Fundoplication Improves Disordered Esophageal Motility

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Patients with gastroesophageal reflux disease (GERD) and disordered esophageal motility are at risk for postoperative dysphagia, and are often treated with partial (270-degree) fundoplication as a strategy to minimize postoperative swallowing difficulties. Complete (360-degree) fundoplication, however, may provide more effective and durable reflux protection over time. Recently we reported that postfundoplication dysphagia is uncommon, regardless of preoperative manometric status and type of fundoplication. To determine whether esophageal function improves after fundoplication, we measured postoperative motility in patients in whom disordered esophageal motility had been documented before fundoplication. Forty-eight of 262 patients who underwent laparoscopic fundoplication between 1995 and 2000 satisfied preoperative manometric criteria for disordered esophageal motility (distal esophageal peristaltic amplitude ≤30 mm Hg and/or peristaltic frequency ≤80%). Of these, 19 had preoperative manometric assessment at our facility and consented to repeat study. Fifteen (79%) of these patients had a complete fundoplication and four (21%) had a partial fundoplication. Each patient underwent repeat four-channel esophageal manometry 29.5 ± 18.4 months (mean ± SD) after fundoplication. Distal esophageal peristaltic amplitude and peristaltic frequency were compared to preoperative data by paired *t* test. After fundoplication, mean peristaltic amplitude in the distal esophagus increased by 47% (56.8 ± 30.9 mm Hg to 83.5 ± 36.5 mm Hg; $P < 0.001$) and peristaltic frequency improved by 33% (66.4 ± 28.7% to 87.6 ± 16.3%; $P < 0.01$). Normal esophageal motor function was present in 14 patients (74%) after fundoplication, whereas in five patients the esophageal motor function remained abnormal (2 improved, 1 worsened, and 2 remained unchanged). Three patients with preoperative peristaltic frequencies of 0%, 10%, and 20% improved to 84%, 88%, and 50%, respectively, after fundoplication. In most GERD patients with esophageal dysmotility, fundoplication improves the amplitude and frequency of esophageal peristalsis, suggesting refluxate has an etiologic role in motor dysfunction. These data, along with prior data showing that postoperative dysphagia is not common, imply that surgeons should apply complete fundoplication liberally in patients with disordered preoperative esophageal motility. (J GASTROINTEST SURG 2003;7:159–163.)

KEY WORDS: Gastroesophageal reflux disease (GERD), fundoplication, esophageal dysmotility, dysphagia

Patients with gastroesophageal reflux disease (GERD) and esophageal dysmotility are the most scrutinized subgroup of surgically treated patients because they may be at increased risk for postoperative dysphagia. A causal relationship between GERD and esophageal dysmotility has been implied by series reporting that antireflux surgery affects demonstrable functional recovery.[1–9] However, pharmacologic acid suppression has not been associated with significant improvement in esophageal motility[5,10–13]; therefore the precise relationship between GERD and esophageal motor function remains controversial.

We recently reported that postfundoplication dysphagia, assessed by a symptom questionnaire, occurred infrequently, even in the presence of preoperative dysmotility and a complete fundoplication.[14]

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However, subjective dysphagia, or lack thereof, does not predict manometric function. The goal of this study was to quantify postfundoplication esophageal motor function in patients with abnormal esophageal function preoperatively.

METHODS

This study was approved by the University of North Carolina at Chapel Hill Committee on the Protection of Rights of Human Subjects. A total of 262 patients with GERD documented by either (1) endoscopic evidence of esophagitis, ulceration, peptic stricture, or Barrett's metaplasia or (2) an abnormal ambulatory 24-hour pH study underwent laparoscopic fundoplication between 1995 and 2000. All patients underwent preoperative esophageal manometry. Forty-eight patients met the preoperative manometric criteria for disordered esophageal motility (distal esophageal peristaltic amplitude $\leq$30 mm Hg or peristaltic frequency $\leq$80%).

Nineteen of the 48 patients with preoperative esophageal motor dysfunction (9 men, mean age 52.2 $\pm$ 12.4 years) had undergone preoperative manometric assessment at our institution and consented to repeat esophageal manometry postoperatively. These 19 patients comprise the study group. Of these, 15 had undergone complete fundoplication and four had partial fundoplication. To measure the effectiveness of fundoplication in the study patients, all were asked to complete a previously validated GERD-specific postoperative symptom survey.[15] Heartburn and dysphagia scores were compared to those in an historical control group comprised of the first 140 patients in our series using the Mann-Whitney U test.

At a mean follow-up interval of 29.5 $\pm$ 18.4 months after fundoplication, all study patients underwent repeat four-channel esophageal manometry. Data were collected by experienced technicians using a water-perfused catheter system (Medtronics, Inc., Shoreview, MN), and all examinations were interpreted by one investigator (N.J.S.). Distal esophageal peristaltic amplitude and peristaltic frequency were compared to matched preoperative data by paired *t* test. Data are reported as mean $\pm$ standard deviation (SD). A *P* value of less than 0.05 was considered significant.

RESULTS

Symptom Survey

After fundoplication, the 19 patients with disordered esophageal motility reported similar heartburn (*P* = NS) and dysphagia (*P* = NS) compared to the historical control group (Fig. 1).

Esophageal Motor Function

Comparison of preoperative and postoperative esophageal manometric data (Table 1 and Fig. 2) demonstrated that mean distal esophageal body pressure increased 47% (56.8 $\pm$ 30.9 mm Hg to 83.5 $\pm$ 36.5 mm Hg; $P < 0.001$), and peristaltic frequency improved 32% (66.4 $\pm$ 28.7% to 87.6 $\pm$ 16.3%; $P < 0.01$). Esophageal motor function normalized in 14 patients (74%) after fundoplication, whereas in five patients the manometric status remained abnormal (2

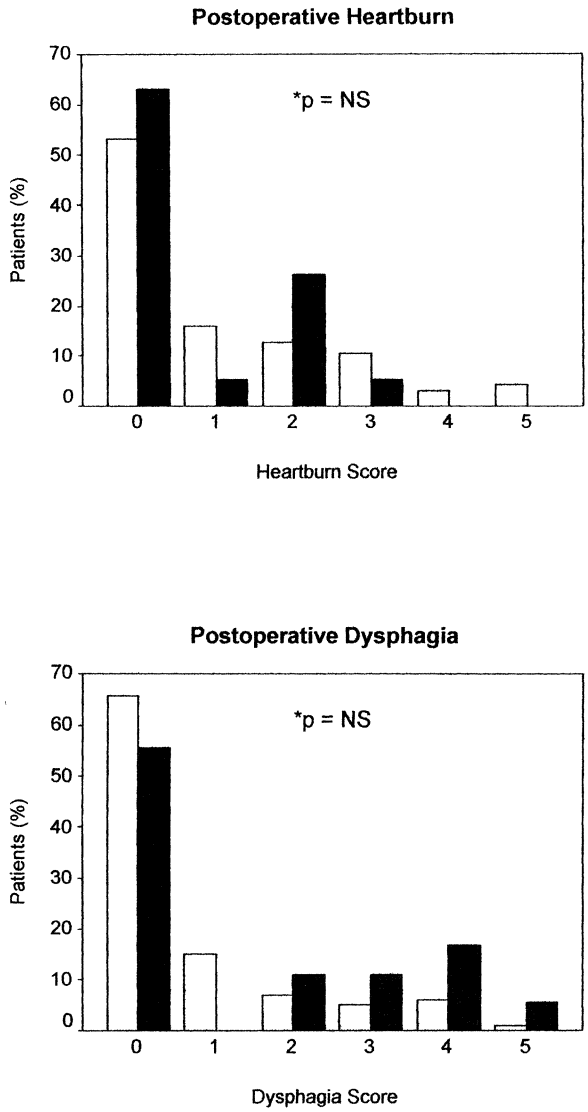

Fig. 1. Comparison of heartburn and dysphagia responses between historical controls and esophageal dysmotility subject group. □ Controls, N = 140; ■ Subjects, N = 19; *Mann-Whitney U test.

Table 1. Individual manometric results after fundoplication

	Amplitude (mm Hg)		% Peristalsis			
Patient	Preoperative	Postoperative	Preoperative	Postoperative	Operation	Result
1	99	138	70	91	Toupet	Normal
2	115.7	148	80	100	Nissen	Normal
3	61.4	87	10	88	Nissen	Normal
4	65	135	50	100	Toupet	Normal
5	88.3	118	80	75	Nissen	Abnormal
6	30.9	69	0	84	Toupet	Normal
7	47.5	69	70	80	Nissen	Abnormal
8	41	31	20	50	Toupet	Abnormal
9	38.3	42	0	100	Nissen	Normal
10	96.5	96	67	100	Nissen	Normal
11	90	120	0	100	Nissen	Normal
12	66.3	69	70	100	Nissen	Normal
13	24	35	100	90	Nissen	Normal
14	29.8	71	92	100	Nissen	Normal
15	29	44	82	100	Nissen	Normal
16	20	49	100	90	Nissen	Normal
17	26	114	90	100	Nissen	Normal
18	25	82	80	58	Nissen	Abnormal
19	85	70	80	58	Nissen	Abnormal
Mean	56.8 ± 30.9	83.5 ± 36.5	66.4 ± 28.7	87.6 ± 16.3	79% Nissen	74% Normal

improved, 1 worsened, and 2 remained unchanged). Three patients with preoperative peristaltic frequencies of 0%, 10%, and 20% improved to 84%, 88%, and 50%, respectively, after fundoplication.

Manometric findings did not differ between patients with complete fundoplication and those with partial fundoplication, but the number of patients who had partial fundoplication is too small for a meaningful comparison.

DISCUSSION

The aim of this study was to determine whether esophageal motor function improves after fundoplication in patients with GERD and preoperative esophageal dysmotility. The normalization of disordered esophageal motor function noted in most patients after fundoplication suggests that a component of esophageal dysmotility occurs secondary to esophageal acid exposure and is often reversible.

GERD and Esophageal Dysmotility

Experimental and surgical clinical data implicate esophageal acid exposure in the pathogenesis of esophageal motor dysfunction. In a baboon model, acute acid-induced esophagitis has been shown to impair esophageal peristalsis.[16] In humans, mucosal injury has been associated proportionally with the degree of esophageal motor dysfunction.[2] Our results agree with those of several surgical series demonstrating improved esophageal motor function after fundoplication. In 1986, Gill et al.[1] demonstrated a 70% improvement in distal esophageal amplitude in 32 patients undergoing open fundoplication. Grande et al.[4] reported that recovery of esophageal motor function after fundoplication continued into the late postoperative period. More recently, other surgical series have reached similar conclusions.[6,17]

Medical studies examining deacidification of esophageal refluxate and one surgical series failed to demonstrate recovery of impaired esophageal motor function. An early study involving nine patients with grade I to III esophagitis treated with H_2-antagonists demonstrated no improvement in esophageal motility.[18] A prospective randomized trial comparing 4 weeks of omeprazole therapy vs. placebo in 31 patients also failed to demonstrate clear improvement in esophageal motility.[11] A prospective surgical trial, which randomized 100 patients with esophageal dysmotility to either partial or complete fundoplication, failed to demonstrate an overall improvement in motor function in either group.[19] However, nearly 20% of patients in this study had evidence of recurrent reflux 4 months postoperatively, indicating that con-

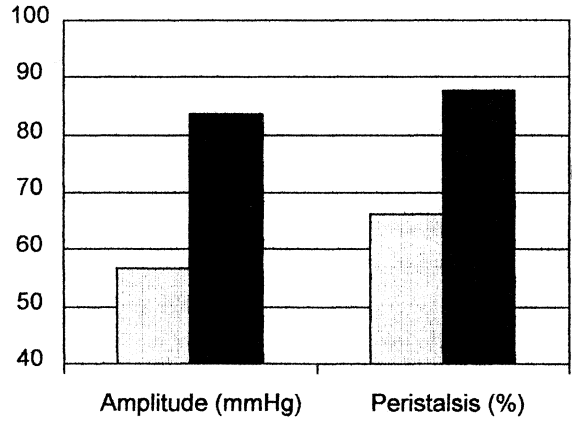

Fig. 2. Observed esophageal amplitude (mm Hg) and peristalsis (%) before and after fundoplication. N = 19; □ preoperative; ■ postoperative.

tinued esophageal acid exposure may have had a significant impact on the conclusions drawn from the study.

The persistent nature of esophageal motor dysfunction in some studies may signify selection bias, inadequate sample size or insufficient follow-up. The chronic nature of GERD may affect functional recovery, with loss of reversibility as acute esophageal inflammation evolves into chronic GERD with fibrosis.[20] Because reflux of gastric acid into the distal esophagus has been shown to occur in medically treated patients despite symptom resolution and endoscopic healing of esophagitis,[21] ongoing acid injury must be considered. In addition, the effect of refluxate composition and volume remains uncertain.

Fundoplication and Esophageal Dysmotility

The selective use of partial fundoplication for patients with esophageal dysmotility assumes that complete fundoplication provides greater resistance to swallowing and that esophageal motor dysfunction persists after surgery. This approach was borne from the results of the initial laparoscopic series, which reported higher rates of dysphagia after complete fundoplication vs. partial fundoplication at early follow-up.[8,22,23] Since then, indirect data (from porcine and human explants) have suggested that complete and partial fundoplications augment the lower esophageal sphincter to a similar degree.[24,25] In addition, manometric assessment in patients revealed resting lower esophageal sphincter pressures of 10 to 15 mm Hg after both complete and partial fundoplications.[26,27] More recent clinical studies with longer follow-up reported that dysphagia rates after complete and partial fundoplications converge at 1 year.[5,28] Some investigators continue to use partial fundoplication exclusively,[29] despite mounting clinical data suggesting that partial fundoplication may provide less durable acid protection, particularly in patients with poor esophageal function.[30,31] Taken together, our previous symptom response study showing that complete fundoplication is not associated with increased risk of postoperative dysphagia,[14] and this report demonstrating that the esophagus recovers motor function when protected from refluxate, refute the use of partial fundoplication as a means to guard against postoperative swallowing difficulties.

CONCLUSION

Our data suggest the following: (1) persistent dysphagia is not obligate after complete fundoplication in patients with abnormal preoperative esophageal motor function; (2) reflux may be an important etiologic factor in preoperative esophageal dysmotility; and (3) limitation of GERD by fundoplication may improve esophageal motor function. Given the evolving evidence for the inferiority of partial fundoplication vs. complete fundoplication in preventing esophageal acid exposure, and the potential for progression of GERD-related esophageal motor dysfunction over time, the rationale of using partial fundoplication to treat patients with esophageal dysmotility is suspect.

As is the case in all studies that focus on a subpopulation of GERD patients defined by an abnormal preoperative variable, small sample size and the possibility that postoperative trends may be explained simply by regression of randomly outlying data toward the mean limit conclusions. We anticipate that well-designed, prospective, randomized studies correlating preoperative and postoperative esophageal acid exposure with manometric function will prove that complete fundoplication is associated with superior acid protection and improved symptomatic and manometric esophageal motor function compared to partial fundoplication.

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Barrett's Esophagus With High Grade Dysplasia: Surgical Results and Long-Term Outcome—An Update

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We updated our surgical results and long-term outcome for prophylactic esophagectomy in patients with Barrett's esophagus and high-grade dysplasia (HGD) and determined the incidence of occult adenocarcinoma. Sixty consecutive patients with HGD who underwent esophagectomy had pre- and postoperative pathology examined at our institution from 1982 to 2001. We reviewed medical records to determine patient characteristics, surgical approach, operative morbidity and mortality, pathology, and length of stay. Patients and/or referring physicians were contacted to determine long-term outcome. Fifty-three men (88%) and 7 women (12%) were followed up for a median of 4.6 years. Transhiatal esophagectomy was performed in the majority of patients (82%). There was one operative death (1.7%) and 15 complications (29%). Median length of stay was 9 days. In 18 patients (30%), invasive adenocarcinoma was detected in the resected specimen. When examined by time periods, 43% (13/30) of patients were diagnosed with occult cancer from 1982–1994, whereas 17% (5/30) harbored occult malignancy from 1994–2001. All patients with adenocarcinoma in the recent interval had stage I disease, as opposed to only 61.5% of patients from the earlier study. Operative mortality declined from 3.3% to 0% over the two intervals as did mean length of stay from 14 days to 10 days. Five-year survival was excellent at 88%. Age and amount of preoperative weight loss were preoperative predictors of survival, whereas major postoperative complications and stage were postoperative predictors of outcome. Barrett's esophagus with high-grade dysplasia continues to be an indication for prophylactic esophagectomy. Overall prevalence of occult adenocarcinoma remains high. We have demonstrated a declining incidence of occult cancer and treatment of earlier stage adenocarcinoma when found in this population of patients treated with esophagectomy. (J GASTROINTEST SURG 2003;7:164–171.)

KEY WORDS: Barrett's esophagus, dysplasia

There is increasing clinical and molecular genetic data supporting the fact that esophageal adenocarcinoma arises from Barrett's mucosa through an intermediate step of epithelial dysplasia. With aggressive anti-reflux treatment, low-grade dysplasia can be converted back to non-dysplastic Barrett's mucosa. However, once high-grade dysplasia (HGD) is attained, there is heightened concern that it will progress to invasive adenocarcinoma. Furthermore, patients with HGD are frequently found to have an unsuspected adenocarcinoma, with an incidence that varies from 0–73%. In 1996, we published our results and outcome in the surgical management of 30 patients with Barrett's esophagus and HGD.[1] We found a 43% incidence of occult malignancy. Operative mortality was 3%. Survival was stage-related, and was favorable for stage I and II disease. Our results prompted us to advocate esophagectomy as primary treatment of patients with HGD. Since that time, the number of HGD patients referred to our institution has increased, prompting this retrospective update of our clinical series.

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MATERIALS AND METHODS

The surgical pathology records at the Johns Hopkins Hospital were reviewed from November 1982 to April 2001, to identify all patients with Barrett's esophagus and high-grade dysplasia, who had undergone esophagectomy. Clinic charts and hospital medical records were examined to identify only those patients in whom the pre-esophagectomy diagnosis was HGD. Sixty consecutive patients were identified. Prior to surgery and inclusion in this study, the diagnosis of Barrett's esophagus with HGD was made or confirmed by our pathologists using histopathologic criteria previously reported.[2,3] As this was a retrospective review spanning almost 20 years, the individuals who comprised our gastrointestinal pathology group varied somewhat. However, the criteria used to make the diagnosis of HGD remained constant. Patients with invasive adenocarcinoma identified preoperatively were excluded.

This study includes 30 patients for whom we previously reported the results for prophylactic esophagectomy from November 1982 to October 1994.[1] That database was included in this study but additional follow-up was obtained through April 2001. For the remaining 30 patients, medical records were reviewed retrospectively to determine patient characteristics, preoperative and postoperative pathology, surgical approach, operative morbidity and mortality, and length of hospital stay. For all patients, referring physicians, primary care physicians, or the patients themselves were contacted for long-term follow-up. Long-term follow-up was for survival only and the following questions were posed: Was the patient alive with no evidence of disease, alive with disease, or dead and what was the cause of death?

Results are determined for the time periods 1982–1994, 1994–2001, and for the entire study period in order to determine trends. Values are expressed as mean ± standard deviation unless otherwise specified. Comparisons between groups were made using unpaired Student's *t* test, Mann Whitney U test, or Fisher's exact test, where appropriate. Kaplan-Meier survival curves were calculated using SPSS statistical software (SPSS, Inc., Chicago, IL). Cox univariate followed by multiple stepwise logistic regression was performed to determine predictors of adenocarcinoma in patients with preoperative HGD and to examine predictors for survival. Significance was accepted at the 5% level.

RESULTS

Patient Characteristics

Demographics are summarized in Table 1. The overwhelming majority of patients were Caucasian (98%) and male (88%). The average age was 60 years. Gastroesophageal reflux symptoms were present in 54 patients (90%), with an average duration of 16 years. A documented previous history of Barrett's was present in 43 patients (72%), with an average duration of 3.3 years. A hiatal hernia was frequently present (78% of patients). In contrast, only a minority of patients had peptic ulcer disease (22%) or a history of preoperative weight loss (18%).

Table 1. Patient demographics

Age (yr)	60 ± 11
Male	88% (53/60)
Caucasian	98% (59/60)
Reflux symptoms	90% (54/60)
duration (yr)	16 ± 14
Barrett's esophagus	
Previous diagnosis	72% (43/60)
Duration (yr)	3.3 ± 2.8
Presence of preoperative weight loss	18% (10/55)
Hiatal hernia	78% (47/60)
Peptic ulcer disease	22% (13/58)
Smoking history	67% (39/58)
Alcohol consumption	70% (38/54)

We compared patient characteristics from those with HGD alone (42 patients) vs. those diagnosed with adenocarcinoma identified postoperatively (18 patients). There were no significant differences between the two groups with respect to age, race, sex, smoking, or alcohol consumption. Previous history of Barrett's esophagus was similar between the two groups, as was the duration of Barrett's with an average of 3.3 ± 2.8 years for each group. Both groups also had a similar incidence of gastroesophageal reflux symptoms; however, the duration of symptoms was significantly longer for patients with adenocarcinoma as opposed to HGD alone, 22.8 ± 12.8 years vs. 12.8 ± 13.8 years, $P = 0.02$. There were no significant differences in the frequency of peptic ulcer disease or preoperative weight loss between the two groups. In contrast, a hiatal hernia was present in all 18 patients with cancer and only 29 of 42 patients (69%) with HGD alone, $P < 0.01$.

We also compared changes in patient demographics across the two time periods, 1982–1994 and 1994–2001. No significant differences were found in patient characteristics, except that significantly more women were diagnosed with HGD from 1994–2001 (7 of 30 patients, 23%) as compared to the initial study from 1982–1994 (1 patient, 3%, $P = 0.02$).

Surgical Results

All patients underwent prophylactic esophagectomy with the results summarized in Table 2. We fa-

vored the transhiatal approach, which was used in 49 patients (82%). In the remainder of patients, three-incision esophagectomy was performed in 1 patient, left thoracoabdominal incision in 4 patients, and Ivor-Lewis approach in 6 patients. There was only one operative death in a 77-year-old woman, who after uneventful initial recovery, suffered an aspiration-associated respiratory arrest on postoperative day eight. Overall, surgical complications occurred in 15 patients (29%)—4 patients with major complications and 11 with minor. Major complications included one superior mesenteric artery thrombosis requiring small and large bowel resection with resultant short gut syndrome, one gangrene of the gastric tube requiring resection and esophageal diversion, one wound dehiscence requiring operative reclosure, and one re-exploration for sepsis. Minor complications included two arrhythmias, two wound problems, one transient hoarseness, two asymptomatic paragastric herniation of a segment of transverse colon, one postoperative delirium, one partial small bowel obstruction, and two readmissions for failure to thrive, requiring nutritional support via jejunostomy tube. Average length of stay was 12.1 ± 9.5 days (median = 9).

We compared our recent results (1994–2001) to those of our previous study (1982–1994) and noted several trends. We have increasingly performed transhiatal esophagectomy and ceased doing the Ivor-Lewis technique. Our operative mortality decreased from 3.3% to 0% and our length of stay decreased from 14 days to 10 days, though not reaching statistical significance. Our overall complication rate remained the same.

Pathology

Pathologic findings are listed in Table 3. The overall incidence of adenocarcinoma diagnosed postoperatively in patients with HGD alone on preoperative endoscopy was 30%. We examined the changes in this incidence over time, from 1982–1994 as published in our prior study, and from 1994–2001. From 1982–1994, 43% of patients were incidentally discovered to have adenocarcinoma, in contrast to 17% of patients from 1994–2001, $P = 0.03$. Also, the distribution of cancer staging has changed. In our earlier study,[1] a significant proportion of patients with adenocarcinoma were advanced beyond stage I disease, 5 of 13 patients (38.5%). From 1993–2001, no patients had stage II or III disease—all 5 patients (100%) were stage I.

We performed binary logistic regression analysis to determine if any of the patient characteristics listed in Table 1 were predictive of Barrett's adenocarcinoma. Length of gastroesophageal reflux disease was the only significant predictor of carcinoma on univariate analysis ($P = 0.03$). Patients with adenocarcinoma had GERD symptoms for 22.8 ± 13.2 years as compared to 12.8 ± 14.03 years for patients with HGD alone. Multivariate logistic regression was unable to be performed with only one predictive variable.

Survival

Kaplan-Meier actuarial survival curve for all patients in the study is depicted in Figure 1. Follow-up was 96.7% complete, with two patients lost to follow-up. Median length of follow-up was 4.6 years, with a range of 0.03 to 15.6 years. Eleven of thirteen patients with stage I esophageal adenocarcinoma are alive with no evidence of disease with a median follow-up of 6.1 years (0.3 to 8.3 years). Two patients with stage I disease died, one died of adenocarcinoma in an incompletely excised HGD segment, and the other died of an unknown cause. Of the four pa-

Table 2. Surgical results

	Overall 1982–2001 (No. of Patients/Total No.)	1982–1994	1994–2001
No. of patients	60	30	30
Surgical approach			
Transhiatal	81.7% (49/60)	70% (21/30)	93% (28/30)
Ivor-Lewis	10% (6/10)	20% (6/30)	0% (0/30)
3-incision	1.7% (1/60)	0% (0/30)	3.3% (1/30)
Left thoracoabdominal	6.7% (4/60)	10% (3/30)	3.3% (1/30)
Complications	29% (15/51)	33% (7/21)	27% (8/30)
Minor	21.6% (11/51)	19% (4/21)	23% (7/30)
Major	7.8% (4/51)	14% (3/21)	3.3% (1/30)
Operative mortality	1.7% (1/60)	3.3% (1/30)	0% (0/30)
Length of stay (days)	12.1 ± 9.5	14 ± 7.9	10.3 ± 10.5

Table 3. Pathology

Incidence of adenocarcinoma	
Overall from 1982–2001	30% (18/60)
Overall from 1982–1994	43% (13/30)
Stage III	7.7% (1/13)
Stage II	30.8% (4/13)
Stage I	61.5% (8/13)
Overall from 1994–2001	16.7% (5/30)
Stage I	100% (5/5)

tients with stage II disease, one is alive with no evidence of disease 15.6 years later, two are dead—one operative death noted previously, the other death from a cardiac arrest at home (41 days after operation), the last was alive at 30 months postoperatively before he was lost to follow-up after 1994. The one patient with stage III disease died at 1.9 years from tumor recurrence and metastatic disease. Overall mean survival for the entire study population was 12.2 ± 0.9 years with a 5-year survival of 87.9 ± 4.7%.

Cox univariate and multivariate regression analysis was performed on the patient characteristics listed in Table 1 to determine preoperative predictors of survival. Age and amount of weight loss were the only significant variables on univariate analysis ($P < 0.1$). Multivariate regression determined that age and amount of preoperative weight loss were independent risk factors for survival ($P = 0.01$ each). Cox analysis of postoperative variables revealed that major postoperative complications and stage were independent predictors of death ($P = 0.005$ and $P = 0.037$, respectively).

DISCUSSION

There is a general agreement that the pathogenesis of Barrett's adenocarcinoma involves a stepwise histologic progression of Barrett's mucosa without dysplasia to low-grade dysplasia, then high-grade dysplasia, and ultimately adenocarcinoma.[4,5] Numerous studies support the pathogenesis from Barrett's mucosa to invasive adenocarcinoma on clinical grounds.[6,7] Hameeteman et al.[7] prospectively followed five patients, showing progression from low- grade to high-grade dysplasia and then to adenocarcinoma over a time period of 1.5 to 4 years. Weston et al.[8] prospectively followed 108 patients newly diagnosed with Barrett's esophagus for a total of 361.8 patient-years. Of 80 patients without dysplasia, one progressed to frank cancer, and 23 to low/intermediate dysplasia; of 20 patients with low- or intermediate-grade dysplasia, four progressed to unifocal high-grade dysplasia; and of eight patients with unifocal HGD, two progressed to cancer and three to multifocal HGD. O'Connor et al.[9] prospectively followed 136 Barrett's esophagus patients for a mean of 4.2 years.

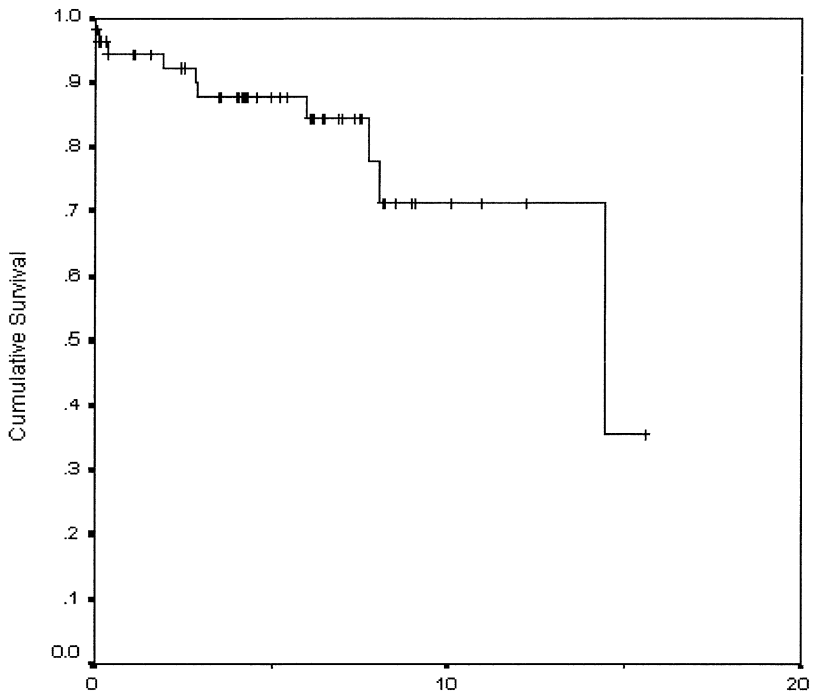

Fig. 1. Kaplan-Meier actuarial survival curve for all patients in the study is depicted. ■ Survival function; + censored.

Low-grade dysplasia developed in 24 patients, high-grade dysplasia in four patients, and adenocarcinoma in two patients, with an incidence of adenocarcinoma of one per 285 patient-years. In addition, flow cytometric abnormalities and mutations in p53 further validate the dysplasia-cancer sequence. Reid et al.[10] correlated flow cytometry and histology, showing that aneuploidy or an increase G2 fraction occurred in 0 of 18 normal patients, in 1 of 34 patients with Barrett's esophagus without dysplasia, in 1 of 4 patients with low-grade dysplasia, and in all patients with high-grade dysplasia or adenocarcinoma. Reid later prospectively evaluated Barrett's patients using objective biomarkers to stratify patients based on their risk of developing cancer.[11,12] Similar studies have been performed analyzing overexpression of p53 protein and neoplastic transformation of Barrett's esophagus.[13,14]

Patients with Barrett's esophagus have a 30–125 fold increased risk of developing esophageal adenocarcinoma, but all will not invariably proceed down the dysplasia-carcinoma pathway. Furthermore, some patients with low-grade dysplasia may regress with treatment of reflux. What is the natural history of high-grade dysplasia? This question has yet to be answered. If patients with documented invasive adenocarcinoma are followed retrospectively, invariably an intermediate stage of HGD is identified. In these studies, the time course for progression from HGD to adenocarcinoma ranges from 0.75–9 years.[15] However, if patients are followed prospectively, then the natural history of HDG is less clear. Weston et al.[16] evaluated prospectively 15 patients with unifocal HGD for 36.8 months. Eight patients (53%) progressed—four (26%) to cancer in 17–35 months, four to multifocal HGD, with or without dysplasia-associated mass or intramucosal cancer after 12–91 months. Schnell et al.[17] prospectively followed 79 HGD patients with endoscopic surveillance. Of 75 patients who remained cancer free after the first year of intensive endoscopic screening, only 12 (16%) patients developed cancer over a mean 7.3-year surveillance period.

In addition to the increased risk of developing adenocarcinoma, patients with HGD have a significant chance of harboring an occult adenocarcinoma, according to several studies.[18] Pellegrini and Pohl[18] compiled 15 studies of the frequency of missed adenocarcinoma in patients diagnosed with HGD alone—of 184 patients who underwent esophagectomy, 80 patients (43%) were found to have adenocarcinoma. The true incidence of "occult adenocarcinoma" is unknown and complicated by several factors. First, most studies investigating the incidence of missed adenocarcinoma are retrospective, including the present study. Second, the majority of gastroenterologists do not have a nationally utilized and accepted consensus as to the approach of endoscopy. Certainly, if endoscopy is performed with four-quadrant biopsy at the level of every centimeter in patients with HGD, the incidence of "missed adenocarcinoma" theoretically should be low. However, missing adenocarcinoma is still possible. Endoscopic biopsy differentiation of HGD and cancer is difficult. Cameron and Carpenter[19] histologically mapped esophagectomy specimens of HGD patients and found that areas of microscopic carcinoma are often small (1.1 cm^2) and easily missed. Therefore, since all gastroenterologists do not routinely perform four-quadrant biopsies at every cm level, the incidence of "occult adenocarcinoma" in this and other studies is that of missed adenocarcinoma.

In most series in which cancer is identified with or develops from HGD, the cancer is early staged. In our original study of 30 patients,[1] however, of 13 patients with adenocarcinoma, two were stage II and two additional patients had stage III cancer. Both patients with stage III disease died later from recurrent cancer. Given the probable pre-malignant potential of HGD (studies which question this are more recent than our study period), a high incidence of occult invasive adenocarcinoma, and a low operative mortality (3.3%) and complication rate (20%), our original study concluded that, in experienced hands, prophylactic esophagectomy was indicated for patients with HGD. We have continued to operate on this premise and since this study, we have seen an increase in the number of patients with HGD referred for surgery. Whether this reflects an increased incidence or an increase in referrals due to heightened awareness is uncertain. However, we were prompted to update our findings.

We have found little change in the patient characteristics—most patients with Barrett's esophagus with HGD are Caucasian men, between 60–70 years of age, with long-standing gastroesophageal reflux, and a hiatal hernia. However, of note, more women have been diagnosed with HGD in the recent years—23% of patients were women from 1994–2001 as compared to 3% in our earlier study. Whether more women are developing Barrett's, an increase in incidence, or more women are being diagnosed with Barrett's esophagus with no change in incidence is unknown. Although our surgical approach has changed to rely almost exclusively on transhiatal esophagectomy, our surgical results continue to demonstrate the safety of prophylactic esophagectomy. Although these did not reach statistical significance, our operative mortality declined from 3.3% to 0%, as did our length of stay from 14 to 10 days. We attribute our

excellent surgical results to three factors: our shift to transhiatal esophagectomy, our high surgical volumes and thus experience with the technique, and our implementation of standardized critical pathways (care maps) for esophagectomy patients.

With respect to pathology, the percentage of patients with occult adenocarcinoma has decreased significantly over time, from 43% in our initial study to 17% during 1994–2001. In addition, recent patients with adenocarcinoma all were resected at an earlier stage, all stage I disease. We and others[1,20,21] have long advocated prophylactic esophagectomy for HGD and in a recent consensus statement on the management of Barrett's esophagus, the panel stated that patients "who have high-grade dysplasia are at substantial risk for harboring an invasive carcinoma and should be considered for esophagectomy."[22] Thus, gastroenterologists may be more likely to refer patients with HGD to surgery rather than to continue surveillance. We believe our declining incidence and earlier presentation of occult adenocarcinoma most likely reflects our gastroenterologists' earlier referral of patients with HGD. However, whether more aggressive endoscopy has resulted in an increase in the number of patients diagnosed with HGD and a decrease in patients with missed adenocarcinoma is unknown. Nonetheless, our overall missed adenocarcinoma rate over the entire study period was 30%. The only predictor of cancer was length of reflux symptoms, which is an imprecise guide. Duration of Barrett's esophagus was not predictive of adenocarcinoma. Until better predictors of adenocarcinoma in HGD patients become available, such as clinical or molecular markers, or more aggressive endoscopic approaches, i.e., four-quadrant biopsies every centimeter, are more uniformly employed and standardized, the frequency of occult cancer will likely remain high.

Patients with Barrett's HGD treated with prophylactic esophagectomy had excellent 5- and 10-year survival rates of 88% and 71% respectively. As would be expected, age was a predictor of survival—older patients at the time of diagnosis were more likely to die sooner. Amount of preoperative weight loss was also an independent risk factor, which likely reflects poorer nutritional status before and/or the presence of cancer in addition to HGD. Postoperative predictors of survival, as expected, were dependent upon presence of major postoperative complications and stage of adenocarcinoma, if found. Long-term sequelae of esophagectomy, such as diarrhea, dysphagia, regurgitation, and weight loss were not examined in this study.

Ultimately, the recommendation for treatment of patients with HGD depends on prospective studies that determine the natural history of HGD. Whether esophagectomy will be the treatment of choice for all HGD patients, for multilevel HGD, or reserved for those patients not willing or able to comply with surveillance protocols has yet to be determined. This study does show that the outcomes of surgery at a center of high volume and experience are excellent in terms of morbidity, mortality, and long-term survival. We feel that the declining incidence and earlier stage of occult adenocarcinoma are the favorable outcomes of our earlier published recommendations advocating an aggressive approach to this disease.

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Discussion

Sanjay Nandurkar, M.D. (Rochester, MN): Could you tell us the proportion of cancers that were detected in newly diagnosed Barrett's compared to those in an established surveillance program? There is data from Sontag's group (and others) suggesting that there is a significant difference in the prevalence of adenocarcinomas within high-grade dysplasia (HGD) depending upon how the patients are analyzed. In patients with HGD detected at index endoscopy (or within a year or two of the index endoscopy) the chance of finding a cancer hidden amongst the HGD is very high. Previous studies reporting on the high number of unsuspected cancers within HGD found only later in the esophagectomy specimens comes from these studies. On the other hand, patients in whom HGD is detected on surveillance many years after index endoscopy, the chance of finding an overt or missed adenocarcinoma is very low. Your data, at least in the second half of the study, showed that the cancers were more often early, stage I. This suggests that they probably came from a surveillance series. And, hence not surprisingly, the chance of detecting incident cancers amidst HGD was low.

Dr. Tseng: Over 50% of our referrals are from outside of our area, and there is no standardized surveillance amongst our referring gastroenterologists. We do not know which patients or how many have been under surveillance protocols. We have, however, seen an increase in referrals from 1994 to 2001. In seven years, which is slightly more than half the time for our original series, we have accumulated the same number of patients. So clearly we have increased referrals. The question, therefore, would be whether the increase in referrals is due to earlier referrals or better surveillance, but unfortunately, we are unable to answer that question.

Dmitry Oleynikov, M.D. (Omaha, NE): I want to compliment the authors on excellent postoperative mortality and morbidity results; certainly, it raises a new bar of excellence. I do have one question, however, regarding the decreased amount of adenocarcinoma found in the pathology specimens. Do you feel that if these patients were, for instance, surveyed instead of taken directly to surgery that perhaps some of those patients would not have ultimate progression to adenocarcinoma, as has been suggested by the Seattle group?

Dr. Tseng: We advocate that patients with high-grade dysplasia be referred to surgery. In a surveillance mechanism, they would have to be surveyed every three to six months and the endoscopies would have to follow very strict guidelines—four-quadrant biopsies, every centimeter, with high-grade dysplasia. High grade dysplasia is difficult to detect and is found often in only a 1 cm^2 area. In addition, 70% to 80% of patients are only diagnosed from one biopsy specimen. The data also suggests that adenocarcinoma can be easily missed. We, therefore, advocate resection, which is curable at the high-grade dysplasia stage and allows removal of adenocarcinoma at an earlier stage when it is found.

Claude Deschamps, M.D. (Rochester, MD): Did you correlate the finding at endoscopy with the incidence of cancer that you have found? We found that when you have nodularity or stricture that there was, naturally, a higher chance of having a cancer. Are you seeing more and more patients that have been treated with PDT?

Dr. Tseng: Thank you for your excellent questions. For your first question, we did not correlate endoscopic findings with the incidence of cancer. Second, the patients that we have been referred have not undergone PDT therapy, but it certainly is a promising therapy that will need to be investigated and compared to the gold standard of surgery.

Thomas R. DeMeester, M.D. (Los Angeles, CA): One of the reasons that there is alternative therapy for these patients, such as photodynamic therapy, etc., is because of the morbidity of esophagectomy, and so I would really like you to tell me, what is your incidence of stricture and how often these people have to be dilated, because is this really where it is going to play out? What is your incidence of dumping and how about the incidence of diarrhea following a vagotomy? Those are critical factors to make this the therapy of choice in patients with such early disease.

Dr. Tseng: We studied early complications; however, we do not have the data on the long-term complications of strictures, dumping, and diarrhea. But you make an excellent point.

Fernando Quijano-Orvananos, M.D. (Mexico City, Mexico): My question is along the line of Dr. DeMeester's questions. What has happened to the cervical esophagus after the gastric pull-up after so many years? Have you noted esophagitis in the cervical esophagus or maybe even Barrett's in the cervical esophagus after years and years of this kind of reconstruction?

Dr. Tseng: Again, an excellent point, which we did not examine in this study. We investigated long-term survival, but not long-term complications.

Invited Discussion—Expert Commentator

Jeffrey H. Peters, M.D. (Los Angeles, CA): The second paper represents an update of a widely quoted paper from the Johns Hopkins Hospital first published in 1996 and reviews 60 patients with HGD at a median follow-up of 4.6 years, 90% of which had transhiatal esophagectomy. There is no doubt that we are seeing increased referrals of early stage lesions, a fact which prompted this study. Thirty percent of patients are presently referred with early stage highly curable lesions. There was one postop death and overall, 30% had invasive carcinoma in the specimen although the rigor of the preoperative endoscopic biopsy protocol is not well described. Operative mortality declines from 3.3% to 0% in the 80s to 90s. Five-year survival was excellent at 88%.

The paper highlights several very important points in the management of patients with high-grade dysplasia, particularly in this era of increasing use of ablative and other non-surgical treatment modalities.

1. A significant percentage of patients will harbor occult invasive carcinoma. This is one of the first papers to demonstrate a trend toward fewer invasive cancers in recent years, likely due to more vigorous biopsy protocols, but the fact that even recently, 20% of patients with high-grade dysplasia have invasive carcinoma in the specimen cannot be ignored.
2. Esophagectomy, which can be performed transhiatally in most such patients, is highly curative. The challenge for the future is to accomplish this with as minimal long-term alimentary disability as possible.
3. The mortality of esophagectomy in the setting of benign disease or early cancer has decreased to near zero in high-volume centers.

These data represent the gold standard against which all other therapies should be measured including laparoscopic and endoscopic treatment methods.

Duodenal Reflux Produces Hyperproliferative Epithelial Esophagitis—A Possible Precursor to Esophageal Adenocarcinoma in the Rat

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Esophageal reflux of duodenal contents converts a rat nitrosamine esophageal cancer model from squamous cell carcinoma to adenocarcinoma. Further, there was a tendency for male rats to have a higher incidence of cancer than female rats. However, chemical castration with the gonadotropin-releasing hormone analog leuprolide did not protect male or female animals from developing cancer. We have identified an early (6-week) hyperproliferative epithelial cell reaction to duodenal reflux. We carried out experiments to assess the specificity of duodenal reflux in producing the hyperproliferative epithelial precursor lesion. Animals underwent specific surgical procedures to produce esophageal reflux of pure duodenal contents, mixed gastroduodenal, or bland intestinal contents. A hyperproliferative mucosal esophagitis developed in the group with duodenal reflux but not in the other groups. Mucosal thickness in the duodenal reflux group reached seven times that of normal mucosa at 6 weeks. These results suggest that esophageal reflux of duodenal contents plays an important role in the pathogenicity of proliferative esophagitis and the potential development of esophageal adenocarcinoma. (J GASTROINTEST SURG 2003;7:172–180.)

KEY WORDS: Gastroesophageal reflux disease (GERD), esophageal cancer, rat, N-acetylcysteine, leuprolide

In the Western world, esophageal adenocarcinoma is appearing more and more frequently.[1,2] The disease has a male predominance with a male:female ratio of 6:1. Developing an animal model of esophageal adenocarcinogenesis has the potential to assist in understanding the mechanisms of carcinogenesis, particularly the metaplasia, which occurs in the esophageal mucosa whereby squamous epithelium is replaced by columnar epithelium, and the possible role of androgens in esophageal carcinogenesis.

Previous work has demonstrated that systemic nitrosamine administration will produce a squamous-type cancer in the rat esophagus.[3] Interestingly, when nitrosamine administration is combined with surgical preparations causing reflux of intestinal contents into the esophagus, adenocarcinoma is seen in addition to the squamous-type lesion.[4,5] This model shares features of the presumed mechanism of carcinogenesis in humans where adenocarcinoma develops more frequently in patients with gastroesophageal reflux disease.[6] In human carcinogenesis, a precursor lesion to adenocarcinoma develops. This is Barrett's metaplasia of the esophageal mucosa in which the squamous epithelium is replaced with columnar epithelium. Our studies were designed to search for a precursor lesion for adenocarcinoma in the rat model. Further, our aim was to determine the relative contributions of

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intestinal, gastric, and duodenal reflux to the development of any precursor lesion that we identified.

When we identified a hyperplastic squamous epithelial lesion produced in the presence of pancreaticduodenal reflux, we evaluated the possible role of oxidative damage in producing this lesion. Our approach was to compare animals with and without a dietary supplement of the antioxidant N-acetylcysteine (NAC).

In addition, we assessed aspects of the role of androgens in carcinogenesis in this model. Most experiments with this model have been carried out using animals of only one sex. We compared males and females. To further evaluate the possible role of gender in this model, half of the animals were given a medical castration with leuprolide, an inhibitor of gonadotrophin-releasing hormone. We hypothesized that there would be a male predominance of adenocarcinoma in the rat model. We further hypothesized that chemical castration with leuprolide would reduce the incidence of esophageal adenocarcinoma in the rat model.

MATERIAL AND METHODS

Male and female Sprague-Dawley rats (250 to 300 g) were purchased from Harlan, Inc. (Indianapolis, IN). All procedures were prospectively approved by the animal care and use committee of Johns Hopkins University. Animals were acclimatized in a central animal facility for 1 week before the experiment. Prior to surgery, animals were fasted for 24 hours. Rats were anesthetized with an intramuscular injection of 50 mg/kg ketamine and 5 mg/kg xylazine. The abdominal wall was shaved and cleaned with 10% povidone iodine solution.

Carcinoma Model

This model combined reflux of duodenal contents with nitrosamine treatment. Seventy-five Sprague-Dawley rats (250 to 300 g) were used (38 females and 37 males). The stomach was removed and the lower esophagus was anastomosed to the duodenum in an end-to-side fashion to produce pure duodenal alkaline reflux. All rats were then given 25 mg/kg doses of the nitrosamine methyl-N-amylnitrosamine (MNAN; provided by Dr. S Mirvish, Creighton University, Omaha, NE) every 4 weeks by intraperitoneal injection. Some animals also received leuprolide (TAP Holdings, Deerfield, IL) at a dosage of 16 mg/kg by depot subcutaneous injection every 3 months starting on postoperative day 30.

Precursor Lesion Surgical Model

We designed experiments to search for a precursor lesion to cancer that would appear within 6 weeks of establishment of reflux. A variety of reflux-inducing surgical procedures were carried out. The aim was to test whether duodenal reflux was particularly and uniquely responsible for producing the precursor lesion.

Through a 3 cm midline laparotomy vertical abdominal incision, animals underwent one of the following procedures:

1. Sham laparotomy (n = 26; Fig. 1, *A*)
2. Pure duodenal reflux: An esophagoduodenostomy was performed after gastrectomy, to cause pure duodenal reflux, the stomach was removed, and the lower esophagus was anastomosed to the duodenum in an end-to-side fashion. This preparation allows the reflux of pure alkaline duodenal contents into the esophagus (n = 30; Fig. 1, *B*).
3. Mixed gastroduodenal reflux: A side-to-side esophagogastroduodenostomy was created to cause a mixed gastroduodenal reflux. A longitudinal incision was made on the antimesenteric border of the duodenum approximately 1.5 cm in length. A second incision was made longitudinally along the lower esophagus anteriorly extending 1 cm distally onto the proximal stomach. A side-to-side anastomosis was fashioned between the two openings using interrupted 6/0 Prolene sutures. This prepara-

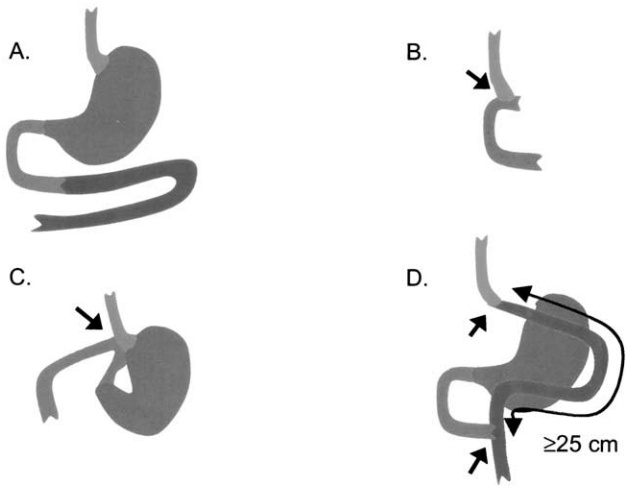

Fig. 1. Surgical techniques used to induce reflux (*black arrows* indicate anastomoses). **A**, Normal esophagus, stomach, duodenum, and jejunum. **B**, Pure duodenal reflux: Esophagoduodenostomy with total gastrectomy inducing pure duodenal reflux. **C**, Mixed gastroduodenal reflux: Esophagogastroduodenostomy causing a mixture of both gastric and duodenal contents. **D**, Intestinal reflux (no pancreatico-biliary component): Roux-en-Y esophagojejunostomy, which prevents both gastric and duodenal reflux.

Table 1. Histological grading parameters

Parameter	Grade
Keratinization	Normal
	Any evidence of increased keratinization
	Keratin layer between two and five times normal thickness
	>5-fold increase
Mucosal thickness	Normal
	Between one and two times normal thickness
	Between two and five times normal thickness
	>5-fold increase in thickness
Papillary hyperplasia	Normal
	Any evidence of increased papillae formation
	Papillae extending the full epithelial thickness
Ulceration	Normal
	One or two small erosions superficial to muscularis mucosa
	Large or multiple ulcers (>2 ulcers) deep to muscularis mucosa
Submucosal inflammation	Normal
	Minimal increase in acute or chronic inflammatory cells in the submucosa
	Large infiltrate of acute or chronic inflammatory cells in the submucosa
Carcinoma	Presence or absence

tion allows free reflux of gastroduodenal contents into the esophagus (n = 14; Fig. 1, *C*).

4. Intestinal reflux: A Roux-en-Y esophagojejunostomy was created leading to reflux of intestinal contents without either gastric or duodenal contents. The lower esophagus was divided avoiding the left gastric artery. A jejunal Roux loop was anastomosed end to end to the lower esophagus with interrupted 6/0 Prolene sutures. The proximal jejunum was then anastomosed end to side to the jejunum 25 cm distal to the esophagojejunostomy, also with the use of 6/0 Prolene interrupted sutures. The peristaltic action of the long Roux loop in the preparation acts as a one-way valve to exclude duodenal and gastric contents from the esophagus (n = 4; Fig. 1, *D*).

Iron dextran has been shown to promote oxidative damage and the incidence of adenocarcinoma in the rat reflux model.[7] To compare animals with and without iron, with regard to the development of the precursor lesion, some of the animals were given 50 mg/kg iron dextran intraperitoneally at the time of reflux surgery (sham, n = 8; esophagoduodenoscopy, n = 7; esophagogastroduodenostomy, n = 3; esophagojejunostomy, n = 4).

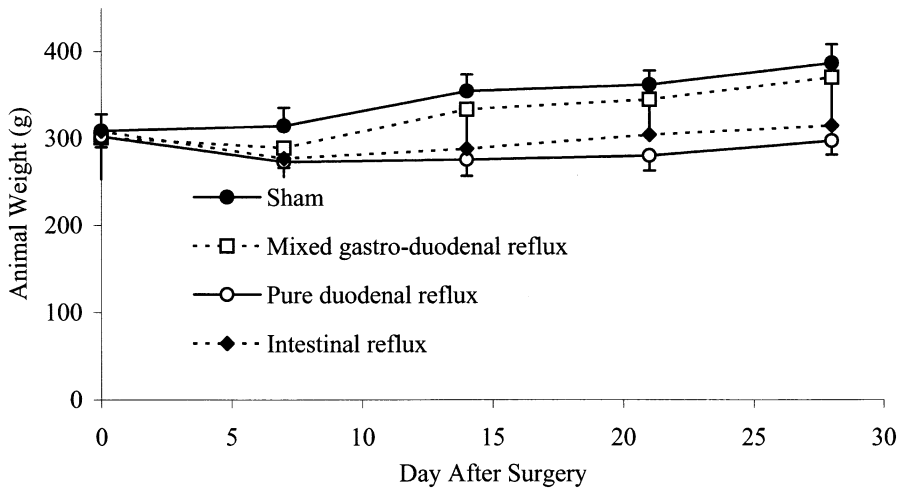

Fig. 2. Comparison of different reflux preparations on the weights of the rats.

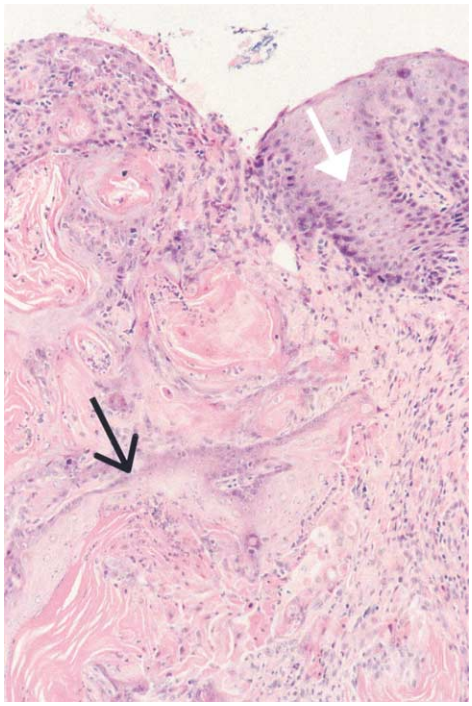

Fig. 3. Squamous cell carcinoma arising in a rodent model. The surface is ulcerated and the adjacent squamous mucosa is hyperproliferative (*white arrow*). The invasive carcinoma displays typical features of squamous differentiation, namely, aberrant keratinization and squamous bridges (*black arrow*). The infiltrative growth pattern indicates that this is an invasive lesion.

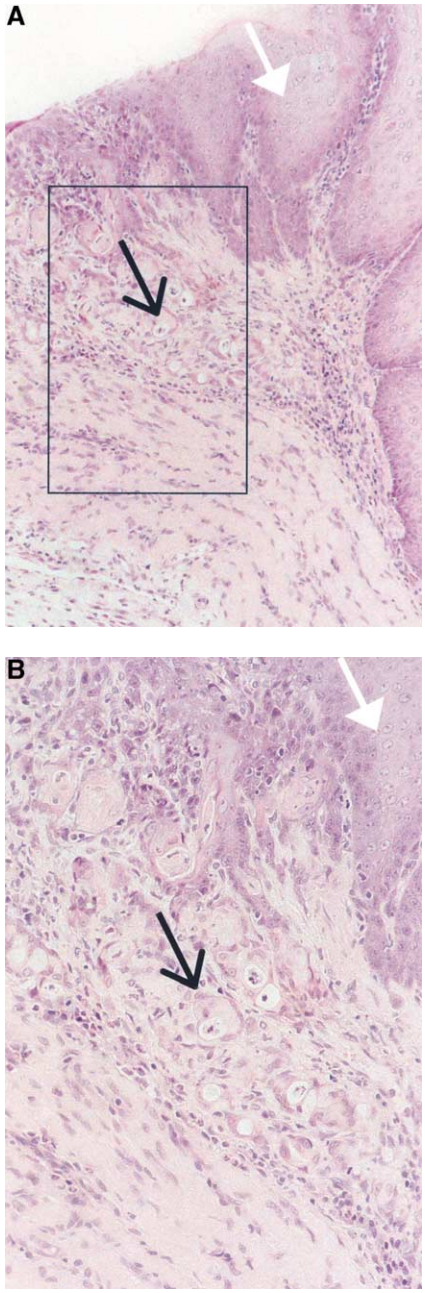

Fig. 4. A, Focus of early invasive carcinoma from the surface. Hypertrophic epithelium is again evident (*white arrow*). In this field the invasive component shows squamous differentiation. Early keratin whorl formation can be seen at the center of the field. **B**, High-power magnification image from the submucosal portion of the lesion depicted in Fig. 4, *A*. Cytologically malignant cells showing glandular differentiation (*black arrow*), as evidenced by the presence of mucin droplets seen infiltrating collagenized tissue (desmoplastic response). These are the features of adenocarcinoma.

Histologic Assessment

Animals were killed between 1 week and 8 months postoperatively. The esophagus was fixed in 3.7% formalin, embedded in paraffin, stained with hematoxylin and eosin, and examined histologically. All sections were graded by two protocol-blinded observers for keratinization, mucosal hypertrophy, papillary hyperplasia, ulceration, submucosal inflammation, and presence of carcinoma (Table 1). Mucosal and muscular thickness was also measured quantitatively using a reticle.

Assessment of Lipid Peroxidation

Thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation and intracellular oxidative stress, were also quantitated spectrophotometrically on esophageal tissue samples that were snap-frozen in liquid nitrogen at the time the animals were killed.[8]

Antioxidant Treatment With N-Acetylcysteine

To assess the effect of an antioxidant on the development of the duodenal reflux–induced esophageal hyperplasia, the antioxidant NAC (Sigma, St. Louis, MO) was administered in the animals' drinking water.[9] Animals were assigned to one of two groups after the induction of duodenal reflux, as previously described. One group (n = 5) was given pure water, and the other group (n = 6) was given NAC in their

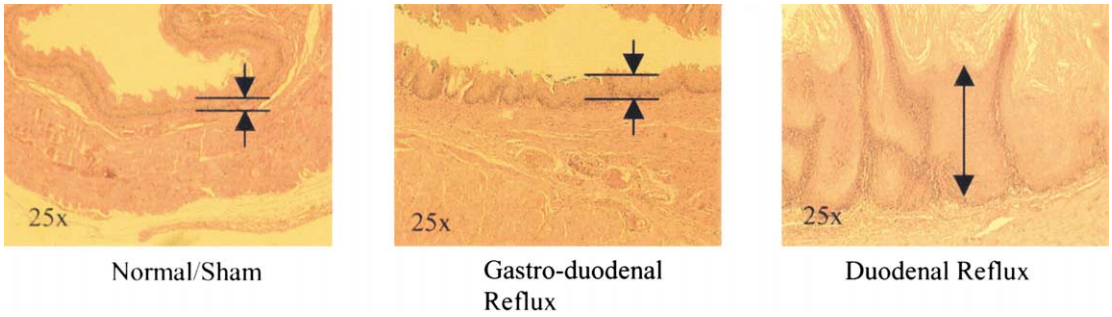

Fig. 5. Photomicrographs of esophageal specimens 6 weeks after the induction of the different forms of reflux.

drinking water at a concentration of 1% weight to volume.[10] This was started on the day of surgery and continued for the 6-week duration of the experiment.

RESULTS

After a transient weight loss, animals in all groups gained weight throughout the course of the experiment (Fig. 2).

Carcinoma

A total of 28 of the 75 animals developed tumors. Eleven had squamous carcinomas and 17 had glandular differentiation (Figs. 3 and 4). All of the tumors were centered on the lower esophagus, never on the liver, pancreas, stomach, or duodenum. In three of those with glandular differentiation, this was clearly contiguous with the squamous epithelium, suggesting that the origin was in the squamous epithelium of the esophagus. Other tumors were located near the anastomosis, so the possibility of an intestinal cell of origin could not be excluded. There was a tendency for male rats to have a higher incidence of cancer than the female rats (17 [46%] of 37 males vs. 11 [29%] of 38 females). The incidence of adenocarcinoma in the male rats (14 of 37) was greater than in the female rats (3 of 38; $P < 0.01$, chi-square analysis). There was a tendency for female rats to have a higher incidence of squamous cancer than male rats (8 of 38 females vs. 3 of 37 males). Leuprolide treatment starting at 30 days into carcinogenesis did not protect the animals from the development of esophageal carcinoma. Twenty-five of 37 male and 18 of 38 female rats received leuprolide. Overall, there was a tendency for the incidence of cancer to be higher in the leuprolide group (47% vs. 25%). In retrospect, starting leuprolide before surgery rather than 1 month after surgery would have assured a more meaningful result. The progression toward carcinogenesis may occur within the first few weeks of exposure to the combination of reflux and nitrosamines, and the animals were not receiving leuprolide until day 30 after the initiation of the reflux. Serum testosterone levels were found to be significantly reduced or undetectable in the leuprolide-

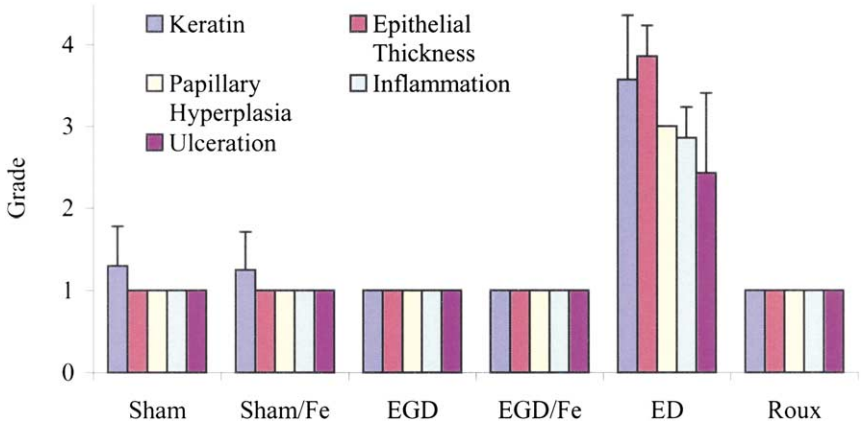

Fig. 6. Histologic grading of the esophageal specimens at 6 weeks.

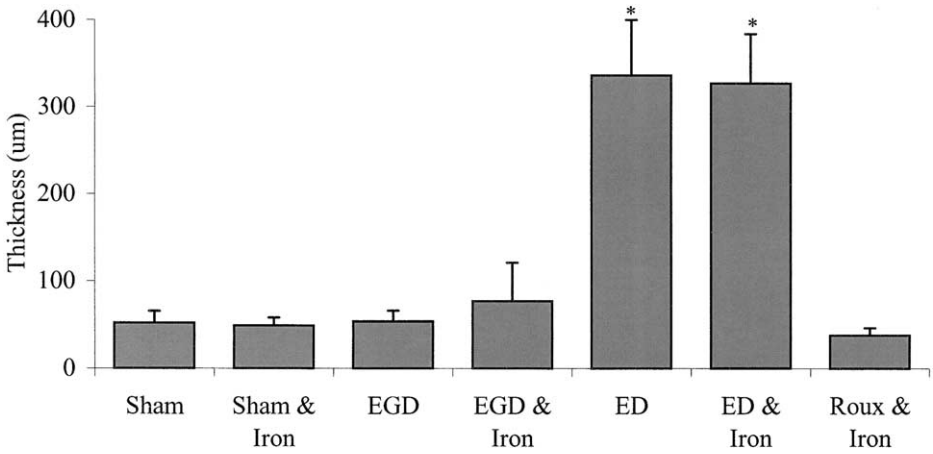

Fig. 7. Epithelial thickness in the different reflux groups at 6 weeks. The duodenal reflux group had significantly increased thickness compared to the control specimens (*$P < 0.05$; analysis of variance).

treated animals, whereas these levels were within the normal range in the nontreated animals, confirming that the leuprolide treatment was effective.

Precursor Lesion: Hyperproliferative Epithelial Esophagitis

Animals with varying types of esophageal reflux were examined at earlier time points than 8 months to look for a precursor lesion that might precede the development of cancer (Figs. 5–8). Ulcerative and inflammatory changes were seen at 1 week after laparotomy in both the gastroduodenal reflux and duodenal reflux groups but not in either the sham-operated or intestinal reflux groups. By 3 weeks postoperatively, however, the group with gastroduodenal reflux had reverted to a normal appearance. Changes in the duodenal reflux group were progressive, leading to marked ulceration, inflammation, and papillary mucosal hypertrophy. After 6 weeks of reflux, only the group with duodenal reflux had marked histologic abnormalities including hyperkeratinization, hyperplasia, inflammation, and ulceration, in contrast to the relatively normal mucosa in all of the other groups. The administration of iron had no effect on these changes (Figs. 6 and 7).

The epithelium gradually increased in thickness from week 1 to week 6 only in the group with duodenal reflux (Figs. 7 and 8) and by 6 weeks averaged more than seven times the thickness of normal mucosa (Fig. 5). The group with duodenal reflux was the only one to demonstrate this hyperplasia. It did not occur in the sham-operated, mixed gastroduodenal, or intestinal reflux groups. As expected, there was no consis-

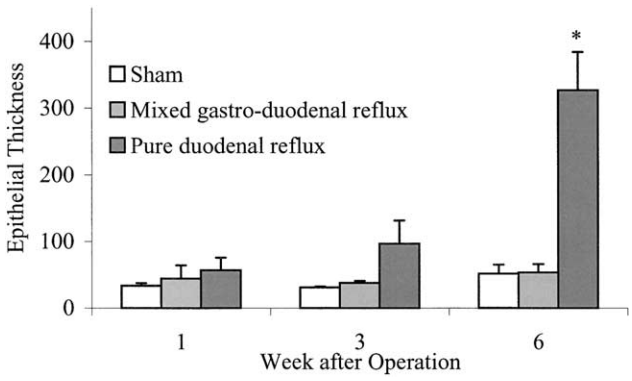

Fig. 8. Epithelial thickness at 1, 3, and 6 weeks (*$P < 0.05$; analysis of variance, compared to sham-operated group).

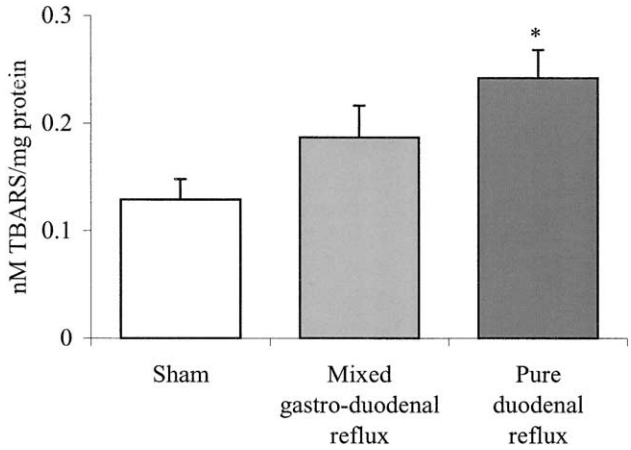

Fig. 9. Oxidative stress as indicated by TBARS in different reflux groups (*$P < 0.05$; analysis of variance, compared to sham-operated group).

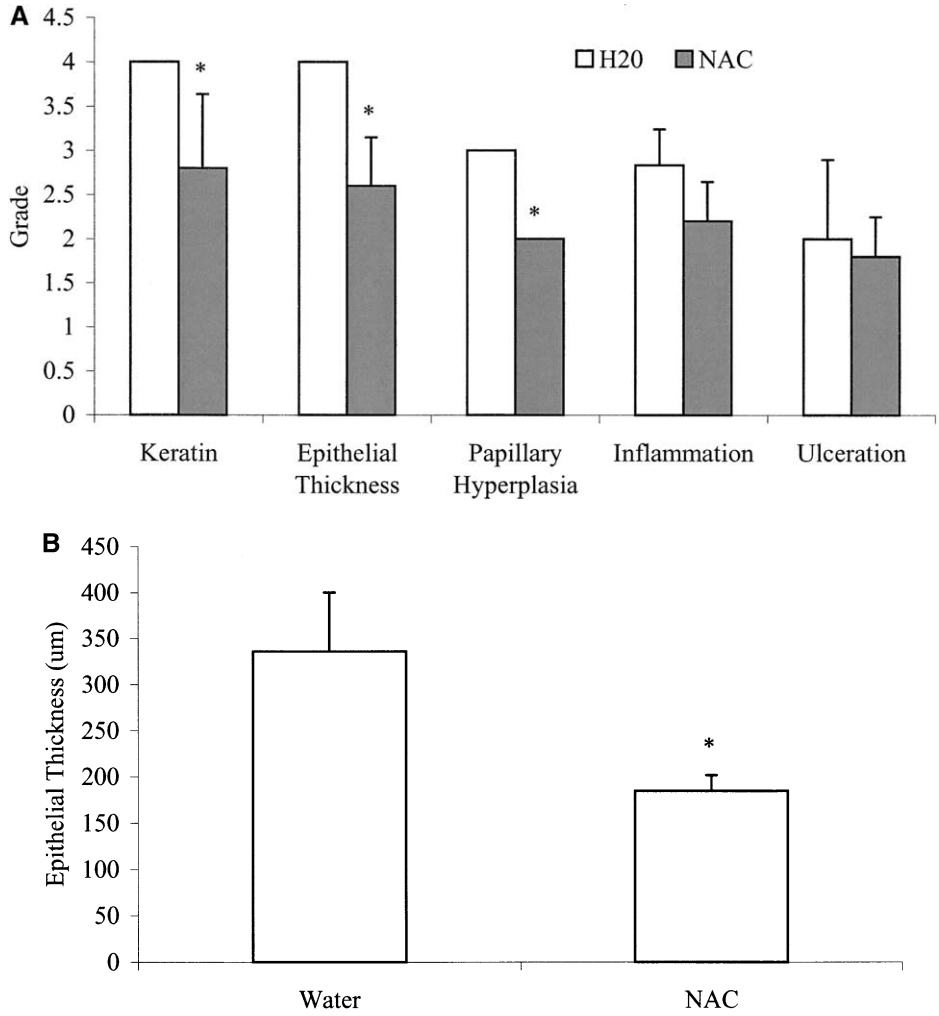

Fig. 10. Effect of NAC on duodenal reflux and induced histologic abnormalities (**A**, $*P < 0.05$; Mann-Whitney rank-sum test, compared to sham-operated group) and epithelial mucosal hyperplasia (**B**, $*P < 0.05$; Student's *t* test).

tent difference in muscle wall thickness among the groups (data not shown). The administration of iron had no influence on the histologic findings.

Oxidative Stress

Thiobarbituric Acid Reactive Substances. There was evidence of increased oxidative stress (TBARS) at 1 week in both the mixed gastroduodenal and duodenal reflux groups; this was maximal in the duodenal reflux group ($*P < 0.028$; analysis of variance). This increase was not seen at later time points (Fig. 9).

N-Acetylcysteine. Animals given NAC in their water (n = 5) had similar weight curves as the control animals (n = 6). After 6 weeks, histologic scoring showed significantly fewer marked abnormalities, including epithelial thickness ($P < 0.008$; Mann-Whitney rank-sum test), papillary hyperplasia ($P < 0.008$), and hyperkeratinization ($P < 0.032$) in the esophagi of animals given NAC when compared to those given only water. Scoring of both inflammation and ulceration, although tending toward an improvement in the NAC group, failed to reach statistical significance. The measurement of esophageal mucosal thickness showed that NAC reduced the inflammatory reaction in the NAC group (336 ± 29) as compared with the control group given pure water (185 ± 8) ($P < 0.001$; Student's *t* test; Fig. 10).

DISCUSSION

The most striking finding in these studies was the identification of hyperproliferative, epithelial esophagitis in the setting of alkaline duodenal reflux. We

suggest that this lesion is a precursor to adenocarcinoma in the rat esophagus. It is seen in the presence of alkaline duodenal reflux prior to the development of adenocarcinoma in this model. The dramatic proliferative response is seen with pure duodenal reflux, but not with combined acid reflux, or bland intestinal reflux showing the specificity of bilious duodenal reflux in producing this lesion.

This study does not identify the component of duodenal reflux that is active in producing the hyperproliferative lesion. However, a proliferative effect of bile acids has been identified in vitro, making bile acids a prime candidate for this role.[11] In mixed gastroduodenal reflux, the presence of acid presumably precipitates the bile acids. Bile acids would be present in mixed reflux, but in the presence of acidity they would be precipitated and therefore inactive as stimulants to mucosal proliferation.[12,13]

The tendency for alkaline duodenal reflux to produce early oxidative damage in excess of mixed gastroduodenal reflux might also be attributed to the presence of active bile salts in solution. The finding that levels of the lipid oxidative product TBARS were elevated in the group with duodenal reflux suggests a role for oxidative damage in the phenomenon. The finding that the antioxidant NAC protected animals from developing the hyperproliferative esophageal lesions provides further evidence for the role of oxidative damage in producing the hyperproliferation. However, NAC may be cytoprotective via other mechanisms besides its antioxidant properties.[9] Nonetheless, these findings regarding the possible role of oxidative damage in reflux esophagitis are consistent with prior work.[14–16] Another line of evidence suggesting that oxidative damage plays a role in esophageal adenocarcinogenesis in the rat model comes from experiments in which iron dextran was shown to increase the incidence of cancer.[7] A recent report suggests that blocking the cyclooxygenase pathway will also prevent development of both proliferative esophagitis and adenocarcinoma in a rat duodenal reflux model.[17]

The present study confirmed the work of others by showing that when nitrosamine treatment is combined with duodenal reflux, a tumor with glandular differentiation is seen after a period of up to 9 months in the lower esophagus.[4,5] Other investigators have found that even in the absence of nitrosamines, duodenal reflux will produce esophageal adenocarcinoma.[17,18] The finding in some of our specimens of carcinoma displaying glandular differentiation streaming from the abnormal hyperplastic squamous epithelium suggests an esophageal epithelium origin for the invasive carcinoma within the squamous epithelium. Pluripotent stem cells may reside in the esophageal mucosa. Interestingly, the fetal esophagus of the rat is lined with columnar epithelium. There is metaplasia during fetal development, whereby this columnar epithelium transforms to squamous epithelium.[19] It is tempting to speculate that a reverse metaplasia occurs during carcinogenesis with the mucosa reverting to the fetal form. It appears that intestinal reflux into the esophagus, particularly duodenal reflux, triggers this metaplasia. Nitrosamine alone does not produce adenocarcinomas. It is in the presence of duodenal reflux that these tumors appear.

With regard to the role of the androgen axis in carcinogenesis in the rat model, our findings were equivocal. There was a tendency for male predominance of adenocarcinoma with 14 of 37 male animals developing adenocarcinoma, whereas only 3 of 38 females developed adenocarcinoma ($P < 0.01$; chi-square analysis). Medical castration with leuprolide had the unexpected effect of tending to increase the incidence of adenocarcinoma in the male animals. Chemical castration by means of leuprolide was confirmed by measurement of serum testosterone levels. Unfortunately the timing of the administration of leuprolide, starting 30 days after surgical creation of the reflux model, may mean that essential progression of carcinogenesis had occurred before the leuprolide was administered. The male predominance seen in the model encourages further evaluation of this phenomenon.

Although tumors with glandular differentiation could be seen emerging in the esophageal squamous mucosa in some of our surgical specimens, it was not always possible to demonstrate such a relationship. Histologic evaluation is limited by the fact that slides are taken at isolated discontinuous points, not continuously throughout the specimen. We may have failed to recognize a site of origin, in some cases, because of incomplete sampling.

In contrast to human esophageal adenocarcinoma, the tumors in the rat model were strikingly submucosal as opposed to mucosal. This could be explained by species variation whereby glandular cells streaming from the pluripotential stem cells in the basilar layer of the esophageal mucosa have a propensity for submucosal growth in the rat, whereas they have a tendency for growth toward the surface of the epithelium in humans. However, we cannot rule out the possibility that the cell of origin for some of our glandular tumors was from some cell line other than the esophageal mucosa. Numerous potential sources for glandular lesions exist in the proximity of the lower esophagus in this model. Gastric and duodenal epithelium, Brunner's glands, pancreatic acinar and ductal cells, as well as hepatic ductal cells, are all possibilities. However, the tumors were always centered grossly in the lower esophagus and never in the duodenum,

stomach, liver, or pancreas. This finding leads us to conclude that the origin was esophageal.

CONCLUSION

These results suggest that esophageal reflux of duodenal contents plays an important role in the pathogenicity of esophagitis and the potential development of esophageal adenocarcinoma.

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Preoperative Intervention Does Not Affect Esophageal Muscle Histology or Patient Outcomes in Patients Undergoing Laparoscopic Heller Myotomy

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Botox injection and pneumatic dilation are common therapies for achalasia. We sought to determine the impact of these preoperative therapies on esophageal muscle histology and outcomes after laparoscopic Heller myotomy. A total of 73 consecutive patients had esophageal muscle biopsies taken from the gastroesophageal junction at the time of myotomy between November 1998 and November 2001. Muscle fibrosis was graded by a senior pathologist who was blinded to preoperative treatments and postoperative outcomes. Patients graded their dysphagia and heartburn symptoms before and after myotomy and graded their outcomes at follow-up. Patients were grouped according to the preoperative endoscopic treatment (dilation, Botox, both, or neither) and the groups were compared. Preoperative therapy did not correlate with esophageal fibrosis or postoperative outcomes, and the degree of esophageal muscle fibrosis was not predictive of outcome. Symptom scores improved significantly for dysphagia (4.5 ± 0.9 vs. 1.6 ± 1.6) and heartburn (2.3 ± 1.8 vs. 1.5 ± 1.4) irrespective of preoperative therapy or fibrosis. Overall, excellent or good outcomes were obtained in 92% of patients at follow-up of 15.7 months ± 14.4. Successful outcomes are highly probable after laparoscopic Heller myotomy regardless of preoperative interventions. The amount of fibrosis in the esophageal muscle is not related to preoperative intervention and is not predictive of outcomes. (J GASTROINTEST SURG 2003;7:181–190.)

KEY WORDS: Esophageal mytomy, laparoscopic Heller myotomy, preoperative therapy

Since its first description by Heller[1] in 1914, esophageal myotomy has proved efficacious in long-term palliation of the symptoms of achalasia. The operation has evolved from two myotomies being undertaken via thoracotomy to a single myotomy completed by means of minimally invasive techniques with little morbidity and a short hospital stay.[2–5] Nonoperative treatments have also evolved to include balloon dilation and botulinum toxin (Botox) injection.[6–9] Despite the apparent superiority of operative myotomy over these less invasive techniques,[10–12] myotomy is still considered by many nonsurgeons as a salvage procedure to be implemented once endoscopic management is no longer effective or possible.[13,14] It has been our perception that myotomy, when it is undertaken in patients who have undergone previous endoscopic therapy, particularly Botox injections, is a more difficult operation because of the adherence of the circular muscle fibers to the submucosa of the esophagus. We hypothesized that this increased difficulty is due to fibrosis induced by dilation or Botox and sought to determine if it would predict outcome following laparoscopic Heller myotomy.

PATIENTS AND METHODS

Laparoscopic Heller myotomy was undertaken in 74 patients between November 1998 and December 2001. The diagnosis of achalasia was confirmed in all

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patients radiographically by barium esophagogram showing tapering of the lower esophagus (i.e., "bird beak") with a dilated proximal esophagus and manometrically by aperistalsis of the esophageal body and incomplete or no relaxation of the lower esophageal sphincter mechanism. Prior to myotomy, the following data were collected: patient age, sex, symptoms, duration of symptoms prior to surgical referral, amount of unintentional weight loss, and previous endoscopic treatments undertaken. Patients were asked to grade their dysphagia and heartburn symptoms on a Likert-type scale from 0 (no symptoms) to 5 (severe/continuous symptoms).

Our techniques for laparoscopic Heller myotomy have been previously reported.[15,16] In short, a five-port approach was used in all patients. Intraoperative endoscopy was undertaken concomitantly, per os, by a gastroenterologist to help identify the gastroesophageal junction, assess adequacy of myotomy, and evaluate for intraoperative perforation. A focused dissection directly onto the esophagus and the gastroesophageal junction was undertaken to avoid disruption of natural antireflux mechanisms. Great care was taken to avoid injuring the vagus nerves or their branches. The longitudinal muscle fibers were divided with a 90-degree angled hook cautery, and the myotomy was begun above the gastroesophageal junction. The myotomy was extended first in a cephalad direction as high as necessary to allow opening of the lower esophagus with gentle insufflation through the endoscope. Similarly, the myotomy was continued caudally until the lower esophageal sphincter was fully disrupted, as seen endoscopically. This myotomy was generally carried just a few millimeters onto the gastric cardia, enough to allow adequate relief of the stenosis. Myotomy was considered adequate when bright transillumination was seen across the myotomized segment, both endoscopically and laparoscopically, without any residual circular muscle bands, when the lower esophagus and gastroesophageal junction were easily distended with gentle insufflation through the endoscope, when the endoscope could be passed into the stomach without resistance, and when perforation or transmural burn was ruled out after thorough examination of the mucosa and gentle insufflation.

Fundoplication was applied selectively in patients with a large hiatal hernia, a patulous esophageal hiatus, or as part of the repair of an intraoperative perforation. Partial posterior (Toupet) fundoplication or, more commonly, anterior (Dor) fundoplication were the fundoplications of choice. When a Toupet fundoplication was to be constructed, circumferential dissection of the lower esophagus with exposure of both diaphragmatic crura was undertaken, along with division of the short gastric vessels with the Harmonic Scalpel (Ethicon Endo-Surgery, Inc., Cincinnati, OH). The crura were plicated posteriorly with sutures prior to construction of the posterior 270-degree fundoplication. Conversely, when a Dor fundoplication was constructed, the short gastric vessels were not divided and the esophageal hiatus was reconstructed as a last step. In this case, the anterior fundoplication was constructed by suturing the anterior gastric fundus over the myotomized segment of the esophagus.

In the early postoperative period (i.e., within 24 hours), the myotomy was evaluated radiographically with water-soluble contrast followed by thin barium if no leak was seen. This study was undertaken to evaluate the integrity of the myotomized segment and to determine the adequacy of esophageal emptying. If no leak was detected and emptying was rapid, patients were immediately started on a liquid diet and advanced to a soft mechanical diet at the time of discharge the following morning. If emptying was slow because of esophageal dysmotility or postoperative edema, diet was delayed until swallowing improved and/or a repeat study documented more rapid emptying. The diet was then advanced as previously described. At discharge, patients were instructed to advance to a regular diet over the ensuing 1 to 2 weeks as tolerated.

After their convalescence, patients completed the same survey as before their operations at clinic visits, by mail, or by telephone inquiry every 6 months. Patients were asked to again grade their dysphagia and heartburn using the same Likert scale. In addition, patients were now asked to report any additional treatments undertaken in the interval after their myotomy and to grade their outcome as excellent (no symptoms or infrequent symptoms), good (greatly improved), fair (slightly improved), or poor (no improvement or worse symptoms) relative to before the operation.

At the time of myotomy, a portion of esophageal muscle was excised for histopathologic examination. Specimens were fixed in formalin and embedded in paraffin blocks. Slides were prepared using routine hematoxylin and eosin (H&E) stains as well as Masson's trichrome stain. Slides were reviewed by a senior pathologist (A.G.), who was blinded as to the patients' names, preoperative therapies, and postoperative outcomes. The degree of fibrosis was graded on a scale of 0 (no fibrosis) to 5 (100% fibrosis).

Data were complete for 73 patients (99%) who underwent laparoscopic Heller myotomy with intraoperative esophageal muscle biopsies. One patient died before outcome data could be obtained and was, therefore, excluded. This patient will be discussed later. Correlations between patient demographics, preoperative endoscopic treatment, esophageal muscle fibrosis, operative complications, and postoperative outcomes were

sought by calculating Spearman correlation coefficients. Any factors with significant correlation (i.e., $P < 0.05$) were subjected to logistic regression analysis. The 73 patients were then grouped as follows on the basis of their preoperative endoscopic therapy: (1) esophageal dilation only; (2) Botox injection only; (3) dilation plus Botox injection; and (4) neither dilation nor Botox injection. Preoperative, perioperative, and outcome data were compared for these four groups. Intergroup differences were compared using one-way analysis of variance (ANOVA) for parametric data and Kruskal-Wallis ANOVA or log-likelihood ratio tests for nonparametric data. Significance was accepted with 95% confidence. Any significant intergroup differences were confirmed by Student's *t* test for parametric data or Mann-Whitney U test or Fisher's exact test for nonparametric data. Intragroup differences were compared using the Mann-Whitney U test (symptom scores) and Fisher's exact test (symptom incidence). Data were maintained on an Excel spreadsheet (Microsoft, Redmond, WA). All statistical analyses were undertaken with True Epistat software (Epistat Services, Richardson, TX). Data are presented as mean ± standard deviation unless stated otherwise.

RESULTS

Demographic, preoperative, perioperative, and postoperative data from the 73 patients undergoing laparoscopic Heller myotomy are summarized in Table 1. Patients waited an average of nearly 9 years after the onset of symptoms to seek or be referred for operative intervention. During this time, 34 patients (47%) reported unintentional weight loss. Previous endoscopic therapy was commonplace in our patients (see Table 1). Esophageal dilation (median two times per patient) was the most commonly applied endoscopic therapy followed closely by Botox injection (median two times per patient) (see Table 1). In 17 patients (23%), dilation was the only form of endoscopic therapy, whereas 11 patients (15%) had undergone only Botox injections. Esophageal fibrosis occurred in less than one third of patients and was generally mild (see Table 1).

There was a significant correlation between preoperative esophageal dilation and postoperative dysphagia score, but dilation did not correlate with outcomes (Table 2). When subjected to univariate regression analysis to evaluate patients for a cause-and-effect relationship, no significant relationship was seen between preoperative dilation and postoperative dysphagia. No correlation was seen between preoperative Botox injection and postoperative symptoms or outcomes. The degree of fibrosis did not correlate with patient characteristics, preoperative endoscopic therapy, postoperative symptoms (see Table 2), or operative complications (data not shown). There was a significant inverse correlation between the degree of fibrosis and outcome. In other words, patients with worsening fibrosis were less likely to have optimal outcomes. Again, by univariate analysis, fibrosis did not directly predict postoperative outcome.

Complications were uncommon, occurring in eight patients (11%). The most common complication was perforation during myotomy, which occurred in four patients (5%). All four perforations were recognized intraoperatively and repaired laparoscopically. Two

Table 1. Preoperative and perioperative data on patients undergoing laparoscopic Heller myotomy

No. of patients = 73	Data
Age (yr)	51.6 ± 16.4
Sex	37 males/36 females
Preoperative symptom duration (yr)	8.7 ± 8.1
Preoperative weight loss (pounds)	13.9 ± 18.2
Preoperative endoscopic therapy*	
Esophageal dilation	52 (71%)
Botox injection	46 (63%)
Dilation + Botox	35 (48%)
Dilation or Botox	62 (85%)
Fibrosis	
Incidence	22 (30%)
Score (per patient with fibrosis)	2.1 ± 0.9
Fundoplication	
Total	22 (30%)
Dor	21 (29%)
Toupet	1 (1%)
Length of stay (days)	2.1 ± 2.1
Follow-up (mo)	15.7 ± 14.4
Dysphagia incidence	
Preop	69 (95%)
Postop	20 (27%)†
Dysphagia score	
Preop	4.5 ± 0.9
Postop	1.6 ± 1.6†
Heartburn incidence	
Preop	36 (49%)
Postop	23 (32%)†
Heartburn score	
Preop	2.3 ± 1.8
Postop	1.5 ± 1.4†
Symptomatic improvement	70 (96%)
Outcome	
Excellent	43 (59%)
Good	24 (33%)
Fair	3 (4%)
Poor	3 (4%)
Would have operation again	71 (97%)

*Percentages do not add up to 100% because of overlap of therapies.
† $P < 0.05$ vs. preop.

Table 2. Correlation matrix for preoperative patient characteristics, esophageal fibrosis, and postoperative symptoms, and postoperative outcomes in patients undergoing laparoscopic Heller myotomy

	Fibrosis	Postop dysphagia	Postop heartburn	Outcome
Age	0.16	0.10	−0.02	−0.16
Symptom duration	0.12	0.16	0.10	−0.12
Preop weight loss	0.00	−0.18	−0.13	0.16
Preop dilation	0.05	0.30*	0.08	−0.12
Preop Botox	−0.16	0.05	0.16	0.01
Fibrosis	—	0.07	0.17	−0.24*

Data are Spearmans *r* values.
* $P < 0.05$.

patients (3%) returned 1 week after discharge from an uneventful operation with fever, cough, shortness of breath, and left-sided pleural effusion. Subsequent workup demonstrated empyema in both cases without evidence of leak on esophagogram with water-soluble contrast medium and barium. One of the two patients underwent thoracotomy with decortication and drainage. She had no stigmata of leak at thoracotomy. She was ultimately discharged home but complains of continued dysphagia. The second patient failed to recover after adequate drainage and died. No leak was identified on repeated esophagograms. This was, and remains, the only perioperative death in our experience. We believe that the empyemas were a consequence of pneumonia. Asystole developed briefly as a vasovagal response after insufflation of the abdomen in one patient (1%). Normal sinus rhythm returned immediately, and the operation was completed without further incident using lower than normal insufflation pressures. This patient had an uneventful postoperative course after a negative cardiac workup. Finally, the pleura was violated in one patient (1%). This was recognized intraoperatively, and the resultant pneumothorax was evacuated with a needle, thereby avoiding tube thoracostomy. Mean hospital stay was 2 days for all patients (see Table 1) with a median of 1 day.

All patients are being followed nearly 16 months after discharge, with significant improvement in symptoms (see Table 1). Dysphagia and heartburn symptoms improved in both incidence and severity after laparoscopic Heller myotomy. Overall, three patients reported poor outcomes. Two of these three patients reported significant improvement in their symptoms during the first year after myotomy. However, they both complained of recurrent dysphagia after 20 and 21 months' follow-up. The third patient has had persistent dysphagia after postoperative empyema, as de-

Table 3. Patient characteristics based on preoperative endoscopic therapy

	Dilation only	Botox only	Dilation + Botox	Neither
No. of patients	17	11	35	10
Age (yr)	51.2 ± 11.9	50.3 ± 23.4	53.8 ± 15.9	47.4 ± 16.8
% Male	47	64	49	50
Symptom duration (yr)	13.1 ± 9.4*	6.4 ± 6.8	8.5 ± 7.8	4.6 ± 5.7
Dilations per patient	2.4 ± 1.3	N/A	2.3 ± 2.3	N/A
Botox injections per patient	N/A	3.3 ± 2.6	2.8 ± 1.9	N/A
Preop weight loss (pounds)	14.3 ± 20.6	11.4 ± 12.3	15.9 ± 19.6	9.2 ± 16.0
Dysphagia				
Incidence	17 (100%)	9 (82%)	35 (100%)	9 (90%)
Score	4.8 ± 0.6	3.9 ± 1.6	4.7 ± 0.5	4.3 ± 1.3
Heartburn				
Incidence	15 (88%)	9 (82%)	18 (51%)	5 (50%)
Score	1.9 ± 1.9	2.5 ± 1.8	2.4 ± 1.9	2.5 ± 1.7
Fibrosis				
Incidence	7 (41%)	2 (18%)	10 (29%)	4 (40%)
Score	2.3 ± 0.8	2.0 ± 0.0	2.0 ± 0.8	2.0 ± 1.4

N/A = not applicable.
* $P < 0.05$ vs. "Neither."

Table 4. Operative characteristics of patients undergoing laparoscopic Heller myotomy based on type of preoperative endoscopic therapy

	Dilation only	Botox only	Dilation + Botox	Neither
Fundoplication	3 (18%)	4 (36%)	12 (34%)	3 (30%)
Complications	2 (12%)	2 (18%)	3 (9%)	0
Perforation	1 (6%)	1 (9%)	3 (9%)	0
Length of stay (days)	2.4 ± 2.4	1.8 ± 1.8	2.2 ± 2.3	1.4 ± 0.7

scribed earlier. Two of these three patients report that they would not undergo the operation again given similar circumstances.

When compared on the basis of preoperative endoscopic treatment, no significant differences were seen in patient age, sex, preoperative weight loss, incidence and severity of dysphagia, incidence and severity of heartburn, or incidence and degree of esophageal muscle fibrosis (Table 3). Patients who had undergone balloon dilation as their only form of preoperative therapy had a significantly longer duration of preoperative symptoms than those who had not undergone any preoperative endoscopic treatment. Patients who underwent endoscopic therapy often had these procedures repeated many times. Preoperative therapy did not affect how often fundoplication was applied, the frequency of perforation, the incidence of total complications, or the postoperative length of hospital stay (Table 4).

There were no significant differences in the length of follow-up between groups (Table 5). Dysphagia was significantly improved in incidence and severity in all groups. Patients who underwent preoperative balloon dilation with or without Botox injection were more likely to have postoperative dysphagia than patients who had never undergone such treatment. The dysphagia scores, however, did not reach statistical significance ($P = 0.07$). Patients who had undergone either balloon dilation or Botox injection prior to myotomy had a higher incidence of heartburn symptoms preoperatively than those who had not undergone these therapies, but this did not reach statistical significance ($P = 0.08$). In addition, these patients had significant reductions in the incidence of heartburn postoperatively (see Table 5). Patients who had never undergone endoscopic therapy were the only patients to have significant reductions in the severity of heartburn symptoms and the lowest incidence and severity of postoperative heartburn of all patients, although this was not statistically significant. Symptomatic improvement and excellent/good outcomes were reported in most patients irrespective of preoperative endoscopic therapy. All but one patient who had undergone balloon dilation with Botox injection

Table 5. Preoperative and postoperative symptoms and outcomes based on type of preoperative endoscopic therapy in patients undergoing laparoscopic Heller myotomy

	Dilation only	Botox only	Dilation + Botox	Neither
Follow-up (mo)	13.2 ± 23.6	19.6 ± 11.8	16.6 ± 9.6	10.7 ± 10.8
Dysphagia incidence				
Preop	17 (100%)	9 (82%)	35 (100%)	9 (90%)
Postop	6 (35%)*†	2 (18%)	12 (34%)*†	0 (0%)*
Dysphagia score				
Preop	4.8 ± 0.6	3.9 ± 1.6	4.7 ± 0.5	4.3 ± 1.3
Postop	1.8 ± 1.8*	0.8 ± 1.2*	1.9 ± 1.6*	0.9 ± 0.7*
Heartburn incidence				
Preop	15 (88%)	9 (82%)	18 (51%)	5 (50%)
Postop	4 (24%)*	3 (27%)*	14 (40%)	2 (20%)
Heartburn score				
Preop	1.9 ± 1.9	2.5 ± 1.8	2.4 ± 1.9	2.5 ± 1.7
Postop	1.3 ± 1.3	1.6 ± 1.7	1.7 ± 1.4	1.1 ± 1.4*
Symptom improvement	16 (94%)	11 (100%)	33 (94%)	10 (100%)
Excellent/good outcome	15 (88%)	11 (100%)	32 (91%)	9 (90%)
Would have again	16 (94%)	11 (100%)	34 (97%)	10 (100%)

* $P < 0.05$ vs. Preop.
† $P < 0.05$ vs. "Neither."

and one who had undergone balloon dilation without Botox injection stated that they would undergo the operation again (see Table 5).

DISCUSSION

Heller myotomy has long been established as a durable means of palliating the symptoms of achalasia. Since the advent of laparoscopy and its application to Heller myotomy, there has been a resurgence of interest among surgeons treating this disease. There is little doubt in the surgical community that laparoscopic Heller myotomy is superior to nonoperative treatment of achalasia, especially in duration of response. Unfortunately, surgical referral is often delayed as patients are relegated to less efficacious therapies such as balloon dilation and Botox injection. Still, outcomes after laparoscopic Heller myotomy, when applied as salvage therapy in these patients, are favorable. Because achalasia is fairly uncommon, few institutions are able to amass a considerable experience with its operative management. It is at these high-volume institutions that we are able to study the pathophysiology of the disease and determine patterns of failure or suboptimal outcomes following laparoscopic Heller myotomy. Previously we reported that preoperative therapy, particularly when balloon dilation was combined with Botox injection, negatively affected postoperative outcomes following videoscopic therapy in our first 110 consecutive patients.[16] After gaining more experience with this operation, we have looked at our more recent experience over the past 3 years. In this study we have shown that preoperative treatment in these patients does not affect the histology of the esophageal muscle or, in contrast to our previous study, significantly alter their postoperative course.

The patients in this study tended to be middle-aged men and women with severe dysphagia who reported nearly a decade of worsening symptoms before seeking, or being referred for, surgical therapy. In the interim, the vast majority of patients underwent balloon dilation, Botox injection, or both with limited success. It was uncommon for patients to be referred for myotomy after just one failure of endoscopic therapy, as most patients had multiple attempts at balloon dilation and/or Botox injection. The few who did not receive such treatment often sought operative intervention earlier at their own discretion. These tended to be younger patients who had a shorter duration of symptoms. Dysphagia was nearly universal and generally severe, often resulting in unintentional weight loss. The incidence and severity of dysphagia was dramatically reduced with laparoscopic Heller myotomy. Interestingly, dysphagia was not the presenting complaint in three patients. Regurgitation of undigested food was the primary complaint in these three patients, all of whom reported excellent outcomes after myotomy. Noteworthy is that symptoms rarely disappear completely after myotomy, underscoring the palliative nature of the operation and ongoing dysmotility of the esophagus. We are very critical of our patients' postoperative symptoms and report any and all dysphagia. Often patients will initially report no dysphagia but when questioned thoroughly, it becomes clear that they do not have dysphagia only because they have significantly altered their eating habits before myotomy. In other words, a patient who reports no dysphagia but avoids breads and meats, requires three or four glasses of water during meals, and must walk around for a period of time after eating is reported here as having severe dysphagia. What may be a more telling indicator of operative success is how patients report their outcomes, which was excellent or good in 92% and significantly improved in 96% in this series.

Interestingly, half of our patients reported heartburn symptoms prior to myotomy. Whether pathologic gastroesophageal reflux truly occurs, and to what degree, in the face of achalasia is a banal discussion that is beyond the scope of this report. Often these symptoms reflect poor esophageal motility with inadequate clearing of normal, physiologic reflux or acidification of retained food particles. As such, nearly a third of our patients continue to report these symptoms postoperatively, although they are significantly reduced in incidence and severity compared to before myotomy. We do not routinely undertake fundoplication at the time of myotomy. Also, it has not been our practice to routinely obtain 24-hour pH monitoring postoperatively because patients generally refuse to undergo this monitoring when given the option. Our practice is to follow patients clinically and to seek further studies as dictated by symptoms and responsiveness to therapy. We believe that all patients should undergo periodic endoscopy, regardless of symptoms, to monitor for esophagitis, esophageal stricture, Barrett's esophagus from occult reflux, and the development of cancer. Patients with severe reflux symptoms undergo evaluations similar to those in patients with primary gastroesophageal reflux and therapy is planned accordingly. In our entire experience with 166 patients through December 2001, only two patients have required fundoplication in the interval following myotomy. Both patients, who were not part of this study and were seen early in our experience, gained significant weight (more than 100 pounds each) postoperatively and developed symptoms of reflux several years after myotomy.

Fibrosis of the esophageal muscle was actually uncommon and did not correlate with preoperative therapy. This was somewhat surprising because these therapies aim to disrupt the lower esophageal sphincter and one would expect fibrosis to occur more commonly. It is possible that fibrosis may occur between the muscle and submucosa or in the submucosa and therefore may not have been sampled in this study. Goldblum et al.[17] found that 69% of patients undergoing esophagectomy for severe, refractory achalasia had notable fibrosis of the muscularis propria, suggesting that fibrosis is associated more with end-stage achalasia than with preoperative therapy, although this association was not directly investigated. Goldblum et al.[18] examined esophageal muscle biopsy specimens from 11 patients undergoing myotomy for "early" achalasia and found only mild fibrosis in three. Although fibrosis did inversely correlate, to a lesser degree, with patient outcomes in our study, a cause-and-effect relationship could not be established. In addition, no relationship was established between the degree of fibrosis and incidence of intraoperative perforation, which was also uncommon. Perforations continue to occur on occasion in our experience during more difficult operations, but are generally of no clinical consequence.

Preoperative treatment did not significantly predict how patients would respond to laparoscopic Heller myotomy. Dysphagia was significantly improved, irrespective of preoperative therapy, but tended to be more common postoperatively in patients who had undergone balloon dilation with or without Botox injection when compared to those patients who had never undergone endoscopic therapy. It is important to note that preoperative dilation techniques were not standardized and, as such, varied from one endoscopist to another and from patient to patient. Patients reported whether or not they had dilation but did not know the specifics of the procedure. We know, based on the practices of our referring gastroenterologists, that balloon dilation was usually undertaken with the intent of cure, but less aggressive dilations may have been undertaken in some patients.

The incidence of heartburn symptoms only improved in those patients who had undergone previous dilation or Botox injections. These patients were more likely to complain of heartburn before myotomy but had a similar incidence of heartburn and scores after myotomy similar to those patients who had never undergone endoscopic therapy. All in all, symptomatic improvement and excellent or good outcomes were the rule, irrespective of the preoperative therapy, such that nearly all patients reported that they would have the operation again, given similar circumstances.

As we continue to gain experience in the treatment of achalasia utilizing minimally invasive techniques, our knowledge of the pathophysiology and the factors that contribute to suboptimal outcomes grows. Although a direct cause-and-effect relationship between preoperative therapy and postoperative outcomes is difficult to establish, mostly because of the high frequency of optimal outcomes, physicians treating patients with achalasia must look at their results critically and modify treatment plans accordingly. It is our belief that balloon dilation may play an important role in the initial management of these patients, particularly early in the course of the disease. However, recurrent symptoms or failure of initial therapy warrants surgical evaluation. Botox injections have a limited duration of effect and should be reserved for those patients who are not candidates for or refuse balloon dilation or myotomy or possibly used as a diagnostic trial to determine potential responsiveness to operative myotomy.

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Discussion

Dr. M. Stelzner (Seattle, WA): I have a question with regard to your fibrosis scoring, because those results are a little surprising. You will agree that fibrosis is not one of the hallmarks of achalasia. However, you showed that the fibrosis scores in those patients who had not undergone any previous treatments was already two and not around zero as one would expect. I would like to know if you think this, in some way, calls into question the validity of your scoring system? Second, you did not find significant increases in fibrosis in your patients after Botox or dilation. If one assumes that each treatment caused even some minor increase in fibrosis, the fibrosis may have increased in an additive manner, and thus significantly, if there were several events preceding the operation. So, in the subgroup that had many previous, for example, four, six, or eight treatments, did you find a higher fibrosis score, and was the score significantly higher in that subgroup?

Dr. M. Bloomston (Tampa, FL): In response to your first question concerning our scoring system, this is a scoring system that was devised by our pathologists and we have not yet validated this scoring system. So it does introduce some questions. When we asked the pathologists to look at these, they initially made statements such as, "there is not going to be any fibrosis, there is no fibrosis in these, or we don't see anything." When we pressed them to really discriminate between these different groups, then they came up with this scoring system, which we have not yet validated. I agree that we may not be sampling the correct part of the muscle; the fibrosis may actually be between the submucosa and the muscle layer. The reason we studied fibrosis was because we found that subjectively this was a more difficult operation for patients who had undergone previous therapies. We assumed fibrosis was the cause of this difficulty. When we looked at the problem objectively, there was not a big difference between our groups. Now, in regard to the increased fibrosis with the number of therapies, we did not see a big difference; only 23 patients had fibrosis, and almost all of those patients already had several therapies when they did undergo surgery. We did not see a correlation between the number of therapies and the degree of fibrosis.

Dr. L.W. Way (San Francisco, CA): My first question is, can you define dilatation? My second question is, every now and then we operate on a patient who has had previous treatment, and it is difficult to separate the muscular layer from the underlying mucosa. In our experience, this kind of esophagus has virtually always had previous treatment. What was the correlation between that type of operative finding and the end point that you are reporting of fibrosis histologically? Also, with regard to the patients who had grade 4 and 5 fibrosis, how many were there and what can you tell us about them? Approaching the data the way you did may create a dilution effect. If, for example, the preoperative therapy created some fibrosis-generating event in, let's say, 1 out of 10 or 1 out of 15 cases, mean scores would not tell you very much, but you could still attribute the occasional severe fibrosis to the previous treatment. We just do not see difficulties in performing the myotomy in patients who have not had previous treatment, and the difficulties in performing the myotomy are not universal in those persons, or even the average experience, but it seems always to be part of the background when it really is a difficult operation and you face severe fibrosis.

Dr. Bloomston: Thank you, Dr. Way. I think we agree on a lot of this. First of all, our definition of di-

lation is not universal. We relied on the reports of the patients themselves as to whether or not they underwent dilation, and how many times they had those dilations. So there are differences in types of dilations from one endoscopist to another and from one center to another, and whether all these dilations were undertaken truly with intent to cure is a matter of debate. Unfortunately, we have a large referral catchment area, so we are not able to really standardize the type of dilation. I agree that it is difficult to determine what is truly a dilation.

Dr. Way: But were these balloon dilatations?

Dr. Bloomston: I would say almost all of them were balloon dilations. Some were just bougienages that were not objectively measured at the time of the dilation. So, again, it is difficult to tell which of those were and the specific techniques, because we relied on patient reporting of those dilations. The next point you brought up was the correlation between the degree of difficulty of the operation and the preoperative therapy, and we agree 100%. It seems as if patients who have preoperative therapy have a more difficult dissection. First of all, it is difficult to grade that in the operating room, because it is truly a subjective finding. So we tried to look at this objectively by noting how many patients had perforations and/or how many patients had poor outcomes. We saw no difference. Maybe it was because we just tried a little harder and took our time when we did encounter a case that looked like it was going to be more difficult, but in the final analysis that did not seem to make a difference. Finally, about the extensive fibrosis, there were only two patients that had grade 4+ fibrosis. There were no patients who had grade 5+ fibrosis. So statistical analysis was really impossible in these patients, and there was no common thread among the patients with 4+ fibrosis.

Dr. E. Zenilman (Brooklyn, NY): This was a very nice presentation. I was struck by the vasovagal reaction that you encountered. Do you know what the incidence of asystole is during insufflation? We have been trained to look for hypotension and bradycardia during that time, but I have never seen asystole. Second, many would perform a Nissen fundoplication after myotomy to prevent gastroesophageal reflux. Do you think your incidence of heartburn postoperatively would have been reduced had you done a wrap, and if you did a wrap, if it had been a tighter one?

Dr. Bloomston: First of all, thankfully the incidence of asystole with pneumoperitoneum is very, very low in laparoscopic cases. This is the only instance where we have had that problem, and it was very transient—only a couple of seconds. Second, would we have improved the heartburn with fundoplication? Twenty-two patients underwent fundoplication: 21 of those 22 had a Dor fundoplication. We apply these selectively in cases where there is a perforation or if we have a patient with a large hiatal hernia or a patulous esophageal hiatus. We have looked at our data. There is no difference in the incidence of heartburn between our patients who have or do not have a wrap. Recognizing that it is a biased group, the patients who receive a wrap may be at higher risk for heartburn postoperatively, but there is no difference between the groups.

Invited Discussion—Expert Commentator

David W. Rattner, M.D.: Dr. Rosemurgy's paper examines the relationship of preoperative Botox injection and balloon dilation to histologic findings and long-term outcomes in a very large group of patients undergoing laparoscopic Heller myotomy. Dr. Rosemurgy's group performs a very high volume of myotomies and on this basis alone their thoughts deserve serious consideration. I must, however, differ with their conclusions. What was measured in this study was histologic change in the esophageal muscle at the site of the myotomy. I am not sure that histologic evidence of fibrosis carries any clinical significance. There was no standardization of the location of the biopsy, and those who perform this procedure know that a portion of the procedure may be quite easy and surgically significant fibrosis encountered only over a small area. Hence the authors need to clarify whether they biopsied the most scarred portion of the muscle or selected the biopsy site on the basis of anatomic locations relative to the squamocolumnar junction or some other landmark. Most surgeons who perform Heller myotomies would be hard pressed to recall a laparoscopic Heller myotomy in a patient who had no prior intervention in whom it was difficult to identify a proper dissection plane—in contrast to the scarring (and I will not use the term "fibrosis" here) seen in patients with previous interventions. In support of this distinction, I would point out that the data from this series show that there were no esophageal perforations in patients who had myotomy as their first intervention (see Table 4). The data also show that patients who underwent presurgical interventions had

more dysphagia postoperatively than those who had surgery as first-line therapy. Although this did not reach statistical significance ($P = .07$), one suspects that it is clinically significant and would be statistically significant as well, if the sample size were larger. The authors do not provide any manometric data on lower esophageal sphincter pressures before and after myotomy, so it is difficult to be sure that the myotomy was comparable in these patients. Recently, a group from Vanderbilt University has shown that a decrease in lower esophageal sphincter pressure of less than 18 mm was associated with incomplete relief of dysphagia. One might also argue that the dysphagia in patients who had prior endoscopic treatment was related to the fact that these patients had a longer duration of disease before they underwent myotomy and hence were more likely to have a dilated esophagus. Regardless of the explanation, it seems hard to justify a recommendation that balloon dilation or Botox be given as initial therapy to patients with achalasia. Although the data in this study demonstrate the outstanding surgical ability of Dr. Rosemurgy's group, they do not support the authors' contention that preoperative intervention has no effect on the outcomes of laparoscopic Heller myotomy.

Cholangitis: Bacterial Virulence Factors That Facilitate Cholangiovenous Reflux and Tumor Necrosis Factor-alpha Production

Lygia Stewart, M.D., Adair L. Oesterle, J. McLeod Griffiss, M.D., Gary A. Jarvis, Ph.D., Lawrence W. Way, M.D.

In previous studies we noted that biliary bacteria produce slime and possess P1-fimbriae. The presence of gram-negative bacteria killed by complement correlated with serious biliary infections and induced more tumor necrosis factor-alpha (TNF-α) production in sera, suggesting a role for cytokine production and complement activation in biliary sepsis. This study examined bacterial virulence factors that facilitate cholangiovenous reflux (CVR) and TNF-α production in a rat model. Twenty-one biliary bacteria and two stool isolates were tested for slime production, sensitivity to complement killing, and hemolysin production. 10^7 Bacterial colony-forming units/ml (or saline control) were injected retrograde into the common bile ducts of Sprague-Dawley rats at a pressure of 30 cm H_2O. Blood was obtained at 5 and 60 minutes after infusion for bacterial culture and TNF-α assay, respectively. The magnitude of slime production correlated inversely with the magnitude of bacterial CVR. Average bacterial colony-forming units were 1.4×10^5, 6.8×10^4, or 2.1×10^3 for bacteria with slime production 0 to 10, 11 to 99, or more than 100, respectively ($P < 0.0001$, analysis of variance). CVR was greater for serum-resistant bacteria (1.2×10^5 vs. 5.5×10^4 [$P = 0.007$, resistant vs. sensitive]), but TNF-α production was greater in serum-sensitive bacteria. TNF-α production as a function of bacterial reflux followed a logarithmic curve ($R^2 = 0.75$) for serum-sensitive bacteria but was linear ($R^2 = 0.60$) for serum-resistant bacteria. These data show how specific virulence factors explain why some bacterial species colonize without causing illness, whereas others colonize and cause sepsis. Although slime production was necessary for colonization, too much slime inhibited CVR. Although complement killing cleared bacteria from the circulation, it was also associated with increased TNF-α production, which can lead to septic manifestations. The most virulent bacterial species (from patients with sepsis) were killed by complement, but they still had significant CVR and were associated with increased TNF-α production. (J GASTROINTEST SURG 2003;7:191–199.)

KEY WORDS: Cholangitis, cytokines, TNF, slime, biliary infections, gallstones

We previously identified bacterial microcolonies within the pigment matrix of gallstones[1] and noted a correlation between the presence of bacteria and increased severity of illness.[2] We characterized these biliary bacteria, noting that they possessed factors that facilitated colonization of the biliary tree, including P1-fimbriae on their surface[3] and the ability to produce slime or glycocalyx.[4] We also noted that although the presence of any biliary bacteria correlated with increased illness severity, not all biliary bacterial species caused clinical infection. Further analysis of patients with bacteria present in the biliary tree revealed that gram-negative biliary bacteria killed by complement were associated with more serious biliary infection, including bacteremia, and gram-negative biliary bacteria killed by complement

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induced more tumor necrosis factor-alpha (TNF-α) production when exposed to sera (using an in vitro model of cultured monocytes).[5] These data suggest a role for cytokine production, possibly mediated through complement activation, in biliary sepsis. Among the various biliary bacterial species, there were differences in the prevalence of bacteremia and the severity of infection. The current study was designed to study these virulence differences in greater detail. Bacterial virulence factors facilitating cholangiovenous reflux (CVR) and TNF-α production were examined using an in vivo rat model.

MATERIAL AND METHODS

Bacterial Characterization

Ninety-three bacterial species were obtained from cultures of gallstones, bile, and blood from patients with biliary infections.[4,5] Clinical data were prospectively collected and correlated with the presence of bacterial virulence factors.[5] Gallstones were collected under sterile conditions, rinsed with normal saline solution, crushed, and cultured in tryptic-soy broth for 24 to 48 hours. Bile was obtained for culture at surgery, endoscopic retrograde cholangiopancreatography, or during percutaneous transhepatic cholangiography. Blood cultures were performed as clinically indicated. Thirteen bacterial species were recovered from stool cultures (12 humans and one rat). All bacterial species were tested for slime production.[4] Gram-negative bacteria were tested for complement-mediated bacterial killing against patient and control sera.[4,5] Hemolysin production by the bacterial isolates was measured by plating the bacteria on sheep blood agar plates followed by overnight incubation at 37° C. Plaques were assessed by visual inspection for the presence and level of hemolysis.

Following the preceding characterization, 23 bacterial species (21 from the biliary tract, one from human stool, and one from rat stool) were chosen for in vivo testing. These bacteria were a subset of the bacterial species previously studied.[4,5] Those selected possessed properties covering the range of three virulence factors: hemolysin production, sensitivity to complement-mediated bacterial killing, and variation in quantitative slime production (Table 1) For clinical correlation, bacterial species were separated into the following three groups based on the clinical manifestations of the patients: (1) no clinical infection = bacteria from patients with a biliary tree that contained bacteria, who had no clinical or laboratory manifestations of infection; (2) infectious manifestations = bacteria from patients who had manifestations of infection including fever, leukocytosis, abscess, cholangitis, and/or bacteremia, who did *not* have organ failure or hypotension; and (3) sepsis = bacteria from patients who had infectious manifestations that included hypotension and/or organ failure.

The gram-negative bacterial species selected for in vivo testing were also tested for complement-mediated bacterial killing against rat serum using our previously described bactericidal assay.[5,6] Bacteria were suspended in Mueller-Hinton broth to an OD_{530} of 0.1 to 0.2 and grown to an OD_{530} of 0.6 (approximately 10^8 bacteria/ml in log-phase growth). They were resuspended in veronal buffer saline (VBS buffer) (OD_{530} of 0.6), and a 1:30,000 dilution in VBS buffer was prepared. A combination of 25 μl of bacterial solution, 25 μl of serum, and 75 μl of VBS buffer was mixed and incubated for 1 hour at 37° C in a shaker bath. This was pour-plated at varying concentrations, incubated overnight in 5% CO_2 at 37° C, and the plates were counted. Growth of bacteria in normal and heat-inactivated serum (60° for 45 minutes) was compared with growth in the absence of serum. Lack of bacterial growth in normal serum, coupled with growth in heat-inactivated serum, documented complement-mediated bacterial killing (serum-sensitive bacteria). Bacterial growth in the presence of serum showed resistance to complement-mediated killing (serum-resistant bacteria).

In Vivo Testing for Cholangiovenous Reflux and TNF-α Production

Male Sprague-Dawley rats were anesthetized (Nembutal, 5 mg/100 g body weight) and the common bile duct (CBD) was ligated. PE-50 tubing was inserted into the CBD, and a catheter was positioned in the superior vena cava. Bacteria (10^7 colony-forming units [CFU]/ml) were infused into the CBD at a constant rate to create a pressure of 30 cm H_2O. The infusion rate determines the biliary pressure. We have previously characterized the pressure obtained at different infusion rates and determined that 30 cm H_2O reliably causes CVR.[7,8] After infusion, blood specimens were obtained from the superior vena cava for quantitative bacterial culture. Blood specimens were pour-plated at varying concentrations (in duplicate), incubated overnight in 5% CO_2 at 37° C, and the plates were counted. A total of 23 bacterial species were studied, and 2 to 11 rats were studied for each bacterial species. A control group (n = 6 rats) that received endotoxin-free sterile saline infusion into the CBD was also studied.

In conjunction with the preceding experiment, plasma was collected in endotoxin-free cell culture tubes at 30 and 60 minutes after the bacterial (or endotoxin-free sterile saline) infusion for TNF-α assay. TNF-α was assayed using a commercially available enzyme-

Table 1. Virulence factors of the bacterial species studied

		Hemolysin		Complement sensitivity		Slime production
Bacterial species	No.	None	Beta or alpha	Killed	Not killed	Range
E. coli	10	5	5	5	5	1.4–82
Klebsiella	2	2	0	1	1	0.8
Enterococcus	3	2	1	0	3	1.7–7.7
Enterobacter	4	4	0	2	2	4.2–73.6
Pseudomonas	4	3	1	2	2	82–200

linked immunosorbent assay kit for rat TNF-α (Genzyme Corp., Cambridge, MA). Assays, which were carried out in duplicate, were in good agreement. All experiments were conducted under an approved animal use protocol.

Statistical Analysis

Statistical analysis was performed using Student's *t* test for continuous variables in a paired and unpaired fashion, or analysis of variance (ANOVA) for multiple comparisons using the Student–Newman-Kuels test. Graphics and R^2 calculations were performed using Excel 2000.

RESULTS

Factors Influencing Cholangiovenous Reflux

Three bacterial virulence factors were examined for their influence on bacterial CVR: hemolysin production, slime production, and sensitivity to complement-mediated bacterial killing. Hemolysin production had no effect on bacterial CVR. Results were as follows: 1.0×10^5 bacterial CFU/ml and 9.0×10^4 bacterial CFU/ml for bacterial species with and without hemolysin production ($P = 0.576$; Student's *t* test).

Increasing bacterial slime production decreased CVR substantially in a logarithmic fashion (Fig. 1). Average blood bacterial CFU/ml for bacteria with slime production 0 to 10, 11 to 99, or more than 100 were 1.4×10^5, 6.8×10^4, or 2.1×10^3, respectively ($P < 0.0001$; ANOVA multiple comparisons) (Fig. 2, and Table 2).

Bacterial CVR was greater for bacteria resistant to complement killing compared with those killed by complement (1.2×10^5 vs. 5.5×10^4 [$P = 0.007$, not killed vs. killed, respectively; Student's *t* test]) (see Table 2). When the contribution from slime production was considered, however, there were no differences in bacterial CVR related to complement killing for bacteria with slime production greater than 10. Complement sensitivity only affected CVR in bacteria with low levels (0 to 10) of slime production ($P = 0.048$, killed vs. not killed; Student's *t* test) (see Table 2).

Bacterial CVR was lower for *Pseudomonas* species compared with the others (average 1.8×10^3 vs. 1.2×10^5 bacterial CFU/ml; $P < 0.0001$, ANOVA multiple comparisons). Differences in CVR among the other bacterial species were not significant. The differences seemed to be related to slime production because *Pseudomonas* species made more slime (average 154 vs. 18; $P < 0.0001$, ANOVA multiple comparisons). Within individual bacterial species (e.g., *E. coli*, *Enterobacter*, etc.), serum resistance and decreased slime production correlated with increased CVR.

TNF-α Production In Vivo

TNF-α production was influenced by the magnitude of bacterial CVR and even more so by bacterial sensitivity to complement killing. TNF-α production as a function of bacterial reflux followed a logarithmic plot ($R^2 = 0.75$) for bacteria killed by complement, whereas the function was linear ($R^2 = 0.60$) for bacteria resistant to complement killing (Fig. 3).

Clinical Correlation

Clinical data (i.e., the clinical manifestations of infection associated with the bacterial species in the patients) were available for all of the isolates tested in the rat. TNF-α production correlated with the severity of infectious manifestations. Average TNF-α production was 300, 550, or 1200 pg/ml for bacterial species causing no clinical infection, infectious manifestations, or sepsis, respectively ($P < 0.0001$ each compared to the other two, ANOVA multiple comparisons). How the level of TNF-α production was achieved was different for bacterial species killed or not killed by complement (Fig. 4). Less CVR was needed to induce the same amount of TNF-α production for bacterial species killed by complement. For example, at an average of 100 bacterial CFU/ml, bacteria killed by complement caused sepsis, whereas

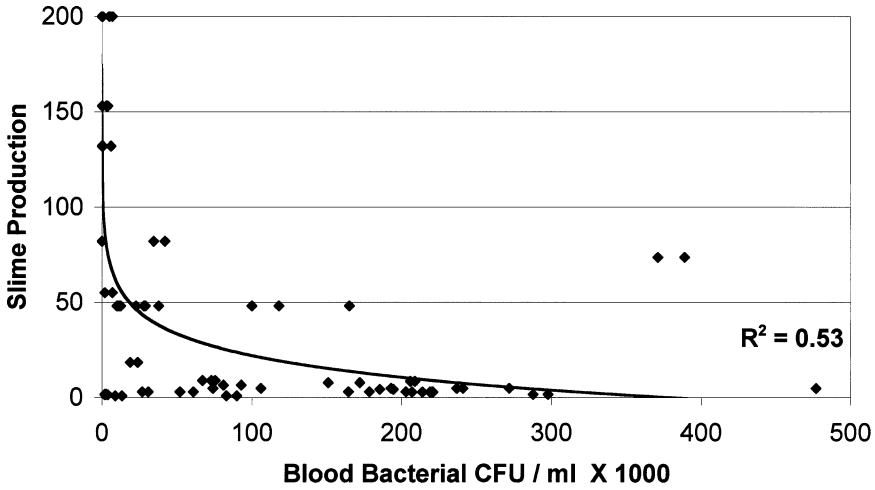

Fig. 1. Slime production correlated inversely with bacterial CVR in a logarithmic fashion ($R^2 = 0.53$). CVR was less for bacterial species that produced larger amounts of slime.

those not killed by complement caused no clinical infection. Infectious manifestations associated with serum-resistant bacteria were only evident at twice this level of CVR (200 bacterial CFU/ml), which is four times the amount (50 bacterial CFU/ml) of reflux needed for serum-sensitive bacteria to cause infectious manifestations.

DISCUSSION

The systemic manifestations of cholangitis result from bacterial reflux from the biliary tree into the systemic circulation (CVR) and the host's response to the invading organisms. This study examined these factors. CVR occurs when bacteria are in the biliary tree and the ductal pressure rises above a threshold of approximately 20 cm H_2O.[9,10]

We previously noted that biliary bacteria possess factors that facilitate biliary tract colonization, including P1-fimbriae and slime production.[3,4] P1-fimbriae and slime allow bacteria to adhere to surfaces. Once adherent, bacterial slime, an anionic glycoprotein, facilitates bacterial survival. The bacteria form microcolonies that are held together and coated by the glycocalyx. Bacterial glycocalyx protects bacteria against antibodies, phagocytosis, antibiotics, and surfactants, and acts as an ion-exchange resin for nutrient transport.[4,11] Slime also facilitates formation of infectious gallstones, and the latter acts as a bacterial reservoir.[1,4] Thus slime production is important for the persistence of bacteria in the biliary tract.

Although slime production facilitates biliary tract colonization, we found decreased CVR among bacteria that produced the greatest amounts of slime. The inhibitory effects of high slime production on CVR may actually be greater than was demonstrated in this study. Bacteria in a microcolony (e.g., as part of a pigment gallstone) must detach from the biofilm and enter bile before reflux into the systemic circulation can occur, and detachment may be inhibited by abundant slime. Even in a planktonic state, however, bacteria that produced more slime demonstrated less reflux, suggesting that bacterial adhesion characteristics influence CVR. CVR may follow an intracellular (transhepatocyte) or paracellular route (with disruption of tight junctions allowing reflux into the spaces of Mall and Disse).[7–9] Increased bacterial adhesion could influence reflux at any of these levels or even at the level of the biliary epithelium, which is known to secrete chemokines, cytokines, express cell adhesion molecules, and function as antigen-presenting cells.[12]

Recovery of bacteria in the blood (CVR) depends on bacterial reflux and bacterial clearance (e.g., complement killing, phagocytosis). Although it is difficult to separate the contribution from these factors, we used different bacterial species, with varying amounts of slime production and complement sensitivity, to elucidate this. One would expect decreased recovery in the blood of serum-sensitive bacteria, which was the case in our study. But there was a great deal of overlap; many serum-sensitive bacterial species showed greater CVR than serum-resistant bacteria. Slime production proved to be more important in determining CVR than complement-mediated killing. There were no differences in CVR related to complement killing among bacterial species that made abundant slime. Only bacterial species with low levels of slime production demonstrated decreased CVR related to complement sensitivity. The influence of slime production to CVR demon-

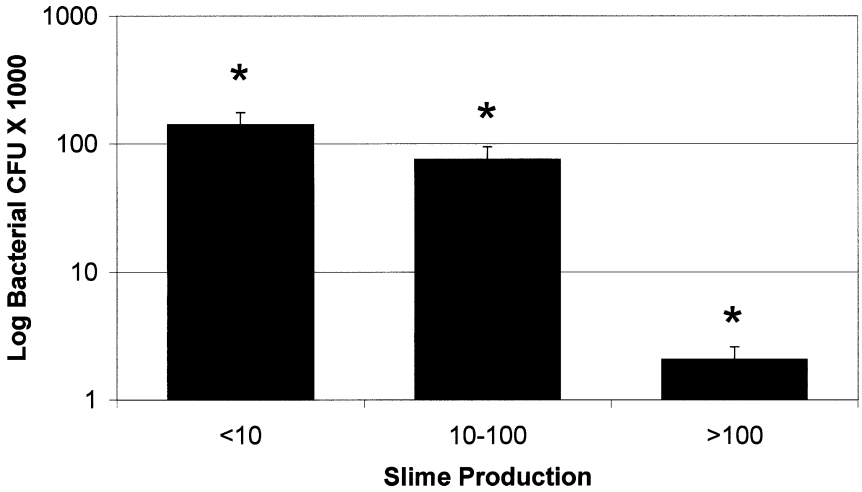

Fig. 2. Average bacterial CVR for bacterial species that produced slime: 0 to 10, 11 to 99, or more than 100. Bacterial CFU/ml for these groups was 1.4×10^5, 6.8×10^4, or 2.1×10^3, respectively ($P < 0.0001$, ANOVA multiple comparisons, Student–Newman-Kuels test). Error bars indicate standard errors of the mean.

strated in the current study (where CVR was measured at one time point after bacterial infusion) agreed with our previous study where CVR was measured at multiple time points during and after bacterial infusion.[13] In that study, *Pseudomonas* species (abundant slime producers) demonstrated decreased CVR compared to *E. coli* (moderate slime producers) at all time points except the start of infusion.[13] Most likely, bacterial slime inhibits bacterial reflux through hepatic pathways, whereas sensitivity to complement increases bacterial clearance. There may be additional factors (e.g., phagocytosis), which were not addressed in this study, that influence bacterial CVR. Although phagocytosis does not have a sizable impact on bacterial clearance at 5 minutes,[14] there could be species differences not yet demonstrated, or other factors influencing bacterial CVR.

Pyelonephritis has many features in common with cholangitis, including ascending infection and infectious stone formation. P1-fimbriae and hemolysin production have been shown to be important bacterial virulence factors associated with pyelonephritis.[15] We previously noted that biliary bacteria also possess P1-fimbriae,[3] but in the current study hemolysin production had no influence on CVR. Bacterial species causing pyelonephritis have also been shown to be serum resistant.[16] We found the opposite with biliary infections and noted that serum-sensitive bacterial strains were associated with more severe infections.[5] This suggests that complement activation and bacterial processing in the liver are important mediators of severity of biliary infections.

Numerous studies have demonstrated a link between complement activation and the development of sepsis. Elevated levels of C3a, C4a, and C5a correlate with the development of septic shock and death.[17–20] C5a induces macrophages, leukocytes, and platelets to produce TNF, interleukin (IL)-1 and IL-8.[21] Destruction of gram-negative bacteria by complement liberates lipopolysaccharide, which in turn induces the

Table 2. Influence of slime production and complement killing on bacterial cholangiovenous reflux*

		Complement-mediated bacterial killing	
Slime production	**Bacterial cfu/ml**	**Killed (bacterial cfu/ml)**	**Not killed (bacterial cfu/ml)**
0–10	$1.4 \times 10^{5\dagger}$	$1.1 \times 10^{5\ddagger}$	$1.6 \times 10^{5\ddagger}$
11–99	$6.8 \times 10^{4\dagger}$	4.0×10^4	1.4×10^5
>100	$2.1 \times 10^{3\dagger}$	1.9×10^3	2.1×10^3
All		$1.2 \times 10^{5\S}$	$5.5 \times 10^{4\S}$

*There were no differences in CVR of bacteria killed vs. not killed, for bacterial species with slime production of 11 to 99 or >100, (Student's *t* test).
†$P < 0.0001$, compared with the other two groups (ANOVA multiple comparisons, Student–Newman-Kuels test).
‡$P = 0.048$, slime production 0 to 10, killed vs. not killed (Student's *t* test).
§$P = 0.007$, all bacterial species, killed vs. not killed (Student's *t* test).

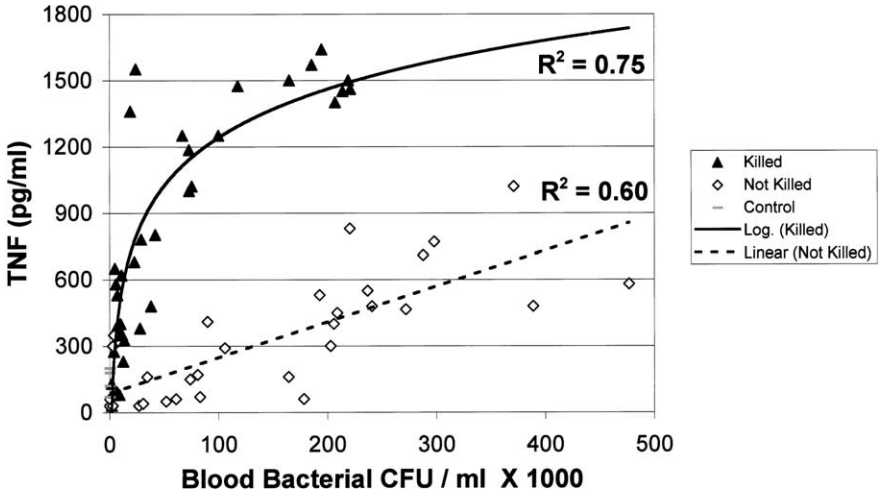

Fig. 3. TNF-α production as a function of bacterial cholangiovenous reflux and serum-sensitivity (bacterial sensitivity to complement killing). TNF-α production vs. bacterial reflux followed a logarithmic curve ($R^2 = 0.75$) for serum-sensitive bacteria, while the function was linear ($R^2 = 0.60$) for serum-resistant bacteria.

release of inflammatory mediators, including TNF, IL-6, platelet-activating factor, oxygen radicals, and nitric oxide. Platelet-activating factor, TNF, and lipopolysaccharide can activate the complement system.[22,23] Thus the inflammatory cascade interacts on many levels, with complement playing a central role. Cytokines are produced by monocytes/macrophages, lymphocytes, fibroblasts, endothelial cells, hepatocytes, and biliary epithelium. We chose to study TNF because it is known to be a key mediator of the exaggerated metabolic and vascular response of septic shock[21,24] and because Kupffer cells represent the largest population of monocytes in the body. Given this, elevated levels of the cytokines produced by monocytes would be expected to be associated with cholangitis, and plasma and bile concentrations of endotoxin, TNF, and IL-6 have been noted to be increased during acute cholangitis.[25,26]

TNF-α is thought to play a fundamental role in the pathogenesis of sepsis for the following reasons: TNF-α levels are increased during sepsis; high TNF-α concentrations are associated with nonsurvival; endotoxin and bacterial challenge cause TNF-α production; TNF-α challenge in humans or animals induces or simulates sepsis and organ failure; and TNF-α neutralization in experimental sepsis causes amelioration of sepsis and increased survival.[21,24,27–29] For these reasons, drugs inhibiting TNF-α were used in several clinical trials of human sepsis. Unfortunately, the results of these trials proved disappointing.[27–29] More recent studies have shown that the sepsis is bimodal with a first phase of proinflammatory mediators (TNF-α, IL-1, IL-6, IL-8, and complement) followed by a second phase of anti-inflammatory mediators (TGF-α, IL-10, and prostaglandin E_2).[27,29] Depending on whether the proinflammatory or anti-inflammatory phase is active, a systemic inflammatory response syndrome or a compensatory anti-inflammatory response syndrome may be present. Clearly TNF-α inhibition would not be useful for patients already in an anti-inflammatory state, and the studies that used TNF-α inhibitors used broad definitions of "sepsis" with no markers to determine the patient's inflammatory state.[27] Thus it is not surprising that the results were poor. Some clinical trials suggest that TNF-α inhibition shows a benefit in patients with evidence of a severe proinflammatory state.[27–29] Depressed HLA-DR levels, a marker for compensatory anti-inflammatory response syndrome, may prove useful in determining appropriate treatment.[29] The liver is thought to play a central role in the initiation of multisystem organ failure in sepsis, but the cytokine response is broad and complex, which may explain why the blockage of only one cytokine seems to be of limited value.[27–31] Despite this, TNF-α is still thought to be an important mediator of sepsis.[27–30]

The findings in this study demonstrate a possible link between complement-mediated bacterial killing and severe biliary infections: increased TNF-α production associated with serum-sensitive bacteria. The study further elucidated the multifaceted nature of biliary bacterial virulence and interactions between virulence factors. Bacterial CVR, per se, or sensitivity to complement-mediated killing did not determine bacterial virulence. A better predictor was the level of TNF-α production. Nevertheless, CVR and serum sensitivity both influenced TNF-α pro-

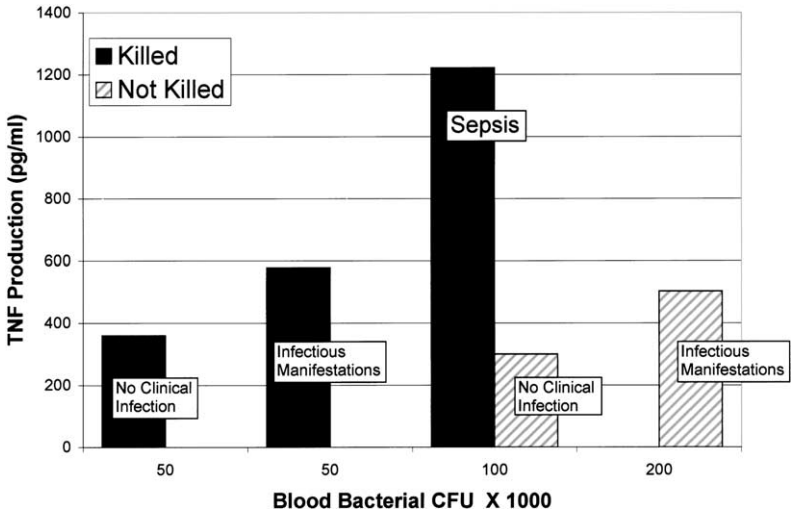

Fig. 4. Average bacterial cholangiovenous reflux and TNF-α production for serum-resistant and serum-sensitive bacterial species that were associated with (1) no clinical infection, (2) infectious manifestations, or (3) sepsis.

duction. Bacteria from patients with no clinical infection induced low levels of TNF-α production coupled with decreased levels of CVR (within serum-sensitive and serum-resistant groups). Bacteria associated with infectious manifestations (but not sepsis) induced intermediate amounts of TNF-α production and were either serum sensitive, with lesser amounts of CVR, or serum resistant, with an increased ability to reflux into the systemic circulation. The most virulent bacteria (from patients with sepsis) were killed by complement but had significant CVR, despite this serum sensitivity, and were associated with increased TNF-α production. Whether the mechanism of increased TNF-α production was related to complement activation or liberation of lipopolysaccharide, or both, will require further study. What did correlate was the presence of high levels of CVR and complement-mediated bacterial killing in bacterial strains causing sepsis. Interestingly, TNF-α production may itself augment CVR, for it increases the permeability of biliary epithelial tight junctions, which would disturb the blood–bile duct barrier.[32]

CONCLUSION

These data explain why some bacterial species colonize the biliary tree but do not cause severe illness, whereas others result in septic manifestations. Slime production is crucial for colonization, but too much can inhibit bacterial CVR. Although complement killing clears bacteria from the circulation, it is also associated with increased TNF-α production, which can lead to septic manifestations. The most virulent bacteria (obtained from patients with sepsis) were killed by complement but still had significant CVR and were associated with increased TNF-α production.

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Discussion

Dr. H.A. Pitt (Milwaukee, WI): As you know, now that we have more patients with indwelling biliary stents, we are seeing more *Klebsiella*, more *Pseudomonas*, and less *E. coli* in patients with cholangitis. Would you put some of your observations into perspective with respect to the bacteria that we see, the *Klebsiella*, the *Pseudomonas*, and *Enterococcus*, for example?

Dr. L. Stewart: Enterococcus is gram-positive, which in these studies induced minimal TNF-α production, and so has to be considered separately. *Pseudomonas* is actually very interesting. *Pseudomonas* species produce large amounts of slime and, because of this, in the animal model they demonstrated the lowest amount of CVR (compared to the other bacterial species), and therefore were associated with a decreased amount of TNF-α production. But, in an in vitro model (using cultured monocytes [published previously]), pound for pound they made more TNF-α than other bacterial species. However, because TNF-α production followed CVR in vivo, *Pseudomonas* species, in fact, were associated with a decreased amount of TNF-α production. Regarding *Klebsiella* species, we noted that they were not as virulent as *E. coli*. *E. coli*, in our studies, was found to be the most virulent bacterial species.

Dr. F.G. Moody (Houston, TX): This is a very interesting study. However, I am not sure exactly what the slime factor is. Also, what is the role of the slime in the intrabiliary pressure? I guess you control for that in your analysis. When we talk about TNF-α, have you selected that particular cytokine specifically because so much comes out of the liver?

Dr. Stewart: I will answer the last question first. You are correct; we did choose TNF-α because it has been demonstrated to be associated with sepsis. Also, the Kupffer cell population, which is the largest monocyte population in the body, is located next to the biliary tree and is a producer of TNF-α. So we thought it was a very good cytokine to study. Now, with regard to slime, we have talked about it previously at this meeting. Slime is a glycoprotein made by

certain bacteria that coats them and allows them to stick to surfaces. It also protects them from antibodies, surfactants, and so on. Therefore slime is important for bacterial colonization. But too much slime can also alter bacterial characteristics important for reflux. More slime seems to make the bacteria stickier, leading to decreased CVR. It is important to note that all of these bacterial species were infused at exactly the same intrabiliary pressure. We characterized this experimental model in previous studies. Dr. S. Raper did some of the earlier work on this. So we knew how to achieve CVR and set up the experiment with pressures that achieved bacterial reflux. But there were differences in the stickiness of the bacterial species, which led to variations in CVR. So, in these experiments, bacterial factors determined the magnitude of CVR (because intrabiliary pressure was held constant).

Invited Discussion—Expert Commentator

David W. Rattner, M.D.: Dr. Stewart and her co-workers have presented an elegant study that further elucidates those factors responsible for bacterial sepsis of biliary origin. Surgeons have been perplexed for many years as to why some patients tolerate bacterial colonization of their biliary tract better than others. In this study, Dr. Stewart has isolated bacteria from human bile and gallstones, and demonstrated that bacterial virulence is ultimately mediated by TNF-α production and release. The magnitude of this surge in TNF production is determined by the innate properties of the colonizing bacteria. Somewhat paradoxically, those bacteria that can be killed by complement are, in fact, the most virulent and likely to reflux from the biliary tree into the venous system. Bacteria that produce slime seem less likely to find their way into the venous system.

With the widespread use of biliary endoprostheses, the mechanisms involved in the development of cholangitis are highly relevant for gastrointestinal surgeons. Dr. Stewart's work may ultimately lead to the design and incorporation of novel stent properties that select more favorable bacteria for colonization. When stents occlude, which at this point in time is an inevitable part of their natural history, the selection of less virulent bacteria as colonizers might diminish the frequency of cholangitis in the interval between stent changes.

Liver Injury During Acute Pancreatitis: The Role of Pancreatitis-Associated Ascitic Fluid (PAAF), p38-MAPK, and Caspase-3 in Inducing Hepatocyte Apoptosis

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We have demonstrated that pancreatitis-associated ascitic fluid contributes to hepatocyte injury during acute pancreatitis; a phenomenon independent of ascites' enzymatic content and Kupffer cell-derived cytokines. Our aim is to characterize the mechanisms of pancreatitis-associated ascitic fluid induced hepatocyte death. NIH mice were injected intraperitoneally with pathogen-free pancreatitis-associated ascitic fluid. Twenty-four hours later, serum AST, ALT, LDH, and hepatocyte apoptosis (TUNEL) were measured. Human hepatocytes (CCL-13) were treated with pancreatitis-associated ascitic fluid ±SB203580 or caspase-3 inhibitor-II. Mitochondrial membrane integrity was determined by DiOC6 staining. Apoptosis was measured by TUNEL staining and flow cytometry after dual labeling with Annexin-V/7-AAD. Data are mean ± SEM of triplicates. Pancreatitis-associated ascitic fluid increased serum AST, ALT, LDH, and apoptotic cells in the mouse liver (all $P < 0.03$ vs. sham). In CCL-13 cells, pancreatitis-associated ascitic fluid induced a time and dose-dependent increase in apoptosis, in addition to p38-MAPK phosphorylation ($P = 0.02$ vs. control), caspase-3 cleavage ($P < 0.03$ vs. control) and decreased DiOC6 mitochondrial staining ($P < 0.01$ vs. control). Both caspase-3 inhibitor-II and SB203580 decreased apoptosis, but the former had no effect on DiOC6 staining. Pancreatitis-associated ascitic fluid induces liver injury and hepatocyte apoptosis by activating p38-MAPK and caspase-3 dependent pro-apoptotic pathways. (J GASTROINTEST SURG 2003;7:200–208.)

KEY WORDS: Pancreatic ascites, hepatocyte apoptosis, caspase-3, p38-MAPK, mitochondrial injury

Acute pancreatitis is a multi-system disease with alternations not only in the pancreas, but also in the liver, lungs, and kidneys, which may lead to distant organ dysfunction and death.[1,2] Although the incidence of liver dysfunction during acute pancreatitis is relatively lower than adult respiratory distress syndrome or renal failure, clinical studies demonstrate a dismal prognosis when liver failure develops in the setting of severe acute pancreatitis.[3] Evidence is accumulating that pancreatitis-related liver injury involves not only necrosis but also programmed cell death.[4,5]

Recently, a number of studies have suggested that pancreatitis-associated ascitic fluid (PAAF) plays a critical role in inducing hepatocyte injury by inducing hepatocyte apoptosis.[4–7] We found that PAAF induces a dramatic increase in cell death/apoptosis in both rat and human hepatocytes, independent of cytokine production and pancreatic enzyme content in the ascitic fluid.[4] In other studies, we showed that p38 mitogen-activated protein kinase (p38-MAPK) plays a central role in inducing liver and pulmonary injury during acute pancreatitis.[8–10] These data strongly suggest the

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possibility that PAAF mediates liver injury through second messenger systems, which are known to activate a wide range of pro-apoptotic pathways. Therefore, we undertook this current study to characterize the mechanisms of PAAF-induced hepatocyte death during acute pancreatitis.

MATERIALS AND METHODS

Animal Care was in accordance with the guidelines of the Department of Laboratory Animal Medicine at the University of South Florida, a facility accredited by Association for Assessment and Accreditation of Laboratory Animal Care.

Production of Pancreatitis-Associated Ascitic Fluid

Severe acute pancreatitis was induced in adult male Sprague-Dawley rats (350–450 g) by retrograde infusion of 4% sodium taurocholate into the pancreatic duct as previously described.[10] Briefly, rats were anesthetized and a #10 polyethylene catheter was inserted into the common bile duct through the duodenal wall. The proximal bile duct was occluded with a noncrushing clamp to prevent reflux of bile acid into the liver. Four percent sodium taurocholate (1.0 μl/g rat) was infused at 20 μl/min and at a constant pressure of < 20 cm H_2O. Eighteen hours later, the animals were sacrificed and ascitic fluid was harvested under sterile conditions. The collected ascites was centrifuged to remove cellular debris, pooled, and stored at −80C until used. This model of pancreatic ascites has been well established for many years, and ascitic fluid collected at the specified time contains negligible amounts of endotoxin and cytokines.[4,10] Sterility was determined by culturing ascitic fluid in Luria-Bertani broth for 48 hours. All contaminated ascites was discarded and subsequent experiments were carried out with endotoxin-free, sterile ascites.

In Vivo Liver Injury

NIH mice (n = 5/group) were injected with PAAF (50 μl/g mouse) intraperitoneally, q4 hour, × 6 injections. Sham mice were injected with saline containing 10% normal rat serum. Twenty-four hours later, the animals were sacrificed and their serum was sampled and stored at −80C. Serum levels of AST, ALT, and LDH were determined using Kodak Ektachem 700 automated analyzer (Kodak, Rochester, NY). The right lobe of the liver was harvested and fixed in 10% formalin buffer for serum AST, ALT, LDH, and hepatocyte apoptosis (TUNEL) staining.

TUNEL Staining of Liver for Apoptosis

Fixed liver tissue was embedded in paraffin, sectioned, and stained with Apop Tag In Situ Apoptosis Detection Kit 7100 (Intergen Company, Purchase, NY) following the manufacturer's instructions. The slides were blinded and examined using Confocal fluorescent microscopy, (490-nm excitation and 520-nm emission) in 10 random sections (40× hpf) per liver.

In Vitro Hepatocyte Death

A human hepatocyte cell line (CCL-13, ATCC Rockville, MD) was grown in Dulbecco's modified Eagle medium (DMEM) (Atlanta Biologicals, Norcross, GA) supplemented with 200 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% fetal bovine serum (GIBCO BRL). CCL-13 cells were seeded in gelatin (1%, Sigma Chemical Co., St Louis, MO) pre-coated 6 well plates at a concentration of 5×10^5/well, and cultured for 24 hours prior to any treatment. CCL-13 cell cultures were then treated with increasing doses of pathogen-free PAAF (6.5–50%) for increasing periods (0.5–6 hours). In addition, CCL-13 cells were pretreated with p38-MAPK inhibitor (SB203580 10 μM, Calbiochem, La Jolla, CA), caspase-3 inhibitor-II (50 μM, Calbiochem, La Jolla, CA), or vehicle (DMSO, Sigma Chemical Co., St. Louis, MO) 2 hours prior to PAAF treatment (20% v/v for 3 hours) for the purpose of measuring PAAF-related apoptosis as described thereafter.

Annexin-V/7-AAD Staining of CCL-13 Cells

Externalization of phosphatidylserine in the cell membrane is an indicator of early apoptosis, which can be quantified by detecting its binding to Annexin-V by flow cytometry.[11] Apoptosis in CCL-13 cells was measured by multiparameter flow cytometry after dual labeling with Annexin-V-FITC (5 μl, Clontech Lab Inc., Palo Alto, CA) and 7-AAD (10 μl, 7-amino-actinomycin D, Calbiochem-Novabiochem, San Diego, CA) following the manufacturer's instructions. The peak emission of 7-AAD is approximately 685 nm; a FL3 photomultiplier tube with a 670 nm-long pass filter was used for measurement.

DiOC-6 Staining of CCL-13 Cells

Reduction in mitochondrial transmembrane potential (MTP) is one of the early events of cell death/apoptosis. Changes in MTP can be measured by flow cytometry techniques that quantify alterations in 3, 3′-dihexyloxacerbocyamine (DiOC-6) uptake.[11,12] CCL-13 cells were pretreated with SB203580 (10 μM),

caspase-3 inhibitor-II (50 μM) or vehicle control (DMSO) 2 hours prior to treatment with PAAF (20% v/v 3 hours). Membrane potential sensitive dye DiOC-6 (0.5 μM, Molecular Probes, Eugene, OR) was added to the medium 30 minutes before concluding the 3-hour treatment phase with PAAF. The reduction of DiOC-6 uptake was determined by flow cytometry.

TUNEL Staining of CCL-13 Cells

A monolayer of CCL-13 cells was seeded in 4 well tissue culture chamber slides (Lab-Tek Chamber Slide system, Inter Med Nunc, Napervill, IL) for 48 hours to allow adherence, and then incubated with 20% PAAF for 24 hours. Another set of CCL-13 cells were treated with PAAF (20% v/v, 3 hours) ± pretreatment for 2 hours with SB203580 (10 μM) or caspase-3 inhibitor-II (50 μM) or vehicle control (DMSO). Apoptosis was detected using ApopTag In Situ Apoptosis Detection Kit 7100 (Intergen Company, Purchase, NY).

Phosphorylated p38-MAPK and Caspase-3 Activation in CCL-13 Cells

CCL-13 cells were seeded in 60 ml tissue culture dish 24 hours before any treatment (10^7 cells/well). CCL-13 cells were incubated with SB203580 (10 μM), caspase-3 inhibitor-II (50 μM), or vehicle 2 hours prior to the treatment with PAAF (20% v/v 45 minutes). Phosphorylated p38-MAPK and caspase-3 activity were determined by immunoblotting. Briefly, protein extracts from CCL-13 cells were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis and electro-transferred to a nitrocellulose membrane. Nonspecific binding was blocked, and the membrane was then immunoblotted overnight at 4C with 1:3000 dilution of rabbit polyclonal phospho-specific p38-MAPK (Cell Signaling Technology, Beverly, MA) or caspase-3 antibodies (Dr. Honggang Wang). Subsequently, the membrane was washed, and incubated with horseradish peroxidase-conjugated anti-rabbit antibody for 2 hours at room temperature. The immunoblot was washed and the bands were detected with an enhanced chemiluminessence kit (LumiGlo; New England Biolabs). Phosphorylated p38-MAPK, caspase-3, and its cleavage subunit were quantified by densitometry (UVP gel documentation system, Upland, CA.).

Caspase-3 Activity in CCL-13 Cells

A monolayer of CCL-13 cells was seeded in 4 well tissue culture chamber for 48 hours to allow adherence, and then incubated with 20% PAAF for 3 hours ± pretreatment with caspase-3 inhibitor-II (50 μM, 2 hours) or vehicle control (DMSO). Caspase activity in these cells was detected using CaspaTag Caspase-3 (DEVD) Activity Kit S7301-100 (Intergen Company, Purchase, NY) following the manufacturer's protocol. The slides were blinded and examined using fluorescent microscopy (Confocal fluorescent microscope, Germany, 490-nm excitation and 520-nm emission) in 10 random sections (40× hpf) per sample.

Statistical Analysis

All experiments were repeated in triplicates. Data are mean ± standard error of the mean (SEM). Means were compared using Student's *t* test. Significance was at $P < 0.05$.

RESULTS

In Vivo Liver Injury: (Parenchymal Enzymes and Apoptosis)

PAAF increased mouse serum levels of AST, ALT, and LDH (183 ± 6 vs. 81 ± 7; 214 ± 6 vs. 36 ± 0.7; 5234 ± 196 vs. 1205 ± 37, all $P < 0.001$ vs. sham, respectively). PAAF increased the number of cells that stained positive by TUNEL. As shown in Figure 1, most of the positively stained cells in normal livers are non-parenchymal; while in mice treated with PAAF, positively stained cells were hepatocytes (36 ± 5 vs. 18 ± 2/hpf, $P < 0.03$ vs. sham, TUNEL).

In Vitro Hepatocyte Death: (Apoptosis)

PAAF induced a time and dose dependent increase in Annexin-V/7-AAD dual-labeled CCL-13 cells (flow cytometry), which peaked at 3 hours (control: 28.5 ±

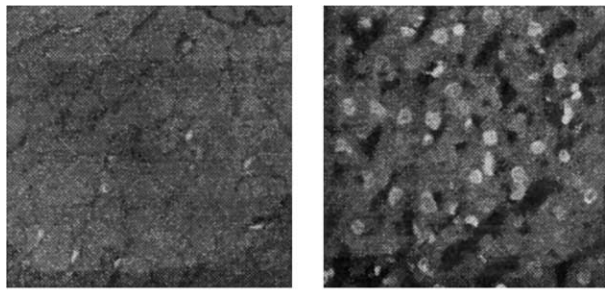

Fig. 1. PAAF increased the number of apoptotic cells in mouse liver tissue (36±5 vs. 18±2/hpf, $P < 0.03$ vs. sham, 63×). Confocal microscopy makes it easy to distinguish apoptotic cells (bright area) in a dark background. In sham mice, positive staining cells are largely non-parenchymal cells.

0.2%; 30 min: 30.2 ± 0.8%; 60 min: 35.1 ± 2%; 180 min: 37.2 ± 3%; 360 min: 60.2 ± 0.8%; $P < 0.01$ vs. control), and 25% v/v ($P < 0.01$ vs. control, Figure 2). Annexin-V staining indicates apoptosis, however some dead cells will stain with Annexin-V and with 7-ADD. Although the percentage of dually labeled cells reached 60.2% of total hepatocyte population at the 6-hour treatment time point, the number of cells that stained with Annexin-V only is 50% less than the positively stained cells in at the 3-hour time point, therefore indicating a larger portion of cells that may be necrotic. Therefore we concluded that 3 hours is the optimal treatment time.

Apoptosis was then confirmed by TUNEL staining; PAAF (20% v/v) increased the number of apoptotic cells from 10.4 ± 1.1 to 20.5 ± 0.9/hpf at 3 hours ($P = 0.001$ vs. control).

p38-MAPK and Caspase-3 Activity in CCL-13 Cells

PAAF (20% v/v, 45 min) increased phosphorylated p38-MAPK (744 ± 46 vs. 436 ± 40; $P = 0.02$ vs. control, Western blot) and caspase-3 cleavage (88 ± 6 vs. 62 ± 2, $P = 0.03$ vs. control, Western blot). SB203580 and caspase-3 inhibitor-II attenuated the increase in p38-MAPK phosphorylation and caspase-3 activation, respectively (480.7 ± 23.2, $P = 0.02$, and 60.7 ± 1.8, $P = 0.03$, Figure 3*A*, *B*). PAAF-induced caspase-3 activation was confirmed by immunofluorescent staining of caspase-3 in CCL-13 cells (49 ± 2.4 vs. 10.7 ± 1.2/hpf, $P = 0.01$ vs. control, Figure 4). Moreover, caspase-3 inhibitor-II decreased this PAAF-induced increase in caspase-3 positive staining in CCL-13 cells from 49 ± 2.4 to 23.5 ± 2.3 ($P = 0.01$ vs. PAAF). Pretreatment with SB203580 (10 μM) or caspase-3 inhibitor-II (50 μM) attenuated the PAAF-induced increase in apoptosis in CCL-13 cells (11.5 ± 2.1/hpf, and 9.75 ± 1.4/hpf, vs. 20.5 ± 0.9/hpf, < 0.002 vs. PAAF, respectively; TUNEL; Figure 5).

Mitochondrial DiOC6 Staining in CCL-13 Cells

PAAF (20% v/v, 3 hours) significantly decreased mitochondrial DiOC6 staining (62 ± 1% vs. 81 ± 1%, $P = 0.001$, PAAF vs. control, Figure 6) indicat-

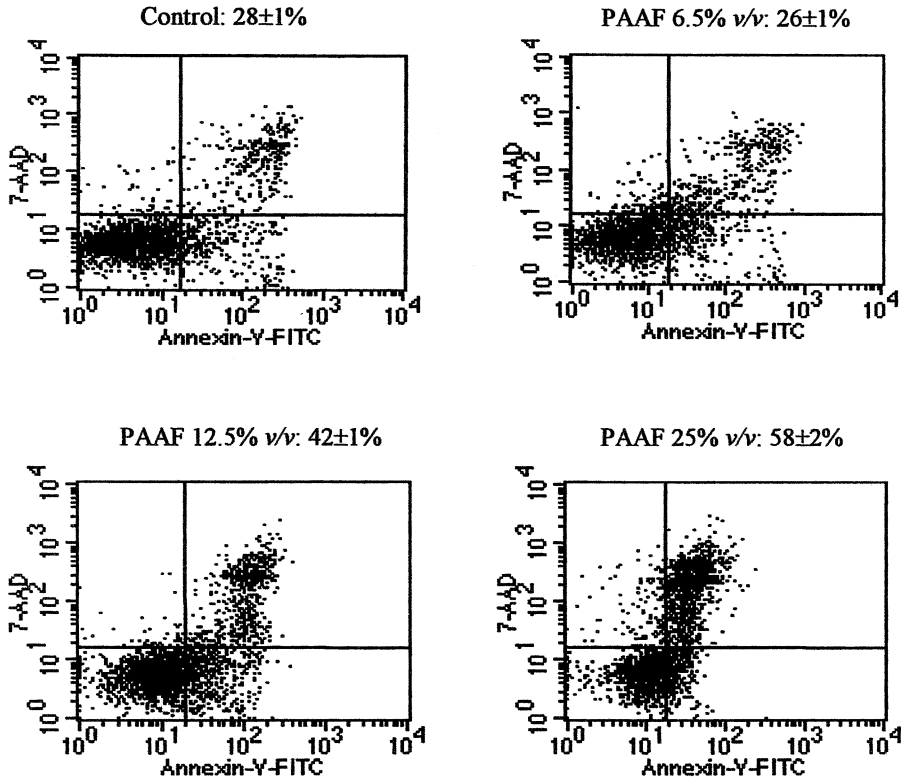

Fig. 2. PAAF induced apoptosis in human hepatocytes (CCL-13) stained with Annexin-V/7AAD in a dose-dependent manner. Apoptotic cells are Annexin-V positive and will stain with 7-AAD at a late stage of cell death. Apoptosis was maximal at PAAF 25% v/v, which is illustrated by the higher percentage of cells that shifted from Annexin-V positive (*right lower quadrant*) to dual staining with 7-AAD (*right upper quadrant*).

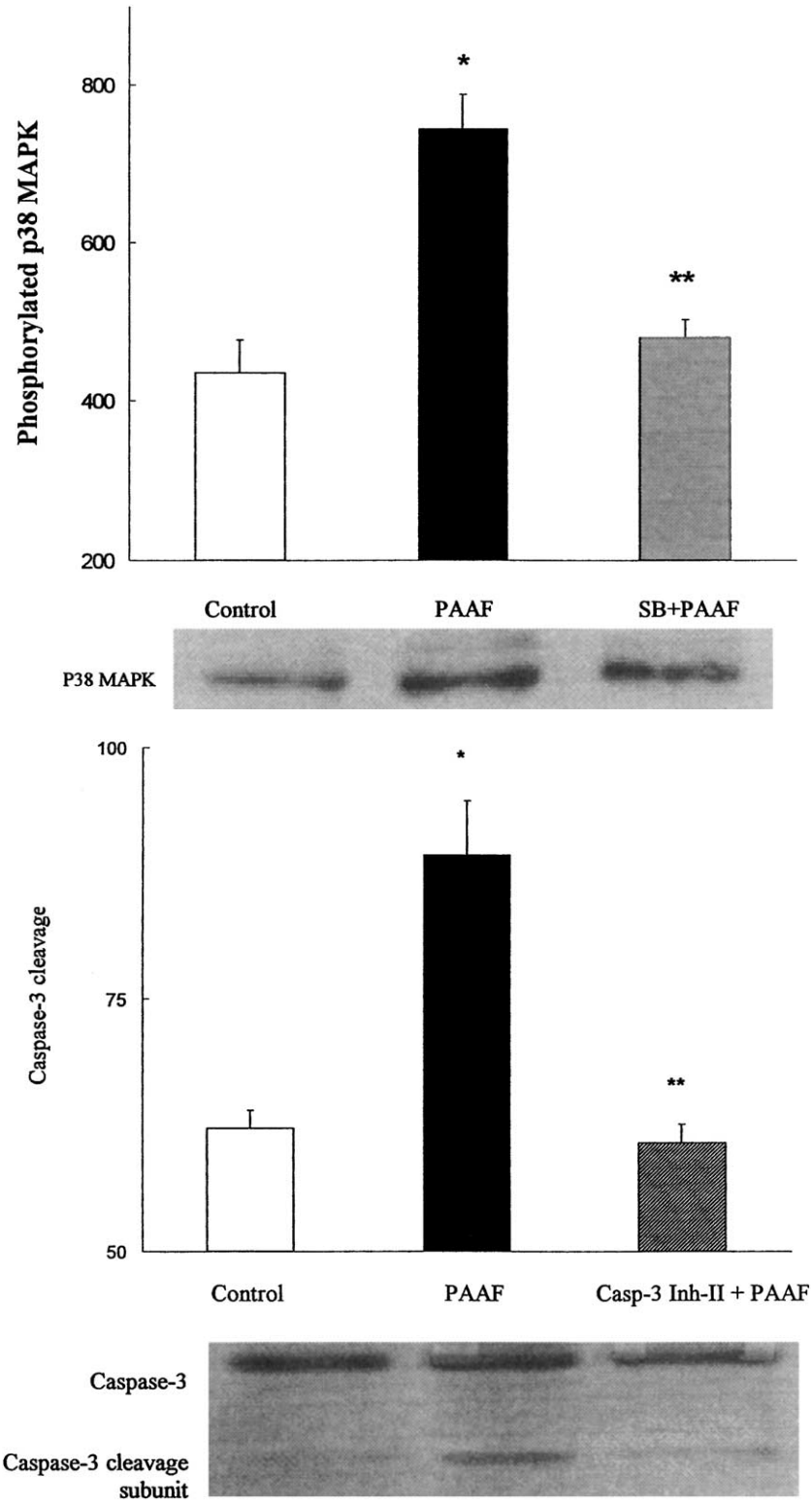

Fig. 3. A, SB203580 significantly attenuated the PAAF-induced phosphorylation of p38-MAPK in CCL-13 cells ($P < 0.02$ vs. control, Western blot, quantified by densitometry). **B,** PAAF induced an increase in activated caspase-3 and its cleaved subunits. Caspase-3 inhibitor-II significantly attenuated the PAAF-induced caspase-3 cleavage in CCL-13 cells ($P < 0.03$ vs. control, Western blot, quantified by densitometry).

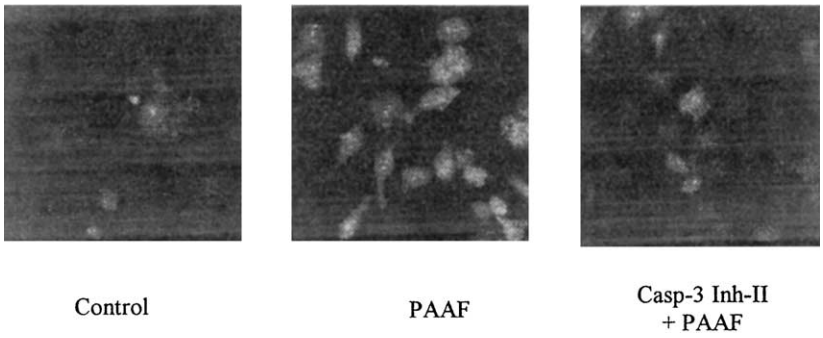

Fig. 4. Caspa Tag fluorescent staining (40× hpf): 20% PAAF induced activated caspase-3 in CCL-13 cells (bright area) as compared to control group ($P < 0.001$), thereby confirming findings in Figure 3*B*. Caspase-3 inhibitor-II attenuated PAAF-induced activation of caspase-3.

ing a reduction in mitochondrial transmembrane potential indicating mitochondrial injury. Caspase-3 inhibitor-II did not attenuate the PAAF-induced decrease in DiOC6 staining in CCL-13 cells.

DISCUSSION

Apoptosis, or programmed cell death, is an evolutionarily conserved, energy-dependent mode of cell death that requires the initiation and regulation of

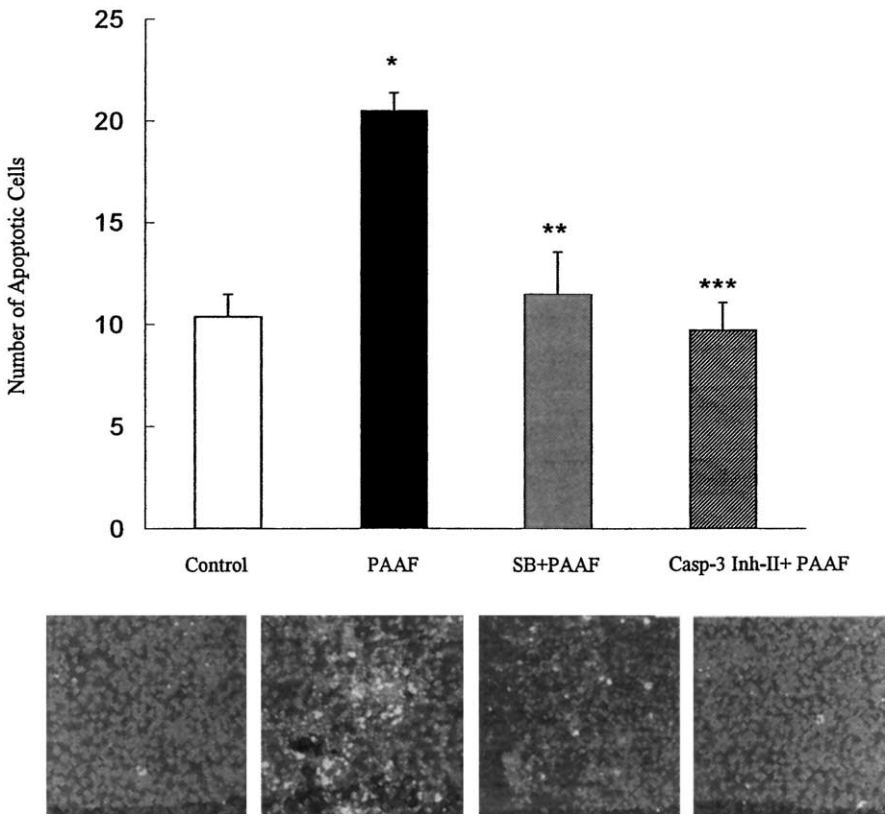

Fig. 5. Bar graph: PAAF significantly increased apoptosis in CCL-13 cells (*$P < 0.001$ vs. control). SB203580 or caspase-3 inhibitor-II significantly decreased this PAAF-induced apoptosis (**, ***$P < 0.02$ vs. PAAF). Photomicrographs: Representative fields of CCL-13 cells treated with PAAF ± SB203580 or caspase-3 inhibitor-II stained by TUNEL and examined with a confocal fluorescent microscope (Original magnification, 40×).

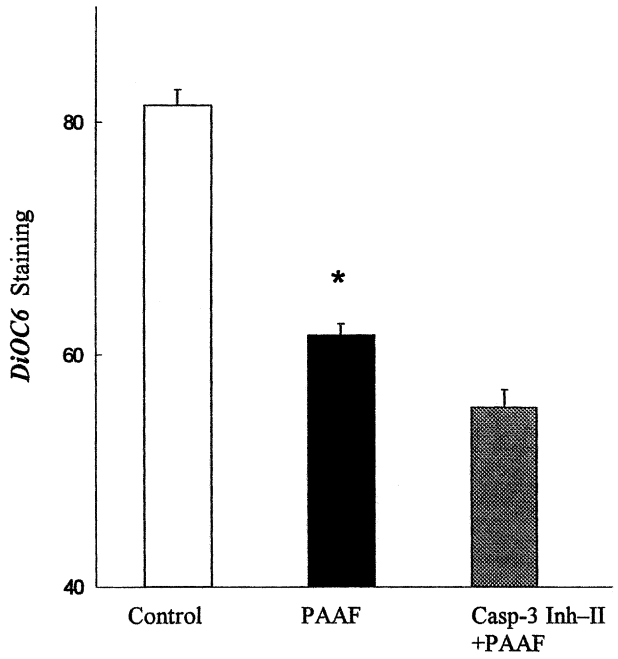

Fig. 6. PAAF induced CCL-13 cell mitochondrial membrane damage and therefore decreased DiOC6 positive staining (flow cytometry, $P = 0.001$ vs. control). Caspase-3 inhibitor-II had no protective effect and did not reverse the loss of mitochondrial transmembrane potential.

complex genetic programs and pathways. In addition to its beneficial effects in keeping a balance between cell regeneration and death, apoptosis can become pathogenic. We, as well as other investigators, have demonstrated that apoptosis was induced in pancreatic acinar cells during experimental pancreatitis through activating the TNF α (p55) receptor.[13,14] Several others have demonstrated that TNF α, which is abundant during pancreatitis, induces hepatocyte apoptosis by binding to death receptors that subsequently initiate pro-apoptotic pathways.[15,16] Blocking pro-inflammatory cytokines has been shown to be protective against the systemic manifestations of acute pancreatitis,[10,17,18] thereby suggesting that pancreatitis-associated end organ dysfunction may be mediated by activation of apoptotic pathways.

We have been interested in pancreatitis-associated liver injury and have demonstrated that Kupffer cell-derived cytokines play an important role in liver injury during acute pancreatitis.[17] Yet, cytokine antagonism did not abolish liver injury. Subsequently, we found that certain heat-stable, non-cytokine, non-protease mediators in PAAF have direct toxic effects on hepatocytes.[4] Therefore, we undertook this study to further characterize the mechanisms of PAAF-induced hepatocyte death.

Pathogen-free and cytokine-free PAAF induced biochemical liver injury in mice, which is indistinguishable from that seen during acute pancreatitis,[4] and increased apoptotic cells in the liver. The magnitude of parenchymal enzyme release however, suggests that hepatocyte necrosis may also be operative.

We utilized a human liver cell line (CCL-13) to further elucidate the mechanism of PAAF-induced hepatocyte apoptosis. PAAF increased Annexin-V/7-AAD dually labeled CCL-13 cells and decreased DiOC6 staining thereby confirming apoptotic cell death. We noticed that CCL-13 cells treated with 20% PAAF for 3 hours yields more Annexin-V positive cells than that at any other time point, specifically more than cells treated for 6 hours. This suggests that apoptosis may be a relatively early event during acute pancreatitis, and hepatocyte necrosis is more likely to occur as late sequelae as others have reported.[19] It follows that for the purpose of the subsequent investigation, we considered treatment with PAAF up to 3 hours optimal to study apoptosis in the CCL-13 cell line.

p38-MAPK, a stress-activated serine/theonine protein kinase that belongs to the MAP kinase superfamily, is reported to play a major role in apoptosis, cytokine production, and transcriptional regulation.[20] p38-MAPK is expressed ubiquitously, with high levels in the liver, spleen, thyroid, placenta, bone marrow, and leukocytes.[21] We previously showed that p38-MAPK inhibition by the macrophage pacifying compound tetravalent guanylhydrazone, CNI-1493, significantly decreased the severity of acute pancreatitis and pancreatitis-associated distant organ dysfunction in experimental animals.[8,9,22] This led us to investigate the potential role that p38-MAPK may play in pancreatitis-induced hepatocyte apoptosis. Our data shows that p38-MAPK was increased in PAAF-treated CCL-13 cells, and SB203580 attenuated p38-MAPK phosphorylation, and significantly decreased the number of apoptotic cells.

Mitochondrial dysfunction, particularly the induction of the mitochondrial membrane permeability transition, has been implicated in the cascade of events involved in apoptosis.[23] In the current study, PAAF induced a significant reduction in DiOC6 staining in CCL-13 cells indicating mitochondrial membrane damage. Moreover, downstream events of cellular apoptosis include p38 MAPK-dependent release of mitochondrial cytochrome C, formation of APAF-1/caspase-9 complex (apoptosome), and activation of effecter caspases, such as caspase-3.[23]

Caspases, which are present in all cells as latent enzymes, are cleaved into subunits at a specific internal aspartate by autoproteolysis or by other caspases. In addition, caspase-3 plays a central role in inducing

apoptosis in mouse hepatocytes.[24] In this current study, caspase-3 was activated in PAAF-treated human hepatocytes; caspase-3 inhibition showed a dramatic protective effect, by attenuating this PAAF-induced apoptosis. However, caspase-3 inhibitor-II showed no favorable effect in attenuating PAAF-induced collapse of mitochondrial transmembrane potential in CCL-13 cells.

These data indicate that PAAF induces hepatocyte apoptosis through mitochondrial and p38 MAPK-dependent pro-apoptotic pathways, with subsequent activation of caspase-3. The lack of effect on DiOC6 staining by caspase-3 inhibitor-II suggests that PAAF-induced mitochondrial dysfunction is an upstream event prior to caspase-3 activation, and warrants further investigation. The strategy in protecting mitochondrial function and blocking p38 MAPK phosphorylation at an early time point may be used to attenuate hepatocyte apoptosis in experimental models of acute pancreatitis.

The authors thank Dr. Hongang Wang for his generosity in supplying the caspase-3 antibody.

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Discussion

Dr. A.S. Gukovskaya (Los Angeles, CA): I have two questions. First, I wonder if you tried to identify what is the inducer of apoptosis in this fluid? And the second question, does this fluid produce the same effect on the pancreas as it produces on the liver?

Dr. Murr: Well, these are very, very interesting questions. I want to start by thanking Dr. Kirkwood for allowing me to close the discussion. This is Dr. Yang's work and he deserves all the credit, and I want to acknowledge the SSAT for its support of this work in the form of my career development award. Now, back to your question. It has been an enigma to what is in the ascites that causes the injury to the lung, liver, or other organs. Other students of the pancreas, who have done this for many, many years, have not been able to isolate one single factor that is responsible for such injury. I think the best way to handle this is to identify one measurable event, and a good tool to measure that event, and then isolate whatever is in the ascites with repeated measurements of that event using that tool. I think one way to do this is hepatocyte apoptosis. I don't have any data, and I don't think anybody has any data, on the effect of the ascitic fluid on the pancreas.

Dr. E.W. Denham III (Chicago, IL): Michel, that was a nice paper, and Dr. Yang did a nice job presenting. I just want to echo a little bit the last question that was asked. It was commented that there were minimal levels of cytokines and endotoxin in the ascitic fluid. Are these completely free of cytokines and endotoxin, and how have you measured those, because those things could be responsible for this injury you see? The other question I have is the specificity of the inhibitors that have been used, and you had a nice demonstration during inhibition there is decreased apoptosis. Are there other pathways that could be involved and have you worked on or done anything else to maybe overexpress p38 MAP kinase or other ways to get at this mechanism to insure that these are the pathways?

Dr. Murr: Thanks, that is a very insightful question. Regarding the question whether the enzymes in the ascitic fluid are responsible for that or not, there may be a small amount of pancreatic enzymes in the ascitic fluid. However, in other experiments that we have presented to the AAS earlier this year, where we heated the pancreatic ascites, or boiled it in order to denature all the protein, we did not see any measurable effect or attenuation of apoptosis in liver cells. We repeated these experiments using antiproteases, which should block all the proteases such as elastase and amylase, and we did not see any difference in hepatocyte apoptosis. So indirectly, there may be some enzyme in the fluid, but I don't think they play any major role. Regarding the specificity of the inhibitors, I think SB203580 is specific for p38 MAP kinase. It is not specific for the other MAP kinases. Now, in putting this all together, I think something with ascitic fluid induces the p38 and causes mitochondrial injury downstream and the upregulation or activation of the effector caspases such as caspase 3.

Invited Discussion—Expert Commentator

Dr. L.W. Traverso: Essentially this paper says that pancreatitis caused by taurocholate injection into the pancreatic duct leads to the release of proapoptotic substances in the peripancreatic transudate or pancreatic ascites. When these ascites were injected intraperitoneally into mice, the authors were able to measure increased apoptosis in hepatic cells along with elevated serum liver enzymes indicating liver injury. The apoptosis in hepatic cells was measured with the gold standard or TUNEL assay and by flow cytometric analysis, the latter, which shows cells in early or late apoptosis. In a human liver cell line apoptosis was also seen after incubation with ascites fluid. The apoptosis was decreased by inhibition of proapoptotic regulatory proteins—caspase 3 and P-38 MAPK. This is not a simple process as there must be hundreds of potential mechanisms biochemically. I would especially like the authors to consider that severe human pancreatitis is usually associated in my experience with little pancreatic parenchymal necrosis and almost all of the actual necrosis leading to the problem is peri-pancreatic necrosis. How does their model simulate this scenario?

Pancreaticoduodenectomy: Role of Interventional Radiologists in Managing Patients and Complications

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Although the mortality rate after pancreaticoduodenectomy has decreased, the morbidity rate remains high. Major morbidity is often managed with the aid of interventional radiologists. The objective of this study was to evaluate the cooperative roles of interventional radiologists and pancreatic surgeons in complex pancreatic surgery, specifically pancreaticoduodenectomy. Our pancreaticoduodenectomy database was reviewed for all patients undergoing pancreaticoduodenectomy between January 1, 1995 and December 31, 2000. The interventional radiologic procedures for each patient were evaluated. A total of 1061 patients underwent pancreaticoduodenectomy. The overall mortality and morbidity rates were 2.3% and 35%, respectively. Five hundred ninety patients (56%) had no interventional radiologic procedures, whereas 471 patients (44%) had interventional radiologic procedures. Of those, 342 (32%) had preoperative biliary drainage (PBD) and 129 (12%) required postoperative interventional radiologic procedures. Percutaneous aspiration/catheter drainage was required in 84 patients for intra-abdominal abscess, biloma, or lymphocele, with 24 requiring two or more abscess drains. Thirty-nine patients underwent postoperative PBD for bile leaks due to anastomotic disruption, undrained biliary segments, or T-tube/bile stent dislodgment. Eighteen patients had hemobilia/gastrointestinal bleeding treated by angiography with embolization. The reoperation rate for the entire cohort of 1061 patients was 4.1% (n = 43). Nineteen of the 129 patients (15%) requiring postoperative radiologic intervention required reoperation. Although 4 of 18 patients who required embolization for bleeding subsequently required surgical intervention for the same reason, only 4 of 84 patients undergoing abscess drainage later required operation for anastomotic disruption or unsuccessful percutaneous drainage. As would be expected, the patients who required postoperative radiologic intervention (n = 129) had a higher incidence of postoperative complications including pancreatic fistula (20% vs. 6%, $P < 0.01$), bile leakage (22% vs. 1%, $P < 0.01$), and wound infection (16% vs. 8%, $P < 0.01$). With the complications in these 129 patients, the postoperative mortality rate was only 6.2% compared to 1.7% in patients who did not require radiologic intervention (n = 932, $P < 0.01$). The median postoperative length of stay was 15 days in those patients requiring postoperative radiologic intervention, 10 days in those not requiring intervention ($P < 0.01$; postoperative interventional radiology vs. no postoperative interventional radiology), and 29.5 days for patients needing reoperation. Interventional radiologists play a critical role in the management of some patients undergoing pancreaticoduodenectomy. Although complications such as anastomotic leaks, abscess formation, and bleeding can result in increased mortality and a longer hospital stay, the skills of the interventional radiology team provide expert management of some life-threatening complications, thus avoiding reoperation, speeding recovery times, and minimizing morbidity. (J GASTROINTEST SURG 2003;7:209–219.)

KEY WORDS: Interventional radiology, pancreaticoduodenectomy, complications

Until the late 1970s, the high morbidity and mortality rates combined with low 5-year survival rate after pancreaticoduodenectomy for pancreatic and periampullary adenocarcinoma led many surgeons to abandon attempts at surgical resection.[1,2] Since then, many centers have reported mortality rates of less than 5%.[3–10] As a result, pancreaticoduodenectomy has been performed with increasing frequency for benign[7,10–12] and malignant disease,[3–10] with more than 200 procedures performed in each of the last

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two calendar years (2000 and 2001) at The Johns Hopkins Hospital.

Despite the decrease in perioperative mortality after pancreaticoduodenectomy, the incidence of postoperative complications remains high, with many centers reporting complication rates exceeding 30%.[7–10,12] Although the complication rates do remain high, many major centers have observed a decrease in the postoperative length of hospital stay.[7,8,10,12] Decreasing lengths of hospital stay coupled with unchanging morbidity rates imply that the management of postoperative complications has improved. Over the past decade, interventional radiologists have become a critical part of the multidisciplinary team caring for patients undergoing pancreaticoduodenectomy. Although their role in providing biliary decompression preoperatively for patients undergoing pancreaticoduodenectomy is controversial,[13–16] it is critical to the management of postoperative complications[17–19] and to patients found to be unresectable at the time they are undergoing surgical exploration for pancreaticoduodenectomy.[20–22]

Complications after pancreaticoduodenectomy such as anastomotic leaks, intra-abdominal abscess formation, and bleeding can increase the mortality rate and lengthen the postoperative hospital stay. The skills of the interventional radiology team provide expert, less invasive management of such life-threatening complications, thereby avoiding reoperation, decreasing recovery times, and minimizing morbidity in this group of patients.

With the exception of case reports and indirect references, a review of the comprehensive role of the interventional radiologist in patients undergoing pancreaticoduodenectomy has not been reported. This report reviews the critical role of the interventional radiologist in managing postoperative complications after pancreaticoduodenectomy.

PATIENTS AND METHODS

Between January 1, 1995 and December 31, 2000, a total of 1061 patients underwent pancreaticoduodenectomy at The Johns Hopkins Hospital. A retrospective review of our prospectively collected database was performed to evaluate the cooperative roles of the interventional radiologist and the pancreatic surgeon in this complex pancreatic surgery. The number of patients undergoing preoperative percutaneous transhepatic cholangiography with percutaneous biliary drainage (PTC/PBD) and the number of patients undergoing postoperative interventional radiologic procedures including PTC/PBD for postoperative bile leakage, percutaneous abscess drainage, and angiography with or without embolization were reviewed and analyzed. Demographic factors, intraoperative data, pathologic diagnosis, and postoperative course were recorded. Many patients included in the current study have been included in previous reports from The Johns Hopkins Hospital.[7,8,11,14,23–38]

Patients in this series underwent pancreaticoduodenectomy for a wide variety of diseases, both malignant and benign. Total pancreatectomy was performed when the pathologic process involved both the right and left sides of the pancreas. Most of the operations were pylorus-preserving procedures, with distal gastrectomy being used for tumors involving the distal stomach/proximal duodenum. As part of a recently concluded randomized trial that was carried out from 1996 to 2001, both distal gastrectomy and retroperitoneal lymphadenectomy were performed more frequently in patients with periampullary adenocarcinoma.[29,35,36] Superior mesenteric or portal venous resections were performed in a small percentage of patients. Tube gastrostomy, tube jejunostomy, total parenteral nutrition, and vagotomy were not routinely used.

After informed consent was obtained, all interventional radiologic procedures were performed under sedation with local anesthesia. For PTC/PBD, the biliary tree was accessed using a percutaneous, transhepatic approach. Typically the right midaxillary area is sterilely prepared and draped. A 22-gauge Chiba (Cook, Inc., Bloomington, IN) needle is advanced percutaneously into the liver, then withdrawn while contrast medium is injected until a bile duct is identified. At this point, cholangiography is performed. Under fluoroscopic guidance, a suitable duct is chosen for accessing with a 21-gauge needle. A guidewire is then placed through the needle and the 6.5 F Jeffries set (Cook, Inc., Bloomington, IN) is advanced. Through the Jeffries sheath, a 5 F angled glide catheter is advanced, using an angled Terumo guidewire (150 cm; Meditech, Natich, MA) to traverse the obstruction. Once in the bowel, the glide catheter is exchanged over a stiffer wire for an 8 F catheter, which is used as the biliary catheter. When draining the left-sided ductal system, a subcostal approach into the left anterior ductal system is preferred.

On identification of a well-defined intra-abdominal fluid collection, the interventional radiologist can aspirate them or place indwelling drainage catheters under fluoroscopic, ultrasound, or CT guidance.

For visceral angiography, the femoral artery is the preferred site of access. A 5 F vascular sheath is placed and secured via the Seldinger technique. The visceral vessel is catheterized with a 5 F catheter. When a bleeding site is identified in a small arterial branch, embolization can be performed after subse-

lective catheterization using (1) injection of a contrast/Gelfoam slurry and/or (2) placement of wire helical coils.

Perioperative mortality was defined as death during the index hospitalization or within 30 days of surgery. The overall incidence of postoperative complications was evaluated. Delayed gastric emptying, pancreatic fistula, and biliary anastomotic leak were defined using previously reported criteria.[8,36,37] For complications such as wound infection, intra-abdominal abscess, pancreatitis, pneumonia, and cholangitis, standard definitions were used.[8] Demographics, pathologic results, intraoperative data, and postoperative complications were compared among those patients requiring interventional radiologic procedures for postoperative complications and those requiring no intervention.

All continuous data are presented as mean ± standard error of the mean (SEM). A chi-square test was used for all comparisons among categorical values, whereas Student's *t* test was used for all comparisons among continuous variables. Significance was accepted at the 5% level.

RESULTS

In the 6 years of this study, 1061 patients underwent pancreaticoduodenectomy. The demographic data and postoperative diagnoses of these patients are presented in Table 1, and the intraoperative data are summarized in Table 2.

There were 24 postoperative deaths in the entire cohort for a perioperative mortality rate of 2.3%. Postoperative complications were seen in 371 patients (35%). A summary of specific complications is presented in Table 3. Forty-three patients (4.1%) required reoperation. Reasons for reoperation were as follows: bleeding in 11 patients; wound dehiscence in seven patients; major hepatico-, pancreatico-, or duodenojejunosotomy disruptions in six patients; local wound problems in five patients; ischemic bowel in four patients; suspected intra-abdominal sepsis (no source found at exploratory operation) in four patients; and intra-abdominal abscess in three patients. The remaining four patients had miscellaneous reasons for reoperation including pulmonary artery rupture secondary to a Swan-Ganz catheter, a perforated duodenal ulcer, a pericolostomy small bowel obstruction, and a hepatic artery thrombosis. Other complications included delayed gastric emptying in 101 patients (10%), wound infection in 92 (9%), pancreatic fistula in 85 (8%), intra-abdominal abscess in 80 (8%), bile leakage in 42 (4%), cholangitis in 28 (3%), pneumonia in 13 (1.2%), and pancreatitis in 11 (1%). The mean postoperative length of hospital stay was 13.0 ± 0.2 days (median = 10 days).

Of the 1061 patients undergoing pancreaticoduodenectomy, 590 patients (56%) had no interventional radiologic procedures, whereas in 471 (44%) such intervention was performed (Fig. 1). Overall, 391

Table 1. Demographics and pathologic diagnoses

Demographics	
Mean age ± SEM (yr)	63.6 ± 0.4
Median age (yr)	66
Sex	54% male
Race	88% White
Pathologic diagnoses	
Periampullary adenocarcinoma	68%
Pancreatic adenocarcinoma	43%
Ampullary adenocarcinoma	12%
Distal bile duct adenocarcinoma	10%
Duodenal adenocarcinoma	3%
Chronic pancreatitis	11%
Neuroendocrine tumor	5%
Intraductal papillary mucinous neoplasm	4%
Benign	2%
Associated infiltrating component	2%
Mucinous cystic neoplasm	3%
Periampullary adenoma	3%
Gastrointestinal stromal tumors	1%
Other	5%

Table 2. Intraoperative data

Type of pancreaticoduodenectomy	
Pylorus-preserving	68%
Classic	32%
Extent of pancreaticoduodenectomy	
Partial	95%
Total	5%
Pancreatic reconstruction*	
Pancreaticojejunostomy	89%
Pancreaticogastrostomy	11%
Extent of lymph node dissection	
Standard	87%
Extended	13%
Estimated blood loss (ml)	
Mean ± SEM	960 ± 50
Median	700
Transfusions (units of PRBCs)	
Mean ± SEM	0.9 ± 0.1
Median	0
Operative time (hr)	
Mean ± SEM	6.4 ± 0.1
Median	6.2

PRBCs = packed red blood cells transfused intraoperatively.
*In the 1007 patients undergoing partial pancreatectomy.

Table 3. Postoperative complications

	Overall group (n = 1061)	Patients requiring postoperative interventional radiology (n = 129)	No postoperative interventional radiology (n = 932)	*P* value
Perioperative mortality rate	2.3%	6.2%	1.7%	<0.01
Overall complications	35%	100%*	30%	<0.01
Reoperation	4.1%	15%	2.5%	<0.01
Delayed gastric emptying	10%	5%	10%	NS
Wound infection	9%	16%	8%	0.01
Pancreatic fistula formation	8%	20%	6%	<0.01
Intra-abdominal abscess	8%	60%	8%	<0.01
Bile leak	4%	22%	1%	<0.01
Cholangitis	3%	9%	2%	<0.01
Pneumonia	1%	4%	1%	<0.01
Pancreatitis	1%	2%	0.8%	NS
Peptic ulcer	0.6%	1%	0.5%	NS
Lymphatic leak	0.5%	2%	0.2%	<0.01
Small bowel obstruction	0.3%	0.7%	0.3%	NS
Postoperative length of stay (days)				
Mean ± SEM	13.0 ± 0.2	20.8 ± 1.3	11.9 ± 0.2	<0.01
Median	10	15	10	

NS = not significant.
*By definition, patients who required postoperative interventional radiologic procedure were considered to have had a complication.

patients had preoperative PTC/PBD; 342 (32%) of these 391 patients had only preoperative PTC/PBD with or without postoperative bile stent changes and required no other interventional radiologic procedures. Complications as a direct result of PTC/PBD included hemobilia in 11 (3%), right pleural effusions in three (0.7%), and inadvertent bile stent dislodgment in three (0.7%). In this group of 342 patients, one or more postoperative bile stent changes were performed in 238 patients (61%) for leakage around the bile stent at the skin site, upsizing to a softer tube, inability to flush the bile stent, or signs and/or symptoms of cholangitis.

Because of complications such as these, practice patterns at our institution have changed over time, particularly with regard to preoperative PTC/PBD. Before 1995, most patients undergoing pancreaticoduodenectomy had preoperative biliary drainage via either PTC/PBD or endoscopic retrograde cholangiopancreatography (ERCP) with placement of an endostent. The percutaneously or endoscopically placed bile stent was used to relieve jaundice prior to surgery. In addition, PTC/PBD provided stenting of the hepaticojejunostomy and access to the biliary tree postoperatively. Before 1996, patients who had not undergone PTC/PBD had intraoperative T-tubes placed for postoperative hepaticojejunostomy stenting and biliary access. The number of patients undergoing preoperative PTC/PBD peaked in 1998 and has subsequently decreased after reports of increased postpancreaticoduodenectomy complications after routine preoperative PTC/PBD or endoprosthesis placement.[13–16] In the current study, 46% of patients underwent preoperative PTC/PBD in 1995/1996, 41% in 1997, and 48% in 1998. This percentage decreased to 33% in 1999 and further decreased to 19% in 2000. The use of T tubes decreased from 52% in 1996 to less than 1% in 2000.

One hundred twenty-nine patients (12%) underwent postoperative radiologic intervention, all for complications occurring after pancreaticoduodenectomy. Postoperative interventional radiologic procedures (Table 4) included the following: (1) percutaneous aspiration of intra-abdominal abscesses, bilomas, or lymphoceles (n = 84, 8%); (2) postoperative PTC/PBD for bile leakage, undrained biliary segments, or T-tube/bile stent dislodgment (n = 39, 4%); and (3) angiography with or without embolization for hemobilia/gastrointestinal bleeding (n = 18, 2%; see Fig. 1). Some patients required two or more procedures. Table 4 presents a summary of the patients requiring postoperative interventional radiologic procedures including mortality rates, reoperation rates, and number of patients in each of the postoperative intervention groups requiring a second intervention.

The 129 patients undergoing postoperative intervention are the focus of the remainder of this report. The demographic and intraoperative data were similar between the groups requiring postoperative interventional radiology and the group not requiring postoperative interventional radiology. Patients requiring

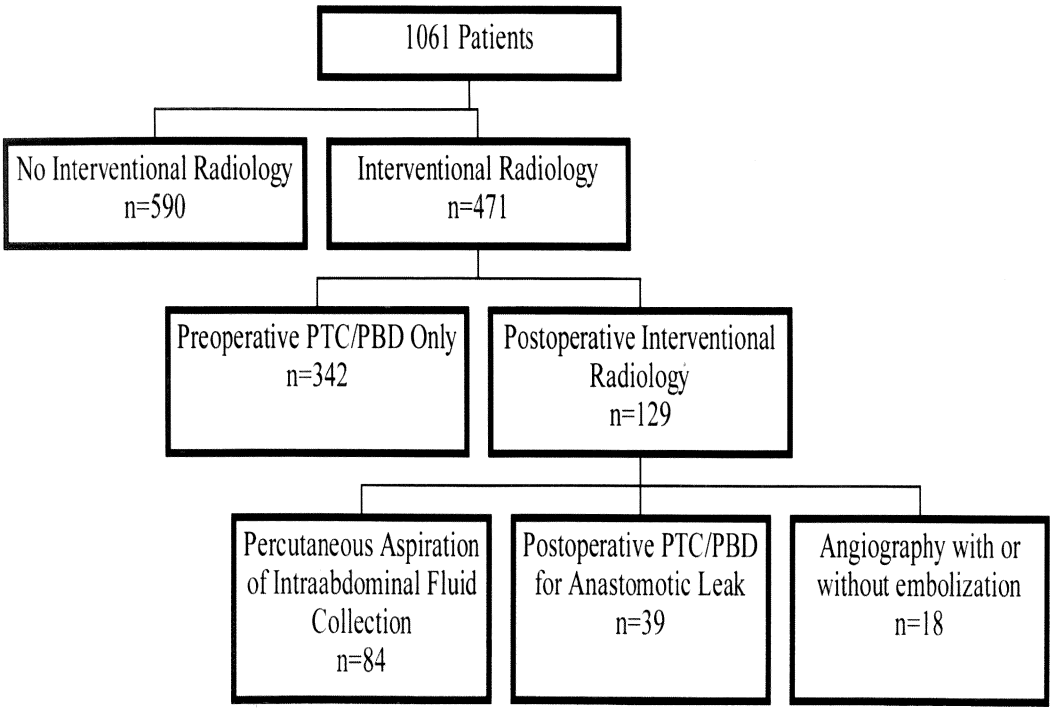

Fig. 1. Breakdown of patients requiring interventional radiologic procedures. Of note, the number of patients requiring different types of postoperative interventions is greater than 129 (total number of patients requiring postoperative interventional radiologic procedures) because some patients had more than one type of procedure (see Table 4).

postoperative interventional radiologic procedures were more likely to have diseases other than periampullary adenocarcinoma when compared to those not requiring intervention (39% vs. 30%, $P = 0.04$).

By definition, all patients requiring interventional radiologic procedures had complications after pancreaticoduodenectomy. As would be expected, the incidence of many specific complications was higher in this group (see Table 3). Patients requiring postoperative interventional radiologic procedures for postpancreaticoduodenectomy complications had a significantly higher perioperative mortality rate (6.2% vs. 1.7%, $P < 0.01$) and a higher incidence of reoperation in the immediate postoperative period (15% vs. 2.5%, $P < 0.01$). In 85% of patients, the aid of the interventional radiologists allowed the operating surgeons to avoid the morbidity of reoperation. The increased complications contributed to a significantly longer length of hospital stay (20.8 ± 1.3 days [median = 15 days] vs. 11.9 ± 0.2 days [median = 10 days]; $P <$ 0.01) in the postoperative interventional radiology group, as compared to the group not requiring inter-

Table 4. Summary of groups requiring postoperative interventional radiology

	Abscess drainage (n = 84)	Postoperative PTC/PBD (n = 39)	Angiography/Embolization (n = 18)
Perioperative mortality	4 (4.7%)	5 (12.8%)	2 (11.1%)
Reoperation	11 (13.1%)	7 (17.9%)	5 (27.8%)
No. requiring second intervention			
Abscess drainage	—	14 (35.9%)	3 (16.7%)
Postoperative PTC/PBD	12 (14.2%)	—	2 (11.1%)
Angiography with or without embolization	3 (3.6%)	2 (5.1%)	—
>1 Abscess drain	24 (28.5%)	12 (30.8%)	2 (11.1%)

PTC/PBD = percutaneous transhepatic cholangiography/percutaneous biliary drainage.

vention. It should be noted, however, that the postoperative length of stay was 30.3 ± 3.2 days (median = 29.5 days) for patients requiring reoperation.

The largest group requiring interventional radiologic procedures were those patients with intra-abdominal abscesses or fluid collections. Eighty-four patients (8%) required percutaneous aspiration (n = 4) or catheter drainage (n = 80) of an intra-abdominal abscess or fluid collection. CT scans before and after percutaneous drainage (Fig. 2, *A* and *C*), as well as images from the interventional procedure (Fig. 2, *B*), for one patient undergoing abscess drainage are shown in Fig. 2. Sixty-five patients had intra-abdominal abscesses (most commonly caused by an anastomotic leak), 15 patients had bilomas as a result of bile leakage, and four patients had lymphoceles. All patients with intra-abdominal abscesses were placed on broad-spectrum antibiotics, which were tailored according to culture results. In successfully treated cases, sepsis usually resolved within 24 to 48 hours after the drainage. However, 24 (28%) of the 84 patients required two or more percutaneously placed drains during their postoperative course for residual collections or ongoing sepsis. There were four deaths and 11 reoperations in this group. Of the 11 patients undergoing percutaneous abscess drainage who required reoperation in the immediate postoperative period, only four were for defined abscesses not amenable to drainage or for anastomotic dehiscences causing widespread peritoneal contamination. Four reoperations were for bleeding and the other three were for wound dehiscence, an abdominal wall complication, and hepatic artery thrombosis (see Table 4).

The second largest group included 39 patients (4%) who required PTC/PBD in the immediate postoperative period. Twenty-two patients in this postoperative PTC/PBD group had a bile leak at the hepaticojejunostomy; six had occluded T-tubes (n = 325 T-tubes used in 1061 patients); five had cholangitis due to an obstructed left-sided ductal system requiring a left-sided stent to be placed; three had aberrant right hepatic ductal systems, which were not recognized and, therefore, not incorporated into the hepaticojejunostomy at the time of surgery (Fig. 3); and three had T-tubes or bile stents that were inadvertently dislodged. A T-tube cholangiogram (see Fig.3, *A*) and postoperative right PTC/PBD (Fig. 3, *B*) for an unincorporated right biliary segment are shown in Fig. 3. Seven patients in this group required reoperation, but none for an intra-abdominal bile collection. There were five deaths in this group among patients who had major anastomotic dehiscences or intra-abdominal bleeding, all of whom required intervention beyond PTC/PBD (Table 4). None of the patients in this group had complications directly related to stent placement.

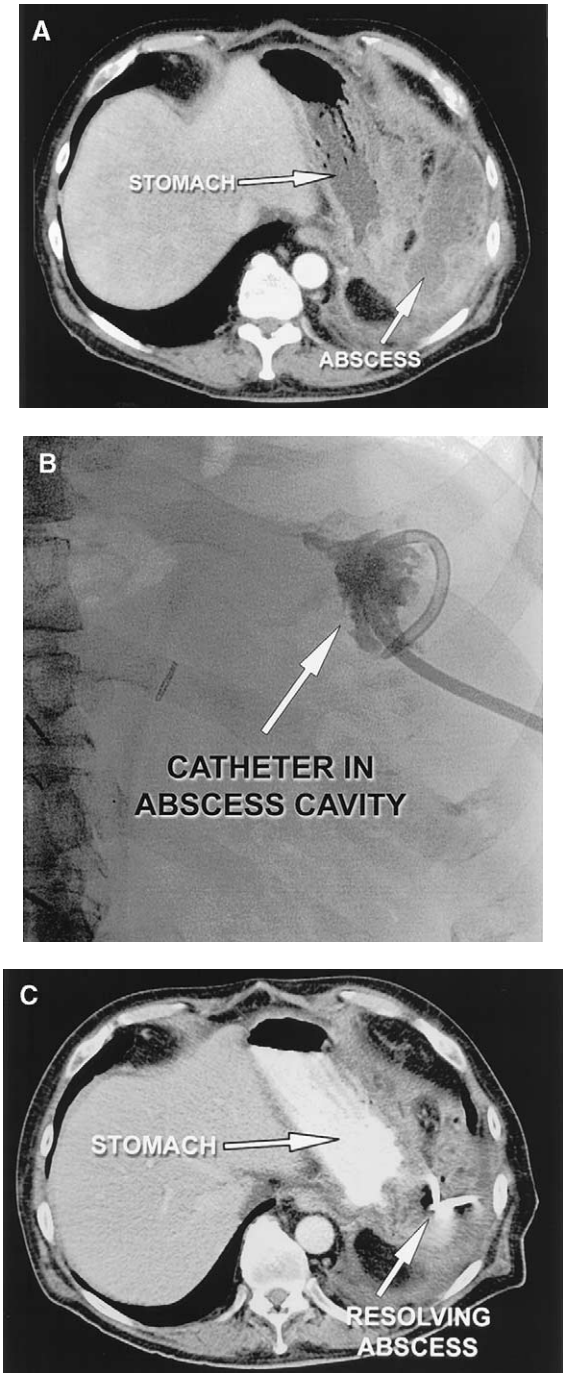

Fig. 2. Intra-abdominal abscess drainage. These images are from a patient who underwent total pancreaticoduodenectomy with en bloc splenectomy and subsequently developed an abscess in the left upper quadrant. **A**, CT scan demonstrating a heterogenous 12 × 4 cm abscess lateral to the stomach in the left upper quadrant. **B**, Image demonstrating percutaneously placed drain. Dye injection through the drain shows filling of the abscess cavity. **C**, Postdrainage CT scan shows drain in place in the left upper quadrant, with marked decrease in the size of the fluid collection.

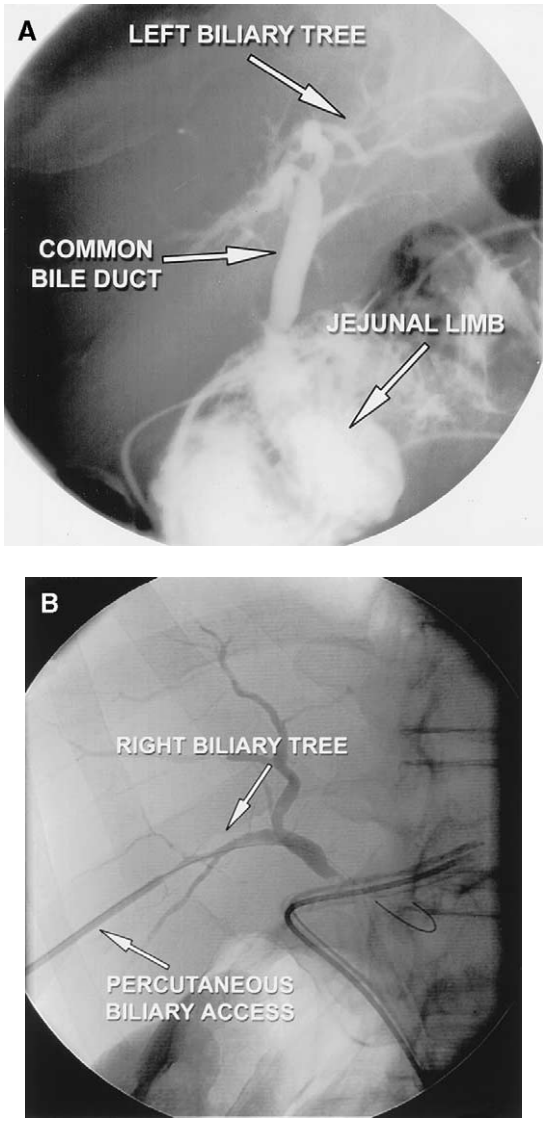

Fig. 3. Postoperative PTC/PBD. **A**, Patient with a postoperative bile leak underwent T-tube cholangiography demonstrating no leakage, but filling of the left-sided biliary ductal system only. **B**, Patient underwent right-sided PTC/PBD showing an unincorporated segment of the right-sided system draining freely into the peritoneal cavity and operatively placed drain. This right-sided system was percutaneously stented and drained to the jejunal limb as a neoanastomosis.

Eighteen patients required postoperative angiography with or without embolization for significant hemobilia or gastrointestinal bleeding. They formed the last major group requiring the expertise of interventional radiologists. Two patients required only upsizing of the indwelling bile stent for tamponade of the venous bleeding, whereas 16 required embolization of a branch of the hepatic artery (n = 10) or gastroduodenal artery (n = 4), celiac axis (n = 1), or portal venous/hepatic artery/biliary fistula (n = 1). In five cases, a pseudoaneurysm was identified as the source of the bleeding. Of the 18 patients who had postoperative bleeding, 12 underwent preoperative PTC/PBD. Of these 12 patients, 10 had bleeding from sites in the right or left hepatic arteries, one had a portal vein/hepatic artery/biliary fistula, and one had bleeding from the gastroduodenal artery. Thus 11 of the 12 cases of bleeding were the result of the PTC/PBD. Of the six patients who did not undergo preoperative PTC/PBD, three had bleeding from the gastroduodenal artery stump, two from branches of the hepatic artery, and one was from the celiac axis. An angiogram from a patient with ongoing hemobilia after pancreaticoduodenectomy shows active bleeding from a distal branch of the right hepatic artery (Fig. 4, *A* and *B*); successful embolization (Fig. 4, *C*) is shown in Fig. 4. Although six patients in this group required reoperation, only four of these operations were for recurrent bleeding after radiologic intervention (Table 4). These patients were either hemodynamically unstable or had other problems, such as anastomotic dehiscences, that required surgical intervention.

Twenty patients had interventional radiologic procedures that did not fall into the previous three categories. Of these, six were preoperative interventions and 14 were postoperative interventions. These miscellanous procedures are summarized in Table 5.

DISCUSSION

"The continued high morbidity associated with pancreaticoduodenectomies is compensated by the ability to treat complications nonoperatively, resulting in a surgical risk that should now be considered medium to low in high-volume centres."[19] This observation of Bassi et al.[19] has been supported by many major centers reporting decreased lengths of stay but unchanged complication rates after pancreaticoduodenectomy.[10,12] In a 1997 report of 650 patients undergoing pancreaticoduodenectomy at our institution between 1990 and 1996, the median length of stay was 13 days,[7] compared to 10 days in the current cohort of 1061 patients undergoing the same procedure.

Interventional radiology is a rapidly expanding field that provides surgeons in both academic and community hospitals with alternative methods of managing some postoperative complications. Interventional radiologists play a critical role in the management of many patients with pancreatic and periampullary diseases occasionally before and commonly after surgery. They may be called on to perform preoperative PTC/PBD in select patients, and they

played a critical role in the palliation of obstructive jaundice in our patients with unresectable disease, which was not addressed in this report. Most important, interventional radiologists play a critical role in the management of several postpancreaticoduodenectomy complications. Although there has been much debate in the literature with regard to the role of preoperative PTC/PBD,[13–16] there has been little focus on the cooperative role of interventional radiologists and their surgical colleagues in the management of these potentially life-threatening complications. There have been numerous reports of outcomes after pancreaticoduodenectomy,[3–10,12,18,19] many of which mention percutaneous drainage of intra-abdominal abscesses and other attempts at conservative management.

Surgical practice and the role of interventional radiologists in the care of patients undergoing pancreaticoduodenectomy has changed over time at our institution. Routine drainage and stenting of biliary anastomoses via preoperatively placed bile stents or intraoperative T-tube placement is no longer performed. There has been much controversy over the role of preoperative PTC/PBD in patients undergoing pancreaticoduodenectomy.[13–16,39] Although the use of preoperative PTC/PBD may decrease the risk of bleeding complications in patients with bilirubin levels above 10 mg/dl,[39] it has been suggested that such therapy be used only in selected patients. A prospective, randomized study by Pitt et al.[13] showed that preoperative PTC/PBD significantly increased both the length of hospital stay and hospital costs. Other groups have reported increased rates of wound infection,[13–15] pancreatic fistula,[14] and intra-abdominal abscesses.[15] Practice patterns at our institution have changed accordingly, with nearly 50% of patients undergoing preoperative PTC/PBD in 1995 and less than 20% in 2000. Currently, preoperative biliary drainage is reserved for patients with ascending cholangitis and for patients with high bilirubin levels in whom there is a delay between the time they are first seen and the time of definitive surgical management. Preoperative biliary drainage is accomplished using an endoscopic approach (endoscopic retrograde cholangiopancreatography with an endoprosthesis) or PTC/PBD. The endoscopic approach is now favored for several reasons. Endoscopically placed biliary stents are less invasive and less painful for the patients and do not have any external hardware for the patient to manage. In addition, they are associated with a lower incidence of hemobilia and pleural effusion.

The focus of the current report is to highlight the contributions of interventional radiologists in our most formidable group of patients—those suffering

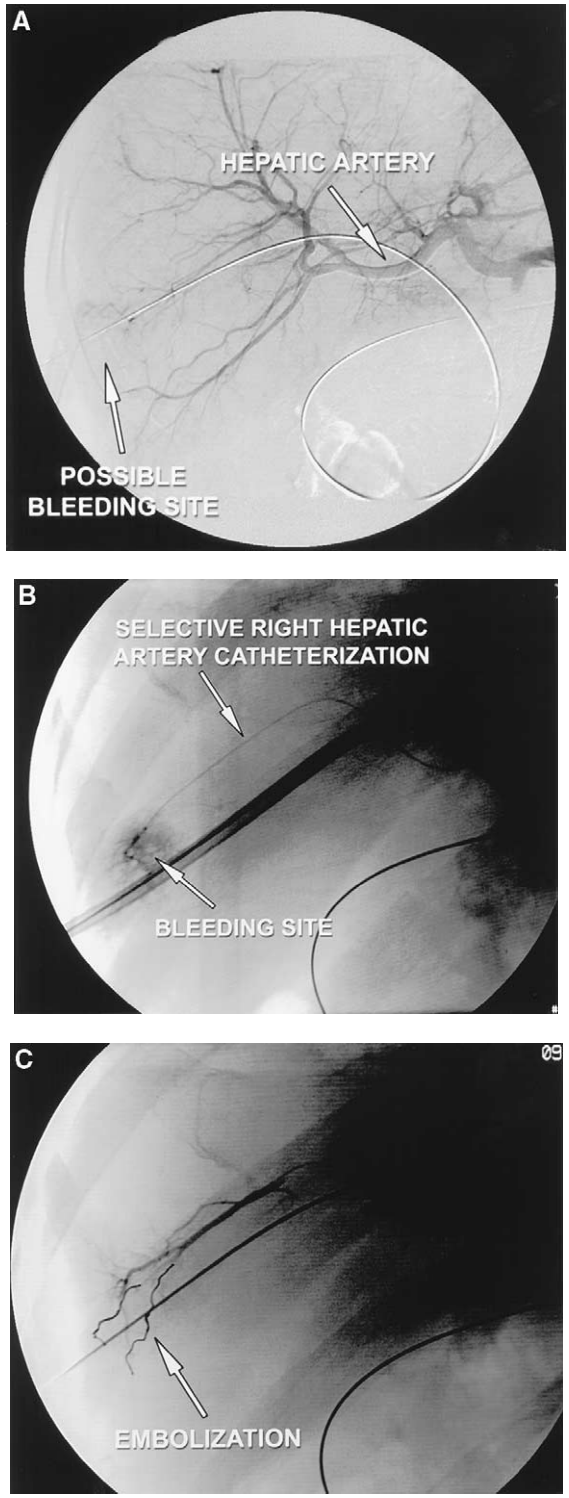

Fig. 4. Angiography with embolization. An angiogram was obtained in a patient with ongoing hemobilia after pancreaticoduodenectomy. **A**, Injection of the common hepatic artery suggests a bleeding site in a distal branch of the right hepatic artery. **B**, Selective injection of this branch demonstrates a clear blush due to leakage of contrast medium from the bleeding site. **C**, Embolization was performed with coils, which successfully stopped the bleeding.

Table 5. Miscellaneous interventional radiologic procedures associated with pancreaticoduodenectomy

Type of procedure	No. of procedures
Preoperative intervention (n = 6)	
Drainage of intra-abdominal abscess(es) before PD	6
From duodenal injury	3
From PTC/PBD	3
Postoperative intervention (n = 14)	
Change of high-output, operatively placed Jackson-Pratt drains to larger drainage catheter	4
Drainage of postoperative pleural effusion	3
Serous	2
Bilious	1
Vascular complications	3
Lysis of common iliac artery clot	1
Lysis of hepatic artery thrombosis	1
Lysis of thrombosed portal vein-saphenous graft	1
Removal of retained intra-abdominal Jackson-Pratt drain tip	1
Drain placement in abdominal wall abscess	1
Common bile duct/intrahepatic stone removal	1
Placement of feeding jejunostomy tube	1
TOTAL	20

PD = pancreaticoduodenectomy; PTC/PBD = percutaneous transhepatic cholangiography/percutaneous biliary drainage.

from potentially life-threatening complications after pancreaticoduodenectomy. In the current series, 129 patients required postoperative interventional radiologic procedures for postpancreaticoduodenectomy complications. In 85% of these patients, the complications were managed nonoperatively. Despite the fact that 60% of patients had intra-abdominal abscesses, 22% had bile leaks, 20% had pancreatic fistulas, 16% had wound infections, and 9% had cholangitis postoperatively; the median length of hospital stay was only 15 days, just 5 days longer than in those who had uncomplicated postoperative courses. This is in contrast to those requiring reoperation whose median postoperative length of hospital stay was 29.5 days.

Patients with pathologic findings other than periampullary adenocarcinoma were overrepresented in the group undergoing postoperative interventional radiology. A higher percentage of patients in this group are more likely to have soft, normal pancreata with small pancreatic ducts, making the pancreatic-enteric anastomosis more challenging and more prone to leakage. Efforts to reduce the formation of pancreatic fistulas have included the use of octreotide[33] and pancreaticogastrostomy instead of pancreaticojejunostomy,[38] but neither has decreased the incidence of fistula formation.

Anastomotic leaks can lead to many postoperative problems including pancreatic fistula and intra-abdominal abscess formation.[19] In the early decades of pancreaticoduodenectomy surgery, the diagnosis of intra-abdominal abscess was difficult to make without reexploring the septic patient without another source. With improvements in imaging technology (dual-contrast spiral CT, ultrasound), this complication is now easier to document. With the aid of CT, ultrasound, or fluoroscopic guidance, most intra-abdominal abscesses and fluid collections can be managed nonoperatively.

Percutaneous abscess drainage has been reported for a variety of disease processes including pyogenic liver abscesses,[40] Crohn's-related abscesses,[41] pancreatic abscesses,[42–44] renal and perirenal abscesses,[45–46] diverticular abscesses,[47] and periappendiceal abscesses.[48] In a review by vanSonnenberg et al.,[46] the investigators suggest that percutaneous drainage of intra-abdominal abscesses has become the procedure of choice. In this series, only 4 of 80 patients undergoing attempted percutaneous drainage of intra-abdominal abscesses required reoperation for persistent abscess or anastomotic dehiscence. It should be noted that in patients with uncontrolled sepsis, complete anastomotic dehiscence, or abscesses not amenable to drainage, early reexploration for drainage and control of sepsis is appropriate.

The placement of percutaneous transhepatic biliary stents in the setting of postoperative bile leaks (usually from the hepaticojejunostomy) allows for external biliary drainage, leaving the biliary tree under low pressure while the anastomosis heals. This prevents the formation or further accumulation of intra-abdominal biloma or abscess, which can result from continued leakage. In cases where bilomas/abscesses have already formed, percutaneous drainage in conjunction with biliary decompression via PTC/PBD allows for nonoperative management.

There have been several isolated reports of small series of patients with visceral artery pseudoaneurysms and hemobilia after pancreaticoduodenectomy.[17,49,50] In our series, embolization of hemobilia/gastrointestinal bleeding was successful in 14 of 18 patients, with four requiring reoperation for recurrent or persistent bleeding. Although angiography and embolization/upsizing of bile stents was successful, it should be noted that the bleeding was likely caused by PTC/PBD in most cases.

CONCLUSION

Because of the nature of the operation, complication rates remain high after pancreaticoduodenectomy. Life-threatening complications such as anastomotic leaks, intra-abdominal abscess formation, and postoperative bleeding can increase the mortality rate and length of hospital stay. Although complication rates remain high, the cooperative role of interventional radiologists and pancreatic surgeons minimizes morbidity and mortality after pancreaticoduodenectomy. The skills of the interventional radiology team provide expert, less invasive treatment of many complications, avoiding the morbidity of reoperation in many cases and reducing recovery times.

We thank the staff of the Cardiovascular Diagnostic Laboratory, who have provided excellent care of our patients over many years.

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Evaluation of Vascular Endothelial Growth Factor Blockade and Matrix Metalloproteinase Inhibition as a Combination Therapy for Experimental Human Pancreatic Cancer

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Blockade of vascular endothelial growth factor (VEGF) and inhibition of matrix metalloproteinases (MMP) are promising therapies for cancer. This study assessed the effects of a neutralizing anti-VEGF antibody (A4.6.1) and an MMP inhibitor (BB-94) on pancreatic cancer (PaCa) in vivo. Five million cells of two human PaCa cell lines (AsPC-1 and HPAF-2) were injected subcutaneously into nude mice; 1 mm^3 fragments of the resulting tumors were implanted into the pancreas of other mice. Animals were randomized into a control group and three treatment groups: A4.6.1 (100 μg intraperitoneally twice weekly); BB-94 (50 mg/kg every other day); and combination (A4.6.1 plus BB-94). Treatment was started after 3 days and continued for 14 weeks. Tumor volume, local and distant spread (score), and ascites were determined at autopsy. Microvessel density as a parameter of neoangiogenesis was analyzed in CD31-stained tumor sections. Both monotherapies reduced tumor volume (HPAF-2: −89% by A4.6.1 and −75% by BB-94; AsPC-1: −48% by A4.6.1 and −72% by BB-94), spread (HPAF-2: −76% by A4.6.1 and −58% by BB-94; AsPC-1: −32% by A4.6.1 and −54% by BB-94), and microvessel density (HPAF-2: −75% by A4.6.1 and −30% by BB-94; AsPC-1: −59% by A4.6.1 and −30% by BB-94), resulting in a tendency toward increased survival (HPAF-2: 8 of 8 animals by A4.6.1 or BB-94 vs. 4 of 8; AsPC-1: 3 of 8 by A4.6.1, 4 of 8 by BB-94 vs. 1 of 8). Combination therapy yielded additional effects in the HPAF-2 group with regard to tumor volume (−95%) and development of ascites (0 of 8 vs. 2 of 8 by A4.6.1 or BB-94 vs. 5 of 8 control mice). Both VEGF blockade and MMP inhibition reduce primary tumor size, metastasis, and angiogenesis, thereby increasing survival in experimental pancreatic cancer. Combination treatment results in additive effects in moderately differentiated HPAF-2 tumors. (J GASTROINTEST SURG 2003;7:220–228.)

KEY WORDS: Pancreatic cancer, matrix metalloproteinases, vascular endothelial growth factor, angiogenesis

Exocrine pancreatic cancer is currently the third most common gastrointestinal malignancy and the fifth most common cause of cancer-related death in the United States.[1] At the time of diagnosis, more than 80% of the patients present with either locally advanced or metastatic disease, without any option for curative surgical resection.[2] The inability to detect pancreatic cancer at an early stage, the aggressive nature of the disease, and the lack of effective conventional treatments, such as radiation therapy or chemotherapy in various combinations, result in a dismal 5-year survival rate of less than 5%.[1,3] It is, therefore, obvious that effective treatment options for human pancreatic cancer are urgently needed.

A variety of novel anticancer strategies have emerged in the past decade. One of the most promising is the inhibition of neoangiogenesis.[4,5] As in all solid tumors, local pancreatic cancer growth beyond

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the size of few cubic millimeters and systemic spread are dependent on the formation of blood vessels mediated by the release of proangiogenic factors such as vascular endothelial growth factor (VEGF).[6,7] Antiangiogenic strategies aimed at inhibiting VEGF yielded therapeutic effects in experimental pancreatic cancer—that is, a neutralizing anti-VEGF antibody reduced tumor growth and metastasis in an orthotopic nude mouse model.[8,9] Similar therapeutic potential was observed after blocking VEGF activity by an antisense molecule.[10] Specific targeting of the tumor vasculature by a VEGF-diphtheria toxin construct resulted in reduced angiogenesis and growth of experimental pancreatic cancer.[11]

Besides the action of endogenous proangiogenic mediators, the proteolytic process of basement membrane invasion and extracellular matrix degradation is crucial not only for angiogenesis but for local and distant spread of the tumor cell itself. It is now believed that matrix metalloproteinases (MMPs), a family of at least 18 naturally occurring degradative enzymes, are primarily responsible for the breakdown of the extracellular matrix and basement membrane during tumor progression.[12,13] A variety of studies employed newly developed synthetic MMP inhibitors in experimental pancreatic cancer, resulting in suppressed MMP activity,[14,15] reduced tumor growth, and increased survival.[16–18]

However, none of the described treatment modalities came close to eradicating tumor disease, even after prophylactic application. The complex and multifactorial regulation of pancreatic cancer growth and progression implies that combination therapies may be more effective than monotherapeutic approaches. The aim of the present study, therefore, was to assess the therapeutic potential of combined VEGF blockade and MMP inhibition in a clinically relevant orthotopic nude mouse model of human pancreatic cancer.

MATERIAL AND METHODS

Drugs

The murine monoclonal neutralizing anti-VEGF antibody A4.6.1[19] (Genentech, Inc., South San Francisco, CA) was dissolved in phosphate-buffered saline (PBS) solution (1 mg/ml) for intraperitoneal injection. BB-94[20,21] (Batimastat; British Biotech, Oxford, UK), a broad-spectrum MMP inhibitor, was suspended at 7.5 mg/ml in PBS and 0.01% (vol/vol) Tween 80 (Sigma Chemical, St. Louis, MO) by sonication.

Cell Lines and Culture Conditions

Two human pancreatic adenocarcinoma cell lines were obtained from the American Type Culture Collection (Rockville, MD): AsPC-1 (poorly differentiated[22]) and HPAF-2 (moderately differentiated[23]). AsPC-1 cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY) and HPAF-2 cells in minimum essential medium (MEM; Gibco). All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco), penicillin G (100 U/ml), and streptomycin (100 μg/ml). The cells were incubated at 37° C in humidified air with 5% CO_2. The medium was replaced twice weekly, and cells were maintained by serial passaging after trypsinization with 0.1% trypsin.

Laboratory Animals and Orthotopic Implantation Technique

Four-week-old male nude mice (Crl:NU/NU-*nu*BR), weighing 20 to 22 g, were obtained from Charles River Laboratories (Wilmington, MA). The animals were housed in microisolator cages with autoclaved bedding, food, and water. The mice were maintained on a daily 12-hour light/dark cycle. All experiments were conducted in accordance with the national guidelines for the care and use of laboratory animals, and the experimental protocol was approved by the Chancellor's Animal Research Committee of the University of California, Los Angeles.

The orthotopic pancreatic tumor implantation technique was previously described in detail[24,25]; 5×10^6 cells from each human pancreatic cancer cell line were injected subcutaneously into the flanks of donor nude mice. The animals were killed after 3 to 4 weeks, when the subcutaneous tumors had reached a size of 1 cm in the largest diameter. The donor tumors were harvested and minced using a scalpel (No. 11) into 1 mm^3 fragments. The abdomens of the anesthetized tumor recipient nude mice were opened by a midline incision under aseptic conditions at a laminar air flow working bench, and the pancreatic tail with the spleen was gently exteriorized. Two small tissue pockets were prepared in the pancreatic parenchyma as an implantation bed with a microscissors (RS-5610 VANNAS; Roboz, Rockville, MD). One donor tumor fragment was placed into each pancreatic tissue pocket in such a way that the tumor tissue was completely surrounded by pancreatic parenchyma. The pancreas was relocated into the abdominal cavity, which was then closed in two layers with 5-0 absorbable sutures (DEXON "S"; Davis & Geck, Manati, Puerto Rico).

In Vivo Treatment

Sixty-four animals (32 per pancreatic cancer cell line) were randomly allocated into one of three treat-

ment groups or a control group. Treatment with A4.6.1 (100 μg intraperitoneally twice weekly), BB-94 (50 mg/kg intraperitoneally every other day), a combination of A4.6.1 and BB-94, or the vehicle (PBS) was started 3 days after orthotopic tumor implantation. The mice were monitored daily to assess their clinical condition, weighed weekly, and killed by a lethal dose of sodium pentobarbital (0.5 mg/g body weight) 14 weeks after the orthotopic tumor implantation. According to the guidelines of the Chancellor's Animal Research Committee of the University of California, Los Angeles, animals had to be killed earlier if one of the following occurred: (1) bulky tumor mass with a visible tumor size greater than 1.5 cm; (2) formation of ascites with visible abdominal distention; or (3) jaundice and/or cachexia associated with a significant clinical deterioration of the animal.

All animals underwent autopsy examination at the end of the observation period. The perpendicular diameter of the primary orthotopic tumor was measured with calipers, and the volume was calculated using the following formula: volume = length × width × depth/2. A dissemination score was developed to assess local tumor infiltration, as well as distant metastasis.[24,26] Local infiltration was determined at the following sites: spleen, stomach, liver (hilus), kidney (hilus), retroperitoneum, diaphragm, mesentery, bowel loops, and abdominal wall. Isolated tumor nodules with no anatomic connection to the primary lesion were judged to be distant metastases. The sites of evaluation included the liver, kidney, spleen, lung, diaphragm, mesentery, retroperitoneum, mediastinum, and the suture line. Tumor dissemination was quantified as follows: every manifestation of tumor infiltration or metastasis was credited with one point. Additional points were awarded for massive local infiltration (e.g., including more than half of the circumference of the spleen), multiple metastatic nodules (>1 in parenchymal organs; >10 on the diaphragm, mesentery, or retroperitoneum), and metastatic nodules larger than 50 mm^3. Clinical consequences of the tumor growth were incorporated into this scoring system: formation of ascites (2 points for volume >5 ml); development of jaundice, ileus, and cachexia. The autopsy data were analyzed by one of us (H.G.H.) who was blinded to the treatment groups.

The primary tumor and all sites of potential infiltration or metastasis were harvested, fixed in paraformaldehyde, and embedded in paraffin. Five-micron thin tissue sections were obtained and stained with hematoxylin and eosin for microscopic examination. The sections were reviewed to confirm the findings of the macroscopic dissemination score.

Microvessel Density

Anti-CD31 was used as endothelial marker to highlight intratumoral microvessels. The human pancreatic cancer xenograft tumors orthotopically grown in the pancreas of nude mice were immediately fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections (3 μm) were deparaffinized and rehydrated, and target retrieval was accomplished by autoclaving tissues at 97° C for 30 minutes in 0.01 mol/L citrate buffer (pH 6.0) followed by a 5-minute treatment in 3% hydrogen peroxide solution to block endogenous alkaline phosphatase activity. After blocking slides for 10 minutes, a purified antimouse CD 31 (PECAM-1) antibody (Pharmingen, San Diego, CA) was applied in a 1:20 dilution and was incubated at 4° C overnight. After thorough rinsing in TBS-Tween solution, slides were incubated with a biotinylated secondary antibody for 20 minutes followed by a 15-minute incubation with streptavidin peroxidase. For color development, slides were incubated for 5 minutes in DAB (3,3′-diaminobenzidine tetrahydrochloride). Microvessel density was quantified as described by Weidner et al.[27,28] Areas of highest neovascularization were found by scanning the sections at low power (×40 and ×100 total magnification). Individual microvessel counts were made on 10 ×200 fields (0.74 mm^2 per field).

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Continuous, normally distributed variables were analyzed by Student's *t* test. Discontinuous variables (dissemination score, microvessel density) were analyzed by the Mann-Whitney rank-sum test. Differences in survival and the development of ascites were analyzed by the chi-square test. $P < 0.05$ was considered statistically significant.

RESULTS

Volume of Primary Tumors

Monotherapy with A4.6.1 and BB-94 significantly decreased the volume of moderately differentiated HPAF-2 tumors to a similar extent (control: 3920 ± 495 mm^3; A4.6.1: 413 ± 71 mm^3; BB-94: 553 ± 27 mm^3; $P < 0.001$, respectively; Fig. 1, *A*). Combination of the anti-VEGF antibody and the MMP inhibitor resulted in a further reduction of primary tumor volume (206 ± 43 mm^3; $P < 0.05$ vs. A4.6.1 and BB-94; see Fig. 1, *A*).

Volumes of poorly differentiated AsPC-1 tumors (control: 1359 ± 148 mm^3) were reduced by either A4.6.1 (709 ± 107 mm^3; $P < 0.001$) or BB-94 (374 ±

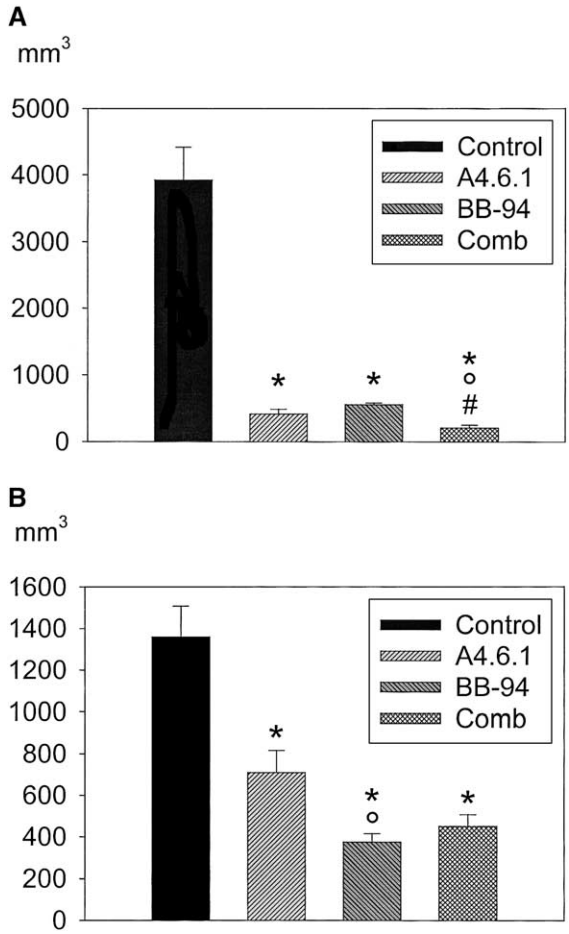

Fig. 1. Volume of the primary tumor in control mice and animals treated with A4.6.1, BB-94, or the combination therapy. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**). $P < 0.05$: *vs. control; ○ vs. A4.6.1; #vs. BB-94.

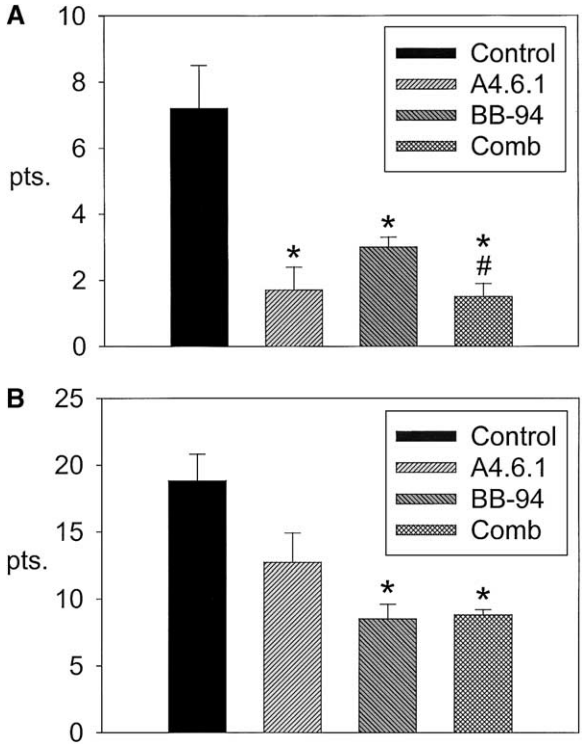

Fig. 2. Dissemination scores, quantifying local and distant tumor spread, in control mice and animals treated with A4.6.1, BB-94, or the combination therapy. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**). $P < 0.05$: *vs. control; ○ vs. A4.6.1; #vs. BB-94.

41 mm^3; $P < 0.001$); the difference between the two regimens was also statistically significant ($P < 0.05$). The effect of combination therapy (450 ± 57 mm^3) was comparable to that of the MMP inhibitor (Fig. 1, *B*).

Tumor Dissemination and Ascites

Local infiltration and distant metastasis were summarized by a dissemination score. Treatment with A4.6.1 (1.7 ± 0.7 points), BB-94 (3.0 ± 0.3 points), and the combination (1.5 ± 0.4 points) resulted in a similar reduction of tumor spread compared to HPAF-2 controls (7.2 ± 1.3 points; $P < 0.001$, respectively; Fig. 2, *A*). Control animals with tumors derived from the poorly differentiated AsPC-1 cell line reached the highest score (18.8 ± 2.0 points), and this was significantly reduced by BB-94 (8.5 ± 1.1 points; $P < 0.001$) and the combination therapy (8.8 ± 0.4 points; $P < 0.001$). The reduction of AsPC-1 tumor dissemination achieved by the anti-VEGF antibody (12.7 ± 2.2 points) did not reach statistical significance (Fig. 2, *B*).

Development of ascites occurred in the majority of HPAF-2 control mice (5 of 8). Fewer animals developed ascites after treatment with either A4.6.1 or BB-94 (2 of 8, respectively). No intra-abdominal fluid collections were found in animals subjected to the combination therapy ($P < 0.05$ vs. control; Fig. 3, *A*). Ascites were found in half of the control animals (4 of 8) with AsPC-1 tumors. Treatment with the anti-VEGF antibody, the MMP inhibitor, and the combination resulted in a tendency toward decreased production of ascites (Fig. 3, *B*).

Survival

Half of the animals in the HPAF-2 control group survived the 14-week observation period. In contrast, all animals in the treatment groups were alive at the end of the observation period (Fig. 4, *A*). Because of the limited number of animals in each group (n = 8), this difference was not statistically significant. The ag-

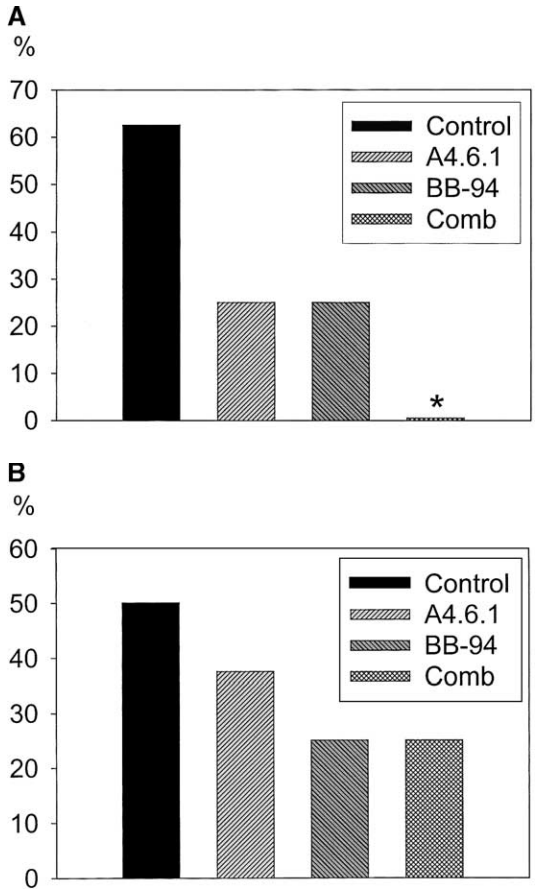

Fig. 3. Development of ascites in control mice and animals treated with A4.6.1, BB-94, or the combination therapy. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**). $P < 0.05$: *vs. control; ○ vs. A4.6.1; #vs. BB-94.

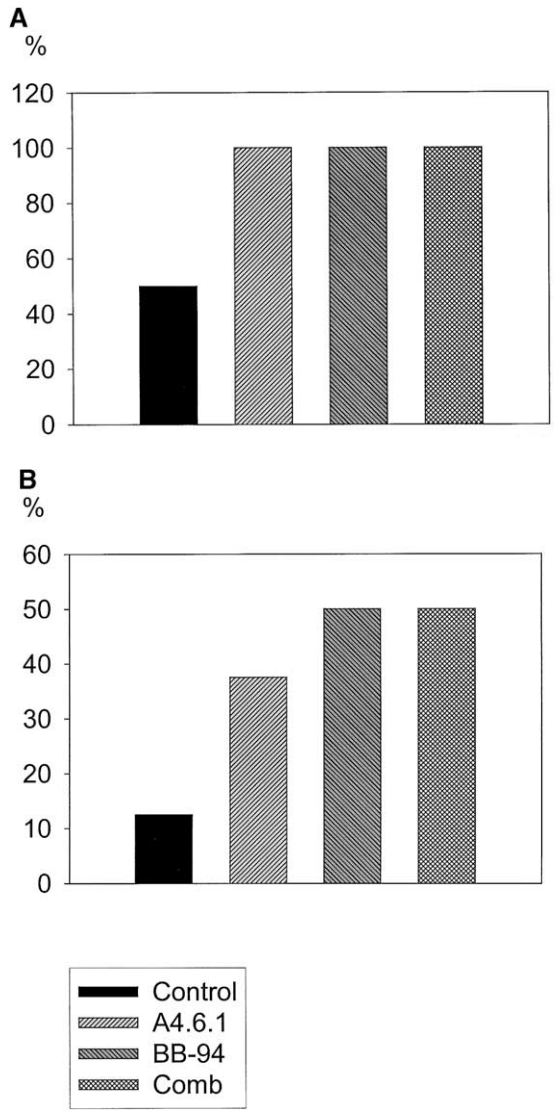

Fig. 4. Fourteen-week survival in control mice and animals treated with A4.6.1, BB-94, or the combination therapy. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**). $P < 0.05$: *vs. control; ○ vs. A4.6.1; #vs. BB-94.

gressive phenotype of AsPC-1 tumors was reflected by a low 14-week survival in the control group (1 out of 8 animals). Treatment with A4.6.1, BB-94, and the combination resulted in a tendency toward increased survival (Fig. 4, *B*).

Microvessel Density in Primary Tumors

Microvessel density as a parameter of angiogenic activity was significantly enhanced in the untreated primary tumors of both tested pancreatic cancer cell lines, compared to normal exocrine pancreas (HPAF-2: 81.9 ± 6.7/0.74 mm^2; AsPC-1: 70.9 ± 5.7/0.74 mm^2; native pancreas: 15.6 ± 1.5/0.74 mm^2; $P < 0.001$).

Neoangiogenesis in HPAF-2 tumors was most effectively reduced by VEGF inhibition (27.0 ± 2.3/0.74 mm^2; $P < 0.001$) and the combination therapy (32.5 ± 5.3/0.74 mm^2; $P < 0.001$). MMP inhibition alone was less effective but still reduced microvessel density compared to control values (56.9 ± 7.5/0.74 mm^2; $P < 0.01$; Fig. 5, *A*). Similar results were found in AsPC-1 tumors; a significant reduction of microvessel density was achieved by all treatment modalities but was most prominent in the A4.6.1 and combination groups (A4.6.1: 29.2 ± 3.2/0.74 mm^2; BB-94: 49.8 ± 4.6/0.74 mm^2; combination: 24.2 ± 2.7/0.74 mm^2; Fig. 5, *B*).

DISCUSSION

Blockade of proangiogenic mediators such as VEGF and inhibition of MMPs have emerged as promising

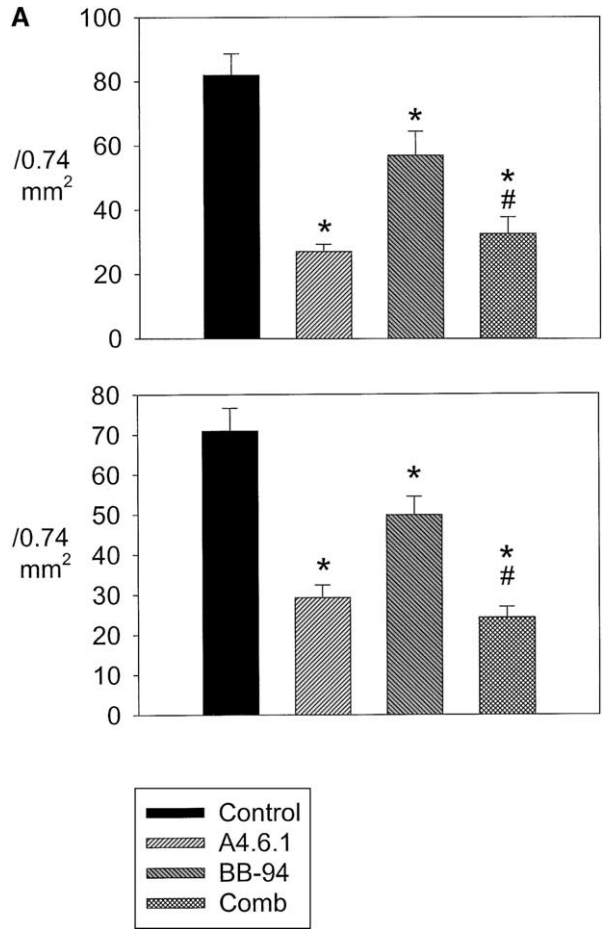

Fig. 5. Microvessel density in control mice and animals treated with A4.6.1, BB-94, or the combination therapy. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**). $P < 0.05$: *vs. control; ○ vs. A4.6.1; #vs. BB-94.

anticancer treatment strategies, especially for devastating malignancies such as exocrine pancreatic cancer, which are virtually noncurable by surgical resection and/or chemoradiation.[29–32] We and others have demonstrated the therapeutic potential of VEGF blockade in experimental pancreatic cancer[8–11,33]; inhibition of MMPs resulted in comparable effects.[16–18] It is a common feature of those monotherapeutic approaches that local tumor growth and metastasis is slowed down but not completely suppressed. This is not surprising, with regard to the complex multistep process of tumor progression in general and angiogenesis in particular, which is influenced by a multitude of regulating factors. Initial clinical studies have confirmed the limited potential of single-agent therapy in patients with advanced pancreatic cancer: the effect of Marimastat, an orally available broad-spectrum MMP inhibitor, on the 1-year survival rate was similar to that of the established cytotoxic deoxycytidine analogue gemcitabine.[34]

According to the National Cancer Institute, a phase II clinical study with an anti-VEGF antibody (vs. gemcitabine) is under way, but results have not been reported so far.

Our study evaluated, for the first time, a combination of VEGF blockade and MMP inhibition in an orthotopic nude mouse model of human pancreatic cancer and compared its effects on local growth, tumor dissemination, neoangiogenesis, and survival with the monotherapies, respectively. We thereby confirmed the similar therapeutic potential of A4.6.1, a high-affinity monoclonal antibody that recognizes all VEGF isoforms,[19] and BB-94, a synthetic hydroxamate with a broad spectrum of activity on MMPs,[35] including MMP-2 and MMP-9, which were detected at high levels in pancreatic cancer tissue.[36] Both single agents significantly reduced microvessel density as a parameter of neoangiogenesis in primary tumors (see Fig. 5), with VEGF blockade being more effective than MMP inhibition. A possible explanation may be provided by recent evidence that MMPs play a more complex role in angiogenesis, exerting not only proangiogenic but also antiangiogenic activity.[37,38] Although a large number of studies have demonstrated the antiangiogenic effect of MMP inhibitors,[39] MMPs have also been implicated in the generation of protein fragments that have angioinhibitory activity. Thus certain MMPs, including MMP-9, are capable of converting plasminogen into the potent endogenous angiogenesis inhibitor angiostatin.[40] The potential of antiangiogenic activities of MMPs in pancreatic cancer is not known; the net effect of MMP inhibition in our animal model still was a reduction of tumor neovascularization.

We studied the combination therapy in two in vivo settings. Tumors were derived from either moderately differentiated HPAF-2 cells or poorly differentiated AsPC-2 cells. VEGF blockade and MMP inhibition resulted in additive effects with regard to primary tumor volume (see Fig. 1, *A*) and development of ascites (see Fig. 3, *A*) in animals bearing HPAF-2 tumors, compared to the monotherapies. Tumor dissemination (see Fig. 2, *A*) and microvessel density (see Fig. 5, *A*) were not different from the most effective single-agent treatment. All three treatment modalities increased the 14-week survival in the HPAF-2 group from 50% to 100% (see Fig. 4, *A*). It was therefore not possible to determine an additional effect of the combination therapy on survival of HPAF-2 animals.

Poorly differentiated AsPC-1 tumors displayed a more aggressive growth pattern, killing seven out of eight control animals (see Fig. 4, *B*) within the 14-week observation period. VEGF blockade and MMP inhi-

bition alone resulted in a comparable tendency toward increased survival. However, because of the limited number of animals, this did not reach statistical significance. A combination of A4.6.1 and BB-94 yielded no additional effect on survival in animals with AsPC-1 tumors. Accordingly, the combined treatment did not exert beneficial effects on primary tumor volume (see Fig. 1, *B*), dissemination (see Fig. 2, *B*), development of ascites (see Fig. 3, *B*), and microvessel density (see Fig. 5, *B*) in the AsPC-1 group.

Possible explanations for the relatively disappointing results of combined VEGF/MMP inhbition in comparison to the monotherapies may include the fact that VEGF and MMPs share common pathways and interact within the process of tumor neovascularization and progression. VEGF has been identified as a MMP regulator, stimulating the production of MMPs in human endothelial cells,[37,41] vascular smooth muscle cells,[42] and certain tumor cells.[43] One may speculate that VEGF blockade will result in reduced MMP expression further downstream, thereby diminishing the therapeutic potential of additional MMP inhibition. Regulation of VEGF by MMPs has also been described: MMP-9 triggers the angiogenic switch in the RIP1-Tag2 transgenic model of pancreatic islet carcinogenesis by releasing VEGF.[44] Another reason for the limited effect of combined VEGF/MMP inhibition is the mode of action of both A4.6.1 and BB-94; these agents are not cytotoxic for pancreatic cancer cells at therapeutic concentrations.[41] Instead they exert cytostatic effects by modulating the environment of the tumor cells. It is probably more effective to combine either the neutralizing anti-VEGF antibody or the MMP inhibitor with cytotoxic agents. Haq et al.[45] recently reported favorable results after combining BB-94 with gemcitabine in a murine model of human pancreatic cancer. The observed differences after combination therapy between the two evaluated tumor types are difficult to interpret. They may be due to the different cellular differentiation. Whether distinct secretion patterns of MMPs and VEGF play a role in our experimental setting remains to be evaluated.

In summary, we have demonstrated that combined VEGF blockade and MMP inhibition exerts limited additional benefit in a (moderately differentiated) subgroup of experimental human pancreatic cancer in comparison to single-agent treatment. Further studies are necessary to systematically identify and evaluate more effective therapeutic combinations for this deadly malignancy.

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Discussion

Dr. K.S. Kirkwood (San Francisco, CA): Have you carried out these experiments for a longer period to see if the survival curves hold?

Dr. H. Hotz: No, we did not extend the observation period. It was obvious that even with the combination therapy we could not eradicate the tumor burden completely, as was found to be the case for monotherapies.

Dr. Kirkwood: In the first cell line, it looked as if the results achieved with the combination therapy were not substantially different from what was achieved with the anti-VEGF antibody alone, and obviously you had variable results with the second cell line. Can you correlate that to characteristics of the cell lines that might then allow you to predict what the results will be?

Dr. Hotz: These cell lines vary in terms of their differentiations. The first one is better differentiated. A possible explanation for that may be that VEGF and MMP share common pathways in terms of their effect on angiogenesis and tumor growth. For example, it has been shown for a variety of cell types that VEGF upregulates MMP, and one may speculate that if VEGF is blocked, MMP inhibition has no further use in this setting.

Dr. M. Korc (Irvine, CA): I was wondering if you had a chance to look at VEGF expression levels in the two cell lines and if you looked at whether it was VEGF-A, B, C, D, and so forth, and does your neutralizing antibody antagonize all of them?

Dr. Hotz: We have previously looked at VEGF expression in those cell lines, and the better differentiated HPAF-2 cell line produces more VEGF than AsPC-1. We have not looked for the other VEGF forms. It is known that this antibody antagonizes all known VEGFA isoforms.

Invited Discussion—Expert Commentator

L. William Traverso, M.D. (Seattle, WA): VEGF is overexpressed in almost all pancreatic cancers (90%). Even though VEGF is an abbreviation for "vascular endothelial growth factor," its effects are not limited to the promotion of growth in the vasculature of the tumor. MMPs promote the spread of tumor. The key to using antibodies to VEGF and MMP is to use the dosage that is just high enough to not cause side effects. Dr. Hotz and his colleagues must have used a dose analysis to achieve these excellent results—that is, they showed that antibodies to VEGF and MMP could slow or prevent the spread of pancreatic cancer and they worked better in combination.

Gene Transfer of Human Manganese Superoxide Dismutase Protects Small Intestinal Villi From Radiation Injury

Hong Liang Guo, Darren Wolfe, Michael W. Epperly, Shaohua Huang, Kaihong Liu, Joseph C. Glorioso, Joel Greenberger, David Blumberg

Small bowel toxicity represents a major dose-limiting side effect of radiation treatment for many malignancies. We examined the effects of overexpressing human manganese superoxide dismutase (MnSOD) in the small intestine in mice to prevent radiation enteritis. Mice were treated with the human MnSOD gene delivered enterally using a nontoxic, replication-defective herpes simplex virus (HSV)-1–based vector. HSV vectors containing the human MnSOD transgene and green fluorescent protein (GFP) transgene, or GFP transgene alone, were constructed and injected intraluminally into a 2cm length of small intestine of C3H/HeNsd mice. Total body irradiation of 15 Gy was delivered to mice inoculated 24 hours earlier with either HSV-MnSOD (10^3 to 10^8 plaque-forming units), control HSV-GFP, or no vector. At 24 or 72 hours after irradiation, mice were killed and villi areas were measured from appropriate segments of the small intestine. Control irradiated mice showed a decreased villi area of 82% by day 3 after irradiation, whereas treatment of mice with HSV-MnSOD 10^8 plaque-forming units led to only a 16% decrease in villi area ($P < 0.001$) before radiation. Similar findings were seen on day 3 and were associated with a significant ($P < 0.001$) preservation of enteric protein content in HSV-MnSOD–treated mice. A dose-dependent effect of MnSOD in preventing radiation-induced small bowel injury was evident. These data demonstrate that overexpression of human MnSOD via a replication-defective herpes viral vector is an efficacious method of protecting the small intestine from ionizing radiation damage. (J GASTROINTEST SURG 2003;7:229–236.)

KEY WORDS: Small intestine, radiation damage, gene therapy, MnSOD

Radiation, administered as total body irradiation, is commonly used to treat patients with hematologic malignancies undergoing bone marrow transplantation. Small bowel injury is a significant complication for these patients and represents a limiting factor to more effective therapy. Acute injury results in a breakdown of gut mucosal barrier characterized clinically by symptoms of nausea, vomiting, anorexia, diarrhea, and electrolyte imbalances. These symptoms may be significant enough to warrant cessation of treatment, thereby limiting the efficacy of therapy. Severe intestinal injury occurs in 3% to 9% of patients undergoing abdominal irradiation and leads to long-term sequelae.[1] Long-term complications occur at a mean of 3.4 years after radiation and include intestinal obstruction (71%), fistula (17%), perforation (10%), and hemorrhage (2%).[1,2] These complications result in mortality rates as high as 42% by 2 years after presentation.[2] Currently there is no effective method for reducing radiation injury to the small bowel.

The etiology of radiation-induced injury is complex and multifactorial. Traditional views hold that acute injury and chronic injury have mutually exclusive etiologies. Acute injury is thought to be the result of mitotic crypt cell death, disrupted epithelial barrier, and mucosal inflammation, whereas delayed chronic radiation injury is believed to result from vascular sclerosis and progressive intestinal wall fibrosis. Several studies have demonstrated that acute mucosal injury can, however, lead to chronic injury

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suggesting that a common etiology may account for acute and delayed radiation-induced small bowel injury.[3–5] Wang et al.[6] recently demonstrated that prevention of acute radiation-induced mucosal injury in rats significantly ameliorated the late-occurring radiation fibrosis seen in this model. Clinical studies in patients also indicate a greater incidence of chronic radiation injury in those individuals suffering acute mucosal injury.[7] Therefore therapies designed to protect the small bowel mucosa from radiation injury may have significant potential in preventing the late effects of radiation injury and decrease the significant morbidity and mortality of patients undergoing total body irradiation.

Several studies have suggested that the toxic acute effects of radiation on small bowel mucosa are mediated by oxygen free radicals.[8–10] Ionizing radiation causes hydrolysis of tissue H_2O giving rise to a hydroxyl radical, a proton, and an electron that is taken up by oxygen to form a superoxide radical (O_2^-).[11] Free radicals such as superoxide may directly cause oxidative cellular damage or serve as secondary messengers for induction of injury. Direct cellular injury may result from free radical–induced lipid peroxidation, depletion of energy stores, and oxidation of protective intracellular thiols such as glutathione.[11] Alternatively free-radicals may act as secondary messengers to initiate cytokine cascades involving interleukin-1, tumor necrosis factor-α, or transforming growth factor-β,[12–14] all of which have been implicated in the fibrosis associated with chronic radiation injury.[4]

Superoxide radicals are normally metabolized by the superoxide dismutases. Superoxide is converted by manganese superoxide dismutase (MnSOD) to H_2O_2, which may then be metabolized to H_2O by glutathione peroxidase or catalase. However, in pathologic conditions such as inflammation and radiation, superoxide production may be dramatically increased and overwhelm the host's detoxifying system resulting in significant pathology. Overexpression of genes regulating superoxide detoxification may provide potential therapeutic strategies to ameliorate pathologic states initiated by oxidative stress. In this study we hypothesized that overexpression of MnSOD would protect the small intestine from the acute effects of ionizing irradiation. We delivered the human MnSOD gene to the small bowel of female C3H/HeNsd mice by a nontoxic, replication-defective herpes simplex vector and showed significant protection of the small bowel from the acute effects of ionizing irradiation. Our results provide an exciting avenue for more effective radioprotection of the intestine during radiation therapy.

MATERIAL AND METHODS

Herpes Viral Constructs

The human MnSOD gene was placed under control of the human cytomegalovirus immediate early promoter (HCMV IEp) and flanked by herpes simplex virus (HSV) U_L41 sequences (93,858-92,230 and 91,631-90,145) to create a vector recombination construct engineered to express MnSOD. MnSOD was excised from the pRF5-MnSOD plasmid,[14] cutting the 3′ end of the transgene with *Pvu*I endonuclease and blunt-ending the 3′ end by incubating with T4 DNA polymerase for 5 minutes at 37° C. The 5′ end of the MnSOD transgene was cut with the restriction endonuclease *Eco*RI and the MnSOD transgene (750 bp fragment) isolated by electrophoresis on a 1% agarose gel and ligated to *Bgl*II linkers (CAGATCTG). After digestion with *Bgl*II, the MnSOD fragment was cloned into the *Bam*HI site of pH41 [15] that contains the UL41 sequences and the HCMV IEp. On conformation of the targeting construct, the plasmid was linearized with the *Not*I endonuclease and cotransfected with QOZHG vector DNA into complementing 7b cells.[16] QOZHG vector contains deletions in the essential immediate early genes ICP4 and ICP27, a promoter modification of the ICP22 and ICP47 genes that confine their expression to complementing 7b cells. An ICP0 IEp:lacZ cassette was inserted into the UL41 locus, and a HCMV IEp:EGFP cassette was inserted into the ICP27 locus. Recombinant viral plaques that did not stain with X-gal were subjected to three rounds of single plaque purification. The genetic structure of HSV-MnSOD-GFP vector, compared to parental QOZHG, was confirmed by Southern blot analysis of viral DNA digested with the *Sph*I endonuclease and probed with the MnSOD gene fragment. Vector stocks (QOZHG and HSV-MnSOD–green fluorescence protein [GFP]) were batch produced in five roller bottles, each of which was infected at a multiplicity of 0.05, harvested when the cytopathic effect was evident (2 to 3 days), and purified on a continuous nycodenze gradient (30%). Ten-microliter aliquots of vector stocks were frozen and stored at −80° C. Plaque-forming unit titers of vector stocks were determined in duplicate on complementing 7b cells using serial dilutions of vector stock.

Determination of Transgene Activity

Female C3H/HeNsd mice underwent laparotomy, and the small intestine was injected with HSV-GFP or HSV-MnSOD-GFP. The mice were killed at 24 hours, and the injected small intestine was excised and immediately frozen in liquid nitrogen. MnSOD activity was measured as previously described[17]: briefly, the small in-

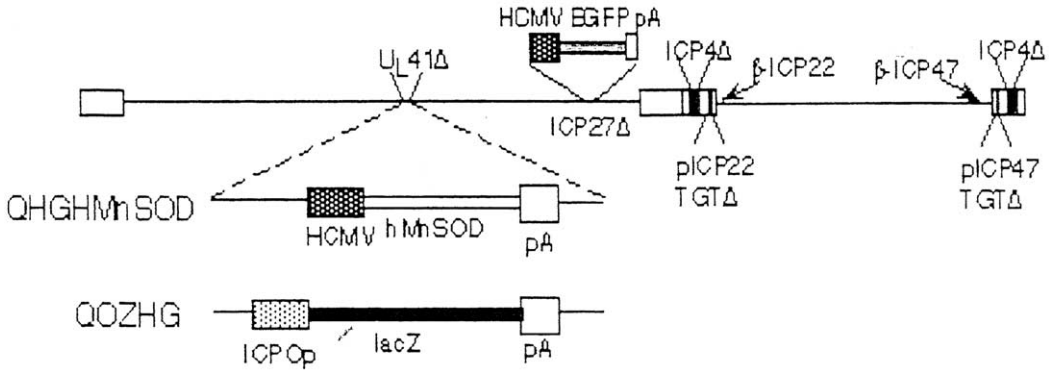

Fig. 1. Schematic of recombinant vectors. QOZHG has deletions in the essential ICP4 and ICP27 genes. The promoters for ICP22 and ICP47 contain deletions of the TATGARAT element (*TGT*Δ) which confines expression to complementing cells. Transgene expression cassettes are inserted into the ICP27 locus (GFP) and UL41 (lacZ for QOZHG; MnSOD for QHGHMnSOD).

testine was thawed and homogenized using a polytron PT2000 homogenizer. Protein (500 mg) was added to assay tubes containing 20 mmol/L Tris (pH 7.6), 1 mmol/L diethylenetraminepentaacidic acid (DEPATAC), 1 U of catalase, 5.6×10^{-8} mol/L nitroblue tetrazolium, 0.1 mmol/L xanthine, 0.05 mmol/L bathocuproine disulfonic acid (BCS), 0.13 mg/ml defatted bovine serum albumin, and 5 mmol/L sodium cyanide. The samples were incubated for 45 minutes at 25° C. Xanthine oxidase was added as the superoxide donor, and the change in absorbance at 560 nm was measured spectrophotometrically. One unit of activity resulted in 50% inhibition of nitroblue tetrazolium reduction.

Histopathology

To evaluate the protection of herpes viral gene transfer of MnSOD, histopathologic examination was performed after female C3H/HeNsd mice were treated with a single dose of radiation (15Gy) with graded concentrations of vector. Mice were instilled with varied concentrations of HSV-MnSOD vector or no vector and irradiated with 15.0 Gy. The site of intestine injected was marked with a suture placed on the adjacent mesentery. Small intestine was harvested at 24 and 72 hours after irradiation. The injected intestinal segment was removed, washed with ornithine carbamoyl transferase (OCT), and placed in a base mold and frozen in OCT. Three vertical sections (5 μ thick) on one slide and three slides for each intestine were prepared using a Shandon AS620E cryotome (Thermo Electron Corp., Waltham, MA). The sections were stained with hematoxylin and eosin by dipping the slides in Harris hematoxylin for 1 minute and 45 seconds, rinsing with tap water until clear, dipping in eosin for 1 minute, and rinsing with water. The slides were allowed to air dry and then dipped twice in 95% ethanol, twice in 100% ethanol, twice in 50:50 ethanol:xylene, and twice in 100% xylene. The slides were mounted using Permamount and cross sections were microscopically examined. The area of intestinal villi was determined using a computerized software program. Protein content (mg/weight tissue) of appropriate sections was measured by Bio-Rad assay (Bio-Rad Laboratories, Hercules, CA).

Statistical Analysis

Comparisons between groups were made by one-way analysis of variance (ANOVA) using SPSS software. Significance was defined as $P < 0.05$.

RESULTS

Detection of MnSOD Transgene Expression

To detect MnSOD expression in small intestine injected with HSV-MnSOD-GFP, MnSOD biochemical activity was measured at 24 hours after intraintestinal injection of HSV-MnSOD-GFP (n = 4 mice) or HSV-GFP (n = 4 mice). MnSOD activity was measured in frozen sections of small intestine by inhibition of nitroblue tetrazolium reduction, with xanthine oxidase as the superoxide generator. Small intestine injected with HSV-MnSOD had a significantly ($P < 0.001$) greater percentage of inhibition (39.33 ± 0.67) than intestine injected with HSV-GFP (25.00 ± 1.00), indicating increased MnSOD activity with MnSOD transgene expression.

Protein Determination

Total body irradiation with 7.5 Gy resulted in a 64% decrease in small bowel protein content from

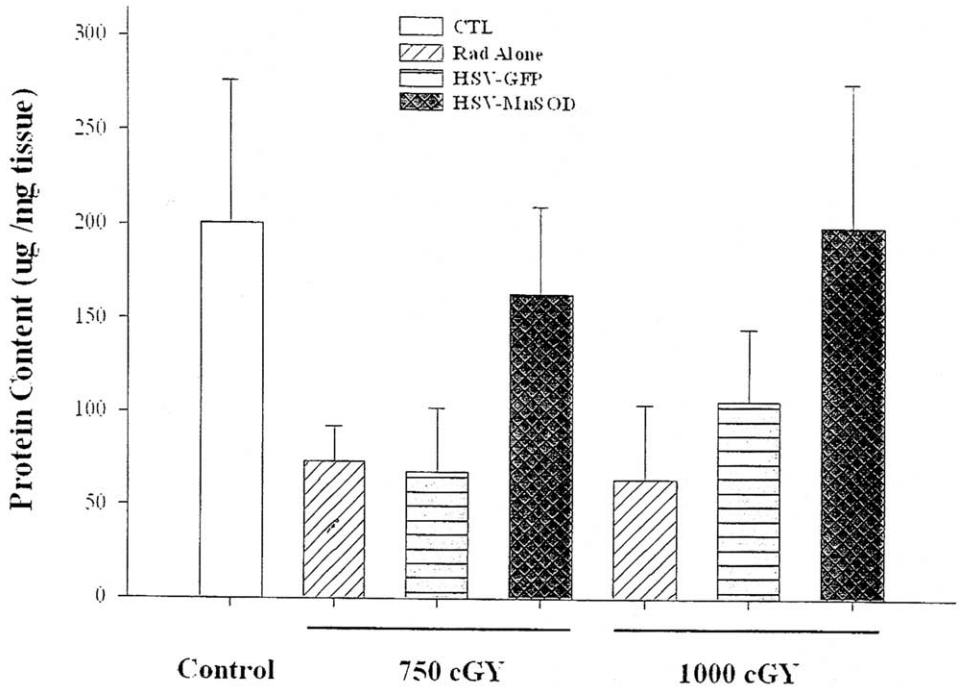

Fig. 2. Protein content (μg/mg tissue) of small bowel (10 cm from terminal ileum) harvested 72 hours after irradiation (7.5 Gy or 10 Gy) in mice treated with HSV-MnSOD, HSV-MnSOD, or no vector and compared to unirradiated control mice. Protein content of the small bowel in mice treated with HSV-MnSOD was significantly ($P < 0.001$) different from mice treated with either HSV-GFP or no vector, but was not different from unirradiated small bowel.

untreated control values ($P = 0.01$; Fig. 2). Treatment of small bowel with HSV-MnSOD before irradiation led to complete preservation of small bowel protein content ($P = 0.52$; HSV-MnSOD vs. untreated control samples). In contrast, treatment of small bowel with HSV-GFP provided no protection from radiation injury with a 66% reduction in protein content after irradiation. Similar results were found with experiments carried out at 10 Gy. At 10 Gy, treatment of small bowel with HSV-MnSOD before irradiation led to no significant change ($P = 0.06$) in protein content compared to untreated control values. In contrast, intraluminal instillation of HSV-GFP before irradiation led to a 47% decrease in protein content, which was similar ($P = 0.59$) to treatment with radiation alone.

Effect of MnSOD Overexpression in Modulating Radiation-Induced Small Bowel Villi Damage

To further evaluate the radioprotection of herpes viral gene transfer of MnSOD, routine histopathologic examination using hematoxylin and eosin was performed on sections of small intestine, and villi area was determined. We examined whether treatment of mice with HSV-MnSOD affected radiation-induced small bowel injury in a dose-dependent manner. Mice (n = 8 per group) were instilled with varied concentrations of HSV-MnSOD vector (10^3 to 10^8 plaque-forming units) or sham laparotomy (n = 8 per group) and then, 24 hours later, they were irradiated with a single dose of radiation (15.0 Gy). Small intestine was harvested at 24 and 72 hours after irradiation and sectioned. Villi area were measured and compared to villi from unirradiated mice (n = 4).

At 24 hours, radiation led to a significant ($P < 0.001$) reduction (81%) in small bowel villi area (sham-operated vs. unirradiated control mice). Intraluminal administration of HSV-MnSOD vector (10^3 to 10^8 plaque-forming units) into the small bowel led to a significant ($P < 0.001$) dose-dependent increase in preservation of mean villi area after irradiation (24 hours) with respective mean villi areas of 47%, 57%, 69%, and 84% of unirradiated control small bowel (Fig. 3). At 72 hours, the mean villi area of mice treated with HSV-MnSOD vector (10^8) was 60% of unirradiated control mice, which was significantly ($P < 0.001$) greater compared to mice irradiated and not receiving HSV-MnSOD (mean villi area = 28% of unirradiated control values). Similar to 24 hours, the effects of intraluminal instillation of HSV-MnSOD (10^3 to 10^8 plaque-forming units) were dose depen-

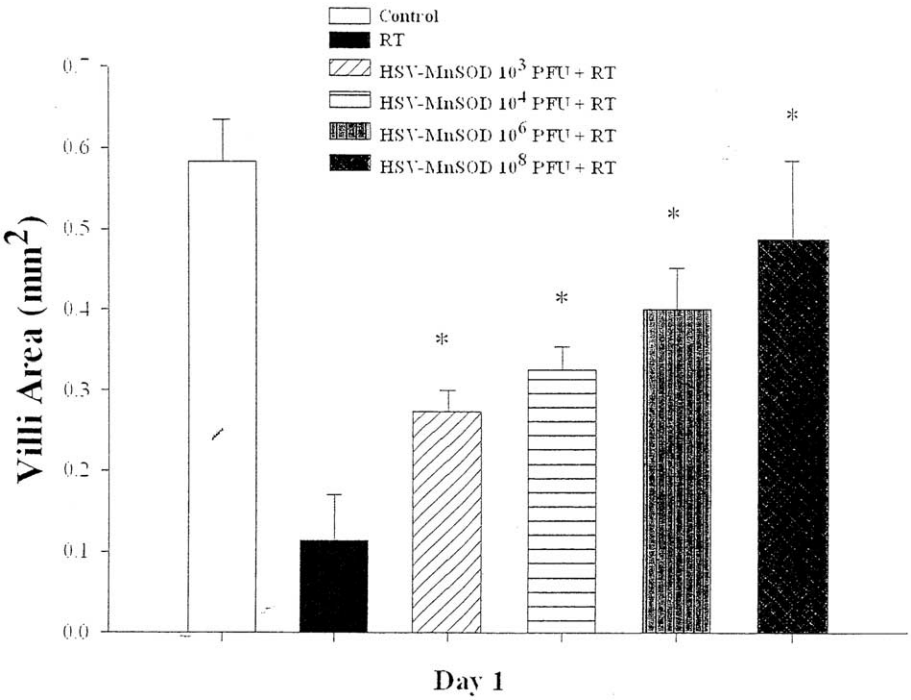

Fig. 3. Villi area 24 hours after irradiation (15 Gy) of mice treated with varying doses of HSV-MnSOD (10^3 to 10^8 plaque-forming units) or radiation alone, and compared to control unirradiated mice. *$P < 0.001$ vs. radiation alone.

dent ($P < 0.001$) at 72 hours in protecting small bowel from total body irradiation (Fig. 4).

DISCUSSION

Small bowel injury continues to be a major complication in cancer patients treated with radiation. Because radiation injury is initiated, in part, by generation of superoxide radicals, and HSV vectors are effective at infection of mucosal tissues,[18] we hypothesized that overexpression of MnSOD from a replication-defective vector could protect the small intestine from radiation. We constructed a herpes viral vector carrying the human MnSOD gene and tested whether overexpression of MnSOD in the small intestine of C3H/HENsd mice could protect against radiation injury. HSV-MnSOD was instilled into the terminal ileum of the small bowel, the segment most commonly injured by abdominal irradiation in patients.[19] Total body irradiation was administered at a dose of 7.5 to 15 Gy, and the small intestine was examined for injury at 24 and 72 hours thereafter. The small intestine was successfully transduced with HSV-MnSOD as evidenced by increased MnSOD biochemical activity in transduced segments.

We hypothesized that MnSOD overexpression might be radioprotective in the small intestine because of overexpression of MnSOD protective of the esophagus and lung from radiation injury in other experimental models.[12–14] However, the small intestine differs from other organs in that the response to radiation injury is often more severe.[20] Despite specific differences of the small intestine in response to radiation injury, we found that overexpression of MnSOD by herpes viral transduction leads to significant protection against radiation injury. Overexpression of MnSOD led to significant small bowel radioprotection, as evidenced by maintenance of small bowel protein content and villi area in mice treated with HSV-MnSOD. By instilling varying amounts of HSV-MnSOD into the small bowel, we further demonstrated that the radioprotection associated with MnSOD overexpression occurs in a dose-dependent fashion. Taken together, our data indicate that herpes viral gene transfer of human MnSOD into the small intestine of mice leads to radioprotection secondary to overexpression of active MnSOD protein.

Because MnSOD overexpression led to significant radioprotection, our data also suggest an important role for the superoxide radical as a mediator of radiation-induced small bowel injury. An alternative mechanism by which MnSOD overexpression may have protected the small intestine from radiation in this model is by downregulating the proinflammatory cytokines. Many studies have implicated a role for various cytokines such

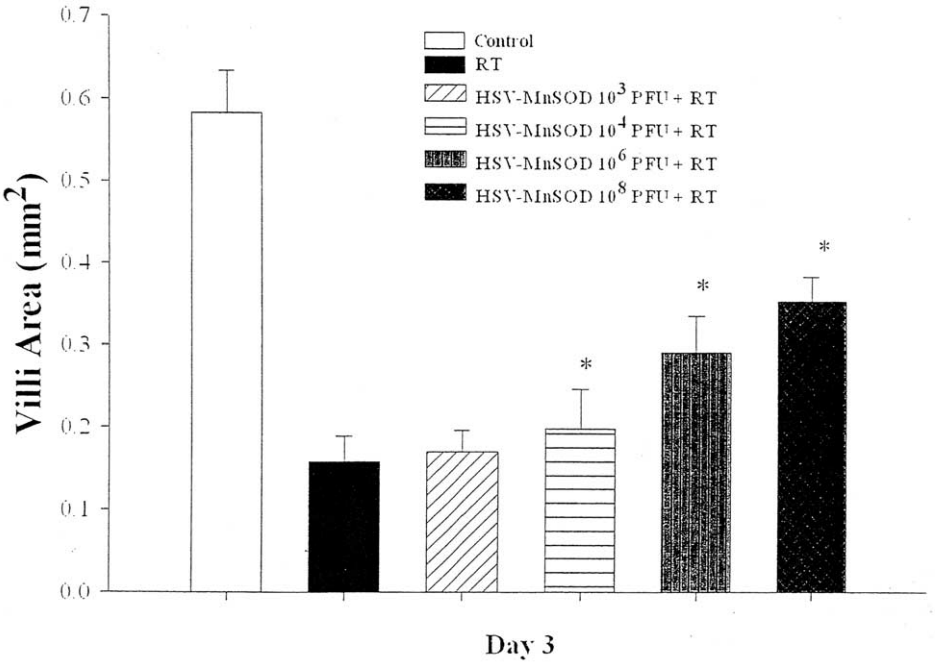

Fig. 4. Villi area 72 hours after irradiation (15 Gy) of mice treated with varying doses of HSV-MnSOD (10^3 to 10^8 plaque-forming units) or radiation alone, and compared to control unirradiated mice. *$P < 0.001$ vs. radiation alone.

as tumor necrosis factor–alpha, interleukin-1, platelet-derived growth factor, and transforming growth factor–beta-1 as mediators of small bowel injury induced by irradiation.[21,22] MnSOD overexpression in other tissues has been associated with suppression of these cytokines.[12–14] Studies examining the effects of MnSOD overexpression on cytokines after injury to the small bowel, however, are sparse.

We have demonstrated that herpes viral gene transfer is an efficacious approach for delivering MnSOD to the small intestine to prevent radiation-induced injury. Overexpression of MnSOD in a segment of the terminal ileum, the most common segment of small bowel affected by irradiation, allows for significant radioprotection. MnSOD overexpression in the small intestine is a promising strategy that has the potential to avert the long-term sequelae of radiation-induced small bowel injury.

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Discussion

Dr. D.I. Soybel (Boston, MA): The use of the GFP marker and the herpes virus transfection from the lumen is really a very nice addition to the armamentarium of the gastrointestinal investigator. My question is, did you look at the microvasculature, since the long-term issues in radiation enteritis are thought to be associated with the vasculitis? I wondered if you had a chance to look at the staining of vascular endothelial growth factor or factor VIII and that sort of thing and see what had happened to the architecture of the endothelium and the microvasculature.

Dr. D. Blumberg: Traditional views hold that acute injury is a mucosal injury, and that chronic injury is the result of damage to the microvasculature of the intestine. However, there are data from a number of studies indicating that if the acute mucosal injury is inhibited, then the small bowel is protected from chronic fibrosis. We are speculating that protection of the mucosa by overexpression of MnSOD will also lead to decreased fibrosis. We have shown that overexpression of MnSOD in the small intestinal villi leads to profound protection of the small bowel in acute experiments. We speculate that the mechanism accounting for the radioprotection induced by overexpression of MnSOD is either scavenging of free oxygen radicals produced by irradiation or suppressing cytokine release. In one recent report (Zheng H, et al. Gastroenterology 2000;119:1286–1296), blockade of transforming growth factor (TGF)–β upregulation in the small intestine ameliorated radiation-induced fibrosis. MnSOD has previously been shown to also inhibit TFG-β upregulation. For these reasons and the fact that delivery of the MnSOD gene intraluminally is technically simple, we speculate that gene delivery of MnSOD intraluminally has much promise in preventing the long-term side effects of radiation in the small intestine.

Dr. M.G. Sarr (Rochester, MN): Have you thought about trying to give some protective substance intravascularly? I had the same question as Dr. Soybel. There is the acute injury and the chronic injury. Your group is wonderful at designing experiments based on your interests in nitric oxide. Could you come up with a "cocktail" that is both intraluminal and intravascular?

Dr. Blumberg: Our next goal is to characterize the long-term effects of intraluminal administration of HSV-MnSOD on radiation-induced fibrosis. It will be interesting to pursue additional studies after this to compare the effects of intravascular delivery of MnSOD to intraluminal delivery. If the herpes vector is given intravenously, we would expect most of it to end up in the liver. Therefore, to study the question you are raising would probably require selective injection of the vector into the splanchnic circulation.

Dr. Sarr: This might actually get at the pathogenesis, because why does the vascular injury progress? Is that something that is induced initially with the radiation? Also, if you can block that initial injury, maybe you will prevent this long-term obliterative endarteritis.

Dr. Blumberg: We are very excited about these preliminary data and will continue to study the long-term effects of overexpression of MnSOD in the small bowel prior to irradiation.

Invited Discussion—Expert Commentator

Richard A. Hodin, M.D.: This work employed a gene therapy approach in mice to try and protect the gut from radiation-induced damage. Because of its rapidly and constantly renewing epithelium, the intestine is among the most damaged end organs in patients exposed to systemic insults such as whole-body irradiation. In an attempt to minimize the intestinal damage in mice, a gene therapy approach was used that involved overexpression of the MnSOD gene. Compared to control mice, the MnSOD intestines demonstrated increased protein content, indicating at least a relative protection from the effects of radiation. These animal studies are very interesting in that a single gene product was successfully delivered to the target organ and beneficial effects were demonstrated. Such an approach could theoretically be used in a variety of clinical settings in which an insult to the intestine is planned to occur (e.g., chemotherapy, vascular procedures, etc.).

In general, the promise of gene therapy has yet to be realized. The enthusiasm of a decade ago has been tempered by all of the complexities and difficulties encountered in this field. However, it is worth pointing out that the gut may be an ideal target for gene therapy. It is readily accessible, could be treated repeatedly if needed, and it has an enormous capacity to repair and regenerate itself. So, one can imagine a successful gene therapy approach in the gut that would be used for a specific window in time, as in the clinical scenario modeled in this paper. Hopefully, we will see more work in the future on gut gene therapy from this group and others.

Transcriptional Activation of the Enterocyte Differentiation Marker Intestinal Alkaline Phosphatase Is Associated With Changes in the Acetylation State of Histone H3 at a Specific Site Within Its Promoter Region In Vitro

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Enterocyte differentiation is thought to occur through the transcriptional regulation of a small subset of specific genes. A recent growing body of evidence indicates that post-translational modifications of chromatin proteins (histones) play an important role in the control of gene transcription. Previous work has demonstrated that one such modification, histone acetylation, occurs in an in vitro model of enterocyte differentiation, butyrate-treated HT-29 cells. In the present work, we sought to determine if the epigenetic signal of histone acetylation occurs in an identifiable pattern in association with the transcriptional activation of the enterocyte differentiation marker gene intestinal alkaline phosphatase (IAP). HT-29 cells were maintained under standard culture conditions and differentiated with sodium butyrate. The chromatin immunoprecipitation (ChIP) assay was used to compare the acetylation state of histones associated with specific regions of the IAP promoter in the two cell populations (undifferentiated vs. differentiated). Chromatin was extracted from cells and cleaved by sonication or enzymatic digestion to obtain fragments of approximately 200 to 600 base-pairs, as confirmed by polymerase chain reaction using primers designed to amplify the IAP segments of interest. The ChIP assay selects DNA sequences that are associated with acetylated histones by immunoprecipitation. Unbound segments represent DNA sequences whose histones are not acetylated. After immunoprecipitation, sequences were detected by radiolabeled polymerase chain reaction, and the relative intensity of the bands was quantified by densitometry. The relative acetylation state of histones at specific sites was determined by comparing the ratios of bound/unbound segments. We determined that in a segment of the IAP promoter between −378 and −303 base-pairs upstream from the transcriptional start site, the acetylation state of histone H3 increased twofold in the differentiated, IAP expressing cells, whereas that of histone H4 remained essentially constant. Additionally, at a distant site, between −1378 and −1303 base-pairs, the acetylation state of H3 and H4 did not change appreciably between the undifferentiated and differentiated cells. We conclude that butyrate-induced differentiation is associated with specific and localized changes in the histone acetylation state within the IAP promoter. These changes within the endogenous IAP gene may underlie its transcriptional activation in the context of the enterocyte differentiation program. (J GASTROINTEST SURG 2003;7:237–245.)

KEY WORDS: Small intestine, differentiation, histone acetylation, chromatin immunoprecipitation

Our understanding of the molecular mechanisms responsible for regulating eukaryotic gene transcription has been rapidly and dramatically expanded by a growing body of evidence supporting the concept of an "epigenetic code" that helps mediate this essential process.[1] This new area of investigation represents a radical departure from the central dogma of cellular biology in that it suggests that information can be

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encoded in the post-translational modifications of proteins and that this code can, in turn, be read and acted on by families of nuclear proteins. The histone proteins that were previously thought to serve merely as scaffolding to package huge amounts of DNA within the confines of the nucleus are now believed to be the central players in storing and transmitting epigenetic information.[2] In fact, it has been known for quite some time that one histone modification, acetylation, is observed in transcriptionally active chromatin or euchromatin.[3] This association was believed to be generalized and nonspecific, because acetylation of histones leads to a "looser" chromatin structure allowing for the binding of transcription factors.[4] However, recent evidence indicates that these modifications represent localized and specific changes that, in turn, regulate gene transcription.[5,6]

In the present work we have used a genetic marker of enterocyte differentiation, the intestinal alkaline phosphatase (IAP) gene, and the relatively novel technique of chromatin immunoprecipitation, to determine changes in the histone acetylation state in specific sequences of the IAP promoter region during differentiation in vitro. We have used a well-characterized model system of enterocyte differentiation, butyrate-treated HT-29 cells. Previous work from our laboratory has pointed to the important role of generalized histone acetylation in this model system.[7,8] The present work is the first description of histone alterations that occur at a specific genetic locus in the context of enterocyte differentiation. Our results suggest that alterations of the histone acetylation state within a specific segment of the IAP gene may play an important role in mediating its transcriptional activity.

MATERIAL AND METHODS

Cell Culture

HT-29 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA) and maintained in standard Dulbecco's modified Eagle medium (Gibco BRL, Rockville, MD) with 10% fetal bovine serum, 2 mmol/L glutamine, and 100 U/ml penicillin-streptomycin (Bio-Whittaker, Walkersville, MD) at 37° C and 5% CO_2. Experiments were performed with cells at 70% confluence. Medium was changed every 3 days and just before each experiment. Differentiation was induced by adding sodium butyrate (Sigma, St. Louis, MO) to the medium to a final concentration of 5 mmol/L for 24 hours.

Chromatin Immunoprecipitation Assay

DNA was cross-linked to its associated proteins by adding formaldehyde directly to the culture medium to a final concentration of 1% volume:volume (v:v) (Sigma) and incubating the cells in the resulting solution at 37° C for 10 minutes. After this incubation, the cells were washed twice with ice-cold 1× phosphate-buffered saline solution, pH 7.4 (Gibco, Grand Island, NY), containing 100 mmol/L each of the protease inhibitors phenylmethylsulfonylfluoride (PMSF) and benzamidine (Sigma). After the second wash, the cells were collected, briefly centrifuged, and resuspended in lysis buffer (1% sodium dodecyl sulfate, weight:volume [w:v]; 10 mmol/L EDTA; and 50 mmol/L tris, pH 8.1, Sigma) for 10 minutes on ice. The lysate was then subjected to sonication using a Fisher model 300 Sonic Dismembrator with a 2 mm microtip (Fisher Scientific, Pittsburgh, PA) for three 10-second pulses at a setting of 30%, with cooling on ice between pulses or to digestion with 500 units/ml micrococcal nuclease (Mnase; Worthington, Lakewood, NJ). The cleaved lysate was then centrifuged at 13,000 rpm for 10 minutes at 4° C, after which the supernate was diluted 20-fold in immunoprecipitation buffer (0.01% sodium dodecyl sulfate, w:v; 1.1% triton-X100, v:v; 1.2 mmol/L EDTA; 16.7 mmol/L tris; and 16.7 mmol/L NaCl, pH 8.1, Sigma) and precleared by adding 80 μl of Salmon Sperm DNA/Protein A Agarose–50% Slurry (Upstate, Lake Placid, NY) and rotating for 30 minutes at 4° C. After a brief centrifugation, the supernate was transferred to a new tube and subjected to immunoprecipitation with 5 μg of the appropriate anti-acetylated histone antibody (Upstate) overnight with rotation at 4° C in accordance with the instructions provided by the manufacturer. These conditions were confirmed as optimum by experiments using different quantities of antibody. The following day, the immune complexes were collected by adding 60 μl of the Salmon Sperm DNA/Protein A Agarose–50% Slurry and rotating for 1 hour at 4° C followed by a brief centrifugation. The supernate was kept as the unbound fraction, and the pellet (containing the bound fraction) was sequentially washed with high-salt, low-salt, and LiCl immune complex wash buffers (Upstate) followed by TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0; Sigma). After a second wash with TE, the immune complexes were eluted in 1% sodium dodecyl sulfate in 0.1 mol/L $NaHCO_3$ (Sigma) by rotating at room temperature twice for 15 minutes and pooling the supernates. The formaldehyde-induced cross-links were then reversed by adding 20 μl of 5 mol/L NaCl (Sigma) to each sample and incubating at 65° C for 4 hours. DNA was then purified by standard phenol/chloroform extraction techniques using 20 μg of glycogen (Sigma) as a carrier.

Polymerase Chain Reaction

DNA purified in the ChIP assay was reconstituted in TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0; Sigma) in various concentrations, which were then used as the template for amplification in polymerase chain reactions (PCRs). Forward and reverse primers were designed based on the published human IAP gene 5′ flanking region and were obtained from Gibco.[9] Primer pairs were designed for approximately 75 base-pair (bp) segments along the length of the 2.5 kilobase (kb) IAP promoter sequence. Each primer pair was tested by using plasmid DNA containing a 2.5 kb segment of the IAP 5′ flanking sequence. Then 100 pmol of each primer was added in a 1 μl volume along with 1 μl of the appropriate amount of template DNA. Preliminary experiments, in which different quantities of template DNA in increasing amounts were added to the PCR mix, were performed to determine the correct amount of DNA with which to obtain the amplified PCR products without reaching the plateau phase of PCR amplification. A total of 45 μl of PCR supermix (Gibco) was added to the primer/template mixture along with 1 μl (10 μCi) each of 33P α-dCTP and ^{33}P α-dATP (10 μCi/μl, PerkinElmer, Boston, MA). Reactions were carried out in a Peltier PTC-200 thermocycler (MJ Research, Waltham, MA) for 30 cycles at 94° C for 30 seconds, 55° C for 30 seconds, and 72° C for 1 minute. Samples were then stored at 4° C until ready for analysis. Amplified PCR products were loaded onto 10% native polyacrylamide gels (10% acrylamide + 2% bis-acrylamide, v:v in 1X TBE (Boston BioProducts, Ashland, MA) cross-linked with ammonium persulfate and TEMED (Bio-Rad, Richmond, CA). The gels were electrophoresed at 10 mV/cm of gel for 4 hours, after which they were dried and subjected to autoradiography.

Densitometry

An Arcus II scanner (AGFA, Ridgefield Park, NJ) was used to scan the autoradiographs of gels, and observed bands were quantified using the National Institutes of Health Image 1.62 software. The results from at least three experiments were then averaged and subjected to statistical analysis using a standard one-way analysis of variance with Dunnet's post-test (InStat software; GraphPad Software, Inc., San Diego, CA) with $P < 0.05$ considered significant.

RESULTS

Design of PCR Primer Pairs for the IAP Promoter

Fig. 1, *A* lists the sequences of the forward and reverse primers used to amplify two different regions of the human IAP promoter. The first pair amplifies a segment between −378 and −303 bp upstream

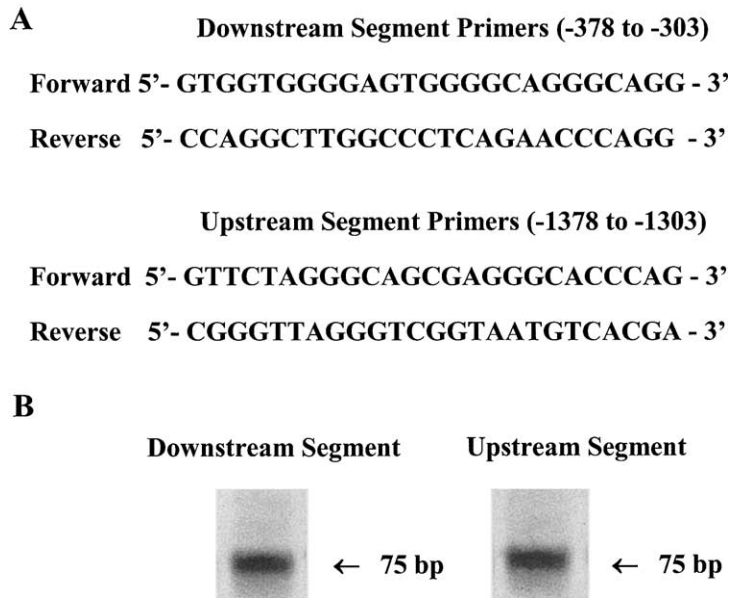

Fig. 1. Amplification of two specific regions of the human IAP promoter by PCR. **A,** The forward and reverse primers used to amplify each segment are listed. Based on the published IAP promoter sequence, both sets of primer pairs are predicted to amplify a 75 bp segment.[11] **B,** The PCR products generated using each primer pair and plasmid DNA containing the human IAP promoter as a template.

from the transcriptional start site, whereas the second pair amplifies between −1378 and −1303. In both cases, the expected PCR product is 75 bp. The primer sequences are based on the published human IAP 5′ flanking sequence.[9] Fig. 1, *B* shows the products of PCR using plasmid DNA containing a 2.5 kb segment of the human IAP promoter. The PCR results of these particular primer pairs are representative of the results obtained for all of the primer pairs designed for the 2.5 kb region of the IAP promoter. These bands were the expected 75 bp in size and demonstrate the efficacy of the primer pairs and PCR conditions.

Verification of Chromatin Cleavage

Extracted chromatin was cleaved by sonication or enzymatic digestion. It is important for chromatin to be cleaved into small enough fragments both for the antibodies against acetylated histones to gain access to their epitopes and to prevent immunoprecipitation (and subsequent detection) of segments far removed from the area of immune complex formation. The ideal size of segments is between 200 and 600 bp, because this size maximizes the efficacy of the immunoselection while at the same time allowing detection of relatively localized sequences. To verify the adequacy of our sonication, PCR was performed using primer pairs that amplify variously sized segments of the IAP promoter on extracted chromatin that was subjected to increasing levels of sonication. Fig. 2 shows the result of increasing cleavage conditions. With a greater degree of cleavage, the larger fragments were unable to be amplified, verifying that the template DNA was being cleaved within the sequence between the more distant primer pairs.

Histone H3 acetylation increases during differentiation-induced IAP activation within a specific proximal promoter sequence. Initial ChIP experiments were performed using primer pairs separated by 200 bp segments along the 2.5 kb promoter sequence. The results of these initial experiments prompted us to focus on a proximal and distal promoter sequence. Fig. 3 demonstrates the results of the ChIP assay performed on butyrate-treated (differentiated) vs. untreated (undifferentiated) HT-29 cells. Shown are representative bands from at least three experiments. Primers designed to amplify a segment of the IAP

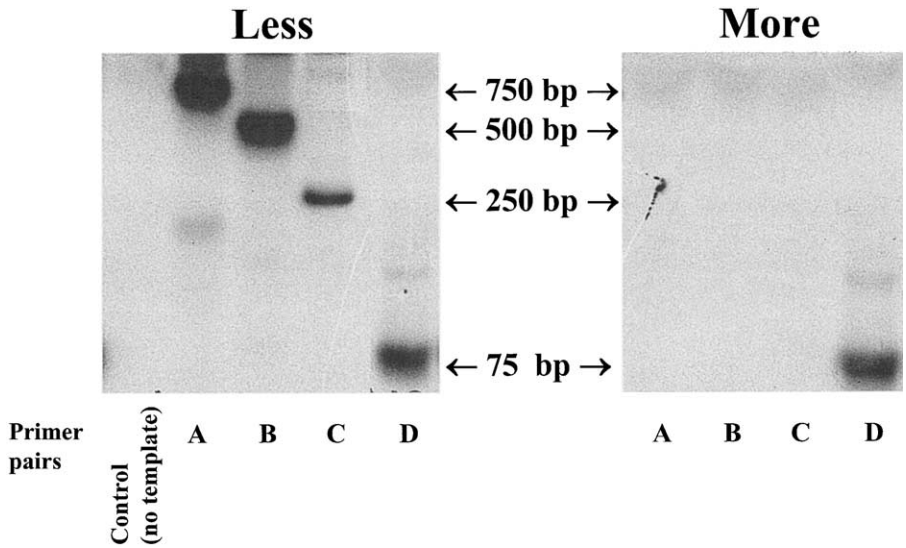

Fig. 2. PCR analysis of the effect of increasing degrees of sonication on the detection of larger segments. Shown are the PCR products using various IAP promoter primer pairs designed to amplify segments of varying size. Primers *A* (forward: 5′-cta cgt agg gcc tct tgt att att t-3′; reverse: 5′-cca ggc ttg gcc ctc aga acc cag g-3′) amplify a segment 750 bp in length between −1977 and −1227 bp upstream from the transcriptional start site. Primers *B* (forward: 5′-tcc tga cct cat gat cca ccc acc t-3′; reverse: same as for primers *A)* amplify a 500 bp segment between −1727 bp and −1227 bp. Primers *C* (forward: 5′-cta act cag caa gat gaa gca gga g-3′; reverse: same as for primers *A*) amplify a 250 bp segment between −1477 bp and −1227 bp. Primers *D* (forward: 5′-gtg gtg ggg agt ggg gca ggg cag g-3′; reverse: same as for primers *A*) amplify a 75 bp segment between −1302 bp and −1227 bp upstream from the transcriptional start site. With fewer pulses, larger segments are amplified (*left panel*), whereas with a greater degree of sonication, these larger segments become undetectable by PCR (*right panel*). Amplification of smaller segments is unaffected by the degree of sonication used.

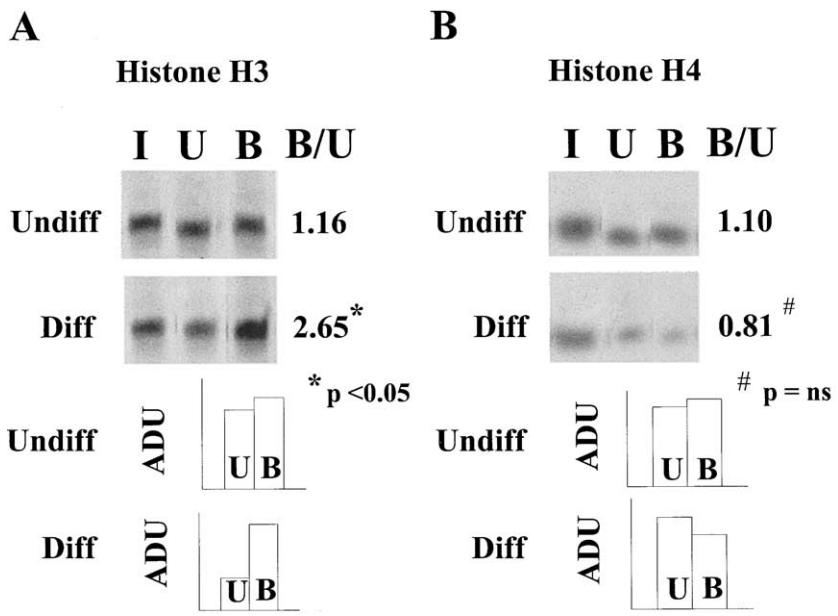

Fig. 3. Results of ChIP followed by PCR-based detection of differentiation-induced changes in the histone acetylation state within a specific proximal IAP promoter sequence. Shown are the products from each cell population (undifferentiated vs. differentiated) using input (total DNA), unbound (nonimmunoselected DNA), and bound (immunoprecipitated DNA) fractions as the templates in the PCR. The bound/unbound ratio represents the relative degree of acetylation of the particular histone within the segment of interest, and these are listed next to each group of the PCR products. A difference in the bound/unbound ratio between the two cell populations represents changes in the acetylation state of the particular histone at that specific site. **A,** In the case of histone H3, the ratio increased from 1.16 to 2.65 ($P < 0.05$), indicating a greater than twofold increase in H3 acetylation at this site. **B,** The ratios observed for histone H4 acetylation at this site were not statistically different in the undifferentiated vs. differentiated cells.

promoter between −378 and −303 bp upstream from the transcriptional start site were used and successfully generated the segment of interest from the total HT-29 genomic DNA in both the control and treated cells (see Fig. 3, lanes labeled "Input" [*I*]). In the case of histone H3, the bound fraction (DNA associated with the acetylated histone) is more intense with respect to the corresponding unbound fraction (DNA associated with the unacetylated histone) in the treated cells compared to the control cells (bound/unbound ratios of number/number and number/number, respectively; n = 3, see Fig. 3, *A*. This difference represents a twofold enrichment in the acetylation state of histone H3 in differentiated cells compared to control cells ($P < 0.05$). Conversely, acetylation of histone H4 at this site was not significantly different in the two cell populations (see Fig. 3, *B*).

Histone H3 and H4 acetylation state at a specific distal IAP promoter site remains constant during differentiation. Fig. 4 shows the results of the ChIP assay used to detect differences in histone H3 and H4 acetylation state at a distal site with the human IAP promoter. Shown are representative bands from at least three experiments, and the ratios are reported as the means ± SEM. The efficacy of the designed primers was confirmed by amplifying the sequence of interest from genomic DNA in both cell populations (see Fig. 4, lanes labeled "Input" [*I*]). At this more distal site within the IAP promoter (between −1378 and −1303 from the start of transcription), the acetylation state of histones H3 and H4 did not change appreciably during differentiation, as determined by comparing the relative ratios of bound/unbound band intensities in the two cell populations (n = 3).

DISCUSSION

For many years, histones were thought to serve merely as "scaffolding" to tightly package DNA in the nucleus. We now know that histones are actively involved in the storage and transmission of information involved in regulating gene transcription. Indeed, the post-translational modifications of these essential proteins may represent an entire "language," much like the genetic code itself, which in turn can

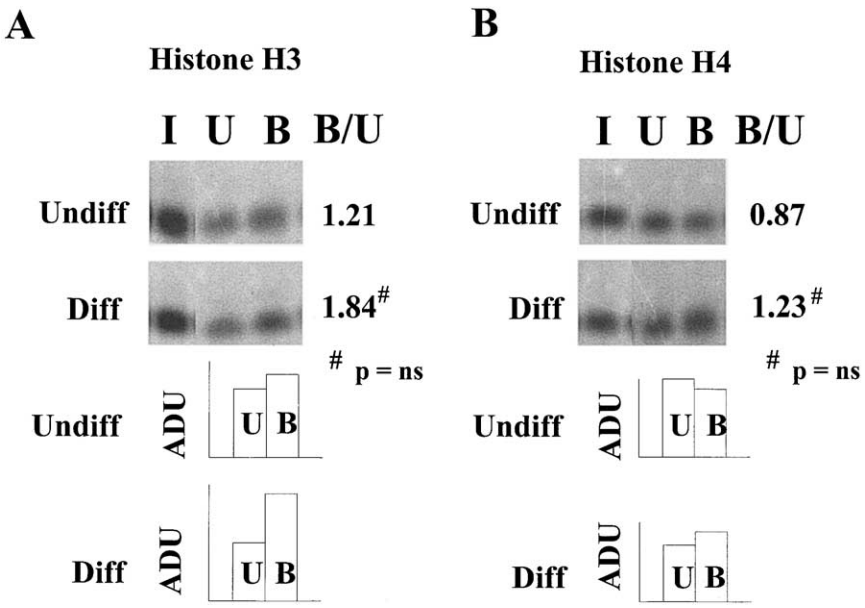

Fig. 4. Results of ChIP followed by PCR-based detection of differentiation-induced changes in the histone acetylation state within a specific distal IAP promoter sequence. The templates used in each PCR were taken from input (*I*; total DNA), unbound (*U*; nonimmunoselected DNA), and bound (*B*; immunoprecipitated DNA) fractions as labeled. The ratios of bound/unbound (*B/U*) band intensity are listed. For both histone H3 and H4, these ratios were not statistically different in the undifferentiated vs. differentiated cells.

direct the molecular activity of the cell, resulting in the regulation of processes such as gene transcription.[10–12] We are only beginning to understand the messages that may be encoded by these covalent histone modifications. The large number of permutations resulting from different covalent modifications raises the possibility of an unprecedented degree of control and fine-tuning within the cell.

Recent evidence from our laboratory has suggested that one type of histone modification, acetylation, plays an important role in mediating enterocyte differentiation.[7,8] Enterocyte differentiation is required to maintain the structure and function of the small intestinal mucosa and to provide for the continuous renewal of the epithelium, which occurs every 3 to 6 days.[13–15] Although the exact mechanisms responsible for enterocyte differentiation are poorly understood, it is generally thought to be the result of the tight transcriptional regulation of a small subset of specific genes. Our previous observations regarding histone acetylation and enterocyte differentiation, taken together with the growing body of evidence supporting an important role of this modification in transcriptional regulation, led us to further explore the potentially critical link between the two.

The ChIP assay allows for the detection of changes in the histone acetylation state associated with specific sequences of DNA under various conditions. This technique offers the important advantage of elucidating molecular mechanisms within the cell's endogenous chromatin, thereby providing information that is likely to be of physiologic importance. The ability to determine the factors involved in controlling gene transcription within the context of the chromosome in situ has become increasingly important with the recognition that many of the elements present in the nuclear milieu (once thought to be "inanimate") may contribute to the regulation of transcription. Most other techniques used to explore gene transcription tend to study DNA in its "naked" state (devoid of histones) outside the environment of the intact nucleus.

In the present work, the ChIP assay was applied to an in vitro model of enterocyte differentiation to demonstrate an association between the histone acetylation state within specific IAP promoter segments and the transcriptional activity of the gene. To make these observations, a method of detecting the DNA sequences of the IAP promoter that were co-immunoprecipitated was required. For this purpose, oligonucleotide primers were designed, based on the published IAP sequence, to amplify the segments of interest.[9] These were first tested on plasmid DNA containing a 2.5 kb segment of the human IAP 5′

flanking region to determine their efficacy and that of the PCR conditions.

Another aspect of the ChIP assay that is critical to its success is the ability to cleave the chromatin into a range of properly sized fragments. If the fragments are too large, the segments of interest may be immunoprecipitated by virtue of remaining in continuity with far distal segments that interact with the anti-acetylated histone antibodies. Such a situation may lead to false positive results because the amplified segment may or may not be associated with histones that are acetylated. Likewise, if the chromatin fragments are too small, the segment of interest may not be able to be detected because a break in the DNA template may have occurred between the location of the forward and reverse primers. In this case, the result would be false negative. We fragmented the chromatin in a nonspecific manner using ultrasonic vibration (sonication). The conditions for sonication were optimized to achieve an enrichment in fragments between 200 and 600 bp in length. Sonicating to this size range eliminates the ability to amplify longer segments of DNA, a desirable result when attempting to detect smaller, localized sequences. This is thought to occur because as the distance between primer pairs increases, so does the likelihood that a break has occurred in the intervening DNA sequence. We were able to achieve chromatin fragments that allowed detection of segments of up to 600 bp in length while at the same time preventing amplification of larger segments and thereby minimizing the chance of false positive results. We also used micrococcal nuclease to achieve enzymatic digestion of the DNA into fragments of approximately 150 to 200 bp. Although we have achieved similar results in the ChIP assay using cleaved DNA from both sonication and enzymatic digestion, we are still in the process of optimizing the conditions in order for the enzymatic digestion to be maximally effective.

Having established the conditions required to properly cleave and detect genomic chromatin fragments, we then applied the ChIP assay to address the specific issue of changes in the histone acetylation state within the IAP promoter. IAP was chosen because it is transcriptionally activated during enterocyte differentiation, making it an excellent tool with which to explore the role that histone acetylation may play in this process. The ChIP assay was initially performed using primer pairs separated by 200 bp segments along the 2.5 kb IAP promoter region. We were particularly interested in the proximal region of the IAP promoter based on previous evidence indicating the importance of this region to IAP transcription.[16,17] We were able to identify that in a small, specific sequence of the IAP promoter near the transcriptional start site, the acetylation state of histone H3 increased with differentiation-induced activation of the gene (see Fig. 3). Interestingly, acetylation of histone H4 at this same site remained constant regardless of changes in IAP transcriptional activity. At the same time we observed that in other segments more distal, the acetylation state of both histones H3 and H4 was unchanged during differentiation-induced activation of IAP. These findings are represented by the data for the distal segment in Fig. 4. These results are intriguing because previous work has implied that the total histone population becomes acetylated during differentiation in this in vitro model.[8] The present work suggests, instead, that the observed histone acetylation events are actually limited and specific. For example, in the two IAP promoter segments studied, we did not observe a change in the acetylation state of histone H4 with differentiation, although when we had previously looked at the total histone H4 population in this model, we had demonstrated an increase in acetylation of this protein.[8] In addition, we have shown that histone H3 becomes more acetylated during IAP activation at one site within the promoter but not at another. Taken together, these results suggest that the histone acetylation seen in this model of enterocyte differentiation occurs in a highly specific manner, both in terms of the location and the subtype of the histones that are modified. Given the fact that each member of the histone family contains several possible sites of acetylation and there are several isoforms of enzymes that accomplish histone acetylation and deacetylation, it is readily apparent that these modifications may represent a highly complex system for fine-tuning the transcriptional activity of genes or gene programs such as enterocyte differentiation. Clearly much additional work will be needed to dissect out the complexity of histone changes that might underlie IAP gene activation.

CONCLUSION

We conclude that butyrate-induced transcriptional activation of the enterocyte differentiation marker IAP is associated with localized and specific changes in the acetylation state of histone H3 within a segment of the IAP promoter near the transcriptional start site. These changes were not observed in a more distant site, and the acetylation state of histone H4 did not appear to vary with transcriptional activation at either site studied. These results suggest that the histone acetylation observed during differentiation in this in vitro model may represent an epi-

genetic code of regulatory signaling, which in turn may play an important role in mediating the control of the overall differentiation process. Further work will be necessary to verify this possibility.

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Discussion

Dr. J.B. Matthews (Cincinnati, OH): You have looked at histone acetylation in the context of the IAP gene. There is, of course, a whole family of genes that are turned on during differentiation. Will each be similar in its dependence on histone acetylation, or will it be different for each gene? What about genes that go down with differentiation, such as some of the markers of differentiation that your laboratory has previously reported?

Dr. B.F. Hinnebusch: We have not applied this assay to any of the other genes. We are still very much in the early stages and actually part of the learning curve, because it is a relatively new assay. I cannot even really speculate because we may expect the changes to be the same since it is an overall program and, as you rightly point out, they do undergo similar changes with differentiation. So it may be a common mechanism to those genes, and we may also see sort of the opposite mechanism in those genes that are, as you say, turned off with differentiation. The real answer is we do not know because we have not yet looked at it in the context of other genes, but we certainly plan to.

Invited Discussion—Expert Commentator

Richard A. Hodin, M.D. (Boston, MA): This paper describes a novel approach to unraveling the gene transcriptional mechanisms that underlie the intestinal epithelial differentiation process. The ChIP assay was used to examine alterations in the histone proteins that are present within the regulatory region of the differentiation marker gene IAP. The unique aspect of this work is that the ChIP approach allows us, for the first time, to examine endogenous chromatin (DNA + surrounding histone proteins), rather than the potentially artificial constructs that are used with techniques such as transfections, gel shifts, and so forth.

It is important to point out that this notion of chromatin structure being important for gene transcription represents a paradigm shift in the gene expression field. For several decades it was assumed that the DNA structure, itself, contained all of the information used by the cell to express its specific phenotype. Now we realize that there is an entirely new or different layer of information that exists—the so-

called "histone code" hypothesis. We are really in the infancy stage when it comes to understanding this histone code. But some scientists believe that the histone code, once it is unraveled, will be just as important and complex as the genetic code with which we are all so familiar. The work that Dr. Hinnebusch and his colleagues have performed should provide a framework for future studies that will focus on how the structure of an endogenous gene (i.e., DNA + histones) relates to its transcriptional activity. I certainly hope that in the years to come we can return to the SSAT meeting having made progress in this exciting project.

Colonic Metaplasia in the Ileal Pouch Is Associated With Inflammation and Is Not the Result of Long-Term Adaptation

A. Brent Fruin, M.D., Ola El-Zammer, M.D., Arthur F. Stucchi, Ph.D., Michael O'Brien, M.D., M.P.H., James M. Becker, M.D.

Ileal pouch–anal anastomosis (IPAA) is the preferred surgical therapy for chronic ulcerative colitis (CUC) and familial adenomatous polyposis (FAP). Previous studies have demonstrated morphologic changes in pouch mucosa such as villous atrophy and crypt hyperplasia. These changes have been labeled "colonic metaplasia." The aims of this study were to determine whether these changes represent "normal" long-term adaptation of the nondiseased pouch or instead are present only in the setting of inflammation. Twenty-four patients were identified, greater than 5 years status post-IPAA for CUC, who underwent pouchoscopy for surveillance and had no history of pouchitis. Thirty-one patients were identified greater than 5 years status post-IPAA for CUC, who had a history of pouchitis and had undergone pouchoscopy at least 5 years status post-IPAA. Eight patients status post-IPAA for FAP were also identified. Biopsy specimens were reevaluated by a single, blinded pathologist for degree of inflammation, the presence of villous atrophy and crypt hyperplasia, and evidence of dysplasia. Among the patients with CUC, the inflammation score was greater in the pouchitis group, 13.2 ± 1.2, compared to the nonpouchitis group, 4.0 ± 0.5 ($P < 0.0001$). Median colonic metaplasia score was greater in the pouchitis group (4 [range 2 to 6]) vs. 2 (9 [range 0 to 6]; $P < 0.0001$). The colonic metaplasia score correlated with the inflammation score (Spearman coefficient $r = 0.83$; $P < 0.0001$). In the eight patients with FAP, the inflammation score was 5.1 ± 0.9 and the median colonic metaplasia score was 1 (range 0 to 4). There was no evidence of dysplasia in any of the biopsy specimens. Patients without a history of pouchitis or symptoms of pouchitis have only a minimal degree of villous atrophy and crypt hyperplasia. These morphologic changes in the ileal pouch are found primarily in the setting of inflammation, and likely represent a reparative response. (J GASTROINTEST SURG 2003;7:246–254.)

KEY WORDS: Pouchitis, metaplasia, atrophy

Proctocolectomy with or without mucosal proctectomy and endorectal ileal pouch–anal anastomosis (IPAA) is the preferred surgical option for chronic ulcerative colitis (CUC) and familial adenomatous polyposis (FAP). Although most patients report a good functional outcome and a significant improvement in their quality of life,[1] pouchitis remains the most common long-term complication after IPAA.[2] Pouchitis, defined as nonspecific, idiopathic inflammation of the ileal pouch, is initially diagnosed on the basis of its clinical presentation. These symptoms generally include increased frequency accompanied by diarrhea, rectal bleeding, cramping, and urgency. However, the diagnosis is established after endoscopic and histologic evaluation of the ileal pouch with findings of edema, granularity, hemorrhage, and ulceration accompanied by histologic features such as villous atrophy, crypt hyperplasia, and a chronic inflammatory cell infiltrate.[2] The cumulative risk at 10 years has been reported to be as high as 46%.[3] The etiology and pathogenesis of pouchitis are poorly understood. Proposed etiologic factors in-

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clude stasis, oxidative stress, bacterial dysbiosis, recurrent ulcerative colitis, or a variant of Crohn's disease. The incidence is more common in patients with CUC who have concomitant sclerosing cholangitis[3] and is negligible in patients with FAP. These facts suggest that a persistent predisposition to inflammation must exist in patients with CUC after IPAA and that this predisposition, in combination with other factors, may result in clinically significant inflammation in the pouch.

Some degree of chronic inflammatory infiltrate is seen in nearly all functioning pouches, even in patients who do not have clinically evident pouchitis.[4] Various studies have described morphologic changes in the ileal pouch mucosal architecture, namely, villous atrophy and crypt hyperplasia. Lerch et al.[5] described total villous atrophy and significant crypt hyperplasia in 12 patients after IPAA and concluded that adaptation of the pouch ileum to its neorectal function results in progressive transformation to colonic-type mucosa. They described pouch mucosa as nearly indistinguishable from normal colonic mucosa. It has been hypothesized that the transformation to colon-like mucosa, or "colonic metaplasia," favors the new development of "colitis" within the pouch[6] and is the major etiologic factor predisposing patients to recurrence of inflammatory bowel disease. Results of subsequent studies have demonstrated that total villous atrophy in the pouch is not a universal finding status post-IPAA.[7,8] Additionally, it was suggested that villous atrophy may be transient[5] and more commonly associated with long-standing pouchitis and pronounced mucosal inflammatory infiltrates.[7] Other studies have not shown a correlation between mucosal inflammatory scores and villous atrophy.[8,9]

The assertion that ileal pouch mucosa undergoes colonic metaplasia, thus favoring the development of recurrent "colitis" in the pouch, has led to concern over the development of dysplasia and potentially neoplasia in the pouch. Periodic surveillance endoscopy and biopsies have been recommended for patients status post-IPAA to screen for the development of dysplasia.[10]

The goals of this study were to evaluate the incidence and degree of villous atrophy and crypt hyperplasia in patients with pouchitis compared to those without pouchitis, in patients who are at least 5 years status post-IPAA for CUC, as well as in patients who are status post-IPAA for FAP. Concurrently we aimed to assess the potential correlation between the degree of mucosal inflammation that is present and the degree of villous atrophy and crypt hyperplasia. In addition, we aimed to assess the persistence or resolution of these mucosal changes over time in relation to ongoing disease activity. Finally, we aimed to assess the incidence of dysplasia in this population.

MATERIAL AND METHODS

Patients

Twenty-four patients were identified who had no history of pouchitis and had undergone endoscopy of the pouch with routine surveillance biopsies at least 5 years status post-IPAA (mean 5.9, range 5 to 8). Thirty-one patients had a history of pouchitis and had undergone diagnostic endoscopy of the pouch with biopsy at least 5 years status post-IPAA (mean 6.5, range 5 to 9). Thirteen had a history of chronic pouchitis necessitating long-term suppressive therapy with maintenance antibiotics. None of these patients with chronic disease had any pathologic features such as perianal disease or fissures suggestive of Crohn's disease. Two of these 13 patients underwent a course of 5-acetylsalicylic acid; one improved and resumed antibiotic maintenance and the other was lost to follow-up. One patient underwent a course of prednisone with improvement and resumed antibiotic maintenance. Twelve patients had prior episodes of successfully treated acute pouchitis with recurrent symptoms (increased stool frequency, cramping, liquid stools). Six patients had had a recent onset of symptoms consistent with pouchitis. An additional eight patients with a history of FAP were identified who underwent endoscopy with biopsy. Seven of these patients underwent endoscopy for surveillance and one underwent endoscopy in the setting of pouch dysfunction and suspected pouchitis. The mean time after IPAA was 4.2 $\pm$ 1 years (range 1 to 9 years).

All of the protocols used in this study were approved by the Institutional Review Board of the Boston University School of Medicine, and the Boston University Medical Center.

Histologic Evaluation

All patients had two biopsy specimens of pouch mucosa taken during flexible endoscopy from the left and right sides of the midposterior portion of each limb of the J-pouch, as well as an ileal biopsy taken 10 to 15 cm proximal to the pouch. Specimens were fixed in formalin, embedded in paraffin, cut by serial microtomy, and stained with hematoxylin and eosin. All specimens were reevaluated by a single gastrointestinal pathologist who was blinded to the clinical information and diagnosis of the patient. An inflammation score was calculated from nine different histologic and architectural components of the mucosa (Table 1). The maximum inflammation score

was 28. Because this was a retrospective study, the scoring system did not contain a clinical or endoscopic component, such as in the pouchitis disease activity index proposed by Sandborn.[2] In the rare cases where there was no uniformity between the two pouch specimens, the sample with the highest inflammatory score was chosen as representative. The degree of villous atrophy and crypt hyperplasia was assessed on a scale of 0 to 3 (with 0 representing normal and 3 representing total villous atrophy and maximal crypt hyperplasia). These two scores were totaled to attain the colonic metaplasia score as shown in Table 1. All specimens were also assessed for evidence of dysplasia using internationally defined criteria[1] and, when available, the ileal segment obtained at the time of colectomy was assessed for evidence of backwash ileitis.

Statistics

Data are expressed as mean ± standard error of the mean (SEM) or median (range), if not normally distributed. Comparisons were made using Student's *t* test or paired *t* test when appropriate with normally distributed data. The Wilcoxon rank-sum test and the signed-rank test were used when data were not normally distributed. Spearman coefficient was used to assess correlation. The chi-square test was used when categorical outcomes were compared. Exact *P* values are reported for each comparison. $P < 0.05$ was considered to represent statistical significance.

RESULTS

The inflammation score in the pouchitis group was significantly greater ($P < 0.0001$) than that in the nonpouchitis group (13.2 ± 1.2 vs. 4.0 ± 0.5; Fig. 1, *A*). The median colonic metaplasia score (total of villous atrophy and crypt hyperplasia) was 4 (range 2 to 6) in the pouchitis group compared with 2 (range 0 to 6) in the nonpouchitis group ($P < 0.0001$; Fig. 1, *B*). The median villous atrophy score was 2 (range 1 to 3) in the pouchitis group compared with 1 (range 0 to 3) in the nonpouchitis group ($P < 0.0001$; Fig. 1, *C*).

Among all patients, the colonic metaplasia score correlated significantly with the inflammation score (Fig. 2, *A*). Similarly, the villous atrophy score correlated significantly with the inflammation score (Fig. 2, *B*), as did the score for crypt hyperplasia ($r = 0.80$; $P < 0.0001$; data not shown). The percentage of biopsies showing a score of 3 for villous atrophy (signifying total villous atrophy) in the pouchitis group was 14 (45%) of 31 compared to 1 of 25 in the nonpouchitis group ($P = 0.0007$). The one patient with total villous atrophy in the nonpouchitis group also had the highest inflammation score in this group. There was no evidence of dysplasia in any of the biopsy specimens.

Fourteen patients with chronic or recurrent pouchitis, who had significant villous atrophy (a score of 2 or 3) and a score of 2 or 3 for crypt hyperplasia during inflammation, were identified as having had subsequent follow-up endoscopy and biopsy. Eight

Table 1. Histologic elements of inflammatory and colonic metaplasia scores

A. Inflammatory Score	
Villi epithelia	
Degeneration: None (0); Mild (1); Moderate (2); Severe (3)	(0–3)
Neutrophilic infiltrate: None (0); Mild (1); Moderate (2); Severe (3)	(0–3)
Lymphocytic infiltrate: Normal (0); Mild (1); Moderate (2); Severe (3)	(0–3)
Crypt epithelia	
Neutrophilic infiltrate: Normal (0); Mild (1); Moderate (2); Severe (3)	(0–3)
Stroma	
Plasmacytoid infiltrate: Normal (0); Mild (1); Moderate (2); Marked (3)	(0–3)
Eosinophilic infiltrate: Normal (0); Mild (1); Moderate (2); Marked (3)	(0–3)
Neutrophilic infiltrate: None (0); Mild (1); Moderate (2); Marked (3)	(0–3)
Vascularity: Normal (0); Mild (1); Moderate (2); Marked (3)	(0–3)
Ulceration	
Ulceration: None (0); Minimal (1); Mild (2); Moderate (3); Extensive (4)	(0–4)
MAXIMUM INFLAMMATORY SCORE	28
B. Colonic Metaplasia Score	
Villous atrophy	
Normal (0); Mild shortening (1); Partial/moderate atrophy (2); Severe atrophy (3)	(0–3)
Crypt hyperplasia	
None (0); Mild (1); Moderate (2); Extensive (3)	(0–3)
MAXIMUM COLONIC METAPLASIA SCORE	6

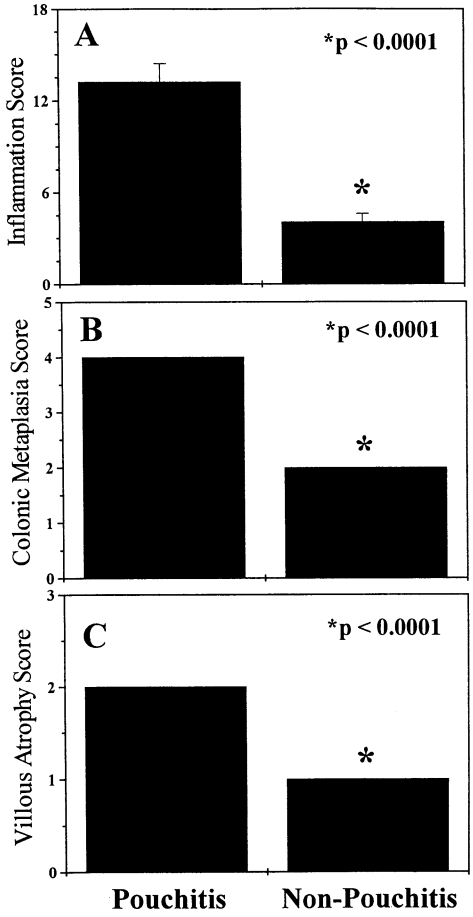

Fig. 1. Inflammation score (**A**), colonic metaplasia score (**B**), and villous atrophy score (**C**) in patients with and without a prior episode of pouchitis. Data in *A* are expressed as mean ± SEM, whereas data in *B* and *C* are medians.

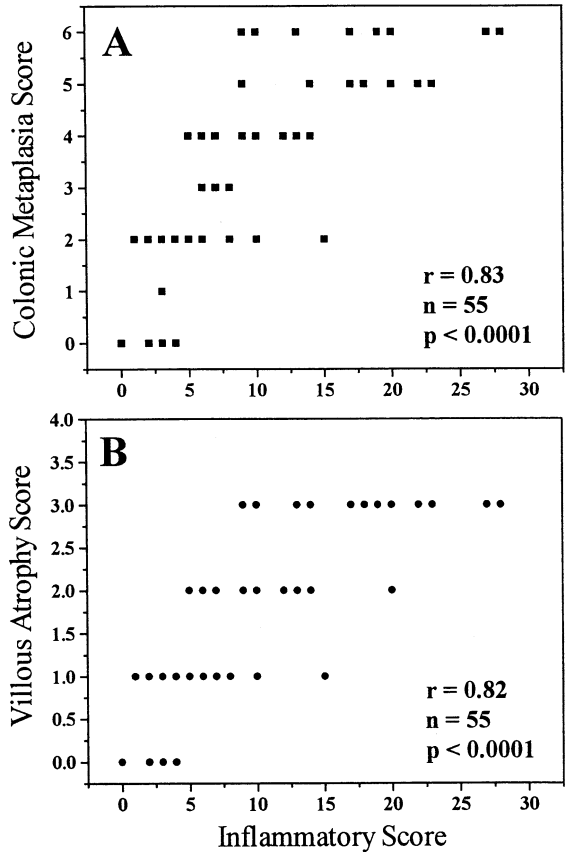

Fig. 2. Spearman correlations between the colonic metaplasia score and the inflammatory score (**A**), and the villous atrophy score and the inflammatory score (**B**).

of these patients were evaluated after improvement or resolution of their symptoms. In this group the inflammation score was significantly reduced from a mean of 19.1 ± 1.6 at the time of initial evaluation to 4.2 ± 1.0 at the time of follow-up biopsy (Fig. 3, *A*). Concomitantly, the score for villous atrophy was also significantly decreased from a median of 3 (range 2 to 3) to a median of 0.5 (range 0 to 1) (Fig. 3, *B*).

The remaining six patients had persistent or recurrent symptoms at the time of follow-up biopsy. There was no significant change ($P = 0.50$) in the inflammation score between the initial visit and the follow-up time points (15.5 ± 1.9 vs. 16.6 ± 2.3; Fig. 4, *A*). There was also no change ($P = 0.99$) in the villous atrophy score (median of 3 [range 2 to 3]) initially compared to the score at follow-up (3 [range 2 to 3]) (Fig. 4, *B*).

The eight patients who were status post-IPAA for FAP had low inflammatory scores (5.1 ± 0.9), similar to patients without pouchitis (Fig. 5). (One of these patients underwent endoscopy in the setting of pouch dysfunction and suspected pouchitis, and had an inflammatory score of 10.) The median villous atrophy score in the patients with FAP was 0.5 (range 0 to 2), and was again similar to patients with no reported history of pouchitis The one patient with probable pouchitis in this group had the highest score for villous atrophy, which was 2.

Fig. 6, *A*, shows a representative ileal pouch biopsy from an asymptomatic patient, status greater than 5 years post-IPAA, who had no history of pouchitis. Villous architecture was normal, and there was no evidence of inflammation or pouchitis. Fig. 6, *B*, shows a representative ileal pouch biopsy from a patient, status greater than 5 years post-IPAA, who has experienced episodic pouchitis. There was mild or partial villous atrophy with crypt hyperplasia and histologic evidence of mild, acute inflammation consistent with mild-to-moderate pouchitis. Fig. 6, *C*, shows a representative ileal pouch biopsy from a symptomatic patient, status greater than 5 years

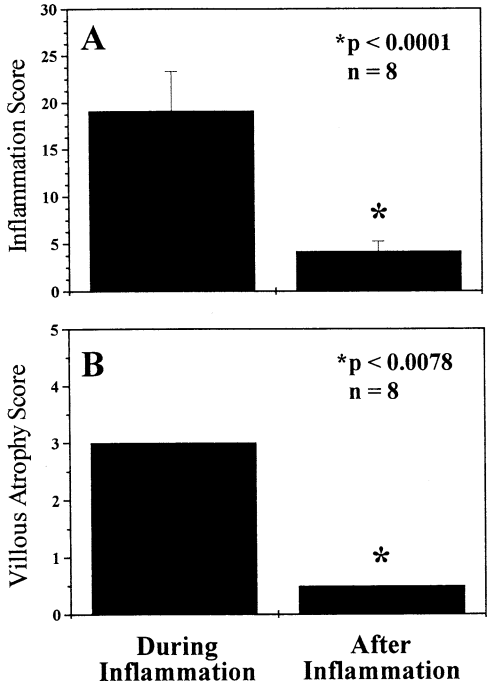

Fig. 3. Inflammation score (**A**) and villous atrophy score (**B**) in eight patients with chronic pouchitis who had biopsies during an inflammatory episode, as well as after inflammation had resolved and clinical symptoms improved.

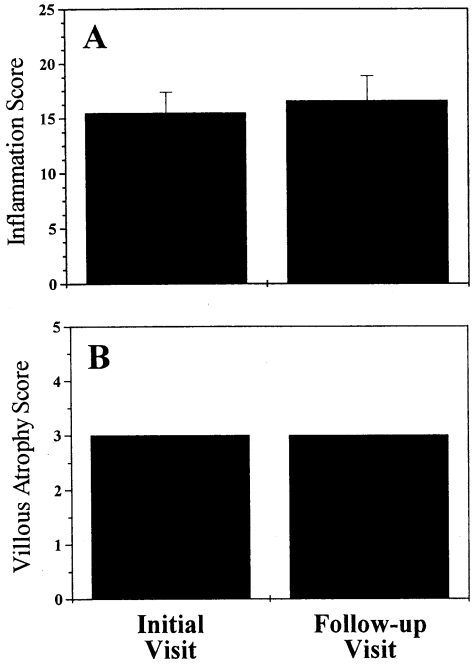

Fig. 4. Inflammation score (**A**) and villous atrophy score (**B**) in six patients with chronic pouchitis who had biopsies at the initial visit, during an inflammatory episode (*Initial Visit*), and at a follow-up visit (*Follow-up Visit*) during which inflammation and clinical symptoms persisted.

post-IPAA, who has experienced chronic episodes of pouchitis. Significant architectural changes included severe villous atrophy and crypt hyperplasia, accompanied by histologic evidence of chronic inflammation consistent with severe pouchitis.

There was no significant evidence of ileitis in any of the ileal specimens obtained from colectomies performed at this institution. Original colectomy specimens from patients who had their surgery performed at other institutions were not available.

DISCUSSION

Patients in this study, greater than 5 years status post-IPAA for CUC, with no history of pouchitis, generally had preserved villous architecture, with a minimal degree of villous atrophy and crypt hyperplasia. The one patient in this group who did have total villous atrophy also had the highest inflammation score in this group. Patients with pouchitis had more pronounced changes in villous architecture, and 45% had total villous atrophy. In both groups of patients, inflammatory scores correlated with the degree of villous atrophy and crypt hyperplasia. The association between degree of inflammation and villous atrophy was suggested by the work of Veress et al.,[7] but was not demonstrated by the results of De Silva et al.[8] and Stallmach et al.[9] Veress et al.[7] observed total villous atrophy and significant crypt hyperplasia primarily in patients with severe acute and chronic inflammation in the clinical setting of chronic pouchitis. Stallmach et al.,[9] with the use of three-dimensional morphometry, found a correlation between crypt depth and degree of inflammation in a series of patients status post-IPAA, but not between villous atrophy and inflammation. However, it should be noted that this study included a limited number of patients (n = 4) with chronic pouchitis, and these patients did have markedly lower villous height, although the findings were not analyzed statistically because of the limited number of observations. De Silva et al.[8] also found no correlation between indices of villous atrophy and inflammatory scores. The ability to detect a correlation between architectural changes and mucosal inflammation in our study may have been attributed to the use of a grading system that assessed inflammatory changes in more detail.

An interesting finding in our study was the restoration of the villous architecture to normal or near-normal morphology in patients with significant pou-

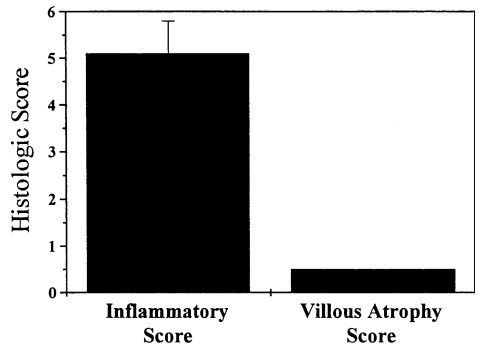

Fig. 5. Inflammatory and villous atrophy scores from eight ileal biopsies obtained from patients with FAP. Inflammatory scores are means ± SEM, whereas the villous atrophy score represents the median.

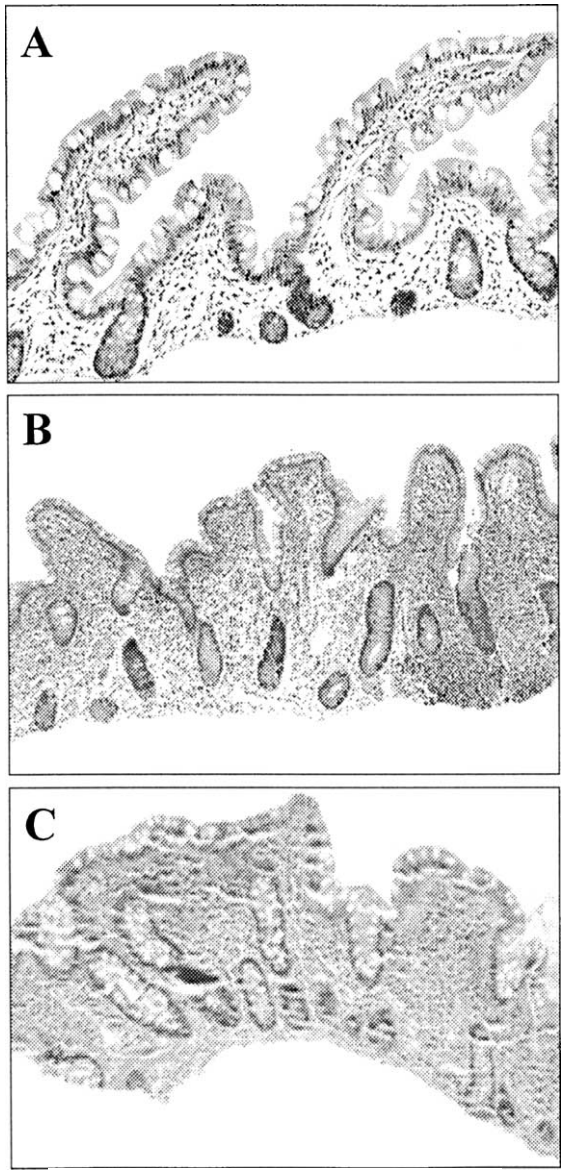

Fig. 6. Representative ileal pouch biopsies showing normal villous architecture and no pouchitis (**A**), mild or partial villous atrophy with crypt hyperplasia and pouchitis (**B**), and severe villous atrophy with crypt hyperplasia and pouchitis (**C**). (Original magnification, ×40.)

chitis who were initially found to have marked villous atrophy and crypt hyperplasia in the setting of significant inflammation. These were patients who had improvement in their clinical symptoms with treatment (primarily consisting of long-term antibiotic therapy). The villous length had increased or returned to normal levels in all subjects, and concomitantly all subjects had markedly lower inflammatory scores than at their initial evaluation. Patients with continued or recurrent symptoms of pouchitis and persistent endoscopic evidence of inflammation at the time of follow-up evaluation still had villous atrophy in the setting of persistent mucosal inflammation. Veress et al.[7] demonstrated that villous atrophy was a transient finding in some patients in a longitudinal follow-up study. Setti-Carrero et al.[12] identified a group of patients who had transient villous atrophy status post-IPAA, as well as a group of patients who developed severe villous atrophy in the setting of inflammation within weeks of ileostomy closure that persisted long term. Hence it appears that the architectural changes such as villous atrophy, crypt elongation, and hyperplasia that occur in the setting of acute and chronic mucosal inflammation of the ileal pouch may, in fact, represent a reparative response. This is evidenced by the increased numbers of mitotic figures observed in crypt epithelia during moderate and severe inflammatory episodes.

Various investigators have attempted to define and describe "colonic metaplasia" via morphologic, immunohistochemical, and functional methods. Investigators have shown increased expression of colonic-type sulphomucin in patients status post-IPAA in the setting of pouchitis.[7,8,13,14] However, it has also been shown that the inflamed colon in CUC has increased expression of small intestinal sialomucin.[8,15] Changes in mucin type in the setting of inflammation may represent a nonspecific response to inflammation rather than an indicator of true metaplasia. A recent study showed that levels of amino-oligopeptidase and maltase activity were decreased in pouch mucosa compared to normal ileum, and were more similar to levels found in the colon.[16] However, all subjects in this study were procured at the time of pouch revision for chronic dysfunction due to elongated spouts, and thus the data may not be directly relevant to the majority of patients who are status

post-IPAA. De Silva et al.[8] demonstrated weak expression of the colonic 40 kD protein in 2 of 25 patients status post-IPAA. However, in the same study, strong expression of sucrase-isomaltase activity, similar to that in the normal ileum, was demonstrated in all 25 patients, which was evidence of preserved ileal function.

Clinically significant inflammation in the ileal pouch of patients status post-IPAA for FAP is rare and has been documented only once in our own series of more than 80 patients with FAP. In a study by Stallmach et al.,[9] in which they analyzed the mucosal architecture of 14 patients status post-IPAA for FAP, only a mild degree of villous atrophy and crypt elongation was detected. The minor architectural changes in these patients were similar to those in the patients with a history of CUC but no pouchitis. Seven patients in our population who had a history of FAP had similar findings in that the inflammatory and villous atrophy scores were very similar to those of patients in our study without a history of pouchitis. The one patient with significant villous atrophy also had the highest inflammatory score among the patients with FAP and is the only patient in our series with FAP who has had a documented case of clinically significant pouchitis. The finding of near-normal small intestinal mucosal architecture in patients with FAP provides further evidence that the villous atrophy and crypt elongation that occur in the setting of inflammation is due to a reparative response, and is not an adaptive response that results simply from the creation of a functional ileal pouch.

No evidence of dysplasia was found in any of the patients in our study. Gullberg et al.[10] reported finding dysplasia in five of seven patients with chronic pouchitis and severe villous atrophy. More recently, Thompson-Fawcett et al.[17] reported only one case of dysplasia among 106 patients evaluated. In their study, no patients with severe villous atrophy had evidence of dysplasia. Only three cases of adenocarcinoma arising from ileal pouch mucosa have been reported, and in two of these cases the pouches had been defunctionalized for most of the postoperative period.[18–20] It remains unclear whether severe, long-term inflammation or villous atrophy are risk factors for dysplasia. As more data from large cohorts become available, it should become possible to further delineate true risk factors and define which patients require extended surveillance.

CONCLUSION

The results of our study suggest that architectural changes of the mucosa, often described as evidence of "colonic metaplasia," occur primarily in the setting of inflammation and often improve when the inflammation is successfully treated. Severe villous atrophy and crypt hyperplasia may represent a reparative response to underlying inflammation, as is commonly observed in other inflammatory diseases of the small intestine, such as celiac sprue. Further investigation of the mechanism by which inflammation causes villous atrophy may be relevant to a better understanding of pouchitis.

Certainly the risk of dysplasia in ileal pouches status post-IPAA for CUC is a rare event, and its relation to pouchitis or severe villous atrophy remains unclear. Continued periodic surveillance may be necessary to more accurately define the risk of dysplasia in the ileal pouch.

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Discussion

Dr. M.T. Dayton: (Salt Lake City, UT): This is a very interesting and provocative work. How did you select patients for inclusion in the study to make sure there was no unintended bias? Would you address how patients were selected for inclusion in the study, because the patients in this study represented a fairly small fraction of your total patient population as you described it. Second, for years many of us have thought this adaptive response was the reason why pouch function improved within the first year after the operation, that is, stool frequency becomes less and leakage becomes less. Do you believe there is any adaptive response at all, as previously described, and if there is, does it account for some improvement in pouch function?

Dr. A.B. Fruin: As to the first question, we evaluated the vast majority of the patients whom we had follow-up with after 5 years. It can be very difficult to maintain long-term follow-up in patients who do not have complications such as pouchitis postoperatively. We attempt to perform surveillance biopsies in asymptomatic patients between 5 and 10 years status post-IPAA, and the "nonpouchitis" group in this study represents those patients who have come back for this surveillance. The pouchitis group, as I said, was somewhat heterogeneous and involved persons with chronic, recurrent, and acute pouchitis, who were evaluated endoscopically at least 5 years status post-IPAA. There is most likely some adaptive response to the pouch environment early in the postoperative course; however, I think in most patients this response manifests itself histologically by mild change in the mucosal architecture.

Dr. C. Groves, M.D. (London, UK): How do you reconcile your findings in light of the fact that approximately 70% of patients who have polyposis with pouches have colonization or phenotypic changes; they have very little inflammation?

Dr. Fruin: We have not seen significant changes in mucosal architecture in our polyposis population. I did not present those data, but the specimens that we do have from patients following IPAA after FAP demonstrate very mild architectural changes. So I do not know if I can really comment on that.

Invited Discussion—Expert Commentator

Robin S. McLeod, M.D. (Toronto, Ontario, Canada): Recently there has been interest in determining whether patients who have ileal pouches are at increased risk for the development of dysplasia and cancer. There have been several case reports of dysplasia and cancer occurring in ileal pouches, although there is no evidence, to date, to suggest that the risk of cancer is increased. Veress and colleagues (see reference 7) reported five cases of dysplasia in a series of 82 patients with pouches. Interestingly, all patients had a history of pouchitis, and all had evidence of severe villous atrophy on histologic examination. These investigators concluded that patients with a history of chronic pouchitis and severe villous atrophy on biopsy may be a group that is at increased risk and requires increased surveillance.

This paper reports on the results from two cohorts of patients—one with ileal pouches without a history of pouchitis (n = 24) and another with a history of pouchitis (n = 31)—in whom routine biopsies were performed. There was no evidence of dysplasia in any of the biopsies from either

group. However, the investigators found that those with inflammatory changes were also more likely to have colonic metaplastic changes, and they concluded that colonic metaplasia does not represent normal adaption of the ileal pouch mucosa but rather is secondary to inflammation.

The findings in this study are interesting and are in keeping with previous evidence that patients with chronic pouchitis and severe villous atrophy may be the group that is at risk for the development of dysplasia. However, dysplastic changes were not seen in any of the patients and the assumption is made that colonic metaplastic changes cause patients to become predisposed to dysplasia. Metaplastic changes of the colon were still seen in patients without inflammation, and thus it is possible that inflammation may not only accelerate metaplasia but may also occur in pouches without evidence of inflammation. The investigators also use the term "colonic metaplasia," but perhaps it would be more correct to refer to the changes as "colonic phenotypic changes" because they have not conducted any histochemical analyses to show that there is a change in mucin quality.

Thus this report is of interest because it provides evidence that dysplasia probably is a rare event in patients with long-standing pouches. It attempts to clarify which patients may be at increased risk for developing dysplasia and may require more careful surveillance but, unfortunately, that remains to be determined.

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Bariatric Surgery Mini-Fellowship Program, February 23–28, 2003; April 20–25, 2003; June 22–27, 2003; August 24–29, 2003; October 26–31, 2003; The University of Texas Southwestern Medical Center at Dallas. Cost: $12,500 (team of 2 physicians and 1 nurse); $6,250 (physician); $1,000 (nurse). For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

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Female Pelvic Floor Disorders, March 14–16, 2003, Sheraton Yankee Trader Beach Hotel, Fort Lauderdale, Florida. Meeting sponsor: Cleveland Clinic Florida. For further information contact: Cleveland Clinic Florida, Office of CME, 2950 Cleveland Clinic Boulevard, Weston, FL 33331. Phone: 954-659-5490; toll free: 866-293-7866 ext. 55490; fax: 954-659-5491; e-mail: cme@ccf.org

Southwestern Center for Minimally Invasive Surgery (SCMIS): Diagnostic Laparoscopy & Ultrasonography, April 11–12, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Bariatric Surgery, May 30–31, 2003; September 26–27, 2003; The University of Texas Southwestern Medical Center at Dallas. Cost: physicians ($300, lecture only; $1050, lecture and lab); UTSW and SCMIS Alumni ($250, lecture only; $950, lecture and lab); nurse ($175, lecture only; $375, lecture and lab). For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Management & Percutaneous Ablation of Small Retinal Tumors, July 25–26, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Management of CBD Stones, August 15–16, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Splenectomy, November 14–15, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

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Journal of Gastrointestinal Surgery

Liver Infection 1

Efficacy Of Combination Therapy (Lamivudine And Famciclovir) in the Treatment of Replicative Chronic Hepatitis B

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Background: Infection with hepatitis B virus (HBV) remains a major cause of morbidity and mortality worldwide. Lamivudine (LVD)is an effective treatment for HBV. Unfortunately, treatment for 12 months or more is associated with development of viral resistance in 24-49% of patients. Patients with high titers of HBV DNA upon initiation of LVD monotherapy have a high rate of developing drug resistance and breakthrough infection. Famciclovir(FCV) is a nucleoside analogue that inhibits the synthesis of HBV DNA. In vivo and in vitro data suggest potential synergy with combination FCV and LVD therapy. Aim: To determine the efficacy of LVD and FCV in the treatment of replicative chronic HBV and its role in reducing the emergence of HBV drug-resistance. Methods: This is a retrospective study evaluating the efficacy of FCV and LVD in the treatment of replicative HBV. Adult patients with replicative hepatitis B and high baseline HBV DNA (viral titer>1,000,000 copies/mL) in a single center were considered for treatment. Replicative HBV was defined as detectable quantitative HBV DNA with or without the presence of HBeAg. HBV DNA titers were measured using the Digene hybrid capture method which has a sensitivity of 142×1000 copies/mL. In three patients, baseline HBV DNA levels were measured in pg/mL using the branched DNA method. These values were converted to copies/mL using the formula: 1pg HBV DNA=283,000 copies/mL). Patients were treated with LVD 100-150mg/day and FCV 500mg 3times/day. Results: Between 6/98 and 3/02, seven patients (4 male, 3 females) were treated with LVD and FCV. Follow up data was available in six patients. Mean age was 51.6 yrs. Mean duration of treatment was 22.5 months(median 23.5 months). Median duration of follow-up was 26 months. At baseline, all patients tested positive for HBsAg and three of them were HBeAg positive. Median baseline HBV DNA titer was 567,000×1000 copies/mL (>2000pg/mL). At baseline, mean ALT, AST and bilirubin were 193 U/L, 186.6 U/Land 2.0 mg/dl respectively. All patients showed undetectable HBV DNA within the first four months of treatment (mean 1.8 months). No single breakthrough infection occurred during the duration of treatment and follow up. Two out of 3 HBeAg positive patients seroconverted within 2 months. One patient continue to be HBeAg positive after 5 months of treatment but with undetectable HBV DNA. At the time of last follow-up: all patients had undetectable HBV titers, mean AST, ALT and bilirubin were 34, 39 and 0.8 respectively. Treatment was well tolerated without adverse events. Conclusion: Combination therapy with LVD and FCV is effective and safe in patients with high baseline HBV DNA titers. Combination therapy reduces the risk of breakthrough infection in these patients. A larger prospective study is needed to confirm these findings.

2

Financial and Clinical Impact of Vancomycin-Resistant Enterococcus (Vre) in Liver Transplant Recipients: A Double Matched Controlled Study

Michele Gearhart, Jill Martin, Leslie Trumbull, Ed Zavala, David Wetzel, Manish Gupta, Todd Merchen, Joseph Morelli, Fred Weber, Jaime Aranda-Michel, Maryetta Bass, Michael J Hanaway, E S Woodle, Joseph F Buell, University of Cincinnati, Cincinnati, OH

Liver transplant recipients are at a significant risk for multi-drug resistant bacterial infections due to their aggressive immunosuppression regimens and broad-spectrum antibiotic use before and after transplantation. The purpose of this study was to evaluate the financial impact of vancomycin-resistant enterococcus (VRE) on transplant recipients, their clinical outcomes and common risk factors. Methods: Liver Transplant recipients demographics and outcomes from 1995-2002 with VRE infections were identified and compared to matched controls based upon age, gender, UNOS status, liver disease and transplant date. Pt demographics, co-infections, prior antibiotic use, length of stay, abdominal surgeries, biliary complications, survival rates and total cost of both inpatient and outpatient therapy were also evaluated. Financial data was obtained through the Health-systems activity-based accounting system (HBOC-Trend Star). Results: There were 19 pts who developed 28 documented VRE infections. There were 38 non-VRE matched control patients. The most common sites of VRE infection were blood (35%), peritoneal fluid (35%), bile (20%), and urine (12%). The median time from transplant to infection was 96 days (range, 4-348 days). The VRE group had significantly increased use of pre-operative antibiotics with vancomycin, altrovafloxacin, and gentamicin. The VRE group experienced more abdominal surgery (p=.0298), biliary complications (p=.018) and a longer length of stay (p=.0056). ICU stay showed a trend to increased length of stay in the VRE group. Survival in the VRE group and non-VRE group was 52% vs. 82% respectively (p=.048). Six of the 19 VRE patients were treated with linezolid for 8 infection episodes with a 66.7% survival. Eight patients were treated with quinpristin/dalphopristin for 9 infections with a 25% survival (p=0.11). Trends for increased financial impact were seen in hospital cost, OR cost, pharmacy cost, blood bank costs, and other miscellaneous costs for the VRE group. The laboratory cost was statistically significance with the VRE cost of $6500 compared to $1,750 (p= 0.02) in the non-VRE group. Conclusion: This data demonstrates that patients with VRE infections have a negative clinical outcome compared to patients without VRE infections. In this small population, improved survival was noted with the use of linezolid, but further trials are necessary. Whether VRE infection is the cause of the increased complications or is simply a marker of poor outcomes is yet to be determined. Overall, the poor clinical outcomes of VRE translated into increased utilization of hospital and financial resources having a negative economic impact.

3

Timing of Bile Duct Ligation Impacts Clearance of Portal Venous Pathogen

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Introduction: Portal venous bacteria is normally well-handled by the liver. Our study is aimed at examining how acute biliary obstruction, an event that we have found to cause specific hepatic cytokine changes, alters clearance of normally innocuous doses of portal venous (PV) bacteria. Methods: C57BL/6 mice were subjected to bile duct ligation (BDL) or sham operation on day -1, 0 or +1 with respect to PV injection of various doses of E coli. Survival was monitored. Choosing D0BDL/D0PV at a dose of 10^5 E coli, bacteria recovered from several organs was measured at four and 24 hours post PV injection. Effect of BDL on hepatic cytokine mRNA and histology was examined. Serum cytokine protein levels were measured by ELISA. Results: Presence of BDL significantly impaired survival of PV-injected animals (see figure). Using the model D0BDL/D0PV, colony forming units of E coli recovered from liver, spleen and lung were similar at four hours after PV injection. However, cfu recovered were significantly greater in the animals subjected to BDL at 24 hours after PV injection. Hepatic IL10 and IL1RA mRNA and IL10 systemic protein levels were significantly greater in the D0BDL/D0PV group compared to the D0sham/D0PV group. Conclusion: BDL results in a significant impairment of clearance of portal venous E coli. This effect is evident even when BDL is performed concurrently with PV injection of bacteria, but wanes if BDL is performed the day after PV injection. We postulate that the mechanism involves a maladaptive increase in IL10 and IL1RA in response to BDL.

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Thermal Fixation: The Histopathology of Hepatocellular Cancer After Radiofrequency Ablation in Liver Transplantation

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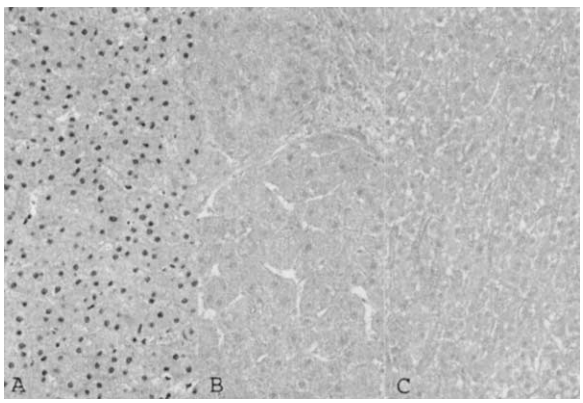
Introduction: Patients who undergo radiofrequency ablation (RFA) of hepatocellular carcinoma (HCC) while awaiting liver transplant (LTx) offer a unique opportunity to evaluate RF lesions of various ages and sizes. The histopathology of RFA has been described as coagulative necrosis. This study evaluates the effect of RFA lesions in this population. Patients and Methods: From 2000 to 2002, 8 patients underwent RFA for HCC. RFA was performed in the least invasive, technically feasible manner with a goal margin of 1 cm. Demographic and logistical data were collected. Total hepatectomy specimens were immediately processed and the gross and microscopic characteristics of the central lesion, transitional and boundary zones, and surrounding nonablated liver were evaluated by a pathologist blinded to patient characteristics. When feasible, ablated lesions were compared with nonablated lesions from the same patient. Results: Patient demographics are shown in Table.

# pts	8
# lesions/pt	Median 1, range 1–3
# Lesion size	Median 3.5 cm
# Rx/lesion	median 2, range 1–2
Initial RFA Rx	Percutaneous 2
	Open 1
	Laparoscopic 5
RFA to Tx interval	Median 8.6 mo, range 4–14 mo

No LTx was altered by the RFA or tumor related findings. Of the 8 patients with LTx, 7 are alive without disease (median follow-up 8.2 months, range 0.7 to 29 months), one patient died from HCC 22 months after LTx. In the acute post-RFA period, a narrow peripheral zone of interstitial hemorrhage occurred. All treated lesions showed thermal fixation with preservation of the tissue architecture and cytologic detail. As the cellular staining characteristics faded with time, the fixed tissue became brittle and resistant to breakdown (Fig.).



In all treated lesions, a minimal wound healing response was seen with a narrow hypocellular fibrotic boundary and scattered peripheral giant cell-type reaction. Coagulative necrosis of the central lesion was not seen. Focally, the fibrotic septae and vascular structures demarcated the ablation front. One lesion was incompletely ablated. There was no evidence of vascular seeding, neovascularization, or needle tract tumor growth. Conclusion: RFA results in "thermal fixation" of the tumor and liver margin in cirrhotic patients with HCC. This mechanism of injury has unique features that distinguish it from classical coagulative necrosis. The completeness of the single treatment session RFA lesion is especially appealing for the treatment of technically feasible HCC in pre-LTx patients. Fig: Thermal fixation. A 4 d; B 5 mo; C 14 mo.

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Resection of Hepatocellular Carcinoma With No Underlying Liver Disease

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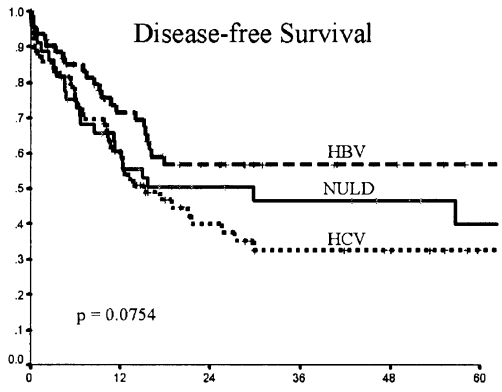
Purpose: To describe the tumor characteristics and overall and disease free survivals of patients undergoing resection of hepatocellular carcinoma (HCC) with no underlying liver disease (NULD), and to compare the results with those of patients with HCC and viral hepatitis. Methods: All patients resected for HCC were tested for hepatitis B (HBV) antigen and hepatitis C (HCV) antibody. Specimens were examined for size of the largest tumor and presence of vascular invasion (VI). Uninvolved liver parenchyma was examined for fibrosis or steatosis. Results: Between 3/87 and 9/02, 189 patients underwent resection for HCC. Of these, 61 tested positive for HBV only and 71 for HCV only. Of the 57 negative for HBV and HCV, 13 had a history of alcohol abuse and fibrosis of the surrounding parenchyma and were excluded, leaving 44 patients with HCC and NULD. Patients with NULD had significantly larger tumors (9.3±4.3 cm) than those with HBV (7.5±4.8 cm) or HCV (6.9±5.1 cm) (p=0.036). Patients with NULD also had a significantly lower incidence of VI (42%) than those with HBV (55%) or HCV (81%) (p=0.001). Five-year overall and disease free survivals (Fig.) were significantly better when comparing HBV to HCV but not significantly different when comparing NULD to HBV or HCV.



When overall survival was stratified to patients with HCC ≤5cm, those with NULD (100%) or HBV (94%) had significantly better 5-year survival than patients with HCV (55%) (p=0.0124). When disease free survival was stratified to patients with HCC ≤5cm, those with NULD (87%) had significantly better 5-year disease free survival than those with HBV (65%) or HCV (36%) (p=0.0272). Conclusions: HCCs in patients with NULD are significantly larger at diagnosis but have a lower incidence of VI compared to those in patients with HBV or HCV. Overall and disease free survivals after resection for HCC are similar in patients with NULD when compared to those with HBV or HCV. In rare cases, when small tumors are detected early in patients with NULD, they do significantly better after resection compared those with HBV or HCV.

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The Number of Colorectal Liver Metastases Treated by Cryotherapy Does Not Predict Survival.

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Nearly 50 percent of patients with colorectal cancer will develop hepatic metastases. Cryoablation of metastases when used alone or in conjunction with hepatic resection is an effective treatment for patients with disease confined to the liver. The effect of the number of hepatic lesions on patient survival remains controversial. The purpose of this study was to determine the prognostic effect of the number of colorectal liver metastases treated by hepatic cryotherapy. There are 2241 patients in our Liver Unit Database. Of these, 1261 patients had CRC. 240 patients have been treated with cryotherapy from 1990-2002. 172 patients had cryotherapy alone or in conjunction with resection. Extra-hepatic disease and lesions greater than 6 cm were considered contraindications for cryotherapy. 146 patients had resections with cryoablation that resulted in complete destruction of all tumors Ro procedure. The number of lesions was determined by IUS. 77 patients or 44.8% had 1-3 lesions, 78 or 45.3% had 4-7 lesions and 17 or 2.9% had more than 8 lesions. There was a single mortality 0.47% and 27.9% morbidity. Median survival in this group was 29 months p=0.0004. 1,2,3,4 and 5-year survival rates were 89%, 65%, 41%, 24% and 19% respectively. Median survival in months by number of lesions was as follows: 1, 32m; 2, 29m; 3, 30m; 4, 31m; 5, 27m; 6-7 37m; 8-12 21m. These differences were not significant. This paper suggests that the number of hepatic lesions is not prognostic of survival. If all disease can be treated Ro procedures, than a combination of resection and cryoablation should be used for multiple liver metastasis. The upper limit of number of metastases that should be treated remains unclear.

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Saline-Linked Surface Radiofrequency Ablation: Factors Affecting Steam Popping and Depth of Tissue Injury in Pig Liver

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Saline linked surface coagulation (TissueLink Medical Inc, Dover NH) is a new technology for hepatic division that has potential for ablation of superficial hepatic tumors and extension of hepatic resection margins. However, RF ablation on the liver surface may result in undesirable steam formation under the capsule, leading to tissue disruption, called steam popping. Also, no studies have been done to optimize tissue destruction by determining the controlling variables. This study was performed to determine how to prevent steam popping while maximizing the zone of tissue destruction. 12 pigs were studied. RF lesions were created from the liver surface using the Floating Ball FB3.0, varying treatment diameter, duration, power and inflow occlusion. After determining the threshold power for steam popping in 90 lesions, another 90 lesions were created with constant power at a particular treatment diameter (1cm/10W, 2cm/15W, 4cm/60W - subthreshold for popping) to assess the effect of time, power and inflow occlusion on depth of destruction. Lesions were sectioned, photographed and measured, followed by histological evaluation of tissue viability by NADH staining. Inflow occlusion, decreasing treatment diameter and increasing power, significantly raised the risk of steam popping, and the thresholds for popping were defined. Working below these thresholds we found that RF ablation using 1cm and 2cm diameters resulted in superficial lesions of 3-5mm depth. However depth could increased to 10mm by using a 4cm diameter. Using inflow occlusion plus 4cm/60W, depth of destruction doubled and was more predictable reaching >20mm. Histology showed that cell destruction in the RF zone was complete. Saline linked RF surface ablation is safe and highly effective in creating zones of destruction in the liver as deep as 2cm. This tool should be helpful in extending resection margins and treating superficial tumors. A human study has been started.

8

Focal Adhesion Kinase (FAK) Inhibition Prevents Growth of Hepatocellular Carcinoma (HCC) Tumors in a Xenograft Model

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FAK is a critical regulator of adhesion signals, and therefore, cellular processes such as motility, proliferation, and apoptosis. FAK is upregulated in human tumors, but its role in HCC is unknown. Inhibition of FAK leads to apoptosis, a process regulated by the C-terminal domain of FAK (called FRNK, a negative regulator of adhesion signaling). We hypothesized that inhibition of these aberrant signals in HCC cells would prevent tumor growth in an in vivo mouse model. Adenoviral vectors expressing green fluorescent protein (GFP, control) or the fusion protein GFP-FRNK were generated. Human HCC cells were infected with GFP or GFP-FRNK (MOI = 4) and 24 hours later 2×106 cells were injected into the flanks of nude mice. Tumor development and volume were evaluated and data analyzed by ANOVA and Chi-Square. Tumor growth was significantly inhibited in GFP-FRNK treated groups ($p<0.01$). Interruption of adhesion signals by inhibiting FAK prevented HCC tumor implantation and growth. This confirms a pivotal role for adhesion signaling in HCC tumorigenesis and suggests that FAK may be a potential therapeutic target.

9

WITHDRAWN

10

Laparoscopic Placement of Hepatic Artery Infusion Pump: Technical Considerations and Perioperative Complications

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Purpose: Perioperative complication rates of 5-36% have been reported after open surgical placement of hepatic artery infusion pump (HAIP). We review our early experience with laparoscopic HAIP (LHAIP) placement in the treatment of colorectal liver metastases. Methods: Between March 1998 and July 2002, 49 consecutive patients with colorectal liver metastases and no extrahepatic disease by imaging were taken to the operating room as candidates for LHAIP. 33 of 49 patients (median age 62, men 20) whose metastases were confined to the liver had LHAIP placement, radiofrequency ablation (RFA) or liver resection. None were candidates for open resection. Results: 33 of 49 (67%) patients had isolated hepatic disease at exploration. 15 (45%) patients with variant HA were successfully treated with LHAIP after vessel ligation (13) or left HA cannulation (2). Injection of methylene blue after pump placement showed extrahepatic perfusion in 3 (9%) patients; 2 treated laparoscopically and one by a postoperative angioembolization. All patients had postoperative scintigraphy with 0% misperfusion. One (3%) patient was converted to an open procedure due to scarring from a prior left hepatectomy. 29 (88%) pumps were used with median 4 cycles (1-13) chemotherapy. One patient died of pulmonary infection on the 15th postoperative day, and one had a nonfatal pulmonary embolus. Catheter thrombosis was seen in 2 (6%) patients, both resolved by thrombolysis. One pump was exchanged due to malfunction and there were no pump infections. The overall perioperative complication rate was 12% at median follow-up of 9.5 months (0.5-30). No differences in complication rates and pump functional times were found between patients with normal and variant HAs ($p>0.607$). Conclusion: LHAIP placement, even with variant HA, was not associated with increased complication rates, although operative times are long. Further studies, with long-term outcomes, are required to elucidate the proper role for LHAIP.

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Transarterial Chemoembolization for Unresectable Hepatocellular Carcinoma in HIV Patients

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Purpose: A quarter of all patients with HIV in the United States are co-infected with Hepatitis B virus (HBV) or Hepatitis C virus (HCV). Hepatocellular carcinoma (HCC) is a major complication of chronic hepatitis and cirrhosis. When unresectable, HCC may be treated by trans-arterial chemoembolization (TACE). We present two HIV patients that underwent TACE for HCC while receiving highly active anti-retroviral therapy (HAAR). Methods: Two male HIV patients in whom an unresectable HCC was diagnosed underwent respectively 3 cycles of TACE using a combination of chemotherapeutic agents, including cis-platinum 50 mg, doxorubicin 50 mg, and mitomycin-C 10 mg; and poly-vinyl alcohol particles as the embolizing agent. At the same time they were receiving HAAR to treat their HIV infection. Their CD-4 counts were recorded before and after TACE. Results: Patients tolerated simultaneous treatment without complications, their CD-4 count was not modified by TACE. One of the patients died 13 months and the other one is alive 34 months after first cycle of TACE. Conclusion: The absence of major complications in the two HIV patients treated with TACE suggests that this treatment warrants further evaluation in this particular population.

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Hepatic Cystadenomas and Cystadenocarcinomas: Diagnosis, Management, and Outcomes

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The aim of this study was to review all patients who had surgery for liver cystadenomas and cystadenocarcinomas at the Cleveland Clinic Foundation. The focus of this study is on long term outcome. These are rare tumors with most series having <10 patients, and they are frequently misdiagnosed as simple liver cysts. Cystadenomas are defined by a pathologic diagnosis: with an epithelial lined cyst and a surrounding ovarian-type stroma. Method: Twenty patients were identified for retrospective review between 1985 - 2002. Sixteen patients had cystadenomas, and four had cystadenocarcinomas. Diagnostic imaging was by CT scan in all patients; ultrasound and/or MRI were also done in 75%. Operative records were reviewed to define the procedures. Follow-up was by clinic visit and/or phone interviews, and ranged from 4 months to 15 years: 13 of the 16 cystadenoma patients had imaging (7 CT scan, 6 U/S) at follow-up. Results: Cystadenomas: All 16 were female, 48 ± 12 years. CT scan showed septated cyst(s) in all patients, which was confirmed with other imaging in some. Eight patients had undergone marsupialization prior to referral - all cysts had recurred in one to four years. Four of the patients referred to us were treated by partial resection prior to 1994: three are asymptomatic, one has documented recurrence, two declined further imaging, and the fourth is lost to follow-up. The other 12 patients have all had total excision, either by enucleation or formal liver resection. All 12 patients have been re-imaged from 4 months to 11 years (6 CT:6 U/S) and only one showed cyst recurrence: at re-operation at 3 years this proved to be a simple cyst. Cystadenocarcinomas: There were 3 female and 1 male patient, age 46 to 75. None had prior interventions and all had complex cysts on CT and/or U/S. All were treated by total excision. Three have recurred at 6 months, 1 and 8 years, and all 3 have died. The single surviving patient had U/S exam at 16 years,

Hepatic artery types and procedures

HA type and procedure	n	Complications, (%)	OR time, minutes	Hospital stay, days	EBL, mls	Pump function, months
All	33	4 (12%)	366 (210–706)	3 (1–14)	100 (25–1200)	8 (0.5–24)
Normal	18	3 (17%)	317.5 (210–528)	3 (1–14)	100 (25–300)	8.5 (0.5–22)
Variant	15	1 (7%)	370 (220–706)	3 (1–9)	150 (50–1200)	8 (1–24)
LHAIP only	10	1 (10%)	300 (220–480)	2.5 (1–9)	175 (25–300)	11 (3–22)
LHAIP & RFA	22	3 (14%)	385 (210–528)	3 (1–14)	100 (50–300)	8 (0.5–24)
LHAIP & Resection	1	0	706	5	1200	4

which showed 2 small cysts. Conclusions: i) Cystadenomas are frequently misdiagnosed as simple liver cysts. The cyst wall should be examined histologically when "simple" liver cysts are marsupialized: if this shows cystadenoma, it should be excised. ii) Total excision of cystadenoma is curative. iii) Cystadenocarcinoma of the liver is rare, should be totally excised, but has a high risk of recurrence and mortality.

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Elevated Tumor Markers in Fluid From Simple Hepatic Cysts

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Accurate preoperative diagnosis of Biliary Cystadenomas is difficult. Case reports have suggested that elevated cyst fluid levels of CEA and CA 19-9 are diagnostic. We report 5 cases where cyst fluid tumor markers were elevated and the final diagnosis was simple hepatic cyst. Four of the patients were women aged 37-54 who presented with vague upper abdominal symptoms. Ultrasound investigations revealed hepatic cysts varying in size from 3 to 10 cm. Three out of the four cysts had septations. Computerized tomography confirmed these findings. Because these lesions were thought to be possible cystadenomas, cyst aspiration for fluid cytology and tumor marker analysis were performed. Cyst fluid CEA ranged from 37.3 to 71.6 ng/ml and CA 19-9 ranged from 1828 to 26627 U/ml. Cytology did not show and malignant cells. One patient had an elevated CA 125 of 2090 U/ml. Two patients had their cyst resected; two had biopsies followed by marsupialization into the peritoneum. Pathology on all specimens revealed a simple cyst. The last patient was an 84 year-old man who underwent aspiration of a cyst while being investigated for Hepatocellular cancer. The cyst fluid CEA was 43 ng/ml and CA 19-9 was 6545 U/ml. This patient was followed for two years with no change in the cyst size. This study suggests that cyst fluid CEA and CA 19-9 levels are not useful in distinguishing simple hepatic cysts from biliary cystadenomas.

14

Promotion of Cell Proliferation Via a Tetratransmembrane Protein Which Is Encoded by a Novel Gene and Is Highly Expressed in Human Hepatocellular Carcinoma

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To further explore the mechanism of carcinogenesis of human hepatocelluar carcinoma (HCC), structural and functional studies of a novel gene highly over-expressed in HCC were performed. The novel gene (AY057051) was cloned in our laboratory and is designated by the international nomenclature committee as LAPTM4-beta. This gene locates at chromosome 8q22.1, composes of 7 exons. The putative amino-acid sequence contains four transmembrane motifs and shows 46% homologous to a tetra-transmembrane protein, LAPTM4-alpha. The expression of LAPTM4-beta is up-regulated in HCC tissues and hepatoma cell lines both in mRNA level showed by Northern Blot and in protein level by Western Blot. The proteins encoded by LAPTM4-beta gene have been identified to have a long form LAPTM4-beta1 with a MW of 35 kDa and a short form LAPTM4-beta2 with a MW of 24 kDa, and to integrate in membranes by immunohistochemistry, Triton X 114 partitional extraction and Western Blot of subcellular fractions. The biological effects of LAPTM4-beta were studied by either anti-sense oligodeoxynucleotide (ODN) treatment or stable transfection. The results showed that the cell attachment/spreading and the proliferation were remarkably reduced in the anti-sense ODN treated hepatoma cells; by contrary, the cell proliferation was promoted via LAPTM4-beta cDNA stable transfection. The LAPTM4-beta cDNA transfected NIH 3T3 cells were tumorigenic when the transfectants were inoculated into NIH mice. Importantly, the LAPTM4-beta protein interacts with the integrin alpha 6 laminin receptor demonstrated by immuno-coprecipitation, and the laminin substrate enhanced the expression of the complex. Tyrosine-phosphorylation of LAPTM4-beta was detected and also enhanced when hepatoma cells were put on laminin substrate. Moreover, the N-terminal sequence of LAPTM4-beta1 contains a typical binding site for SH3 domain of signaling molecules in cytoplasm. These results taken together suggest that the up-regulated expression of LAPTM4-beta plays some role on aberration of HCC cell proliferation triggered by laminin and the signal transduction thereof. It was reported that laminin and the integrin alpha 6 beta 1 receptor were highly expressed in HCC, and the higher the expression the worse the prognosis of patients with HCC. Further study on interactions between LAPTM4-beta proteins and their binding proteins may further reveal the mechanism of HCC growth and metastasis promoted by laminin and the integrin receptor.

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Radiofrequency Thermoablation in the Treatment of Hepatocellular Carcinoma

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Aim: Radiofrequency thermoablation (RFTA) is one of the options in treatment of hepatocellular carcinoma (HCC). We have studied the safety and efficacy of this method Material and methods: Between April 12th, 2001 and October 1st 2002 24 patients with HCC in cirrhotic liver were treated using RFTA. In 22 pts tumors were inoperable due to tumor extension and/or poor liver function, 2 patients refused surgical intervention. In all patients postnecrotic liver cirrhosis was present, 8 patients were in class C, 14-B and 2-A according to Child-Pugh classification. In 2 cases tumors were above 10 cm on diameter and on another 2 smaller than 3 cm. 1 patient had previously hepatic resection (2 times) and one - alcohol installation. In all patients RFTA was done percutaneously using "CoolTip" equipment (Radionics, Burlington, MA). Results: No intra- or early postoperative complications were observed. In one case hepatic abscess was treated with percutaneous drainage. The perioperative hospital stay was 3 days for 20 patients, 3-6 days for the last 4. The observation period is 3-17 months (mean 8,7). 4 patients died: 3 due to cirrhosis complications not associated with the procedure, one due to neoplasm spread. Large tumors (more than 10 cm in diameter) were treated in 5 and 6 sessions. In one of these patients tumor seems to be completely destroyed, in the other one the therapy was interrupted due to abscess formation (still alive 6 months after RFTA start). In one patient previously treated surgically we observe tumor dissemination. 2 tumors about 5 cm in diameter were destroyed partially and needed second RFTA session, in one patient second metachronous HCC focus was found and destroyed using RFTA. In other patients control CT investigations show only avascular space in the region. Conclusion: RFTA seems to be safe and efficient method in the treatment of HCC in cirrhotic liver.

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Selective Internal Radiation Therapy (Sirt) for the Treatment of Advanced Colorectal Liver Metastases

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Selective internal radiation therapy is a relatively novel treatment being applied to patients with non-resectable liver tumours. The treatment involves hepatic arterial delivery of 90Y microspheres, which because of the blood supply of liver tumours, are relatively selectively delivered to all liver tumour sites. The aim of this study was to document the results of SIRT in 100 consecutive patients with advanced, non-resect-

able, colorectal liver metastases treated between Feb 1997 and Feb 2002. Estimated liver involvement was <25% in 60 patients, 25-50% in 21 and >50% in 19. A single dose of between 2.0-3.0 GBq of SIR-spheres was injected into the hepatic artery via a subcutaneous port and followed at 4-weekly intervals by regional chemotherapy with 5FU. 1 patient died of radiation hepatitis 9 weeks after SIRT and 8% developed duodenal ulceration. SIRT was otherwise well tolerated. Responses to SIRT were assessed by serial CEA measurement and CT scans. Median CEA 1 and 2 months after SIRT (expressed as % of initial CEA) were 24 and 13 respectively. Patients were assigned to two groups based on the development or not of extrahepatic disease within 6 months of SIRT. Median survival from SIRT for Group 1 (no EHD) (n=49) was 12.6 mo (1.2-56) with estimated survival ± sd at 6, 12, 18 and 24 months of 75.5%, 63%, 51%, and 40.8% respectively. For Group 2 (EHD) (n = 51) median survival was 8.3 mo (1.0-28.8) and estimated survival at 6, 12, 18 and 24 months was 74.5%, 41%, 25% and 7.8% respectively. This difference is statistically significant by Log-Rank test (p[0.001). SIRT is a highly effective and well tolerated regional treatment for extensive, colorectal liver metastases. Tumour marker data suggests that substantial destruction of liver tumour can be achieved in over 90% of patients by a single treatment. Survival times, particularly for those who do not develop extrahepatic metastases for some time, appear to be extended. SIRT warrants further use and investigation in patients with advanced colorectal liver metastases.

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Resectable Colorectal Liver Metastases: Is There a Role for Chemotherapy?

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Objective: There is a need to improve outcomes in patients with resected colorectal liver metastases. The addition of chemotherapy to surgical therapy is a logical extension from the experience with node-positive colorectal carcinoma. Unfortunately, the utility of chemotherapy as an adjunct to hepatic resection in the treatment of metastatic colorectal carcinoma is still unproven. Methods: Patients undergoing liver resection for colorectal metastases at a regional tertiary care hospital were examined. Demographic, clinical, pathological, and outcome data was collected for 113 patients who underwent resection between January 1990 and July 2002. One hundred and thirty-five resections were performed with a surgical mortality rate of 2.7%. Analyses were made comparing overall and disease free survival between those who received peri-resection (adjuvant and neo-adjuvant) chemotherapy (n=54) and those who did not (n=45). Specific analysis was also made of adjuvant chemotherapy. Results: Five year actuarial survival was 36% for the combined cohort, with a median survival of 4.5 years (95% CI 3.34-5.65). Overall disease-free survival was significantly improved in those that received either neo-adjuvant or adjuvant chemotherapy (log-rank p=0.011). These results were similar to those derived when judging patients who received adjuvant chemotherapy versus all other resected patients (log-rank p=0.01). In addition, there was a trend (p=0.08) toward improvement in overall survival for patients who received either adjuvant or neo-adjuvant chemotherapy versus those which did not. Conclusion: This retrospective study demonstrates a significant improvement in disease free survival with the addition of chemotherapy to liver resection for colorectal liver metastases. This is an important and re-assuring finding as it is unlikely that future prospective studies will include a no chemotherapy arm.

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Heparin Inhibits Colon Cancer Adherence to Hepatocyte Spheroids

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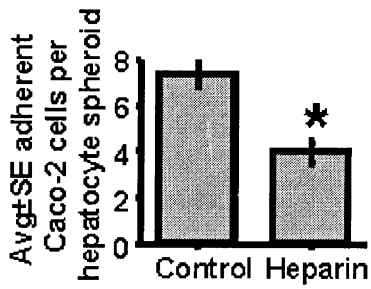
Hepatic colon cancer metastases remain a cause of morbidity and mortality, but the interactions between colon cancer cells and hepatocytes are poorly understood. These interactions may be key to hepatic metastasis. The purpose of this study was to investigate the role of heparan sulfate proteoglycans (HSPG) in interactions between colon cancer cells and hepatocytes. HSPG are cell surface proteins that have covalently attached glycosaminoglycans chains, including chains of heparan sulfate (HS). Binding to HSPG is primarily mediated by HS, and can be inhibited by heparin, an HS analog. We hypothesized that heparin would inhibit adherence of Caco-2 colon cancer cells to hepatocyte spheroids. Hepatocytes, when cultured in a spinner flask, form spheroids, which are hepatocyte aggregations that recapitulate the hepatic lobular structure, form normal junctions and bile ductules, have enhanced metabolic function as compared to dispersed hepatocytes, and may better reflect the characteristics of hepatic parenchyma than dispersed hepatocytes. Caco-2 cells were stained with DiI (1,1-dilinoleyl-3,3,3∋3∋-tetramethylindocarbocyanine), a fluorescent membrane dye, and incubated with primary hepatocyte spheroids (1:6, Caco-2:hepatocytes) with or without 100 µg/ml heparin. Spheroids were examined by fluorescence microscopy, and adherent Caco-2 cells enumerated. Heparin inhibited the Caco-2 cell adherence to hepatocyte spheroids, $P < 0.01$ Student's t-test. These results suggest that the interactions of colon cancer cells with hepatocytes may be mediated in part by HSPG. Further investigations are required to define the structural determinants necessary for HSPG-mediated colon cancer cell adherence to hepatocyte spheroids. This line of research may suggest novel prophylactic and therapeutic measures for colon cancer metastasis to the liver.



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Role of Radiofrequency Ablation In Multimodality Management of Unresectable Liver Tumours

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Methods A prospective study of all the patients with unresectable liver tumours who are suitable for Radiofrequency ablation (RFA) was carried out over two years. Data were collected prospectively in a computerised database. Patients details included age, sex, details of the disease including radiological findings, histology, and previous treatments. All patients underwent radiofrequency ablation of their liver lesions as well as other treatment including systemic and/or regional chemotherapy as decided in multidisciplinary meetings. Details of the procedures were recorded and patients were followed up regularly in a dedicated RFA clinic. Results There were 50 patients, 32 male and 18 females. Mean age of the group was 64.7 years. Total 110 lesions were ablated during 78 procedures.. There were 17 patients with hepatocellular cancer (HCC), 27 had colorectal liver metastasis, and other lesions included metastasis from renal cell carcinoma, breast carcinoma metastasis, metastasis from granulosa cell tumour and cholangio carcinoma. Fifty-one procedures were carried out percutaneously under ultrasound guidance, 21 were open procedures, 5 were laparoscopic procedures and 1 procedure was carried out under CT guidance. Twelve patients with HCC underwent transarterial chemoembolisation, 20 patients with colorectal liver metastasis had systemic chemotherapy. Seven patients underwent

liver resection along with RFA of the other liver lesions. Follow up varied from 3 to 48 months with median follow up of 15.5 months. Twelve patients had complication out of 110 ablations (9.1%). Two patients died within 30 days of the procedure. Thirty seven patients are alive (72.5%) at the end of follow up and 9 are disease free (17.6%). Conclusion Radiofrequency ablation along with transarterial chemoembolisation and/ or systemic chemotherapy can be effective in controlling malignant tumour in liver in selected group of patients. A randomised trial is needed to find out the influence of RFA on long-term survival.

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Hepatic Resection for Non-Colorectal Metastases: Is it Worthwhile?

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The benefit of surgical resection of hepatic metastases from non-colorectal cancer is unclear. We conducted a retrospective review of all patients who underwent surgical therapy (resection or ablation) of non-colorectal metastases. The records of 28 patients who underwent liver resection or tumor ablation for metastatic non-colorectal malignancies between 1988 and 2002 were reviewed. Histology of the primary tumor was neuroendocrine (n=8), gynecologic (n=5), breast (n=4), sarcoma (n=4)adenocarcinoma of unknown primary (n=3), renal cell (n=2), lung (n=1), and parotid (n=1). Extent of liver resection included wedge/segmental (n=10), hemi-hepatectomy (n=11), and extended hemi-hepatectomy (n=4). In 4 patients tumor ablation was performed with cryotherapy (n=2), alcohol injection (n=1) and radiofrequency (n=1). Four patients underwent repeat procedures for recurrent disease, cryotherapy (n=2) and wedge resection (n=2). There was one peri-operative death (4%). For the 8 patients who are deceased the median overall survival, excluding the one post-operative mortality, was 29 months, range 11 to 78 months. Hepatic recurrences were present at the time of death in all of these. Of the 20 patients still in follow up, median survival is 19 months, range 7 months to 140 months. Ten of these patients are free of hepatic disease. Thus far, 8 patients have survived 3 years or longer with 5 of these 8 surviving longer than 5 years. Primary tumor histology in these 8 patients includes 4 with neuroendocrine varieties and one each of breast, ovarian, renal cell and sarcoma. Actuarial survival based on life-table analysis is 88%, 68%, 62%, 55% and 47% at 1, 2, 3, 4 and 5 years respectively. Follow-up is ongoing. Conclusion: The development of hepatic metastases is generally considered an indicator of advanced disease and is associated with a poor prognosis. Resection of non-colorectal hepatic metastases, in selected patients, may result in prolonged survival.

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Improved Functional Quality of Life and Survival After Resection of Liver Metastasis From Carcinoid Tumor

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Purpose: Despite the growing number of treatment modalities available, five-year survival for patients with liver metastasis from carcinoid (LMC) tumors is reported to be as low as 20-30%. Retrospective studies suggest that patients undergoing resection (RSX) have improved survival in comparison to those with LMC who are not treated with RSX. The purpose of this study was to measure longitudinal functional performance and survival, and to describe other clinical outcomes following RSX for LMC. Methods: We retrospectively reviewed the records of patients undergoing RSX for LMC during the past two decades at our institution. Outcome measures included functional performance, survival, symptoms, and biochemical tumor markers (Chromogranin-A, neuron-specific enolase, and 5-HIAA). Functional performance was determined (prior to RSX, at 3, 6 and 18 months following RSX, and annually thereafter) via the Karnofsky index, a widely used proxy for functional health-related quality of life. Data were analyzed via Kaplan-Meier and repeated measures analysis of variance methods, and statistical significance was interpreted at $p < 0.05$. Results: Twelve patients underwent a total of 16 resections at a mean ($\pm$ SEM) age (at the initial RSX) of 50 $\pm$ 9 years. Ten patients (83%) had preoperative symptoms, and seven (58%) had carcinoid syndrome. Of the 10 symptomatic patients, 80% had complete or near complete relief of all symptoms. Of these, 63% had symptomatic recurrence at 23 $\pm$ 15 months. Seven patients (58%) had at least one elevated tumor marker pre-RSX. Of these seven, four patients had normalization of all tumor markers post-RSX and remained within normal limits at 102 $\pm$ 27 months post-RSX. The other three remained elevated post-RSX, but two of the three experienced decreases in marker levels of two and three fold. Statistically significant improvement in functional performance was observed in the first three months following RSX in all patients. Nine patients survived greater than one year following RSX. Functional performance increased significantly in these patients (from 81 $\pm$ 5 pre-RSX to 91 $\pm$ 4 at 54 months post-RSX), with specific significant improvements (versus baseline) at 6, 18, 30, and 42 months. The mean survival time for all patients was 126 $\pm$ 21 months. One-, five-, and seven-year cumulative survival was 83.3%, 83.3%, and 62.5%, respectively. Conclusions: Hepatic resection for liver metastasis from carcinoid tumors is associated with significantly improved and sustained functional quality of life and, probably, with improved survival.

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Lessons Learned in an Initial Experience With Laparoscopic Hepatic Surgery: Operative Results

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Since the advent of laparoscopic cholecystectomy, surgeons have attempted to adapt these techniques to solid organ surgery including hepatic resection. This report reviews our initial experience with laparoscopic liver surgery. Methods: We retrospectively reviewed all laparoscopic hepatic cases performed from July '00 to Oct '02. Patient demographics, intraoperative and postoperative results were examined Results: Twenty-five patients underwent exploration for: solid masses (n=17) and cystic masses (n=8). Patient demographics noted a mean age 52.5 $\pm$ 14.7 yrs, 12 M and 13 F, and 13 (52%) with cirrhosis. Preoperative diagnosis was variable: 13 HCC, 7 simple cysts, 3 metastases, 1 bleeding adenoma and 1 hemmorhagic cyst. Thirteen (52%) underwent resection, 8 (32%) received radiofrequency ablation and 4 (16%) were biopsied and closed without intervention. No operative conversions were required. In the resection group: 3 lateral segmentectomies, and 3 segmental resections (VIII/VII, V,II/III and II/III) and 7 cyst excisions were performed. In the RF group the mean size of the lesion treated was 3.0 $\pm$ 0.8 cm with 6/7 (86%) cirrhotic. In one pt five carcinoid tumors were treated. In the 4 pts biopsied: 1 had carcinomatosis from HCC, 1 had diffuse multifocal disease from HCC, 1 had 5 benign lesions (FNH) and the remaining pt had no lesion on lap US and localized biopsy. The mean operative time for all procedures was 195 mins with a mean blood loss of 250 cc. Complications included: laparoscopy for rebleeding (2/25;8%), ascites formation (9/25:36%), hernia (1/25;4%) and one death (1/25;4%) from hepatic failure in the pt with 2 hepatomas resected. Conclusions: 1) bleeding has not impacted the ability to perform laparoscopic hepatic surgery 2) Ascites is a common complication especially in cirrhotic patients 3) despite the limited approach, death from liver failure may occur. However, the mortality rates are lower in cirrhotics then contemporary reports of open surgical procedures. Our initial experience in laparoscopic hepatic surgery has confirmed the safety and efficacy of this procedure. This experience provides a foundation for utilizing these techniques in more extensive procedures.

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Prognostic Significance of CEA Levels in Hepatic Resection for Metastatic Colorectal Cancer

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Objective: Carcinoembryonic antigen (CEA) level is amongst the many prognostic factors affecting hepatic resection for colorectal cancer metastases. To determine the prognostic significance of pre-operative CEA levels and post-operative drop of CEA values, we studied 115 out of 229 consecutive liver resection patients in our prospective database who underwent hepatic resections for colorectal cancer metastases. Methods: All data were recorded prospectively. Of the 115 patients in the study, 81 patients had both pre-operative and post-op CEA levels measured. In addition, we also correlated CEA values to other known prognostic factors of survival using both univariate and multivariate analyses. These factors include Dukes staging, margins of resection, R0/R1/R2 resection, presence of extra-hepatic disease, intra-operative blood loss, use of auto-transfusion, number of lesions, DNA index, tumor differentiation, and intra-arterial chemotherapy. Results: Pre-operative CEA values ranged from 1 to 4,000 ug/L. Post-operative CEA values ranged from 0 to 180 ug/L. We divided patients into two groups based on pre-operative CEA levels of < 10 ug/L vs. $\pm$ 10 ug/L. Mean survival was significantly better in the group with pre-op CEA < 10. We also divided patients into two groups based on lowest post-operative CEA levels within the first 4 weeks of < 5 ug/L vs. ≥ 5 ug/L. Again, the mean survival was significantly better in the group with lower post-operative CEA levels (Table).

	Survival (mo)	Mean Survival (mo)	p value
Pre-op CEA <10	0.23–106	36.9	<0.05
Pre-op CEA ≥10	0–173	23.3	<0.05
Post-op CEA <5	0.2–173	45.1	<0.001
Post-op CEA ≥5	7.37–41.2	25.8	<0.001

To ascertain that other common prognostic factors as mentioned in Methods did not skew the observed results, we found that these factors were equally distributed among the four groups of patients divided according to CEA levels. Drop of CEA levels was observed from post-operative day 1 to week 4, but the nadir of CEA levels was observed between week 3 to week 4 after the surgery. In order to see the importance of other known prognostic factors in post-operative survival, we correlated them with survival time and did not find any stastically significant difference in survival on univariate analysis. However, on multivariate analysis of groups of prognostic factors, viz., early Dukes stage, R0 resection, absence of extra-hepatic disease, and a segmentectomy were statistically correlated with a better survival. Conclusion: Pre-operative and post-operative CEA levels are important prognostic factors in hepatic resection for metastatic colorectal CA. The ideal time for measuring post-operative CEA level is during post-operative week 3 to week 4 when the level is at its nadir.

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Correlation of Molecular Markers and Echogenicity in Colorectal Cancer Liver Metastases

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Differences in the echogenicity of colorectal cancer liver metastases (CRCLM) have been shown to be prognostic both after curative resection and after i.a. chemotherapy. Molecular impact of different markers on hypo- or hyperechoic appearance defined during intraoperative ultrasound has been studied immunhistochemically using tumor samples. Standard immunohistochemical techniques were used to stain sections of 67 patients CRCLM for Ki-67, Laminin-5, MMP-9, VEGF, MUC-2, CD3, CD31 and CA19-9. 26 CRCLM with hypoechoic and 41 CRCLM with hyperechoic appearance were analyzed. All patients had undergone curative resection. Uni-, multivariate and survival analyses were performed using SPSS 10.0 for Windows. Mucin staining was identified significantly more often in patients with hypoechoic CRCLM compared to hyperechoic CRCLM (84% vs 44%, $P = .004$). There was a significant overall survival difference between hypoechoic CRCLM patients (med. survival 19.5 months) and hyperechoic ones (41.1 months, $P < .002$) after a mean follow-up of 37 months. Recurrence-free survival was also significantly different (8.8 vs 23.0 months, $P < .001$). In regards to the above mentioned investigated markers a significant correlation with hypoechoic appearance of CRCLM was identified for laminin-5 ($P < .05$), CA 19-9 ($P < .01$) and mucin ($P < .001$). Mucin expression was a significant survival predictor ($P < .05$) irrespective of echogenicity. Cox regression analysis revealed echogenicity ($P = .005$) and mucin ($P = .03$) independently influencing survival in this patients group. Echogenicity seems to be consistently related to mucin content of CRCLM. Patients with hypoechoic CRCLM and thus high mucin expression have a worse prognosis. Tissue remodeling markers and factors involved in neoangiogenesis are not influencing echogenicity. A possible role of chemoresistance to 5-fluorouracil based chemotherapy in mucin producing tumors has to be further explored.

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Incidence of Liver Metastases in Patients Receiving Adjuvant Chemotherapy After Lymph Node Positive Primary Colon Cancer

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The incidence of liver metastases during follow-up of colon cancer patients under current adjuvant chemotherapeutic protocols following primary tumor resection is essentially unknown. We prospectively documented the incidence of colon cancer liver metastases (CCLM) in an adjuvant clinical trial of lymph node positive colon cancer patients (Studie 90, ABCSG). 598 Dukes C colon cancer patients were randomized to receive one of four treatment regimes after curative colon cancer resection: 5-FU, 5-FU+Levamisol, 5-FU+Interferon-a, 5-FU+Levamisol+IFN-a. Patients were equally distributed among the four treatment groups. Uni- and multivariate analysis of possible risk factors for the development of liver metastases was performed. The Kaplan-Meier method was used for survival analysis. After a median follow-up of 57 months 146 patients (25%) had died. Median overall survival has not been reached, 1-, 3- and 5 year survival were 94%, 84% and 74% respectively. There were 10% T1, T2 tumors, 74% T3 and 16% T4 tumors; 56% patients were N1 positive, 29% N2 and 15% had N3 lymph nodes involved. 69% were well or moderately differentiated, 31% had G3 or G4 tumors. 34% had right sided, 66% left sided colon cancer. Liver metastases were the most frequent recurrence site, in total 94 patients or 15.7%, 29 patients developed local recurrence, 20 patients lung metastases. 46% of the patients with liver metastases underwent surgical resection. Tumor stage significantly influenced the occurrence of liver metastases in uni- and multivariate analysis ($P = .003$). There was no difference in regards to different treatment groups. Treatment strategy of patients developing liver metastases had a significant impact on survival with liver resection prolonging survival most (median 32 months vs 10 months after other treatments, $P < .0001$). Liver resection remains the treatment modality with the most promising overall survival, if patients develop liver metastases during follow-up of colon cancer. Current adjuvant chemotherapeutic approaches seem to reduce the occurrence of liver metastases compared to untreated historical controls. Regular study monitored follow-up of colon cancer patients enable early detection of liver metastases allowing a high percentage of resectabilty.

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Outcomes of Hepatic Resection for Breast Cancer Metastases

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Introduction: Hepatic resection is not a standard of care to treat patients with metastases from breast cancer. However, it has been suggested a possible a role for selected patients with no other sign of cancer recurrence. More information is necessary to define the potential benefit of partial hepatectomy in this setting. Methods: The records of all consecutive patients undergoing hepatic resection for breast cancer metastases between 1980-2001 were reviewed. Patients were offered a surgical resection when an R0 resection was anticipated preoperatively and extrahepatic disease was ruled out by imaging studies. Follow-up was complete by outpatient visits or mail correspondence. The Kaplan-Meier method was used to generate overall and recurrence-free survival. Results: Fourteen patients underwent partial hepatectomy to treat breast cancer metastases; mean age was 57 yr (SD 12). The liver was the first site of metastases in 10 patients. The median interval-free period (prior to hepatectomy) was 57 months (range 10-106) and in 9 patients was more than 24 months. An R0 resection was achieved in 10 patients, and extrahepatic disease was found intraoperatively in 3 (peritoneal implants, portal nodes). Median operative time was 165 minutes (120-250), transfusion requirement was 0 units (0-5), and length of stay was 6 days (4-8). Two patients experienced postoperative complications; there was no mortality. Median overall survival was 70 months (5-yr:63%) and recurrence-free survival was 28 months (5-yr:34%). In patients with interval-free period greater than 24 months, they were 70 months (5-yr:63%) and 32 months (5-yr:44%), respectively. Most recurrences were located in the liver (4 out of 8). Conclusions: Partial hepatectomy offers an extended survival in very selected patients with metastatic breast cancer if the liver is the only site of disease. An extended preoperative interval-free period appears to select the best candidates for surgical therapy.

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Ablation of Hepatocellular Carcinoma (HCC) Prior to Transplantation — Pathologic Analysis of the Explant

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Background: Radiofrequency Ablation (RFA) and Percutaneous Ethanol Injection (PEI) ablation techniques have found utility for treatment of HCC. Transplantation has become the preferred treatment for patients with HCCs that lie within size and multiplicity criteria; however, the donor shortage has limited its widespread application. Since 1999, PEI and RFA have been used to control tumor progression in selected patients prior to hepatic transplantation for HCC. Pathologic analysis of the explanted liver in those transplanted provides a unique opportunity for the assessment of the oncologic effects of ablation. Methods: From Jan 1999 to Sept 2002, 37 cirrhotic patients were transplanted for known HCC. 14 patients (38%) underwent ablative procedures on 15 lesions prior to transplantation. PEI was performed on 6, RFA on 8, and one patient had both. Two patients had repeated ablations for recurrence. Ablated lesions were surveilled radiologically q3-monthly until transplantation. Mean interval between last imaging and transplant was 2.2 months. Hepatectomy explants were thoroughly analyzed for evidence of residual tumor at the site of ablation, as well as other findings. Results: Mean size of lesions pre-ablation was 29 mm (range 10 to 51 mm). Mean interval between ablation and transplant was 6.5 months. Seven patients had apparent radiographic control of their tumors, 6 suggested residual or recurrent tumor, and one was transplanted prior to post-ablative imaging. Pathologically, gross residual tumor, with varying degrees of necrosis or hemorrhage, was evident in all 15 ablation sites. Other incidental foci of cancer, or satellite lesions were identified in 7 (50%) patients. Mean post-transplant survival is 22.5 months for the ablation cohort: 2 patients (14.7%) have died, one having an aggressive recurrence of HCC two months following transplant. Survival in the non-ablation cohort was 20.7 months. Conclusions: Radiographic assessment of tumor recurrence appears to underestimate residual disease and the completeness of tumor ablation. These findings challenge the concept that current ablative techniques achieve complete eradication of tumor and are curative. The patients in this analysis, however, represent the early experience with PEI or RFA as a bridge to transplantation. Although residual tumor was identified, it is of unknown clinical significance. Long-term follow-up is needed to assess value of ablation for HCC.

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Hepatic Artery Infusion - Pitfalls and Benefits

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Introduction: Hepatic artery infusion provides a major method of controlling hepatic metastases in patients with non-resectable disease either as a singular technique or to supplement RFA or cryo-ablation. It also has suggested benefit as adjuvant therapy for patients with resectable disease. There are problems with pump management, but the benefit appears to outweigh the pitfalls. Material Methods: We have evolved a technique of HAI catheter placement with the goal to maximize the efficiency of the catheter placement. Patients are monitored with arteriography, magnetic resonance arteriography and dye distribution status by methylene blue injection or on-table arteriography coupled with ultrasound tumor localization and measurement and measurement of post therapy targeted liver lesions to define anti tumor response.

Results—Outcome

			Survival Outcome		
	Patients	Measureable Antitumor Response	Response Median	2-year	5-year
HAI (only)	26	67%	17 mo	23%	4%
+RFA	8	<60–80%	31 mo	52%	28%
+Resection	13	~100%	Not reached	66%	55%
Vs. resection alone	87	~100%	35 mo	73%	21%
Systemic RX	139	~15–20%	11 mo	13%	1%
Historic control*	136	Unknown	6 mo	8%	1%

*Kaplan-Meier Historic

Complications include misperfusion in 4 patients (all with negative nuclear scan) consisting of catheter migration with alteration of liver perfusion to the tumor (2 pts) and fracture of catheter in 1 patient. Conclude: HAI provides an important strategy to control hepatic metastases. Although complications occur, most are manageable with close attention to details. HAI has potential to significantly augment survival after resection or tumor ablation and, when used alone in advanced disease, may provide meaningful disease control and prolong overall survival.

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Radiofrequency Ablation for Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is a world-wide disease usually associated with poor outcomes. Systemic chemotherapy is ineffective. Hepatic artery chemoinfusion or chemoembolization has had mixed

results. Hepatic resection continues to be the treatment of choice for this disease. Unfortunatley many patients because of poor liver function or significant co-morbidities are not candidates for hepatic resection. Radiofrequency ablation has become an increasing treatment modality for unresectable hepatic tumors. This study retrospectivley reviews 36 patients with unresectable hepatocellular carcinoma from 8/98 to 9/02. Mean follow up is 25 months (1 to 50 mo) All patients were treated with a cooled-tip cluster radiofrequency probe to achieve tumor margin temperatures of > 70C. Patients were treated via percutaneous or operative approaches. 46 lesions were treated in 36 patients. Mean tumor size was 5.3 cm (6 mm to 15 cm). There were two mortalities within 30 days of ablation (CVA, cardiac arrhythmia). Morbidities included 2 patients with hepatic abcess, 2 transient liver dysfunction, 1 hepatic artery to portal venous fistula, and 2 segmental hepatic infarcts. Overall survival was 1 yr 76.7% (23/30), 2 yr 70.6% (12/17), 3 yr 50% (6/12), 4 yr 50% (1/2), 5 yr. NA. Two patients underwent orthotopic liver transplant following ablation with no viable tumor of the explanted specimen. Conclusion: Hepatic radiofrequency ablation is a treatment option which offers improved survival for patients with unresectable hepatocellular carcinoma.

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Treatment Of Unresectable Primary Hepatic Malignancies Using Hyperthermic Isolated Hepatic Perfusion (IHP).

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Background: Primary hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Isolated hepatic perfusion (IHP) is a locoregional treatment technique that isolates the liver in order to deliver high dose chemotherapy, biologic agents, and hyperthermia directly to hepatic parenchyma. This study presents our experience using IHP with melphalan with or without tumor necrosis factor (TNF) to treat nine patients with hepatocellular carcinoma or adenocarcinoma of hepatobiliary origin. Methods: Nine patients with unresectable primary hepatic malignancies underwent a 60-minute IHP with 1.5 mg/kg melphalan with or without 1.0 mg/kg TNF. Four patients failed one or more previous treatment regimens and the mean hepatic replacement was 41% (range 10-75%). Patients were monitored for response, toxicity, time to recurrence, and survival. Results: Six of nine patients (67%) experienced a >50% regression of tumor on objective radiographic imaging with an additional patient having a 45% reduction in tumor burden. Mean time to recurrence was 6.6 months for those who responded to treatment. Patients who had a response to therapy had an average overall survival of 16.3 months. In five patients hepatic progression was the only sight of disease at death. In three of the remaining four patients, progressive hepatic disease accompanied systemic metastases. A single patient died of progressive pulmonary metastases, without evidence of liver progression. Conclusions: IHP can be performed safely and has significant anti-tumor activity in patients with unresectable primary hepatic malignancies. Hepatic progression continues to be the dominant factor influencing survival in this group of patients.

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Hepatic Artery Chemoembolization for Isolated Colorectal Metastases to the Liver

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Introduction: Surgical resection is the preferred treatment for most hepatic malignancies, but is an option in only 25% of patients due to tumor characteristics or patient comorbidities. Hepatic artery chemoembolization (HACE) is an alternative therapy for these more advanced tumors, but its safety and efficacy for isolated hepatic colorectal metastases (CRM) has not been proven. We reviewed patients with isolated hepatic CRM who underwent HACE at our institution and compared their survival with both surgery for CRM and HACE for other malignancies. Methods: Data evaluated from CRM patients who underwent HACE between 1992 and 1999 included demographics, treatment details, and length of survival (LOS). These survival data were compared to LOS after surgical resection for CRM (n=135) as well as LOS after HACE for hepatocellular carcinoma (HCC) (n=40) and metastatic carcinoid (n=16). These data were analyzed using Kaplan-Meier and log rank methods. Results: Twenty-three patients with isolated hepatic CRM having an average age of 59.8 ± 12.1 years (57% male) underwent 44 HACE treatments. Length of hospital stay after HACE ranged from 1-15 days with an average stay of 2.9 days. Minor morbidities (nausea/vomiting, abdominal pain, fever) were reported in 21 patients (91%). Major morbidities included access site hematomas (n=3) and neutropenia (n=2). There was one mortality secondary to sepsis and multi-system organ failure. LOS after the initial HACE treatment for CRM was significantly shorter than after surgery for CRM (median 9.3 vs. 36.2 months; $p<0.001$). LOS after HACE for CRM was significantly shorter than after HACE for carcinoid (median 9.3 vs. 14.3 months; $p<0.05$), but was equivalent to LOS after HACE for HCC (median 9.3 vs. 7.9 months; n.s.). Conclusions: HACE for CRM is safe and well-tolerated, but survival after HACE was worse than survival after surgical resection for CRM. Results from HACE for CRM are comparable to those for HCC but worse than for carcinoid.

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Intra-Arterial Yttrium-90 Sir-Spheres for Metastatic Disease to the Liver

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Purpose: To evaluate the safety and efficacy of Yttrium-90 Sir-Spheres resin for the treatment of metastatic liver disease. Materials and Methods: 24 patients were treated with intra-arterial Yttrium-90 Sir-Spheres. All patients received 2 treatments on a lobar basis at 28-35 day intervals. Indications for treatment included metastatic liver cancer from the pancreas (n=2), colon (n=14), breast (n=3), carcinoid (n=1) and unknown primary (n=4). The average lobar volume was 1163 cc; the average dose of Y90 was 1.1 GBq. Patients had baseline liver function tests, tumor markers, CT and PET scans on or before on the day of treatment. Clinical follow-up, liver functions, CT scans were obtained at 30, as well as PET at 90 days. All patients were off chemotherapy at the time of treatment. Results: 22 of 24 patients received treatment on an outpatient basis and were discharged 6 hours after catheterization. 30 and 90 day clinical, laboratory, and CT follow-up was available in 18 of 24 patients. PET follow-up was available in 16 patients. 21 of 24 (88%) patients complained of fatigue for 7-14 days. 4 patients experienced transient but very severe burning in the area of treatment during the injection of Y90. On CT imaging, 15 of 18 patients had an average decrease in tumor size of 33%. 3 of 18 showed no change on CT. PET showed complete, partial and no response in 7, 7 and 2 patients respectively. Average tumour marker drop (CEA, CA19-9, CA15-3) in the 18 patients was 51% at day 90 following 1st treatment. The patient with carcinoid syndrome had complete resolution of symptoms. Conclusions: Sir-Spheres hepatic unilobar infusion for metastatic liver disease appears to represent a new and efficacious therapy with mild toxicity in a

patient population whose disease is refractory to other forms of treatment. Patients experience less toxicity than with chemoembolization. Survival data in this salvage patient population is extremely promising. Laboratory, CT and PET data are all considered integral in the follow-up process. Combinatorial trials with chemotherapy are warranted.

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Treatment of Unresectable Hepatocellular Carcinoma Using Intra-arterial Yttrium-90: Imaging, Laboratory and Clinical Response

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Background: Recently, Yttrium-90 has become available for the treatment of hepatocellular carcinoma. Yttrium-90 is a beta emitter with an average penetration of 2.5 mm. The half-life is 64.2 hours. Since it is administered via the hepatic arterial route, it can be viewed as internal beam radiation. Methods: Between June 2001 and June 2002, 32 patients underwent 48 administrations to the right and/or left hepatic artery. All patients carried the diagnosis of hepatoma. The average lobar volume was 1020 cc; the average dose of Y90 was 2.2 GBq, average absorbed dose of 135-150 Gray. Patients had baseline liver function tests and tumor markers on the day of treatment. Clinical follow-up and laboratory values were obtained at 30 day interval. Follow-up CT scans of the liver were obtained at 30 days, and subsequently at 90 day intervals. Results: 31 of 32 patients received treatment on an outpatient basis and were discharged 6 hours after catheterization. 1 patient experienced sudden onset chills and fever, but was discharged at 48 hours after a negative fever workup. 30 and 90 day clinical, laboratory and CT follow-up was available in 27 of 32 patients. 22 of 27 (81%) patients complained of fatigue for 7-10 days. 2 patients experienced transient but very severe burning in the area of treatment during the injection of Y90. On CT imaging, 15 of 27 patients had an average decrease in tumor size of 38%. Average AFP drop in the 27 patients was 51% at an average of 90 days. Median survival based on available data in 25 patients is 274 days after initial treatment. Conclusions: Yttrium-90 microspheres hepatic unilobar infusion for hepatocellular carcinoma appears to represent an efficacious therapy with minimal toxicity in a patient population whose disease is refractory to other forms of treatment. It can be safely performed on an outpatient basis. Survival data in hepatocellular carcinoma is extremely promising.

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Evaluation of Response using CEA, CT and PET in Patients Receiving Yttrium-90 (Y-90) for the Treatment of Metastatic Colorectal Cancer

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Background: Recently, Yttrium-90, a beta-emitter, has become available for the treatment of hepatocellular carcinoma. It is delivered by the hepatic arterial route. There is growing interest in using this treatment modality for colorectal liver metastases. Methods: Between June 2001 and July 2002, 105 patients underwent 185 administrations of Y-90 to the right or left hepatic artery. Of these patients, 34 carried the diagnosis of colorectal cancer metastatic to the liver. All patients had failed at least 2 polychemotherapy regimens, including 5FU, LV, ± CPT-11. Some patients had tried capecitabine (Xeloda). None of the patients were on chemotherapy at the time of infusion. The average lobar volume was 936 cc; the average dose of 90 Y was 2.88 GBq, average absorbed dose of 142 Gray. Patients had baseline liver function tests, CEA, CT and PET within a few days preceding the day of treatment. Clinical follow-up, CEA, and CT were obtained at 30 and 90 days. Follow-up PET scan was obtained at 90 days and was available in 24 patients. Results: 32 of 34 patients received treatment on an outpatient basis and were discharged 6 hours after catheterization. 2 patients experienced sudden onset chills and fever within 6 hours of the infusion. 30 and 90 day clinical, CEA, and CT follow-up were available for 27 of 34 patients. Average ratio of post to pre treatment CEA was 0.87 (range: .22-1.9) after 1 infusion, and 0.63 (range: 0.04-1.6) after the second. 3 patients CEA were excluded from the analysis because of the development of new intra and extrahepatic metastases. On CT follow-up, a mixed response was seen, with some lesions enlarging while others manifesting a decrease in size. PET imaging demonstrated a metabolic response in 20 of 24 (83%). Conclusions: Y90 lobar hepatic treatment can be performed safely on an outpatient basis in patients with colorectal metastases to the liver. Treatments are well tolerated, most will complain of fatigue. Response to Y90 in these patients is supported by the decrease metabolic activity on PET and in CEA. CT imaging findings can be misleading and should be correlated with PET. Based on this data, combinatorial trials with systemic chemotherapy are warranted.

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Perioperative Complications in Patients Undergoing Major Liver Resection With and Without Neoadjuvant Chemotherapy

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Introduction: Systemic chemotherapy is increasingly used prior to resection of hepatic colorectal metastases. Previous reports have indicated an increased risk of perioperative complications associated with the use of systemic chemotherapy prior to resection. The purpose of this study was to investigate perioperative complications in patients receiving systemic chemotherapy consisting of 5-FU and Leucovorin (LV) with or without CPT-11 versus those who did not, prior to major liver resection. Methods: Patients with colorectal metastases undergoing major liver resection (resection of ≥ 3 segments) with curative intent at the M.D. Anderson Cancer Center from June 1997 to June 2002 were identified by retrospective chart review. Patients who received preoperative regional therapy or regimens other than 5-FU+LV+/-CPT-11 were excluded. Perioperative parameters measured included the use of neoadjuvant therapy, type of resection, estimated blood loss (EBL), characteristics of the resected tumors, margin status, and 30-day mortality and morbidity rates. Fisher's Exact and Kruskal-Wallis analyses were used to test for statistical significance. Results: For the 5 year period, 108 patients were analyzed. 47 patients (44%) had no chemotherapy within 6 months of resection, 27 patients (25%) received a mean of 24 weeks of systemic 5-FU/LV within 6 months of resection, and 34 (31%) received a mean of 15 weeks of systemic 5-FU/LV/CPT-11 prior to resection. Of the entire group, 81 (75%) underwent right or extended right hepatectomy and 27 (25%) underwent left or extended left hepatectomy. A significantly higher number of patients in the group undergoing preoperative 5-FU/LV/CPT-11 had multiple tumors. Patients in this group also tended to have smaller tumors, less complications and a higher R0 margin resection rate, but these were not statistically significant. Median blood loss and length of hospital stay were also not significantly different. There were no perioperative deaths. Conclusions: The use of CPT-11 and fluoropyrimidine-based chemotherapy prior to major liver resection is not associated with increased morbidity or mortality. This strategy may therefore provide an effective therapeutic option, particularly in patients with multiple colorectal metastases.

Group	n	Median EBL (cc)	Multiple tumors	Max size (cm)	R0 resection	Complications	Median LOS (days)
No pre-op	47	425	19 (40%)	4.3	42 (89%)	23 (49%)	8
5FU/LV	27	400	10 (37%)	4.0	26 (96%)	10 (37%)	7
CPT11/5FU/LV	34	500	25 (73%)*	2	33 (97%)	10 (29%)	7

*P=0.003 vs other groups by Fisher's Exact test.

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Treatment of Unresectable Hepatic Malignancies With Intra-arterial Brachytherapy

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Purpose: To review the safety and efficacy of intra-arterially delivered brachytherapy for the treatment of primary and metastatic tumors in the liver. Methods: Patients having been treated for malignant tumors of the liver with radioembolization with yttrium-90 containing particles were reviewed. The patient demographics and the results of treatment were studied with special note made of any complications observed. Unilobar embolizations were performed and repeated for treatment of both lobes. Results: 68 patients were reviewed (42 men, 26 women) with a mean age of 62 (range 32 to 89 years). There were 22 HCC treated and 46 metastatic tumors (28 Colorectal cancer primaries, 10 breast cancer, 4 neuroendocrine, 4 other primaries). The mean follow up was 9 months with 78% of patients surviving. ECOG performance status at the entrance of the study was an average of 1.8 and at the end of 18 months 1.2. Complications included 3 patients with gastroesophageal ulcers and no misadministrations or overdosages. Post procedural toxicity was primarily lethargy and mild pain. Mean survival was not reached during the performance of this study. Conclusion: This is an effective treatment for unresectable tumors with minimal toxicity.

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Single Center Comparative Analysis of LDT Versus OLT for Hepatocellular Carcinoma

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Live donor transplantation (LDT) has been promoted as a means of facilitating transplantation in patients with hepatocellular carcinoma (HCC), but it is not clear that LDT confers a survival advantage compared to orthotopic liver transplant (OLT). We analyzed outcomes after LDT in a cohort of patients with HCC, in comparison with conventional OLT. Results: 278 pts. underwent LT (83 LDT) between 1999-2002, including 41 with HCC (14.7%). 32% of adult LDT pts. had HCC, compared to 11% of OLT pts. Overall, 39% of pts. with HCC received LDT, 61% OLT. Disease free survival was 85% overll (median f/u 360d). 5 pts. developed recurrence (4 LDT/1 OLT) with a mean time to recurrence of 303d. 30 pts. had known HCC at the time of transplant, including 5 who developed HCC while awaiting transplant. HCC was incidental in 11 pts. All except one with known HCC received pretransplant therapy (chemoembolization, resection, and/or ablation). There were no significant differences between LDT and OLT groups in preop AFP, proportion of patients receiving preop therapy, or disease free survival. Grouping by stage is illustrated in the table. Time from diagnosis to transplant was 117.5d in the LDT group versus 246d in the OLT group (p=0.008 Mann Whitney U). Conclusions: In patients with HCC undergoing LT, there is no difference in survival based on the donor source (LDT vs. OLT), despite a shorter waiting time for LDT. OLT pts. tended to have a greater likelihood of decompensated liver disease compared to LDT, assessed by Okuda and CLIP scores. Although the cohort is small, there are a greater proportion of LDT patients with recurrent disease (25%) compared to OLT (4%). It is possible that the mandatory waiting time imposed by OLT may in fact serve an observational screening purpose in assessing tumor aggressiveness prior to transplant.

	N	OKUDA I	OKUDA II	OKUDA III	CLIP 0/1	CLIP 2/3	CLIP 4/5
LDT	11	5	4	2	2	9	0
OLT	19	3	9	7	6	10	3

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Multi-modality Tumor Control Prior to Liver Transplantation in Patients With Hepatocellular Carcinoma (HCC)

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Liver transplantation (LTx) has recently become an acceptable mode of treatment in selected patients with unresectable HCC. However, due to the scarcity of donor organs and long waiting times, it is desirable for patients, not being transplanted soon, to have some form of tumor control prior to transplantation. We at our institution use a multi-modality approach to the management of HCC. In our institution, 33 LTxs were performed between June 1999 and September 2002. Of these, 14 recipients (11M, 3 F) had 23 HCC. In these patients the average number and size of HCC was 1.57 (1-4) and 2.14 (0.3-4.5) cm respectively. In five out of the 14 patients, these tumors were incidental, and discovered only on the pathology exam of the explanted liver. Therefore, nine patients had known HCC prior to LTx. Two patients received right lobes donated from relatives and another two received cadaveric graft soon after listing, so did not required tumor control therapy. The remaining five patients waited for 12.6 (4-26) months before LTx. Two patients had previous resection and presented with unresectable recurrence. Three patients underwent microwave coagulation therapy, and one had 6 treatments of alcohol injection. One patient received 6 cycles of the novel PIAF (Cisplatin, Interferon, Adriamycin and 5-FU) chemotherapy followed by selective internal irradiation (SIR) treatment with Yttrium 90 microspheres. One patient received conformal radiation therapy. There was only one postoperative death on one of the two patients who had liver resection. All the remaining 13 patients are doing well with no evidence of tumor recurrence with a mean follow up time of 16.1 (3.9-34.5) months. In conclusion, patients with HCC who need to wait for LTx should be treated with aggressive multi-modality therapy. The type of therapy should be tailored to the individual patient.

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One Hundred Adult-to-Adult Live Donor Liver Transplantation Using Right Lobe Graft Containing the Middle Hepatic Vein - A Study of Donor Safety

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Background. It is recognized that adequate venous drainage of segments V and VIII is necessary for optimum graft function in right lobe adult-to-adult live donor liver transplantation (LDLT). Inclusion of the middle hepatic vein (MHV) in the graft provides the best drainage but the risk to the donors has not been adequately assessed. The present study evaluated the outcome of 100 donors to determine the safety of donors donating right lobe grafts that contained the MHV. Methods. From 1996 to 2002, 100 right lobe LDLTs were performed. All right lobe grafts except one contained the MHV. The CT scans of the donors were studied thoroughly with respect to the anatomical arrangement of the hepatic veins and their branches. By CT volumetry, donors with left lobe volume >30% of the total liver volume were accepted for donor operation. During the donor operation, attention was paid to preserve the segment IV hepatic artery and to avoid prolonged rotation of the right lobe (which could induce ischemic injury to the left lobe). The MHV was transected proximal to a major segment IVb hepatic vein whereas possible in order to preserve the venous drainage in the liver remnant. RESULTS. By CT volumetry, the left lobe volume was 368 (range 217-594) ml or 33.9 (range 23.6-45.2) % of the total liver volume. On CT scan, the segment IVb hepatic veins, one to two in number, drained into the MHV (n=83), left hepatic vein (n=31), or junction of the middle and left hepatic vein (n=1). In 71 donors, an umbilical vein (which drained segment IV) was obvious on CT scan. In 48 donors, at least one branch of segment IVb hepatic vein was preserved in the left lobe. After graft retrieval, Doppler study of the segment IV portal vein showed normal flow direction, static flow and reversed flow direction in 59%, 20% and 20% of donors, respectively. There was no donor death. The donor operation complication rate was 25%, mostly minor wound infection. Major postoperative complications included cholestasis (n=4), bile duct stenosis (n=2), bleeding duodenal ulcer (n=1), intestinal obstruction (n=1) and incision hernia (n=1). The postoperative INR of donors who had all segment IVb hepatic veins sacrificed was slightly higher on POD 1 (P=0.054) but, in all donors, the liver function was largely normal by POD 7. The postoperative serum bilirubin and aspartate aminotransferase levels of the donors were not different from those of the reported series of right lobe grafts without the MHV. Conclusion. Right lobe donation including the MHV is safe provided that utmost care is given in preserving the liver remnant function.

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Influences of Right Anterior Sector Congestion on Whole Graft Function After Right Lobe Adult Living Donor Liver Transplantation (RL-LDLT)

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Severe venous congestion of the right anterior sector is occasionally encountered during RL-LDLT. As we need to consider not only the volume, but the function, of the graft when discussing the issue of "small-for-size" graft, we evaluated the clinical impact of the right anterior sector congestion in the right lobe graft. Subjects and methods: From June 2001 to July 2002, fifteen RL-LDLTs were performed in 15 adult patients who had advanced liver cirrhosis (4 with UNOS status 2B, and 11 status 3)at our institute. Recipients were divided into two groups according to the degree of right anterior sector congestion at the end of transplant surgery. Group A consisted of 6 patients with severe venous congestion more than 50% of the anterior sector, and Group B comprised 9 patients with slight or nil congestion. Ordinary liver function tests and bile production rate which reflects the energy state of liver cells were compared between the groups. Results: The graft to recipient weight ratio (GRWR) and graft volume/recipient standard liver volume (GV/SV) ranged between 1.16 and 1.75%, 54 and 85%, respectively, in Group A, and 0.86 and 1.25%, 47 and 72%, in Group B. One hospital death was observed in Group B (GRWR-0.87, GV/SV-49). The graft bile production rate (daily bile discharge(ml)/100g of liver graft) was substantially suppressed by more than half immediately after the transplant in patients with severe congestion vs Group B (7.87±4.83 vs 19.04±11.54ml/100g/24hr, mean±sd, on day1, p<0.05) and (9.43±6.19 vs 16.47±11.66 on day2, p=0.20), but recovered within a week (19.13± 13.91 vs 22.3±14.30 on day6). There were no differences between the groups with respect to daily ascites discharge (157±137ml vs 370±475ml on day 6), and total bilirubin levels except alanine aminotransferase levels on day 3 and 7 (698±411 vs 251±115 and 286±113 vs 154±28U/L, p<0.05). Conclusion: Although all our patients with severe congestion (40% of whole patients) tolerated RL-LDLT uneventfully, probably due to large graft volume (GRWR>1.16 and GV/SV>53), the graft function substantially decreased in the immediate postoperative period by more than half, compared with the one without significant congestion. Special attention should be paid against congestion of the right anterior sector during and immediately after RL-LDLT, particularly when using marginal or "small-for-size" graft.

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Aberrant Arterial Anatomy Is Not Associated with Higher Arterial Complications in Liver Transplantation

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Introduction: Arterial complications (ACOMP) after liver transplantation (OLT) can be associated with a multitude of factors. In this study, we review our experience with aberrant arterial anatomy (AAA) and examine its potential role in ACOMP and its association with biliary complications (BCOMP). Methods: This is a single center retrospective review from a tertiary transplant center. Patient records were reviewed for demographic information, arterial anatomy, ACOMP (thrombosis, stenosis), BCOMP (leak, stenosis) and final outcomes. Descriptive statistics, Chi-Square and Pearson correlation were used where appropriate. Results: Between January 1990 and July 2002, 231 primary adult OLTs were performed at our institution. There were 155 males and 76 females, with an age of 50.5±10 years. Accurate detail of AAA was noted in 191 OLTs. There were 53 cases (22.9%) of AAA; most commonly 26 replaced right and 14 replaced left. Of 231 OLTs, there were 25 ACOMP (10.8%) that occurred at 2.03±0.55 months after OLT; 17 were stenoses and 8 thromboses. There was no correlation between AAA and ACOMP, p=0.88. Arterial patch (AP) was used in 167 OLTs (72.3%) and did not correlate with ACOMP, p=0.33. BCOMP occurred in 58 OLTs (25.1%) and was simultaneous to an ACOMP in 8. There was a strong correlation between ACOMP and BCOMP, p=0.005. Lastly, AAA did not correlate with BCOMP, p=0.27. Conclusions: 1-AAA is common after OLT, 2-AAA does not correlate with ACOMP or BCOMP, 3-ACOMP correlates with BCOMP, the latter should warrant an arterial assessment for occult ACOMP.

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Effect of ATP on Mitochondrial Calcium Uptake Is Mediated By P2Y Receptor-Like Mechanism

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Primary graft non-function in liver transplantation is in part associated with cellular damage due to cold ischemia/reperfusion injury (CIR). Cold ischemia and subsequent reperfusion lead to a marked increase in intracellular calcium (Ca2+) concentration. Mitochondrial Ca2+ uptake, among other Ca2+ homeostatic mechanisms, attempts to protect cells by scavenging extra Ca2+ from the cytoplasm, but, mitochondrial Ca2+ overload leads to mitochondrial permeability transition and triggers apoptotic pathways. Intracellular adenosine 5'-triphosphate (ATP) regulation of mitochondrial Ca2+ uptake is important for cell survival during CIR, but mechanisms of this regulation are unknown. We hypothesized

that ATP regulates mitochondrial Ca2+ uptake via a receptor-like mechanism. Methods. Rat livers were perfused with UW solution via the portal vein, harvested and homogenized. Mitochondria were separated from liver homogenate by differential centrifugation and mitochondrial Ca2+ uptake was determined by using 45Ca2+. After incubation at 37C in the presence 0.2 μM 45Ca2+, 1mM pyruvate and 1 mM malate, the mitochondrial suspension was filtered and Ca2+ uptake was calculated from the radioactivity of the filters. A non-hydrolysable analog of ATP, AMP-PNP (100μM), Ruthenium Red (inhibitor of mitochondrial Ca2+ uniporter, RR, 10μM) and P2Y receptor antagonist reactive blue-2 (RB-2, 300μM) were added to the incubation medium to determine their influence on Ca2+ uptake. The experiment was repeated on 3 animals and each measurement was performed in triplicates. Statistical analysis was performed using Student's t-test, with $p<0.05$ taken as significant. Results: 1. ATP analog AMP-PNP significantly activates mitochondrial Ca2+ uptake. 2. RR completely inhibits Ca2+ uptake by itself and in combination with AMP-PNP. 3. P2Y inhibitor RB-2 completely inhibits mitochondrial Ca2+ uptake in combination with AMP-PNP. Conclusions. 1. The effect of AMP-PNP suggests that intracellular ATP at concentration lower then normal activates mitochondrial Ca2+ uptake. 2. Inhibition of this effect by RR suggests that intracellular ATP activates Ca2+ uptake via the calcium uniporter. 3. The ability of RB-2 to cancel the effect of AMP-PNP and depress Ca2+ uptake below baseline suggests that ATP influences Ca2+ uptake via a P2Y receptor mechanism.

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Biliary Complications in 96 Right Lobe Living Donor Liver Transplants

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Introduction: Biliary reconstruction during right lobe living donor liver transplantation (RL LDLT) is the most technically challenging aspect of the procedure. This study documents the incidence, nature, and outcome of biliary complications following RL LDLT. Method: Between June 1999 and January 2002, 96 RL LDLT were performed in our center (91 adults; 5 children). We retrospectively reviewed the records of these recipients, noting the number of bile duct anastomosis; type of reconstruction; incidence, timing, treatment, and outcome of leaks and strictures; and patient survival. Results: Multiple ducts (≥ 2) were found in 58 grafts (60.4%). Roux-en-Y reconstruction was performed in 53 cases (55.2%), duct-to-duct in 39 (40.6%), and both in 4 cases (4.2%). Thirty-nine recipients (40.6 %) had biliary complications: 21 patients had leaks and 22 had strictures (4 patients had both). Six patients had multiple biliary leaks requiring multiple operations. Patients with ≥ 2 biliary anastomosis had a higher incidence of bile leaks (19% vs. 5.5%, p=NS). The incidence of leaks was higher with Roux-en-Y compared to duct to duct (18.2% vs 7.3%; p=NS); the opposite was for strictures (16.3% vs 31.7%; p=NS). Leaks occur at a median of 12 days after transplant, while strictures presented at a median of 178.5 days. Freedom from biliary complications was 55% at 2 years. Two-year survival for patients with and without biliary leaks was 65% and 85%, respectively (p=0.07). Overall 2-year patient and graft survival was 81% and 77%, respectively. Conclusion: The bile duct is still the Achilles' Heel of the liver transplant, with a complication rate of 40% in RL LDLT. Leaks seem to be more common with multiple anastomosis and Roux-en-Y reconstructions; duct-to-duct reconstruction is more prone to stricture. Bile leaks require aggressive treatment to improve outcome.

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Liver Transplantation for Hemangioendothelioma

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Hemangioendotheliomas are exceedingly rare liver tumors that are often multi-focal and incompatible with resection. Liver transplantation has been anecdotally utilized in patients but significant series have not been reported. Methods: We retrospectively reviewed our transplant tumor database for all hemangioendothelioma patients who underwent liver transplantation. Results: Eighteen patients received liver transplants for the diagnosis of hemangioendotheliomas. The mean age of the group was 33.3 ± 13.2 years (range 3-57 yrs) and was comprised of 12 females and 6 males. There were 16 Caucasians, 1 African American, and 1 Asian. The diagnosis was made by preoperative biopsy and the presence of lymph node invasion confirmed at the time of explantation. Antibody induction was utilized in 3 patients while IL-2 receptor antibody was utilized in 2 other patients. Maintenance immunosuppression consisted of prednisone (n=18), cyclosporine (n=10), tacrolimus (n=8), azathioprine (n=7), and Mycophenolate mofeltil (n=4). Post-operative adjuvant chemotherapy was given to three patients (2 patients with node + disease). Mean follow-up was 27.2 ± 35.8 months (range 6 to 110 months). Node positive disease was encountered in 3 patients. Recurrent disease was observed in three patients (16.7%), two of these had node + disease. The overall patient survival was 77% at 3-years. When compared the survival in the node + group was 33% with the 2 deaths occurring in the first six months post-op. While in contrast, survival in the node negative group was 75%, p=0.001. The mean survival was 9.2 ± 5.4 months for those with recurrent disease. Conclusions: Transplantation for hemangioendotheliomas can be performed safely with excellent 3-year survival. Unfortunately, node positive disease portends a high-risk for recurrence, which significantly diminishes patient survival.

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Does Estrogen Protect Against Reperfusion Injury in the Liver?

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Organ ischemia followed by reperfusion injury impacts many areas of surgical care. Ongoing research is directed at limiting or preventing reperfusion injury. Prior studies have shown female rats to have decreased lung injury after hemorrhagic shock followed by resuscitation. The purpose of our study was to investigate whether estrogen would protect against reperfusion injury in the murine liver. Twenty male C57BL/6 mice were divided into 2 groups: control and experimental. Each group underwent midline laparotomy and ligation of the vascular pedicle of the left lateral lobe of the liver for one hour. After one hour of ischemia, the left lateral lobe was allowed to reperfuse for 5 hours. After 5 hours of reperfusion, the carotid artery was cannulated and 0.5 cc of blood withdrawn. The abdomen was then re-opened and a biopsy of the left lateral lobe was taken to be stained with TUNEL reagent. The animal was then sacrificed. Mice in the experimental group were given a subcutaneous injection of estrogen twenty-four hours prior to laparotomy. Mean AST in the control group was 2368. Mean AST in the experimental group was 514. There was not a statistically significant difference between these two groups (p=.1365). Mean ALT in the control group was 3767. Mean ALT in the experimental group was 861. Again, there was not a statistically significant difference between these groups (p=.2025). Tissue specimens from neither the control group nor the experimental group had TUNEL positive cells indicating the start of apoptosis. Although mean transaminase levels between the control and experimental groups are not equal, the difference was not statistically significant.

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Impact of Donor and Recipient Risk Factors on Survival and Quality of Life After Liver Transplantation

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Background: In an effort to expand the donor pool, marginal donors (with presumed marginal grafts) are increasingly used. The aim of this study was to compare graft and patient survival as well as health related quality of life (HRQOL) on the basis of optimal versus marginal donor organs and by other potential risk factors. Methods: 430 cadaveric liver transplants in 402 recipients between 1991 and 2002 at Vanderbilt University Medical Center were analyzed for graft and patient survival. Additionally, Karnofsky functional performance (FP) and HRQOL (SF-36 and Psychosocial Adjustment to Illness Scale) were measured in 75 recipients. Potential risk factors influencing these outcomes were assessed including donor age and weight, warm and cold ischemic time, recipient UNOS status and age, and gender matching. Data were analyzed via Kaplan-Meier techniques, Cox regression, and analysis of variance methods. Results: Graft survival (mean + SEM) was 44 + 8 versus 96 + 4 months when donors were $>$ 60 versus $<$ 60 years, respectively ($p< 0.01$). Patient survival was 62 + 9 versus 106 + 3 months for these donor age groups ($p=0.01$). Cold ischemic time (CIT) greater than versus less than 12 hours was associated with shorter graft survival (78 $\pm$ 8 versus 97 $\pm$ 3 months, $p= 0.01$). A comparable pattern was seen for patient survival in relation to CIT ($p=0.03$). Cox regression demonstrated that UNOS "status 1", donor age, and CIT were independently associated with shorter graft survival (model $p<0.001$, all predictors $p<0.05$). Similarly, UNOS "status 1" and donor age were adversely related to patient survival (model $p=0.01$, all predictors $p\leq0.05$). FP and HRQOL improved over time following transplantation, but this improvement was not affected by donor or recipient characteristics, or CIT. Conclusions: This study demonstrates the effects of donor age, recipient urgency status, and CIT on survival following liver transplantation. However, these factors do not affect the trajectory of improvement in FP and HRQOL following liver transplantation.

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Live Donor Liver Transplantation for Acute-on-Chronic Hepatitis B Liver Failure

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Aim: The survival results of patients who suffered from acute-on-chronic liver failure (United Network for Organ Sharing priority status 2a) and received live donor liver transplant (LDLT) have been reported to be poor. The aim of the present study was to evaluate the survival outcomes of patients who underwent LDLT using right-lobe liver grafts for acute-on-chronic hepatitis B liver failure. Patients and Methods: The study comprised 32 patients who had acute-on-chronic hepatitis B liver failure and underwent LDLT using right-lobe liver grafts from June 1996 to March 2002. The mean ($\pm$ SEM) MELD scores before liver transplantation was 36 $\pm$ 1.8. LDLT using right-lobe liver graft including the middle hepatic vein was performed after informed consent was obtained from voluntary donors and preoperative evaluations were completed. Oral lamividine 100mg daily was started before transplant and maintained indefinitely afterwards for hepatitis B prophylaxis, and hepatitis B immune globulin was not used. Results: The mean preoperative intensive care unit stay was 2.4 days and the mean ($\pm$ SEM) postoperative hospital stay was 38.1 $\pm$ 5.8 days. At a median follow-up of 23 months, both patient and graft survival was 88%, respectively. Four recipients died after LDLT, and the causes of death included systemic candidiasis (n = 1), necrotising pancreatitis (n = 1), empyema thoracis (n = 1), and biliary sepsis (n = 1). The survival results were not different from those of 49 patients who underwent LDLT for elective conditions during the same study period (graft survival = 82%, p = 0.55; patient survival = 84%, p = 0.75). Two (6.3%) patients developed recurrent hepatitis B resulting from viral breakthrough 47 and 53 months, respectively, after transplantation, but remained well after treatment with adefovir. Post-operative complications occurred in 8 (25%) donors, but most of them were minor complications. The mean ($\pm$ SEM) hospital stay of the donors was 12.0 $\pm$ 1.0 days. There was no donor mortality. Conclusion: When cadaveric organ donation is scarce, LDLT using right-lobe liver grafts represents a timely and effective therapeutic option for patients with acute-on-chronic hepatitis B liver failure. It results in satisfactory survival outcomes comparable to patients who receive LDLT for elective conditions.

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Protective Effect of N2-Mercaptopropionylglcycine, on Liver During Ischemia/Reperfusion Process

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N2-mercaptopropionylglycine (N2-MPG), among other properties, is a powerful super oxide synthesis inhibitor and was tested as a preventive agent of metabolic and structural damage of hepatic parenchyma, in the ischemia/reperfusion process. Twenty-two rats and twenty-two dogs were divided into four groups: Group I: rats that received I.V. saline 0.9%; Group II: rats that received 100mg/kg of N2-MPG; Group III: dogs that received saline I.V. 0.9% and Group IV: dogs that received 100mg/kg N2-MPG. Ten minutes after the saline or drug administration, each group was submitted to left lobe normothermic liver ischemia for 25 minutes followed by reperfusion. Biochemical studies 24hrs. after reperfusion revealed a significantly low elevation of transaminase in animals of groups G-II (AST=271$\pm$182; ALT=261$\pm$161) and G-IV (AST=101$\pm$45; ALT= 123$\pm$89) when compared in the controls G-I (AST=2144$\pm$966; ALT=1869$\pm$1040 00) and G-III (AST=18002.10$\pm$76.51; ALT=277$\pm$219), all in UI/dl. Histology study demonstrated a significantly minor aggression to animals of G-II and G-IV when compared to G-I and G-III. These results suggest an actual and significant release of free radicals of oxygen and that N2-MPG may have a significant protective effect on the liver parenchyma when submitted to normothermic ischemia/reperfusion process. Descriptors: Ischemia, Reperfusion, Liver, N2-Mercaptopropionylglicyne, antioxidants, Free Radicals.

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Liver Transplantation for Neuroendocrine Tumors

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Introduction: Liver transplantation for the treatment of metastatic neuroendocrine tumors (NETs) is radical. While cure is not impossible, it is certainly improbable. The world's experience with transplantation for this indication is limited to less than 150 cases with widely varying results and few 5-year disease-free survivors. We reviewed our experience with transplantation for patients with NETs. Methods: Since 1992, 43 patients with NET liver metastases have been evaluated at the Mount Sinai Hospital. Fifteen (34.9%) patients received only medical therapy. Sixteen (37.2%) patients underwent hepatic resection, either for localized tumor in hopes of cure, or for debulking of symptomatic metastases. Fourteen (32.6%) patients were evaluated and listed for transplantation. In general, the decision to proceed with transplantation was made in physically fit patients with unresectable tumors with uncontrollable symptoms due to tumor bulk and/or tumor hormone production. Two patients listed for transplantation underwent prior hepatic resection. Orthotopic liver transplantation was performed in the standard fashion with primary cyclosporine or tacrolimus immunosuppression, except in one living donor case performed without immunosuppression in identical twins. Results: Of the 14 patients evaluated and listed for transplant, 11 (78.6%) underwent liver transplantation, 3 with living donor grafts. Among the 3 patients listed but not transplanted, one was lost to follow-up, one died 14 months after listing, and one remains waiting over 4 years. There were

six (54.6%) non-functioning tumors, 3 (27.3%) carcinoids, and 2 (18.2%) VIPomas. There were 4 (36.4%) men and 7 (63.6%) women with a mean age of 51.2±6.3 years. Three patients had distal pancreatectomies and 1 patient had a Whipple procedure at the time of transplant. Three patients had prior distal pancreatectomies, three had prior intestinal resections and 1 patient's tumor was an incidental finding in the explant after transplant. The 1 and 5-year survival among these patients is 73% and 36%, respectively (range 0-119 months), with a median follow-up of 62 months. Of the 3 patients surviving >5 years, only 1 was disease free. Conclusion: In carefully selected patients with biologically favorable (i.e., less aggressive) metastatic neuroendocrine tumors, liver transplantation may be an appropriate option. Long-term survival is possible in these patients, however, cure is highly unlikely.

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Efficiency of Recipient Screening and Donor Evaluation in Adult Living Donor Liver Transplantation

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Living donor liver transplantation has become a valuable tool to overcome the shortage and limits of cadaveric organ donation, and it has been shown to significantly reduce the mortality in children while waiting for transplantation. To assess the efficiency of recipient screening and donor evaluation in our adult liver transplant program we reviewed our early learning phase. Since initiation of the living donor liver transplant program at our institution in March 2000 a total of 61 adult patients were listed for cadaveric donor liver transplantation with Eurotransplant. At this point of time, the option of living donation was discussed with 41/61 recipients (72 %). In principle, a potential living donor was available and identified in 16/41 patients (39 %), remained unclear in 15, and was not available in 10 cases, respectively. Meticulous donor evaluation following a four-step protocol with thorough workup of medical history, physical examination, extensive laboratory tests, magnetic resonance imaging studies, laparoscopy with liver biopsy, endoscopic retrograde cholangiography, arteriography, psychology and other consults was completed in 15/16 cases (94 %). One donor reconsidered living donation, and finally refused to complete the evaluation because the recipient suffering from primary sclerosing cholangitis and ulcerative colitis had developed a colon carcinoma in the meantime. Living donor right hemihepatectomy and partial transplantation were performed and completed in 14/15 patients (94 %). One procedure had to be aborted since the recipient developed a septic shock during the operation. Thus, 14 of all 61 recipients seen during this period of time were able to receive a living donor liver transplantation resulting in an efficacy rate of 23 %. This figure corresponds well with our preoperative waiting list mortality rate of 25 % before the start of the living donor program. Our preliminary experience which is well in accordance with data published from other centers demonstrates that recipient screening and identification of potential living donors as well as their evaluation is efficient, and can result in a significant increase of organ donors for liver transplantation thus reducing also the risk of adult patients to die before transplantation can be realized.

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Living Donor Liver Transplantation : Is the Morbidity Underestimated?

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Transplant units have unreliably evaluated donor outcome in LRLT, as evidenced by the wide incidence of complications identified in the medical literature and in the NIH survey. Health authorities are catching up by implementing registries world-wide. It remains however the responsibility of the medical community to promote accurate reporting of donor complications. Between January 1995 and August 2002, 102 living-related donors were operated in our centre including left lateral sectoriectomies (LLS=30), left hepatectomies (LH=35) and right hepatectomies (RH=37, including 19 with the middle hepatic vein) as part of a pediatric (n=58) or an adult (n=44) transplantation. Donor and recipient operations were performed independently, without cross over. Donors included 45 women and 57 men with a mean age of 36±9 years (range, 18-73). All intra- and postoperative complications were recorded prospectively by a specialised liver surgeon not involved in either donor or recipient procedures. Patients underwent a medical and an independent psychological evaluation 6 and 12 months after surgery. Donor mortality was nil. Duration of surgery and blood loss were 373±54 min and 577±237 mL. These figures were not significantly influenced by the extent of resection. There was one serious intraoperative adverse event (displacement of right hepatic vein clamp). Incidence of surgical complications was 9% (6 biliary leaks including 4 that required percutaneous drainage, 1 abdominal hernia, 1 reoperation for chylous ascites and 1 reoperation for bleeding). Incidence of major medical complications was 12% (3 pneumonia, 3 pulmonary embolism, 1 pneumothorax, 1 compression of the brachial plexus, 2 symptomatic pleural effusions that required drainage and one significant but transient kidney failure). No patient experienced overt liver failure. Incidence of other complications revealed by the prospective postoperative assessment was 24% including asymptomatic pulmonary embolism in 3, asymptomatic large pleural effusions in 4, gastroparesis in 2 and 16 cases of fever > 38.5. Several of these complications had not been previously recognized following liver resections for other indications. Overall incidence of complications was 45%. Overall duration of in-hospital stay was 12±3 days and was influenced by the extent of resection. Hospital readmission following initial discharge was required in 4 patients. One, 6 and 12 months after donation there is not evidence of major and important sequelae and all donors had normal liver tests. In conclusion the prospective inclusion of all events following liver donor resection revealed a high incidence of complications. Some of these complications have not been recognized or have been underestimated following resections for other indications.

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Laparoscopic Procurement Model for Living Donor Liver Transplantation

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Introduction and Objectives: Noting the contribution to renal transplantation by the introduction of the laparoscopic approach to donor nephrectomy, we investigated the possibility of performing a laparoscopic hepatic lobe procurement with the goal of performing a live donor liver transplantation. We describe our technique and determine its feasibility for such a goal. Methods: The surgical technique was developed over a series of 12 adult female pigs and adapted in two human cadavers. The technique included pneumoperitoneum with CO2, mobilization of the liver, and transection of the parenchyma into right and left lobes with a cavitron ultrasonic aspirator. The vascular inflow and outflow structures (hepatic artery, portal vein, hepatic veins) of the anatomical specimen being procured were preserved undisturbed during the hepatic transection. No temporary

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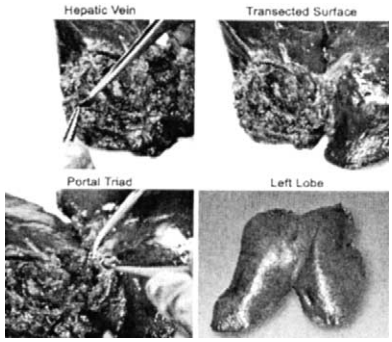
vascular occlusion techniques were utilized. The vascular structures were stapled and sectioned just prior to removal of the specimen. Results: Hepatic lobectomies were successfully performed laparoscopically. Parenchymal tissue viability was demonstrated by histologic and clinical examination. Vascular and biliary structures were preserved in order to allow for subsequent transplantation. Operative time from establishment of pneumoperitoneum to lobe procurement was under 4 hours. Conclusions: This study demonstrates the feasibility of laparoscopic living donor procurement for liver transplantation, both from a technical and a physiological perspective.



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Liver Transplantation for Hepatocellular Carcinoma: Should Patients With Stage III Disease Receive Allocation Priority?

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Introduction: Recent reports suggest that liver transplantation may provide an effective treatment strategy for carefully selected patients with unresectable hepatocellular carcinoma (HCC). Under guidelines adopted in February, 2002 by the United Network for Organ Sharing allocation schema, patients with stages I or II HCC receive priority for transplantation, but not patients with higher tumor staging. The current study was undertaken to investigate long-term outcomes for transplantation based on HCC tumor staging in a single center. Methods: From 1985 to 2001, 48 (8%) out of 605 primary liver transplants were performed in our center in patients with HCC. Data was prospectively collected and retrospectively reviewed. Transplant procedures for HCC were evenly distributed over the 16-year period of review. Chemoembolization and tumor ablation were performed while patients were awaiting transplantation, however the effect of neoadjuvant therapy was not assessed as part of the current study. Tumor staging was based on TNM classification from pathologic data obtained from the explanted specimen. Kaplan-Meier survival curves were generated for purposes of this analysis. Results: The majority of patients undergoing transplantation during the period of study were male (40 patients, 83%), with a mean age of 52.2 years (range 22-68). Median follow-up was 4.7 years. Overall patient survival rates are shown below. No statistically significant survival differences were noted for patients with stages I, II, and III tumors compared with patients undergoing liver transplantation during the same time interval but without HCC. Similarly, patients with stage III HCC had a similar outcome to patients with less advanced disease. However, patients with stage IV HCC had a significantly worse survival compared with patients with stages I-III HCC. Conclusions: Liver transplantation is an effective treatment for patients with unresectable HCC. In this single-center report with long-term follow-up, transplantation for stage III disease appears to yield results that are similar to stages I and II. While the current MELD-based liver allocation algorithm gives extra priority to those with stages I and II disease, consideration should be given to including patients with stage III disease.

Survival rates by stage of disease

		Survival		
Pathological stage	**N**	**1-year**	**3-year**	**5-year**
I	12	80%	70%	70%
II	11	75%	55%	55%
III	9	78%	78%	52%
IV**	16	56%	31%	25%

**$p<0.05$ compared with I–III.

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Methoxypolyethylene Glycol Modified-Albumin Enhanced the Cold Preservation Properties of Uw Solution in Liver Grafts

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Liver grafts preserved in cold undergo changes mainly manifested by morphological changes of the sinusoidal endothelium. Swollen and fragmented cytoplasm translates into poor portal blood flow, increase release of liver enzymes and low bile production upon liver reperfusion. Studies were performed to determine if the addition of higher molecular weight polyethylene glycol modified albumin to the University of Wisconsin (UW) preservation solution ameliorates the cold preservation injury of liver grafts. Mehtoxypolyethylene glycol 5000 activated with cyanuric chloride was covalently coupled to human albumin (Peg-Alb) at multiple sites. The Isolated Perfused Rat Liver model was used (IPRL).

IPRL results of grafts preserved with UW solution and UW solution plus Peg-Alb. Values are given after 60 minutes of perfusion with a sanguineous perfusate.

Group (n=4) (preservation time in h)	**Portal Blood flow ml/g of liver/min**	**AST units/g of liver**	**Bile production ml/g of liver**
Control neg UW (1h) Mean±SD	0.93±0.033**	2.1±1.08**	10.5±5.97
Control pos UW (30 h)	0.19±0.010	14.4±0.34	0±0
PEG-Alb & UW (30 h)	0.98±0.005**	28.4±1.03	3.5±7.54
Alb & UW (30 h)	0.05±0.007	26.9±2.45	0±0

**$p<0.05$ by ANOVA.

The addition of high molecular albumin to UW preservation solution appears to ameliorate endothelial injury of cold preserved liver grafts as judged by both better portal vein blood flow and bile production. However, further morphological and molecular studies are needed to define its role.

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The Role of Platelets in Murine Hepatic Ischemia/Reperfusion Injury

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Ischemia Reperfusion(I/R) Injury is a leukocyte mediated event that results in tissue infiltration by the leukocytes with a resultant increase in the inflammatory mediators and tissue damage. The mechanism of injury is initial sequestration in the vasculature, transendothelial migration and injury to the parenchymal cells. This process uses several cellular events that involve cell adhesion molecules and chemical mediators. Sev-

eral studies have demonstrated leukocyte-endothelial interactions in various I/R models. Recently, Drs. Maguire and Zibari have demonstrated that antiplatelet antibody induced thrombocytopenia attenuated the effects of I/R. The purpose of our experiment is to determine the platelet interactions with the endothelium in the murine hepatic I/R injury model. Forty C57BL/6 wild type mice, 6 to 8 weeks of age were divided into two groups of 20 each. One group received donor platelets that underwent I/R and the other group received platelets that did not. Each group is then subdivided into the control group that received a sham laparotomy with no ischemia and the experimental group that received a laparotomy, with 30-minute ischemia of the left lateral lobe of the liver and a one-hour reperfusion period. The donor platelets were isolated, tagged with fluorescent Rhodamine, centrifuged and rid of leukocytes, RBCs and plasma. This donor platelet rich suspension is infused into the control and experimental mice over 2 minutes using the external jugular vein cannulation. The left lateral lobe of the liver was placed on the video microscope and the terminal hepatic venules were observed for platelet-endothelial interaction. A 100 micrometer length was isolated and its flow recorded for 2 minutes. The platelet interactions with the endothelium were then divided into rolling, saltation and adherence. The unpaired t test was used to analyse the data. Our study showed no statistical significance in the platelet interaction with the endothelium between the control and the experimental groups irrespective of the donor platelets being activated or not. There may not be any direct platelet-endothelial interactions resulting in the ischemia/reperfusion injury. Although our study did not demonstrate any direct platelet-endothelial interactions, multiple studies showed that the leukocytes and the platelets are both necessary to mount the ischemic reperfusion injury. It is possible that the platelets may indirectly cause the leukocytes to sequester either through chemical mediation or upregulation of certain adhesion receptors, somehow priming the leukocytes to adhere to the endothelial wall eventually causing parenchymal damage. This may be one of several factors that are involved in the I/R injury, which is yet to be clearly understood.

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Preoperative Surveillance for Hepatocellular Carcinoma in Liver Transplant Candidates: Image Isn't Everything

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Background: The optimal screening protocol for hepatocellular carcinoma (HCC) before liver transplantation (OLT) is controversial. Aim: To evaluate the ability of our Program's screening protocol to preoperatively detect HCC, and to examine the impact of this on patient survival. Methods: A retrospective review of 424 consecutive adult OLT at our institution was performed. Preoperative HCC screening in these patients consisted of abdominal ultrasound (US) every 6 months and serum alpha-fetoprotein (AFP) every 3 months until OLT. Patients with suspicious lesions on US or AFP>20 ng/ml were further evaluated with biphasic, contrast-enhanced helical computed tomography (CT) or with magnetic resonance imaging (MRI). Patient demographics, etiology of liver disease, results of preoperative imaging and AFP, and explant pathology were recorded. Results: Seventeen of 424 (4%) OLT recipients had HCC identified on pathologic examination of the explant. There were 15 men and 2 women, mean age 45.8 ± 15 years (range 18.9-61.9), with a mean follow-up of 17.4 months ±19.9 months (range 0.43-78.6). The most common indications for OLT in patients with HCC were hepatitis C (53%) and Laennec's cirrhosis (17.6%). There was a trend toward more hepatitis C infection in patients with HCC (53%) compared to those without HCC (32%), p=0.07. Screening US and AFP detected (DET) only 8/17 HCC preoperatively (47.1%). Nine of 17 HCC were discovered incidentally (INC) in the explant (52.9%). Seven of 8 patients with DET HCC had a liver mass identified on US; 6 of these underwent confirmatory CT or MRI. One of 8 patients with DET HCC had a normal US and an elevated AFP, with subsequent confirmation of a liver mass on MRI. AFP correlated poorly with presence of HCC, with only 3/17 patients demonstrating elevated preoperative AFP, all in the DET group. Fifty six percent of INC and 75% of DET HCC were either stage I or II (p=0.14). Despite a surprisingly higher (but statistically insignificant) percentage of stage III tumors in patients with INC HCC, 1- and 3-year actuarial survival was comparable between INC and DET. Conclusions: Despite the poor ability of US and AFP to detect HCC preoperatively, the similar long-term survival between patients with INC and DET HCC suggests that routine surveillance with CT or MRI would be unlikely to significantly alter management strategy for the vast majority of OLT candidates. In this series, only 2/424 patients (0.5%) would have been excluded as OLT candidates had surveillance imaging detected their advanced HCC preoperatively. Better screening and risk stratification tools are needed to more accurately predict which patients require more extensive imaging in order to most efficiently utilizes diagnostic resources.

Kaplan-Meier Patient Survival

	1 year	3 years
INC	63%	63%
DET	72%	72%

p=0.9.

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Prevention of Hepatitis B Virus Infection After Liver Transplantation With Hepatitis B Immunoglobulin and Lamivudine

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The outcome of liver transplantation (LT) in patients with hepatitis B related liver diseases improved significantly by introduction of passive immunoprophylaxis and antiviral drugs. But there was no consensus concerning dosage and duration of these agents for prevention of hepatitis B recurrence after LT. The aim of this study is to investigate the efficacy of HBV prophylaxis with HBIG and lamivudine. (Patients and methods) Between 1999 and 2001, LT was done in 59 patients who have positive HBsAg. Twenty two of them carried hepatocellular carcinoma (HCC) also. Imunnosuppression were FK 506 or cyclosporine based protocol. Prophylaxis against HBV related disease was started in the anhepatic phase with application of 10.000 IU HBIG and consisted of HBIG infusion of 10000 IU per day for seven days postoperatively and following infusion of 10000 IU per week for three times. After then, hepatitis B antibody titer was checked and maintained above 350 IU/L by intermittent infusion of HBIG. Also all HBV associated patients received lamivudine 100 mg po daily for one year. After stopping lamivudine, hepatitis B antibody titer was maintained above 250 IU/L. Mean follow up period was 19.9±10.2 months. Recurrence of hepatitis B was found in 3 patients. But in 2 patients, HCC had recurred and after that HBIG was not administered. Only one patient with proper administration of HBIG and lamivudine (at that time no lamivudine was administered because 1 year has passed) as protocol became HBsAg positive and HBV DNA probe positive at 14 months postoperatively. Then lamivudine was administered. But he has normal liver function test profile and no subjective symptoms. Combination prophylaxis with hepatitis B immunoglobulin and lamivudine is safe and highly effective in prevention of HBV recurrence after liver transplantation.

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Outcome of Liver Transplantation Using the Grafts From the Living Donor With Isolated Hyperbilirubinemia

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Aims: Shortage of cadaveric donor increased living donor liver transplantation (LDLT). Our aim was to determine whether isolated unconjugated hyperbilirubinemia of the donors affects the short-term outcomes of both recipients and donors in LDLT. Materials & Methods: From January 1999 to September 2002, 140 cases of LDLT was performed at Seoul National University Hospital. Fourteen donors had unconjugated hyperbilirubinemia (1.4-3.5mg/dl) in the absence of other abnormal liver chemistry tests. Hemolysis, viral hepatitis and biliary tract obstruction were excluded through preoperative studies. The mean age of donors was 31.6±6.6 years. The recipients composed 5 children and 9 adults. The original disease was 8 hepatitis B related liver cirrhosis (including 2 hepatocellular carcinoma), 3 biliary atresia, 2 fulminant hepatitis and one Alagille syndrome. Three cases of operative mortality were excluded in this study. Mean follow up period was 6.0±7.6 months. Results: The postoperative course in donors was not eventful. The mean hospital stay was 11.6±2.4 days. The mean peak level of serum bilirubin was 4.4±2.1 (range 1.9 to 8.1) mg/dl and at the 7th postoperative day it decreased down to 1.8±0.9 (range 0.8 to 3.3) mg/dl. One month later, it improved to preoperative level (1.3±0.6, range 0.9 to 2.2), except one with small remnant liver that was 30.0% of the total liver volume. The recipient showed variable course of recovery. The serum bilirubin level decreased to the preoperative level at the 14th postoperative day except 2 cases, one with bile duct stenosis which was resolved with endoscopic drainage procedure due and the other one showing slow recovery to the normal range at 9 month later. There was no differences in postoperative recovery of these recipients and the other recipients using grafts from donors without preoperative hyperbilirubinemia. Conclusion: LDLT from donors with isolated hyperbilirubinemia will be feasible for both donors and recipients that recovered without complication associated with isolated hyperbilirubinemia.

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Reduced Survival in Patients Undergoing Liver Transplantation for HCV Cirrhosis Despite Reduced Immunosuppression

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Background: Chronic liver failure resulting from HCV infection is a main indication for liver transplantation (LTx) worldwide. HCV recurrence, indicated by viremia and liver histological changes, is almost universal. Progression of hepatic injury post-LTx appears to be more rapid than in the non-transplant setting, with 20%-30% of patients developing allograft cirrhosis 5 years after surgery. Despite these observations, post-LTx patient and graft survival has not always been affected adversely when compared to patients transplanted for alternative indications, although follow-up in prior studies may not have been sufficient to detect differences in outcomes. Aim: Compare long-term survival in patients undergoing primary LTx for chronic liver disease from HCV infection versus patients with alternative etiologies of liver failure at our center. Methods: All patients who underwent primary LTx between Jan. 1997 and Dec. 2000, surviving greater than 3 months after surgery, were included in the analysis. Standard immunosuppression consisted of tacrolimus and corticosteroids utilizing a reduced immunosuppressive protocol with a rapid taper of prednisone (10mg by 1 month, 5 mg by 6 months post-LTx) and target trough tacrolimus levels between 4 and 10 ng/mL whenever tolerated. Results: A total of 131 primary transplants were performed; 26 patients were excluded from analysis because of early graft failure or mortality. The remaining 105 patients (49 HCV/56 non-HCV) provided the basis for survival analysis. Patient groups were not significantly different with regards to age, gender, and severity of illness as estimated by Child-Pugh or MELD scores. Mean follow-up was 31±15 months. Kaplan-Meier actuarial survival based on graft loss or death was calculated for the groups listed in the table below. There were 15 deaths in the HCV infected subgroup, 11 (73%) related to graft failure. Conclusions: Our data reveals a longer-term survival disadvantage in patients undergoing LTx for cirrhosis secondary to HCV infection when compared to patients without HCV-related liver disease, even when employing a reduced immunosuppressive protocol.

Kaplan-Meier Survival - Graft Loss or Death

	1 year	2 year	3 year
All LTx patients	94%	85%	78%
Non-HCV patients (n=56)*	98%	96%	89%
HCV patients (n=49)*	90%	74%	65%

*p=0.002 (log rank comparison of HCV vs non-HCV survival curves).

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Reversal of Calcineurin Inhibitor Induced Nephrotoxicity in Liver Transplant Recipients: Early Results of Prospective Single Center Trial

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Background: Immunosuppression with calcineurin inhibitors (CNI) has made liver transplantation a highly successful procedure with 1 year graft survival rates of >90%. CNI use is often associated with progressive nephrotoxicity. In our center 22 of 24 solid organ transplant recipients needing renal replacement had end stage renal disease secondary to CNI use. Six of these were post-liver transplants. While the acute renal effects of CNI are reversible, chronic effects are thought to be irreversible. Alternate, less toxic, yet effective immunosuppressive agents are desirable to prevent such permanent renal damage. Rapamycin, a newer agent with immunosuppressive properties similar to CNI but with no nephrotoxicity may provide such an alternative. Aims: 1. To evaluate the effects of CNI withdrawal on renal function in stable liver transplant recipients with evidence of renal dysfunction. 2. To evaluate the efficacy of rapamycin based immunosuppression in stable liver transplant recipients with renal dysfunction. Methods: Liver transplant recipients with stable liver function > 6 months post transplant, with evidence of renal dysfunction were included in this study. Following informed consent, the CNI was discontinued and Rapamycin was commenced orally at a dose of 3-5mg/day. Rapamycin levels were maintained at 6-10 ng/mL. Baseline renal (BUN and creatinine) and liver function (bilirubin, ALT, AST and Alk Phos, serum lipid profile) were determined at the time of change of immunosuppression, and monitored weekly for one month, every two weeks for one month, then monthly thereafter. Renal function was assessed by a 24-hour creatinine clearance (CrCl) prior study entry. Results: Fifteen recipients were enrolled between June and September, 2002. There were 11 males and 4 females (age 47 to 66 years). The mean CrCl was 55ml/min at the time of enrollment(range 32-72). Mean follow-up was 2 months (range 1-4.5). The improvement in CrCl was 14 mL/min at one month (range 5 to 38, $p<0.001$). Rejection was diagnosed in one recipient, and treated successfully with a steroid recycle. No recipients required resumption of CNI. Three of the 15 recipients showed alteration in lipid profile necessitating dietary modifications or lipid lowering agents. No infectious complications were noted. Conclusions: 1. Withdrawal of CNI based immunosuppression arrests progression of renal dysfunction and results in variable recovery of renal function in liver transplant recipients who are > 6 months post transplant. 2. Rapamycin allows successful withdrawal of CNI in stable liver transplant recipients in early follow-up.

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Utility of Diagnostic Laparoscopy Prior to Planned Hepatic Resection in Patients with Hepatic Metastasis from Colorectal Cancer: Experience in Over 250 Cases

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Introduction: Laparoscopy is a useful staging tool in patients with intra-abdominal malignancies, although recent advances in imaging technology have reduced its yield and its role has become less clear. This study examines the utility of laparoscopy prior to planned resection of hepatic colorectal metastases and proposes a means of targeting high risk patients in an effort to improve the yield of the procedure. Methods: Data on patients (n=258) undergoing diagnostic laparoscopy prior to planned hepatic resection for metastatic colorectal cancer between 1997 and 2002 was collected in a prospective database and retrospectively analyzed. A previously published clinical risk score (0-5) was determined for each patient based on 5 pre-operative variables: lymph node positive primary tumor, disease free interval < 1 year, CEA > 200ng/ml, > 1 hepatic tumor, largest tumor size > 5cm. One point was given for each parameter and the sum represented the score. In this study patients underwent a "non-therapeutic celiotomy" if they underwent open exploration with no tumor directed therapy having been rendered. Results: Mean age of patients in the study was 62 years and 51% were female. Patients were evaluated pre-operatively with a mean of 2.4 radiologic studies. Laparoscopic exam was complete in 172 patients (64%); adhesions from prior celiotomy limited the laparoscopic examination in 95 patients (35%). Overall, celiotomy was avoided in 27 patients (10%) because of identification of extra-hepatic disease (n=18) or additional intra-hepatic disease (n=9) at laparoscopy. A non-therapeutic celiotomy was performed in 21 patients following laparoscopy (8%). The most common sites of disease missed at laparoscopy were in perihepatic lymph nodes (n=10) and additional intra-hepatic disease (n=5). Complete risk score data was available in 88% of patients. An increasing risk score was associated with a significantly ($p<0.01$) higher yield of laparoscopy (see table). Intra-operative complications related to laparoscopy occurred in only 4 patients (1.5%). Conclusion: Pre-operative risk stratification of patients identifies patients at high risk for occult unresectable disease and therefore identifies those most and least likely to benefit from staging laparoscopy.

Clinical risk score	# patients	% spared celiotomy
0–1	56	0
2–3	160	11
4–5	35	26

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Stapled Laparoscopic Left Lateral Segment Liver Resection

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Introduction: Minimally invasive applications to liver surgery have been limited to relatively minor wedge resections, minor ablations and cyst unroofing. Technological advances in instrumentation and the use of hand assistance has led to the success of increasingly significant liver resections. We hypothesized that the use of endoscopic staplers would facilitate the success of totally laparoscopic left lateral segment liver resection. Methods: Five consecutive patients undergoing liver resections for a variety of reasons underwent diagnostic laparoscopy and laparoscopic ultrasound to determine the feasibility of complete laparoscopic resection. Criteria for stapled resection included: Expected margin greater than one centimeter for malignancies, liver parenchymal thickness between the ligamentum venosum groove and the falciform ligament less than three centimeters. The procedure was performed with three, five millimeter ports and one 1.5 centimeter port for stapler application. The left lateral segment was completely mobilized, the ligamentum venosum was thinned and minimal portal dissection was performed using blunt and sharp dissection. The liver parenchyma and vascular structures were divided using one staple load of the Endo GIATM Universal Stapler with a 60 mm by 4.5 mm staple load to include the portal structures and another to include the left hepatic vein. All specimens were removed intact. A detailed video of the technique will be integrated into the presentation. Results: The results for each patient are described in Table 1. There were no complications and no deaths. All margins were negative on final pathology. Conclusions: Patients with soft, normal liver parenchyma that is less than three centimeters in thickness can undergo stapled laparoscopic left lateral segment liver resection with a short length of stay and outcomes consistent with those seen in open resection.

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Laparoscopic Hepatectomy

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This paper reviews the experience of 41 laparoscopic anatomical hepatic resections performed November 1999 - August 2002. Forty-one patients aged 31 - 75 years, 25 females/16 males, have undergone right hepatectomy (13), left hepatectomy (3) and segment 2/3 resections (25). Pathology was colorectal metastasis (26), FNH (4), cystadenocarcinoma (2), adenoma (2), HCC (2), cystadenoma (1), hydatid (1), cholangiocarcinoma (1), haemangioma (1), no pathology (1). A 6 port technique and 30 degree laparoscope were used. Hepatic duct and artery are clipped and portal vein divided with harmonic scalpel and GIA. Branches of the IVC are clipped and hepatic veins divided, usually from below, with Endo GIA. Specimens are removed through a small RUQ incision. Three patients were converted - bleeding (2) and anatomy (1). Operating time was 3.5 hours (1.8 - 5.2 hours). Seven patients had a blood transfusion (1 - 4 units). Hospital stay was 5.4 days (2 - 21 days); pulmonary embolism (0); hospital and 30 day mortality (0). Two patients were reoperated - wound dehiscence (1), infected haematoma (1). There were 2 bile leaks, dry by day 8 and 10 and 1 R pneumonia. Three patients have tumour recurrence - HCC at 8 months (still alive), colorectal 8 and 12 months - both with multiple liver tumours and peritoneal disease (both died). A third patient died of metastatic melanoma 9 months after

Results

Patient	Age (yr)	Sex	Diagnosis	EBL (cc)	Case length (min)	Length of stay (days)	Return to work (days after discharge)
1	56	F	Metastatic colorectal cancer	50	210	3	7
2	47	M	Metastatic colorectal cancer	50	150	2	7
3	54	M	Recurrent pyogenic cholangitis	30	180	1	7
4	81	M	Metastatic nasopharyngeal carcinoma	25	240	2	7
5	29	F	Focal nocular hyperplasia	50	130	3	7

colorectal metastatic resection. There are no port site recurrences. Laparoscopic hepatic resections are feasible in experienced hands.

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WITHDRAWN

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En-Bloc Resection of Hepatocellular Carcinoma With Right Atrial Extention Using Cardiopulmonary Bypass

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The incidence of hepatocellular carcinoma is increasing in the United States. Tumor extension through a hepatic vein and the suprahepatic inferior vena cava into the right atrium is an uncommon presentation of this disease. Patients are at risk for cardiopulmonary as well as oncologic complications. This situation is often considered beyond resectional treatment, particularly in cirrhotic patients. There have been scattered reports of surgical resections of such tumors, including both staged thoracic and abdominal operations as well as simultaneous procedures with separation of the hepatic and cardiac portions of the tumor. We report three cases of very large hepatocellular carcinomas in non-cirrhotic young women where resection was successfully undertaken through a combined bilateral subcostal incision and median sternotomy. Institution of a cardiopulmonary bypass circuit allowed combined hepatic vascular and right-sided cardiac exclusion. The extensive exposure along with the protective effects of lowered core temperatures allowed precise en bloc resection of these masses with initial inflow control, parenchymal transection and a right atrial incision extended down the IVC to the appropriate hepatic vein. Once the tumors were excised the resulting atrial and vena caval defects were reconstructed using pericardium or allograft tissue before restoring hepatic flow and weaning from cardiopulmonary bypass. We discuss the technical details of these procedures and review the literature regarding this disease presentation and its management.

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Rigth Trisectionectomy for Primary Liver Cancer

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This study is to evaluate the feasibility and effect of right trisectionectomy for primary liver cancer. From Jan. 1987 to Nov. 1999, 32 cases of right trisectionectomy were performed out of 1017 primary liver cancer patients during this period of time, (26 cases with hepatocellular carcinoma, 2 cases with cholangiocarcinoma, 4 cases with mixed tumor). All of right trisectionectomy were performed under normothermic interruption of the porta hepatis for single time and the period of interruption lasted in between of 15-40 minutes. 2 cases of right trisectionectomy were performed without blood transfusion. Results: The 1, 2, 3, 4 and 5 year survival rates were 56.6%, 38.5%, 26.1%, 23.5% and 23.1%, respectively. The longest survival time after right trisectionectomy is 14 years. The patient is atill alive with free cancer. Main complications were present in 5 patients. Mortality within I month was 3.1% (1/32). Conclusion: Right trisectionectomy is safe and effective in treatment of primary liver cancer if the indications and operative techniques are mastered properly.

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Perioperative Factors and Outcome Associated With Massive Blood Loss During Major Liver Resections

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Mortality and morbidity from major liver resections has dramatically decreased over the past 25 years. This is due to a better understanding of liver anatomy and introduction of new operative techniques, but also to improved anesthetic perioperative support. Certain cases are still associated with voluminous blood loss. These patients may be at higher risk for postoperative problems and longer hospital length of stay (LOS). We have retrospectively reviewed 115 patients undergoing major hepatic resection (3 or more anatomic segments) with respect to operative blood loss (EBL). Those with an EBL $>$ 5000 ml (Group 1) (n=39) were compared to those with an EBL $<$ 2000 (Group 2) (n=42). Type of resection, age ($>$ 70), tumor size, mortality, morbidity, and hospital LOS were examined. Operative reports were also examined for any explanation for excessive blood loss. The EBL for Group 1 was 7692+3848 ml and for Group 2 1359+514 ml. Primary liver tumors were resected in 20 patients in Group 1 and in 18 patients in Group 2. The balance of resections was for metastatic tumors, primarily colorectal in origin. In Group 1 13/39 patients had a left hepatectomy compared to 10/42 patients in Group 2 (p=0.34). Overall mortality was 5/115. Four deaths occurred in Group 1 and one in Group 2 (p=0.16). Two deaths in Group 1 were intraoperative (hemorrhage, air embolism). There was no difference in number of patients with complications, 12/39 in Group 1 and 8/42 in Group 2 (p=0.22). In Group 1 2 patients required reoperation for bleeding, there were none in Group 2. Largest tumor size did not differ between the two groups (p=0.08), nor did the proportion of patients 70 or older (p=0.06). There was no difference in hospital LOS (10.54+6.1 vs 8.90+4.7 days, p=0.21). Review of operative notes in Group 1 indicated no unusual problems in 13/39, large tumors or proximity to the inferior vena cava (IVC) in 10/39, and bleeding from the middle hepatic vein (MHV) in 7/39. Three patients in Group 1 required total vascular exclusion for tumor removal, there were none in Group 2. The use of rapidly infused, warmed fluids during surgery prevented prolonged periods of intraoperative hypotension and hypothermia. In summary, massive EBL during major liver resection seems to be provoked by tumors near the IVC or major hepatic veins or injury to the MHV during operation and not with patient age, tumor size alone, or type of hepatectomy. However, with rapid transfusion of warmed blood intraoperatively, the perioperative course of these patients does not differ from those with much less EBL.

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A Combined Transmesenteric/Transjugular Approach to Intrahepatic Shunting Can Overcome Portal Vein Thrombosis

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Surgical treatment of portal hypertension involves shunting of portal venous blood to the systemic venous system. Standard approaches involve creation of a surgical shunt (extrahepatic or intrahepatic) or replacement of the liver. Extrahepatic shunts are performed in patients with well-preserved hepatic function. Liver transplant is reserved for individuals demonstrating liver failure. Treatment of portal hypertension in patients with poor hepatic function who are poor surgical candidates or those awaiting liver transplant undergo transjugular intrahepatic portosystemic shunting. Some patients present with extensive mesenteric and portal venous thrombosis (PVT) without significant liver dysfunction. These patients lack a vessel of adequate caliber to undergo a conventional shunting procedure. Intrahepatic shunting carries significant risk in this setting as there is no clear portal venous target at which to aim. Anterograde recanalization of the portal vein can provide a portal target for placing an intra-hepatic shunt in the standard trans-jugular manner. We have used minilaparotomy and anterograde cannulation of the mesenteric venous system to enable placement of intra-hepatic shunts in patients with PVT who were not candidates for any conventional shunting procedure. Over a 14-month period, eight patients presented with history of massive upper gastrointestinal bleeding secondary to portal hypertension. All had complete occlusion or cavernous transformation of the portal vein by ultrasonography. All eight patients underwent minilaparotomy with cannulation of a peripheral venous branch in the small bowel mesentery, providing access to the portal vein. Anterograde recanalization using a endovascular reconstruction technique of glidewires, catheters, and balloon angioplasty allowed the identification of a target in an intrahepatic segment of the portal vein which could be reached by a transjugular shunt. All eight patients underwent successful reduction in the portosystemic pressure gradient to below 12 mm Hg. Four of eight shunts thrombosed within the first 24 hours but were successfully recanalized percutaneously using conventional transjugular technique. Median follow-up was 11.5 months (range 2-16 months). A total of 10 revisions were required for the eight shunts (an average of 1.25 per shunt). There were no episodes of re-bleeding. Patient survival was 87.5% (7/8), with no deaths directly related to complications of portal hypertension (one from sepsis). The combined transmesenteric/transjugular approach is an effective treatment for patients with portal hypertension and either cavernous transformation or complete occlusion of the portal vein, which would otherwise preclude these patients from undergoing a shunting procedure.

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Radiofrequency Assisted Liver Resection: A New Technical Device

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Liver resection remains the best therapy for liver tumour. A new approach employs liver surgery and radiofrequency to obtain a direct coagulation of the tissue of the line of resection, which is treated by multiple applications of radiofrequency. Once obtained the coagulation of the tissue, it is sectioned with a scalpel without stitches and clips. The blood loss is reduced to the minimum with consequent benefits in term of mortality and morbidity. This technique does not require clamping of the hepatic pedicle, particularly useful in case of resections on chirrotic. Moreover the line of resection treated by radiofrequency presents a coat of 1 cm. of coagulated tissue that is a warranty for a complete resection of the neoplastic disease. The costs of this technique are very low. The limit of this approach is for lesions which are at 1.5 centimetres or less from the main portal pedicles, because of the risk of main biliary ducts injury. Thus it is recommendable to employ the standard technique when close to hilum and the radiofrequency assisted technique for the other areas. The procedure is longer than the usual techniques, because it requires lots of applications before the parenchymal resection. Some Centres in Europe have begun to employ this approach, mainly for liver resection, but also for partial kidney resections. Six patients underwent this procedure (Habib technique). There were four females and two men, mean age was 65.7 years (range 61-72). Two patient presented an hepatocellular carcinoma, while the other four had colorectal liver metastasis. A total of 11 lesions were treated, mean dimension was 25.9 millimetres (range 8-60 mm.). Patients received two left bisegmentectomies, four segmentectomies and seven wedge resections. Mean operative time was 266.7 minutes (range 170-420), the resections required a mean of 30 (range 20-50) radiofrequency application, each of them lasted about four minutes. Blood loss was 38.3 cc. (range 5-70), any further device than radiofrequency was required to get haemostasis and no patients needed blood transfusion. All patients presented a postoperative raising of transaminase blood level and bilirubine, which normalised in 7-10 days. After one month a patient developed an abscess in the site of the resection which required a percutaneous drainage. Mean hospital stay was 6.3 days (range 5-8 days). We believe that this new approach integrates with other techniques to improve the results in liver surgery. It permits to reduce the blood loss, to obtain negative margin of resection and presents reduced costs. Anyway it presents limits when near to main portal pedicles and operating time is longer than standard techniques. Further progresses in technical devices and the development of the learning curve will reduce the operating time and improve the efficacy of this procedure.

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Surgical Exposure For 1431 Partial Hepatectomies: A Comparison of Complications for a Chevron Versus Hockey Stick Incision

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Introduction: Optimal exposure for partial hepatectomy can be achieved through a number of different incisions (i.e. chevron, midline, hockey stick, and thoracoabdominal). The short term and long term results from these types of incisions have been reviewed for other procedures, but never for partial hepatectomy. The aim of this study is to compare the chevron with midline extension (CM) to the extended right subcostal with midline extension (i.e. hockey stick (HS)) incision for both perioperative and long term wound complications. Methods: A review from 1/1995 to 6/2001 identified 1431 patients who underwent exploration and partial hepatectomy. Patients undergoing bile duct resection/reconstruction or colon resection were excluded. Demographics, comorbidities, body mass index, operative and transfusion data, perioperative complications, early (seromas, infections, ascitic leak and dehiscence), and late wound complications (hernias) were analyzed. Results: From the 1431 patients explored, 861 resections were performed through a CM, and 570 resections through a HS incision. The median age of patients resected through a HS incision (62 (range16-86 years) was significantly older than patients with a CM (61 (range 16-88 years) ($p=0.001$). The median body mass index was similar for both groups (26.4 CM vs 26.6 HS). The prevalence of diabetes, pre-op steroid use, and atherosclerotic disease was similar for both groups. The extent of hepatic resections was similar with 63% of the CM and 59% of the HS patients undergoing resection of a lobe or more. The estimated blood loss was significantly greater in the patients resected through a CM (>1000cc in 30%) incision when compared to the HS (16%) incision ($p=0.007$).

The overall complication rate (42% CM vs 43% HS), and the perioperative wound complication rate (15% CM vs 12% HS) were similar for both incision types. The post-operative hernia rate was significantly less in the patients resected through a HS (5%) incision when compared to patients resected through a CM (10%) (p=0.002) with an odds ratio of 2.1 (95% CI: 1.3, 3.4) for CM incisions. Conclusion: Major hepatic resections can be performed safely through an HS incision. The overall wound complication rate was lower with the HS incision, with a significantly reduced hernia rate when compared to the CM incision.

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Anatomic Studies of the Hepatocaval Ligament

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The hepatocaval ligament is a key anatomic landmark for safe exposure of the hepatic veins. It is a vestige of the intrahepatic development of the superior part of the inferior vena cava and hepatic veins from the right vitalline vein. We studied the anatomy of this ligament on 20 fresh adult cadavers and 30 hepatic resections where the hepatic veins were dissected. In-situ casting studies were done. The ligament extended from the upper lateral aspect of segment 7 around the inferior vena cava to the caudate lobe. It tended to be wide on the right with fibers splaying out over the vena cava and becoming narrower on the left. Mean dimensions were: width right 1.75+0.72 cm, width left 0.69+0.43 cm, and length 3.2+0.7 cm. There were three basic types of ligaments: type 1 - all ligament (64%), type 2 - part ligament part tongue of liver on either end (24%) and type 3 - (12%) all ligament. A small caudate vein was found directly behind the ligament on the right in 69% of cases. Histology of the ligament showed fibroconective tissue and occasional bile ducts. Knowledge of the exact anatomy of the hepatocaval ligament will aid surgeons in their dissection of this area.

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Early Results of Radiofrquency Thermoablation of Liver Malignancies

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Radiofrequency thermoablation (RFTA) is nowadays one of treatment methods for inoperable hepatic tumors. Aim: The aim of the study is to evaluate short-term results of RFTA in the liver Material and methods: Between April 12th, 2001 and October 1st 2002 127 patients with liver malignancies were treated using RFTA. 24 of them suffered from hepatocellular carcinoma, 103 - had liver secondaries from colorectal, breast, ovarian pancreatic and kidney cancer, malignant melanoma and carcinoid tumor. In all cases, except ovarian cancer, the primary focus was successfully treated before RFTA. In patients with ovarian cancer RFTA was done to obtain maximal cytoreduction before chemotherapy. 367 tumors were treated in 173 ablation sessions. 1-4 tumors were ablated during one session. All tumors were treated percutaneously under direct US control using "CoolTip" equipment (Radionics, Burlington, MA). The postoperative quality of life were measured using the SF-36 protocol Results: No serious intraoperative complications were observed. In one case transient bradycardia, probably due to vagal nerve agitation during ablation, occured. In the early postoperative period pain in the right upper abdominal quadrant and/or right shoulder was the main problem, but on only 25 patients opioids were necessary. The hospital stay was 2-3 days in 156/173 ablation sessions and less than 7 days in all other. 3 hepatic abscesses were observed - one connected with colon perforation due to thermal lesion. In this case an open operation (abscess drainage and colostomy) was performed. In two other cases percutaneous drainage was succesfull. After 126/173 sessions transient (up to two weeks) temperature elevation was observed. No biliary complications occured, but, if ablation of hilar lesion was necessary, the biliary tree was cooled through nasobiliary drain. Conclusion: Radiofrequency thermoablation is a safe method for treating malignant hepatic lesions with low rate of complications and good postoperative quality of life.

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Liver Resection in the Treatment of Polycystic Liver Disease (PCLD)

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Liver resection and fenestration of the remnant liver is claimed to be more effective than fenestration and less invasive than transplantation in the treatment of extensive PCLD. This treatment has however not gained wide acceptance. The aim of the present study was to assess : 1) if it is always possible, 2) its risk and long term results, 3) if the outcome can be predicted. Between 1995 and 2001, all patients referred with symptomatic Type II or III PCLD (Ann Surg 1997 ; 225 : 286) were considered for resection if they had incapacitating symptoms, no end-stage kidney failure and neither ascites nor denutrition. Sixteen patients fulfilled these criteria. Seven patients had Type II and 9 had Type III PCLD. Eight patients had associated PCKD including 6 with creatinin clearance comprised between 30 and 60. One patient without PCKD had a clearance of 26. Mean time since diagnosis of PCLD was 10 yrs and 8 had failed previous attempt at treatment by fenestration. Mean ECOG performance status was 1.9. All patients could undergo resection. Mean number of segments removed was 4 (2 - 6) and mean duration of surgery was 6.3 hrs. Mortality was nil but morbidity was 94 % mainly due to pleural effusion (10), ascites (9), bleeding (5), biliary leak (3). Each of these complications were markedly higher than after comparable resections for other indications. One patient required reoperation at 9 mo for persistent pleural effusion and one patient died following thoracocentesis at 4 mo. After a mean follow-up of 54 months, two patients have experienced recurrent symptoms, due to expansion of residual cysts, successfully treated by ethanol injection in one and kidney/liver transplantation in the other with terminal kidney failure. Incidence of bleeding and biliary leak was higher in patients with Type III than Type II cysts (66 % vs. 0 %, p = .01). Even slightly impaired kidney dysfunction was the main risk factor for prolonged pleural effusion and ascites (57 vs. 0%, p = .02). Recurrent symptoms were not correlated with the extent of the disease nor with the presence of PCKD. Liver resection proved always possible in this series. Type III cysts and even slight kidney dysfunction are risk factors for postoperative complications. Due to donor shortage, resection may however still be considered as the first choice treatment.

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Differentiation of Normal and Radio Frequency Ablated Liver Tissue using an Optical Based Feedback System

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Introduction: Radio Frequency Ablation (RFA) is an evolving technology used to treat patients with unresectable hepatic tumors. RFA causes coagulative necrosis by local tissue heating. There is currently no way to measure ablation margins in real-time. Optical characteristics of liver can be measured using fluorescence and diffuse reflectance spectroscopy. We hypothesize that changes in optical characteristics can be used to detect the ablation margin during liver RFA. Methods: Three dogs with normal livers underwent midline laparotomy. Spectroscopic measurements were made using a micro interrogation probe (MIP) which was connected to an optical recording system. Following placement of the RFA probe, the MIP was placed ultrasonographically within the expected zone of ablation (ZOA). Four ablations were performed per dog, 2 using diffuse reflectance

spectroscopy and 2 using fluorescence spectroscopy. Ablations were performed by delivering a constant power of 80W. RFA was stopped when changes in the optical feedback characteristics changed. At that time, the MIP tract was marked with India ink, and following animal sacrifice each ablation zone was excised. The relationship of the MIP to the ZOA was examined grossly, and microscopically. Results: On gross inspection, the tip of the MIP was at the advancing edge of the ZOA when RFA was stopped. Histology confirmed that the MIP tip was completely surrounded by coagulative necrosis. Conclusion: (1) Fluorescence and diffuse reflectance spectroscopic changes occur within liver parenchyma during tissue destruction from RFA. (2) The changes in spectral feedback correlated directly with the propagating ZOA. These results indicate that an optical based feedback system using fluorescence and diffuse reflectance spectroscopy can accurately define ablation margins in real-time.

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Implications of the Normal Variations in Segmental Liver Volumes for Hepatic Resection and Transplantation

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Background: Extended hepatic resection leaving a small liver remnant and liver transplantation with partial donor liver grafts are being performed with increasing frequency. Remnant volume and donor graft volume correlate with surgical outcomes. Spiral computed tomography (CT) permits accurate determination of the preoperative liver volume. Systematic study of the relative contribution of hepatic segments to total liver volume (TLV) could improve preresection and pretransplant planning. Methods: Total liver volume (TLV) and segmental hepatic volumes were measured by CT in 102 patients without liver disease who underwent imaging for conditions unrelated to the liver or biliary tract. Results: On average, the right liver (segments 5, 6, 7, and 8) (997 $\pm$ 279 cm^3) accounted for two thirds of the TLV (1518 $\pm$ 353 cm3), while the left liver (segments 2, 3, and 4) (493 $\pm$ 127 cm^3) accounted only for one third of the TLV. Segments 2 + 3 contributed about half of the volume of the left liver (242 $\pm$ 79 cm^3) and 16% of the TLV. The right liver represented 45% to 80% of the TLV, the left liver represented 15% to 45% of the TLV and segments 2 + 3 represented 10% to 25% of the TLV. Segments 2 + 3 represented less than 20% of the TLV in more than 75% of patients, and the left liver contributed less than 20% of the TLV in more than 10% of patients. Conclusions: Important interpatient variations in hepatic volumes exist. The left liver represents one third of the TLV. In the absence of appreciable hypertrophy, we recommend measurement of the left liver (2 + 3 $\pm$ 4) prior to extended right hepatectomy or left liver donor hepatectomy.

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Perioperative Liver Function Tests in Hepatic Resection

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Background: Surgical resection of primary and metastatic hepatic malignancy confers survival advantage over alternative therapeutic modalities and remains the treatment of choice. In this retrospective review we establish a framework for evaluation of perioperative liver function tests in these patients. Methods: The records of 45 consecutive patients who underwent a successful hepatic resection (using the same technique of parenchymal transection and intermittent inflow occlusion) over the past 4 years were reviewed for repeating trends in commonly measured hepatic parameters. Each patient was assigned to one of the following four groups: formal right hepatic lobectomy (1,n=15), left hepatic lobectomy (2,n=5), left lateral lobectomy (3,n=10), and a segmentectomy (4,n=16). The groups were individually analyzed and then brought together for a back-to-back comparison. The data was plotted as a mean+/-2SD to reflect the expected intra-group variability. Results: In the first group, which underwent right hepatic lobectomy, SGOT and SGPT ($\pm$1SD) peaked by 24hrs at 730$\pm$421 U/ml and 652$\pm$438 U/ml, respectively. Both tapered off over the next 3 days. Alkaline phosphatase fell from 135$\pm$63 to 96$\pm$34 U/ml on day one and subsequently began a steady clime. Similarly, albumin fell from 4.24 to 2.84g/dl in the first 24hrs and leveled off at that level. There was no measurable change in PTT over the duration of hospital stay, whereas PT peaked at 13.8$\pm$1.9s on POD1 and over the next three days gradually came down to 11.6$\pm$1.4s. Total bilirubin reached 1.9$\pm$0.9 mg/dl on day 2, and then began a downward trend. Direct bilirubin continued to rise through the observation period reaching 0.6$\pm$0.3 mg/dl on day four. Blood glucose levels rose 160% by day one trending down to 120% of the baseline by day 4. Of note, none of the patients had episodes of hypoglycemia. Finally, after a slight fall in the first 48hrs platelets returned to baseline by day 5. Groups 2, 3, and 4 had little inter-group variability, and followed trends similar to group one with smaller deviations from the baseline. Significant differences from group 1 were noted for SGOT and SGPT, which peaked at 310$\pm$225 U/ml ($p=0.003$) and 326$\pm$193 U/ml ($p<0.001$) respectively, and PT that rose to 12.1$\pm$1.8s ($p=0.020$) on day 2 before returning to the baseline. Conclusion: These results establish a framework for objective evaluation of hepatic parameters in four types of liver resection, the procedure that caries serious complications. The patients undergoing left hepatic lobectomy, left lateral lobectomy, and segmentectomy have little inter-group variability and can be evaluated against a single standard, whereas right hepatic lobectomy requires a different standard. Given a good outcome of all of the subjects in the study, departure from these standards may be an early sign of looming problem.

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Comparision of Laparoscopic and Open Surgical Management of Hepatic Cysts

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Hepatic cysts are often benign masses of the liver that cause symptoms. Surgical management has varied from fenestration to resection, with the utilization of laparoscopy occurring in the last three years. Methods: Retrospective analysis of pt, and tumor demographics, intraoperative and postoperative results comparing laparoscopic (7/97-8/02) versus open resection (7/94-8/02) were performed. Results: Thirty patients were managed for cystic disease of the liver. The median age in this series was 61.2 yrs (range 36-80 yrs), with the majority female 25 (83%), and Caucasian 25 (83%). The majority of patients (23/25;88%) presented with symptoms: pain (n=25), bloating or early satiety (n=6) while few were asymptomatic (n=3). The majority of lesions (18/30;60%) were found in the right lobe of the patient. Twelve patients were managed with a laparoscopic approach while 18 underwent traditional resections. Median age, gender and race were similar in both groups with the median size of the laparoscopic lesions 9.7cm (range 5 - 22 cm) and the open 9.2 cm (range 7-18cm). Similar side distribution was noted 5/12;41% were left sided in the laparoscopic group while 6/11;38% in the open group. Operative technique in the laparoscopic group included resection (n=6), and fenestration (n=6) compared to the open management group lobectomy (n=2), non-anatomical resection (n=4), fenestration (n=11) and simple aspiration (n=1). Mean operative time was longer in the open group (240 $\pm$ 20 vs. 180 $\pm$ 12$<$0.05) while EBL was similar. Pathology was similar with the laparoscopic group consisting of simple benign cysts (n=9) and bile duct cysts (n=3) and the

open group with simple benign cysts (n=12), bile duct cysts (n=2), bile duct cyst adenomas (n=2), hydatid cyst (n=1) and an echinococcal cyst (n=1). No recurrences have been noted in the laparoscopic group with a mean follow-up of 8 months. However, complications were significantly higher in the open management group (6/18;33% vs. 0/12;0%p<0.02). The complications noted in the open group were: bile leak (n=2), hepatic abscess (n=2), ascites (n=1) and abdominal infection (n=1). Conclusions: The laparoscopic approach to hepatic tumors appears to provide a safe and efficacious manner to manage symptomatic cystic liver tumors. This approach is not limited by the size or location of the tumor and in this limited series appears to have a lower morbidity than the open approach.

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A High Short Term Survival Justify Extended Resection for Hepatocellular Carcinoma With Major Portal Vein Involvement

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Untreated patients with hepatocellular carcinoma (HCC) associated with macroscopic portal vein invasion have short expected life span and are considered as contraindication for transarterial chemoembolization, percutaneous treatment and liver transplantation. It is still unclear whether surgical treatment for patients with HCC with major portal vein invasion is beneficial and safe, and therefore justified. Thirty-three patients out of 410 resected patients with HCC and macroscopic portal vein thrombosis diagnosed preoperatively were selected (PVT Group). Eight (24%) patients had undergone palliative resection (bilateral tumors, associated extra-hepatic procedure, positive ascitic fluid cytology) and 25 had curative resection. Aims of this retrospective study were a) to assess the safety of surgical treatment b) to compare short and long term outcome of these patients with characteristics - matched HCC patients without vascular invasion (Control Group, n=25) and c) to assess the clinico-pathological factors affecting survival. The mean follow up was 15.5 months. The morbidity rates in PVT and Control Groups patients were 36% and 24% (ns). One patient (3%) died in the PVT Group and there was no mortality in the Control Group. The overall survival of the Curative Resection and the Control Groups were 75%, 41%, 28% and 73%, 69%, 68% at 1, 2 and 3 years respectively ($p<0.05$) and the disease free survival were 38%, 26%, 24% and 53%, 44%, 40% at 1, 2 and 3 years respectively (ns). The median survival of the patients who underwent palliative resection was 5 months (range 2-14). The recurrence pattern was different in Curative Resection and Control Groups (47% vs. 25% systemic recurrence and 52% and 75% isolated intrahepatic recurrence, respectively). Degree of differentiation of the tumor and extent of involvement of portal thrombus were significantly associated with overall survival whereas only degree of differentiation was found to be significantly associated with disease free survival. The presence of cirrhosis was not found to influence the prognosis. Surgical treatment for HCC with macroscopic portal invasion is safe in selected patients and immediate results are comparable with matched controls. An overall good short term survival justified resection when compared to the poor spontaneous survival for untreated patients. Hence, surgical treatment should be considered for patients with HCC with major portal vein invasion if it can be performed with a curative intent.

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Resection of Metachronous Liver Metastases from Ovarian or Fallopian Tube Carcinoma

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Introduction: Patients who develop metachronous hepatic metastases after initial treatment of ovarian or fallopian tube carcinoma often have disease refractory to chemotherapy and carry a poor prognosis. Surgical resection is rarely performed, so the purpose of this study was to determine the survival of these patients following surgical resection. Methods: From 1988-2001, 24 patients with recurrent ovarian or fallopian tube carcinoma who underwent complete gross resection of their hepatic metastases were identified from prospective databases at a single institution. Results: The median age of patients was 53 years old, and the median interval between primary diagnosis and liver resection was 69 months. All patients had primary surgical resection or debulking and chemotherapy as part of their initial treatment. Subsequent hepatic resections included trisegmentectomy (2), lobectomy (2), segmentectomy (17), and wedge resection (3). Additional resection of disease outside the liver was performed in 18 patients (67%). Resection of all gross disease was possible in all cases, with 14 R0 resection and 10 R1 resections. Eighteen patients recurred with sites of initial recurrence including the abdomen and pelvis (14), spine/paraspinal region (2), lung (1), and liver (1). Overall survival was 27 months after hepatic resection with a range of 6-94 months. Fourteen patients were alive at the time of last follow-up, and 10 had no evidence of disease. No significant prognostic factors could be identified on univariate analysis. Conclusion: In highly selected patients with recurrent ovarian or fallopian tube carcinoma involving the liver, hepatic resection along with resection of other gross disease may lengthen survival and should be considered as a treatment option.

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Extended Hepatic Resections — Increased Risk with Biliary Resection and Reconstruction

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Background: Advances in anesthetic and intensive care, coupled with a better understanding of the hepatic anatomy and improved surgical technique, have rendered liver surgery safer over the last two decades. This has led to an increase in the application of extended resections, defined as 3 or more segments plus the middle hepatic vein. These resections may also include the extrahepatic biliary tree with reconstruction. The purpose of this analysis is to determine the morbidity of the extended resection with or without biliary excision vs traditional hemihepatectomy. Methods: From July 2001 through mid-October 2002, 145 hepatic resections were performed. There were 51 hemihepatectomies and 31 extended resections (twice the rate performed in our center one decade ago). Ten of extended resections (32%) included biliary resection and/or reconstruction. Outcomes analyzed for the three groups included morbidity, mortality (3 mo), estimated blood loss, blood transfusion requirements, postoperative liver function patterns, and length of stay (LOS). Results: Patient demographics and preoperative co-morbidities were similar between the groups. Extended resection with biliary tree was more commonly performed for cholangiocarcinoma and gallbladder cancer. Outcomes appear in the table. Trends towards liver dysfunction were seen with greater incidence in both extended resection groups as measured by peak bilirubin and INR values, but did not reach statistical significance. Cholangiocarcinoma was the diagnosis in each of the 3 deaths with extended resections. The two deaths in the extended with biliary resection group were directly related to sepsis from the biliary reconstruction. Conclusion: The early outcomes of extended hepatectomy without the biliary tree are similar to those of the traditional hemi-hepatectomy. An increase in the morbidity and mortality of an extended hepatic resection with biliary excision appears to be related to the biliary tract reconstruction

Outcomes of extent resection

Resection type	Mean EBL	# Transfused	PRBC > 2 Units	LO S	Mortality
Hemihepatectomy	1576 cc	13/51 (25%)	11/51 (22%)	10 d	1/51 (2%)
Extended Resection	1633 cc	6/21 (29%)	2/21 (10%)	11 d	1/21 (5%)
Extended Resection plus Biliary Tree	1415 cc	3/10 (30%)	1/10 (10%)	20 d	2/10 (20%)
p-value	NS	NS	NS		0.05

rather than extension of the parenc hymal transection beyond the middle hepatic vein.

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Reduced Blood Loss using the Hydro-Jet Technique for Hepatic Parenchymal Dissection

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Background: High-pressure water-jet dissection technology (Hydro-Jet, ERBE) was originally developed for applications in the steel and glass industries where ultra-precise cutting and engraving was desirable. This technology has recently been adapted for medical applications with favorable results. To date, the Hydro-Jet has been successfully employed in procedures performed on such diverse organs as the kidney, prostate, synovium, gallbladder, and the parotid gland. The advantages of this thin, laminar liquid jet effect include precise, controllable, tissue-selective dissection with excellent visualization of, and minimal trauma to, surrounding fibrous structures. This is an analysis of the intial use of the Hydro-Jet in a sequential, but overlapping series compared with the CUSA device. Methods: All 141 consecutive liver resections performed over a 15-month period were included. The parenchymal transection techniques employed were: CUSA (n=78 from 7/01 through 6/02), Hydro-Jet (n=51 from 3/02 to 10/02) and Other (n=12 from 8/01 to 8/02). This small "Other" group was omitted from further analysis. Extent of resection was classified as: One or two segments (Seg1-2 group; 11 HJ and 19 CUSA), and 3 or more segments (Seg3+ group; 40 HJ and 59 CUSA). Analyses were performed of operative time, estimated blood loss, blood product requirements, post-op liver enzyme patterns, and three-month mortality. Results: Demographics, disease process, preoperative co-morbidities, and case type were equivalent between the CUSA and Hydro-Jet groups. In the Seg1-2 resections there were no deaths and all outcomes were similar between Hydro-Jet and CUSA. Outcomes for Seg3+ resection groups for CUSA and Hydro-Jet appear in the table. For these major Seg3+ resections the operative times, LOS, and postoperative liver biochemistry were similar between the groups. Conclusions: The Hydro-Jet dissection system provides safe and accurate dissection of the hepatic parenchyma. This initial experience suggests use of the Hydro-Jet may result in less blood loss with decreased number of patients requiring transfusion, and fewer cases of large blood volume loss (> 2 units PRBCs transfused). Larger numbers will be required to confirm these initial findings.

Outcomes in Seg3+ resections

Technique	Mean EBL	# Pts transfused	PRBC > 2 units	Mortality
CUSA	1733 cc	19/59 (32%)	11/59 (19%)	5/59 (8.5%)
Hydro-Jet	1180 cc	6/40 (15%)	2/40 (5%)	0/40 (0%)
p-value	NS	0.04	0.04	0.07

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Increased Ischemic Injury in the Old Liver: A Novel Pathway of Injury

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Ischemic injury is common during liver resection, shock or trauma. Due to our aging population and increased need for organs for transplantation liver surgery is increasingly performed in older patients. However, the effect of age on ischemic injury of the liver is unknown. Methods: 60 minutes ischemia of 70% of the liver was performed in C57BL/6 mice of 6 weeks and 60 weeks of age (life expectancy 50 to 90 weeks). Differences in steatosis or fibrosis between the two groups were evaluated by a blinded pathologist (JW). Liver injury was determined by AST levels 4hr after surgery. Apoptosis was evaluated by caspase 3 activity and the TUNEL test. TNF, a central mediator of apoptosis in the normal liver, was determined in the liver tissue 4hr after resection. Necrosis was investigated 24hr after reperfusion by H&E staining. Finally, ischemic preconditioning (10 minutes ischemia and 15 minutes reperfusion) was performed in old and young mice as a novel strategy against reperfusion injury. Results: Mice of 60 weeks of age had significantly higher AST levels after 60 minutes ischemia and 4hr of reperfusion than young mice of 6 weeks of age (12500U/L vs 8200 U/L; $p<0.05$). Caspase 3 activity was higher in old mice than in young animals (98 vs 67 AUF/mg $p= 0.04$). In addition, old mice had significantly more TUNEL pos. hepatocytes 4hr after reperfusion when compared to the young control mice (55% vs 77%; $p<0.05$). In contrast, TNF? was 3 times lower in old mice when compared with mice of 6 weeks of age (1.8 vs 0.6 pg/mg; $p= 0.03$). 24hr after reperfusion old and young mice had similar degrees of necrotic tissue (20% vs 15%; $p= 0.23$). Ischemic preconditioning dramatically reduced AST levels in young mice (8200 vs 3200 U/L), while no protection was observed in old animals (12500 vs 14300 U/L). Similar, while preconditioning reduced the number of Tunel pos. cells in young mice (55% vs 15%) identical Tunel staining was found in old mice with or without preconditioning (77% vs 75%). In addition, caspase 3 levels were reduced in young mice by preconditioning (67 vs 44 AUF/mg $p= 0.04$), while increased caspase 3 levels were present after preconditioning in old mice (98 vs 134 AUF/mg $p= 0.03$). Conclusion: Livers from older animals tolerate ischemic injury poorly. The decreased tolerance for ischemic injury of old mice is related to higher susceptibility to apoptosis. While hepatocyte apoptosis is mediated by the local release of TNFA in the normal liver, the low TNFA levels observed in old mice indicate that increased activation of the apoptotic pathway occurs through a novel - TNFA independent - pathway. Old livers are not protected by ischemic preconditioning and require other strategies to prevent reperfusion injury.

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Surgery for Portal Hypertension

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Hypothesis: Surgery for portal hypertension has evolved widely in the past decades. Selection criteria and the type of operation have evolved because of the appearence of other therapeutic alternatives, such as pharmacotherapy, endoscopic therapy, transjugular intrahepatic portosystemic shunt and liver transplantion. We believe the surgical alternative has a therapeutic role in a select patient population. Design: Retrospectic review of the medical records of patients operated on for bleeding portal hypertension in the past 53 years. Setting: An academic tertiary care university hospital. Patients and Methods: In a 53 years period, 1121 operations for the treatment of bleeding portal hypertension have been done, including shunts and devascularization procedures. We have two periods of time, from 1949 to 1973 and from 1973 to 2002. In the first period 161 operations were done and in the second 960 operations were done. In the past years in low-risk (Child-Pugh classification A) selected patients, only elective portal blood flow-preserving operations have been done. Results: Non-portal blood flow-preserving procedures had a wide spectrum of results, with a high encephalopathy rate and short long-term survival. The results with portal blood flow-preserving procedures in the past five years are as follows: operative mortality, 1.6%; postoperative encephalopathy, 8%; rebleeding, 6%; and shunt obstruction, 6%. Conclusions: Portal hypertension surgery has a role in a elective operations and in low-risk selective patients when portal blood flow-preserving procedures are done. The type of operation is selected according to the individual characteristics of each patient.

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Regeneration of the Old Liver: New Aspects for Liver Surgery in Elderly Patients

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The liver is the only solid organ with the capacity to regenerate. After major liver resection or partial liver transplantation patient survival often depends on the ability of the liver to restore the tissue loss. Due to presumed impaired regeneration, older livers are excluded from split liver transplantation. Additionally, major liver resection is increasingly performed in the elderly. However, data regarding the effects of age on liver regeneration are lacking. Methods: 70% liver resection was performed in C57BL/6 mice of 6 weeks, 30 weeks and 60 weeks of age. The degree of steatosis & fibrosis in each group was blindly determined. Regeneration was investigated 2 days after liver resection by 3 independent markers of the mitotic cycle (PCNA, BrdU and mitotic index) in 30 high power fields (HPF). IL-6, a key mediator of regeneration, was determined in liver tissue 4hr after surgery. Results: No differences regarding steatosis or fibrosis were found in the liver tissue among the different age groups. Each marker of regeneration was dramatically decreased in older mice. Two days after 70% liver resection mice of 6 weeks of age had significantly more PCNA staining (102/ HPF), when compared to mice of 30 weeks (70/ HPF) or mice of 60 weeks of age (40/ HPF) ($p< 0.01$). Similarly, young mice had higher BrdU staining (15/HPF) than mice of 30 weeks (8/HPF) or 60 weeks of age (2.4/HPF) ($p< 0.01$). Finally, mitosis was more frequent in mice of 6 weeks (2,6/HPF) than in mice of 30 weeks (1,7/ HPF) or 60 weeks of age (0.5/HPF) (p[0.05). IL-6 was higher in young mice of 6 weeks (18.4pg/mg) when compared with mice of 60 weeks (7.5mg/pg) ($p=0.03$). Conclusion: Advanced age is associated with an impaired ability of the liver to regenerate. Decreased regeneration is associated with low levels of hepatic IL-6 indicating impairment at the level of the signaling growth factors. Future strategies should include restoring IL-6 in elderly patient to normalize the regenerative capacity of the liver.

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Extending Right Hepatic Trisectionectomy — How Far Can We Go?

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Aims: The most major right liver resection is right trisectionectomy: removal of segments 4,5,6,7,8 ± 1 with a resection line to the right of the falciform ligament. However, in some cases extending the resection is necessary to achieve tumor clearance. We aimed to assess the outcomes following such a resection and effects on hepatic synthetic function. Methods: Considering patients at our center with at least one year follow-up, betweeen January 1993 and October 2001, 112 patients underwent right trisectionectomy for malignancy. Of these, 37 patients underwent a right trisectionectomy extending beyond the falciform ligament for metastases (30 colorectal, 4 sarcoma, 2 neuroendocrine, 1 esophageal). This extension was done either in contiguity or as a segment 2/3 metastasectomy (M2, M3 or M2+3). In contiguity (IC) resections of either segment 2 (IC2) or 3 (IC3) or both (IC2+3) were done to achieve clearance of the tumor but taking care to avoid injury to the left portal structures in the umbilical fissure. All patients had their liver function tests monitored regularly post-operatively for at least 1 week (bilirubin, ALT, alkaline phosphatase, albumin, prothrombin time). Results: Of 37 patients (M:F 16:21, median age 60, range 33-79 years) who underwent a right trisectionectomy extending beyond the falciform ligament, 17 had an IC resection (IC2 n=5, IC3 n=8, IC2+3 n=4). 7 patients had an IC resection and a metastasectomy (IC + M2 n=1, IC + M3 n=3, IC + M2+3 n=3). 13 patients had a segment 2 or 3 metastasectomy alone (median 2, range 1-5 metastases). The median hospital stay was 10 days, range 6-23 days. There was 1 (2.7%) in hospital death from multiorgan failure. 3 patients developed a bile leak (treated by percutaneous drainage) and 2 had post-operative hemorrhage requiring re-exploration. 13 patients (35%) had significant transient hepatic dysfunction that recovered. 6 patients have died with recurrent disease and 2 from other causes. 29 patients are currently alive, 5 with recurrent disease. Conclusion: Right trisectionectomy can be extended across the falciform ligament safely, enabling clearance of the tumour with acceptable mortality, although a third of patients developed transient hepatic dysfunction.

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Impact of Multiple Surgical Procedures on Liver Control in Patients With Metastases From Colorectal Cancer

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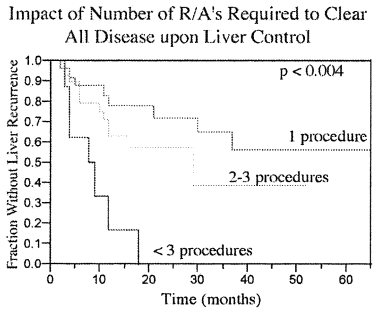
Introduction: In the era of expanding options for treatment (e.g. resection, ablation, regional infusion) predictors of liver-specific control following surgical treatment of hepatic colorectal metastases is important for clinical decision making. Objective: The purpose of this study is to identify predictors of disease recurrence following liver surgery for colorectal metastasis. Materials and Methods: A retrospective database was assembled from an NCI-designated Comprehensive Cancer Center. Information was collected regarding patient characteristics, surgical technique, tumor-related variables, and recurrence. Collected data was analyzed with the appropriate statistical methods. Results: 127 liver resections or ablations (R/A) were performed on 64 patients in as many operations between 1995 to the present. There was 1 peri-operative death. Twenty-nine patients underwent a single R/A (40%), 28 had two or three R/As (44%), and 7 (11%) had more than three R/As. Twenty-three patients (36%) had a single metastatic deposit, the remaining 41 (64%) having multiple lesions. After a median 23 months of follow up, 27 (42%) suffered hepatic recurrence. Factors found to be associated with shorter liver-specific disease free survival were number of R/As required ($p<0.0004$) and the presence of multiple liver metastatic deposits ($p < 0.03$). Factors found not to affect median liver specific disease free survival includes advanced patient age (> 70 years), gender, primary

nodal positivity, synchronous versus metachronous presentation, and extent of hepatic resection (lobectomy or greater resection versus lesser R/As). Conclusion: Hepatic control by R/A is adversely affected by increasing number of lesions and procedures required to clear disease.



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Laparoscopic Management of Benign Hepatic Cysts and Cystic Neoplasms

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Benign cystic lesions of the liver include a wide spectrum of disease processes, including benign simple cysts, polycystic disease, and cystic neoplasms. The purpose of this video presentation is to illustrate the laparoscopic approach to benign cystic lesions of the liver. In this presentation, the laparoscopic treatment of a simple hepatic cyst, fenestration of polycystic liver disease, and excision of a giant hepatic mucinous cystadenoma will be demonstrated. Principles of laparoscopic treatment of benign cystic lesions include adequate preoperative imaging and the routine use of intraoperative laparoscopic ultrasound. We routinely use three to four trocars and a 30 or 45 degree laparoscope. Cyst and hepatic parenchymal division is performed with the Harmonic scalpel and electrocautery. For simple hepatic cysts, the cyst wall is excised such that at least 50% of the diameter of the cyst is unroofed. For cysts with a large intrahepatic component, the omentum is mobilized and placed within the cyst to facilitate closure. For polycystic liver disease, cysts are unroofed, resected, and/or fenestrated. If biliary communication is identified, the involved duct is suture ligated from within the cyst cavity. For cystic neoplasm, laparoscopic enucleation and resection are performed. Intraoperative frozen section evaluation of the cyst wall for malignancy is necessary. The postoperative course is similar to that for laparoscopic cholecystectomy. In summary, benign hepatic cysts and cystic neoplasms are effectively treated with a laparoscopic approach.

LIVER
Other

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The Development of a New Bioartificial Liver and its Application in 12 Acute Liver Failure Patients

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Background: Bioartificial liver is a hope of supporting liver functions in acute liver failure patients. Using polysulfon fibers, a new bioartificial liver was developed. The aim of this study was to show whether this bioartificial liver could support liver functions or not. Material and methods: Hepatocytes were procured from swine using Seglen's methods. The bioartificial liver was constructed by polysulfon bioreactor and more than 1010 hepatocytes. It was applied 14 times in 12 patients, who were divided into 7 cases of simultaneous HBAL and 5 cases of non-simultaneous HBAL. Each BAL treatment lasted 6 hours. The common state of the patients and the biochemical indexes were studied. Results: After treated with bioartificial liver, ammonia, prothrombin time and total bilirubin index showed significant decrease. 2 days later, only ammonia still showed significance. In one month period, 1 case (1/7) in simultaneous group died while in non-simultaneous group 2 cases (2/5) died. Significance was shown in two groups. The total mortality rate was 25%. Conclusions: The constructed bioartificial liver can support liver functions in acute liver failure. The simultaneous HBAL is better than non-simultaneous HBAL.

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Focal Adhesion Kinase Signaling in Hepatic Regeneration

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Introduction: The liver has considerable ability to regenerate in response to injury of many types. Following partial hepatectomy (PH), a rapid and specific series of biochemical events occur prior to cellular proliferation. Maximum DNA synthesis occurs within 24 hours of PH and regeneration is completed by 10 days. Post resection c-Jun, c-fos and liver regenerating factor-1 (LRF-1) mRNA levels are elevated within 30 minutes of the procedure. One critical regulator of cellular proliferation is adhesion mediated signals. These are regulated by focal adhesion kinase (FAK) following its activation by the interaction of integrins with the extracellular matrix. Yet the role of FAK and other adhesion signal mediators are unknown in liver regeneration (LR). Activated FAK results in increased cell motility, proliferation and suppresses apoptosis, all important in organ regeneration. We hypothesized that FAK would play a pivotal role in LR as evidenced by elevated protein levels and activation following resection. Method: Balb/C mice were divided into four treatment groups. (1): 24 hours after sham PH (n=6), (2): 1 hour after 1/3 PH (n=6), (3): 24 hours after 1/3 PH (n=6) and (4): 288 hours after 1/3 PH (n=2). All animals had a general anesthetic, a midline laporatomy and procedure noted above performed. At the study time indicated, the remainder of the liver was removed. A second group of animals underwent 1/3 PH, then at 1, 5, 10, 15, 30, and 60 minutes later, the remaining liver was resected. Liver cell lysates were analyzed by immunoblotting for FAK expression and activation. Results: FAK (total and activated) is downregulated in the post resected samples as compared to pre resection at 1 hr, 24 hrs and 288 hours. As this was unexpected, we analyzed the FAK family protein PYK2, and found its expression inverse to that of FAK. Activated FAK is increased very early after PH (maximum at 5-10 min), and then returned to baseline, whereas PYK2 stayed elevated until 30 minutes post resection and decreased to normal by 60 minutes. Downstream effectors of adhesion signaling also were activated early. Conclusion: LR stimulates changes in adhesion mediated signals, suggesting their involvement in hepatic growth. Regulation is early, within minutes, with FAK transiently increasing before being suppressed, whereas its related family member, PYK2 is upregulated for a more sustained time after PH. Further studies are warranted to elucidate the role of adhesion signaling in LR and hepatocellular carcinogenesis.

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Resection for Symptomatic Hepatic Cysts: Is It Worthwhile?

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Background: Symptomatic liver cysts can be managed surgically by fenestration or by hepatic resection. Although fenestration procedures are preferred, this is associated with a higher recurrence rate. Conversely, hepatic resections are rarely associated with recurrence and are becoming a safer procedure in specialised units. Methods: 40

patients with symptomatic liver cyst (non-parasitic and non-malignant) surgically treated by fenestration or resection were identified from a prospectively collected database. A retrospective analysis of primary outcome measures including operative parameters, morbidity and mortality rates, length of post-operative stay and recurrence rates in months was carried out to determine the better treatment option. Results: 27 patients had undergone fenestration (17 by open method and 10 by laparoscopic method) and 13 patients had undergone resection for treatment. Cyst and patient parameters were comparable in all three treatment arms. There was no mortality in our series. Morbidity was less in the fenestration group but in the resection group morbidity was mainly for minor complications like wound infection, chest infection and paralytic ileus. At median follow-up of 20 months, there were no recurrences in the resection group but 6/27 (22%) in the fenestration groups (3 in laparoscopic and 3 in open fenestration). Two required a hepatic resection and the other four were observed as they were asymptomatic radiological recurrences. Conclusion: The best result for our patients was obtained with resection. Although it was associated with a longer post-operative recovery and morbidity, resection can be carried out safely for both primary and recurrent cystic lesions no recurrence. Fenestration procedures provided adequate treatment with a quicker post-operative recovery but also a trend towards a higher recurrence rate, especially when carried out by laparoscopy.

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Peripheral Cholangiocarcinoma in Mexico

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Introduction: Peripheral Cholangiocarcinoma (PCC)is an extremely rare tumor. In Mexico, it represents about 1.5% of all malignancies. It affects primarily male adults in the fifth decade of life. The most common symptoms are pain and weight loss, together with a palpable abdominal mass at physical examination. The treatment is usually difficult, being hepatic resection the most viable choice for the physician. The prognosis is still poor, with an overall survival of 5% at 5 years of follow up. We present here a series of PCC in a Cancer center at Mexico city. Patients and methods: We reviewed the clinical files in the Instituto Nacional de CancerologÌa at Mexico city of patients with the diagnosis of Peripheral Cholangiocarcinoma during the period of June 1992 to June 2002. Results: 11 patients were diagnosed with Peripheral Cholangiocarcinoma, 3 of them were male (28%), the other 8 were female (72%). The mean age at the time of diagnosis was 61 years (range 39-87). The most common symptoms were abdominal pain (81%), weight loss (63%), nausea and vomiting (27%). Abdominal mass was found in 6 patients (54%) and jaundice was reported in only one patient (9%). ACE was high in 6 out of 8 (75%) patients evaluated (mean: 104.6 ng/dl, range: 2.4-150 ng/dl). CA19-9 was also measured in 8 patients, 7 (87.5%) of which were reported with elevated values (mean: 45,922.5 ng/dl, range: 150-146,440 ng/dl). All patients were submitted to surgery; left hepatectomy was performed in 4 of them (36.36%), right hepatectomy, left trisegmentectomy and right trisegmentectomy in 1 patient (9.09%). 4 patients could not be resected (36.36%) due to disseminated disease. 3 patients (27.27%) were initially proposed for intrarterial chemotherapy, because of non-resectable lesions, one of them was further submitted to surgical resection; the other two presented tumor succession. Only one patients was in for palliative radiotherapy. Post chirurgical complications were reported in 5 patients (45%). 2 patients died as a result of these complications. Conclusions: Peripheral Cholangiocarcinomas are even less frequent than their perihiliar counterpart. In Mexico, PCC occurs regularly in females. Hepatic resection is the only curative treatment available to date.

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Resection of Non-Colorectal Liver Metastases

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Background: The liver is a common site of metastases in the spread of tumors. Hepatic resection in patients with colorectal metastases has been accepted as a therapeutic option. In contrast, treatment in patients with non-colorectal liver metastases is poorly documented in the world. Aims: To analyze results of hepatic resection in patients with non-colorectal liver metastases. Methodology: A retrospective analysis was performed with the database of patients with hepatic metastases from non colorectal origin that were resected from October 1995 to September 2002 in the Instituto Nacional de Cancerología. Results: From 150 hepatic resections performed, 34 were from non-colorectal liver metastases in 33 patients. 20 were females and 13 males with age from 15 to 70 years (mean: 39 years). The primary tumor originated from the breast in 7, testicular in 5, ovarian in 4, stomach in 4 and carcinoid tumors in 3, from melanoma, kidney and sarcomas in 2 and 4 miscellaneous. 17 patients presented symptoms (palpable mass, pain, weigh loss, jaundice). In 3 cases (10%) liver resection was performed at the time of the resection of the primary lesion. The mean tumor size was 6.8 cm (range from 2.3 to 30 cm). Nine right hepatectomies were performed, left hepatectomies (9), right trisegmentegtomies (6), non anatomic resection (3), left lateral segmentectomies (5), caudate lobe resection (1), resection of segment VII (1) and resection of segment II (1). Median operative bleeding was 1560 mL (range: 100-4000mL). Mean surgical time was 256 min (range: 120-390 min). Eight patients presented complications (23.5%): hepatic failure in 3, hemorrhage in 2, ascites, biloma and pleural effusion in one. There was one perioperative death (2.9%). Nineteen patients are still alive at a median follow up of 22 months (range 1 to 65). Conclusions: The presence of a low morbidity and mortality in this group of patients justify the option of treatment in selected patients with non colorectal liver metastases. The survival rate obtained in this series is similar of that obtained in colorectal liver metastases.

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WITHDRAWN

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Surgical is Better than Conservative Therapy in Alveolar Echinococcosis: Long Term Follow-up in 90 Consecutive Patients

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Aim: Alveolar Echinococcosis is an infection caused by Echinococcus multilocularis characterized by local tissue invasion and destruction similar to a malignant tumor. The most frequent site of infection is the liver, although primary extrahepatic localization has been rarely described. We analyzed 90 consecutive patients treated for alveolar Echinococcus in our institution with the aim of comparing the outcome between surgical vs. non surgical therapies. Method: 90 consecutive patients treated for Echinococcus alveolaris at our institution since 1976 were analyzed. Results: Of the 90 patients, 41 underwent resection: 9 were liver Echinococcus debulking procedures and 2 involved resection of extrahepatic lesions; the rest were curative anatomic liver resections. A total of 49 patients were treated non-operatively with benzimidazole chemotherapy. Criteria for inoperability were bilateral or large central liver lesions and major vascular involvement. Within the surgical treatment group there were 5 deaths (12%) during the complete follow-up, only 1 of which was related to progression of Echinococcus disease (2%). This involved a patient who had undergone a debulking liver resection procedure with incomplete Echinococcus lesion removal. In the nonoperative treatment group, a total of 20 patients died (41% mortality) of which 6 were related to progression of Echinococcus disease (12%). The median survival of the resection group was 23 yrs (SEM 13.2 months) and of the non-operative group 13 yrs (SEM: 28 months) ($p=0.0007$). The median follow-up of all patients was 10.5 yrs [range: 6 months to 25 yrs]. Conclusion: Although experience with Echinococcus alveolaris indicates that radical surgery can only be performed in 20% to 30% of patients, surgical resection should be considered as the therapeutic first option whenever possible.

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Laparoscopic Surgery in the Cirrhotic Population-Is It Truly Safe?

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Since the advent of the laproscopic cholecystectomy the use of laproscopic technique has become increasingly diverse. As experience and technology has increased so have the complexity of patients considered for laproscopy. Still, little data has been published on laproscopy in cirrhotics. Methods: We retrospectively reviewed demographic and outcome data on cirrhotics who have undergone laproscopic surgery. Results: 25 patients were identified with a mean age of 50.6 (33 -77). 12 males (48%) and 13 females (52%) were noted. 18 patients (72%) were Caucasian, 6 (24%) were African-American and 1 (4%) was Asian. The etiology for cirrhosis included: Hepatitis C 64% (n=16), alcohol 12% (n=3), Hepatitis B 8% (n=2), and others 20% (n=5). Most patients had advanced cirrhosis: Childs class B 40% (n=10), Childs class C 40% (n=10), and Childs class A 20% (n=5). Fifty-six percent (n=16) had undergone prior abdominal surgery. The majority of patients underwent laproscopic cholecystectomy with liver biopsy 40% (N=10), followed by radiofrequency ablation with laproscopic ultrasound 28% (n=7), laproscopic ultrasound with liver biopsy 16% (n=4), laproscopic liver resection 8% (n=2), laproscopic appendectomy 4% (n=1), and laproscopic ventral hernia repair 4% (n=1). Estimated blood loss was less than 50ml in 64% (n=16) of patients, between 100ml and 250ml in 24% (n=6), and greater than 500ml in 12% (n=3). Two patients required conversion to an open surgical procedure. One case returned to the operating theater due to bleeding that was controlled laparoscopically. The most common complication was ascites 64% (n=16). The remaining complications included: incisional hernia 12% (n=3), urinary tract infection 8% (n=2), bile leak 4% (n=1), mild encephalopathy 4% (n=1), pneumonia 4% (n=1), bleeding requiring return to the operating room 4% (n=1), bacteremia 4% (n=1), and Clostridium difficile infection 4% (n=1). The mortality rate was 4% (n=1). Conclusion: Due to concerns over high morbidity and potential mortality, cirrhotic patients have presented a daunting dilemma when being considered for surgery. In our series most were at high risk due to advanced Childs classification and were further complicated by previous abdominal surgery. Despite these challenges, blood loss, complication rate, and mortality rate were minimal and comparable to most open series. We believe this data confirms that laparoscopic surgery in advanced stage cirrhotics can be performed safely.

PANCREAS

Acute Pancreatitis

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Role of ERCP in the Management of Predicted Mild Acute Biliary Pancreatitis

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Objective: The role of endoscopic retrograde cholangiopancreatography (ERCP) in mild acute biliary pancreatitis is controversial. This study aims to examine the results of ERCP in patients with predicted mild acute biliary pancreatitis, and analyze biochemical and imaging findings with relation to the occurrence of ductal pathology. Patients and Method: 172 consecutive patients, admitted between January 1998 and December 2000 with the diagnosis of acute pancreatitis, were included. They were treated according to a departmental management protocol. All patients were investigated with transcutaneous ultrasound study and ERCP if biliary etiology was suspected. Several biochemical and imaging parameters were analyzed for prediction of any biliary pathology. Results: Biliary calculus was the etiology in 63% of patients (n=108). Amongst these 108 cases, 86 patients (79.6%) suffered from predicted mild disease. There were only 80 patients undergoing ERCP as five patients refused and one was operated before ERCP investigation. The overall incidence of choledocholithiasis in those underwent ERCP was 45% (36/80) and all ductal stones could be successfully removed endoscopically. The incidence of ductal stone was only 4.8% (1/21) if serum bilirubin and alkaline phosphatase were normal and there was no ductal dilatation on ultrasound study. Conclusion: Routine ERCP is not recommended in all cases of predicted mild acute biliary pancreatitis. In case there is no biochemical derangement or ultrasound evidence of dilated biliary system, intraoperative cholangiography, instead of preoperative ERCP, should be the choice during cholecystectomy. If cholecystectomy has already been performed, other less invasive imaging methods such as magnetic resonance cholangiopancreatography (MRCP) should be considered.

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Activation of Nociceptive Neurons at T9 and T10 Correlates With the Severity of Acute Pancreatitis

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Background: Mechanisms of pain signal transduction in pancreatic disease are poorly understood. Expression of the protein c-fos in the dorsal horn of the spinal cord is a useful marker for detecting activation of nociceptive neurons. We recently showed that c-fos expression is increased in rats with mild interstitial pancreatitis induced by

subcutaneous injection of the secretagogue, cerulein. In the present experiments we examined whether c-fos expression correlated with the severity of pancreatic inflammation. Methods: Rats were anesthetized,the biliopancreatic duct was cannulated and infused with 100µl of 10 mmol glycodeoxycolic acid (GDOC) or saline (NS) under pressure-controlled conditions (20 mmHg). The animals then received continuous intravenous infusion of cerulein (5µg/kg/hr) or saline (NS). After 6h, serum amylase, pancreatic edema (wet:dry ratio), and spinal cord c-fos expression (immunoreactive nuclei/section dorsal horn gray matter) were measured. Staining of c-fos antibody at T9-T10 was compared to internal controls at cord levels T6 and T12. An average of 20 spinal cord histologic sections were evaluated per rat (3-5 rats/group). T-test was used for statistical analysis and results are expressed as mean+SEM. Results: Control animals treated with intravenous + intraductal saline (NS-NS) showed no evidence pancreatic inflammation. Animals treated with cerulein + intraductal saline had findings consistent with mild interstitial pancreatitis with marked hyperamylasemia (mg/dl) (7,700 ± 600 vs 4,400 ± 600, $p<0.05$) and pancreatic edema (7.9 ± 0.5, vs 4.3 ± 0.7, $p<0.05$). As expected, animals treated with cerulein + intraductal GDOC had severe hemorrhagic pancreatitis (amylase 12,400 ± 600, $p<0.05$; wet:dry 6.9 ± 0.6, $p<0.05$). Expression of c-fos correlated with the severity of pancreatitis as shown in Table 1.

c-Fos immunoreactivity

	NS-NS	NS-Cer	GDOC-Cer
T9	15±4	26±2**	38±10*
T10	11±2	21±3**	37±12*

*p=0.05, **p<0.05 vs. NS-NS.

The findings were most pronounced at spinal cord levels T9 and T10, with insignificant increases in c-fos expression at levels T6 and T12. Increased c-fos expression was localized to superficial laminae I and II and deep laminae V, VI, and X. Conclusions: Expression of c-fos in the dorsal horn of the rat spinal cord correlates with the severity of acute pancreatitis. The spinal cord levels involved (T9-10) and the distribution of c-fos expression within the spinal cord are characteristic of pancreatic nociceptive neuronal activation. These data enhance our understanding of pain pathways in pancreatic disease.

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N2-Mercaptopropionylglycine(N2-MPG) in Experimental Acute Pancreatitis

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Background: N2-Mercaptopropionylglycine(N2-MPG) is known as an antioxidant thiol that reduces the abnormal production of xantine-oxidase. The aim of this research is to analyze the strenth of this substance to offer some protection to pancreatic tissue in the mild and severe acute pancreatitis (AP). Methods: AP was achieved by two methods: a) mild: supramaximal dose of cerulein; b) severe: infusion of 2.5% sodium taurocholate into the biliopancreatic duct of the rat. Thirty-six male Wistar rats (220-270g) were submitted to AP with cerulein (Two parenteral doses of 20mg/Kg; one hour interval) in two groups: GI: nineteen rats previously treated with N2-MPG(100 mg/Kg IV) ten minutes before and GII (control), of seventeen animals that received only saline 0.9%. AP with taurocholate 2.5% (0.5 ml into the main biliopancreatic duct) in other two groups: GIII: of eleven rats previously treated with N2-MPG (100 mg/Kg IV) ten minutes before AP and GIV (control): of fifteen animals which received only saline 0.9%. The albumin leakage into the pancreatic interstice, a significant inflammatory parameter, was measured through Evans-Blue (EB) dye, that links totally with serum albumin after injection into the pancreatic tissue, immediately before induction of AP and can be measured within the first hour. The rats were sacrificed one hour after. Water tissue content was also measured. Results: There was a relevant reduction of EB leakage in GI (344±27 µg/g tissue) when compared to GII (729±84 µg/g tissue), $p<0.01$, and in GIII (386±52 µg/g tissue) when compared to GIV (543±53 µg/g tissue), $p< 0.05$. There was no difference in tissue water content between GI (88.2± 0.6%) and GII (87.4±0.9%), but certainly between GIII (77.7±2.1%) and GIV (82.8±1.2%), $p<0.05$. The amilase levels did not show any difference among the four groups. Conclusions: These results suggest that the use of the antioxidant N2-MPG offers a protective action, at least in rats, reducing the severity of AP induced by supramaximal dose of cerulein, and even in a more severe AP such as produced by sodium taurocholate at 2.5%, although apparently not interfering with its pathogenesis. It also strengthens the actual participation of free radicals of oxigen in the physiopathology of acute pancreatitis.

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Percutaneous Lavage for Infected Pancreatic Necrosis

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Purpose: Infected pancreatic necrosis has classically been treated by surgical debridement. Both open and closed operative approaches are associated with 10-30% mortality rates and significant morbidity. We present the results of a less invasive therapeutic approach for this disease entity - serial percutaneous lavage. Methods: Between 1993 and 2001 all 34 patients presenting to our service with infected pancreatic necrosis underwent percutaneous debridement as initial therapy. Mean age was 48 years (range 20-85). All patients had documented infected necrosis by culture of initial drainage. Four of the patients were critically ill initially, requiring mechanical ventilation at the time of presentation. These were termed Group A. Group B consisted of the 30 extubated and non-critically ill patients. Briefly, our treatment protocol consists of placing one to four catheters percutaneously into the necrosis, and sequentially increasing the catheters in size to 16 French. Aggressive lavage and debridement of infected necrosis, as well as removal of debris, is carried out through these large bore catheters. Procedures take place one to three times per week in the Radiology Suite. Patients are discharged once stable and percutaneous debridements are continued on an outpatient basis. Treatments persist until radiologic resolution of the disease process, at which time all catheters are removed. Results: One Group A patient and no Group B patients died, for an overall mortality in our population of 2.9%. The overall success rate of percutaneous debridement was 65%, however the success rate differed significantly between the two groups. All four patients in Group A failed percutaneous management and ultimately required surgical necrosectomy. Five Group B patients (16.6%) required surgical intervention. These included two necrosectomies with marsupialization for failed therapy, a negative laparotomy for suspected peritonitis, drainage of a paracolic gutter collection by flank incision, and pancreaticojejunostomy for a persistent pancreatic duct fistula. The mean inpatient stay did not differ significantly between the two groups (78 ± 47 for Group A and 53 ± 31 for Group B, $p=.19$). The mean outpatient treatment days for the patients treated successfully by catheter lavage was 37 days (range 0-98). This represented 41% of the total time of lavage therapy. Conclusions: Percutaneous lavage and debridement is a safe, less invasive option with zero mortality for stable patients with infected necrosis. A large portion of the treatment can be conducted on an outpatient basis. At this time, percutaneous lavage

is not recommended as primary therapy in critically ill patients with infected pancreatic necrosis.

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Time Course of Gastric Ileus in a Conscious Mouse Model of Acute Pancreatitis

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The pathophysiology of gastric ileus in pancreatitis is unclear. We have developed a conscious mouse model that uses food consumption to monitor the progression of ileus. Pancreatitis was induced in fasted mice by hyperstimulation with the 3 or 7 hourly doses of cerulein. Food consumption was monitored every 12 hours. Serum amylase peaked 6 hours after induction of pancreatitis (10389±233 U/lit) and returned to normal levels (2135±338 U/lit) after 24 hours. Histologically, pancreatic edema was evident within 1 hour of induction and persisted for at least 4 days. Inflammatory cell infiltration did not appear in histological samples until 12 hours after induction of pancreatitis. For the highest cerulein dose, food consumption was significantly reduced 12, 36 and 60 hours after induction (Table).

Nocturnal feeding (g12 hours ± SD)

Hours	Saline	3×50 μg/kg Cerulein	3×100 μg/kg Cerulein	7×100 μg/kg Cerulein
12	3.3 ± 0.1	2.9 ± 0.4	2.7 ± 0.2	2.3 ± 1.0*
36	4.0 ± 0.3	3.3 ± 0.4*	3.2 ± 0.3*	2.1 ± 1.1*†
60	4.0 ± 0.4	3.8 ± 0.3	3.8 ± 0.4	3.0 ± 1.0*†
84	3.5 ± 0.5	3.5 ± 0.4	3.6 ± 0.1	3.9 ± 0.4

*P<.04 vs. saline control, †P<.04 vs. lower doses by ANOVA. Hours indicate time after final cerulein or saline injection.

For the lower doses, food consumption was significantly reduced only at the 36-hour time point. Summary: In cerulein pancreatitis, 1) Hyperamylasemia recovers before food consumption returns to normal, 2) Pancreatic edema persists well after the recovery of normal feeding behavior, and 3) The onset of pancreatitis induced ileus correlates most closely with the appearance of inflammatory cells within the pancreas.

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Apoptosis in Severe Necrotizing Pancreatitis

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Existence and the degree of apoptosis in pancreatic parenchyme in severe necrotizing pancreatitis seems important for analyzing the high mortality of this disease. Materials and Methods: Two patients with massive necrotizing pancreatitis were studied. For them distal pancreatectomy was necessary in early phases of the disease because of the worsening against intensive care. One survived and another died. Control group consists of three patients. The analysis was made mainly based on histological findings: 1) Light microscopical findings with heamtoxylin-eosine staining and/or the TUNEL method, and 2) Electronmicroscopic findings with or without the TUNEL. The degree of apoptosis was calculated. Results: 1) In both cases apoptosis cells were found occupying relatively large areas. 2) Apoptosis cells usually located around the necrotic tissue or between normal tissue and necrosis. 3) In the patients who survived (relatively moderate degree of parenchymal necrosis) had relatively large amount of apoptosis cell (17%) and that of the patient who died with massive necrosis (more than half of the total parenchyme) occupied smaller part(apoptosis occupied approximately 5% of the parenchyme). Inflammatory cell infiltration into the apoptotic or necrotic areas was minimal in the severest necrotic patient. There were several cells found which showed phagocyte function of the apoptotic nuclei/cells in the survived case. Discussion and Conclusions: Several hypotheses of the significance of apoptotic pancreatic cells were proposed, which are as follows: Apoptosis cells seems to have an intermediate role which is different from the necrotic cells, considering the location and the amount of a patient who survived, which may suggest that apoptosis may not be harmful. Inflammatory cell infiltration and phagocyte function were thought to be other important factors influencing the mortality, because they seem to reflect the existence of physical response. For patients with massive apoptosis(with minimal necrosis), conservative intensive care may be superior. On the contrary, surgical procedure may be justified for patients with massive necrosis which resist against intensive care. Developing a new method for detecting the degree of apoptosis may be expected.

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Single Operative Debridement for Pancreatic Abscess

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Purpose: We hypothesized that optimal treatment of pancreatic abscess is best performed with a single, rather than multiple, operative debridements. Methods: During the period 1988 to 2002, 20 patients were treated for pancreatic abscess by a single surgeon at a university hospital setting. Operative treatment involved drainage and thorough debridement of the pancreatic abscess when possible via the base of the transverse colon mesentery. A 1.5-2.0 inch left flank incision served as an exit site for multiple drains placed into the pancreatic bed. Patient records were reviewed in a retrospective fashion to obtain data regarding operative and hospital treatment and outcomes. Statistics were performed using a Chi square analysis. Results: There were 17 males and 3 females, with an average age of 50.9 years (range 22-73). Nine had a history of previous pancreatitis. Gallstones were the precipitating etiology of pancreatitis for 7 patients, alcohol for 4, hypertriglyceridemia and ERCP for one patient each, and for 7 patients the etiology of the pancreatitis was unknown. Sixteen of the 20 patients were successfully treated with a single stage operation for debridement of a pancreatic abscess. Three patients underwent a second procedure, and one had three operations. Average blood loss for the operations was 1259 ml (range 100-6000). Average number of drains placed was 2.4 (range 0-5). Of the 25 operative procedures in 20 patients, pancreatic necrosis was found during 19, whereas in 6 the gland was firm or edematous but no necrosis was present. Sixteen of the 20 patients had pancreatic necrosis debrided. Additional procedures included cholecystectomy in 4 (two gangrenous), bowel resection in two, and splenectomy in one patient. Average hospitalization was 35.1 days (range 7-90) and average postoperative stay was 21.9 days (range 5-45). Four patients died. Of these, three were found not to have significant pancreatic necrosis at the time of their surgery ($p<0.01$). Of the 16 patients who survived, all but one had significant pancreatic necrosis debrided at their first (14) or second (2) operation. Postoperative complications included two pancreatic fistulae, only one severe wound infection, and no enterocutaneous fistulae. Conclusions: We conclude that this technique of single operative drainage, when performed in the setting of pancreatic necrosis and abscess, is highly effective for the treatment of that problem. It is associated with a high rate of survival and a low rate of postoperative fistulae or severe wound problems.

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Peroxisome Proliferator-Activated Receptor-Gamma Agonists Decrease the Severity of Acute Pancreatitis

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Peroxisome proliferator-activated receptors (PPAR) are ligand inducible transcription factors and members of the Class II nuclear hormone receptor superfamily. PPARs regulate cellular energy and lipid metabolism. PPARs have potent anti-inflammatory properties through down regulation of early inflammatory response genes, including the NF-kB, AP-1, and JNK/p38 MAPK pathways, via a cross coupling mechanism. We have previously shown that PPAR-gamma is expressed in pancreatic acinar cells in vivo. We hypothesized that PPAR-gamma activation decreases the severity of acute pancreatitis. PPAR-gamma expression was studied in pancreatic tissue sections from male C3H mice with immunofluorescence using monoclonal antibodies specific for PPAR-gamma. The cerulein model of acute pancreatitis was used to evaluate expression of PPAR-gamma in pancreatitis and the effect of PPAR-gamma agonists on the severity of acute pancreatitis. Severity of pancreatitis was determined by serum amylase and histology. In acute pancreatitis, PPAR-gamma expression was absent 1-hour and 6-hours after the last injection of cerulein (three hourly i.p. injections of 100mcg/kg). At 12-hours post-injection, PPAR-gamma expression recovered to baseline and was overexpressed by 24-hours. Return of PPAR-gamma expression paralleled the time course of amylase elevation in the cerulein model with a peak amylase level at 6-hours and return toward baseline by 24-hours. Pretreatment with a single i.p. injection of 15d-prostaglandin-J2 (15d-PGJ2), a potent PPAR-gamma agonist, restored the expression of PPAR-gamma that was absent early in acute pancreatitis. Pretreatment with 15d-PGJ2 resulted in significant reductions in serum amylase (3% reduction at 1-hour post cerulein injections, 33% at 6-hours*, 39% at 12-hours*, 44% at 24-hours*; *$p<0.006$ 15d-PGJ2 pretreated versus carrier pretreated by one-way ANOVA). Pretreatment with a single i.p. injection of the specific PPAR-gamma agonist, ciglitazone, reduced serum amylase content by 39% at 6-hours ($p<0.001$). 15d-PGJ2 pretreatment reduced tissue edema, polymorphonuclear cell infiltration, and acinar cell necrosis compared to control. In summary, PPAR-gamma expression is decreased in acute pancreatitis. This decrease in expression corresponds to a rise in serum amylase levels and tissue injury. Pretreatment with selective PPAR-gamma agonists restores PPAR-gamma expression and decreases the severity of acute pancreatitis.

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The Adjuvant Use of Percutaneous Drainage of Pancreatic Necrosis Prior to Operative Drainage

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Infected pancreatic necrosis requires debridement because percutaneous drainage catheters are small diameter and thick necrotic tissue cannot pass through them resulting in plugging of the catheters. We have used percutaneous catheters in an adjuvant fashion to improve the overall condition of the patient prior to operative debridement. Our hypothesis is that percutaneous drainage of peripancreatic fluid in the face of necrosis improves APACHE and Ranson scores prior to operation and therefore make patients better risk for surgical intervention. We retrospectively reviewed the records of 35 patients that underwent treatment for infected pancreatic necrosis. We analyzed the APACHE II score and Ranson Criteria on admission and immediately preoperatively. We also evaluated the patients for complications and mortality. Results: All patients with percutaneous drainage of peripancreatic fluid and infected pancreatic necrosis required operative debridement. The average number of drains was 4.6 with a range of 1-13. The APACHE score on admission was 11/7.8 (nonsurvivor/survivor) and increased to 16.8/8.95 (nonsurvivor/survivor) immediately prior to surgery. The Ranson criteria on admission were 4.8/1.95 (nonsurvivor/survivor) and decreased to 4/1.4 (nonsurvivor/survivor). The morbidity for the hospitalization was 77% and the mortality was 17%. The average intensive care unit stay was 28 days. We conclude: 1- percutaneous drainage of peripancreatic fluid in patients with infected necrosis did not avoid exploration and debridement. 2- percutaneous drainage in this setting improves Ranson scores but not APACHE scores prior to exploration. The role of percutaneous drainage of peripancreatic fluid collections in patients with infected pancreatic necrosis remains unclear. Further studies are required to define subsets of patients that might benefit from urgent operation versus delayed operation with adjuvant use of drains.

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Percutaneous Strategy for Management of Necrotizing Pancreatitis

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Background: Necrotizing pancreatitis is the most severe complication of acute pancreatitis, complicating approximately 10-20% of cases of severe pancreatitis. The role of surgery remains a controversial topic in the management of acute necrotizing pancreatitis and pancreatic abscess. Over the last quarter of a century, advances have developed leading towards non-operative management of necrotizing pancreatitis and pancreatic abscess. We hypothesize that percutaneous radiologic management of necrotizing pancreatis and pancreatic abscess has acceptable mortality and complication rates. Methods: We performed a retrospective review of data collected at LSUHSC-Shreveport and University of California Medical Center-San Diego over a 10 year period. Cases were equally distributed between institutions. Data was restricted to patients diagnosed by computed tomography or ultrasound with necrotizing pancreatitis or pancreatic abscess. Surgical and radiologic services at the institutions collected the data. 67 cases of necrotizing pancreatitis and pancreatic abscess were identified. Data points included: sex, age, etiology, indication for drainage, complications, death, and catheter characteristics(i.e. size and number). Data are presented as percentages and simple means. Results: We observed that 66% of patients were male and that mean age of presentation was 42 years with a range of 6-74 years. Alcohol and gallstones comprised 55% of cases. Trauma and previous upper abdominal surgery comprised 30%. The indication for drainage in all cases were sepsis or sepsis syndrome. Catheters averaged 2.3 per case with a range of. Percutaneous management alone was successful in 83% of cases. Surgical intervention was required in 4 cases. Mortality rate was 13%. Complication rate was 21% and represented persistent sepsis, incomplete drainage, pancreatic and enteric fistulae, and diabetes mellitus Conclusion: We conclude from this study that percutaneous management of necrotizing pancreatitis and pancreatic abscess was successful in 83% of these patients, and has an acceptable mortality and complication rate in our institution when viewed in light of previous surgical literature.

PANCREAS

Tumors

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Local Resection for Peri-Ampullary Lesions

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Transduodenal resection of lesions localized to the Ampulla of Vater is an alternative to pancreaticoduodenectomy (PD). The efficacy of this more limited resection, particularly in patients with familial adenomatous polyposis (FAP) or small ampullary carcinoma, is not well defined. Between 1992 and 2002, nineteen patients underwent a transduodenal ampullectomy at a multidisciplinary, University-based, hepatobiliary referral center. A full-thickness resection of the wall of the duodenum with the ampulla with reconstruction of the transected

bile and pancreatic ducts was used. Average age was 64.2 years (range 33-84), with a mean follow-up of 45 months (range 2-100). Eleven patients had benign ampullary adenomas including 4 with FAP. Seven patients had localized adenocarcinomas. Two patients had frozen section confirmation of cancer and were converted to PD resections. Two patients had false negative frozen sections, with permanent sections revealing adenocarcinoma; these patients had R0 resections and therefore did not undergo further surgery. Three other patients underwent RO resections via TDA, and not PD because of prohibitive medical conditions/risks. One patient had a benign stricture. The 3-month mortality was zero and morbidity was: 2 (11%) minor complications, and 5 (26%) major complications. Fourteen patients (74%) remain disease free at a mean of 40 months. Three of the patients with FAP (including the two who had high-grade dysplasia) have locally recurrent adenomatous change. Two of those with carcinoma (29%) have disease recurrence with metastatic disease involving the liver in both cases. Local resection appears to be an effective option for benign lesions, including patients with FAP; however, those with high-grade dysplasia must be surveilled closely for local recurrence. It may be adequate for selected patients with very localized adenocarcinoma, particularly those whose risk for pancreaticoduodenectomy is high.

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Caspase-3 Induces Apoptosis in Pancreatic Cancer Cells After Treatment With Gemcitabine

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Pancreatic cancer remains a highly chemoresistant malignancy. Gemcitabine, today's most effective first-line agent, acts by disrupting cellular replication. Caspases belong to a family of proteases that function as key components of the apoptotic death machinery. We investigated the mechanisms by which gemcitabine blocks proliferation and whether it can induce apoptosis in pancreatic cancer cells. Methods: Quiescent pancreatic cancer cells (BxPC-3) were stimulated to proliferate (10% FCS) with or without gemcitabine, PS-341 (26S proteasome inhibitor) or both. Proliferation was measured by MTT assay and apoptosis by propidium iodine staining. To determine activation of the apoptotic regulatory cell proteins, caspase-3 and cleavage of poly (ADP-ribose) polymerase (PARP) into its 85 kDa fragment were assessed by Western Blot. Results: Gemcitabine at even low doses (10uM) significantly inhibited cellular proliferation, while PS-341 (10nM) had no effect. With combined treatment, PS-341 potentiated the antiproliferative effects of gemcitabine ($p=0.001$). At 48 hours, the apoptotic fraction was greatly enhanced by the presence of PS-341 compared to gemcitabine alone. Caspase-3 accumulated as early as 30 minutes and was associated with cleavage of PARP to its apoptotic fragment. Conclusions: Gemcitabine, a nucleoside analogue, may in part exert its antiproliferative effects by directing pancreatic cancer cells to a default pathway of apoptosis. 26S proteasome inhibition potentiates this effect, suggesting its potential clinical value against chemoresistance in pancreatic cancer.

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Late Pancreatic Metastasis: Role of Surgery (Oral Presentation)

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Background: The pancreas is rather an uncommon site for metastasis; moreover, an exclusively intrapancreatic localization of a secondary tumor is quite an unlikely event. Such lesions are often discovered as accidental findings. A few selected cases are best approached by surgical resection, as it seems to provide best survival rate and longer disease-free interval. The aim of this study is to describe 3 cases we have observed, in order to better define the role of surgery in the treatment of this pathology. Method and materials: In a four year term, (Sep 98/Aug 02), we have observed at the 3rd unit of dept. of Surgery of the S. Spirito General Hospital in Pescara, 3 cases of single pancreatic metastasis, out of 81 cases of pancreatic neoplasms; the primary lesions were in the lung, in the ovarian and in the kidney. Two patients were male and one was a woman, and they were aged 65, 71 and 72 respectively The patient with primary tumor in the lung (SCLC) had also, at the diagnosis, a sincronous metastasis in the pancreas, and was treated by chemiotherapy alone. The patient with primary tumor in the kidney underwent surgery, then was regularly followed up by times; five years later he developed a single pancreatic metastasis, that was resected according to Whipple; he is actually surviving since 10 months being free from disease. The patient with ovarian tumor underwent resection too, and then was regularly checked up by times; 21 years later was discovered a single metastasis in pancreas that was enucleated surgically; she is actually surviving since 7 months being free from disease. Discussion: The Pancreas is quite an uncommon localization for isolated metastasis; kidney and lung cancer are the tumors that more frequently give rise to these secondary lesions, while the ovarian is rather a rare source. The presence of pancreatic metastasis in patients with clinical history of cancer may be suspected by imaging findings: CT, EUS and MRI may show ipervascularization, that is an infrequent feature of the ductal adenocarcinoma, while it is common in neuroendocrin tumors. Confirmation of diagnosis is always achieved by FNA. A few selected patients are best treated by surgical resection. Best candidates for surgery are the patients with optimal performance status, who develop a single metastasis from renal cancers or sarcomas many years later the treatment of the primary tumor. Conclusion: The Pancreas may be the site for a late onset metastasis arising from some types of tumors. For some selected cases, the most reliable therapeutic option available is the surgical resection, as it provides longest survival and optimal palliation of clinical picture.

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Non Functioning Islet Cell Tumors of the Pancreas: Case Report, Our Experience and Management (Oral Presentation)

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Background: The aim of the study is to present a clinical case regarding Non functioning islet cell tumors of the pancreas (NIT), to emphasize the anomalous presentation and give our contribution to the management of the illness, according to the latest acquisition and our experience. Case report: An asympotmatic 67 year old female patient was admitted to our unit for an abnormal dilatation of the Wirsung duct , occasionally showed by abdominal US, due to a NIT of the head of the pancreas, as suspected by CT scan and Octreoscan study. Intraoperative US and histological specimen confirmed the diagnosis. The patient underwent a Pancreaticoduodenectomy according to Whipple procedure. In the thirteenth postoperative day, after uneventful course, she was discharged. Between 1998 and 2002 6 patients , 1 male and 4 females with a median age of 57 years (DS+-7), have been treated by surgical therapy for Islet Cell Tumors of the pancreas at the III Unit of the Dept. of Surgery in S. Spirito General Hospital in Pescara (Italy), 3 of these with NIT. Preoperative diagnosis was made in all cases by CT scan, EUS and Octreoscan. All patients were treated with aggressive surgery: Whipple resection(3), distal pancreasectomy(3), body-tail pancreatectomy with resection of liver metastasis (1). All patients are still alive. Discussion: Non Functioning Islet Tumors of the Pancreas represent 45-50% of the endo-

crine pancreatic neoplasms , and 7-8% of the pancreatic tumors overall. The largets extent of them usually have symptoms related to the pancreatic mass (pain, intestinal bleeding or obstruction , jaundice), but not to the excessve hormone secretion. Classification: neuroendocrine tumors well differentiated (A, benign B, an incert behavior), well differentiated endocrine carcinomas, poor differentiated endocrine carcinomas, and mixed esocrine-endocrine carcinomas. They have great dimension, 2-20 cm (average 7 cm), solitary, rarely small and multiple, and in this case the suspect of MEN 1 syndrome is mandatory. Women predilection , with range of age interested between 40-50 years. Slow growth of expansive type; 60-90% are of malignant nature. Recent imaging studies (CT scan, MRI, EUS, Octreoscan, PET), even in association with fine-needle aspiration, are able to visualize the primary lesion and liver metastasis too with accuracy of 90%. Surgery is therapy of choice. The high grade of resectability is a peculiarity of this neoplasms. Pancreatic resection is always compulsory even in presence of liver metastasis. Resection of liver metastasis can improve survival. Somatostatine analogues, alpha-IFN and chemiotherapy, singly used or in association, are effective in those patients with advanced metastatic disease.

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w-3 Fatty Acid Induction of In Vitro Pancreatic Cancer Cell Cycle Arrest

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Background: Omega-3 fatty acids(w-3 FA),have anti-inflammatory and anti-proliferative effects. Recently, a commercially produced and clinically used European lipid emulsion, (Omegaven) has become available. The anti-inflammatory activities of this w-3 FA emulsion have been demonstrated to occur via cytokine downregulation and inhibition of IkB phophorylation. The potential for this emulsion as an anti-proliferative agent has been described but not demonstrated. Hypothesis: Incubation of Mia-Paca pancreatic cancer cells with w-3 FA emulsion will result in cell cycle arrest. METHODS: Mia Paca cells were maintained in D-MEM at standard conditions. Cells were seeded in 6-well plates and treated with 10, 50, and 100 uM Lipovenos (w-6 lipid emulsion) and Omegaven for 24, 48, and 72 hours. FA emulsions were diluted in serum-supplemented media and administered in a continuous fashion. Cells were harvested, fixed in 70% ethanol and stained with propidium iodide. Cells were incubated in the dark at 37C for 30 minutes and analyzed by flow cytometry for FL-2 area, with a minimum of 104 events per sample. Results: At all timepoints (24, 48, 72 h) and all concentrations (10, 50, 100 uM) Lipovenos treated cells did not differ from the control (non-treated) cell cycle profile in terms of G1-, S-, and G2-content. At all timepoints, 10 uM Omegaven did not show difference compared to control cells and Lipovenos negative controls. At 24 hours, 50 uM Omegaven treated cells showed 12% reduction of cells in G1, no difference in S-phase content, and 13% increase of cells in G2, as compared to Lipovenos at the same concentration. At 24 hours, 100 uM Omegaven treated cells showed 15% reduction of cells in G1, 5% increase of cells in S-phase, and 10% increase of cells in G2. At 48 hours, 50 uM Omegaven treated cells showed 11% reduction of cells in G1, 5% increase of cells in S-phase, 7% increase of cells in G2, and a sub-G1 population. At 48 hours, 100 uM Omegaven treated cells showed 17% reduction of cells in G1, 6% increase of cells in S-phase, 12% increase of cells in G2, and a sub-G1 population. Conclusion: In this model, w-3 FA emulsion causes both dose-dependent and time-dependent cell cycle arrest. Arrested cells progress to cell death as evidenced by the sub-G1 population. These results have clinical implications since this w-3 FA emulsion has both anti-inflammatory and anti-proliferative effects which are treatment specific.

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Insertion of a Double-Lumen Gastrojejunostomy Tube Improves Outcomes After Resection of Periampullary Tumors

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Background. Delayed gastric emptying is a frequent postoperative complication after pancreaticoduodenectomy (PD) or total pancreatectomy (TP) for periampullary tumors. This often results in increased length of stay and a prolonged need for nutritional support. As many patients with periampullary tumors have little nutritional reserve because of malnourishment or cachexia, such an event may also inhibit full recovery from the procedure. Recently, double-lumen gastrojejunostomy tubes (GJT) have been developed, which enable simultaneous gastric decompression and enteral feeding. We postulate that insertion of GJTs during resection of periampullary tumors will decrease the average length of stay and reduce the need for total parenteral nutrition (TPN). Patients and Methods. At the University of Miami, thirty-six patients with periampullary tumors were randomized at the time of resection (35 PD, 1 TP) to insertion of a GJT (N= 20) or control (N=16). Patients in the GJT group received Sandosource Peptide (Novartis) at prescribed rates, beginning on the first or second postoperative day. Controls were managed according to the routines of the participating surgeon. Outcomes were followed prospectively.Results. Prior to surgery, there was no difference in the proportion of patients who suffered weight loss or jaundice. Insertion of a GJT was not associated with increased blood loss, although there was an increase in operative time (416 ± 128 min vs. 327 ± 55 min; P = 0.007). Complications associated with the GJT include hyperglycemia (N = 1) and bloating (N = 1) secondary to enteral feeding, and inadequate gastric decompression (N = 1). Prolonged gastroparesis (defined as an inability to tolerate oral feeds for longer than 14 days after surgery) occurred in 4 controls (25%) and in none of the patients who had a GJT. TPN was administered to 9 patients in the control group (56%), and 1 patient in the GJT group (5%). Mean length of stay after surgery was significantly longer in controls, compared to patients who had a GJT (15.8 ± 7.8 d vs, 11.5 ± 2.9 d, respectively; P = 0.01). Hospital charges were $80,896 ± 58,025 in the control group, and $52,589 ± 15,964 in the GJT group (P = 0.03). Conclusions. In patients undergoing resection of periampullary tumors, insertion of a GJT is safe. Moreover, insertion of a GJT improves length of stay and decreases the need for parenteral nutrition, making this maneuver cost-effective. At the time of resection of periampullary tumors, GJT insertion should be considered, especially given this is a patient population in which weight loss and cachexia are frequent.

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Cyclooxygenase-2 Inhibitors Suppress Growth Proliferation Primarily via Independent Pathways in Cyclooxygenase-Depleted Pancreatic Cancer Cells

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While increased expression of cyclooxygenase-2 (COX-2) occurs in pancreatic cancer, the antitumor efficacy of COX-2 inhibitors continues to be debated. Recent reports implicate both cyclooxygenase-2-dependent and COX-2-independent pathways in the antitumor activity of COX-2 inhibitors. To evaluate the relative contribution of these two pathways, we examined whether depletion of COX-2 expression prior to inhibitor exposure modulated its antitumor activity. Mehtods: Quiescent, BxPC-3 pancreatic cancer cells were transiently transfected with either COX-2 antisense oligonucleotide (AS ODN),

COX-2 sense oligonucleotide (S ODN), or no oligonucleotide (No ODN). Cells were then stimulated to proliferate in 10% FCS, a known inducer of COX-2 expression, with varying concentrations of NS-398, a COX-2 specific inhibitor (0 to 500uM). COX-2 expression was confirmed by Western Blot, and cell proliferation was determined by MTT assay. Proliferation indices were analyzed by ANOVA and Tukey's HSD where appropriate. Results: Antisense-mediated COX-2 depletion was observed as early as 24hrs after transient transfection. At 96hrs, no significant difference in growth inhibition was noted in either the antisense transfected cells or controls. Conclusions: In this cell proliferation model, the antiproliferative effect of cyclooxygenase-2 inhibitors is mediated primarily by mechanisms independent of COX-2 expression.

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Outpatient Mini-Laparoscopy for Staging of Periampullary Neoplasms

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An expeditious evaluation of a suspected periampullary carcinoma should include a cost effective determination of local resectability and metastatic spread. Most patients unfortunately present with inoperable disease, and early detection of metastatic spread would truncate the evaluation process, and eliminate the need to assess local unresectablity. The goal of the pilot protocol was to evaluate the feasibility of an outpatient mini-laparoscopy with monitored sedation. Patients with no metastatic disease by mini-laparoscopy and local anesthesia subsequently underwent standard laparoscopy with laparoscopic ultrasound under general surgery. Twelve patients have undergone mini-laparoscopy staging under the supervision of an anesthesiologist in an outpatient surgery center for this initial phase. There were nine men and three women, with a mean age of 65 (43-83) years. All patients had dedicated pancreatic spiral computed tomography which revealed a periampullary mass in all, but no peritoneal or liver metastases. These scans also demonstrated local unresectability in three. A 2mm forward viewing laparoscope was used, and peritoneal washing performed. Unsuspected liver metastatic disease was pathologically confirmed by forceps biopsy in four (30%): all four had liver metastases, two also had peritoneal metastases, and three also had positive peritoneal washings. The median operative time was 12 (7-40) minutes. Anesthesia/analgesia included: midazolam in all twelve patients (mean 2mg, range 2-6 mg), with meperidine in seven patients (mean 118 mg, range 75-150 mg) or fentanyl in four patients (mean 343 mcg, range 250-370mcg). A dose of 30 mg of ketorolac was given in seven patients to minimize post procedure discomfort. On a visual analogue scale of 0-10, preoperative pain scale ranged from 0-8 (mean 2) and postoperatively 0-9 (mean 4.5). There were no procedure-related complications. No metastatic disease was found at standard laparoscopy. A Whipple procedure was ultimately performed in seven (58%). Outpatient mini-laparoscopy is accurate for staging of metastatic disease and can be performed with minimal sedation. Its use can potentially be expanded to an office based procedure with nurse-monitored anesthesia.

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Deletional Inactivation of the p16/MTS1 Tumor Suppressor Gene in Hamster Pancreatic Tumors: Analysis by Multiplex Real-Time PCR

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Pancreatic cancer is this fifth most common cancer causing death in the United States and current treatment modalities are inadequate. The p16/MTS1 tumor suppressor gene is known to be inactivated in up to 98% of human pancreatic cancer specimens and represents a potential target for novel therapeutic intervention. Chemically induced pancreatic tumors in Syrian golden hamsters have been demonstrated to share many biological similarities with human pancreatic tumors and represent a potentially suitable model for the evaluation of therapies targeting p16/MTS1. The purpose of this study was to determine the rate of p16/MTS1 inactivation, by homozygous deletion, in a sample of Syrian golden hamster pancreatic tumor specimens. Pancreatic ductal adenocarcinomas were chemically induced in Syrian golden hamsters by the weekly subcutaneous administration of N-Nitrosobis 2-oxopropyl) amine (BOP). Genomic DNA was extracted from parafin-embedded tissues after micro-dissection. A total of 30 tumors were analyzed for p16/MTS1 homozygous deletions using a real-time based deletion assay. Multiplexing with the control gene, a-cardiac myosin heavy chain, was used to control for potential contamination with non-tumorigenic DNA. Specimens noted to have $\Delta\Delta$Ct values less than 35% of known wild-type controls were considered to harbor homozygous deletions. Homozygous deletion of p16/MTS1 was identified in 11 of 30 (36.7%) specimens analyzed. Analysis of DNA extracted from normal hamster pancreas was unremarkable. These results indicate a similar rate of p16/MTS1 homozygous deletions in human tumors and BOP-induced hamster tumors. This would suggest that the model is potentially suitable for comparative studies evaluating therapeutic interventions targeting p16/MTS1. Analysis for p16/MTS1 mutation and/or promoter region hypermethylation may demonstrate an increasing association with known inactivating events of p16/MTS1 in this experimental model.

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Clonal Progression in the Carcinogenesis of Intraductal Papillary-Mucinous Tumor of the Pancreas

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Purpose: Intraductal papillary-mucinous tumor of the pancreas (IPMT) is a relatively new entity in pancreatic disease that has unique features including various grade of histological abnormality within a tumor. The histological variety within a same tumor raised the question whether IPMTs may originate from their benign conditions (adenoma and/or hyperplasia) subsequent progress to the malignant conditions (carcinoma). The aim of this study was to clarify the relationship between histological abnormalities and genetic background to know the mechanism of the carcinogenesis in IPMT. Materials and Methods:Twenty-three cases of IPMT including 6 invasive carcinomas, 3 non-invasive carcinomas, 5 border line tumors, and 9 adenomas were studied. Firstly, the each epithelial lesion within a tumor was classified into four grade which includes benign (adenoma/hyperplasia), borderline (dysplasia), carcinoma (non-invasive), and carcinoma (invasive). Secondly, genomic DNA was extracted from the each microdissected specimen, and then the K-ras mutation and LOH at 9p21 (p16/INK4a gene locus), and LOH at 17p13 (p53 gene locus) were investigated. Results: The K-ras mutations were frequently seen without relationship to histological grade (4/6 invasive carcinomas, 3/3 non-invasive carcinomas, 4/5 border line tumors, and 4/9 adenomas). The LOH at 9p21 was observed increasingly with the degree of histological abnormality (5/5 invasive carcinomas, 1/3 non-invasive carcinomas, 1/5 border line tumors, and 1/9 adenomas). The LOH at 17p13 was seen only in cases of invasive carcinomas (6/6 invasive carcinomas). The K-ras mutation and the LOH at 9p21 was observed from benign (adenoma/hyperplasia) lesions and mostly conserved during the progression to the malignant condition. The LOH at 17p13 was observed from borderline (dysplasia) lesions, and also conserved during the evolution from benign lesion to carcinoma. Conclusion: The K-ras mutation, LOH of p16/INK4a and

p53 gene locus, and their clonality observed during the progression would be important events in the carcinogenesis of IPMT.

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Thymidylate Synthase (TS) Expression Predicts the Response to 5-FU Based Adjuvant Therapy in Pancreatic Cancer

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Thymidylate synthase (TS) is the target enzyme for 5-fluorouracil (5-FU), and TS expression may determine clinical response and survival following therapy with 5-FU in colorectal cancer. 5-FU is also widely used in the adjuvant therapy of pancreatic cancer. Therefore, we examined the role of TS expression in determining prognosis and predicting the response to adjuvant therapy in pancreatic cancer. Paraffin sections from 132 resected patients were used to build a pancreatic cancer tissue microarray. TS expression was determined using immunohistochemistry. High and low intratumoral TS expression were present in 72 of 132 (55%) and 60 of 132 (45%) tumors, respectively. The presence of nodal metastases ($p=0.01$), poorly differentiated histology ($p=0.02$), management with adjuvant therapy ($p=0.05$), and high TS expression ($p=0.03$) were each predictors of decreased overall survival by univariate Cox analysis. Median survival among patients with low intratumoral TS expression (17 months) was longer than among patients with high TS expression (12 months). TS expression predicted survival independent of pathological stage. TS expression did not predict overall survival among all patients receiving adjuvant therapy (n=94) or among patients receiving 5-FU based adjuvant therapy (n=71). However, the risk of death was significantly reduced by adjuvant therapy (risk ratio=0.30; 95% CI=0.14-0.65; $p=0.002$) among patients with high intratumoral TS expression. Furthermore, the beneficial effect of adjuvant therapy was greatest among patients with high TS expression receiving 5-FU (risk ratio=0.23; 95% CI=0.10-0.54; $p=0.0007$). In contrast, overall survival was similar in patients with low intratumoral TS expression managed with either resection or resection plus adjuvant therapy. In conclusion, high TS expression is a negative prognostic marker in resected pancreatic cancer. Patients with high intratumoral TS expression benefit from 5-FU based adjuvant therapy.

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Trastuzumab (Herceptin) and Gemcitabine (Gemzar) for Metastatic Pancreas Cancers that Overexpress HER2/Neu

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In a previously reported retrospective Brown University study of 154 pancreatic cancer patients, 28% of 75 patients with metastatic pancreatic cancer were found to over-express HER2. The patients with metastatic pancreatic cancer and HER2 overexpression, retrospectively, were found to have worse survival outcomes than those who did not overexpress HER2. When used in combination with systemic chemotherapy, trastuzumab (Herceptin) has been used to improve survival in breast cancer patients with HER2 overexpression. Therefore, we initiated a Phase II study of trastuzumab (Herceptin) and gemcitabine (Gemzar) for patients with metastatic pancreatic cancer. In this prospective study, 34 patients with metastatic pancreatic cancer were found to have 2+ to 3+ HER2 overexpression. These patients were treated with gemcitabine 1 g/m2/week for 7 of 8 weeks, and a loading dose of trasuzumab 4 mg/kg, and then trastuzumab 2 mg/kg/weekly. Thirty two patients were available for evaluation of toxicities and complete follow-up. Grade 3 /4 toxicities included neutropenia (n=10), thrombocytopenia (n=5), and decline in LVEF (n=1). Three of 32 patients (9%) had a confirmed partial response. Thirteen of 38 had either a radiographic response, or >50% reduction of CA 19-9. The median survival was 7 months. The one-year survival was 19%. From this institution, historical controls with metastatic pancreas cancer had a survival of 5-6 months when treated with gemcitabine alone. Trastuzumab (Herceptin) and Gemcitabine (Gemzar) combination is well tolerated, and offers a modest survival benefit in patients with metastatic pancreas cancers that overexpress HER2.

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Plasma S-Adenosylmethionine Levels: A Methyl Donor as a Potential Aid in the Diagnosis of Pancreatic Cancer

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Background: S-adenosylmethionine (AdoMet) is a critical biochemical intermediate, a methyl donor in myriad reactions and a precursor in polyamine synthesis. Prior work showed that plasma AdoMet levels were elevated in transgenic mice with pancreatic tumors. We hypothesize that Adomet levels are also elevated in human gastrointestinal (GI) malignancies, including pancreatic cancer. We suggest that determination of plasma Adomet levels may aid in the diagnosis of pancreatic cancer. Methods: Plasma AdoMet levels were measured in triplicate by high pressure liquid chromatography in 29 patients with malignant (n=17, including 6 with pancreatic tumors), benign neoplastic (n = 2) or benign inflammatory (n = 10) processes of the GI tract. In preliminary work, the mean level of AdoMet in 12 healthy persons was 106 nM ± 13. Wilcoxon test was used for stastistical analysis. Results: There was no overlap in AdoMet levels between patients with malignant disease and those with benign inflammatory processes. AdoMet levels were significantly elevated in 17 patients with GI malignancies (median 785 nM, range 350-3456) as compared to 10 patients with benign inflammatory GI conditions (median 160 nM, range 132-252, $p < 0.001$). AdoMet levels were elevated in six patients with pancreatic tumors (median 665 nM, range 350-956) as compared to five patients with benign pancreatico-biliary inflammatory conditions (median 139 nM, range 132-176, $p < 0.001$). Two patients with benign neoplastic lesions had elevated levels of 1590 and 1140 nM, respectively. Conclusion: Our data demonstrate that plasma levels of Adomet are significantly elevated in patients harboring malignant tumors of the GI tract, including tumors of the pancreas, as compared to those with benign inflammatory conditions of the GI tract. AdoMet plasma concentrations may prove to be a useful tool for the diagnosis of pancreatic cancer. In addition, the role of AdoMet in abnormal methylation of tumor cells merits further investigation.

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Does Venous Resection During Whipple Procedure Safely Increase Resectability Rates and Improve Outcomes?

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Background: Surgical resection is both a highly effective palliative therapy and provides the only chance of cure for periampullary malignancies. Unfortunately, many patients are deemed not candidates for resection because of locally advanced disease. In order to improve resectability rates for such patients we, as others, have adopted an aggressive surgical approach to the treatment of periampullary malignancies including resection of involved portal (PV)or superior mesenteric vein (SMV). Design: A retrospective review of 28 consecutive Whipple procedures and a review of the published literature regarding PV/SMV resection for periampullary malignancies. Results: Overall there were no significant intraoperative complica-

tions or peri-operative mortalities. Twelve patients (43%) underwent a Whipple procedure and required a concomitant portal or SMV resection due to venous involvement. Operative time and blood loss was increased in the venous resection group. Peri-operative complications were observed in three of 12 patients who also underwent portal or SMV resection and 5 of 16 patients who underwent Whipple alone. Four of the 12 patients in the venous resection group and 3 of 16 in the Whipple alone group succumbed to recurrent disease at one-year follow-up. Four previously published series also support similar survival rates for patients undergoing Whipple procedure alone or concommitant venous resection. Conclusion: Adding portal or SMV resection to a Whipple procedure may be performed safely and allows a significantly greater number of patients with periampullary malignancies to benefit from surgical resection.

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Late Intra-Abdominal Surgical Complications after Pancreas Transplantation With Roux-en-Y Enteric Drainage

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Background: Pancreatic transplantation (PTx) is associated with considerably high incidence of surgical complications. The incidence and characteristics of early post transplantation intra-abdominal infectious complications is well discussed in the surgical literature. However, delayed presentation of peri-pancreatic abscesses with or without graft duodenal leak is less documented and understood. Patients and methods: The records of all PTx recipients, who were hospitalized for late (> 3 months post transplantation) intra-abdominal complications, were reviewed systematically. Donor, recipient, operative and post-operative clinical, imaging and laboratory data were analyzed. All cases were of simultaneous kidney—pancreas cadaveric transplantation and in all, pancreatic exocrine drainage was enteric with doudeno-enterostomty to a Roux-en Y limb, together with systemic venous drainage. Results: Eight out 44 K-PTx recipients, who had an initial un-eventful surgical course, were re-hospitalized due to delayed intra-abdominal surgical complications. Two were explored for mechanical small bowel obstruction and are excluded from this report. The other six (4 females, 2 males, mean age, 44) were treated for peri-graft abscesses (n=5) or delayed anastomotic leaks (n=1). The mean interim from the PTx was 16 months (range, 4-43). There was no association with rejection episodes, graft pancreatitis or other systemic infection or CMV disease. Management included surgical drainage (n=5) together with graft tube doudenostomy (n=2) or percutaneous drainage alone (n=1). One patient died of sepsis. Three developed pancreatic-cutaneous fistulae, which were resolved within few weeks to months. Conclusions: PTx with enteric drainage is associated with late peri-(graft)-pancreatic surgical complications. The mechanism, etiology and risk factors are not clear, but seem to be different than those of the early post-operative period.

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Obesity is Associated with a Higher Rate of Anastomotic Leak in Enteric-Drained Simultaneous Pancreas and Kidney Transplantation

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Aim: The influence of body mass index (BMI) on outcome of simultaneous pancreas and kidney transplantation has not been reported. Methods: A retrospective review of 88 consecutive primary SPK at our institution from 3/15/95 - 8/28/01 was performed. All patients received antibody induction and maintenance immunosuppression with tacrolimus, mycophenolate mofetil, and steroids. Systemic - enteric pancreas implantation was performed in all cases. Primary endpoints were patient, pancreas, and kidney survival. Secondary endpoints were rates of anastomotic leak, pancreas thrombosis, major infection, rejection, relaparotomy, and length of stay. Results: Fifty two patients (59.1%) were non-obese (NO) with BMI $\leq$ 24.9. Thirty six patients were obese (OB) with BMI > 25. Distribution of recipient age, gender, and ethnicity was similar between groups. There was a trend toward longer pancreas anastomotic time in OB(OB: 31.8 $\pm$ 8.4 min, NO: 28.1 $\pm$ 9.9 min, p = 0.07). Kidney and pancreas anastomotic times and preservation times were not different between NO and OB. Actuarial patient and graft survival were similar between NO and OB and are depicted below. The mean rates of pancreas thrombosis, major infection, pancreas rejection, kidney rejection, relaparotomy, and length of stay were similar between groups. The mean number of anastomotic leaks in OB (0.17 $\pm$ 0.38) was higher than in NO (0.02 $\pm$ 0.14), p = 0.014. Eight six percent of all leaks (6/7) occurred in OB patients. Mean BMI in the 7 patients with leak (26.8 $\pm$ 1.9, range 23 - 27.6) was significantly higher than in patients that did not develop a leak (23.9 $\pm$ 3.7), p = 0.05. Conclusions: Although the overall anastomotic leak rate in enterically-drained SPK is low, the presence of obesity is associated with a significantly higher rate of leak. All but 1 leak in this series (86%) occurred in OB patients. Suspicion for the presence of enteric anastomotic leak should therefore be especially high in OB SPK recipients.

Kaplan Meier Patient and Graft Survival

	Patient		Pancreas		Kidney	
	1 Year	5 Year	1 Year	5 Year	1 Year	5 Year
NO	94%	94%	78%	70%	90%	90%
OB	94%	87%	77%	62%	97%	89%
P value	0.73		0.58		0.75	

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Investigation of Non-Heart Beating Donors as a Potential Source of Pancreatic Islets

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Background: A recent report from Edmonton indicates that type-1 diabetes can consistently be reversed by isolated islet transplantation. However in this report of 7 patients, insulin independence was achieved only after islet transplanted from 2 or 3 donors. In view of the donor shortage, isolated islet transplantation is unlikely to become the treatment of choice for diabetes if each recipient requires islets from several donors, since whole pancreas transplantation requires only one donor. A source of donor pancreata currently considered unsuitable for whole organ transplantation are those from non-heart beating donors (NHBDs), in which unlike traditional brain dead donors (BDDs), withdrawal of support and cardiac arrest occur prior to organ recovery. Whether NHBDs can provide an adequate number of functional islet equivalents (Ieq) for successful transplantation is unknown. Methods: From 2/00 to 11/01 we isolated islets from 107 human pancreata, including 10 NHBDs. Islets from NHBDs were compared with those from heart beating BDD by in vitro assays (insulin release (IR) and perifusion) and by their ability to reverse diabetes when transplanted to nude mice. Results: The results of the 10 NHBD isolations are compared to a comparable group of BDD isolations in the table below. These results encouraged us to

utilize islets from a NHBD for transplantation to a diabetic patient (C-peptide < 0.5). 446,320 islet equivalents (8,500 IEq/kg recipient BW, 50% purity) were transplanted by percutaneous portal vein infusion. The patient rapidly developed islet function (C-peptide 0.7-2.2) and remains insulin independent 10 months later. NHBD islet function was similar to that observed in three other diabetic patients treated successfully with islets from BDDs. Conclusions: We determined in experimental assays that NHBDs provide an excellent source of large numbers of normally functioning islets and report the first successful human islet transplant using a NHBD.

Donor type	Donor age (years)	Cold ischemia (min)	Warm ischemia (min)	Islet yield (Ieq)	IEq's/ gm panc	Purity (%)	Mice cured
NHBD	42.9	350	N/A	385,085	4412	52.0	12/14
BDD	43.5	409	22.9	492,080	6052	51.7	16/16

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Predicting Resectability of Periampullary Tumors With 3-Dimensional Computed Tomography

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Purpose: While helical CT carries an overall 70-80% accuracy for determining periampullary tumor resectability, dynamic dual phase contrast-enhanced 3-dimensional volume-rendered CT (3D-CT) improves the radiographic assessment of local vascular invasion and extent of tumor burden. To determine the reliability of preoperative 3D-CT in predicting a margin-negative resection for periampullary cancer, a prospective study was carried out. Methods: Intraoperative findings from exploratory laparotomy, performed for potential pancreaticoduodenal resection, were gathered prospectively on 120 patients who were deemed resectable after undergoing a preoperative 3D-CT. Comparisons were made between 3D-CT imaging and intraoperative findings, specifically for the extent of tumor burden, presence of local or distant tumor spread, and tumor invasion of the local venous or arterial systems. Statistical analyses were performed to determine the sensitivity, specificity, predictive values, and overall accuracy of 3D-CT in predicting operative findings. Results: Of the 120 patients who were thought to be resectable after 3D-CT and were explored, 105 patients (87.5%) underwent pancreaticoduodenectomy. Eighty percent of the pancreatic resections were performed for suspected periampullary masses, and pancreatic adenocarcinoma was present in 53/105 (51%), followed by pancreatic neuroendocrine tumors (11%) and ampullary cancer (9%). The average size of the resected tumors was 3.4 cm (range, 0.5-12cm). The extent of local tumor burden involving the pancreas and peripancreatic tissues was accurately predicted by 3D-CT in 72% of all patients. Among the resected pancreatic specimens, margins were found free of disease in 84% of cases. The statistical accuracy of 3D-CT in predicting intraoperative findings is shown in the table. Fifteen of the 120 patients (12.5%), who were thought to be resectable on preoperative 3D-CT, did not undergo a pancreatic resection due to the intraoperative discovery of local vascular invasion (n=9) or unsuspected hepatic metastasis < 1 cm (n=6). Conclusions: Compared to helical CT, 3D-CT significantly improves the prediction of a margin-negative resection (accuracy = 85%) in patients with a presumed periampullary cancer. The accuracy of 3D-CT is a result of its enhanced assessment of the extent of local tumor burden and involvement of the adjacent portal-mesenteric vascular anatomy.

Accuracy of 3D-CT in predicting intraoperative findings

Anatomic findings	Pos pred value	Neg pred value	Sensitivity	Specificity	Overall accuracy
Invasion of SMV or PV	84.2%	87.3%	85.7%	85.9%	85.8%
Invasion of SMA	78%	91%	41.2%	98.1%	90%
Vascular anomlies of HA	100%	93.6%	58.8%	100%	94.2%
Tumor respect-ability	94.2%	47%	92.4%	40%	85.8%
Margin-negative resection	93.9%	52.2%	87.5%	70.6%	84.8%

SMV, superior mesenteric vein; PV, portal vein; SMA, superior mesenteric artery; HA, hepatic artery

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Radical Antegrade Modular Pancreato-Splenectomy: A New Approach to Resection of Adenocarcinomas of the Body and Tail of Pancreas

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Introduction: Retrograde distal pancreatectomy with splenectomy is the standard procedure for cancers of the body and tail of the pancreas. However, this procedure has limitations in terms of visibility during dissection, the posterior extent of resection, and the ability to achieve a complete N1 node resection. Low node counts and positive margins in resected specimens are common. Methods: A new procedure was developed that provides improved visibility by performing the dissection in antegrade manner. This entails early division of the neck of the pancreas and dissection from right to left. This step also permits early control of the vasculature and adjustment of the depth of the posterior extent of resection based on the CT scan and intraoperative findings. The posterior extent may either be ante or retro-adrenal. The lymph node dissection is based on N1 lymph node drainage of the body and tail of the pancreas as described by O'Morchoe (Lymphatic system of the pancreas. Microscopy Research & Technique 1997;37:456-77). It entails removal of the "ring" of nodes around the body and tail and the vertical chain of nodes along the left side of the celiac and superior mesenteric arteries. RESULTS Ten patients, six with adenocarcinomas of the body of the pancreas, have undergone the procedure since 1999. Two were asymptomatic on discovery. Median tumor size was 4cm (2-15cm). In four the retro-adrenal dissection was done. Nine of ten patients had negative resection margins and the median node count in patients who did not receive neoadjuvant radiation was nine nodes. Operative times (median 5.2 hr) and blood loss (median 400m) were similar to large series of standard distal pancreatectomies. Three patients developed complications; there were no postoperative deaths. Four of six patients with adenocarcinoma are alive with no evidence of disease at 3mo, 6mo, 36mo and 36mo after surgery. Conclusions: The procedure is a logical development that parallels changes in pancreaticoduodenectomy that have occurred in the past decade. Early results with the operation are encouraging.

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Binding Pancreaticojejunostomy: 150 Consecutive Cases Without Leakage

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Background: Pancreatojejunal anastomostic leakage is a major cause of morbidity and mortality after pancreaticoduodenectomy. To prevent the development of pancreatic fistulae, we have designed a special technique named Binding pancreaticojejunostomy. Objective: To verify the safety of a (the) new technique, binding pancreaticojejunostomy, in a prospective cohort study. Methods: Binding pancreaticojejunostomy means binding 3 cm of the serosa-muscular sheath of the jejunum to the intussuscepted pancreatic stump. In brief, this procedure is performed as follows: First, the cut end of the pancreatic remnant is isolated at a distance of 3cm. Three cm of the distal cut end of the jejunum is then everted, and the exposed jejunal mucosa is destroyed by electric coagulation. Next,the pancreatic stump and the everted jejunum are brought together and sutured with silk, intermittently or continuously. The mucosa is only sutured, and care must be taken to avoid penetration of the serosa and muscular layers.The everted jejunum is then returned to it's normal position to wrap over the pancreatic stump , and sutured to the pancreas with few stitches for fixation. Lastly, 1cm from the cut end of the jejunum, a catgut tie is looped around the entire circumference of the anastomosis. A bundle of vessels is spared for maintaining blood supply to the distal end of the binding ligature. Pancreatic leaks were defined as a significant increase in the volume or change in the character of the effluent from the surgical drains, or the persistence of amylase-rich drain output in the excess of 50 ml per day and amylase more than 1000IU/L. Results: From Jan 1996 to May 2001, 150 consecutive patients received this type of pancreaticojejunostomy. (In this study,)There were 107 male and 43 female, with age ranging from 36 to 78 years, with a mean age of 62 years. None of the cases developed pancreatic fistula. Conclusions: Binding pancreaticojejunostomy is a safe, simple and effective technique. The success of this technique is resulted from three safety measures. First, the serosa-muscular sheath of the jejunum was bound to the pancreatic remnant. Second, the anastomotic suture needle penetrated only the inner mucosa layer, keeping the muscular and serosa layer intact. Third, the jejunal mucosa covering the pancreas is (was) destroyed to avoid secretions and promote healing.

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Cystic Pancreatic Neoplasms: Enucleate or Resect?

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Background: Cystic pancreatic neoplasms are being detected with increasing frequency as incidental findings on newer sophisticated imaging studies. The senior author has previously reported that enucleation of mucinous cystic neoplasms can be performed safely without recurrence (Ann. Surg. 1998). When compared to resection, however, enucleation was associated with a higher incidence of pancreatic fistula (50%) and a longer length of stay (19.5 days). Over the past five years, the indications for enucleation have expanded, and the procedure has been modified to include intra-operative ultrasound and, when indicated, closure of the pancreatic defect. This retrospective analysis was performed to determine whether these modifications have improved operative outcome. Methods:Twenty-six patients with pancreatic cystic tumors were included in this study, 14 with mucinous cystic neoplasms (MCN), 8 serous cystadenomas (SCA), and 4 cystic islet cell tumors (ICT). Enucleation was performed in ten patients (7 MCN, 1 SCA, 2 ICT) whereas 16 patients had their tumor resected (8 pancreatoduodenectomy, 8 distal pancreatectomy). These two groups of patients did not differ with respect to mean age (56 years), gender (69% female), presentation (58% incidental), or site (50% head, neck or uncinate). However, patients undergoing enucleation did have smaller tumors (1.9 cm $\pm$ 0.3 vs 5.0 cm $\pm$ 0.7, $p<0.01$) on preoperative CT scan. Results: Data are presented as mean $\pm$ SEM and are compared by Student's t test or the Mann-Whitney rank sum test as appropriate. Operative and postoperative outcomes are presented in the following table. Conclusions: This analysis suggests that enucleation of benign cystic pancreatic neoplasms reduces operative time and blood loss without increasing postoperative complications or length of stay. We conclude that enucleation should be the standard operation for small cystic pancreatic neoplasms.

Operative and Post-Operative Outcomes

	Operative time (min)	Blood loss (ml)	Pancreatic fistula	Length of stay (days)
Enucleation	195±17*	110±15*	20%	13.2±3.0
Resection	301±28	423±13	25%	15.9±3.1

*$p<0.01$ vs resection.

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The Value of Prophylactic Intraperitoneal Drainage With Pancreaticoduodenectomy for Pancreatic Head and Periampullary Neoplasms

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Background: Although the use of intraperitoneal closed-suction drains with pancreaticoduodenectomy (PD) is usually routine, such usage has recently been challenged. Our aim was to determine the value of intraperitoneal drains with PD for neoplasia. Methods: Medical records of all patients from 1995-1998 undergoing PD for pancreatic head or periampullary neoplasms at our institution were reviewed. Data were collected to ascertain the presence, management and outcome of anastomotic complications. Follow-up was complete in 97%. All data were collected regarding hospital course, evaluation and management of anastomotic complications. Results: A total of 267 consecutive patients were identified. At the time of PD, all patients had drains placed near the pancreatic and biliary anastomoses. Anastomotic complications included fistula in 41 (15%), perianastomostic abscess in 4 (1.5%) and anastomotic bleeding in 11 patients (4%). Anastomotic fistula occurred at the pancreaticojejunostomy (n= 39), choledochojejunostomy (n=1), or both (n=1), and were diagnosed at a mean of 8.4 days (range: 4-19) postoperatively. Anastomotic fistula or abscess presented with a combination of fever, leukocytosis, or abdominal pain/tenderness in 43 patients (96%), however drain fluid amylase levels were abnormal ($>3 \times$ serum amylase) in only 28 patients (62%). Operatively placed drains were sufficient to manage the anastomotic fistula or perianastomotic abscess in only 15 patients (33%). The majority of these complications required either percutaneous drainage (52%) or reoperation (15%). Conclusions: Prophylactic intraperitoneal perianastomotic drains with PD for pancreatic head or periampullary neoplasms are of limited value. They contribute positively to the management of anastomotic complications in only one third of patients, representing only 6% of all patients undergoing PD. Whether these drains prevented other complications or prolonged otherwise insignificant anastomotic leaks cannot be determined by this study.

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Major Hepatectomy With Simultaneous Pancreatectomy for Advanced Hepatobiliary Cancer

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Introduction: Major hepatectomy with pancreatectomy (MHP) is a unique surgical technique in the treatment of hepatobiliary cancer. Few reports have discussed the usefulness of this procedure and its indications. There are a small number of patients diagnosed with hepatobiliary malignancy in which a very aggressive surgical approach is indicated. The two major risk factors for patients undergoing MHP are hepatic failure and pancreatic leak. The aim of this study was to review our experience with MHP and attempt to identify which patients benefit from an MHP for hepatobiliary malignancy. Methods: A review of the prospective Hepatobiliary database from 1/1994-7/2000 identified 17 patients who had undergone an MHP. Pre-operative radiographic, laboratory, intraoperative findings, hospital outcome and long term follow up was obtained. Results: Over 4000 patients with hepatobiliary malignancies were evaluated at our institution, with 1823 patients undergoing resection and 17 patients undergoing a combined MHP. Eleven women and 6 men were identified with a median age of 58 (range 24-76) years. Histology was as follows, 8 patients with neuroendocrine, 3 sarcomas, 2 cholangiocarcinoma, 1 ampullary carcinoma, 1 gallbladder, 1 gastric recurrence, and 1 benign fibrosis. All 17 patients underwent resection of two or more Couinard segments with 9 patients undergoing distal pancreatectomy and 8 undergoing pancreaticoduodenectomy. Median operative time was 6.5 hours (range 4 to 8 hours), with median blood loss being 1050cc (range 150 to 2500). Postoperative complications occurred in eight patients, and accounted for three deaths. Eight patients are free of disease with a median follow up of 54 months, 6 patients have recurred with 2 dead of disease with a median disease free interval of 8 months. Conclusion: Combined MHP is an appropriate procedure in a select population of patients with a variety of hepatobiliary malignancies. Long term survival can be achieved in the select patient population regardless of histology if a complete resection can be achieved.

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Do Preoperative Pancreatic Stents Increase Complications After Lateral Pancreaticojejunostomy?

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Introduction: Recent studies suggest that preoperative placement of endoscopic biliary stents increases postoperative morbidity after pancreatic surgery. In order to evaluate the influence of endoscopically placed pancreatic stents on surgical outcome after lateral pancreaticojejunostomy (LPJ) in patients with chronic pancreatitis, we compared the operative results in patients who underwent preoperative pancreatic stenting with those who received ERCP only. Methods: The records of 129 consecutive patients who underwent LPJ for chronic pain associated with chronic pancreatitis from 1995 through 2001 were retrospectively reviewed and analyzed. Results: There were 68 patients (37 men, 31 women, mean age 47 years, range 14 to 72 years) who had preoperative pancreatic stenting. The remaining 61 patients (32 men, 29 women, mean age 48 years, range 4 to 72 years) had ERCP preoperatively without placement of a stent. The two groups had a similar stage of disease progress measured by incidence of pancreatic duct strictures, pancreatic duct stones, pseudocysts, terminal biliary stenosis, need for prescription narcotics, insulin dependency, need for pancreatic enzymes, and symptom duration. There was no difference in operative time (201 $\pm$ 64 minutes vs. 215 $\pm$ 67 minutes) or intraoperative blood loss (250 vs. 300 ml) between the two groups. The overall postoperative morbidity was higher in the stented group (30.9% vs. 11.5%, $p<0.05$). Intraabdominal complications occurred more frequently in the stented group (19.1% vs. 4.9%, $p<0.05$), while there was no difference in extraabdominal side effects in the two groups (11.8% vs. 6.6%, NS). Mortality (0% vs. 2.2%) was not significantly different. The mean length of follow-up was 24 months. Postoperative hospital length of stay was similar in both groups (8.0 vs. 7.0 days). In the pancreatic stent group 8.8% required reoperation for failure of LPJ to improve chronic pain. In the other group 9.8% underwent reoperation with pancreatic resection for LPJ failure. Conclusion: Comparison of patients who undergo pancreatic duct stenting prior to LPJ with those who undergo only ERCP preoperatively showed significant difference in postoperative intraabdominal complications (e.g. abdominal abscesses, anastomotic leaks). Extraabdominal complications (e.g. wound infection, pneumonia) and mortality were not different. Failure rates as measured by the need for reoperation were similar in both groups. An increase in intraabdominal complications after LPJ in patients with pancreatic stents may be related to factors associated with stent occlusion and pancreatic ductal bacterial colonization.

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Efficacy of Venous Reconstruction in Patients with Adenocarcinoma of the Pancreatic Head

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Pancreaticoduodenectomy (PD) is often avoided when there is tumor involvement of the portal/superior mesenteric vein (PV/SMV) confluence due to the perception that venous resection is complex, morbid, and has poor long-term survival. The aim of this study was to compare patients who required venous reconstruction as a result of tumor involvement to patients who required no venous reconstruction during a PD for adenocarcinoma of the pancreatic head. Methods: Retrospective analysis was completed on fifty-four consecutive patients who underwent a PD for adenocarcinoma of the pancreatic head from 1993 - 2002. From this total, 14 patients (26%) required PV/SMV resection while 40 patients (74%) had a standard PD without venous reconstruction. Duration of surgery, intraoperative blood loss, margin status, ICU days, hospital length of stay (LOS), morbidity, mortality, Kaplan-Meier life table analysis of survival were assessed for all patients. Data is expressed as mean $\pm$ standard deviation. Two-tailed t-test and Fishers-exact test were used for analysis. Results: See table below Conclusions: Results from this study suggest that PV/SMV resection in select patients during PD increases the length of ICU stay, but does not significantly add to the complexity of the operative procedure or the incidence of positive histologic margins. Kaplan-Meier life table analysis shows similar survival curves to patients who do not undergo venous reconstruction.

	PV/SMV resection (n=14)	No venous resection (n=40)	p value
Surgery duration (min)	405 $\pm$ 103	380 $\pm$ 113	0.46
Intraop blood loss (cc)	1520 $\pm$ 1000	1210 $\pm$ 1200	0.39
Transfusions (Units)	2.7 $\pm$ 3.1	2.0 $\pm$ 2.9	0.46
ICU stay (days)	0.7 $\pm$ 0.5	0.3 $\pm$ 0.5	0.01*
Hospital LOS (days)	14 $\pm$7	12 $\pm$ 7	0.23
Mortality rate	7%	2.5%	0.32
Morbidity rate	22%	27.5%	0.42
Histologic margin +	30%	16%	0.42
Median survival (mos)	10	13	

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Cystic Neoplasms (CN) of the Pancreas and Their Surigcal Management

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Introduction: Because of increased awareness and the widespread use of abdominal ultrasound and computerized tomography (CT), an increasing number of CN of the pancreas are being identified. CN of the pancreas are divided into four main groups: 1. Serous cystic neoplasms (SCN). 2. Mucinous cystic neoplasms (MCN). 3. Solid papillary cystic neoplasms (SPN). 4. Intraductal pancreatic mucinous neoplasms (IPMN). Methods: We have retrospectively reviewed our experience with CN at our institution between the years 1992-2002. Charts were reviewed for age, gender, clinical signs and symptoms, diagnosis, surgical treatment, morbidity, mortality and histology. Results: Between 1992 and 2002, 69 patients underwent surgical resection of CN. There were 48 females (F) and 21 males (M) ranging in age from 20-85 years (mean 56 years). The most common presenting symptoms were abdominal pain, abdominal mass, nausea, vomiting, weight loss, jaundice, and recurrent pancreatitis. CT was the most useful diagnostic test. Seventeen (25%)of the patients were diagnosed between the years of 1992 and 1995 and 52 (75%) of the patients were diagnosed between the years of 1996 and 2002. The table below summarizes our surgical data. PD-Pancreaticoduodenectomy DP-Distal pancreatectomy TP-Total pancreatectomy CP-Central pancreatectomy AD-Adenoma CA-Carcinoma. Mortality was 4.3% (3/69). Two of three deaths were in patients who had TP for IPMN. Pancreatic fistula occurred in 10.1% (7/69) and intraabdominal abscess 2.8% (2/69). Conclusions: 1. CN of the pancreas are being recognized more frequently. 2. CN are best treated with surgical resection. 3. Mortality in this series was 4.3% (3/69). 4. Two of three patients who died had TP for IPMN. 5. Central pancreatectomy appears appropriate for small benign cystic lesions of the neck of the pancreas.

ENTITY	NO	M/F	PD	DP	TP	CP
SCN	24	7/17	6	16	0	2
MCN-AD	18	3/15	2	15	0	1
MCN-CA	5	1/4	1	4	0	0
SPN	5	0/5	1	3	0	1
IPMN	17	10/7	9	6	2	0

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Influence of Preoperative Endoscopic Biliary Drainage on Postoperative Infectious Morbidity of Duodenopancreatectomy for Cancer

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The utility of preoperative biliary drainage (PBD) before duodenopancreatectomy (DP) in jaundiced patients is debated especially because of the heterogeneity of studied patients and techniques for drainage. The aim of this study is to report the influence of endoscopic PBD on postoperative morbidity (especially infectious) in jaundiced patients with resectable pancreatic head cancers treated with DP. Between September 1997 and 2001, among 198 patients who underwent pancreatic resection in our center, 25 had endoscopic PBD with plastic endoprosthesis (7-12F) before DP. Among these 6 were excluded for benign disease (n=5) or metallic prosthesis (n=1). Postoperative complications in 19 patients with PBD were compared to 21 matched patients who underwent DP for pancreatic head cancer without PBD during the same time period. Groups were comparable for all clinico-pathological characteristics except preoperative bilirubin level, which was higher in the PBD group. The overall complication rate of PBD was 21% (1 cholangitis, 1 pancreatitis, 2 endoprosthesis replacement). The mean hospital stay after PBD was 5 ± 3 days. Pancreatic fistula (26% vs. 19%) and mortality rates (5% vs. 5%) were identical in the two groups. The number of patients with one or more postoperative complications was similar (12 (63%) vs. 9 (43%), p=0.2), however the overall incidence of complications was significantly greater in the PBD group when stent-related complications were included in the analysis (74% vs. 43%, p=0.05). Further, the number of patients with one or more infectious complications, including infected collections (superficial, wound, and/or profound) after DP was significantly higher in the PBD group (47% vs. 5%; p=0.001). Total hospital stay (including stay for PBD) was significantly longer in the PBD group (34 ± 13 vs. 24 ± 9 days; p=0.03). Finally, in patients who had PBD, the bacteriological analysis of infected collections revealed a close correlation between the bacteria present in the bile (collected at the time of DP) and the culture from infected collections. These data demonstrate that endoscopic PBD with plastic endoprosthesis before DP for patients presenting resectable pancreatic head cancers increases the risk of postoperative infectious complications and prolongs the overall hospital stay. We therefore recommend selective PBD in jaundiced patients with resectable pancreatic head cancers, in patients with cholangitis or those who will undergo preoperative neoadjuvant treatment.

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Postoperative Jejunal Feeding and Outcome of Pancreaticoduodenectomy

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Complications following pancreaticoduodenectomy are common partly due to a poor preoperative nutritional status. Previous studies have not shown benefit from perioperative TPN in majority of patients. The aim of this study is to evaluate impact of early postoperative enteral tube feeding in patients undergoing Whipple operation. A retrospective chart review of 180 consecutive patients who underwent Whipple operation between 1994 and 2000 was performed. Patients were divided into two groups; those with early postoperative enteral tube feeding via a nasojejunal or a gastro/jejunal tube placed at surgery versus no planned enteral feeding. Use of enteral feedings was based on surgeons preference. Outcome variables were compared using t-test and included length of hospitalization, early and late complications and readmissions within 30 days of discharge. 98 patients (54%) received early postoperative jejunal feeding while 82 patients (46%) did not. Both groups were comparable in age (mean 66 vs 60 yrs), gender (59% vs 60% male), diagnosis of pancreatic adenocarcinoma (42% vs 56%), preoperative albumin (3.7 vs 3.7 g/dl) and type of procedure (pylorus sparing 84% vs 85%). The mean operative time (406 vs 360 min) and incidence of blood transfusion (44% vs 18%) were higher in the jejunal feeding group while intraoperative complications were similar (1.0% vs 1.2%). Both had similar length of hospital and ICU stay (14 vs 15 and 1.3 vs 1.1), while ICU readmissions (6% vs 10%), length of stay due to delayed gastric emptying (1.5 vs 2.4) and use of TPN (6% vs 27%) were less in the jejunal feeding group (Table 1). Jejunal feeding was delivered to 98 patients via bridled nasojejunal tube in 55 patients (56%) and jejunostomy tube in 43 (44%). Jejunal feeding was started on POD 2 and lasted a mean of 12.7 days. 21 patients (21%) were discharged with home feedings. Duration of NG tube decompression (5.6 vs 6.1 days), prokinetic agents use (55% vs 51%), and time of starting regular diet (POD 9.5 vs 10.4) were similar in both groups, while vomiting after NG tube removal was less in the enteral feeding group (10% vs 29%). There was a lower rate of readmission within 30 days of discharge, and a lower incidence of early and late complications in the jejunal

feeding group (Table 1). Tube-related complications occurred in 6 out of 98 patients, and all were gastrojejunal tubes. Early postoperative jejunal tube feeding in patients undergoing pancreaticoduodenectomy is associated with a significantly lower rate of TPN use, hospital readmissions, and late complications. These data support routine use of a bridled nasojejunal feeding tube and early enteral nutrition.

	Enteral feed (n=98)	No enteral feed(n=82)	p value
TPN	6 (6%)	22 (27%)	0.0003
Readmit	12 (12%)	22 (27%)	0.015
Early complications	51 (52%)	51 (62%)	NS
Late complications	12 (12%)	25 (31%)	0.003

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Neoadjuvant Gemcitabine (Gemzar), Paclitaxel (Taxol) and Radiation may Downstage Locally Advanced Pancreas Cancer Facilitating Resection

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Introduction: Surgical resection for pancreatic cancer is the most effective treatment for this disease. Only 15% of patients at the time of diagnosis are candidates for resection. Forty percent of cases present with locally advanced, non-metatstatic disease, traditionally, not amenable to pancreatic resection. Neoadjuvant chemoradiation to downstage locally advanced pancreatic cancer for resection is evolving. The 5FU based chemoradiation only rarely achieves an adequate response to allow for resection. The Brown University Oncology Group (BRUOG) has evaluated paclitaxel (Taxol)/radiation and Gemcitabine (Gemzar)/paclitaxel/radiation treatment in a series of trials for medical effectiveness. We retrospectively reviewed our data to determine if these neoadjuvant modalities can downstage locally advanced pancreatic cancer to be resected. Results: 42 patients who initially had locally advanced disease were treated on a phase II trial of Taxol 50 mg/m2/wk with 50.4 Gy radiation. A significant number were downstaged, however were not medically cleared for surgery. Ultimately, 14 underwent surgical exploration. Four patients were resected, one had fibrosis with negative biopsy, and 9 had unsuspected liver metastases. Of the patients who were resected, 3 had partial responses and one had stable disease following chemoradiation. One patient, who initially had SMA encasement, is disease free at 6 years follow up. Gemcitabine was subsequently added to paclitaxel/radiation treatment in a phase I trial. Twenty patients were enrolled in the trial and 10 were treated at the maximally tolerated dose (MTD): gemcitabine 75 mg/m2/wk, paclitaxel 40 mg/m2/wk, and 50.4 Gy radiation. Of the 10 patients treated at the MTD, four patients had downstaging of disease such that they underwent exploration for resection. Prior to neoadjuvant treatment, one had portal vein thrombosis, the second had portal vein invasion, the third had superior mesenteric artery (SMA) encasement, and the fourth had both inferior vena cava and SMA involvement. A pancreaticoduodenectomy was performed on 3 of 4 patients with negative margins. A fifth patient was treated off protocol after the study was completed. This patient had post-chemoradiation resection with negative margins. Conclusion: The role for neoadjuvant chemoradiation for locally advanced pancreatic cancer is evolving. The BRUOG studies show that neoadjuvant paclitaxel based chemoradiation regimens can downstage some patients with locally advanced disease, making their tumors amenable to resection. Paclitaxel/gemcitabine/radiation appears to be more effective in downstaging pancreatic cancer than 5FU based chemoradiation. The ultimate impact on survival remains uncertain.

BILIARY

Gallstones

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Outcome of Laparoscopic Cholecystectomy for Presumed Biliary Pancreatitis

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Anatomic and clinical data have long supported the concept of gallstone migration as one cause of acute pancreatitis. Calculi should be excluded since cholecystectomy is potentially a curative treatment for this cause of pancreatitis. Similarly, cholecystectomy may also represent definitive treatment for idiopathic pancreatitis presumptively due to microlithiasis. A retrospective review of patients who underwent laparoscopic cholecystectomy for known calculus or idiopathic pancreatitis was conducted to assess outcome. A cholecystectomy database was queried for patients operated for pancreatitis between July 1995 and June 2000. A total of 94 patients underwent cholecystectomy, 72 of which were operated on for documented calculi by transcutaneous ultrasound. Twenty-two patients underwent surgery for presumed idiopathic pancreatitis based on a negative ultrasound and history. Patients with known calculi included 42 women and 30 men with a mean age of 62 (range 21-80) years. Common duct calculi were documented in 16 patients (17%). Pre-operative ERCPs were performed in 31 patients (43%) and was diagnostic of common duct stones in 8 (27%). Intraoperative cholangiography was successful in 68 of 72 patients (94%), and common duct stones were found in 10 (15%). Conversion to open was done in three (4%). At a mean follow-up of 57 months, pancreatic complications occurred postoperatively in 4 patients (7%): two with acute recurrent pancreatitis, one acute pancreatitis due to pancreatic divisum, and one ampullary stenosis. Patients with idiopathic pancreatitis presumed to have microlithiasis included 11 women and 11 men with a mean age of 45 (range 34-75) years. Preoperative evaluation included crystal analysis in two patients, ERCP in 9 (41%), and MRCP in two (9%). Open cholecystectomy was required in two (9%). Unsuspected calculi were discovered by pathology in six (23%), including two patients with common duct stones at operative cholangiography. At a mean follow-up of 47 months, subsequent biliary pancreatic disease occurred in six (27%); four with chronic idiopathic pancreatitis, one each with acute pancreatitis due to pancreatic divisum and sphincter of Oddi dysfunction by abnormal manometry. In summary, laparoscopic cholecystectomy confers long-term success in preventing pancreatic complications when calculi are diagnosed preoperatively. Patients with presumed idiopathic pancreatitis should be investigated for ductal abnormality with non-invasive testing. Empiric cholecystectomy is advised for patients with idiopathic pancreatitis and a normal pancreatic duct to achieve high clinical success by removing unsuspected calculi.

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Cholesterol Crystal Nucleation in Heterozygous Leptin-Deficient Lean Mice

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Background: Cholesterol gallstones result from cholesterol supersaturation of bile, biliary stasis and crystal nucleation. Obesity is a known risk factor, but a complete understanding of this phenomenon is lacking. Leptin, a hormone produced by adipocytes, has been implicated in the pathogenesis of both obesity and gallstone formation. We have demonstrated that lean heterozygous leptin-deficient mice have an intermediate gallbladder response to neurotransmitters and cholesterol saturation similar to both lean control and obese leptin-deficient mice. However, cholesterol crystal formation in heterozygous leptin-deficient mice has not been studied. Therefore, we hypothesized that bile from heterozygous mice would form cholesterol crystals at an intermediate rate between lean control and homozygous leptin-deficient obese mice. Methods: Seventy-five lean control (C57BL/6J), 54 lean heterozygous (C57BL/6J Lephet) and 36 obese (C57BL/6J Lepob) female mice underwent cholecystectomy at 12 weeks of age. Bile was collected, pooled, filtered and incubated at 37° C. Cholesterol monohydrate crystal formation was observed daily under polarized light microscopy for 17 days. Crystal observation time (COT) in days, crystal growth (slope) and crystal mass (total number) were measured and analyzed with the Mann-Whitney Rank Sum Test. Results: Crystal formation data are presented in the table. Conclusions: Lean heterozygous leptin deficient mice have crystal observation time, growth and mass similar to lean controls rather than homozygous leptin-deficient obese mice. Lean heterozygous leptin-deficient mice have serum leptin levels that are intermediate between lean control and leptin-deficient obese mice. We conclude, therefore, that serum leptin correlates with gallbladder response to neurotransmitters but not with cholesterol crystal formation.

Cholesterol monohydrate crystal formation

Strain	Pools	COT	Crystal growth	Crystal mass
Lean control	9	7.0 ± 0.3	0.9 ± 0.1	37 ± 1.7
Lean heterozygous	8	7.1 ± 0.3	1.0 ± 0.1	39 ± 1.3
Obese homozygous	6	5.7 ± 0.6*	3.2 ± 0.2†	137 ± 6.0†

*$p < 0.05$, †$p < 0.001$ vs. Lean control and heterozygous.

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Reduced Biliary Cholesterol Crystal Formation in Leptin-Resistant Obese Mice

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Background: Obesity is a major risk factor for cholesterol gallstone formation, but the pathogenesis of this phenomenon remains unclear. Previous studies from this laboratory have demonstrated that both leptin-deficient and leptin-resistant obese mice have diminished gallbladder motility. Leptin-deficient obese mice also have enhanced cholesterol crystal formation in-vitro despite normal biliary cholesterol. However, the majority of obese humans are leptin-resistant. Our lab has recently demonstrated that leptin-resistant obese mice have low biliary cholesterol levels. Therefore, we tested the hypothesis that leptin-resistant obese mice would have reduced cholesterol crystal formation. Methods: Eight week old lean control (C57BL/6J, n=96) and leptin-resistant (Lep Db, n=59) female mice were fed a non-lithogenic chow diet for four weeks. All animals then underwent cholecystectomy, and bile was collected, pooled, filtered, and maintained in a water bath at 37° C for 17 days. Bile was observed daily for cholesterol crystal formation under polarized light microscopy. Birefringent liquid and solid cholesterol monohydrate crystals were counted in ten high power fields. Crystal observation time (COT) in days, crystal growth rate (slope), and crystal mass (total number) were determined. Data were compared using the Mann-Whitney Rank Sum Test. Results: Liquid crystal results are presented in the table. Solid cholesterol crystals formed only in the lean control animals with a COT of 15.3 + 6.7, a slope of 1.8 + 1.5, and a crystal mass of 395 + 273 ($p < 0.03$ for COT, slope, and mass vs leptin-resistant mice). Conclusions: These data suggest that leptin-resistant obese mice have delayed cholesterol crystal observation time, decreased crystal growth rate, and diminished crystal mass. These observations are consistent with the lower biliary cholesterol levels observed in leptin-resistant obese mice. Therefore, we conclude that a defect in gallbladder motility, as opposed to cholesterol metabolism, is primarily responsible for increased gallstone formation in leptin-resistant obesity.

Liquid Crystals in Lean and Leptin-Resistant Mice

Study group	COT (days)	Crystal growth (slope)	Crystal mass (number)
Lean control	7.7 ± 1.2	210.7 ± 98.2	3204 ± 1635
Leptin-resistant	15.2 ± 1.7*	1.8 ± 1.5*	16 ± 10*

*$p<0.05$ vs lean controls.

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Leptin-Resistant Obese Mice Have Paradoxically Low Biliary Cholesterol Saturation

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Background: Human obesity is associated with leptin resistance, elevated serum glucose and lipids, hepatic steatosis and cholesterol gallstone formation. These gallstones are thought to result from hypersecretion of biliary cholesterol as well as biliary stasis. We have previously demonstrated that leptin-deficient C57BL/6J Lepob obese mice have abnormal biliary motility and are prone to cholesterol crystal formation despite normal biliary lipids. However, leptin-resistant C57BL/6J Lepdb obese mice, which are know to have elevated serum leptin, glucose and lipids as well as hepatic steatosis, should be a more appropriate model for human gallstone formation. Therefore, we tested the hypothesis that leptin-resistant mice would have increased gallbladder volume and biliary cholesterol saturation. Methods: C57BL/6J lean control mice (n=60) and leptin-resistant Lepdb obese female mice (n=60) were fed a non-lithogenic chow diet for four weeks. At 12 weeks of age, the mice were fasted overnight and then underwent cholecystectomy. Gallbladder volumes (GBV) were measured, and the bile was pooled for measurement of total bile acids (TBA), phospholipids (PPL) and cholesterol (XOL). Cholesterol saturation index (CSI) was calculated. Serum leptin and cholesterol levels were determined. Hepatic fat vacuoles were counted per high-power field (hpf). Data were analyzed by the Mann-Whitney Rank Sum Test. Results: Fasting GB volume in lean controls and Lepdb obese mice was 14.1 ± 0.6 vs 39.0 ± 1.6 μL, $p<0.05$. Serum leptin in lean control and Lepdb obese mice was 3.3 ± 1.0 vs 191.9 ± 14.8 ng/mL, $p<0.05$. For Gallbladder bile lipids, serum cholesterol and hepatic fat vacuoles see table. Conclusions: These data suggest that leptin-resistant Lepdb obese mice have 1) increased gallbladder volume and 2) decreased biliary cholesterol saturation despite elevated serum cholesterol and hepatic steatosis. We conclude that the link between obesity and gallstone formation does not require hypersecretion of biliary cholesterol.

Strain	TBA mol%	PPL mol%	XOL mol%	CSI	Serum XOL mg/dl	Fat vacuoles/hpf
Lean C57BL/6J	81.9 ± 0.4	15.3 ± 0.5	2.8 ± 0.3	0.50 ± 0.10	61.1 ± 3.0	28.2 ± 5.9
Obese Lep^{db}	84.2 ± 0.7*	13.8 ± 0.7	2.0 ± 0.1*	0.37 ± 0.01*	125.8 ± 3.8*	109.5 ± 26.6*

*$p<0.05$ vs C57BL/6J lean controls.

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Management of Complex Biliary Calculi With a Holmium Laser

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The management of complex biliary tract calculi is fraught with complications and treatment failures. This is exemplified in patients with primary intrahepatic calculi (PIC) who develop recurrent cholangitis. Standard surgical and endoscopic approaches often fail to clear all calculi and many patients undergo unnecessary liver resection. The success of the Holmium laser for urologic calculi led us to adapt treatment strategies for PIC and secondary biliary tract calculi (SC). Our goals were to remove all calculi, prevent recurrent sepsis and preserve hepatic parenchyma. Methods: Thirty patients with complex biliary calculi were treated. After controlling sepsis and evaluating the extent of calculi, appropriate access to and drainage of the biliary tract was obtained. Holmium laser lithotripsy was performed under video guidance using 7.5 or 10 Fr flexible choledolchoscopes and a 200 micron laser fiber generating 0.6-1.0 J at frequencies of 6-10 Hz. Lithotripsy procedures were repeated until cholangiography and choledochoscopy confirmed clearance of calculi. Results: 21 patients of Asian descent with PIC (14 female, 7 male; age 28-79 years) and 9 patients with SC (5 female, 4 male; age 29-88 years) were treated. PIC patients presented with pain (6), pain and fever (8) or cholangitis (7) whereas SC patients presented with pancreatitis (1), jaundice (2) or cholangitis (6). Prior procedures in PIC patients included none (6), cholecystectomy without (4) or with drainage procedure (11). Seven SC patients had prior cholecystectomy and two had no previous biliary surgery. Access to the biliary tract in PIC patients was via percutaneous transhepatic catheters (PTC-8), surgically placed T-tubes (TT-10) or a combination (3). For SC patients access was via PTC (6), TT (1) or cystic duct during laparoscopic cholecystectomy (2). Biliary drainage in PIC patients was by choledochoduodenostomy (CDD-3), hepaticojejunostomy (HJ-14) or endoscopic sphincterotomy (ES- 4). Biliary drainage for SC patients was HJ-1 and ES-8. Complete stone clearance required an average of 3.9 procedures (range 1-15) for PIC and 2.1 (range 1-9) for SC patients regardless of stone composition. (cholesterol, Ca-bilirubinate or mixed bile pigments). No patient required hepatic resection. Complications included hepatic subcapsular hematoma (1), infected hepatic cyst (1) and arrythmia (3). No complications or deaths were attributed to the Holmium laser. During follow-up (range 2 months to 3 years) no patient presented with cholangitis, one PIC patient was retreated for recurrent stones. Two PIC patients died of cholangiocarcinoma (1) and cirrhosis (1). Conclusion: Complete clearance of calculi can reliably and safely be achieved with a Holmium laser regardless of stone composition or location while preserving hepatic parenchyma and preventing recurrent sepsis.

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Tumors

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Gallbladder Cancer: Are We Doing Any Better?

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Background: The aim of this study was to evaluate contemporary outcomes associated with the management of gallbladder cancer. Methods: The medical records of 48 consecutive patients with gallbladder cancer treated at our institution from 1/1981 through 11/2001 were reviewed. Survival was analyzed using the Kaplan-Meier method (mean follow-up period: 24 months). Prognostic factors were analyzed (univariate Log-Rank test). Results: Mean patient age was 68±12 years. Twenty-nine (60%) patients were female. Sixty-seven percent of patients presented with right upper quadrant pain, 27% with jaundice and 19% with weight loss. Thirty-nine (81%) patients underwent laparotomy or laparoscopy; among these patients the preoperative diagnosis was gallbladder mass in 44%, cholelithiasis in 28%, and acute cholecystitis in 28%. Eighteen (38%) patients underwent curative resection (10 simple cholecystectomies and 8 radical cholecystectomies). There were no procedure-related mortalities. Prognostic factors are shown in the table. Overall 1-year and 5-year survival rates were 40% and 13%, respectively. Patients who underwent complete resection had higher 1-year and 5-year survival rates (73%, 31% respectively) than patients who underwent palliative surgery or no surgery (12%, 0% respectively, $p<0.05$). Of 18 patients who underwent curative resection, 17% had T1 tumors, 50% had T2 tumors, and 33% had T3/4 tumors. T1/T2 tumors were associated with better survival rates than T3/T4 tumors (92% vs 50% at 1-year, 46% vs 0% at 5-year, $p<0.05$). For patients who underwent simple cholecystectomy, 1- and 5-year survival rates were 60% and 20%. For patients who underwent radical cholecystectomy (cholecystectomy, wedge resection of liver bed, and lymph node dissection), 1- and 5-year survival rates were 100% and 60%, respectively. The presence of lymph node metastases was an adverse prognostic factor among these patients (0% vs 75% 2-year survival rates for patients with positive lymph nodes vs negative lymph nodes, respectively, $p<0.05$). Of the 30 patients who did not undergo curative surgery, 37% underwent biopsy alone, 27% had a simple cholecystectomy and 33% had a biliary bypass or drainage. Median survival for these groups was 5.6, 8.0, and 5.6 months, respectively. Conclusion: The overall survival for patients with gallbladder cancer remains poor. Although radical surgery is safe, it is associated with long-term survival only in a highly select subset of patients with gallbladder cancer.

Univariate analysis: factors associated with poorer overall survival

Factors	p value
Age >50	<0.05
Male gender	<0.05
Jaundice	<0.05
Tumor unresectability	<0.05
Microscopic positive margin	<0.05
T (T1/2 vs T3/4)	<0.05
N (N1/2 vs N0)	0.18
M (M1 vs M0)	<0.05
Grade (Poorly differentiated vs Moderately or well differentiated)	0.33
Stage (stage 1/2 vs stage 3/4)	<0.05

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Improved Survival With Aggressive Surgical Resection for Gallbladder Cancer: A Twelve-Year Experience

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Many physicians have a relatively nihilistic approach to the treatment of gallbladder cancer. Recently, there has been a paradigm shift toward ex-

tended, R0 resections for this disease. We hypothesize that this increase in R0 resections has resulted in improved survival for these patients. A retrospective review of all patients admitted with gallbladder cancer in the past 12 years to a multidisciplinary, hepatobiliary referral center was performed. A total of ninety-nine patients were identified. Average age was 64 years (range 37-88). The twelve-year period of review was divided into 2 time periods, those treated in the first 6 years and those treated in the past 6 years. Thirty-five patients were treated in the first time period, and 64 in the second time period. Disease stratification by stage was similar in the two time periods. Overall, 38 patients underwent an operation with curative intent. Of these, 17 underwent a wedge resection of the liver, 9 had a resection of segments 4 and 5, and 9 underwent an extended right hemihepatectomy. Six (17%) patients in group one had some form of liver resection, while 29(45%) patients in group two had a liver resection. In this same group, 22 patients had a complete resection of the extrahepatic biliary tree. Nine (26%) R0 resections were performed in time period one and 24(38%) in time period two. Both the number of liver resections and extrahepatic biliary resections were greater in the second time period (both $p < 0.04$). In both time periods, an R0 resection was associated with a statistically significant improvement in survival ($p < 0.02$ time period one, $p < 0.0001$ time period two). Overall survival of all patients in time period two versus period one was significantly greater ($p < 0.03$). Median survival in period one was 9 months and 17 months in time period two. In conclusion, a complete RO resection leads to improved survival in patients with gallbladder cancer. As well, the shift towards a more aggressive surgical approach (greater number of liver and biliary tree resections) in the last 6 years of our study versus the first 6 years has led to a significant improvement in overall survival.

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Cytokeratin is a Superior Marker for Detection of Micrometastatic Biliary Carcinoma

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Background: Regional lymph node metastasis is one of the most significant poor prognostic factors in patients with biliary tract carcinoma. The incidence of lymph node micrometastases in these patients is unknown, but may be beneficial for staging. We sought to evaluate the utility of three antibodies for immunohistochemical (IHC) detection of micrometastatic disease. Methods: Surgical specimens from 35 patients with biliary tract carcinoma (15 bile duct, BD, and 20 gallbladder, GB) were evaluated. Specimens were separated into those with histologically positive or negative nodes based on routine histology obtained at the time of resection. In order to assess the degree of positive staining in histologically involved tissue, primary tumors and histologically involved lymph nodes were stained with the following antibodies using standard IHC techniques: cytokeratin (AE1:AE3), CEA (carcinoembryonic antigen) and EMA (epithelial membrane antigen). The antibodies with the highest positivity were then used to evaluate the lymph nodes of patients with histologically negative nodes. A total of five 4 μm thick sections taken at intervals of 40 μm were obtained from each histologically negative lymph node. Micrometastatic disease was defined as clustered atypical cells 2 mm in size detected only with the use of IHC. Results: Of the 35 specimens, lymph nodes were histologically positive in 20 (8 BD and 12 GB), while 15 (7 BD and 8 GB) were histologically negative. All of the primary tumors and histologically positive lymph nodes demonstrated bright staining with cytokeratin and CEA antibodies, whereas only 83% were positive for EMA (Table). Therefore, cytokeratin and CEA antibodies were used to evaluate histologically negative lymph nodes in order to detect occult micrometastatic disease. In the 15 patients with negative nodes, anti-cytokeratin immunostaining detected micrometastatic disease in two patients with BD cancer. Staining with anti-CEA was negative in all specimens. Therefore, 2 of 7 patients with BD cancer and 0 of 8 patients with GB cancer were found to have occult micrometastases in histologically negative lymph nodes. Conclusion: Cytokeratin immunostaining enables detection of micrometastases in nodal specimens found to be negative by routine histology in patients with biliary tract carcinoma. Prospective protocols incorporating cytokeratin staining of the lymph nodes may help determine the clinical significance of occult micrometastatic disease in these patients.

Immunostain	Sensitivity for detection of biliary tract carcinoma	Detection of micrometastatic disease
Cytokeratin	100%	Yes
CEA	100%	No
EMA	83%	—

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Intrahepatic Biliary Cystadenoma (IBC): Role of Cyst Fluid Analysis and Surgical Management in the Laparoscopic Era

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Recent interest in cyst fluid (CF) analysis for CA19-9 and CEA and the introduction of laparoscopic surgery (LS) in the management of hepatic cysts have resulted in sporadic reports of elevated CA19-9 and CEA levels in IBC CF and the application of LS in the management of simple cysts. However, the role of CA19-9 and CEA in the diagnosis of IBC and the role of LS in the management of IBC have not been previously defined. We have studied 22 patients with IBC at a single institution (7 years). In the last 10 patients, we prospectively analyzed CF for CA19-9 and CEA and used LS for the diagnosis and treatment of IBC. We compared this experience with 12 previous patients with IBC who were managed with open surgical intervention. We found that in 10/10 patients with IBC who had elevated CA19-9 (range 3,136-1,757,510, N<33 U/ml) and mildly elevated CEA (range 3.7-212, N<3 ng/ml), the cyst lining consisted of biliary epithelium with or without mesenchymal stroma (MS). In 1/10 patients (highest CA19-9 level), the cyst lining also contained mucinous epithelium without MS. In contrast, patients with simple cysts had normal CF values of both CA19-9 and CEA. In the previous 12 patients managed with laparotomy, 6/12 patients had biliary epithelium alone and the other 6 also contained mucinous epithelium. 1/6 patients with mucinous epithelium had intestinal metaplasia and a small focus of carcinoma-in-situ. Our approach to IBC has evolved significantly over the past 7 years. Currently, we feel that all patients with IBC should undergo cyst wall tissue sampling to determine whether a pre-malignant (mucinous intestinal epithelium ± MS) or malignant diagnosis that requires resection exists. Our recent data suggest that using a combination of CF aspiration and LS, open surgical management can be avoided in the vast majority of patients with IBC.

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Induction of MIC-1/GDF-15 Following Bile Duct Injury

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Background: Macrophage inflammatory peptide-1 (MIC-1)/GDF-15 is a divergent member of the transforming growth factor-fl superfamily cloned by others and us. MIC-1/GDF-15 is expressed in both liver and normal colon. MIC-1 is known to inhibit proliferation of certain colon cancer cell lines in vitro and suppresses tumor formation in vivo. MIC-1 also limits production of pro-inflammatory cytokines. We have previously demonstrated MIC-1 expression is rapidly induced following a wide variety of acute and chronic liver injuries. We, therefore, hypothesized that MIC-1 may also be a mediator of biliary tract injury and could play a role in regulation of bile duct proliferation. Experimental Design and Results: C57/Bl6 mice underwent liagtion of the common bile duct. A time-dependent induction of MIC-1 mRNA expression was observed by Northern blot

analysis. Densitometry demonstrated an eight-fold induction of MIC-1 message at 6 hours. In situ hybridization of liver sections following bile duct ligation demonstrated a peri-portal expression pattern consistent with expression of MIC-1 in bile ducts and small peri-ductal hepatocytes. Examination of human livers with sclerosing cholangitis also demonstrated enhanced expression of MIC-1/GDF-15. Conclusion: MIC-1/GDF-15 is expressed following bile duct injury. Taken together with the documented anti-tumor and inflammatory effects our results suggest MIC-1/GDF-15 may play a role in control of bile duct growth and biliary tumor formation.

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Surgery
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A Single-Center Experience on the Surgical Treatment of the Middle and Lower Bile Duct Carcinoma

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Aim: To optimize surgical treatment of the middle and lower bile duct carcinoma, we analysed prognostic factors of these cancer patients based on a single center experience. [Methods] The records of 48 patients with the primary middle and lower bile duct cancer between January, 1989 and December, 2000 at our department were reviewed retrospectively. Data were collected by chart review including review of surgical records, pathological specimens and clinical information. Results: The overall 5-year survival rate of was 34.2% and the 5-year survival rate of patients whom surgical Curability A was accomplished was 44.5%. No metastatic lymph node status and no vascular invasion were predictors of favorable outcome ($p<0.05$). For the middle bile duct cancer, the prognosis of patients treated with pancreatoduodenectomy with/without pylorus preservation was better than that of patients performed bile duct resection under the condition of Curability A operation ($p<0.05$). For the lower bile duct cancer, the 5-year survival rates of no invasion to the pancreas parenchyma, a little invasion to the pancreas and severe invasion to the pancreas were 60%, 55% and 25% respectively ($p<0.05$). Conclusions: Pancreatoduodenectomy or pylorus-preserving pancreatoduodenectomy with extended lymphadenectomy should be performed for the patients with middle and lower bile duct cancer. Patients with metastatic lymph nodes, vascular invasion and/or invasion to the pancreas should receive systemic multimodal therapy (chemotherapy, radiation, etc.).

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Biliary Complications a Decade After Introduction of Laparoscopic Cholecystectomy

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Purpose: When laparoscopic cholecystectomy was first introduced a decade ago, multiple investigators reported an increasing incidence of bile duct injury. We studied the current incidence of direct biliary complications following cholecystectomies, ten years after the wide introduction of LC. Method: A retrospective chart review of 1465 consecutive primary cholecystectomies performed between 7/1/98 and 6/30/2001 was performed. Procedures were classified as laparoscopic (LC), open (OP), or laparoscopic converted to open (CON). Using intent to treat model, LC and CON groups were combined and compared to LC in terms of complications Results: 1465 consecutive cholecystectomies were performed: 1232 LC, 143 CON, and 90 OP. The overall biliary complication rate was 1.8%. The rate in the LC/CON group was 1.6% vs. 4.4% in the OP group. There was one major CBD injury in the LC/CON group (0.07%). The remainder of complications were minor. In the LC/CON group there were 10 bile leaks (0.7%) and 11 (0.8%) retained stones. 4 bile leaks required no intervention, 4 required ERCP, 1 required CT guided drainage, and 1 required operative drainage. All 11 retained stones required ERCP, and 1 required subsequent operative management. In the open group there were 2 bile leaks and 2 retained stones (2.2%). 1 of these bile leaks required ERCP, while both cases of retained stones required ERCP. One of these required subsequent open common duct exploration. There were a total of 7 deaths, 4 (0.3%) in LC/CON and 3 (3.3%) in OP. The majority of deaths (3 in OP and 2 in LC/CON) were due to overwhelming sepsis, which was present prior to the cholecystectomy in these 5 cases. One LC patient died from unrelated C. difficile colitis. The other death was related to a failing lung transplant complicated by cholangitis. Conclusion: At our institution, the current incidence of biliary complications following LC is as low as the pre-laparoscopic era.

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Open Laparoscopy: New Technique of Treatment of Choledocholithiasis

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Background: An open laparoscopic operation with the use of the ring retractor is a new minimally invasive technique of surgical treatment of a pathology of biliary system. Purpose: To evaluate feasibility and outcomes of open lapa- roscopic operations with application of a minilaparotomy up to 4 cm in treatment of choledocholithiasis and ampullary stenosis. Patients: 586 patients were observed, an average age is 57.9 years (17-90). Cholecystitis accounted for over 90% of cases: 48% of the patients had acute form and 53% had chronic form of cholecystitis. 10 patients had history of previous biliary tract surgery. Stones of biliary tree and/or of ampullary stenosis were found in 78% of patients; 26% had obstructive jaundice. Other complications of cholelithiasis included purulent cholangitis (n=65), pericystic infiltrate (n=36), peritonitis (n=20), bilio-digestive fistulas (n=10), Mirizzi syndrome (n=9), etc. Methods: A two-stage surgical approach was applied to 321 patients. On the first stage of treatment, open laparosco- pic cholecystectomy (OLCh n=221), endoscopic papillotomy (EPST n=88) or cholecystostomy (n=12) were performed. 108 patients had a T-tube drainage of a common bile duct, 111 - drainage of a cystic duct by Halstedt technique. The transformation into open laparotomy was necessary in 2 cases. On the second stage of treatment EPST was performed on 216 patients (EPST failed in 2 of them), OLCh with choledocholititomy and T-tube drainage of common bile duct - on 51, OLCh alone - on 39. 4 patients had OLCh with choledocholititomy and choledochoduodenostomy (1 of them required transduodenal papillotomy) and 2 patients had a balloon dilatation of a Vater papilla. Transformation into open laparotomy was necessary in 3 patients. Patients with previous surgeries of bile ducts (n=6) received minilaparotomy, choledocholititomy with a T-tube drainage of a common bile duct. A single-stage approach was used in 247 patients. OLCh with a T-tube drainage of a common bile duct is done at 38 patients, OLCh with a drainage of a cystic duct - at 123, OLCh with choledocholititomy and T-tube drainage of common bile duct - at 39 patients. 11 patients required papillotomy, 2 - choledochoduodenostomy, 1 - Roux-en-Y hepaticojejunostomy. In 33 patients OLCh was converted into open laparotomy. Results: Average hospital stay was 23.2 days, with 15.9 days in post-operative period. Intraoperative complications were encountered in eight cases. The complications in the postoperative period were observed in 7.75% patients with a mortality rate of 2.1%. Conclusion: open laparoscopy with the use of a ring retractor allows to perform minimally invasive operations for a wide spectrum of biliary tract pathology, including repeated restorative - reconstructive interventions. The frequency of conversion into open laparotomy in this series was 6.2%.

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Patterns of Primary Recurrence after Resection for Gallbladder Cancer and Hilar Cholangiocarcinoma

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Objective: Gallbladder cancer and cholangiocarcinoma are often considered to be different manifestations of the same disease, but recent data suggest that they are distinct neoplasms with different invasive and meta-

static properties. The present study addresses biologic differences between gallbladder cancer and cholangiocarcinoma by examining primary recurrence patterns after resection. Methods: Patients with either hilar cholangiocarcinoma (HCCA) or gallbladder carcinoma (GBCA) submitted to a potentially curative resection were identified from a prospective database. Pathological diagnosis of adenocarcinoma was confirmed. Specific sites of recurrence, pathological data, and overall and disease-free survival were analyzed. Local/regional recurrence was defined as recurrence at the resection site or within the porta hepatis or retroperitoneal lymph nodes. Distant recurrence was comprised of peritoneal, discontiguous liver, or extra-abdominal metastases. Results: Of all patients who underwent potentially curative resection for GBCA or HCCA between May 1990 and October 2000, complete recurrence data were available for 80 patients with GBCA and 76 patients with HCCA. The median time to recurrence was significantly less for patients with GBCA compared with HCCA (12 vs 21 months, $p=0.007$). The proportion of patients who recurred was similar with 52 (68%) HCCA patients and 53 (66%) patients with GBCA developing recurrence over the follow-up period. Of those who recurred, an isolated local/regional recurrence occurred in 59% (26 of 44) of HCCA patients, compared with only 15% (7 of 48) of GBCA patients ($p<0.001$). By contrast, initial recurrence at a distant site, with or without concomitant local/regional recurrence, occurred in 85% (41 of 48) of GBCA patients, compared with 41% (18 of 44) of HCCA patients ($p<0.001$). This pattern of recurrence was diagnosis specific regardless of stratification by other clinicopathologic factors, including tumor stage. Factors predictive of any recurrence on multivariate analysis included a positive resection margin ($p=0.03$), node positive disease ($p=0.01$), and a moderately/poorly differentiated tumor ($p=0.01$). A diagnosis of HCCA and GBCA was specific to the development of local/regional only recurrence and distant recurrence, respectively. Conclusions: After complete resection, HCCA is more likely to recur at local/regional sites, whereas GBCA has a greater propensity for distant metastatic spread. GBCA is also associated with a much shorter time to recurrence. These results strongly suggest that GBCA and HCCA are distinct clinical entities with different tumor biology. The results further suggest local/regional adjuvant treatment strategies, such as radiation therapy, are unlikely to be helpful after resection of GBCA.

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WITHDRAWN

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Multidisciplinary Approach to Recurrent Pyogenic Cholangitis

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Introduction: Recurrent Pyogenic Cholangitis (RPC), formerly known as Oriental Cholangiohepatitis, appears to be increasing in prevalence in North America. Advances in radiologic and endoscopic therapies and resective surgery emphasize the need for a multidisciplinary approach in its management. Methods: From January 1992 to October 2002, 23 consecutive individuals with RPC were treated at a North American University Teaching Hospital setting. Results: Of the 23 patients, 12 were male and 11 were female with a mean age of 59 years (SD 16.6). Sixteen (70%) patients were Asian, 6 (26%) were Caucasian and one (4%) was Hispanic. Imaging studies included an abdominal ultrasound (US) for every patient and an abdominal CAT Scan (CT) for 21(91%). In comparison to US, CT was more sensitive in identifying atrophic hepatic segments (6 vs. 4 patients), intrahepatic stones (19 vs.15 patients) and liver abscesses (3 vs. 2 patients). All patients underwent biliary tree manipulations to attempt stone clearance. Endoscopic Retrograde Cholangiograms (ERCP), Percutaneous Transhepatic Cholangiograms (PTC) or both was performed in 6 (26%), 7(30%) and 10 (43%) patients respectively. The following biliary duct manipulations with stone extractions were performed: 46 PTCs, 20 ERCPs with papillotomies, and 12 PTC with Lithotripsy (PTCL). Surgery was performed on 13 (56%) patients. Common bile duct exploration (CBDE) with Hudson loop hepatico-jejunostomy was performed in 12 (92% of surgical patients) and synchronous hepatic resections for impacted stones was performed in 3 of these patients (right hepatectomy in two and segment 2/3 resection in one). An intraoperative diagnosis of metastatic cholangiocarcinoma was made in 1 patient and no procedure was performed. Post-operatively, 5 of the 12 Hudson loop patients (41%) patients underwent further stone retrieval through the loop for recurrent symptomatic hepatolithiasis. There was no peri-operative (90 day) mortality. Wound infections developed in 2 (15%) patients, subphrenic abscess and stenosis of the hepaticojejunostomy in 1 (7%) patient. One patient developed liver insufficiency after 4 years from CBD exploration and Hudson's loop reconstruction requiring a cadaveric orthotopic liver transplantation. Conclusions: The prevalence of RPC appears to be increasing in the West. Physicians practicing in Europe and North America need to be familiar with its presentation, diagnosis and therapy. This multidisciplinary approach which considers ERCP,

PTC or PTCL as first-line therapy for stone extraction, reserves surgical intervention for those who fail these manipulations.

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Choice of the Optimum Periods of the Operative Treatment Sick With Cholangitis

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The ubiquitous growing of the diseases biliary ways, and as effect this - a growing of the number by sick with acute cholangitis, which vastly complicate the main disease and quite often (16-23%) bring about lethal upshot. Under observation there were 242 sick with diseases biliary ways, complicated by acute cholangitis for period since 1998-2001 gg. In age structure dominated the person senior 60 years. The primary nature to obstructions beside 172 sick (71.1%) was choledocholitiasis. Beside 212 sick (87.6%) obstruction biliary tree matched with changes to wall bilious bubble. The retrospective analysis history diseases has shown that typical clinic choldngitis in the manner of triads Charcot existed only beside 26.9% sick. As a rule, diagnosis cholangatis was fixed during operative interference. For the reason searching for of the new methods of the diagnostics of the inflammatory process in biliary fetter, alongside with clinic-laboratory methods of the diagnostics, us was analysed complex of the factors, characterizing immunological end biochemical status (linking centre albumin) beside 70 patients in comparison with practically sound persons (n=15) in speaker of the disease. The results of complex immunological and biochemical studies have installed that they are indicative of far change the inflammatory process, breaches of the functional condition liver and intoxication sick at more early periods. The results have served the ground for scientific motivation more active tacticians operative interference beside sick with diseases biliary ways, complicated cholangitis on 1-2-e day from moment of the arrival in permanent establishment. The surgical treatment concluded is liquidations of the reasons obstructive cholangitis and unload biliary ways. Such approach to treatment sick has allowed to reduce lethality with 5% before 1.5%, reduction to duration of intoxication, jaundices and liver insufficiency, of the purpose of the main medicinal forms and amount complications ($p<0.01$). So, execution operation beside sick with diseases biliary ways, complicated by acute cholangitis at early periods of the disease prevents liver insufficiency and intoxication - a main reasons to deaths beside given contingent sick.

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Metallic Stents (MS) Bridging the Biliary Confluence: A Surmountable Surgical Obstacle

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MS are being increasingly used to bypass biliary strictures. In some patients however, surgery may be secondarily considered because of stent obstruction (75 - 100 % at 5 years) or when the diagnosis of unresectability is revised upon reevaluation. The aim of the present study is to report our experience in this situation when MS bypass the biliary confluence. Between 1999 and 2001, four patients underwent surgical cure of a hilar stenosis previously treated by MS (5% of resection of hilar stenosis during the study period). Primary indication for surgery was MS obstruction or attempt at cure of a presumed hilar malignancy. Surgery aimed at removing all MS by a combined biliary and hepatic resection. The four patients had overall 10 MS (2-4/ patient). Eight MS were obstructed. Three patients were jaundiced and two had cholangitis that required preoperative biliary drainage. The largest MS-free bile duct was a second order branch in three patients and was filled with stones in one. Two patients had hemi-liver atrophy and the two others underwent preoperative portal vein embolization. Three patients had associated vascular injuries on preoperative work-up (one disruption of right hepatic artery, two pylephlebitis of the left portal vein). All but one MS were encased in the bile duct wall and proved unextractable. Surgery consisted in a major liver (4 to 6 Couinaud's segments / patient) and biliary resection removing all MS and a Roux en Y anastomosis on 1 - 4 bile ducts. The patient with a normal preoperative angiography had such dense adhesions between the stented bile duct and portal vein that resection of the portal bifurcation was required. Morbidity was 75 %. After a follow-up of 18 months, all patients are symptoms free with normal liver function tests. No patient in fact had malignancies. Hilar obstructions initially managed by MS can still be surgically cured provided a major liver resection is performed, as for hilar malignancies. Simple extraction of the MS should not be regarded as a realistic anticipation. The procedure is demanding but the only alternative to liver transplantation.

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Acute Bile Duct Injury:The Need for a High Repair

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Although it has been stated that the first repair is the best option for the acute lesion of the bile duct, repair of the injuries in the acute setting (within minutes to hours of the lesion), represents a challenge, because it is usually done in small ducts, whose viability can not always be determined. A review of the charts of patients acutely repaired. Methods: Among 204 patients repaired between 1989 and 2002 a total of 30 cases were repaired within minutes to hours after the injury. These patiens were divided in 2 groups: I) Patients with Roux en Y hepatojejunostomy below the hepatic junction. II) Patients with Roux en Y hepatojejunostomy at the junction level. Results: Twenty-eight of the referred cases were secondary to a laparoscopic approach, the remaining two were conse- quence of an open cholecystectomy.All patients has a Strasberg E type of lesion (junction preserved). In all cases a Roux en Y hepatoyeyunostomy was done, with a transhepatic stent. Twelve patients were included in group I and 18 in II. Three patients in group I (25%) and one in group II (5%) developed anastomosis dysfunction with a mean follow up of 56 months for group I and 52 for group II. Two cases in group I required reoperation (16%) and none in group II (0%). Conclusion: In the acute setting, complex lesions should be treated with a high bilioenteric anastomosis (junction level) in the first attempt of repair. Anastomosis done at a lower level have a higher dysfunction rate, probably related to ischemic injury of the anatomosede duct. High repair allows an anastomosis at a level in which the ducts have an adequate blood supply.

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Strategy for Advanced Type 4 Hilar Bile Duct Carcinoma

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To achieve curative resection for hilar bile duct carcinoma with lower morbidity and mortality is still a difficult problem. Recent advances of diagnostic modalities such as helical CT and MRCP (magnetic resonance cholangio-pancreatography), 3D images of bile duct and vascular involvement can be obtained easily, which is helpful for pre-operative simulations and for decision of operative procedure. Pre-operative management such as PTPE (percutaneous transhepatic portal embolization) improves prognosis after major hepatectomies. By pre-operative diagnosis and management as mentioned before, rationale and safe operation for hilar bile duct carcinoma can be possible, and we report our recent experiences for hilar bile duct carcinoma classified as type 4 by Bismuth-Corllette classification, which is the most difficult type, in this paper. Twenty-one patients with hilar bile duct carcinoma underwent operation between 1996 and 2002. Hilar bile duct carcinomas classified as type 4 by Bismuth-Corllette classification were 8 cases in those patients. Pre-operative PTPE was performed in two patients. Right trisectionectomy was performed in 3 cases, left trisectionectomy in one, extended left hepatectomy in 2, segment 4, 5, 8 resection in one, and exploratory laparotomy in one. Segment 1 resection was performed in all cases, portal vein resection in one case and pancreaticoduodenectomy in two cases. There was no operative death or hospital

death. R0 resections were possible in 5 cases. Helical CT, MRCP, angiogram, and precise cholangiogram were employed for pre-operative diagnosis. We show a typical case to show how to decide operative procedure. The case is 67 year-old-male. Posterior sector bile duct and lateral sector bile duct had stenosis and invasion to left portal vein bifurcation was diagnosed. Left t trisectionectomy or segment 4, 5, 8 resection with portal vein resection was planned at first. By 3D-CT volumetry, posterior segment was small, so we selected segment 4, 5, 8 resection. Right portal vein was reconstructed by end to end anastomosis, and left portal vein by side to end anastomosis using external iliac vein graft. Three posterior sector bile ducts and three lateral sector bile ducts were anastomosed to jejunum with a Roux-Y loop. All bile duct margins were free from cancer microscopically. The maximum serum total bilirubin level was 10.9 mg/dl on the first operative day. The post-operative course was uneventful. We reported our recent experiences of type 4 hilar bile duct carcinoma. Rationale operation can be possible with precise pre-operative diagnosis and simulation.

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Extrahepatic Bile Duct Injury During Laparoscopic Cholecystectomy

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Background: Despite clear advantages of laparoscopic cholecystectomy, the injury of hilar structures, particularly of the common bile duct, remains the subject of continuous discussion. Materials/Methods: From April 1998 till September 2002, 33 patients with damage of extrahepatic bile duct during laparoscopic cholecystectomy were referred to our institution. All patients underwent elective surgery because of symptomatic cholelithiasis in a hospital elsewhere. In 5 of these patients, the bile duct damage was diagnosed at the time of laparoscopic procedure leading to conversion to open surgery. In 3 other patients the bile duct damage was recognized early postoperatively and endoscopic bile duct stenting was performed subsequently. All 33 patients underwent laparotomy in our institution. Results: Complete transection of the common bile duct was present in 29 patients. In 2 patients iatrogene stenosis of the common bile duct was found. Two patients had significant tangential duct lesions. Of the patients with complete bile duct dissection, in 4 individuals damage of the common hepatic artery and right hepatic artery, respectively, took place. Reconstructive surgery included Roux-en-Y bilioenteric anastomosis in 31 cases, in 3 of them over endoluminal drain. Additionally, in 2 of the patients, reconstruction of the hepatic artery was performed. Two patients required right hepatectomy. In one of these patients a Klatskin tumor not recognized at the time of laparoscopy was disclosed. The postoperative outcome was uneventful in 30 patients. Intraabdominal abscess required relaparotomy in one patient. Two patients with complex bile duct and arterial damage died postoperatively, one in septic organ failure and the other one due to hepatic insufficiency while waiting for liver transplantation. Conclusion: Injury of common bile duct presents a dangerous and potentially lethal complication of laparoscopic cholecystectomy, in particular in cases of concomitant vascular injuries. Early recognition of the lesion is extremely important. It is advisable to treat these patients in specialized centers since their management frequently requires advanced knowledge in hepatobiliary surgery.

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The Implications of Male Gender on Outcomes of Cholecystectomy

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Background: Little data exists regarding the impact of male gender upon the outcome of cholecystectomy for symptomatic cholelithiasis. Objective: The purpose of this study is to determine the impact of male gender on outcome of cholecystectomy in terms of the incidence of acute and gangrenous cholecystitis, the rate of conversion from laparoscopic to open cholecystectomy, and the incidence of biliary complication. Setting: A university-affiliated urban hospital. Methods: All patients undergoing cholecystectomy at a single institution from January 1996 to March 1999 were included in this analysis. Data was tabulated from hospital records and analyzed with appropriate statistical methods. Results: 1070 patients underwent cholecystectomy over the study period. 908 (84.9%) patients were female (M: F ratio = 1:5.6). Patient ethnicity was Hispanic in 829 (77.5%), White in 73 (6.8%), African-American in 34 (3.2%), Asian in 28 (2.6%), and not defined in 106 (10%). 817 (76.4%) procedures were laparoscopic, 204 (19.1%) were converted from laparoscopic to open and 49 (4.6%) were open. The indications for cholecystectomy were as follows: 471 (44%) biliary colic, 380 (36%) acute cholecystitis, 145 (13.6%) gallstone pancreatitis, 63 (5.9%) choledocholithiasis, and 11 (1.3%) other. Biliary complications were comprised of bile leak and biloma in 25 (2.3%) cases and bile duct injury in 10 (0.93%). In univariate analysis, men were more likely to undergo cholecystectomy for acute cholecystitis (39.5% vs. 23.3%, p = 0.0001), develop gangrenous cholecystitis (25.3% vs. 11.2%, $p=7\times10^{-6}$), require conversion to open cholecystectomy (42.8% vs. 16.1%, $p = 10^{-16}$) and suffer biliary complication (7.4% vs. 2.6%, p=0.04). Male gender was found to be independent of age, race, surgical indication, and length of preoperative hospitalization in a multivariate analysis. Conclusion: Male gender is associated with acute cholecystitis, gangrenous cholecystitis, conversion to open cholecystectomy and biliary complication.

Results of Multivariate Analysis

Endpoint	Odds ratio with male gender	95% CI
Acute cholecystitis	2.08	145–2.97
Gangrenous cholecystitis	2.57	1.69–3.91
Conversion to open	3.24	2.23–4.70
Biliary complication	2.79	1.36–5.75

All p-values less than 0.001.

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Biliary Reconstruction: Can Classic Techniques Meet New Challenges?

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Introduction: Classic techniques in biliary surgery were developed to treat common duct strictures after cholecystectomy. In the past decade, the expansion of hepatic surgery has required more complex reconstructions using either primary or bilio-intestinal anastomosis. We analyzed the incidence of leak and stricture as a function of the complexity of the reconstruction in all cases requiring biliary reconstruction. Methods: Between 1/1/98 and 12/1/01, 231 reconstructions were performed 62 pediatric and 169 adult cases in 4 indications: 181 primary transplants (PT), 23 transplant revisions (TR), 19 benign obstructions (BO), 8 malignant obstruction (MO). Among PT, 120 were standard OLT while 34 were right lobe grafts (RL), 14 left lobe (L), and 31 left lateral segment (LL). Biliary intubations (T-tube or stent) were used in 30 (13%). Fifty-five had multiple duct anastomoses (MDA)(24%), with 2 duct (37:67%), 3 ducts (13:24%), or 4 ducts (3: 5%). Results: Leaks occurred in 12.5% of MO, 5% of BO, 12.7% of PT and 0% TR (p=.25). Neither leaks (12.7% vs 11%) nor strictures (12% vs 16%) were more common in MDA. Leaks were most common (34%) after RL transplants (p=.019). Strictures occurred in 12.5% of MO, 5% of BO, 16% of PT and 15% TR (p=.77). Strictures were most common (29%) after LL transplants (p=.0012). Interventional (25%) or endoscopic procedures (5.6%) were required in 67 patients overall (29%). The actuarial stricture or leak free

survival was 68% at 48 months and only 3 patients (1.3%) died, or lost transplants due to biliary complications. Conclusions: These results indicate that classic biliary reconstruction is sufficiently robust to accommodate the demands of partial grafts and extensive tumor resections. Up to 4 duct anastomoses were constructed with a complication rate not different from single anastomosis. Generous use of interventional techniques was essential to successful long term outcomes. The impact of this analysis is somewhat limited by a relatively short follow-up period.

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Major Iatrogenic Injury of Biliary Tract During Laparoscopic Cholecystectomy

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The laparoscopic cholecystectomy is nowadays teh gold satndard treatmenr for cholelitiasis. Despite the expertise gained in performing the procedure, biliary tract injury remains the most serious complication. It is important for the affecte patient's prognosis not only to recognize the lesion during the operation or at least at the earliest moment but also to choose the best therapeutic modality for the biliary tract reconstrution. Through a anonymous qustionnaire patients who underwent laparoscopic cholecystectomy between March 1994 and Novermber 1999 were analuzed. A series of 17 patients presenting with major iatrogenica biliar tract injury was available. Parameters analyzed were mechanismos of injury, methods of diagnosis, therapeutic modalties and early and long term results. The iatrogenic injury was mainly in elective surgery, without factors reisk, anatomical anomalies or bleeding. It was intraoperatively identified in 52,9% of the patientes and in its majority the procedure was converted for repair.The abdominal ultrasound and endoscopic retrograde cholangiopancreatography are important methods for the diagnosis of iatrogenic injury in the postoperative period. The lesions were classified according to STRASBERG. The repair was either endoscopically or surgically made being the hepatic-jejuno anastomoses with Y of Roux the treatment with best results, independente of lesion level.

BILIARY
Other

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ERCP, Papillotomy and Internal Stenting: The Treatment of Choice of Bile Leak Following Complex Hepatic Trauma

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Introduction: Trends toward non-operative treatment and judicious usage of "damage control" techniques improved dramatically the outcome of complex hepatic injuries. Bile leak secondary to hepatic trauma (blunt or penetrating) is a challenging complication. Application of ERCP in this setting was suggested before but its role in the "conservative approach" is not yet established. Patients and methods: Accumulated experience in the management of traumatic bile leak is presented. All patients were initially treated according to the ATLS principles. Bile leak was diagnosed by fistula to surgical wounds or intra-abdominal drains or by HIDA scan. ERCP, papillotomy and temporary (6-10 weeks) trans -papillary stenting were performed within 24 hours after diagnosis. Enteral feeding was maintained. Recovery was defined as the cessation of leakage. Results: Between 1996 and 2001, 6 patients were treated accordingly. The clinical data is summarized: Conclusions: ERCP, papillotomy and temporary internal stenting, together with percutaneous drainage of intra-abdominal or intra-hepatic collections is safe and efficient in the management of bile leak following both blunt and penetrating hepatic trauma. It is recommended as the treatment of choice in patients who were managed non-operatively and also in those who were operated before to control bleeding or other intra-abdominal injuries.

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Prooxidant-Antioxidant State in Rat Plasma During the Creation of Supraduodenal Cholestatic Condition

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Aim: We investigated the prooxidant-antioxidant state in rat plasma under the conditions of supraduodenal cholestasis for 3 or 10 days. Material and methods: Experiments were performed in 28 male rats Wistar (250,0 (10,0 g). In 14 treated animals the common bile duct was ligated near its inflow into the duodenum, with its complete dissection between two silk ligatures (no duct ligation in 14 control animals). After 3 and 10 days (n = 7 treated and 7 control rats for both times) the conjugated diene (CD), malondialdehyde (MDA) and Schiff base (SB) concentrations, superoxide dismutase (SOD) and catalase (CAT) activities and antioxidant vitamin (α-tocopherol and retinol) levels were determined according to V.B. Gavrilov et al. (1988), R.S. Timoshin et al. (1987), B.L. Fletcher et al. (1973), S.I. Chevary et al. (1985), M.A. Koroliuk et al. (1988) and V. Ch. Chernyuskene (1984), respectively. Results: 3 days of cholestasis resulted into higher MDA content (27.57 $\pm$ 3.2 nmol/ml vs. 17.57 $\pm$ 2.96 in control; $P < 0.05$). SB levels insignificantly increased from 13.01 $\pm$ 2.55 to 20.43 $\pm$ 10.21 units/ml, however CD concentration decreased from 38.57 $\pm$ 4.57 to 24.77 $\pm$ 5.74 nmol/ml ($P < 0.05$) - perhaps, due to the lower contents of their precursors (polyunsatu-

Age/ mechanism	Hepatic injury, co-injuries	Initial treatment	Presentation, leakage site	Outcome
21/ GSW	Tear, massive bleeding, ACS	Packing, decompression	E-C fistula, right hepatic duct	Complete recovery
45/ GSW	Tear, massive bleeding, gastric perforations	Laparotomy (X2) LHA ligation	E-C fistula, left hepatic duct	Complete recovery
60/ MVA	Intra-hepatic hematoma, tear	Observation, percutaneous drainage	Bile ascites, sepsis, R lobe–peripheral	Complete recovery
31/ MVA	Tear	Packing, pe-operation (X2)	Bile in drain, left hepatic duct	Complete recovery
29/ MVA	Tear	Observation	Biloma, sepsis, R lobe–peripheral	Complete recovery
34/ Stab wound	Tear, gastric perforation, pancreatic tear	Laparotomy, distal pancreatectomy	Bile in drain, R lobe–peripheral	Complete recovery

ACS, abdominal compartment syndrome; E-C, entero-cutaneous; LHA, left hepatic artery.

rated fatty acids) in plasma lipids and phospholipids. SOD activitiy decreased from 78.48 ± 3.44% to 58.66 ± 7.25% comparing with the level without inhibition, and CAT activity diminished from 280.91 ± 32.23 to 79.42 ± 20.44 micromol(ml^{-1})(min^{-1} (both $P < 0.05$). α-tocopherol content fell by a factor of 1.3 and retinol content insignificantly decreased. 10 days of cholestasis lead to even higher activities of the free radical processes, with significant rise in SB, lowering of SOD and CAT activities by the factors of 1.4 and 1.5 respectively; (-tocopherol and retionl contents decreased by the factors of 1.6 and 2.1 respectively. Conclusion: We observed that the degree of antioxidant system inhibition enhanced with a time of supraduodenal cholestasis. By our opinion, such enhancement was due to: 1) inhibition of antioxidant enzymes by lipid peroxidation products (supported by more significant increase in SB production); 2) lower contents of antioxidant vitamins resulting from their accelerated utilization for the free radical inactivation and/or from their decreased intestinal absorption caused by disturbed enterohepatic bile acid circulation.

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Adjuvant Therapy for Biliary Malignancies: International Trends and Possibilities

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Major surgical resection of Stage II, III and IVA gallbladder cancer and hilar cholangiocarcinoma has become standard around the world. However, these malignancies still have a poor prognosis after resection and are uncommon even at tertiary hepatobiliary referral centers. As a result, no prospective randomized trial of adjuvant therapy has ever been performed. Therefore, we distributed a biliary cancer questionnaire to hepatobiliary oncology experts in the Americas, European and Asian/Pacific regions to determine 1) interest in participation in an international trial and 2) current experience with respect to patients and adjuvant therapy. Questionnaires were received from 331 specialists at 262 centers in 39 countries on six continents. Responses came from 164 centers (63%) in North, Central and South America, 45 centers (17%) in Europe/Middle East/Africa, and 53 centers (20%) in the Asian/Pacific region. Eighty-eight percent of specialists and 85% of centers were interested in participating in an adjuvant therapy protocol. However, only 25% of the centers resect gallbladder cancers in 6-15 or more patients per year, and 30% of centers resect hilar cholangiocarcinoma in 6-15 or more patients per year. Similarly, the ability to randomize 6-15 or more patients was considered feasible in 15% of centers for gallbladder cancer and 21% of centers for hilar cholangiocarcinoma. Infusional chemotherapy was available in 99% of centers. However, conformal radiation could be delivered in only 88% of centers with regional differences, Americas - 93%, Asia/Pacific - 84%, and Europe - 73%. When asked about current treatments, only 2% of centers use photodynamic therapy, and 32% use brachytherapy. Current use of adjuvant chemotherapy, external beam radiation therapy (EBRT), and chemoradiation can be seen in the table. The results of this questionnaire suggest that 1) regional differences exist in the current management of biliary malignancies, 2) the vast majority of hepatobiliary oncologists want to participate in an adjuvant therapy trial, and 3) adequate patients and facilities are available in approximately 50 centers around the world. We conclude that a prospective, randomized trial of adjuvant therapy in resected biliary malignancies is feasible with international cooperation.

	Americas	Europe	Asia/Pacific	Total
Chemotherapy	66%	68%	79%	68%
EBRT	70%*	29%	40%	59%
Chemoradiation	71%*	29%	55%	63%

*$p<0.05$ versus other groups

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Urinary Excretion of Organic Anions and Cations in Bile Duct-Ligated Rats

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Purpose: In patients with complete bile duct obstruction, the only pathway of the elimination of cholephilic compounds is through the urine. Although the changes of various transporters in the liver and kidney in cholestasis have been elucidated, little is known about how effectively the elimination of these compounds is compensated by the urinary excretion. Therefore, in the present study, the urinary excretion of various cholephilic compounds was compared in bile duct-ligated rats for 3 days (BDL) and Eisai hyperbilirubinemia rats (EHBR), an Mrp2-deficient rats. Methods: Male SD rats (250 g) were anesthetized, and the common bile duct was ligated. Three days later, BDL, EHBR and control rats were anesthetized again, and the urinary bladder and the femoral vein were cannulated. Thirty min after the urinary bladder cannulation, radiolabeled organic anions and cations were intravenously injected, and urine samples were collected every 1 h for 4 h. The radioactivity in the urine and in the serum and liver samples after the experiments was counted. Urinary excretion was shown as the cumulative % dose per 4 hr. Results: Among organic anions, pravastatin (86±4% in BDL, 36±2% in EHBR and 6±1% in controls) was more effectively excreted into the urine than leukotriene C4 (59±7% in BDL and 18±3% in control) and temocapril (51±1% in BDL and 38±4% in EHBR and 22±2% in controls) in BDL. The efficacy of the urinary excretion of pravastatin was in the order of BDL>EHBR>controls. Between organic cations, erythromycin (49±5% in BDL and 19±4% in controls) was more effectively excreted into the urine than vinblastine (26±1% in BDL and 6±3% in controls) in BDL. The radioactivity in the serum and liver at the end of the experiments was about 0-3% with all materials. Conclusions: Although the elimination of the cholephilic compounds was compensated by the urinary excretion in BDL, the degree of the urinary excretion was different among the compounds, possibly due to the affinity of these compounds to the renal transporters.

HPB Endoscopy

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Day Case ERCP

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Aim: Many centres admit patients for ERCP. This is contrary to cost containment. The purpose of this study was to identify factors, which precluded Day case ERCP, its safety and complications associated. Methods: Medical records of patients undergoing ERCP (Both Day

case and Inpatients) in the year 2001were reviewed retrospectively. Day case patients were discharged the same day following observation for a mean period of 4 hrs and bloods checked. Results: A total of 160 patients between the age of 40yrs - 90 yrs underwent ERCP. The success rate was 153/160. OPD Patients: n=88. ERCP + sphincterotomy: 32, ERCP: 51, Stent removal / changing: 3, Failed: 2. Three patients stayed in (post-procedure pancreatitis = 2, Periampullary carcinoma = 1). Post procedure Hyperamylasemia (Amylase 1 and 1/2 times normal) was encountered in 3 patients. Inpatients: n=72. ERCP + Sphincterotomy: 14, ERCP: 32, Stenting: 4, Failed: 5. Of these 36 patients were discharged the following morning. Patients staying in were: Post procedure pancreatitis: 1, Malignancy detected in 24 patients, gallstone pancreatitis: 9. Hyperamylasemia was encountered in 12 patients. No other complication was encountered nor was any morbidity. Conclusion: Post procedure hyperamylasemia does not influence the practise of Day case ERCP nor does the diagnosis or Age. Patients with pre / post procedure pancreatitis stayed in for convalescence (n = 9 and 3). Patients with diagnosis of malignancy were kept in for further treatment. These findings show that Day case ERCP is safe and effective with significant cost savings.

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Self-Expandable Stents in Palliative Treatment of Malignant Obstructive Jaundice

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Endoscopic, transduodenal insertion of self-expandable stents (SES) in palliative treatment of and biliary malignant strictures opened a new approach in endoscopic surgery. According to recent publications SES show their superiority over standard PTFE stents in permeability period. This paper presents results of application of SES in palliative treatment of obstructive jaundice caused by tumors of pancreatic-biliary area and infiltrating gastric carcinoma. Between 08.1999 and 06.2002 49 procedures of SES stenting of extrahepatic biliary ducts were performed.26 females and 23 males were treated, average age of 64 y.o (47-87 y.o.) Most common indication was advanced Klatskin tumor (KT)- 32 cases (group A). 17 patients were treated due to obstructive jaundice caused by pancreatic , gallbladder or gastric carcinomas. All patients were previously disqualified from radical surgical treatment or underwent palliative or diagnostic laparotomies.We analyzed lab parameters of obstructive jaundice, permeability period, need of additional procedures to alleviate the jaundice and intra- and postoperative complications. Results: In all 49 patients significant decrease in laboratory parameters of jaundice (bilirubine level, alkaline phosphatase) was observed.Among patients in group A (KT) 4 (12.5%) required additional treatment due to stent overgrowth. In group B - 6 (35.3%) patients required additional treatment. In 1 case in group B, patient with advanced pancreatic cancer, infiltrating the duodenum and CBD had the duodenal SES introduced, then CBD SES was passed through duodenal stent.No serious intraoperative nor postoperative complications were observed. Conclusion: Self-expandable stents in palliative treatment of malignant obstructive jaundice offer significant alleviation of jaundice lab parameters as well as long permeability time compared to patient survival time. Lack of serious complications makes SES endoscopic application safe alternative to palliative surgical treatment.

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Carcinoma of the Papilla of Vater - Are Endoscopic Appearance and Endoscopic Biopsy Discordant?

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Carcinoma of the papilla of Vater is classified as periampullary cancer; 5% of all gastrointestinal tract malignant. Early and accurate diagnosis is important for those patients with tumor of the papilla as the prognosis is more favorable than others periampulary neoplasms. Endoscopically obtained biopsies from suspicious papillas can establish an early and immediate preoperative diagnosis, although even for skilled pathologists it is difficult to distinguish carcinomas from non-invasive lesions on the basis of forceps biopsies. The purpose of this study was to asses the preoperative diagnostic sensitivity of duodenoscopy appearance and biopsy in all suspicious of tumor. Twenty eight patients with suspicious of carcinoma of the papilla of Vater and with final diagnosis established by specimen from duodenopancreatectomy were included in this retrospectively study .In each case, a comparison was made between endoscopic biopsy and duodenoscopic appearance. A final diagnosis was established by surgical specimen. After surgery, the resected tumors of the papilla of Vater were definitely diagnosed as adenocarcinomas in 96.4% (27) and inflammatory non-neoplastic lesion in 3.6% (01). Duodenoscopic appearance sensitivity for malignancy was 85.1% (23) .One case was diagnosed falsely as positive by duodenoscopic appearence. Endoscopic biopsy sensitivity was 70.3% (19). When we compared both methods, we noticed that concordance value was 53.6% (15/28) and discordance value was 46.4% (13/28). Preoperative diagnosis of carcinoma of the papilla of Vater is mandatory for making therapeutic decisions. In this casuistic, endoscopic biopsy was more limited than endoscopic appearence in preoperative diagnosis of carcinoma of the papilla of Vater.

HPB Imaging

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WITHDRAWN

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Positron Emission Tomography to Assess Response of Hepatocellular Carcinoma to Yttrium-90 Theraspheres Treatment

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Purpose: The use of Yttrium-90 Theraspheres provides localized radiation therapy to unresectable hepatocellular carcinoma (HCC). The microspheres often induce necrosis of tumor tissue without reducing lesion size, making assessment of therapy difficult with conventional imaging modalities. We reviewed our experience using positron emission tomography (PET) to monitor treatment response. Methods: The medical records and radiologic studies of 9 patients with HCC who were treated with Yttrium-90 Theraspheres between February 2002 and July 2002 were reviewed. Six patients have undergone both pre- and post-treatment PET and CT scans. All imaging studies were reviewed by a single nuclear medicine physician (K.M.) to determine standardized uptake values (SUV) and CT scan correlation. Results: There were 3 women and 3 men with a median age of 61 years (range, 49-78). There were a total of 10 hepatic tumors treated. The average pre- and post-treatment SUV of 10 hepatic lesions in these patients were 3.27 (range, 1.8-7.1)) and 1.92 (range, 1.4-4.1) respectively, with an average decrease of 1.35 (p=.047). Background SUV of the liver ranged from 0.8 to 2.1. Only 1 hepatic lesion demonstrated an increased SUV after Theraspheres treatment. For all 6 patients, CT scan findings post-treatment were equivocal for improvement or progression of disease. In the 4 patients in whom alpha-fetoprotein levels were available pre- and post-treatment, 3 patients had increased tumor marker levels when PET scans demonstrated decreasing SUV, while the fourth patient with an increased post-treatment SUV had no significant change in alpha-fetoprotein levels. A fifth patient had a normal alpha-fetoprotein level before Theraspheres treatment and no further levels were collected. Review of pathology indicated 3 patients had moderate to well-differentiated carcinomas. Conclusions: PET scanning after Yttrium-90 Theraspheres treatment for HCC demonstrates significantly decreased metabolic activity. Correlation with tumor marker levels and clinical outcome is necessary to determine if the use of functional imaging is accurate in the assessment of treatment response and efficacy.

HPB
Other

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Operative Experience of U.S. General Surgery Residents: Liver and Pancreas 1989–2001

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Background: Paradigm shifts in the care-strategies of patients with hepato-pancreatico-biliary (HPB) diseases have occured in the last decade. Limited data is available to quantify how these have affected the U.S. general surgery resident HPB experience over the same period. The aim of this study was to determine the liver and pancreatic operative experience reported by chief residents of U.S. general surgery residencies. We hypothesized that the U.S. chief resident operative experience in liver and pancreatic diseases has increased over the past decade. Methods: The Resident Statistic Summaries (Report C) of the Residency Review Committee (Surgery) for 12 academic years from 1990 through 2001 were obtained. The reported pancreatic and liver-related operative experience for all U.S. surgical chief residents were compiled and analyzed. Results are expressed as the average number of operations performed, the standard deviation and the percentage of total operative cases. Results: The average number of residents per general surgery program has increased by 13% since 1990. Since 1990, the average total chief resident reported liver and pancreatic operative cases have increased by 50% and 40%, respectively. In regards to liver-related operations, chief residents are reporting an increase in lobectomy and segmentectomy from an average of 1.2 in 1990 to 2.3 in 2001 with a standard deviation of 1.6 and 3, respectively. For pancreatic-related operations, the average number of Whipple operations reported by chief residents has increased from 1.1 to 2.6 operations with a standard deviation of 1.27 and 3, respectively. In contrast, the reported surgical drainage procedures for pancreatic abscesses and pseudocysts have diminished by about 33%. Conclusions: U.S. surgical chief residents have a widely variable experience in liver and pancreatic surgery. However, their experience in all liver and pancreatic surgery has increased since 1990. While the surgical experience in surgical drainage procedures for pancreatic pseudocyts have decreased, this has been balanced by a large increase in the number of pancreaticoduodenectomies performed over the past 10 years. Additionally, the majority of U.S. surgical chief resident liver-related experience consists of lobectomy or segmentectomy.

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Microbiology of Nosocomial Infections in a Unit of Hepato-Pancreatic-Biliary Surgery

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Introduction: Nosocomial infections (NI) make up an important problem Public Health Care. NI are an important cause of hospital morbility and mortality, and contribute, also to increase mortality, causing to important social and economic costs. In our environment, among a 3 and 14% of patients that enter in hospitals of sharp they develop one NI. From an etiologic point of view they are characterized by their constant evolution over time, experiencing in the last years a resurgence of the grampositivos. Aim: The aim of this work is to know microorganims of NI having happened during 1 year in a HPBS unit with medical staffs and nurses's very small number. Material and Methods: The patients included in the study have been those entered or transferred, programmed or urgent, of other units to the HPBS. We was considered infection according to the CDC approaches. We was counted daily during one year: it dates of entrance, discharged, surgical intervention, ASA, intrinsic and extrinsic risk factors, nosocomial infections, its microbiology, reoperations. The data were picked up by a single doctor during that whole time. Results: 468 patients were analyzed during a period of one year. The mean age was of 59.78 years. 50.54% of the series was male and 12.45% bigger than 80 years. Of the 422 interventions, the surgery in 53.68% was clean-contaminated. There were 112 NI, what supposed a rate of accumulated infection of 23.93%. The NI more frequent was surgical infection (SI) (45.54%) and inside the same ones the surgical of organ or space (45.10%). In 75% of those NI it was microorganims responsible. The frequently isolated microorganism was the SARM (21.43%) after Escherichia coli (14.29%), Pseudomona aeruginosa and other Streptococcus (7.14%). In clean- contaminated surgery the most frequent isolated germ was SARM, in the contaminated one it was E. Coli and in dirty it was Streptococcus others. 54.35% of the SI was caused by grampositives coco. Conclusions: The rate of infections incidence is elevated in comparison with other surgical departments but inside the limits of the NI in pathology of the HPBS. It is appreciated, the same as in other series a resurgence of grampositives like responsible for NI and, in short of the SI. This could rebound in the empiric antibiotic politics of this unit, although the appropriate prevention was a protective factor statistically significant.

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Are Benefited Patients of Unit Hepato-Pancreatic-Biliary Surgery of Predeposit Self-Transfusion Program? Yes

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Introduction: HPBS has a high morbility and mortality. The anemia is a habitual discovery in perioperatory, being frequent hemoderivates consumption (of the 23 to 41%). It is known the possible adverse effects of the use of allogenic blood. We present our first and half year of our program PST. Patient and Methods: A prospective study was carried out from May of 2001 to April of 2002 of the patients remitted to

our Blood Bank for their inclusion in the program of PST (rejected and admitted) and of those in those that it was reserved units of allogenic blood before surgery. They were not included: the deaths neither the reoperates since alone they happened in the allotransfunsed group, neither preoperative transfused neither those that didn't enter in way programmed to the hospital. The following variables were analyzed: sex, age, illness, requested units, obtained units, transfused units, use of alogenic blood, surgical time, hospital stay, ICU and InCU stay, morbility, hemoglobin levels and hematocrite. Results: Of the 50 included patients 22 patients were remitted to the program of PST (44%), but 6 were rejected (27%): 2 for hepatitis, 2 for cardiopaty, 1 bad veined access and 1 for epilepsy. A patient rejected surgery once completed the whole program of PST. 24 were transfused (42.9%), 66% of those rejected and 53% of those admitted. 33% of the series was neoplasic diseases. 38 units were requested and 36 were obtained (94.7%). 42.7% was used. 33.3% of the included patients the PST received allogenic units. The mean stay of transfused patients with allogenic blood in the ICU and hospital it was of 6.4 and 29 days in front of 2.5 and 7 days of the rest. When having the patients stays in ICU and InCU and to compare both groups the result it was of 5.2 and 39.2 days in front of 1.2 and 10.9 days. The alone bigger complications happened in those patients that were almost allogenic transfused with a frequency 7 times more. Comments: Our data of PST show that it is an effective method and insurance to avoid and to diminish the exhibition to allogenic blood of selected patients that they will be subjected to HPBS. Possibly, they would benefit of a rate smaller than complications and of some stays in ICU and Hospital smaller.

CASE REPORTS: LIVER

Infection

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Amebic Liver Abscess in a Neonate: Report of a Case

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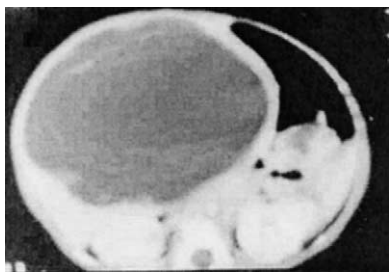
Introduction: The amebic liver abscess (ALA) is produced by the invasion of the Entamoeba hystolytica in the hepatic parenquima. It is considered as one of the major complications of the amebic infestation. The prevalence of the ALA is higher in areas of poor economic and sanitary conditions. We present a case of a ALA that presented in a neonate. Case Report: A neonate of 20 days of life that presented mucous diarrheal stools during the last 4 days. At the physical examination, the patient was febrile, and with signs of dehydration. The abdomen was tense, and an abdominal mass was palpated in the right upper abdominal quadrant. CT scan reported a cystic hypodense image of 7.4 × 9.7 cm in the caudate, and part of the right lobe (Fig 1).



CT scan of the neonate, presenting an hypodense cystic image in the hepatic right lobe.

The patient was admitted and the mass was surgically resected. Histopathologic analysis confirmed the diagnosis of ALA. At the fouth postoperative day, the patient was reoperated presenting an acute abdomen with clinical signs of peritonitis, dying in the early postoperatory. Discussion: ALA is a serious disease common in our country, due to the endemic presence of the E. hystolytica. The spread of the trophozoite is from the colon, through the gut circulation to the liver and other tissues, even the fetal circulation. It presents usually between the third and fourth decade, but rare cases had been reported in scholars, and young children. Cases in neonates are the rarest. It appears in children with a deteriorated health condition, making the prognosis very poor. The diagnosis of ALA is based on unspecific clinical signs like fever, abdominal tenderness, abdominal mass, cough, vomit, diarrhea, and jaundice. In our case, the treatment of choice was the surgical resection because of the size and risk of rupture with spread of its content.

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An Unusual Presentation of Hepatic Amebiasis During the Puerperium

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Amebic liver abscess caused by the Protozoan organism, Entameba Histolytica, is endemic to many parts of the developing world. Invasion of the colonic mucosa results in amebic dysentery, and in some cases dissemination to the liver results in abscess formation. Amebic liver abscess is a rare complication of pregnancy and there are few reports in the word literature. We present a unique case of amebic colitis during pregnancy resulting in cecal perforation in multiple liver abscesses immediately post partum. A 19-year-old gravida 2, para 2, female presented during her second trimester with fever, abdominal pain, and diarrhea. She was evaluated at a local ER, however, her symptoms resolved spontaneously and no specific treatment was instituted. The remainder of her pregnancy was unremarkable, and at term, she had an uncomplicated cesarean section. Four days after discharge, she presented with right lower abdominal pain. She was evaluated in the local E.R. and was discharged home. However, her symptoms continued and she again developed diarrhea. She returned to the hospital with hypotension, marked leukocytosis and fever. Due to clinical deterioration, she was taken to surgery and was found to have a perforated cecum with frank pus in the peritoneum. A right hemicolectomy and ileostomy was performed. Postoperatively, the patient had persistent fever and a repeat CT scan on postoperative day 5 revealed multiple liver abscesses. She was transferred to our Liver Disease Center and on arrival was febrile, hypotensive, and tachypneic. One large abscess beneath the pericardium was percutaneously aspirated for fear of impending rupture. Gram stains were negative for bacteria and the diagnosis of amebiasis was suspected. The patient was treated empirically with metronidazole. Subsequent analysis of the aspirate revealed amebic trophozoites. The patient slowly improved and was discharged home on oral metronidazole for one month. Outpatient follow-up revealed a young, healthy mother and child. To our knowledge, this is the first description of multiple hepatic amebic abscesses with bilobar distribution in the puerperium. This case demonstrates the difficulty in the diagnosis of intraabdominal pathology during pregnancy and the puerperium. The clinician must have high index of suspicion to establish the correct diagnosis.

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Intrahepatic Cholangiocarcinoma Masked as Fever of unknown Origin

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Intrahepatic Cholangiocarcinoma (IHC) is rare liver tumor, representing 5% of primary hepatic malignancies. Typically, tumors of this origin present with vague abdominal pain, pruritus, or jaundice. Surgical resection remains the main stem of therapy. Herein, we describe an unprecedented case of a patient with an anplastic IHC who presented with a fever of unknown origin (FOU). The patient was a 64 year old Caucasian gentleman who presented with fever of unknown origin for several weeks. History and physical examination were unremarkable, particularly for abdominal pain, pruritus, or jaundice. The only physical finding was a persistent elevated temperature (100 F). Laboratory finding confirmed a subtle elevation of the liver function tests. A CT of abdomen demonstrated a left hepatic hypodense lesion suspicious for an abscess. A CT- guided aspiration was performed and revealed an anaplastic carcinoma with necrosis. An extensive metastatic evaluation was negative, with exception of a marginally elevated CA 19-9. Left hepatectomy was performed without complication. The intraoperative findings included a large mass within segments 2 and 3 with extension to segment 4. Tumor extended into the hepatic base of the falciform ligament, while was completely resected. Histopathology revealed a 13 cm IHC with an anplastic component. A single focus of soft tissue involvement was confirmed at the supero-medical margin. Postoperatively, the patient did well and was referred for adjuvant treatment. IHC associated with FUO is extremely rare and represents a diagnostic dilemma. The lack of associated clinical findings may add to the complexity of the scenario. Persistent diagnostic strategies and prompt surgical evaluation are warranted.

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Liver Transplantation for Hereditary Hemorrhagic Telangiectasia

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Introduction: Hereditary hemorrhagic telangiectasia (HHT), or Osler-Weber-Rendu syndrome, is an autosomal dominant disorder characterized by mucocutaneous and visceral vascular malformations. While liver involvement is less common, its manifestations can be severe. We recently performed orthotopic liver transplantation (OLT) for HHT and believe this should be considered the primary approach for carefully selected patients with significant liver involvement. Case Report: A 50-year-old woman referred with HHT had a history significant for skin telangiectasias since childhood, recurrent episodes of epistaxis, and anemia secondary to GI bleeding. Two brothers had documented HHT. Physical examination showed multiple mucocutaneous telangiectasias, jugular venous distension, a systolic murmur with hyperdynamic precordium, an enlarged, pulsatile liver with a bruit, and ascites. Evaluation included: right heart catheterization showing a high cardiac output; normal pulmonary arteriography; MRI, CT, and ultrasound of the abdomen showing splenomegaly and hepatomegaly with multiple shunts and early filling of the hepatic venous system; and vena caval oximetry showing an oxygen saturation step-up from 67% to 87% between the infra and suprahepatic IVC. Based on these findings, the patient underwent OLT. She required two units of blood during the uncomplicated procedure and was discharged on postoperative day 4. Her course was complicated by an episode of acute rejection that responded to pulsed steroids. Summary: HHT affects approximately 1/500-1/8000 persons. The classic picture is a familial pattern of mucocutaneous telangiectasias and epistaxis. Symptomatic liver involvement is found in 8-31% of patients. Telangiectasias may be present with or without cirrhosis or fibrosis. Liver failure may be present, and large vascular malformations can lead to high output cardiac failure. Many authors have described embolization or ligation of the hepatic artery to limit shunting. Results have been mixed, with mortality rates up to 20%, and no long-term follow-up has been published. Complications include hepatic and biliary necrosis, sepsis, and GI bleeding, and recurrence is common. OLT represents a definitive treatment for symptomatic HHT from liver involvement. Since 1995, 14 patients have been reported who have undergone OLT for HHT, many where OLT was used as rescue therapy after failed embolization, with good results in follow-up of 9-65 months. Conclusion: OLT should be considered as initial therapy in patients with significant liver involvement from HHT. OLT reduces the risks of high output cardiac failure and may reduce GI bleeding from mucosal vascular malformations by eliminating portal hypertension. While theoretically attractive, embolization does not appear to be a successful approach for this disease.

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Accessory Hepatic Lobe Torsion Simulating Acute Cholecystitis: Laparoscopic Treatment

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The liver is not subject to grade or frequent to deviation from its usual form in relation. It has been found without any division into lobes. On the other hand, a case has been recorded in which adult liver was divided into twelve lobes. In similar cases excess lobe has been observed. The authors describe a case of a 30 years old female patient presenting right side abdominal pain with nausea and sinals of peritoneal irritation. Laboratorial exams showed no alterations. Abdominal ultrasound reveled acute cholecystitis alithiasis, thickened with dense liquid content as well as inflammation secretion around. During laparoscopic approach, the gallblader was turned out to be deleted with discret thickened wall. In the hepatic lobes, an accessorie hepatic was identified, twisted and necrotic. The base of the lobe was then dissected and ressected using ultrasound cautery. Acute cholecystitis was present due to isquemic and cholecystectomy was performed. The successful outcomes was achieved by resecting the torsed accessory lobe. The patient remained asymptomatic. It was recorded a very rare anatomic abnormality with a small number of cases described in the literature.

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Portal Vein Thrombolysis

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Purpose: To evaluate the safety and efficacy of catheter directed thrombolysis for treatment of portal vein thrombosis. Materials and Methods: Three patients (2 female, 1 male; age range 20 - 58) presented with abdominal pain and imaging documented progression of portal vein thrombosis despite anticoagulation. Two patients had portal vein thrombosis of unknown etiology. One patient had thrombosis post splenectomy. All three patients had catheter directed portal vein thrombolysis with improvement in symptoms. Two patients had intravascular stents placed as part of their treatment. Results: All patients had decrease in clot burden and improvement in flow after catheter directed thrombolysis. All three patients had improvement in their abdominal pain sysmptoms. Conclusions: Catheter directed thrombolysis can aid in the management of portal vein thrombosis when anticoagulation has failed. Intravascular stents may be necessary to restore adequate flow.

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Grey Turner's Sign and Cullen's Sign in Severe Acute Pancreatitis

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Subcutaneous manifestations (Grey Turner's sign, Cullen's sign, and disseminated fat necrosis) of severe acute pancreatitis (SAP) are often

discussed but seldom observed. We experienced a patient with gallstone SAP presenting with Grey Turner's sign and Cullen's sign, in which signs were improved following a successful intensive therapy consisting of continuous intra-arterial infusion therapy (CAI) and interventional endoscopy (IVE). The patient was a 34-year-old female. She was admitted to the hospital on account of severe epigastric pain, severe acute pancreatitis was diagnosed. Her APACHE II score was 10 points. An abdominal CT scan revealed the swollen pancreas and the fluid collection and fat necrosis around the pancreas extended to the pelvic cavity. The CT also revealed bilateral pleural effusions, cholecystolithiasis, and inflammatory changes involved the anteriolateral abdominal wall. Immediately she was received endoscopic retrograde bilialy drainage (ENBD) and bile duct clearance. Subsequently CAI consisted of pancreatic enzyme inhibitors and antibiotic agents was performed for 9 days to subside the pancreatic inflammation. She had been supplied parental nutrition through the central vein. As a result, the patient's general condition improved, and these ecchymosis resolved within 7 days of presentation. Furthermore she underwent lapaloscopic cholecystectomy, she completely recovered from SAP. Bodywall ecchymosis in the loins (Grey Turner's sign) and periumbilical region (Cullen's sign) are frequently mentioned in the clinical literature as the signs of severe hemorrhagic pancreatitis. These signs predict the development of severe complications during the course of the disease and, in particular, there is an association with pseudocyst formation. Computed tomography depiction of these signs is sparsely documented. These observations help confirm the precise anatomic pathways by which the extravasated pancreatic enzymes and their effects lead to these cutaneous discolorations. Briefly, we will present here the course for a case of gallstone SAP complicated with the Grey Turner's sign and Cullen's sign with some reviews of literatures.

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Middle Segment Pancreatectomy for Intraductal Papillary Mucinous Tumor of the Pancreas: Case Report

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We report a case of middle segment pancreatectomy for intraductal papillary mucinous tumor in A 52-yr-old male patient. He presented with a seven year history of diabetes mellitus and hyperlipidemia. A laparoscopic cholecystectomy for gall bladder stone was performed three years ago. At this time, hospital treatment for acute pancreatitis was undergone. The abdominal ultrasonography and the abdominal computed tomography showed dilatation of pancreatic duct. Endoscopic retrograde cholangiopancreatography (ERCP) showed diffuse dilatation of the entire pancreatic duct. Filling defects of the pancreatic duct were found. During the ERCP, mucin was extruded through the papilla. Sono-guided aspiration cytology of mucin revealed no malignancy. Mesenteric angiography demonstrated no atypical findings. According to these examinations, we suspected an intraductal papillary mucinous tumor. A middle segment pancreatectomy was performed. No lymph node swelling was found. In operation, pancreatoscopy was performed and we found very clear papillary structure. The pancreatic segment containing the papillary structure was resected. The remaining body and tail of the pancreas was anastomosed to the stomach. Microscopically, a diagnosis of non-invasive intraductal papillary adenocarcinoma of the pancreas was performed.

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Synchronous Colon Cancer and Pancreatic Solid-Cystic-Papillary Tumor

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Background: Solid-cystic-papillary Tumor(SCPT) is a special type of pancreatic neoplasm. It is low grade malignant and the prognosis is good. At present time, there is no report of synchronous SCPT with colon cancer. Objective: To sum up the experience in the diagnosis and treatment of synchronous SCPT with colon cancer. Methods The clinical courses, surgical treatments and morphological findings of one patient with SCPT are analyzed. She is a 15 year old female patient who had a large tumor in the tail and the body of pancreas with multiple metastasis in the liver. She also had synchronous sigmoid adenocarcinoma and received radical resection of sigmoid and pancreatic tumors including biopsy and alcohol injection of the liver metastasis. Results: After the surgery, the patient recovered and survived for 37 months. Conclusion: Good patient survival is achievable when take with aggressive treatment of synchronous SCPT with colon cancer. It needs aggressive surgical treatment even when there is liver metastasis.

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Solid and Papillary Epithelial Neoplasm of the Pancreas: A Clinicopathologic Study

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Solid and papillary epithelial neoplasm of the pancreas (SPEN) is extremely rare accounting for only 0.17 to 2.7 per cent of all nonendocrine tumors of the pancreas. This neoplasm usually occurs in young women and often remains asymptomatic until becomes manifest as an abdominal mass. The authors describe three cases of the SPEN and present pathologic characteristics. The medical records of three patients were reviewed retrospectively from 1996 to 2002. All patients were operated on at Sao Paulo Hospital. Patients were female with a mean age of 32 (range, 19 to 50 years old) . One patient had a large asymptomatic abdominal mass and the others had large abdominal mass and gastrointestinal complaints. The tumor range in size from 8 to 20 cm by abdominal ultrasonography and computed tomography. A biopsy specimen obtained by fine needle aspiration cytology guided by endoscopic-ultrasonography was performed in one case and it was consistent with SPEN. All pacients underwent resection with included one distal pancreatectomy and two local excisions. There was no evidence of liver metastasis. Pathology demonstrated solid and cystic-papillary epithelial neoplasms. Neoplastic cells were diffusely positive for vimentin, alpha-1-antitrypsin and for neuron-specific enolase in immunohistochemical study. The mean follow up was 4.3 years. SPEN is an unusual malignant neoplasm of the pancreas with distinct clinicopathologic entity and low malignant potential. Even though it is locally invasive, a high cure rate by surgical resection alone is possible.

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A Novel Approach to Sealing the Cut Pancreatic Duct During Distal Resection

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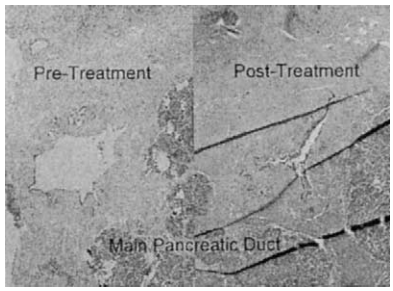
Distal pancreatectomy is performed for various pancreatic and extrapancreatic processes. The procedure carries significant morbidity mostly due to complications from the cut end of the pancreas. Fistula and abscess rates approach 20-40%. We evaluated a novel approach to seal the pancreatic stump using the topical application of radiofrequency energy. Radiofrequency energy can be applied to the cut end of the pancreas to cause a permanent seal of pancreatic ducts by causing collagen shrinkage. Data: On three patients undergoing pancreatico-duodenectomy we evaluated the pancreatic duct histology on the non-treated resection margin and the specimen margin which was treated using the TissueLink Dissecting Sealer 3.0(DS3.0) for hemostasis. Routine and trichrome stains were performed. Significant collagen shrinkage was noted. The main pancreatic duct walls were co-

apted by application of the DS3.0 (Image 1). We have since treated the cut surface of the pancreas in two patients undergoing resection. The first a 63 yo male who underwent spleen sparing distal pancreatectomy for IPMT. He had no evidence of pancreatic leak by drain amylase or clinical outcome. Drain amylase was 14IU/l and the patient was discharged home on the ninth postoperative day. The time course of his hospitalization was due to underlying cardiac and pulmonary disease. The second is a 73 yo female who underwent a wedge resection with distal pancreatico-jejunostomy and the pancreatic stump in the head was treated only with the DS3.0. The patient had return of bowel function on POD#2 and was tolerating a regular diet by POD#3. Patient was monitored in hospital till POD#5 and was discharged. In both cases no sutures, clips, staples, or sealants were used on the cut end of the pancreas. Application of the DS3.0 was the sole means of closure of the pancreatic remnant. Application of this technology shows promise in pancreatic surgery. Prospective trials of this technique should be undertaken.



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Laparoscopic Treatment of an Insulinoma of the Pancreas Tail

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The insulinoma is a rare tumor, composed of islet cell tissue that occasionally occur in the pancreas, most frequently in the body and tail and commonly solitary. Insulinomas are more likely to be benign than malignant. Only 10% are multiples and only 10% are malignant. Laparoscopic enucleation is an appropriate operative technique. The authors present a video of a laparoscopic technique for enucleation of the insulinoma in the pancreas tail. A case of a 14 year-old man with hypoglycemia and hyperinsulinism is described. Ultrasound exam showed a 2 cm lesion protruded from the lower border of the tail of pancreas, confirmed by CT and endoscopic ultrasonography. During surgical procedure, the patient was placed in the low lithotomy position. A 10-mm trocar was inserted near the umbilicus after a small laparotomy, and a zero grade laparoscope was then inserted via that trocar. Three other 5-mm trocars were placed around the upper abdomen under direct vision. The retroperitoneal cavity was wide opened and the solitary tumor was then resected with laparosonic coagulating shears. Examination of the resected specimen confirmed that the tumor had been completely removed. Histologically, it was proven to be a benign insulinoma. The patient had a satisfactory outcome after the operation, with a low debt pancreatic leak healed in the tenth postoperative day.The laparoscopic enucleation of benign insulinomas seems to be a feasible and safe procedure.

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Laparoscopic Resection of Pancreatic Cystic Lesion

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Objective: To report the clinical result and experience of laparoscopic resection of pancreatic cystic lesions. Methods: In 2001,two cases were admitted to Sir Run Run Shaw Hospital because of the recurrence left abdominal discomfort. The CT scan showed a cystic lesion 5cm in diameter located at the body and the tail of the pancreas. They were resected with laparoscopy. Results: The operation was performed successfully, and it took about 60 mins, with less than 50 ml of blood loss. The patients were able to ambulate after after 6 hrs and were discharged on the 4th day of surgery. The symptoms had completely disappeared and no recurrence during the follow up period of 15 and 19 months. Conclusion: It is possible to resect pancreatic cystic lesions with laparoscopy and it has the advantages of less trauma and quick recovery.

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Solid and Papillary Epithelial Neoplasm of the Extrahepatic Bile Ducts

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We recently treated a young woman referred to us for a presumed cholangiocarcinoma. Gross and histologic features of the tumor as well as its clinical behavior were identical to that of a solid and papillary epithelial neoplasm (SPEN) of the pancreas. No heterotopic pancreatic tissue was detected. The occurrence of a SPEN in the extrahepatic bile ducts has not been previously reported and forms the basis of this report. A 24-year-old black female was evaluated for acute onset abdominal pain. Initial lab work up revealed slightly elevated liver function tests prompting an abdominal ultrasound. The ultrasound revealed a cyst-like mass located in the base of the liver in association with the extrahepatic bile ducts. An abdominal CT scan confirmed a 2×2 cm mass located in the porta hepatis. No other lesions were identified. A presumptive diagnosis of a cholangiocarcinoma was made and a CT guided biopsy of the mass was attempted. It was however non-diagnostic as no cellular elements were obtained. A surgical resection for definitive histologic diagnosis and biliary reconstruction were planned. At surgery, the resected specimen revealed a solid mass engulfing the bifurcation of the common bile duct, with extension into both hepatic ducts as well as the cystic duct. On cut section the mass had both solid components as well as areas of hemorrhagic necrosis. The portions of the hepatic ducts within the tumor were patent without evidence of intraductal extension. All resection margins were free of neoplasm. No adenopathy was noted in the region. The patient was reconstructed using a Roux-en-Y hepaticojejunostomy with a subfascial jejunal stoma (Hutson-Russell procedure) to allow future biliary access if necessary. Histology was consistent with a SPEN. The patients postoperative course was uneventful. She did develop an anastomotic stricture requiring dilatation. At a recent three year follow up she remains free of any evidence of recurrence. The clinicopathologic features of the patient presented in this report are consistent with that of SPEN. To our knowledge this represents the first report of a solid and papillary neoplasm of the extrahepatic bile ducts in the absence of heterotropic pancreatic tissue.

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Mucin-Producing Carcinoma of the Gallbladder (Mucinous Adenocarcinoma) With Obstructive Jaundice

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A 66-year-old woman was admitted to our hospital because of upper abdominal discomfort and nausea in 2001. She was pointed out cholelithiasis in 2000, but didn't have any therapy because of no symptom. On admission she had hyperbilirubinemia (4.1 mg/dl). US showed moderate dilatation of the intra and extrahepatic bile duct

and debris-like echoes in the gallbladder. ERCP was performed, and the cholangiogram demonstrated a filling defect in the choledochus due to debris, stone or mucinous material. CT and MRI showed thickness of the G.B. wall and stone, no abnormal findings of the pancreas. We suspected mucin-producing carcinoma of the gallbladder or chronic cholecystitis. So we tried laparoscopic method for diagnosis. After laparoscopic method,we suspected mucin-producing carcinoma of the gallbladder. We converted open method and performed cholecystectomy with partial hepatectomy, resection of the common bile duct and lymphnodes dissection. Papillomatous tumor with mucin was occupied at the body of the gallbladder and histologically it was mucinous adenocarcinoma with invasion to sub serosa of the gallbladder. There are a few reports of mucin-producing carcinoma of the gallbladder with obstructive jaundice.

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The Worlds First Long Term Survivor After Ex-Vivo Liver Resection and Partial Autotransplantation for Advanced Hilar Cholangioccarcinoma

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Bismuth type IV cholangiocarcinoma (CC) carries a poor prognosis. In-situ resection is risky and tumor clearance is usually not possible. Ex-vivo liver resection and autotransplantation (Atx) is theoretically an option. There are only 5 previously reported cases of this procedure for CC in the English literature, and most of them died early in the postoperative period. The only long-term survivor reported previously died of tumor recurrence at 13 months. We are reporting a patient who has survived for 17 months without any sign of tumor recurrence. This potentially represents the worlds first cure for CC using this technique. This patient is a 26-year-old female who was transferred to our unit with obstructive jaundice. Ultrasound and CT examination revealed a hilar liver mass. Bilateral PTBD were inserted and a Bismuth type IV CC was confirmed by laparoscopic ultrasound examination. Angiogram showed involvement of the portal vein by the tumor at the confluence. In situ resection was deemed impossible due to the tumor location. In view of the patients young age, ex-vivo resection of segments 5, 6, 7, 8 and part of 4 was performed followed by a partial liver Atx. The left portal vein was connected to the main portal vein trunk using an internal jugular vein interposition graft. Biliary drainage was reconstituted using a roux-en-Y jejunal loop anastomosed to the individual bile ducts draining segments 2, 3, and 4. She was discharged on postoperative day 45. The pathology specimen demonstrated CC with clear margins. MRI and CT examinations done over the following 17 months show hypertrophied remnant liver with no evidence of recurrence. On follow up she is doing well and has returned to her daily routine. In conclusion, ex-vivo liver resection and Atx can be considered as a viable option for cure among highly selected patients with CC.

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Hepatic Artery Pseudoaneurysm Obscured by Closed Suction Drain

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NH is a 64 year old female with a h/o retroperitoneal Leiomyosarcoma. In 6/1999 she underwent right nephrectomy and IVC resection. In December, 2001 she had segment 7 of her liver resected for recurrence. In April, 2002 MRI revealed a mass in her porta hepatis that enhanced on PET scan. NH underwent resection of the porta hepatis mass. The mass displaced the portal structures anteriorly, and it involved a hepatic artery originating from the celiac axis. The dominant hepatic artery emanated from the SMA and was situated at the posterolateral aspect of the hepatoduodenal ligament. On POD 7 NH was discharged home with a drain since a small amount of bilious output was present. On POD 15 she presented with acute abdominal pain and with blood in her JP. Until this time the abdominal drain fluid had remained low in quantity (<20cc/day) and its character had not changed since her discharge from the hospital. She was diaphoretic, tachycardic, and had significant abdominal tenderness. She stabilized hemodynamically after transfusion of blood. An abdominal CT scan revealed a large amount of blood around the liver with no active extravasation. An arteriogram revealed no active bleeding source and no pseudoaneurysm. Due to the degree of hemoperitoneum, exploratory laparotomy was performed and the perihepatic hematoma was evacuated. No active source of bleeding was identified. On POD 9 following exploration, NH developed recurrent bleeding in her JP drain. This bleeding was self-limited, ending within minutes. Subsequently NH became orthostatic and developed melena. EGD revealed hemobilia. NH then underwent arteriography revealing a hepatic artery aneurysm from a branch of her right hepatic artery. For treatment of this aneurysm, her right hepatic artery was coil-embolized. The etiology of this aneurysm was likely a consequence of her initial operation when her portal mass was resected. During this resection, her right hepatic artery may have been injured but was not clinically evident immediately. Over the ensuing two weeks, however, a small area along the artery probably broke down leading to the formation of a pseudoaneurysm which was likely responsible for her initial episode of bleeding. The immediate question becomes "Why was the pseudoaneurysm not appreciated on the initial arteriogram?" The small pseudoaneurysm was not identified on abdominal exploration. This is not unusual especially in a previously operated field. It does seem unusual, however, that a bleeding pseudoaneurysm was not appreciated on the initial arteriogram. On reevaluation of the studies, it was appreciated that her original JP drain was sitting immediately adjacent to the right hepatic artery. It therefore may have been involved in both trauma to the artery as the vessel beat against this foreign body and in obscuring detection of the bleeding hepatic artery pseudoaneurysm.

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Use of a Polytetrafluoroethylene Tube and Patch in the Repair of a Difficult Duodenal Stump

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Introduction: Special care must be taken to avoid any complication in the duodenal stump such as suture dehiscence. We wish to communicate the successful results obtained with a polytetrafluoroethylene tube and patch used to repair a duodenal stump that suffered dehiscence of the sutures several times. Case Report: This is a case of a 35-years-old woman in whom a hepaticojejunostomy was performed previously, due to an iatrogenic choledocus injury. Because of bile leakage drainage, a second celiotomy was indicated, and a duodenal wall defect with edematous borders was found. The primary repair was unable, so a partial section of the duodenum was made. During the next 24 days, the patient suffered dehiscence of the sutures in the duodenal stump 3 times. Because of the difficulty in closing the borders, a polytetrafluoroethylene tube was sutured to the duodenal borders, and exteriorized through the lateral abdominal wall. The patient improved, and 30 days later the duodenal wall was not edematous. The tube was withdrawn, and a polytetrafluoroethylene patch was sutured covering the duodenal stump. The patient had a good evolution and was discharged with periodical checkouts. Discussion: Primary repair is the first choice of treatment for gut wall defects. In our patient primary closure was not possible because of the inflammation of the duodenal wall, a leading factor in the incompetence of the stump sutures. Clinical and experimental trials had tested the effectiveness

of the polytetrafluoroethylene in the repair of the digestive and biliary tract. In this case the use of a polytetraflouroethylene tube allowed duodenal contents drainage leading to a successful stump healing, and posteriorly, a safe duodenal repair with a patch of the same material.

VIDEO PRESENTATION: LIVER
Infection
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Laparoscopic Splenectomy for Interferon-Induced Thrombocytopenia in Patients with Hepatitis C Cirrhosis

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Interferon can be curative for patients with chronic hepatitis C. Yet, many patients are unable to complete a full course of interferon therapy due to severe exacerbations of baseline thrombocytopenia related to hypersplenism. Reversal of platelet consumption may correct thrombocytopenia and allow for the resumption of interferon therapy. Laparoscopic splenectomy offers a low-morbidity alternative for these patients. This video demonstrates our techniques for laparoscopic and hand-assisted laparoscopic splenectomy in patients with hepatitis C cirrhosis and portal hypertension.

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Benign and Malignant Laparoscopic Liver Resections: A Video Presentation

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Laparoscopic liver surgery has evolved more slowly than many other areas of abdominal surgery. However, significant progress has been made in recent years and these procedures are performed with increasing frequency. The application of laparoscopic techniques is currently reserved for highly selected patients, and usually performed by experienced laparoscopic and hepatobiliary surgeons. Certain specifically adapted instruments facilitate laparoscopic hepatic surgery. Laparoscopy may be used for staging malignant diseases; guided biopsies with or without ultrasound; for evaluation of hepatic trauma; aspiration, unroofing and marsupialization of simple or multiple cysts; wedge resections and formal resections of benign and malignant lesions. Laparoscopic techniques have also been used for cryoablation and radiofrequency ablation (RFA) of tumors. Identification and control the portal triad (the Pringle maneuver) can be safely achieved laparoscopically. This video demonstrates many of the techniques currently used in laparoscopic liver surgery. The pathology shown includes: excision of two hemangiomas; excision of two hepatic adenomas (one hand-assisted); and a wedge resection of one colorectal metastatic lesion. Also demonstrated are multiple biopsies; cryoablation and RFA; laparoscopic staging; and trauma evaluation. Potential complications include: hemorrhage, CO2 embolization, and exfoliation of tumor cells. All complications that are inherent to laparoscopy may also be encountered with this approach. Appropriate training in laparoscopy and hepatic surgery can minimize the frequency and significance of these complications. The procedures shown were performed at Duke University Medical Center and Hospital Italiano de Buenos Aires. Conclusions: Laparoscopic liver surgery is performed with increasing regularity and can be accomplished safely when performed by well-trained surgeons on appropriately selected cases.

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Demonstration of the Hydro-Jet Technique for Hepatic Parenchymal Dissection

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High-pressure water-jet dissection technology (Hydro-Jet, ERBE) was originally developed for applications in the steel and glass industries where ultra-precise cutting and engraving was needed. With the ability to control the impact force of the dissecting stream, this technology has recently been adapted for medical applications with favorable results. To date, the Hydro-Jet has been successfully used in procedures performed on such diverse organs as the kidney, prostate, synovium, gallbladder, and the parotid gland. The advantages of this thin, laminar liquid jet effect include controllable, precise, tissue-selective dissection of the parenchymal structures, with excellent visualization of, and minimal trauma to, surrounding fibrous structures. We have recently applied this modality to hepatic parenchymal transection at our Hepatobiliary and Liver Transplantation unit. From March to Oct 2002, the Hydro-Jet dissector has been used in 51 patients undergoing liver resection, including twelve patients undergoing right hepatectomy as a living donor for transplantation. The objective of this video is to demonstrate this instrument for dissection of the hepatic parenchyma. The principles of high-pressure water dissection, the different techniques, and the perceived advantages of this technology will be introduced. The video demonstrates the use of the Hydro-Jet device in a donor right hemi-hepatectomy for living-related liver transplantation. Particular emphasis will be placed on the careful identification and precise dissection of the course of the middle hepatic vein, which is frequently resected with the donor graft. In addition, the use of the Hydro-Jet on hepatic parenchyma of various consistencies (normal, cirrhotic, and fatty) is shown. Direct comparison with other hepatic parenchymal dissection modalities including cavitron ultrasonic aspiration (CUSA), and the floating-ball instrument is demonstrated. Finally, a brief overview will be offered of our initial clinical experience employing this device in over 50 resections over an eight-month period.

VIDEO PRESENTATION: PANCREAS
Surgery
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A Case Report of a Lateral Pancreaticojejunostomy in a Patient With Idiopathic Chronic Pancreatitis

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Introduction: The main goal of surgical intervention for patients with chronic pancreatitis is the relief of medically intractable abdominal pain. Several procedures have been described for this purpose, including a standard Whipple resection, pylorus-preserving pancreaticoduodenectomy, distal pancreatectomy and a duodenum-preserving pancreatic head resection. The alternative approach is drainage of the dilated main pancreatic, technique known as the Puestow procedure or lateral pancreaticojejunostomy. This video illustrates a modified Puestow procedure in a young patient with a history of idiopathic pancreatitis. Methods: An 18-y/o female presented with a history of idiopathic pancreatitis and a distal common bile duct stricture previously stented by ERCP. On the day of the surgery, the posterior wall of the stomach was dissected free of the anterior surface of the pancreas over the distance of the body and tail of the gland. The electrocautery was then used to divide the gland down to the level of the main pancreatic duct. The duct was opened in a longitudinal direction for a segment of approximately 3 cm. The jejunum was then transected approximately 25 cm distal to the ligament of Treitz and

the Roux limb of jejunum was brought through the transverse colonic mesentery to perform a pancreaticojejunostomy in a side-to-side fashion using interrupted Vicryl sutures. Continuity of the GI tract was restored approximately 45 cm distal to the end of the Roux limb with a side-to-side enteroenterostomy, which was performed using the GIA stapling device and the enterotomy was then closed with a TA-55 stapler. A 10 French feeding jejunostomy tube was then placed distal to the jejunojejunostomy and brought out through a separate incision in the right lower quadrant. Two Blake drains were placed, one near the site of the pancreaticojejunostomy and the other near the site of the cholecystojejunostomy. Postoperatively, the patient developed episodes of mild abdominal pain that were successfully treated with medications. Subsequent radiologic examination revealed a significant decreased pancreatic inflammation.

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Laparoscopic Management of Pancreatic Pseudocysts

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Pancreatic pseudocysts are important sequelae of both acute and chronic pancreatitis. Controversy exists regarding the relative merits of observation, percutaneous drainage, endoscopic drainage, or surgical drainage of pseudocysts. The purpose of this video presentation is to illustrate our evolving laparoscopic approach to pancreatic pseudocysts. In this presentation, a laparoscopic cystgastrostomy and Roux-en-Y cystjejunostomy will be demonstrated. Principles of laparoscopic treatment of pancreatic pseudocysts include adequate preoperative imaging and the routine use of intraoperative laparoscopic ultrasound. The conduit into which the cyst is drained is a function of the anatomic relationship between the cyst and surrounding viscera. For lesions in the lesser sac with close approximation between the anterior cyst wall and the posterior wall of the stomach, a transgastric cystgastrostomy is preferred. In this technique, an initial 5 mm umbilical and 12 mm ultrasound trocar are placed. Once the anatomy is confirmed, the stomach is insufflated to permit placement of two to three additional 2-5 mm trocars directly into the stomach. The cyst is seen bulging up into the posterior stomach. A long needle or gallbladder aspirator is placed into the cyst to verify cyst location. Once confirmed, a full thickness incision is made through the posterior stomach, entering the pseudocyst. Both electrocautery and the Harmonic scalpel are effective in creating this anastomosis. If separation between the cyst wall and stomach is encountered, the cyst wall is sewn to the stomach with interrupted absorbable suture. The retroperitoneum is debrided by extracting the necrotic debris and passing it through the pylorus. The length of cystgastrostomy must be approximately 50% of the diameter of the cyst. For the transgastric approach, the trocars are removed from the stomach and gastrostomy sites closed with interrupted absorbable suture. The principle indication for cystjejunostomy is location of the pseudocyst more inferiorly within the lesser sac. These lesions are seen bulging through the transverse mesocolon and are not in proximity to the posterior stomach. The cyst location is confirmed as above. A small incision is made into the cyst wall through the mesocolon. A Roux limb is established by dividing the bowel approximately 20 cm from the ligament of Treitz. A side-to-side cystjejunostomy is created with an endoscopic linear stapler. The enterotomy is closed with interrupted absorbable suture. A side-to-side jejunojejunostomy is made. Most patients tolerate a liquid diet the day of surgery and are discharged within 24-48 hours. In summary, pancreatic pseudocysts are effectively treated with a laparoscopic approach.

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The Use of the Hepatic Round Ligament in Pancreatic Surgery

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Pancreatic resection continues to be a challenge in modern surgery. One risk of pancreatic surgery is a post-operative leak. Leak rates following pancreatico-duodenectomy in current series averages 10 to 15%. Pancreatic leak rates following distal pancreatectomy and tumor enucleation are not insignificant. This video demonstrates the use of the hepatic round ligament (ligmentum teres) as a vascularized pedicle to support pancreatic anastomoses and closures from pancreaticoduodenectomy, distal pancreatectomy, and insulinoma enucleations. Our data demonstrate decreased pancreatic leak rates for these procedures when this technique is used.

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Central Pancreatectomy

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To present a videotape on the technique for central (middle segment) pancreatectomy. Video presentation of central pancreatectomy performed for a cystic lesion of the neck of the pancreas. A comprehensive surgery of the reported data on central pancreatectomy in the English Surgical literature will be presented aslo on the video. Central Pancreatectomy should be considered for benign lesions of the neck of the pancreas to avoid pancreaticoduodenectomy or distal pancreatectomy with or without splenectomy.

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Radiofrequency Energy Assisted Pancreatico-Duodenectomy

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Advances in surgery and anesthesia have led to significantly decreased morbidity and mortality from pancreatico-duodenectomy at major centers. The procedure still requires meticulous dissection and care to maintain hemostasis. Recent technologies for liver resection using precoagulation have led us to adapt the TissueLink Dissecting Sealer 3.0 (DS3.0) for use in pancreatic surgery. Utilizing this technology a nearly bloodless resection can be performed with a handful of suture ligatures and without the use of other operative adjuncts such as topical hemostatic agents, tissue glues, staplers or clips. This video highlights the application of the DS3.0 for pancreatico-duodenectomy, its use and areas of caution. The resection shown is in a patient with alcoholic cirrhosis in which the procedure was performed with less than 200cc of blood loss. The attached image demonstrates the ability to dissect the uncinate process off the superior mesenteric artery and seal larger branches without the need for ties.

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How I Do It: Organ-Preserving Total Pancreatic Head Resection—Total Pancreatic Head Resection While Preserving Alimental Tract And Biliary Tract (Video)

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A duodenum-preserving total resection of the head of the pancreas with subsequent pancreatoduodenostomy is a new trial for pancreatic surgery.

The major points for this operation is to maintain the duodenal blood supply. For this purpose, we found it necessary to preserve the posterior superior pancreatoduodenal artery and mesoduodenal vessels. There are eight steps in this operation. (1) Resect gastroepiploic artery/vein in front of the pancreas. (2) Division of the pancreas over the portal vein. (3) Resect anterior superior pancreatoduodenal artery along the duodenum (Preserve posterior pancreatoduodenal artery). (4) Isolate the head of the pancreas from the duodenum and the bile duct. (5) Resect vessels along the removing pancreas. (6) Resect the proximal pancreatic duct just before it merge into the bile duct. (7) Preserve the duodenal papilla. (8) Conduct an end-to-end pancreatoduodenostomy. From October 1988 to August 2002, total resection of the head of the pancreas was performed for 22 patients. As for postoperative complications, although there were transient cholecystitis in one, pancreatitis in one, duodenal obstruction in two cases, these conditions improved after conservative treatment. No operative mortality. Postoperative endoscopic pancreatography confirmed the opening of the pancreatic duct to the duodenum. The advantage of this operation is preservation of the sphincter function of the duodenal papilla to allow drainage of pancreatic/bile juice into the duodenum, which is more physiological than Beger's operation.

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Bilateral Thoracoscopic Splanchnicectomy - Operative Technique

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This video abstract demonstrates the exposure and technique we use for bilateral thoracoscopic splanchnicectomy as a treatment for chronic abdominal pain in patients with chronic pancreatitis. In the video production, we review the anatomic variability of the greater splanchnic nerve and provide data on operative morbidity and long-term follow-up.

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Minimizing Operative Time, Ischemia Time, and Blood Loss in Major Hepatic Resections: A Prospective Evaluation of the Transparenchymal Use of Vascular Linear Cutting Staplers.

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Introduction: Prolonged warm hepatic ischemia time and excessive blood loss can lead to increy rates following liver resection. We prospectively evaluated a novel ultrasound-directed technique of major hepatic resection using transparenchymal application of vascular staplers intending to minimize blood loss, operative time, and warm hepatic ischemia time. Methods: Beginning in 1998 formal hepatic resections were performed with ultrasound-directed transparenchymal application of the vascular GIATM stapler to control the portal pedicle as well as divide the hepatic parenchyma. The Endo-GIATM stapler was used for control of the hepatic veins. The video demonstrates localization of the lesion as well as the resection margin using surgeon performed intraoperative ultrasound (IOUS). After identification of the lesion the vascular anatomy is examined with IOUS following which a large clamp is used to encircle the portal pedicle and a silk suture is brought through the liver as a guide for the stapler. The stapler is then fired until the parenchyma and the portal pedicle is divided. The hepatic vein is divided with the Endo-GIATM. Finally, the small amount of residual parenchyma is divided with the ultrasonic dissector and hemoclips or another application of the stapler to complete the resection. The inflow is occluded at this point with a Rummel tourniquet if necessary. One hundred and eighty-six patients undergoing hepatic resection from December 1998 to May 2002 utilizing this technique were identified from a prospective hepatobiliary tumor surgery database. These patients were reviewed for blood loss, transfusion requirement, inflow occlusion (Pringle maneuver) time, and operative time. All patients underwent resection for primary or metastatic hepatic tumors. Results: The average blood loss for these patients was 446cc. This is considerably less than recently reported blood loss of 900-1500cc. The inflow occlusion time was 13.6 minutes with a total operative time of 170.4 minutes. These times were also considerably less than the ischemia times (30-80 min) and operative times (300-600 min) reported for major hepatic resections. The above data included additional liver-related procedures performed in 55% of the patients (103 of 186). These included radiofrequency ablation of contralateral tumors in 82, additional segmental resections in 13, and hepatic artery pump placement in 15. Conclusions: Ultrasound-directed transparenchymal application of vascular staplers to control inflow and outflow during major liver resection minimizes blood loss, warm ischemia time, and operative time compared to published reports of patients undergoing resection utilizing other techniques.