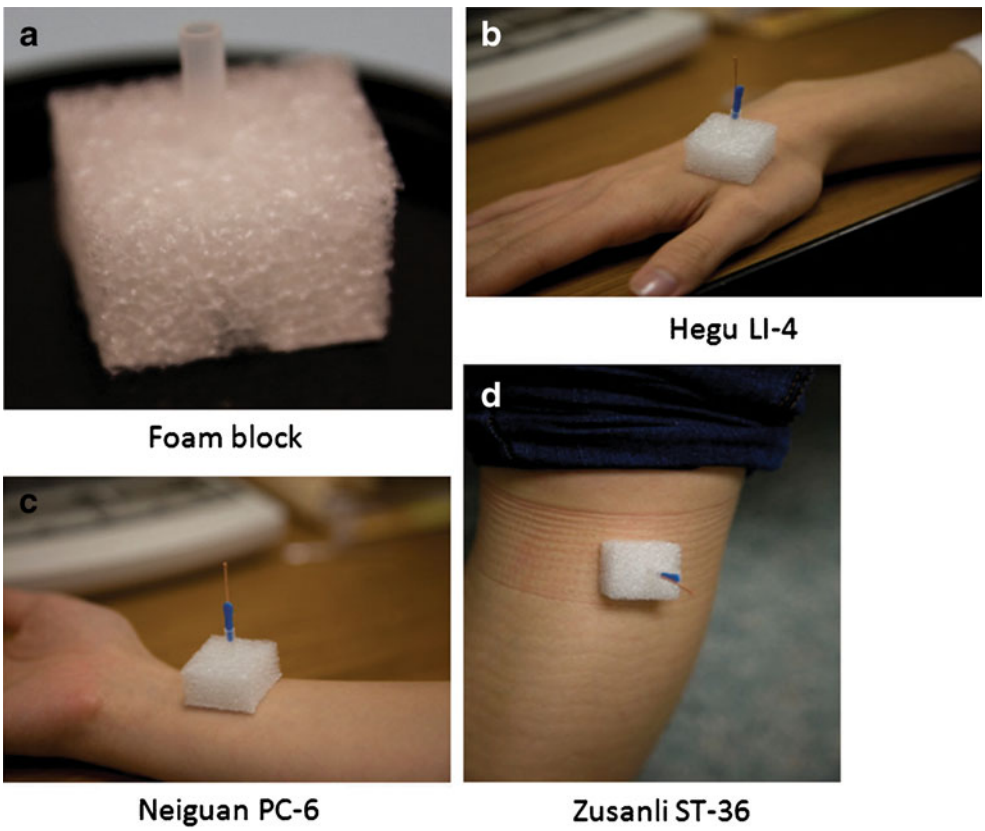

Fig. 2 A needle through **a** a homemade foam block over **b** Hegu LI4, **c** Neiguan PC6, and **d** Zusanli ST36

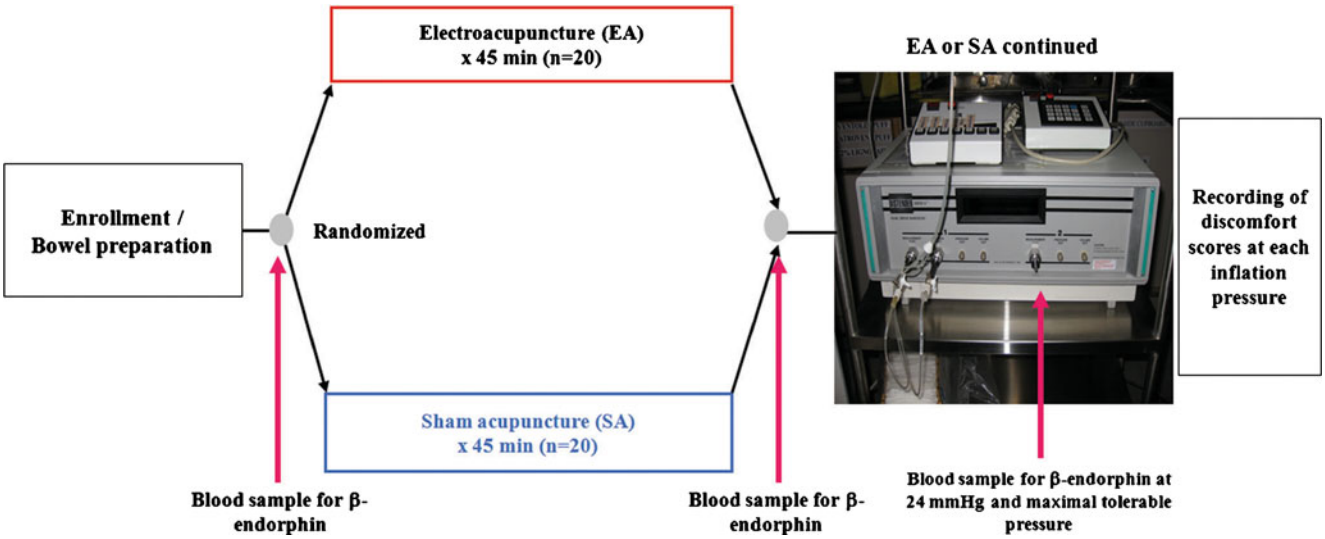

Fig. 3 A flow chart illustrating the study procedures

removed, and gentle pressure was applied with a sterile gauze for 3 min to minimize bleeding.

Outcome Measures

Subjective Discomfort Scale

During each 60-s phase of sustained incremental pressure, the subject was asked to rate the colorectal sensation using an electronic panel attached to a computer, indicating either "no perception", "first perception of distension", "urge to defecate", "discomfort or pain", and "extreme pain". The subject was also asked to rate the degree of colorectal discomfort using a visual analog scale (VAS); a 10-cm ungraduated line, with words "no discomfort at all" anchored to one end and "discomfort cannot be tolerated" anchored to another. The VAS and subjective discomfort scales were recorded by an independent research assistant who was blinded to the acupuncture intervention allocation.

Table 1 Demographic data of subjects in the electroacupuncture (EA) and sham acupuncture (SA) groups

	EA group (n=20)	SA group (n=20)	P value
Age (years)	51.5±4.6	50.8±5.8	0.69
Gender (male/female)	5/15	8/12	0.311
Body weight, (kg)	59.0±8.28	59.3±13.5	0.917
Body height (m)	1.62±0.06	1.62±0.08	0.858
Body mass index (kg/m^2)	22.4±2.17	22.4±4.00	0.979

Data are mean±standard deviation, except for gender which is in number of male/female subjects

Beta-endorphin Measurement

Venous blood (3 mL) was drawn (from a cannula inserted into the cubital vein of each subject under aseptic technique) before the randomization process, immediately after the 45-min intervention, at a distension pressure of 24 mmHg, and at the conclusion of the intervention. The blood samples were stored in EDTA tubes and transferred in a 4-°C ice box to the biochemistry laboratory of the involved hospital, and batch was analyzed for beta-endorphin (Human) using EIA kit (ELISA; Phoenix Pharmaceuticals Inc, 330 Beach Road, Burlingame, CA94010, USA) to ensure similar enzyme kit reaction. Figure 3 shows the experimental procedure.

Data Analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS version 15.0 for Windows, SPSS Inc., Chicago, IL USA). Repeated-measures ANOVA was used to determine the change in sensation at different bag inflation pressures. Between-group sensation at each pres-

Table 2 Mean pressure (mmHg) at which various levels of discomfort were perceived in the electroacupuncture (EA) and sham acupuncture (SA) groups

Perception	EA group (mmHg)	SA group (mmHg)	P value
First sensation	21.0±4.8	14.2±7.6	0.02
Urge to defecate	29.8±5.3	22.3±7.7	0.001
Discomfort/pain	36.9±2.9	29.3±7.2	<0.001
Severe pain	43.5±6.6	35.6±8.8	0.007

Table 3 Number of participants in the electroacupuncture (EA) or sham acupuncture (SA) group who tolerated a distension pressure of 40, 44, and 48 mmHg

Pressure	EA (n=20)	SA (n=20)	P value
40 mmHg	18 (90%)	9 (45%)	0.006
44 mmHg	16 (80%)	6 (30%)	0.004
48 mmHg	12 (60%)	4 (20%)	0.022

sure was compared using independent sample t test for parametric data and Pearson chi-squared test or Mann–Whitney U test for non-parametric data. A statistical significant level was set at p less than 0.05.

Results

Forty healthy subjects (13 male, 27 female; mean age, 51.1±5.18 years) were recruited and randomized to either the EA or SA group (n=20 in each group). The demographic data for both groups were similar (Table 1). All subjects completed the procedure, and no adverse effects were observed during the intervention. All subjects also completed the subsequent colonoscopy screening in the afternoon (at 3–4 h after the Barostat intervention), and reports for all subjects in this cohort were normal.

The "first perception of distension" sensation recorded was at a significantly higher pressure in subjects in the EA group (21.0±4.8 mmHg) compared to the SA group (14.2±7.6 mmHg; Table 2). The maximal tolerable pressure was also significantly higher in the EA group compared with the SA group (43.5 mmHg±6.6 mmHg and 35.6 mmHg±8.8 mmHg, respectively, p=0.007). The number of subjects who could tolerate a higher rectal distension pressure (at 40, 44, and 48 mmHg) was significantly higher in the EA group (Table 3). At each inflation pressure, the VAS score reported by the EA group was lower than that reported by the SA group (Fig. 4), but this difference was only significant at 24 mmHg (2.80 vs. 4.74 for EA and SA respectively, p=0.013).

The beta-endorphin levels in both groups were similar at baseline level (0.83±0.25 and 0.82±0.17 ng/ml, respectively, p=0.915) but significantly higher after 45 min of electroacupuncture in the EA group (1.10±0.46 ng/ml) compared to the SA group (0.76±0.11 ng/ml; p=0.005). At a distension pressure of 24 mmHg, the beta-endorphin levels for the EA and SA groups were 1.25±0.45 ng/ml and 0.79±0.17 ng/ml, respectively, p<0.001. At maximal tolerable pressure, the levels were 1.19±0.48 ng/ml and 0.73±0.08 ng/ml, respectively (p=0.001). There was no significant change in beta-endorphin level from baseline in the SA group.

Discussion

This study shows that electroacupuncture over LI4, PC6, and ST36 significantly reduced the level of colorectal discomfort induced by Barostat distension when compared to sham acupuncture. Subjective discomfort reduction was accompanied by a significant increase in beta-endorphin level.

The Barostat procedure was designed initially to evaluate gastrointestinal smooth muscle tone but has also been used to evaluate visceral sensitivity.[17] The pressure generated by the balloon in the colorectal region induces an unpleasant sensation and is thus considered an appropriate objective means to induce rectal discomfort in our subjects in this study.

This is the first controlled study which investigates the effect of acupuncture on rectal discomfort in humans. The acupuncture points used in this study (LI4, PC6, and ST36) for control of rectal discomfort were those commonly reported for reduction of abdominal and colonic pain.[14–16] The study shows that acupuncture stimulation can reduce the discomfort associated with colorectal distension induced by a Barostat. The role of acupuncture in the

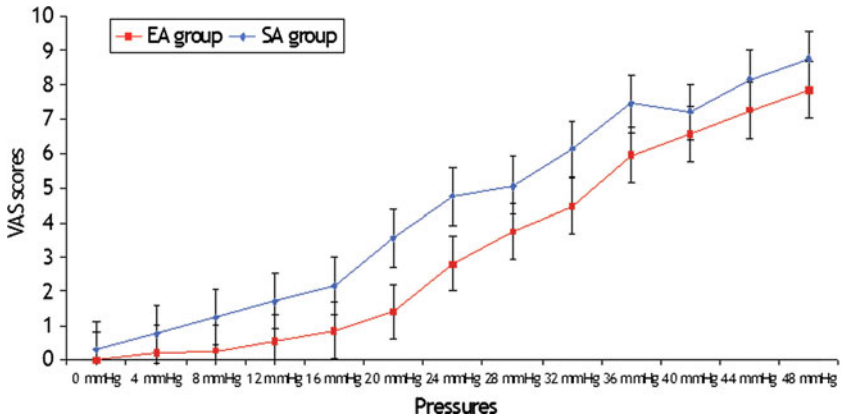

Fig. 4 Visual analog scale (VAS) for rectal discomfort at different distension pressures

management of chronic pain has been extensively investigated, [18,19] but the effect on visceral pain cannot be inferred because unlike somatic pain fibers, the visceral nerves are unmyelinated.[20] Xu and coworkers [21] induced visceral pain in rats and reported an opioid release with EA. EA also increased the pain threshold in rats with chronic hypersensitive visceral pain, accompanied by an increase in 5-hydroxytryptamine.[22]

Our study also suggests that the effect of EA on rectal discomfort is associated with a temporal increase in blood beta-endorphin levels of as much as 50% at 24 mmHg inflation pressure. The VAS score recorded significantly less discomfort in the EA group compared to the SA group at this pressure, suggesting a mechanistic relationship. The beta-endorphin level declined at the maximal inflation pressure, suggesting that the increase in beta-endorphin induced by EA possibly peaked at about 25–30 min post stimulation (the time when inflation pressure reached 24 mmHg) and then commenced to decline with accommodation to the pain stimulus. These changes were not apparent in the SA group.

It is postulated that EA can forward signal to the hypothalamus and thalamus via myelinated nerve fibers.[23] It has long been proposed that acupuncture analgesia has been associated with the release of endogenous opioids, especially beta-endorphin and endomorphins.[24,25] Theoretically, low-frequency (2 Hz) EA induces the release of beta-endorphin via stimulation of the mu and delta opioid receptors, while high-frequency (100 Hz) EA triggers the kappa opioid receptor, inducing the release of dynorphin.[26–28]

It has also been suggested that EA may induce pain relief by means of an anti-inflammatory role affecting hormonal and neuronal systems via stimulation of the hypothalamus.[29] Inflammatory markers were not measured in this study, but intuitively, it seems unlikely that the acute discomfort induced by colonoscopy would be sufficient to induce a rapid change in inflammatory blood markers.

Limitations of the Study

The site of discomfort during colonoscopy is often in the sigmoid colon due to mesenteric stretching as well as lumen distension; however, the Barostat balloon induces rectal rather than sigmoidal discomfort. Insertion of the balloon to the level of the sigmoid carries an unacceptable risk of colonic rupture, particularly when colonic pathology has not been excluded at the time of the study.

We did not measure the amount of analgesia required by the subjects during their colonoscopy because the colonoscopy procedure was scheduled 3–4 h after the EA/SA intervention. In such circumstances, the endorphin-induced analgesic effect associated with EA would have declined to zero. The aim of this pilot study was to determine the role of EA and a mechanism of action in relieving rectal discomfort. Our encouraging results have spawned further investigation into the appropriateness of EA during colonoscopy.

This study employed a design whereby the surgeon, subjects, and the investigator responsible for outcome measurements were all blinded to the allocated intervention; a control group with no intervention was however not included. In consequence, any placebo effect of the sham acupuncture cannot be excluded. Nevertheless, this study showed a significant between-group effect for both subjective pain relief and plasma beta-endorphin levels following electroacupuncture, supporting an inferred relationship.

Application of acupuncture required an acupuncturist. Whether this cost can be offset by the reduced analgesic usage and nursing care to manage opiate side effects was not investigated. The positive findings of this pilot study justify further investigation into the role and cost–benefits of EA during colonoscopy.

Conclusion

This study showed that 45 min of electroacupuncture over LI4, PC6, and ST36, compared to sham acupuncture, significantly reduced the level of colorectal discomfort induced by Barostat distension. Subjective discomfort reduction was accompanied by a significant increase in beta-endorphin level. The role of electroacupuncture in reduction of rectal discomfort during clinical colonoscopy warrants further investigation.

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ORIGINAL ARTICLE

Sigmoid Diverticulitis in Young Patients—A More Aggressive Disease than in Older Patients?

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Abstract
Introduction There is controversy over whether sigmoid diverticulitis (SD) is more aggressive with a higher risk of perforation in younger than in older patients. The aim of this study was to assess the clinical presentation and outcome of patients ≤40 and >40 years old with acute diverticulitis.
Patients and Methods Consecutive admissions of all patients with acute SD were prospectively recruited from January 1998 to June 2010.
Results A total of 1,019 patients were included: 513 (69 ≤40 years and 444 >40 years) presented with their first episode, while 506 (20 ≤40 years, 486 >40 years) had a prior history of SD. The percentage of patients with severe SD did not differ between the two age groups either for the first (covered perforation, 30.4% vs. 29.5%, p=0.875; free perforation, 26.1% vs. 23.9%, p=0.69) or for the recurrent episode (covered perforation, 15% vs. 8.2%, p=0.287; free perforation, 5% vs. 4.1%, p=0.846). Furthermore, the rate of emergency surgery did not differ between both age groups either for the first (26.1% vs. 23.9%, p=0.690) or the recurrent episode (5% vs. 4.1%, p=0.846). No differences in the rate of Hartmann's procedure (52.6% vs. 68.3%, p=0.180) and failure of conservative treatment (3.4% vs. 4.9%, p=0.607) were observed between younger and older patients.
Conclusion Acute SD in younger patients is not more aggressive and has no higher risk of perforation or need for emergency surgery compared to older patients.

Keywords Acute diverticulitis · Age · Emergency surgery · Severe diverticulitis · Free perforation

Introduction

Sigmoid diverticulitis (SD) is one of the most common diseases in the Western world, and its incidence is increasing with the increase in average age of the population.[1, 2] About 10–25% of these patients develop acute sigmoid diverticulitis.[3–5] Its clinical spectrum extends from asymptomatic diverticulosis with recurrent courses to symptomatic disease with potentially fatal complications.[6] The indication for surgical intervention depends on several factors. Whether the age of the patients is a risk factor in itself is controversially discussed, especially in younger patients (<40 years) with acute sigmoid diverticulitis.[7–16] Originally, the European Association for Endoscopic Surgery[17] and the American Society of Colon and Rectal Surgeons[18] recommended surgical intervention in young patients with acute sigmoid diverticulitis because these patients had a high long-term risk of severe complications on account of the recurrent nature of the disease. These recommendations have been called in question by numerous publications in recent years, and controversy persists about the stages at which colectomy is really indicated.[13, 19] Large patient studies performed in recent years have

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demonstrated that the risk of recurrence after the first episode of acute sigmoid diverticulitis is altogether lower than anticipated, and that the risk of recurrence or complications is not higher in younger than in older patients.[20, 21]

It remains unclear though whether younger patients present with a more aggressive and fulminant type of acute sigmoid diverticulitis that would suggest the need for surgery in this patient group.

The aim of this study was to assess the clinical presentation and outcome of younger (≤40 years) and older (>40 years) patients with acute diverticulitis.

Patients and Methods

Patients and Therapeutic Strategy

Consecutive emergency admissions of all patients with acute diverticular disease were prospectively recruited from January 1998 to December 2009 in the Department of Surgery at Campus Benjamin Franklin, Charité–Universitätsmedizin Berlin. The diagnostic procedure was standardized and based on clinical examination, blood tests, and an abdominal computed tomography (CT) scan with oral, intravenous, and transrectal contrast within 12 h after hospitalization for all patients. Criteria for an abdominal CT scan were suspicion of diverticulitis by history and physical examination or unspecific abdominal pain associated with leukocytosis or elevation of C-reactive protein (CRP). The Hansen and Stock classification was used to assess the clinical picture in the CT scan[22] (Table 1). Here, stage 0 denotes irritation-free diverticulosis with detection of only gas- or contrast-filled diverticula and no inflammatory signs in the CT. Stage I is the stage of acute uncomplicated diverticulitis. The CT morphological correlative here is bowel wall thickening. Stage II designates acute complicated diverticulitis and is further subdivided into stages IIa, IIb, and IIc according to the severity of inflammation. Stage IIa involves peridiverticulitis or phlegmonous diverticulitis with spread of the bowel inflammation to the pericolic fatty tissue. The CT shows inflammatory infiltration of pericolic fat. Stage IIb is characterized by CT morphological detection of a mesocolic or retroperitoneal abscess or an abscess in the minor pelvis. In stage IIc, the CT detects free air and/or fluid as the correlative of free bowel perforation. Stage III is the stage of chronic recurrent diverticulitis, and classification here is based not only on the CT but also on the patient's history. The typical CT sign of chronic inflammation is bowel wall thickening, sometimes with stenosis or fistulas. The CT examination enabled the admitting physician to establish the treatment concept before initiating therapy. Patients with signs of acute diverticulitis but no evidence of free perforation or diffuse peritonitis received i.v. antibiotic treatment (1 g of sulbactam and 2 g of ampicillin) until infection parameters had normalized for at least 7 days. Any intra-abdominal abscess detected was interventionally drained right away, when appropriate. Any additional diseases were excluded in all patients by colonoscopy at least 4 weeks after acute inflammatory attack if none had been performed in the last 24 months. Patients with free perforation or diffuse peritonitis (Hinchey III and IV, Hansen–Stock IIc) were treated by emergency surgery. When emergency surgery was required, the first-choice procedure was sigmoid resection with primary anastomosis. Hartmann's procedure was performed only in high-risk patients with immunocompromised status or preoperative organ failure, or in patients with fecal peritonitis. Patients with exacerbation of infection under antibiotic treatment (failure of conservative treatment) underwent acute operative treatment. Indications for elective surgery were: first attack in immunosuppressed patients and repeated attacks of recurrent diverticular disease (Hansen–Stock stage III). The degree of inflammation was assessed only during the immediate attack in cases of recurrent diverticulitis. Patients with a diagnosis of colorectal cancer during the admission period or a known history of colorectal cancer were excluded from this study. To record the number of previous SD episodes, patients were asked how often they had already received in- or outpatient antibiotic treatment for acute SD based on distinct clinical and laboratory parameters.

The following data were prospectively recorded: age, gender, comorbidity (including drug intake), previous

Table 1 Hansen and Stock classification[22]

Stage	Description	CT scan
0	Diverticulosis	Gas- or contrast-filled diverticulum
I	Acute uncomplicated diverticulitis	+ Intestinal wall thickening
II	Complicated diverticulitis	
IIa	Peridiverticulitis, phlegmonous diverticulitis	+ Inflammatory reaction in pericolic fatty tissue
IIb	Abscess diverticulitis, covered perforation, fistulation	+ Mesocolic or retroperitoneal abscess, lower pelvic abscess
IIc	Free perforation	Free air, free fluid, abscesses
III	Chronic recurrent diverticulitis	Intestinal wall thickening, stenosis or fistula

abdominal surgery, diagnostic findings (stage of disease; uncomplicated vs. complicated diverticulitis), white blood cell count, C-reactive protein, number of diverticulitis attacks, and type of treatment (conservative and/or surgical). Two groups were set up to analyze the influence of age on the severity of diverticulitis: group I, comprised patients ≤40 years and group II included patients >40 years.

Surgical Procedure

Sigmoid resection was performed by laparoscopy as a routine procedure without loop ileostomy in all patients without free perforation, diffuse peritonitis, or extensive surgery in the lower abdomen. Four experienced surgeons performed the operation on patients in the lithotomy position. The detailed procedure has been described elsewhere.[10–12] When emergency surgery was required, the first-choice procedure was sigmoid resection with primary anastomosis, if needed with loop ileostomy. Hartmann's procedure was performed only in high-risk patients with immunocompromised status or preoperative organ failure, or in patients with diffuse fecal peritonitis. The surgical technique remained unaltered during the entire study period.

Abdominal CT Examination

CT examination was performed with oral (30 ml of Gastrografin®, Schering, Berlin, Germany in 1 L of H_2O), intravenous (150 ml of Ultravist 300® (Schering, Berlin, Germany)), and transrectal contrast (200 ml of Peritrast®-RE/36). An initial scout view (topogram) was used for planning the actual examination. During intravenous contrast application (flow velocity 3 ml/s), spiral acquisition was performed from the diaphragm to the pelvic floor. Examination parameters were: the computer tomography scanner Somatom Sensation 16 (Siemens AG Medical Solutions, Erlangen, Germany), a slice thickness of 16× 1.5 mm, and secondary reformatting in coronary orientation. CT morphological staging was done by Hansen–Stock classification (Table 1).

Statistics

Statistical analysis was done using SPSS 17.0 software (SPSS, Chicago, IL). Statistical significance was calculated by using the Mann–Whitney U test for quantitative variables and the χ^2 test for categorical data ($p<0.05$=statistically significant). If not otherwise specified, the values are means±standard deviations. Multiple logistic regression was performed by incorporating factors with a p value ≤0.1 in the univariate analysis (age, gender, comorbidity, and previous episodes). Statistical significance was defined as $p<0.05$ (two-sided).

Results

Patient Characteristics

A total of 1,019 patients with acute diverticulitis were identified. Findings at the index admission are shown in Table 2. Eighty-nine patients (8.7%) patients were 40 years or younger, with a mean age of 37.46 years, while 930 patients (91.3%) were older than 40 years, with a mean age of 61.34 years ($p<0.001$). Significantly, more men with acute diverticulitis were found among the younger patients compared to the older ones (70.8% vs. 51%, $p<0.001$). Sixty-nine (77.5%) of the patients <40 years had suffered a first episode compared to 444 patients (47.7%) over 40 years ($p<0.001$). A recurrence was seen in 20 patients <40 (22.5%) compared to 486 patients >40 years (52.3%; $p<0.001$). No significant differences between younger and older patients were observed regarding mean CRP concentration and mean white blood cell count on admission or among the percentage of obese patients. Chronic renal failure, diabetes, ischemic cardiopathy, and hypertension were seen significantly more frequently in the older group ($p<0.05$).

CT Examination and Degree of Diverticulitis

All Patients (First Episode and Recurrence)

Based on the total number of patients (first episode and recurrence), patients ≤40 years accounted for significantly fewer cases of uncomplicated diverticulitis. The percentage of phlegmonous diverticulitis did not differ between the two age groups whereas severe diverticulitis was seen significantly more frequently in the younger group (covered perforation, 27% vs. 18.4%, $p=0.049$; free perforation, 21.3% vs. 13.5%, $p=0.044$), provided the diverticulitis episode (first or recurrent episode) was not taken into account. In comparison, the older group had a significantly higher percentage of radiologic findings such as stenosis or fistula (Table 3). Table 3 also shows the degree of diverticulitis in relation to the first and recurrent episodes of SD.

Patients with a First Episode of SD

Comparing only patients with a first episode of SD yielded no differences between the two age groups with regard to the degree of inflammation in diverticulitis (Table 3). The percentage of uncomplicated diverticulitis (2.9% vs. 3.8%, $p=0.703$) and especially the percentage of severe diverticulitis (covered and free perforation) did not differ for the first episode of SD (covered perforation, 30.4% vs. 29.5%, $p=0.875$; free perforation, 26.1% vs. 23.9%, $p=0.69$) when

Table 2 Findings in 1,019 patients at index admission

	Age ≤40 years (n=89)	Age >40 years (n=930)	p value
Sex			<0.001
Male	63 (70.8%)	474 (51%)	
Female	26 (29.2%)	456 (49%)	
Age (years)	37.46	61.34	<0.001
Diverticulitis events			<0.001
First episode	69 (77.5%)	444 (47.7%)	
Recurrence	20 (22.5%)	486 (52.3%)	
Mean CRP (mg/dl) at admission (range)	6.7 (0.1–20.4)	7.9 (0.4–44.0)	0.264
Mean white blood cell count ($\times 10^9$/l) at admission (range)	11.9 (4.2–21.1)	11.9 (4.3–21.8)	0.862
Comorbidity			
Chronic renal failure	1 (1.1%)	89 (9.6%)	0.007
Diabetes	1 (1.1%)	121 (13%)	0.001
Ischemic cardiopathy	3 (3.4%)	102 (11%)	0.024
Hypertension	10 (11.2%)	353 (38%)	<0.001
Obesity (BMI >35 kg/m^2)	5 (5.6%)	112 (12%)	0.071
Comorbidity (>1)	24 (27%)	426 (45.8%)	0.001

comparing the two age groups. Furthermore, the percentage of phlegmonous SD (40.6% vs. 41.7%, p=0.865) as well as the percentage of strictures or fistulas (0% vs. 1.1%, p= 0.376) did not differ during the first episode between the two age groups.

Patients with a Recurrent Episode of SD

The percentage of uncomplicated diverticulitis (0 vs. 12.1%, p=0.097) and especially the percentage of severe diverticulitis (covered and free perforation) did not differ

Table 3 CT stages in relation to first and recurrent episodes of SD for group aged ≤40 years compared to group aged >40 years

Degree of diverticulitis	Age ≤40 years n=89 (F 69, R 20)	Age >40 years n=930 (F 444, R 486)	p value
Uncomplicated (stage I)			
Overall	2 (2.2%)	76 (8.2%)	0.045
First episode	2 (2.9%)	17 (3.8%)	0.703
Recurrence	0	59 (12.1%)	0.097
Phlegmonous (stage IIa)			
Overall	44 (49.4%)	443 (47.6%)	0.745
First episode	28 (40.6%)	185 (41.7%)	0.865
Recurrence	16 (80%)	258 (53.1%)	0.018
Covered perforation (stage IIb)			
Overall	24 (27%)	171 (18.4%)	0.049
First episode	21 (30.4%)	131 (29.5%)	0.875
Recurrence	3 (15%)	40 (8.2%)	0.287
Free perforation (stage IIc)			
Overall	19 (21.3%)	126 (13.5%)	0.044
First episode	18 (26.1%)	106 (23.9%)	0.690
Recurrence	1 (5%)	20 (4.1%)	0.846
Stricture/fistula (stage III)			
Overall	0	114(12.3%)	<0.001
First episode	0	5 (1.1%)	0.376
Recurrence	0	109 (22.4%)	0.017

F first episode, *R* recurrence

for the recurrent episode of SD (covered perforation, 15% vs. 8.2%, p=0.287; free perforation, 5% vs. 4.1%, p=0.846) between the two age groups. In comparison, phlegmonous SD was found significantly more frequently in the younger age group (80% vs. 53.1%, p=0.018), while strictures or fistulas were seen more often in the older patients (22.4% vs. 0%, p=0.017) during recurrent episodes of SD.

Treatment and Outcome

Treatment is summarized in relation to the age group in Table 4. The overall percentage of patients receiving conservative treatment did not differ between the two groups (55.1% vs. 61.3%, p=0.250). Overall, there were also no significant differences in the rate of failure of conservative treatment (3.4% vs. 4.9%, p=0.507) between younger and older patients during the first (4.3% vs. 7.9%, p=0.297) or recurrent episode (0% vs. 2.3%, p=0.496).

The overall percentage of patients receiving elective surgery did not differ between the two groups (23.6% vs. 25.2%, p=0.745). Only elective surgery was found significantly more frequently in the younger age group during the first episode compared to the older patients (17.4% vs. 9.5%, p=0.046), but not during recurrence (45% vs. 39.5%, p=0.623).

On the whole, the rate of emergency surgery was found significantly more frequently in the younger age group (21.3% vs. 13.5%, p=0.044), but no differences were seen between the two age groups either for the first (26.1% vs. 23.9%, p=0.690) or the recurrent episode (5% vs. 4.1%, p=0.846), provided the diverticulitis episode was taken into account.

When emergency surgery was required, most patients were treated by a Hartmann's procedure: 10 (52.6%) of 19 patients <40 compared to 86 (68.3%) of 126 patients >40 years (p=0.180). Sigmoid resection with primary anastomosis without ileostomy was performed in 3 (15.8%) patients <40 compared to 14 (11.1%) patients >40 years (p=0.555), and 6 (31.6%) patients <40 compared to 26 (20.6%) patients >40 years underwent a sigmoidectomy with primary anastomosis and protective ileostomy (p=0.284).

The mean hospital stay was significantly shorter in the younger group than in the older group (10.7 days vs. 16.6 days, p=0.009). When only successful conservative treatment was considered, the mean hospital stay was 7.19 days in the younger group and 7.81 days in the older group (p<0.001).

Of 43 patients under 40 years who underwent surgery, 1 patient (2.3%) had a major complication compared to 15 of 406 patients (6.4%) in the older age group (3.7%) (p=0.283). The rate of anastomotic leakage was 2.3% in the younger age group and 3.7% in the older age group (p=0.643). No differences in the rate of minor complications were observed between younger (7.0%) and older (8.4%) patients (p=0.748).

The mortality rate was zero in the younger group and 1.1% (10 patients) in the older group. All of those who died had been admitted because of a first episode of diverticulitis. Six of the deaths occurred after emergency surgery. The cause of death was related to the previous comorbidity and the severe degree of peritonitis.

Multiple Logistic Regression

Several possible prognostic factors were examined here for their influence on the risk of perforation. Univariate analysis revealed no gender influence (p=0.123). Thus, gender was not incorporated into the logistic regression analysis due to a p value >0.1. However, age, comorbidity (more than 1), and the first episode of SD proved to be risk factors for a free perforation in the univariate analysis

Table 4 Treatment in relation to age groups at index admission (first episode vs. recurrence)

	Age ≤40 years n=89 (F 69, R 20)	Age >40 years n=930 (F 444, R 486)	p value
Conservative treatment			
Overall	49 (55.1%)	570 (61.3%)	0.250
First episode	39 (56.5%)	296 (66.7%)	0.100
Recurrence	10 (50%)	274 (56.4%)	0.573
Elective surgery			
Overall	21 (23.6%)	234 (25.2%)	0.745
First episode	12 (17.4%)	42 (9.5%)	0.046
Recurrence	9 (45%)	192 (39.5%)	0.623
Emergency surgery			
Overall	19 (21.3%)	126 (13.5%)	0.044
First episode	18 (26.1%)	106 (23.9%)	0.690
Recurrence	1 (5%)	20 (4.1%)	0.846

F first episode, *R* recurrence

($p<0.05$; Table 5), but only the comorbidity and the first episode of SD are also risk factors in the multiple logistic regression (first episode, odds ratio (OR) 8.364 (95% confidence interval (CI) 5.097–13.725), $p<0.001$; comorbidity (>1), OR 2.115 (95% CI 1.446–3.094), $p<0.001$).

Discussion

The prevalence of diverticulosis has been increasing in the Western world in the past few years.[1, 2] About 10–25% of the diverticulosis patients develop acute sigmoid diverticulitis in the course of their lives.[3–5] Epidemiological data show a disproportionately increased incidence in young patients.[14, 20, 23] The course and therapeutic regimen for these patients are still controversially discussed.[7–16, 24, 25] The current literature offers a similar number of studies favoring conservative treatment in patients under 50,[14, 20, 26–28] while others recommend surgery.[21, 26, 27, 29–32] However, one drawback of many of these studies is that they do not differentiate between first episode and recurrence of diverticulitis. This is an important parameter since diverticulitis is usually most aggressive during the first episode, while the risk of perforation decreases with the number of episodes.[14, 20]

Our own data show that the rate of perforation in both age groups is significantly higher after the first episode of SD than after a recurrence (≤40 years, 26.1% vs. 5%; >40 years, 23.1% vs. 4.1%). This may lead to overestimating the risk of a free perforation in younger patients since the probability of a first episode is by nature higher in that age group than in the older one. Thus, besides age, it is also important to differentiate between first episode and recurrence. In our patient cohort, nearly 75% of the patients <40 (77.5%) had suffered a first episode. This was significantly more frequent than in patients >40, with a first episode rate of 47.7%.

In our study, a comparison of the degree of inflammation in younger and older patients revealed that the younger patients as a whole seemingly have a higher risk of a covered or free perforation. However, differentiating here between first episode and recurrence, the disease was not more aggressive in younger than in older patients. If the diverticulitis episode is taken into account, the percentage of patients with severe diverticulitis did not differ between the two age groups for both the first (covered perforation, 30.4% vs. 29.5%, $p=0.875$; free perforation, 26.1% vs. 23.9%, $p=0.69$) and the recurrent episodes (covered perforation, 15% vs. 8.2%, $p=0.287$; free perforation, 5% vs. 4.1%, $p=0.846$). In comparison, radiological findings such as stenosis or fistula were seen more often in older patients ($p<0.001$). Thus, there were also no significant differences between the therapeutic regimens. The overall percentage of patients receiving conservative treatment (55.1% vs. 59.2%, $p=0.443$) or elective surgery (23.6% vs. 25.2%, $p=0.745$) did not differ between the two age groups. Only elective surgery was found significantly more frequently in the younger age group during the first episode compared to the older patients. This fact could possibly be explained by surgeon bias because our study is non-randomized. On the whole, the rate of emergency surgery was found significantly more frequently in the younger age group (21.3% vs. 13.5%, $p=0.044$), while no differences were seen between the two age groups either for the first (26.1% vs. 23.9%, $p=0.690$) or the recurrent episode (5% vs. 4.1%, $p=0.846$). These data are in agreement with the results obtained by Hjern et al.,[33] who were also able to demonstrate that the first episode in younger patients does not take a more aggressive course. Here, the rate of severe diverticulitis (2%) was even significantly lower compared to 11.9% in patients >50. Thus, the indication for surgery in the younger patient group of this study was lower, but the difference was not significant (2% vs. 6.8%).

Table 5 Univariate analysis of prognostic factors for perforated diverticulitis

Risk factor	Free perforation ($n=145$)	No perforation ($n=874$)	*p value*
Gender			0.123[a]
Male	85 (58.6%)	452 (51.7%)	
Female	60 (41.4%)	422 (48.3%)	
Mean age (years)	37.46	61.34	<0.001[b]
Age			0.044[a]
<40 years	19 (13.1%)	70 (8%)	
>40 years	126 (86.9%)	804 (92%)	
Comorbidity			0.031[a]
None	69 (47.6%)	500 (57.2%)	
>1	76 (52.4%)	374 (42.8%)	
Diverticulitis events			<0.001[a]
First episode	124 (85.5%)	389 (44.5%)	
Recurrence	21 (14.5%)	485 (55.5%)	

[a] Chi-square test

[b] Mann–Whitney test

Furthermore, in our study, both age groups evidenced similar success rates for conservative treatment with no differences in the rate of failure of conservative treatment (3.4% vs. 4.9%, p=0.507). This is in agreement with the data of other authors.[34] No differences were observed between the two age groups with regard to comorbidity (more than one secondary disease) or morbidity and mortality. In both the univariate and the multivariate analyses, the risk analysis for perforation revealed a comorbidity of more than one relevant secondary disease (CHD, COPD, IDDM, renal insufficiency, and immunosuppression) and a first episode as the only risk factor for a perforation. On the other hand, an age under 40 years had no influence.

To our knowledge, our study is currently the largest single-center study with more than 1,000 patients that compares the extent of diverticulitis in younger and older patients. A limiting factor in the assessment of our study results is that there was no follow-up of the patients. Obviously, the decision for surgery is always influenced by a number of significant factors, as for example, the degree of inflammation and perforation of the intestinal wall, but this decision must also include clinical factors and the influence of individual life circumstances. Only long-term course observations can definitely clarify whether surgery is actually recommended in younger patients with a decade-long smoldering disease and the potential risk of recurrences and other complications. There is currently no conclusive evidence from large cohorts or prospective studies as to whether diverticulitis is more prone to develop complications requiring early intervention in younger than in older patients. However, at this point, it is impossible to deduce an indication for the operative treatment of sigmoid diverticulitis solely on the basis of a younger age. Indeed, the actual episode must be re-evaluated in each case. Moreover, it is important to keep in mind that the risk of perforation decreases with the increasing number of episodes.[14, 20] The indication for elective sigmoid resection under the premise of a possible future perforation is thus questionable.[14]

In summary, based on the CT stage and the data obtained in our patients, patients ≤40 years do not have a more aggressive or fulminant diverticular disease than older patients, and both younger and older patients have a similar risk. Our findings demonstrate that the indication for surgery is not dependent on age but on the actual clinical findings.

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ORIGINAL ARTICLE

Gene Mutations and Prognostic Factors Analysis in Extragastrointestinal Stromal Tumor of a Chinese Three-Center Study

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Abstract
Background Most gastrointestinal stromal tumors (GISTs) have gain-of-function mutation of the *c-kit* gene, and some have mutation of the platelet-derived growth factor receptor-α (*PDGFR-α*) gene. Extragastrointestinal stromal tumors (EGISTs) are mesenchymal tumors that occur outside the digestive tract. But the clinicopathologic characteristics of EGISTs are still poorly understood.
Methods Paraffin-embedded tissues from 25 cases of EGIST were analyzed for CD117, CD34, Ki-67, S-100, smooth muscle actin, and desmin expression by immunohistochemical method. These cases of EGISTs were also evaluated for the presence of *c-kit* exons 9, 11, 13, and 17 mutations and *PDGFR-α* exons 12 and 18 mutations. Survival analysis was used to evaluate the prognostic factors.
Results *c-kit* mutations were detected in 44% of EGIST patients and all were exon 11 mutations. *PDGFR-α* mutations were found in 12% of the 25 cases and all were exon 18 mutations. Survival analysis indicated that mitotic count and Ki-67 labeling index (Ki-67 LI) were significant predictors of survival.
Conclusion The pattern of *c-kit* and *PDGFR-α* mutation in EGISTs was essentially similar to that in GISTs. From the molecular genetics aspect, EGISTs may be a special subtype of GISTs. The results also show that the combination of mitotic counts and Ki-67 LI may be useful for predicting the prognosis of EGISTs.

Keywords Extragastrointestinal stromal tumor · Proto-oncogene protein c-kit · Platelet-derived growth factor receptor-α · Gene mutation · Prognosis

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most frequent mesenchymal tumors of the digestive tract. The diagnosis of GIST is based on histomorphological features and, in most cases, histochemical immunoreactivity to the tyrosine kinase receptor of KIT (CD117).[1–3] Most GISTs have oncogenic *c-kit* mutations that engender constitutive activation of this receptor tyrosine kinase, resulting in increased cell proliferation and survival, and such mutations appear to play key roles in the pathogenesis of many GISTs.[4] Moreover, recent studies have described the mutations of platelet-derived growth factor receptor-α (*PDGFR-α*) at the juxtamembrane domain (exon 12) and tyrosine kinase domain (exon 18) in some populations of GIST.[5,6]

Recently, mesenchymal tumors resembling GISTs and positive for KIT have been found outside of the digestive tract, especially in the soft tissues of the abdomen and

retroperitoneum.[7] Those tumors are called extragastrointestinal stromal tumors (EGISTs). Furthermore, Yamamoto et al. have described the frequency of *c-kit* and *PDGFR-α* mutations and the clinicopathologic importance of such mutations in 39 cases of EGISTs.[7] However, the clinicopathologic features, prognostic factors, and gene mutation in EGISTs are still poorly understood, especially in the Han Chinese population. In this study, we aimed to characterize the clinicopathologic features in 25 cases of EGISTs in three Chinese medical centers (Hangzhou First People's Hospital of Zhejiang Province, Taizhou Hospital of Zhejiang Province, and The Second Affiliated Hospital of Zhejiang University) and then elucidated the frequency of *c-kit* and *PDGFR-α* mutations and their clinical significance for predicting the patients' prognosis.

Materials and Methods

Patients and Tumor Samples

Fifty-eight cases of soft-tissue tumors from the abdominal cavity, retroperitoneum, and pelvic cavity were stored in the files of our department. These tumors were originally diagnosed as leiomyomas, leiomyosarcomas, schwannoma, and leiomyoblastomas. All tumors were reexamined histologically using hematoxylin and eosin staining, and one to 20 sections were examined in each case. Immunohistochemical staining was performed on all tumors by using antibodies to the following antigens: CD117, CD34, Ki-67, S-100, smooth muscle actin (SMA), and desmin. EGISTs were defined as tumors fulfilling the following characteristics: (1) tumors showing histologic resemblance to homologous GISTs; (2) tumors that originate from the soft tissues of the abdominal cavity, retroperitoneum, and pelvic cavity, but display no connection to the wall of the gastrointestinal tract and the pelvic organs; (3) tumors with CD117 immunopositivity[7]; (4) exclusion of metastatic GISTs through remote clinical history until 15 years before, especially clinical history of an apparently benign tumor (leiomyoma, etc.) history. In this study, the majority of EGISTs were diffusely and strongly positive for CD117. Four cases with weak CD117 immunopositivity were included in this study because they showed weak but diffuse immunoreactivity for CD117, diffuse expression of CD34, and similar histologic features to those of other EIGSTs with strong immunoreactivity for CD117.

Finally, the study group included 25 cases of EGISTs from Hangzhou First People's Hospital of Zhejiang Province, Taizhou Hospital of Zhejiang Province, and The Second Affiliated Hospital of Zhejiang University during the period from 1995 to 2008. Each specimen was rechecked by two pathologists, respectively. Each EGIST was evaluated for clinicopathologic and histologic features, including cell type (epithelioid, spindle, or mixed), mitoses, and the presence of necrosis and hemorrhage. Mitoses were counted and summed from 50 high-power fields (HPF). To avoid potential impact on survival, all selected patients have never accepted STI-571 (imatinib mesylate; Gleevec) treatment.

Immunohistochemistry and Evaluation

Paraffin-embedded, formalin-fixed tissue was used for immunohistochemical analysis. Four-micrometer representative sections of the specimens were deparaffinized with xylene and rehydrated in graded alcohols. Tissue sections were incubated with the primary antibodies for 4 h at room temperature. Antibody against CD117 (Dako Corporation, Glostrup, Denmark; polyclonal antibody, dilution 1:200), CD34 (Santa Cruz Biotechnology, Santa Cruz, CA,USA; monoclonal antibody, dilution 1:50), Ki-67 (Dako Corporation, Glostrup, Denmark; monoclonal antibody, dilution 1:100), S-100 (Dako Corporation, Glostrup, Denmark; polyclonal antibody, dilution 1:400), SMA (Santa Cruz Biotechnology, Santa Cruz, CA, USA; monoclonal antibody, dilution 1:400), and desmin (Santa Cruz Biotechnology, Santa Cruz, CA,USA; monoclonal antibody, dilution 1:100) were commercially available. The subsequent development of antibody bridge labeling was performed by the streptavidin–biotin–peroxidase method with hematoxylin counterstaining.

Result Assessment Immunohistochemical results were assessed independently by two pathologists in a semiquantitative manner. Localization of positive signals was accurate and the staining of background was light. For CD117 and CD34, positive signals were shown in cytoplasm and/or cell membrane, whereas Ki-67 and S-100 were presented in nucleus but SMA and desmin were shown in cytoplasm. The quantity of immunoreactive tumor cells was estimated according to the following scheme: positive tumor cells <10% (−); positive tumor cells ≥10% (+). For Ki-67,based on the Ki-67 labeling index (Ki-67 LI) of the neoplastic nuclei, the tumors were divided into three groups: <1%, 1–5%, >5%.

c-kit and *PDGFR-α* Gene Mutations Detection

The study group included 25 cases of GISTs. The control group included nine cases of leiomyomas, leiomyosarcomas, and schwannoma. These specimens were rechecked by two pathologists, respectively. *DNA extraction*: To isolate DNA from formalin-fixed, paraffin-embedded tumors, representative paraffin blocks were cut at 8 μm

using a clean disposable microtome blade. The paraffin sections were transferred directly into 1.5-ml Eppendorf tubes and incubated in 1 ml of xylene at 50°C for 15 min and then pelleted at 12,000 rpm for 5 min; this step was repeated. Then the products were resuspended in 1 ml of absolute alcohol at room temperature, spun down, and dried at room temperature. The pellets were then processed using digestive liquid (500 mmol/L Tris, 20 mmol/L EDTA, 10 mmol/L NaCl, 1% SDS, 500 μg/ml proteinase K; pH 8.0) 500 μl overnight at 55°C. Thereafter, the digestive products were extracted by phenol–chloroform–isoamyl (25:24:1) two or three times. Finally, the extracts were washed with 1 ml of absolute alcohol and spun down. The final extracts were dissolved in TE buffer and kept at −20°C for later PCR use. Primers were designed by the software Primer Premier5.0 to amplify exons 9, 11, 13, and 17 of the *c-kit* gene and exons 12 and 18 of the *PDGFR-α* gene (Table 1). PCR amplification include: (1) 94°C denaturizing 5 min; (2) 94°C denaturizing 30 s, annealing 30 s, 72°C elongating 35 s; 30–35 cycles; (3) 72°C elongation 10 min. ddH_2O replaced template as negative control. PCR products were purified and sequenced using the ABI PRISM BigDye terminator cycle sequencing ready reaction kit and ABI Prism 377 genetic analyzer. Mutations were blasted with *c-kit* and *PDGFR-α* gene orders on NCBI GenBank. All mutations were confirmed by a second independent round of PCR and sequencing.

Follow-up and Statistical Analysis

Follow-up was carried out by outpatient surveillance, telephone, and letters. The end point was any death from EGIST for the analysis of disease-specific survival (DSS). SPSS for Windows (version 15.0) was used for statistical analysis. Univariate survival analysis was carried out using Kaplan–Meier plots and the log rank test. Multivariate survival analyses were carried out using Cox regression analysis with forward and then backward stepwise progression instead. A difference in probability (P) values of <0.05 was considered significant.

Results

Clinical and Pathologic Characteristics

There were 15 men and 10 women, with a median age of 58 years (range, 35–76 years; Table 2). Nine cases of EGISTs were located in the mesentery, seven in the omentum, six in the retroperitoneum, and three in the pelvic cavity. The tumors ranged from 5 to 28 cm in size (mean, 12.7 cm). Grossly, most tumors presented as circumscribed or lobulated firm masses. Cystic change was recognized in several cases. According to the proportion of spindle cell and epithelioid cell, 25 cases of EGIST could be divided into three subsets: spindle cell type (12 out of 25); epithelioid cell type (five out of 25); mixed cell type (eight out of 25). EGISTs of spindle cell type (Fig. 1a) were composed typically of relatively uniform eosinophilic cells arranged in short fascicles or whorls. The tumor cells have paler eosinophilic cytoplasm than smooth muscle neoplasms, often with a fibrillary, syncytial appearance where nuclei tend to be uniform in appearance and more ovoid or shorter than those of a smooth muscle cytoplasm, often with vesicular chromatin. EGISTs of epithelioid type were composed of rounded cells with variably eosinophilic or clear cytoplasm. In cases with clear cytoplasm, often "retracted" eosinophilic cytoplasm could be seen around or adjacent to the tumor cell nuclei. Epithelioid lesions, similar to spindle lesions, tend to have uniform round-to-ovoid nuclei with vesicular chromatin. Lesions of mixed cell type may exhibit an abrupt transition between spindle cell and epithelioid areas. Mitotic counts varied from 0 to 35 per 50 HPF. According to the evaluation criteria of Fletcher et al.,[3] 25 cases of EGIST could be divided into four subsets: very low-risk group (VLR, zero out of 25);

Table 1 Primer sequence for *c-kit* exons 9, 11, 13, and 17 and *PDGFR-α* exons 12 and 18 and the corresponding annealing temperature (TA) and the size of expected PCR products

Exon	Primers	Primer sequence 5′→3′	TA (°C)	Product size (bp)
c-kit 9	9-F	ATTTATTTTCCTAGAGTAAGCCAGGG	59	305
	9-R	ATCATGACTGATATGGTAGACAGAGC		
c-kit 11	11-F	CCAGAGTGCTCTAATGACTG	56	190
	11-R	ACTCAGCCTGTTTCTGGGAAACTC		
c-kit 13	13-F	GCTTGACATCAGTTTGCCAG	56	193
	13-R	AAAGGCAGCTTGGACACGGCTTTA		
c-kit 17	17-R	TACAAGTTAAAATGAATTTAAATGGT	55	228
	17-F	AAGTTGAAACTAAAAATCCTTTGC		
PDGFR-α 12	12-F	AAGCTCTGGTGCACTGGGACTT	59	251
	12-R	ATTGTA AAGTTGTGTGCAAGGGA		
PDGFR-α 18	18-F	TACAGATGGCTTGATCCTGAGT	60	212
	18-R	AGTGTGGGAGGATGA GCCTG		

Table 2 Clinicopathologic characteristics in 25 cases of EGIST

Case no.	Age (years)	Gender	Location	Diameter (cm)	Cell type	*c-kit* mutation	*PDGFR-α* mutation	Ki-67 LI	Mitosis (/50 HPF)	Follow-up (months)
1	51	F	Mesentery	11	Spindle	No	No	3.6	3	NA
2	60	F	Retroperitoneum	13	Spindle	No	No	25.6	35	10 (DOD)
3	48	F	Retroperitoneum	9	Mixed	No	No	0.5	3	140
4	35	M	Pelvic cavity	14	Spindle	Yes	No	1.2	1	44
5	70	M	Retroperitoneum	18	Spindle	No	No	9.2	4	19
6	65	M	Omentum	5	Mixed	Yes	No	2	0	78
7	76	F	Mesentery	18	Epithelioid	No	No	4.4	3	39 (DOD)
8	51	M	Mesentery	23	Epithelioid	No	Yes	3.6	6	9
9	57	F	Omentum	9	Mixed	No	No	7.3	15	28 (DOD)
10	62	F	Retroperitoneum	7	Spindle	Yes	No	4.7	1	27
11	58	M	Retroperitoneum	21	Spindle	Yes	No	8.2	9	84 (DOD)
12	73	M	Mesentery	10	Epithelioid	No	No	10.3	4	18 (DOD)
13	47	M	Mesentery	16	Spindle	Yes	No	0.7	2	133
14	63	F	Pelvic Cavity	8	Mixed	No	No	2.8	5	65
15	41	M	Mesentery	13	Mixed	Yes	No	10	3	49 (DOD)
16	59	F	Pelvic Cavity	11	Spindle	No	No	1.4	1	47
17	69	M	Retroperitoneum	13	Mixed	No	No	0.5	3	31
18	54	M	Mesentery	28	Epithelioid	No	Yes	8	3	35 (DOD)
19	55	F	Omentum	6	Spindle	Yes	No	7.8	10	8 (DOD)
20	49	M	Omentum	17	Mixed	No	No	14.7	7	56 (DOD)
21	64	F	Mesentery	9	Spindle	Yes	No	1	1	15
22	53	M	Mesentery	8	Spindle	Yes	No	15.7	4	24 (DOD)
23	60	M	Omentum	18	Mixed	No	Yes	4.6	32	38
24	62	M	Omentum	6	Spindle	Yes	No	10	4	NA
25	52	M	Omentum	7	Epithelioid	Yes	No	0.5	1	106

LI labeling index, *DOD* died of disease, *NA* not available

low-risk group (LR, one out of 25); intermediate-risk group (IR, seven out of 25); high-risk group (HR, 17 out of 25).

Immunohistochemistry Results

Of 25 cases of EGISTs, all were positive for CD117 (Fig. 1c). Among these, 21 tumors were strongly and diffusely positive for CD117, four cases showed weak but diffuse staining for CD117, 18 (72%) cases of EGISTs were positive for CD34 (Fig. 1b), four (16%) were positive for S-100, two (8%) were positive for SMA, and two (8%) were positive for desmin. For the Ki-67 LI (Fig. 1d), seven (28%) were <1%, 11 (44%) were 1–5%, and 7 (28%) were >5% (Fig. 1d).

c-kit and *PDGFR-α* Gene Mutations Analysis

Of 25 cases of EGISTs, *c-kit* mutations were identified in 11 cases of EGISTs which were all in exon 11. No mutations was identified in exons 9, 13, and 17. Most of mutations were heterozygous. These mutations included five cases of EGISTs with in-frame deletion (two cases of which were codons WK(Trp–Lys)557–558 deletion; Fig. 2a). Five cases with point mutations and one case with frame shift mutation. All the mutations clustered between codons 556 and 561, i.e., a hotspot located in the proximal part of exon 11. *PDGFR-α* mutations were identified in three cases of EGISTs which were all in exon 18 (D842V point mutation; Fig. 2b). Moreover, there was no concomitant *c-kit* and *PDGFR-α* mutations in a same individual EGIST case. In the control group, no *c-kit* and *PDGFR-α* mutations were identified in nine cases of non-EGIST tumors.

Survival Analysis

At the end of the study, March 2009, follow-up information was available for 23 patients. The median follow-up time was 38 months (range, 9–140 months). Ten patients died during follow-up. The DSS of the 23 cases of EGIST was 91.3% at 1 year, 73.9% at 3 years, and 60.9% at 5 years. Survival analysis indicated that mitotic count and Ki-67 LI

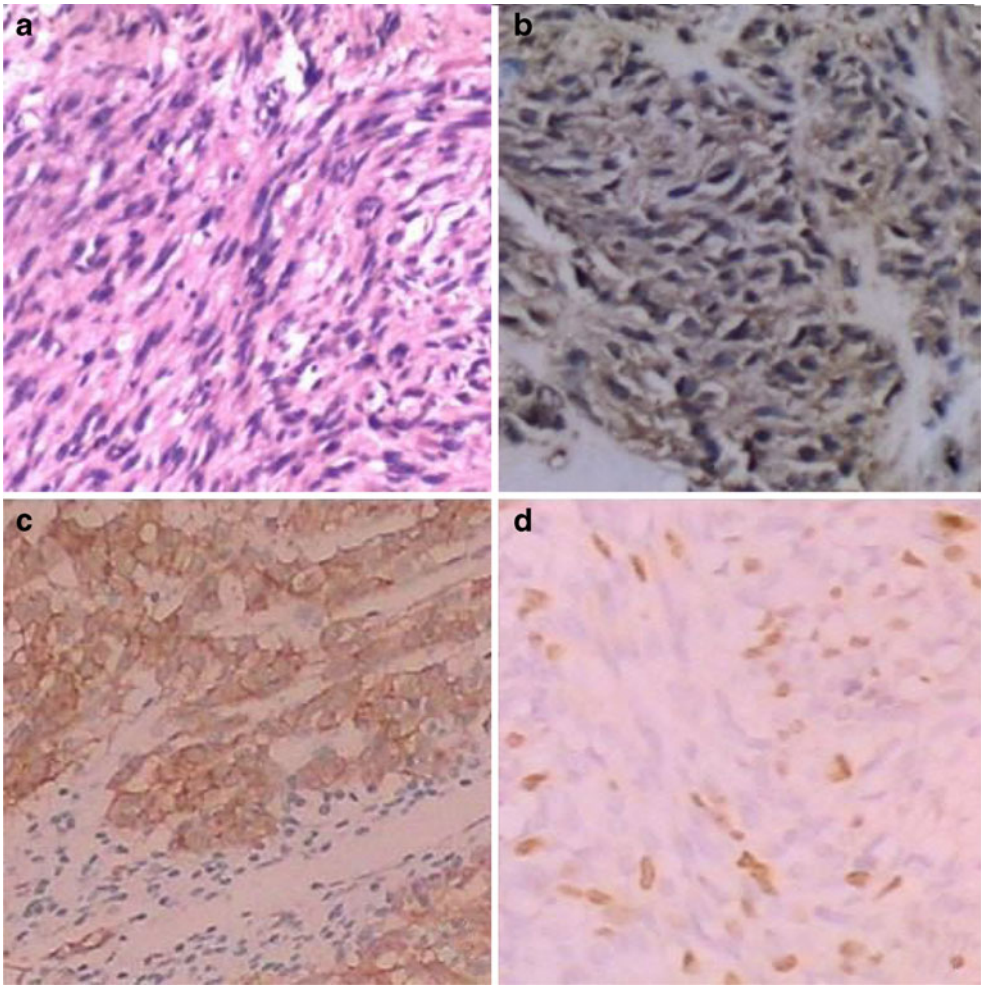

Fig. 1 **a** EGIST of spindle type, H&E stain; **b** CD34 immunohistochemistry shows strong diffuse cytoplasmic and cell membrane expression in EGIST; **c** CD117 immunohistochemistry shows strong diffuse cytoplasmic and cell membrane expression in EGIST; **d** Ki-67 immunopositivity shows nuclei expression in EGIST

were significant predictors of DSS (P=0.025 and P=0.032, respectively). However, tumor size, primary location, Fletcher's risk category, and *c-kit* and *PDGFR-α* gene mutations had no significant relationship with DSS of EGISTs. From the results of this study, it is reasonable to define EGISTs as three categories according to mitotic count and Ki-67 LI: LR group (<5/50 HPF with <5% Ki-67), IR group (≥5/50 HPF with <15% Ki-67 or <5/50 HPF with ≥5% Ki-67), HR group (≥5/50 HPF with ≥5% Ki-67). Based on the above grading criteria, 10 cases were classified as low risk, eight as intermediate risk, and five as high risk. This risk grade was significantly associated with DSS (P=0.028).

Discussion

GISTs are the most common mesenchymal tumor of the digestive tract and characterized by the expression of CD117. Most GISTs have oncogenic *c-kit* mutations and

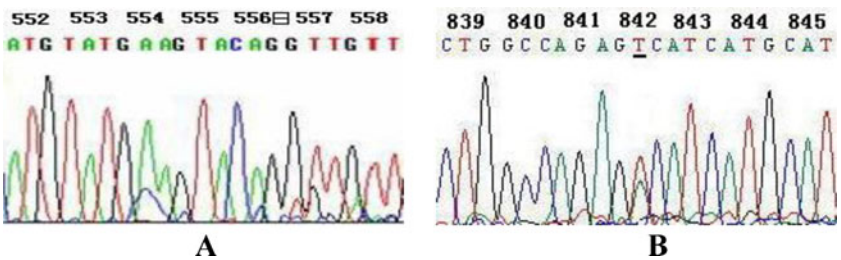

Fig. 2 Genomic evaluations of *c-kit* and *PDGFR-α* mutation by sequencing. **a** Sequence analysis of *c-kit* gene demonstrates a deletion of codons 557–558 WK(Trp–Lys) 6-bp of exon 11 in one EGIST. **b** Sequence analysis of *PDGFR-α* gene demonstrates a heterozygous exon 18 A>T point mutation D842V in one EGIST

such mutations appear to play a key role in the pathogenesis of many GISTs. The mutation rate of *c-kit* is about 53.8–92%.[8–10] In GISTs, *c-kit*-activating mutations cluster frequently in a "hotspot" located in the proximal part of exon 11 between Gln556 and Glu561. A majority of them represent in-frame deletions (one to several codons). However, missense mutation has also been reported in approximately 10% of cases. Mutations in exons 9, 13, and 17 are 5–10%, 0.6–2%, and 0.6%, respectively, and less frequently than in exon 11 (57–71%). But their functional significance appears to be similar to that of the typical mutational "hotspot".[9–11]Recently, a subset of GISTs have been found to have *PDGFR-α* mutations rather than *c-kit* mutations. Mutations in exons 18 and 12 of *PDGFR-α* have been reported. Rubin et al. have reported mutation frequencies in exons 18 and 12 of *PDGFR-α* gene were 5.6% and 1.5%, respectively.[12]

Recently, Reith et al. have reported KIT-positive tumors primarily in the omentum and mesentery, and such tumors are designated EGISTs. EGISTs are histologically and immunohistochemically similar to their gastrointestinal counterpart.[13] Furthermore, Sakurai et al. have described a *c-kit* gene mutation at exon 11 in five cases of GISTs primarily in the omentum.[14] Yamamoto et al.[7] examined the clinicopathological features, prognostic factors, and *c-kit* and *PDGFR-α* mutations in 39 cases of EGISTs. The *c-kit* mutations included point mutations and deletion at exon 11 (juxtamembrane domain) in 12 of 29 cases (41.4%), and the identical tandem duplication of Ala and Tyr at codon 504 of exon 9 (extracellular domain) in two of 29 cases (6.9%), but no mutation was found in the kinase domain (exons 13 and 17). In their study, two EGISTs had the *PDGFR-α* mutation; one had V561D (exon 12) and the other had Del DIMH842–845(exon 18).

In the current study, *c-kit* mutations were identified in 11 cases of EGISTs which were all in exon 11. No mutations was identified in exons 9, 13, and 17. Most of mutations were heterozygous. All the mutations clustered between codons 556 and 561, i.e., a hotspot located in the proximal part of exon 11. *PDGFR-α* mutations were identified in three cases of EGISTs which were all in exon 18 (D842V point mutation). No *c-kit* and *PDGFR-α* mutations was identified in nine cases of non-EGISTs of the control group. The current study results indicated that the clinicopathological features and *c-kit* and *PDGFR-α* mutations of EGIST were similar to their gastrointestinal counterpart (GIST). From the aspect of molecular genetics, EGISTs and GISTs may be from the same origin. Furthermore, the fact that EGISTs were histologically and immunohistochemically similar to GISTs suggests that EGISTs were the distinctive entity, distinguished from leiomyosarcoma and neurogenic tumor.

In addition, the frequency (44%) of *c-kit* mutation in our EGISTs seems to be lower than that in GISTs, particularly compared with those in recent reports. One possible explanation is the difference of the type of tissue used for DNA extraction. Miettinen et al.[15] studied 1,765 cases of GIST and found that the frequency of *c-kit* mutations could be as low as 28%. Moreover, studies using frozen specimens have yielded higher mutation frequencies than using formalin-fixed, paraffin-embedded tissue (because of the more broken DNA fragment). In addition, such mutation frequency increased toward the more recent specimens. Most of our study specimens were collected 5 years ago, so the low mutation rate was explainable. Our previous study[16] found that *PDGFR-α* mutations were more likely seen outside the gastrointestinal tract and of epithelioid or mixed cell type. In the current study, the frequency (44%) of *PDGFR-α* mutation in 25 EGISTs was 12%, higher than in GISTs. This result suggests that the *PDGFR-α* mutation may play an important role in the tumorigenesis of EGISTs. But it should be investigated further.

Survival analysis indicated that mitotic count and Ki-67 LI were significant predictors of DSS. However, tumor size, primary location, Fletcher's risk category, and *c-kit* and *PDGFR-α* gene mutations had no significant relationship with DSS of EGISTs. The most acceptable and easily applicable morphologic criteria for defining risk of aggressive behavior in GISTs are Fletcher's risk criteria (based on tumor size and mitotic count). According to these criteria, tumors with size more than 5 cm may be regarded as having more aggressive behavior. But it may not be applicable in EGISTs. Moreover, in the current study, EGISTs were often large-sized due to their anatomic site, having enough space to grow and presenting clinical symptoms only after a long time. From the results of this study, it may be reasonable to define EGISTs as three categories according to mitotic count and Ki-67 LI: LR group (<5/50 HPF with <5% Ki-67), IR group (≥5/50 HPF with <15% Ki-67 or <5/50 HPF with ≥5% Ki-67), HR group (≥5/50 HPF with ≥5% Ki-67). These risk criteria were significantly associated with DSS.

In summary, we studied the clinicopathologic features, prognostic factors, and *c-kit* and *PDGFR-α* mutations in 25 cases of EGISTs in three Chinese medical centers. The study results indicated that the clinicopathological features and *c-kit* and *PDGFR-α* mutations of EGIST were similar to their gastrointestinal counterpart. But *PDGFR-α* mutation was slightly higher than in GISTs. From the aspect of molecular genetics, EGISTs and GISTs may be from the same origin. EGISTs may be a special subtype of GIST. The results of this study also showed that the combination of mitotic counts and Ki-67 LI may be useful for predicting the prognosis of EGIST.

But there is a question worth noticing. Agaimy et al. demonstrated that true EGISTs were extremely rare, and most so-called EGISTs were probably mural GISTs with

extramural growth towards the peritoneal cavity with eventual loss of their connection to the gut wall. It may be better to re-evaluate surgical reports and remote clinical history and search for residual muscular tissue from the gut wall in the tumor pseudocapsule.[17] But few tumor pseudocapsules of specimens from the pathology archives of these three Chinese medical centers were well preserved. It is impossible to use the immunohistochemical index desmin (as evidence of origin from the gut wall) to evaluate the tumor margins. This was considered as a potential drawback of this study.

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Conflict of Interest Statement There are no conflicts of interest to declare.

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ORIGINAL ARTICLE

Measurement and Interpretation of Patient-Reported Outcomes in Surgery: An Opportunity for Improvement

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Abstract
Background Surgery may have a profound effect on patients' health-related quality of life (QOL). To be optimally useful, trials that seek to guide clinical decision making should measure outcomes that are important to patients and report the results in a clinically meaningful way. We sought to explore how researchers currently measure and interpret QOL in surgical trials, using gastric cancer as a case study.
Method We performed a systematic review of randomized controlled trials (RCTs) of gastric cancer surgery published between 1966 and 2009 that included at least one patient-reported outcome (PRO). Investigators assessed trial eligibility and extracted data in duplicate using standardized forms, then resolved disagreements by consensus.
Results Our search identified 87 RCTs of gastric cancer surgery, of which 11 (13%) included at least one PRO. Ten RCTs measured one or more validated PROs, although six also included ad hoc measures. All manuscripts presented the results as raw scores and nine of the 11 trials identified a statistical difference between groups. All 11 manuscripts prominently reported the PRO results in the abstracts and conclusions, but only one discussed the clinical significance of the differences between groups.
Conclusions Most RCTs of gastric cancer surgery do not include measures of QOL and those that do suffer from important limitations. RCTs would be more useful to surgeons and patients if authors measured PROs and utilized existing approaches to present the results of PROs in ways that provide an intuitive sense of the magnitude of effects.

Keywords Surgery · Quality of life · Minimal important difference · Patient-reported outcomes · Systematic review · Clinical trial design · Gastric cancer · Gastrectomy

Abbreviations

PRO	Patient-reported outcome
QOL	Quality of life
RCT	Randomized controlled trial

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Introduction

Surgical practice has progressed considerably over the past decades, with corresponding improvements in mortality and morbidity from most major procedures.[1–4] As surgeons seek to minimize pain and suffering associated with surgery and maximize patient satisfaction, the focus and priorities of surgical research has evolved. Terms such as "quality of life" (QOL) are now common in the surgical literature, and patients' values and beliefs are carefully considered. This has changed the scope of surgeon practice, with more time

being spent discussing treatment options and expectations with the patient.

Given the potential profound impact of surgery on patients' QOL, research studies that seek to guide clinical decision making should incorporate outcome measures that are important to, and ideally reported by, patients. Although randomized controlled trials (RCTs) including QOL outcomes are becoming more common in surgery, most authorities appear not to rely on QOL data when making recommendations.[5–7] This may be due to methodological limitations in the RCTs, statistically insignificant results, lack of confidence in the measurement tools, or surgeons' inability to interpret the QOL data in a clinically meaningful way. If these potential barriers to the clinical incorporation of QOL data could be identified and overcome, patients and surgeons would be better able to make shared treatment decisions that appropriately incorporate individual values and preferences.[8]

We sought to explore how researchers currently measure and interpret QOL in surgical trials, using gastric cancer as a case study. Over the past several decades, the overall mortality rate from gastric cancer has decreased substantially both in the United States and worldwide, in part due to early diagnosis and aggressive multimodality therapy including surgery, chemotherapy and, in select settings, radiation therapy.[9,10] Although multimodality treatment of gastric cancer improves the chances of survival, each intervention carries with it significant morbidity. In particular, gastric cancer surgery may result in a variety of chronic symptoms including lack of appetite, early satiety, gastroesophageal reflux, abdominal bloating, cramping, pain, diarrhea, and lightheadedness. While almost all patients suffer, to some degree, these symptoms following gastrectomy, approximately 20% find them debilitating.[11] Gastric cancer surgery represents an area where high-quality research to improve QOL is desperately needed.

Methods

To identify all potentially relevant RCTs, we searched the electronic databases Medline, EMBASE, CINAHL, and PsychINFO from 1966 until September 16, 2009 using combinations of the following MESH terms and keywords: "gastrectomy, gastric resection, gastric, stomach, cancer, neoplasm, malignancy, surgery". We conducted a sensitive search strategy using the "Clinical Queries" feature of Medline and EMBASE and restricted our search to articles published in English.

Two investigators (KB and SJ) independently reviewed the combined titles and abstracts (when available) identified from the databases and flagged citations that they felt were potentially relevant. The investigators then reviewed the full text of each study identified by either individual, applying explicit eligibility criteria for inclusion in the systematic review (Table 1). The reviewers met and discussed all disagreements, reaching a final consensus about the trials to include. We measured agreement between reviewers using the Kappa statistic.

Two reviewers (KB and PK) independently extracted data from each eligible manuscript using standardized forms. Methodologically, we assessed the following features of each trial: allocation concealment, blinding, specification of primary outcome, and loss to follow-up. We evaluated the quality of life methodology based on the criteria suggested by Efficace.[6] We recorded the specific patient-reported outcomes (PROs) that were measured, whether the results were statistically significant and how the authors interpreted the results. Reviewers resolved disagreements by consensus.

Results

Our initial search yielded 898 unique citations, of which one or both reviewers considered 119 to be potentially eligible (Fig. 1). We excluded 32 based on our review of the full manuscripts, yielding 87 RCTs of gastric cancer surgery. Of these, 14 (16%) included at least one PRO. Three of these trials were updates or duplicate publications, yielding 11 unique RCTs eligible for this review. Reviewers achieved good agreement in the application of eligibility criteria (kappa 0.56 for gastric cancer surgery RCTs and 0.89 for RCTs including PROs).

Only three of the 87 RCTs identified were conducted in North America, and all of the trials that measured PROs were performed in Europe or East Asia, with the majority taking place at single institutions (Table 2). All RCTs

Table 1 Criteria to include studies in the systematic review

Full article published in English
Randomized controlled trial (or quasi-randomized)
Patients with invasive gastric cancer of any stage
At least one of the interventions being studied is surgical or endoscopic
Includes at least one patient-reported outcome

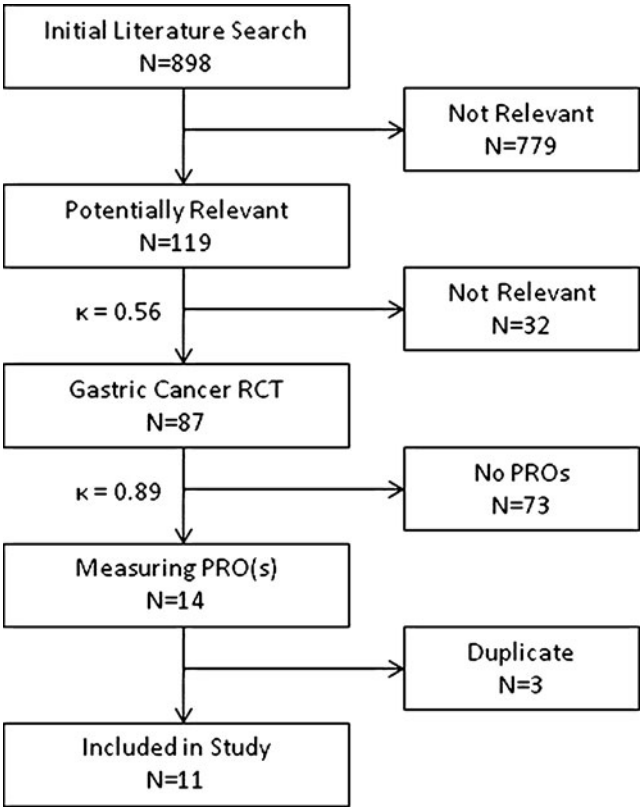

Fig. 1 Overview of trial identification and selection process. *RCT* randomized contlled trial, *PRO* patient-reported outcome, *k* kappa

included patients with localized gastric adenocarcinoma, although three trials included patients with metastatic disease and two included histologies other than adenocarcinoma (lymphoma and gastrointestinal stromal tumor). Nine of the eligible trials explored different techniques of reconstruction following gastrectomy, one trial compared laparoscopic with open resection, and one trial examined the extent of lymph node dissection.

Most trials suffered from important methodological limitations (Table 3). Only one described central randomization to ensure concealment of allocation, no trial incorporated blinding of patients, clinicians, or data analysts, and only one trial blinded the individuals assessing outcomes. Authors explicitly defined a primary outcome in three of the trials. The six trials that reported loss to follow-up succeeded in following over 95% of patients.

Ten RCTs measured one or more validated PROs; one trial included seven instruments (Table 4). The most commonly used measures were the Gastrointestinal Quality of Life Index (GIQLI), the Gastrointestinal Symptom Rating Score (GSRS), the Spitzer QOL index, and the Visick Score. Six of the trials included at least one ad hoc (not systematically developed or validated) PRO. Four authors provided the rationale for choosing the specific PRO(s). Three of the manuscripts discussed the psychometric properties of the instruments, and two discussed the

Table 2 Characteristics of trials included in this review

Author	Year	Location	Centers	Subjects	Patients	Interventions
Fein[12]	2008	Germany	Single	138	Localized or metastatic gastric cancer	Total gastrectomy reconstructed with Roux-en-Y vs. Roux-en-Y with a pouch
Fuchs[29]	1995	Germany	Multicenter	120	Localized or metastatic gastric adenocarcinoma	Total gastrectomy reconstructed with Roux-en-Y vs. jejunal interposition
Hoksch[30]	2002	Germany	Single	48	Localized gastric adenocarcinoma	Total gastrectomy reconstructed with jejunal interposition vs. interposition with a pouch
Horvath[31]	2001	Hungary	Single	46	Localized gastric cancer	Total gastrectomy reconstructed with Roux-en-Y vs. Roux-en-Y with a pouch
Kim[32]	2008	Korea	Single	164	Localized gastric adenocarcinoma	Laparoscopic-assisted distal gastrectomy vs open distal gastrectomy
Kono[33]	2003	Japan	Single	50	Localized gastric adenocarcinoma	Total gastrectomy reconstructed with Roux-en-Y vs. Roux-en-Y with a pouch
Schwartz[34]	1996	Germany	Single	60	Localized or metastatic gastric adenocarcinoma	Total gastrectomy reconstructed with Roux-en-Y vs. Roux-en-Y with a pouch
Svedlund[35]	1999	Sweden	Single	64	Localized gastric adenocarcinoma	Total gastrectomy with Roux-en-Y vs. Roux-en-Y with a pouch vs. subtotal gastrectomy
Troidl[36]	1987	Germany	Multicenter	38	Localized gastric adenocarcinoma	Total gastrectomy reconstructed with Braun esophagojejunostomy vs. Roux-en-Y with a pouch
Wu[37]	2008	Taiwan	Single	221	Localized gastric adenocarcinoma	D1 vs D3 lymph node dissection
Zhang[38]	2009	China	Single	149	Localized gastric adenocarcinoma	Proximal gastrectomy reconstructed with end–side vs. end–end anastomosis

NR not reported

Table 3 Methodological characteristics of trials

Author	Allocation	Patients blinded	Clinicians blinded	Outcome adjudicators blinded	Data analysts blinded	Loss to follow-up	Primary outcome specified
Fein[12]	Central	No	No	No	No	<5%	Yes
Fuchs[29]	NR	No	No	No	No	<5%	No
Hoksch[30]	NR	No	No	No	No	None	Yes
Horvath[31]	Envelopes	No	No	No	No	Unclear	No
Kim[32]	NR	No	No	No	No	<5%	Yes
Kono[33]	NR	No	No	No	No	None	No
Schwartz[34]	NR	No	No	No	No	Unclear	No
Svedlund[35]	NR	No	No	Yes	No	5–20%	No
Troidl[36]	Envelopes	No	No	No	No	Unclear	No
Wu[37]	NR	No	No	No	No	<5%	No
Zhang[38]	NR	No	No	No	No	None	No

NR not reported

Table 4 Measurement of patient-reported outcomes (PROs) in trials

Author	Patient-reported outcomes	A priori hypothesis	Rationale for PRO	Psychometric properties described	Similarity of patients to those involved in instrument development	Adequacy of domains
Fein[12]	Gastrointestinal Quality of Life Index (GIQLI)	No	No rationale provided	No	No	No
Fuchs[29]	Spitzer Index Visick Score	No	Conceptually most relevant	No	No	Yes
Hoksch[30]	EORTC C-30 Ad hoc measure(s)	No	Conceptually most relevant	Yes	Yes	Yes
Horvath[31]	Gastrointestinal Quality of Life Index (GIQLI)	No	No rationale provided	No	No	Yes
Kim[32]	EORTC C-30 EORTC STO-22	Yes	No rationale provided	No	No	Yes
Kono[33]	Gastrointestinal Symptom Rating Score (GSRS)	No	No rationale provided	No	No	No
Schwartz[34]	Ad hoc measure(s)	No	No rationale provided	Yes	Yes	Yes
Svedlund[35]	Gastrointestinal Symptom Rating Scale (GSRS) Body Symptom Scale	No	No rationale provided	No	No	Yes
	Comprehensive Psychopathological Rating Scale					
	Mood Adjective Check List					
	Sickness Impact Profile					
	Karnofsky Performance Status Scale					
	Structured and Scaled Interview to Assess Maladjustment					
	Ad hoc measure(s)					
Troidl[36]	Visick Score Ad hoc measure(s)	No	Conceptually most relevant	No	No	Yes
Wu[37]	Spitzer QOL index Ad hoc measure(s),	Yes	No rationale provided	Yes	No	Yes
Zhang[38]	Spitzer QOL index Ad hoc measure(s)	No	Conceptually most relevant	No	No	No

similarity of patients in the trial to the patients used to develop or test the instrument.

All manuscripts presented the PRO results as raw scores, changes in raw scores from baseline, or graphs of raw scores. All trials reported the results of at least one of the PROs in the abstract of the manuscript. The authors justified their conclusions at least partially on the results of the PROs in all cases. Nevertheless, though nine of the 11 trials compared PRO scores between groups and identified a statistical difference, only one of the manuscripts discussed the importance of the difference to patients.[12]

Discussion

Patients who have undergone surgery may experience debilitating symptoms that have a profound effect on their QOL. As practitioners of evidence-based surgery, contemporary surgeons require high-quality evidence about the effects of surgical interventions on patients' QOL. Although RCTs of surgical interventions are becoming more common, only 16% of the trials we identified in this review included one or more PRO.[13] Most surgical interventions in patients with gastric cancer are intended to cure the disease and prolong life, so trials are appropriately focused on measures like overall survival or disease-free survival. However, given the impact these interventions may have on QOL, investigators should include PRO measures to allow patients and surgeons to make fully informed decisions about therapy.

In general, the methodological quality of the trials we identified was poor. Only one of the trials described an adequate method of concealed allocation, and the majority did not report how participants were allocated to groups. Only one of the trials reported blinding individuals who assessed outcomes, a safeguard that is often possible even when patients and clinicians cannot be blinded.[14,15] It is possible that trials incorporated some of these methodological safeguards but failed to report them, since we relied on the published manuscripts to assess the methodology. However, simple checklists of features that should be reported in manuscripts of RCTs exist and have been endorsed by most medical journals, so authors should refer to these checklists when preparing reports of their trials.[16–18] Prior reviews using criteria from these checklists have identified deficiencies in the reporting of surgical RCTs.[19–22] The limitations we identified in the RCTs included in this study are likely an indication of a pervasive problem in surgical trials rather than a specific limitation of trials measuring QOL.

In order to understand the impact of different surgical interventions on QOL and to convey this information meaningfully to patients, investigators and surgeons require reliable, valid, and interpretable instruments that measure outcomes that are important to patients. Patient questionnaires that are not formally developed and tested (ad hoc questionnaires) may seem to pose clinically reasonable questions, but unless they are properly developed and psychometrically tested, they may be misleading.[23] Fortunately, researchers have developed QOL instruments that are applicable to patients with a variety of malignancies and to patients with gastrointestinal disorders specifically.[24] Seven of the 11 trials we identified included gastrointestinal-specific instruments such as the GIQLI, the GSRS, or the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-STO22. Table 5 summarizes the characteristics of these three instruments. Six investigators also incorporated generic instruments including the Spitzer QOL Index or the EORTC QLQ-C30. Both generic and specific instruments are appropriate for QOL studies, and they may provide complementary information. Over half of the trials, however, included at least one ad hoc measure, which may provide misleading information.

Most manuscripts did not provide the researchers' rationale for selecting the PRO instruments used in the trials. Furthermore, most authors did not comment on the psychometric properties of the instruments or on the similarity (or differences) between the participants in the trials and the patients involved in the instrument's development. In order for a PRO measure to be appropriately included in a trial, it must adequately address the domains of interest with acceptable reliability, validity, and responsibility when tested in a group of patients similar to those being studied.[6]

The reporting and interpretation of PROs in trials deserve special attention. Even if a methodologically sound trial identifies a statistically significant difference in the most relevant QOL instrument, the clinical relevance must be interpretable by surgeons in order to be useful. Measures of QOL typically yield continuous scores: most commonly, a lower score indicates a worse quality of life than a higher score. For example, the EORTC QLQ-C30 global QOL domain scores range from 0 (the lowest possible overall QOL) to 100 (the highest possible QOL).[25] The simplest summary measure of such continuous data is the mean or median of the values. Indeed, all of the trials identified in this systematic review presented the PRO results as raw scores, changes in raw scores from baseline, or graphs of raw scores over time. One benefit of using mean values is the ease of statistical testing; almost all of the RCTs reported statistical tests comparing PRO scores between groups, and a statistically significant difference was identified in all except one of these trials.

Table 5 Characteristic of quality of life instruments appropriate for patients following gastric cancer surgery

Instrument	Format	Development/validation	Content	Population
European Organisation for Research and Treatment of Cancer Stomach Module (EORTC STO-22)[39,40]	Patient self-administered questionnaire consisting of 22 questions. Used in conjunction with the EORTC QLQ-C30 core questionnaire (30 questions). Divided into five scales and four single items.	Items from literature review, interviews with patients, health professionals. Tested in 219 patients. Available in many translations.	Dysphagia Pain Reflux Eating Anxiety Taste Hair loss Dry mouth Body image	Specific for gastric cancer
Gastrointestinal Quality of Life Index (GIQLI)[41]	Patient self-administered questionnaire consisting of 36 questions, specific and generic. Divided into five domains.	Items generated from literature review and experts, tested on 70 patients to construct initial tool. Refined with testing on 204 patients. Further validated in other populations.	Core symptoms Physical items Psychological items Social items Disease-specific	Intended for patients with upper or lower gastrointestinal disease
Gastrointestinal Symptom Rating Scale (GSRS)[42]	Patient self-administered questionnaire consisting of 15 questions. Combined into five symptom clusters.	Developed in patients with irritable bowel syndrome and peptic ulcer disease. Further validated in other populations.	Reflux Abdominal pain Indigestion Diarrhea Constipation	Intended for patients with upper or lower gastrointestinal disease

The major limitation in reporting raw scores is the difficulty in interpretation. For example, investigators comparing Roux-en-Y reconstruction following total gastrectomy with or without a pouch reported a mean difference in GIQLI QOL score of 16.2 at 30 months following surgery.[12] This difference was highly statistically significant (p=0.009); but how important is it to patients? Few surgeons will be able to discern the extent to which a raw score represents a trivial, small but important, or large difference. As a result, surgeons may either disregard the PRO results or rely exclusively on the statistical tests. Only one of the manuscripts included in this review discussed the patient importance of the PRO scores in the manuscript; presenting normal reference ranges for the QOL scores.[12] In contrast, all of the trials presented the PRO results in the abstract, and in each manuscript, the conclusions were based partially or exclusively on the PRO results, relying instead on the statistical tests or visual graphs. Researchers may choose from several options of presenting PRO results that are more interpretable than raw scores. A full discussion of these approaches is beyond the scope of this manuscript, but interested readers may find them well described elsewhere.[26–28]

Our systematic review was limited to surgical trials in gastric cancer, but the results are likely generalizable to other areas of surgical oncology. We chose to study gastric cancer because of the profound impact that gastric surgery may have on patients' QOL and the need for studies focused on QOL to guide management. Trials designed to improve outcomes in patients undergoing major surgery for other gastrointestinal malignancies, including pancreatectomy and proctectomy, should also include patient-important outcomes such as QOL.

None of the trials included in this study was conducted in North America. Indeed, only three of the 87 RCTs identified in our initial systematic search were performed in North America. In contrast, in a study of QOL assessment in patients with prostate cancer, a large proportion of trials were conducted in North America, where this disease is more prevalent.[6] The lack of studies measuring QOL in gastric cancer patients is most likely due to the relatively low incidence of gastric cancer in North America relative to the remainder of the world, rather than a reflection of differing levels of interest in QOL assessment between North American and international surgeons.

In summary, most RCTs of gastric cancer surgery do not include measures of QOL and those that do suffer from important methodological limitations. When statistically significant differences exist between interventions, authors assume that the differences are clinically significant and report the results prominently in the abstracts and conclusions. RCTs would be more useful to surgeons and patients if authors utilized existing approaches to present the results of PROs in ways that provide an intuitive sense of the magnitude of effects.

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HOW I DO IT

Antegrade En Bloc Distal Pancreatectomy with Plexus Hanging Maneuver

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Abstract
Introduction Although antegrade en bloc distal pancreatectomy is appropriate for invasive distal pancreatic malignancies, this technique is not easy to perform because the end-point of deep vertical resections cannot be controlled. This report describes the usefulness of the application of hanging maneuver in performing the radical surgery.
Methods A tape for guidance is passed in a space behind the bundles of the left celiac and mesenteric plexus, followed by sagittal resection of the distal pancreas exposing the root of the celiac artery and superior mesenteric artery. After dividing the pancreas down to the level of the roots of the celiac and superior arteries, the distal pancreas is dissected from the retroperitoneum in medial to lateral fashion.
Results This technique was applied in six patients with distal pancreas malignancies, without any positive cancer cells at the resected margin. The mean tumor size was 3.0±0.9 cm. The mean duration of surgery and intraoperative blood loss were 258±71 min and 226±240 ml, respectively.
Conclusion Antegrade en bloc distal pancreatectomy with plexus hanging maneuver is an appropriate technique for treating distal pancreatic malignancies.

Keywords Hanging maneuver · Distal pancreatectomy · Pancreas cancer · Nerve plexus

Abbreviations

ADPPH	Antegrade en bloc distal pancreatectomy with plexus hanging maneuver
CeA	Celiac artery
DP	Distal pancreatectomy
NP	Nerve plexus
RAMPS	Radical antegrade modular pancreatosplenectomy
SMA	Superior mesenteric artery

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Introduction

Distal pancreatectomy (DP) is the standard treatment for pancreas cancer originating in the body or tail of the pancreas. Such procedures are widely performed using a lateroposterior approach, whereby dissection proceeds from mobilization of the spleen to dissecting the posterior plane from left to right direction.[1] However, these conventional approaches have some limitations, particularly during mobilization, including possible spillage of cancer cells by compression and possible exposure of the cancer cells on the retropancreas dissection plane.[2] To avoid such risks, Strasberg et al.[3] reported a rational anterior approach, called radical antegrade modular pancreatosplenectomy (RAMPS) in 2003. However, en bloc resection of the thick nerve plexus (NP), including lymph nodes around the root of the celiac artery (CeA) or superior mesenteric artery (SMA), is still not easy in RAMPS. Furthermore, the end-point of the vertical resection in radical DP is the roots of CeA and SMA, which are covered by the thick NP. Hanging maneuver was developed by Belghiti et al.[4] for liver

surgery. In this maneuver for hepatectomy, a tape is passed behind the liver to guide the hepatic resection via the anterior approach. We have since applied the maneuver to outline the proper sagittal dissection plane in performing antegrade en bloc DP.

Methods

Via a bilateral subcostal incision with midline extension, the gastrocolic ligament is opened, followed by the division of the root of the splenic artery. A blunt Pean clamp is passed across the cranial surface of the CeA, followed by division of the cranial side of the celiac NP and exposure of the origin of the CeA. A vessel sealing system (VSS; LigaSure Atlas and V™, Valleylab Inc., Boulder, CO, USA) is used for the division of the thick nerve tissues. Secure ligations or the use of ultrasonic coagulation sheers (SonoSurg®, Olympus Surgical & Industrial Inc., Tokyo, Japan) might be the alternatives. Even under the use of VSS, the distal end of the thick nerve plexus should be ligated off and divided to prevent chylous leakage. All the short gastric vessels are also divided using the VSS.[5]

After performing the full right-side visceral rotation, the NP on the dorsal side of the proximal SMA is opened, and the origin of the SMA is exposed and controlled. Then, a blunt Kelly clamp is passed cranially through a space between the root of the SMA and the left NP lateral to the SMA (Fig. 1a), reaching a space between the CeA and left NP lateral to the CeA (Fig. 1b). The anatomical relationship between the SMA, CeA, and the aorta should have been be checked by CT prior to the procedures. Recognition of the clockwise position of the origins of the CeA and the SMA from the aorta may help the secure passing of the clamp along the roots of such major arteries. A 10-Fr plastic tape (ATOM™ tube, ATOM Medical Inc., Tokyo, Japan) is passed through the newly created tunnel, and this tape guides the end-point of the sagittal resection process during the antegrade en bloc DP.

The common hepatic artery is isolated from the pancreas, and the dissection is continued to expose the caudal surface of the CeA from the NP. The anterior surface of the superior mesenteric vein is identified at the root of the transverse mesocolon. A blunt Pean clamp is passed across the superior mesenteric vein behind the pancreas neck, which is divided by electrocautery. The main pancreatic duct should be securely ligated, and the cut surface of the remnant pancreas is heat-coagulated using soft coagulation system (VIO 300™, ERBE Inc., Marietta, GA, USA).

The pancreatomesocolic ligament is divided to the left. Then, the plane of the dissection proceeds vertically, dividing the Toldt's fusion fascia, and the left renal vein

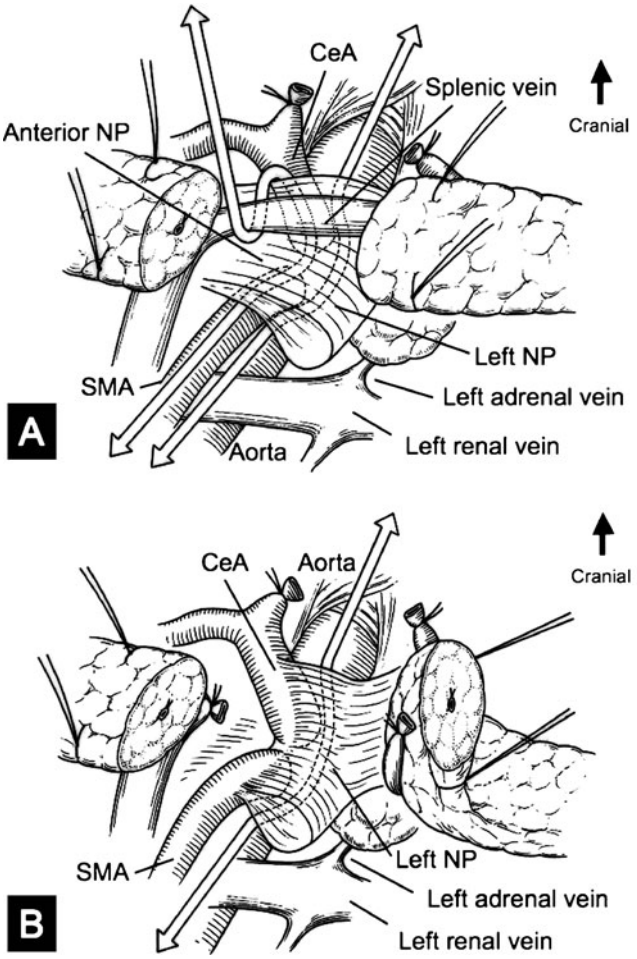

Fig. 1 The anterior NP is pulled up and divided, and the splenic vein is preserved (**a**). The left NP is pulled up by the tape, which was previously passed through a space between the origin of the major arteries and the left NP (**b**). *Black arrows* indicate the cranial side. *CeA* celiac artery, *NP* nerve, *SMA* superior mesenteric artery

is exposed as the marker of the caudal border of the en bloc DP. Without dividing the splenic vein to prevent congestive bleeding from the distal pancreas, another plastic tape is passed along the anterior surface of the SMA to the caudal surface of the CeA (Fig. 1a). By pulling up the tape, the thick NP lying anterior to the SMA is safely divided. The left NP is divided at the level of the left renal vein. The plastic tape within the space between the left NP and the origins of the SMA and CeA is hanged to safely divide the left NP (Fig. 1b).

At this point, the dissection plane goes laterally. The division of the left celiac ganglion enables us to identify the anterior surface of the adrenal vein. If the tumor appears to invade the retropancreatic fat tissue, the left adrenal vein should be ligated and the dissection plane goes under the adrenal gland and across the renal capsule. The anchor sutures, which were made on the cut edge of the distal pancreas, need to be pulled up to visualize the retroperitoneal dissecting plane

visible. After dissecting the splenorenal ligament, the complete total distal pancreas, including vessels, nodes, NP, and retropancreas tissues, are excised en bloc.

Results

Between April 2009 and May 2010, ADDPH was performed in six patients (four males and two females) with the mean age of 67 years. The indication for DP included invasive ductal carcinoma (n=4) and invasive mucous carcinoma (n=2). Two patients had prior acute pancreatitis with pseudocyst formation. The other two patients had received radiation therapy before surgery. The mean tumor size was 3.0±0.9 cm. The mean duration of surgery and blood loss were 258±71 min and 226±240 ml, respectively. For three of the six patients, a deeper posterior plane was needed to remove the left adrenal gland and expose the anterior surface of the left kidney.

Although none of the patients died during surgery, four patients had postoperative complications, including chylous ascites (n=3) and delayed gastric emptying (n=1). Tumor invasion was seen in the posterior peripancreatic fat close to the adrenal gland in four (65%) patients. Perineural invasion was present in four (65%) patients, and the tumor invasion was present in the NP around the CeA and SMA in one (17%) patient. Negative surgical margins were obtained in all six patients (100%). Although the observation period was short, all the patients are alive and without recurrence at 2, 5, 8, 11, 12, and 13 months, respectively.

Discussion

The basic principles in surgery for malignancies include no-touch isolation technique, negative cancer cells at the resected margin, and en bloc resection.[6–8] In previous reports, the positive resection margin ranged from 25% to 28% for DP performed using conventional lateroposterior approach.[9,10] Moreover, in the conventional approach, the thick plate of the NP and celiac ganglion around the CeA, SMA, and aorta block the inward retroperitoneal dissection during mobilization, and it becomes difficult to perform en bloc resection of the structures. Therefore, the anterior approach in DP is a rational technique, as Strasberg et al.[3] reported. However, the application of radical anterior approach is potentially difficult because the end-point of the sagittal resection process cannot be controlled in the original approach. The end-point of the vertical resection process in radical DP is the origins of CeA and SMA, which are covered by the thick NP. Thus, a vertical division via the anterior approach down to the level of anterior surface of the aorta may cause serious injury of the vessels or organs. Although previous randomized trials in pancreatoduodenectomy for pancreas head cancers revealed no beneficial impacts of extended para-aortic lymph nodes dissection on survivals,[11] it has also been reported that only regional R0 resection can only offer the chance of survivals.[7,12] The current technique aims for en bloc R0 resection under no-touch technique.

Antegrade en bloc distal pancreatectomy with plexus hanging maneuver (ADPPH) is a secure technique, offering advantages in DP via the anterior approach over the classical technique. First, the tape positioned at the left side of the roots of the CeA and the SMA helps the surgeon to identify the optimal line for the vertical pancreas resection easily. This concept, using the hanging maneuver, was previously reported for complex hepatic resections.[13,14] The most significant role of the hanging maneuver in ADPPH is the tape that elevates the thick nerve plexus on the root of the CeA and SMA and outlines a proper dissection plane from the anterior to posterior. It also protects the structures behind it: CeA, SMA, left renal vein, and aorta. A surgeon can simply follow the tape for deeper dissection, and the division of the tissue anterior to the tape results in the completion of the sagittal resection down to the root of the CeA and SMA. Moreover, by using the hanging maneuver to divide the NP anterior to the SMA, the splenic vein could be kept open until the final stage of the surgery. Occlusion or division of the splenic vein in an early stage of DP sometimes causes intraoperative congestive bleeding because the vein is the only major drainage vein from the distal pancreas. We believe that the small amount of blood loss during radical ADPPH (226±240 ml) may be due to preservation of the splenic vein.

The incidence of chylous ascites was high (50%) ascites in the current technique. However, daily output of the chylous ascites from the abdominal drain was less than 100 ml in all the three patients, and non-per oral for several days cured all the chylous ascites. In ADPPH, the left-side half of the periarterial plexus along the CeA and SMA is completely cleared. Therefore, we speculate that the division of the small lymphatic nests around the proximal SMA resulted in such complications.

In summary, the use of hanging maneuver is useful when performing radical DP via the anterior approach. DPAH is an innovative, safe, and rational technique and is applicable for the resection of distal pancreas malignancies.

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REVIEW ARTICLE

Humans, Mice, and Mechanisms of Intestinal Atresias: A Window into Understanding Early Intestinal Development

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Abstract
Introduction Intestinal atresias have long been hypothesized to result from either failure of recanalization of the intestinal lumen or in utero vascular accidents. Recent work in animal models is now calling for a reassessment of these widely held paradigms.
Purpose In this review, we will examine the data that led to the original hypotheses and then evaluate more recent work challenging these hypotheses. Furthermore, we will discuss how defining the mechanism of atresia formation in animal models may provide insight into early intestinal development and the mechanism of lengthwise intestinal growth.
Conclusion Such insight will be critical in developing regenerative therapies for patients with intestinal failure.

Keywords Intestinal atresia · Mechanism · Intestinal growth · Hypothesis · Intestinal development · Vascular hypothesis · Epithelial plug · Duodenum

Introduction

Intestinal atresia is a phrase used to describe a segmental defect of the intestine which disrupts the luminal continuity of the intestinal tube during development. This is frequently accompanied by a loss of the surrounding mesoderm and blood supply to the affected region. A simple way of thinking about an atresia is that if the developing intestine is a series of pipes connected to one another end-to-end, in atresia, one of these segments of pipe disappears. Intestinal atresias can occur anywhere throughout the intestine, from the duodenum to the colon, and are one of the most common causes of neonatal intestinal obstruction, with an incidence between 0.57 and 6.6 per 10,000 live births (Table 1).[1]

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An Early Hypothesis Based on an Interesting Observation

Intestinal atresias were first described in 1684.[2] The etiology of these defects were ascribed to any number of causes, including psychiatric fits of the mother, a lack of bile secretion, peritonitis, improper axial rotation of the intestine, compression by the transverse mesocolon as well as obliterative embryonic events.[3] In 1900, a Viennese anatomist, Julius Tandler, published a hypothesis on the origins of intestinal atresia based on his studies of normal duodenal development. Tandler observed that the endoderm of the duodenum proximal to the "umbilical loop" (or C-loop of the duodenum) undergoes, "A remarkable thickening of the epithelium… until finally the duodenum becomes a solid string, in which no lumen at all is to be found." This occlusion occurs at day 42 of development. Thereafter, the duodenum recanalizes between days 44 to 46 by forming multiple, small channels in the epithelial string which later coalesce into a single lumen. Tandler postulated that:

> "If one keeps in mind the fact that on one hand the epithelial occlusion of the duodenum represents a normal event, but on the other hand that it is exactly in this place that most pathologic occlusions of the

Table 1 Incidence of intestinal atresias versus allelic incidence of factor V Leiden

	Region/country	Incidence of small intestine atresia	Allele frequency (%) of factor V Leiden
1	Spain (2001–2005)	0.57	3.33
2	Ireland (1995–2005)	0.86	6.95
3	Norway (1974–2005)	0.9	3.7
4	Chile (2002–2005)	0.95	2.5
5	Hungary (2001–2005)	1.03	4.9
6	UK	1.04	3.61
7	Finland	1.12	4.2
8	USA–Utah (2001–2005)	1.32	4.2
9	Italy (ISMAC) (2001–2005)	1.5	2
10	N. Netherlands (1985–2005)	1.62	2.9
11	Wales	1.84	4
12	Slovak (1995–2005)	2.02	4
13	New Zealand (2001–2005)	2.27	3.8
14	Germany (Northeastern)	2.29	7
15	Sweden (2001–2005)	2.71	7.8
16	Czech Republic (1995–2002)	2.81	5.1
17	Saudi Arabia	2.88	1.3
18	Australia	3.14	3.6
19	South America (2001–2005)	3.2	1.6
20	Canada	3.84	5
21	Japan (2001–2005)	6.63	0

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> intestine occur, the question does not appear unjustified to ask whether these processes relate to each other, that is, whether they are causally related. It would not be impossible that in rare cases the physiologic atresia remains and develops into a congenital atresia…. It is clear to me that the opinion represented here does not exceed the status of a new hypothesis, and it is not meant to exceed this."[3]

In spite of a cautiously worded qualification, Tandler's hypothesis has become dogma in the pediatric surgical community; however, researchers have begun to challenge this hypothesis over the last two decades. In his original paper, Tandler reported that, like humans, the duodenum of rats undergoes a similar developmental phase of luminal obliteration as a result of proliferation. More recent histological studies in rats indicate that this species do not undergo this developmental event.[4] It remains unknown whether mice also fail to form a proliferative endodermal solid core or plug during development; however, duodenal atresias can be generated in mice by mutating either the gene for fibroblast growth factor receptor 2IIIb (Fgfr2IIIb) or its ligand Fgf10.[5,6] If mice, like rats, fail to form a proliferative endodermal plug, it would indicate that this developmental event is not required for duodenal atresia formation.

Do Atresias Arise from Mechanical Events?

Fifty-five years after Tandler's work was published, surgeons J.H. Louw and Christiaan Barnard hypothesized that a "vascular accident" was the major etiology of intestinal atresias of the jejunum and ileum,[7–9] an idea originally proposed by Spriggs in 1912.[2] Their hypothesis was based on the clinical observation that thrombi were present in vascular arcades of the proximal intestine adjacent to the atretic region. They then tested whether segmental occlusion of the arterial blood supply to the intestine in utero would result in atresias. They successfully performed fetal surgery on two near-term canine fetuses, ligating arteries in the mesentery adjacent to the small intestine. Both pups developed atresias. Based on these observations, they concluded that interruption of the vasculature in utero (possibly from a thromboembolic event) was a major etiology of atresia formation.[7–9]

Louw and Barnard's work provided experimental evidence that mechanical compression of the arterial blood supply to the intestine can give rise to atresias, and there is clinical evidence that *some* intestinal atresias may arise from this mechanism. For example, gastroschisis and volvulus are two congenital defects that have a high association with atresia formation. In gastroschisis, a rupture occurs on the right side at the junction of the

umbilical cord and the abdominal wall resulting in a ring-like abdominal wall defect. The intestine then herniates through the ring into the amniotic cavity. Seven percent of these patients will present with an atresia,[10] which likely results from mechanical compression of the intestine by the ring-like defect. In volvulus (as a result of improper rotation and fixation or from a focal twist of the intestine around an adhesive band), intestinal blood flow is impaired resulting in involution of the affected segment of the intestine,[8,9] loss of intestinal continuity, and atresia formation (Fig. 1). Not surprisingly, Louw and Barnard's hypothesis was based on observing an atresia arising from a focal volvulus around an adhesive band.[7]

Cystic fibrosis (CF) has a high association with intestinal atresia formation but the mechanism is unknown. In fact, findings consistent with CF were associated with intestinal atresia long before CF was identified as a separate, distinct disease. Tandler credited the first description of this association to an investigator named Forer.[3] Forer observed that atresia formation was associated with a lack of bile in the meconium, giving it the white or gray appearance commonly found in children with CF and intestinal atresias. Several studies have demonstrated a significantly higher incidence of CF in patients with jejunal–ileal atresias than would be expected in the general population.[11,12] Cystic fibrosis with meconium ileus is reported to account for 11% of jejunoileal atresias and 5% of all intestinal atresias.[13] If CF is included as a mechanical cause of atresia formation, together with gastroschisis and volvulus, these conditions account for approximately 50% of jejunoileal atresias and only 20% of all atresias.[13] *While the mechanism by which atresias occur in the setting of* CF *remains unclear, the majority of intestinal atresias (80%) do not appear to be associated with mechanical events.*

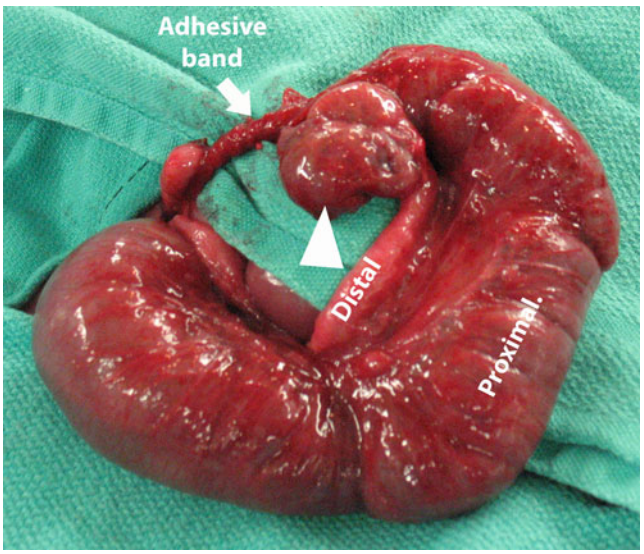

Fig. 1 Atresia of the mid-ileum resulting from an adhesive band and subsequent volvulus. Proximal and distal limbs are indicated. Adhesive band is marked by a *white arrow*. The volvulized segment of the bowel is indicated by the *white arrowhead*

Is There Epidemiological Evidence of In Utero Thromboembolic Events as a Cause of Intestinal Atresias?

Since the publication of Louw and Barnard's work, there has been a noticeable lack of genetic, molecular, or developmental evidence to support a "vascular accident" as an etiology of atresia formation. One paper was published that suggests a relationship between a predisposition to hypercoagulable states and atresia formation. That paper reported an associated increase in the allelic frequency of either factor V Leiden or the R353R mutation of the polymorphic region of factor VII in 28 patients with intestinal atresias.[14] Whether any of the patients in this study were hypercoagulable at the time of their presentation was not determined. Factor V Leiden is very rarely associated with arterial thrombosis, the putative mechanism of the "Vascular hypothesis." The R353R mutation results in increased levels of factor VII leading to coronary artery thrombosis in adults; however, gestational and perinatal levels of vitamin K-dependent clotting factors (including factor VII) are very low in infants due to a deficiency in vitamin K.[15] Therefore, it is very unlikely that the R353R allele can result in atresia formation in utero. Finally, if either factor V Leiden or R353R were causative in atresia formation, then populations with high allelic frequencies of these mutations would have an increased incidence of intestinal atresias. This, however, is not the case. The Centers for Disease Control issues a report every year on the rate of congenital defects, including intestinal atresia, for a number of countries.[1] Based on data from reporting countries, there is *no* statistical correlation between the incidence of intestinal atresia and the allelic incidence of factor V Lieden (correlation coefficient of −0.36) (Table 1; Fig. 2) or R353R (correlation coefficient of 0.24) (Table 2; Fig. 3).[1,16–39,18,40–50] This analysis strongly suggests that neither mutation plays a role in, nor is associated with, intestinal atresia formation.

What Does the Clinical Presentation of Intestinal Atresias Reveal About Their Etiology?

Intestinal atresias present over a range of severity. The current categorization scheme reflects this range with types I, II, IIIa, and IIIb, organized by the amount of tissue absent and type IV reflecting the presence of multiple defects

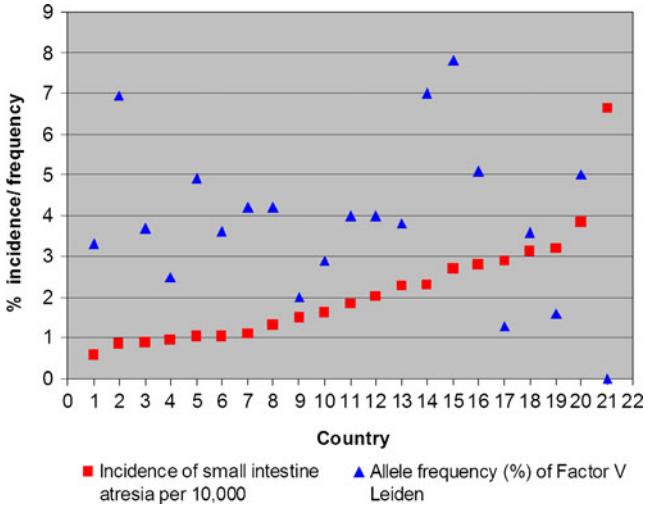

Fig. 2 Graph of the incidence intestinal atresia versus allelic incidence of factor V Leiden

(Fig. 4). These five types of atresia occur with a relatively equal incidence and distribution throughout the intestinal tube. These characteristics are similar to other developmental defects that arise from genetic mutations that disrupt a common developmental process (cleft palate, omphalocele, and limb defects).[51–55] Following this rationale, a type IIIb atresia would reflect an early disruption in this common developmental process in the embryonic gut, whereas less severe defects would reflect a later disruption in the same developmental process. Additionally, atresias primarily affect a limited area of intestine and are rarely hereditary.[56]

These clinical characteristics argue that atresias arise from a somatic mutation that disrupts a common developmental process in the embryonic gut.

In What Tissue Would a Disruption in a Common Developmental Process Have to Occur for an Atresia to Develop?

Further examination of the clinical presentation of atresias points to a defect in endoderm development as a leading event in atresia formation. This is most evident in duodenal atresias. Duodenal atresias, in addition to having a significant association with Down syndrome, also present with a high incidence of anomalies of other midline structures, including the esophagus, pancreatic duct, bile duct, heart, and rectum.[56,57] Like the duodenum, these other structures initiate development in the midline of the embryo, and with the exception of the heart, are all derived in part from endoderm. The heart is not composed of endoderm, but cardiac myoblasts come into direct contact with the foregut endoderm during their migration out of the heart fields prior to forming the heart tube.[58] It appears that this developmental interaction with the endoderm is critical in programming these cells in the proper formation of the heart.[58] The common thread running through all these defects is that the endoderm plays a central role in their development. Thus, disruptions in endoderm development would appear to be central to the etiology of these defects.

Table 2 Incidence of Intestinal atresia versus allelic incidence of R353R mutation for factor VII

	Region/country	Incidence of small intestine atresia	Allele frequency (%) of factor V Leiden
1	Spain (2001–2005)	0.57	3.33
2	Norway (1974–2005)	0.9	3.7
3	Chile (2002–2005)	0.95	2.5
4	UK	1.04	4.4
5	USA–Utah (2001–2005)	1.32	4.2
6	Italy (ISMAC) (2001–2005)	1.5	2
7	N. Netherlands (1985–2005)	1.62	2.9
8	Wales	1.84	4
9	Slovak (1995–2005)	2.02	4
10	New Zealand (2001–2005)	2.27	3.8
11	Germany (Saxony)	2.29	8
12	Sweden (2001–2005)	2.71	7.8
13	Saudi Arabia	2.88	2.5
14	Australia	3.14	3.6
15	South America (2001–2005)	3.2	1.6
16	Canada	3.84	5

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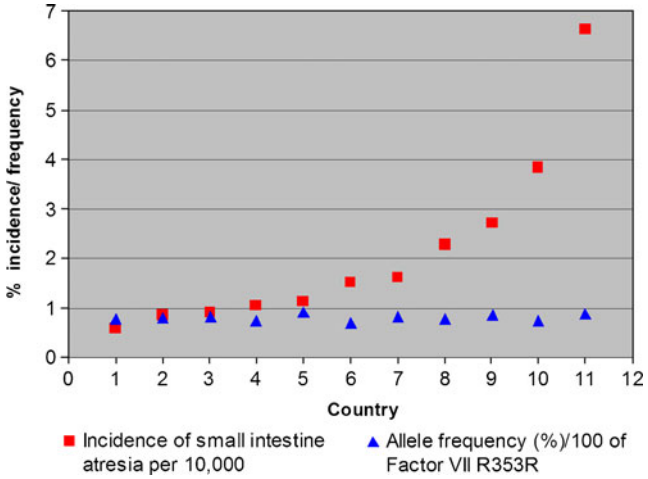

Fig. 3 Graph of the incidence intestinal atresia versus factor allelic incidence of R353R mutation for factor VII

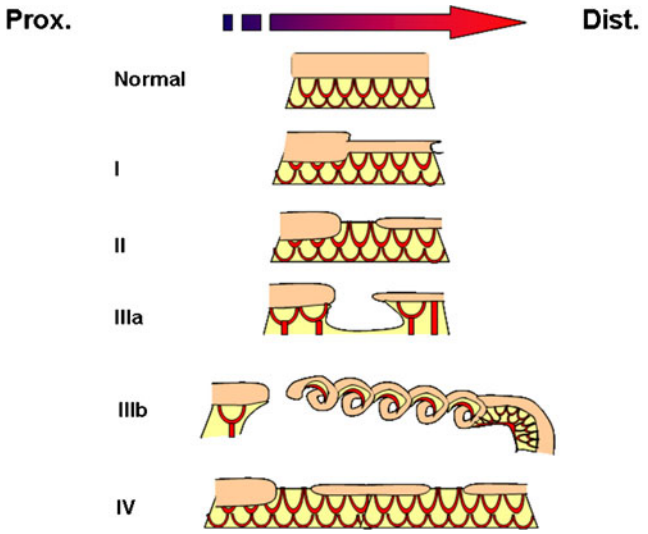

Fig. 4 Atresias range in severity from a segmental narrowing or stenosis of the intestine to much more severe defects where there is an absence of a segment of the intestine and its blood supply. They are categorized based on increasing severity of the defect determined by three factors: (1) loss on intestinal tissue, (2) disruption of the accompanying intestinal blood supply, and (3) number of defects present. Type I atresias are a defect in which the intestinal mesoderm remains in continuity, but the lumen is obstructed by a diaphragm of tissue. In some cases, there is a small central opening, which allows for the restricted passage of contents. This variant includes the defect known as intestinal stenosis. Type II atresias are a defect wherein a small solid core of tissue connects the proximal and distal portions of the intestine; the core of tissue entirely lacks a lumen. In type IIIa atresias, the affected segment of intestine disappears and a V-shaped defect is visible in the adjacent mesentery or blood supply to the intestine. Type IIIb defects have an extensive gap in the blood supply between the proximal and distal limbs of intestine and they are frequently referred to as "apple peel defects" or "Christmas tree defects" because the distal limb of the bowel coils around its own mesentery. Type IV atresias have multiple points of interruption in the intestine, but the intestinal mesentery remains largely intact

What Do Animal Models Tell Us About Atresias?

Recent data from animal models contests the view that the leading event in atresia formation is vascular. These data, instead, suggest that a disruption in endoderm development results in atresia formation. For example, mutation of *Fgfr2IIIb* or the gene encoding its ligand *Fgf10* results in both colonic and duodenal atresias. This suggests that the mechanism of formation of both atresias is the same.[5,6,59–61] The *Fgfr2IIIb* gene encodes for a membrane-bound tyrosine kinase receptor that is thought to be expressed in the endoderm, but not the mesoderm, of the developing intestine.[62] Mutation of *Fgfr2IIIb* or *Fgf10* results in the loss of receptor signaling function leading to atresia formation. The leading cellular events in this model are increased in cell death and decreased proliferation specifically, and exclusively, in the endoderm.[61] These endodermal cellular events precede any changes in the vasculature by at least a full day in the mouse (the equivalent of 4–6 days in a human). Second, disruption of Hedgehog signaling in the results in atresia formation in mice.[64] Hedgehogs are signaling proteins that are generated by the endoderm and act on targets in the mesoderm. Mouse embryos that are homozygous for mutation in *Shh* develop a stenosis of the duodenum and a variant of imperforate anus, both of these defects fall within the spectrum of intestinal atresias.[63] Finally, it has been shown that mutation of a gene encoding a transcription factor expressed exclusively in the colonic endoderm (*Cdx-2*) after embryonic day 12 in mice also results in atresia formation.[64] Taken together, these data appear to refute the vascular hypothesis and instead point to a disruption in endoderm development or endoderm to mesoderm signaling as the leading events in atresia formation.

A New Round of Questions

The accumulating evidence favoring a disruption in endoderm development as the leading event in atresia formation raises a number of important questions. First, when do atresias occur? Second, what genes are involved? Third, what are the downstream morphogenetic events that result in the improper development of the affected intestinal segment? Finally, what do these defects reveal about the normal processes of intestinal growth and development?

Based on data from mouse models, intestinal atresias begin forming very early in development between E10.5 and E12.5 (the equivalent of days 33–48 in humans); however, the likelihood of demonstrating similar timing of these events in humans either from existing embryo collections or with the current prenatal imaging technologies seems remote at best. The animal models implicate disruptions in several molecular pathways in atresia

formation, including the Fgfs and the Hedgehogs. Encouragingly, work in humans has demonstrated that mutations in the *Fgfr2* coding region are associated with duodenal stenosis.[65] Work in mice has demonstrated that mutations in a number of members of the Hedgehog signaling pathway genes (*Gli-1*, *Gli-2*, *Gli-3*, *Ihh*, and *Foxf1*) do not result in intestinal atresias but in some cases can cause variations in imperforate anus.[63,66] These results suggest that formation of atresias may require disruptions of other signaling cascades in addition to the Hedgehog pathway. Unraveling the cellular events downstream of the initial insult to the endoderm will be challenging because these events will involve cellular differentiation, movement, and organization into specific tissue layers. These processes in early intestinal development have not been well defined.

What atresias may reveal about intestinal development and lengthwise intestinal growth could be far more profound. In mice, atresias begin forming at E10.5, the beginning of the most rapid phase in linear intestinal growth which runs from E10.5 to E15.5 and is equivalent to weeks 4.5 to 12 in the human.[61] At the beginning of this phase, the intestinal length is approximately one third that of the embryo. By the end of this phase, the intestine is five to six times the length of the embryo.[67] During this period, the lengthwise growth of the intestine outpaces that of the embryo by a factor of nearly 15 to 1. The characterization of cellular events during atresia formation should provide critical insight into normal intestinal development during this most rapid phase of lengthwise growth. These insights will be essential if we are to develop much needed novel therapies to stimulate lengthwise intestinal growth for some 30,000 patients in the U.S.A. with intestinal failure.

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GI IMAGE

Gallstone Ileus Causing Perforation of the Sigmoid Colon

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Abstract A rare case of a cholecystocolonic fistula causing gallstone ileus with perforation of the sigmoid colon is described.

Keywords Gallstone ileus · Sigmoid perforation

An 87-year-old woman came to the emergency department reporting severe abdominal pain since the morning. The pain initially started in the right upper quadrant and migrated to the left lower quadrant. The patient had a history of calculous cholecystitis which was treated conservatively (Fig. 1). Currently, physical examination showed a distended abdomen with peritoneal irritation at the left lower quadrant. Blood counts revealed inflammation and elevated alkaline phosphatase. Liver enzymes and bilirubin were within normal range. Plain abdominal radiography revealed dilated small bowel loops with air-fluid levels and caecal dilatation. A subsequent CT scan showed a cholecystocolonic fistula and a perforation of the mid-sigmoid with a large gallstone (4.2×3.1 cm) at the level of perforation (Fig. 2). The caecum also contained several smaller gallstones. At laparotomy, a perforation of the distal sigmoid and dilation of the caecum up to 12 cm was found. Given the presence of purulent free fluid and the ASA 3 score of our patient, a Hartmann's procedure was performed (Fig. 3). Decompression of the caecum by colostomy and drainage allowed us to simultaneously remove the caecal gallstones. Histology showed no evidence for malignancy of the colon.

Gallstone ileus is an unusual but well-known cause of intestinal obstruction. Migration of a gallstone through a cholecystoduodenal fistula and subsequent impaction at the site of the terminal ileum is described in the majority of

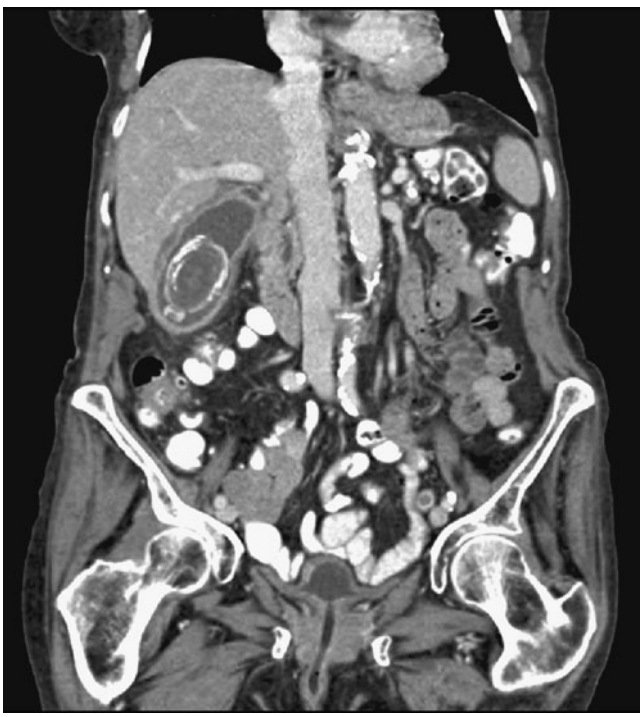

Fig. 1 CT scan 5 months prior to colonic perforation showing the large gallstone within the gallbladder

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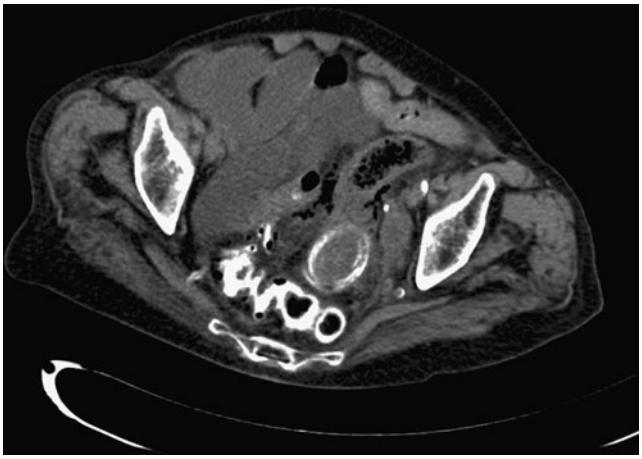

Fig. 2 The large gallstone is migrated through a cholecystocolonic fistula to the sigmoid causing a large bowel obstruction and perforation of the sigmoid

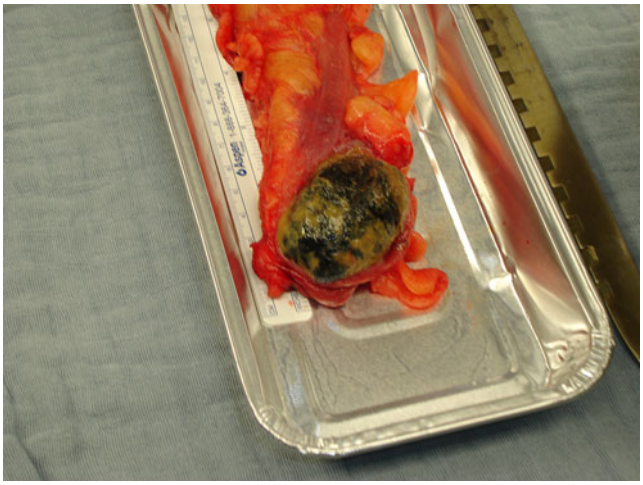

Fig. 3 Resected sigmoid with gallstone

cases.[1–4] Impaction at the site of the sigmoid is very rare. Narrowing of the lumen as a result of previous sigmoidal polypectomy could explain the site of impaction in our patient.

As clinical presentation is often aspecific and a history of calculous cholecystitis, a useful pointer towards a pre-operative diagnosis, is present in only half of the cases, diagnosis is often delayed. In combination with the predominance of gallstone ileus in the elderly population, diagnostic delay contributes to the high mortality rate (15–18%).[1] Unreserved use of imaging techniques can decrease the diagnostic delay.[3] Signs of bowel obstruction, airobilia and an atypical migrating mineral shadow are characteristic findings on plain abdominal X-ray. Although this "Rigler's triad" is considered pathognomonic, these features are present in only one third of the patients.[2] CT scan has been reported to be a powerful tool in obtaining an early and definitive pre-operative diagnosis.[3–5] The mainstay of surgical treatment for gallstone ileus is prompt relief of the bowel obstruction by removing the gallstone. To date, there is no consensus on the most appropriate approach and the extent of surgery. With regard to the presented case, timely and minimal extended surgery was necessary to remove the blockage and to prevent severe faecal peritonitis, two main objectives in the emergency setting.

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