"How I Do It" Session: Imaging of Benign and Malignant Pancreatic Disease: The Year 2001

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INTRODUCTION

This year's "How I Do It" session of the Pancreas Club dealt with the topic of imaging of benign and malignant pancreatic diseases, updating the field to the year 2001. It was thought that this topic was particularly relevant because of technologic advances in the fields of computed tomography (CT), endoscopic ultrasonography EUS), magnetic resonance imaging (MRI), and positron emission tomography (PET).

The session featured four speakers: (1) Karen M. Horton, M.D., from The Johns Hopkins Medical Institutions, discussed the current state of CT imaging, focusing on multidetector CT and three-dimensional imaging of the pancreas; (2) Maurits J. Wiersema, M.D., from The Mayo Clinic in Rochester, Minnesota, discussed the use of EUS, focusing on chronic pancreatitis, cystic pancreatic neoplasms, and pancreatic adenocarcinoma; (3) Caroline Reinhold, M.D., M.Sc., from the McGill University Health Center in Montreal, discussed MRI, focusing on anatomic variants, chronic pancreatitis, and pancreatic neoplasia; and (4) Naomi Alazraki, M.D., from the Atlanta VA Medical Center and Emory University, discussed imaging of pancreatic cancer with the use of fluorine-18 fluorodeoxyglucose PET. Brief synopses of these four presentations follow.

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Multidetector CT and Three-Dimensional Imaging of the Pancreas: State of the Art

Karen M. Horton, M.D.

Since the introduction of CT scanners in the late 1970s, there have been significant advancements in both scanner technology and computer software, which have led to dramatic improvements in pancreatic imaging. When CT scanners were first introduced, only 10 mm slices were obtainable with an interscan gap of 8 mm. Each slice took at least 1 minute to acquire, which resulted in respiratory motion artifact and limited resolution. Also, at that time, only ionic intravenous contrast agents were available and they were administered slowly over time. Pancreatic masses could only be visualized if they were large and infiltrating. In the 1980s improvements were made in scanner technology with faster dynamic scanners. Slices in the range of 2 to 5 mm were then obtainable. Each slice took approximately 2 to 3 seconds to obtain, thus decreasing the amount of respiratory motion artifact. Also, in the 1980s nonionic contrast agents were introduced, which decreased the number of minor reactions. These faster scans allowed visualization of small masses but were still of limited use for staging.

Spiral CT scanners were first used clinically in the late 1980s. This technology allowed much faster scanning (approximately 1 second per rotation), and information was obtainable as a volume data set. Slices of 1 to 2 mm were possible over short distances. At that time power injectors were also introduced, which allowed faster administration of the bolus contrast. It was at that time that three-dimensional imaging of the abdomen became feasible because of the increased resolution and narrow collimation. The ability to detect and stage pancreatic cancer improved significantly. However, the most exciting recent advancement in CT technology was the introduction of the multidetector CT in the late 1990s. This scanner uses multiple rows of detectors and a faster rotation speed of 0.5 second per rotation. Depending on the manufacturer, this scanner is eight times faster than a single-detector spiral scanner and four slices are obtained per rotation. Slice thicknesses of 0.5 mm are now possible. The introduction of multidetector CT scanners has revolutionized CT scanning by greatly improving resolution, speed, and therefore the quality of the three-dimensional images. This presentation will describe state-of-the-art CT scanning in the year 2001, which uses fast intravenous contrast injection, multidetector CT technology, and the latest in three-dimensional imaging.

SCANNING TECHNIQUE

In the year 2001, state-of-the-art CT scans of the pancreas include multidetector CT acquisition. Dual-phase imaging is performed in both the arterial and venous phases of enhancement. This improves visualization of the arteries and veins during staging of pancreatic cancer. Also, water is now used as an oral contrast agent. Water is well tolerated and will not interfere with visualization of vessels during three-dimensional imaging. Nonionic contrast medium (120 ml) is routinely administered through a peripheral intravenous catheter at the rate of at least 3 ml per second. We routinely obtain 1.25 mm slices through the pancreas. This requires one approximately 20-second breath hold, which most patients tolerate well. Again, the pancreas is imaged during both the arterial and venous phases. If visualizing the study on film, 3 to 5 mm slices are printed on film. However, the 1.25 mm slices are then sent to a three-dimensional workstation for review. For three-dimensional imaging of the pancreas, multiple software platforms are available. We use the Siemens Virtuoso (Siemens, Iselin, NJ). This allows near real-time volume rendering of the three-dimensional-volume set. Volume rendering is superior to other platform-rendering algorithms such as shaded surface. With volume rendering, the user is able to adjust the window level, center, opacity, and brightness as necessary to best visualize the structures. In

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addition, this software allows multiple cut planes and excellent editing tools to maximize visualization of the desired structures. The addition of three-dimensional viewing of the data sets improves the detection, staging, and surgical planning. It can often obviate the need for additional studies such as angiography.

CT STAGING OF PANCREATIC NEOPLASMS

At our institution we routinely perform dual-phase multidetector CT scanning of the pancreas for detection and staging of pancreatic tumors (Fig. 1). The thinner collimation and increased resolution obtainable with multidetector CT and a good bolus injection allow detection of smaller tumors that do not necessarily obstruct the pancreatic duct or common bile duct, or do not definitely result in a contour abnormality of the gland. Adenocarcinoma of the pancreas typically appears as a low-density mass within the pancreas. It can often be visualized best during the venous phase of enhancement. The tumor may result in obstruction of the pancreatic duct and or common bile duct depending on the location. Other primary lesions, such as islet cell tumors, typically appear hypervascular and enhance brightly on the early-phase images. Cystic tumors of the pancreas have low density and may contain enhancing solid elements.

Once the primary tumor is detected, staging can be performed on the same scan. Because of the dual-phase acquisition, the arteries and veins are well visualized. Tumor encasement occurs when the tumor causes narrowing of the adjacent arteries and veins. At times the superior mesenteric artery or celiac axis is involved. Vascular invasion can also involve the portal vein, superior mesenteric vein, or splenic vein, often resulting in involvement of collateral vessels. The three-dimensional study is especially helpful for visualizing the inferior branches of the mesenteric arteries and veins, as these structures are small and are not optimally visualized on axial scans.

In addition to vascular encasement, lymph nodes can be detected on CT. Smaller nodes can be identified with thinner collimation obtainable with the multidetector CT scanner. However, it is a persistent limitation of CT that tumor involvement of nodes cannot always be determined. Tiny nodes measuring less than 5 mm can harbor tumor, whereas large nodes in the range of 1 to 2 cm can be benign.

Finally, in addition to diagnosing the primary tumor, vascular invasion, or nodal metastases, liver metastases can also be detected by CT. The thinner collimation possible with the multidetector scanner should improve the detection of smaller metastases to the liver.

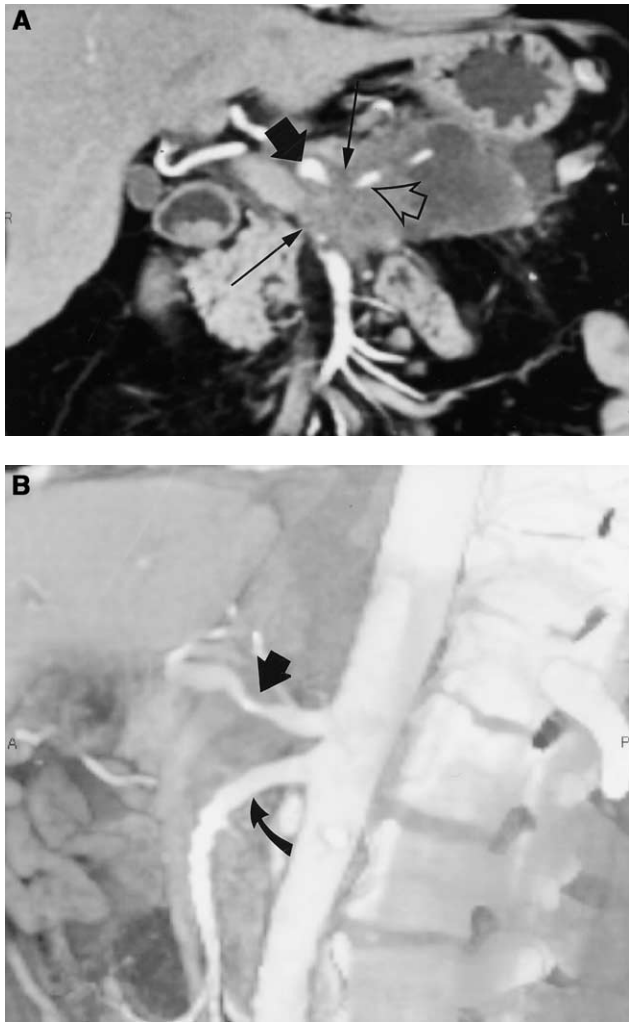

Fig. 1. A, Coronal three-dimensional multidetector CT image obtained from a patient for staging of pancreatic cancer demonstrates a mass (*long, thin arrows*) in the neck of the pancreas encasing the splenic artery (*open arrow*) and common hepatic artery (*solid arrow*). **B,** Sagittal three-dimensional multidetector CT image from the same patient as in **A** shows narrowing of the celiac axis (*solid arrow*) compatible with tumor encasement. The superior mesenteric artery (*curved arrow*) is normal in caliber. In this image, the three-dimensional settings were adjusted to accentuate the vasculature.

CONCLUSION

In the year 2001, state-of-the-art CT scanning of the pancreas includes dual-phase acquisition during the arterial and venous phase of enhancement, water as oral contrast medium, thin collimation (1 mm), a good intravenous contrast bolus, and the addition of

three-dimensional imaging to better visualize the primary tumor and to improve staging. As these scanners have only been in clinical use for a little more than a year, good prospective studies have not yet been performed. These will likely demonstrate the improved detection and staging of tumors as a result of the increased resolution, thinner collimation, and better three-dimensional imaging available today.

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Endoscopic Ultrasonography

Maurits J. Wiersema, M.D.

Endoscopic ultrasonography (EUS) is an established technique for imaging the pancreas. The close proximity of the transducer to the region of interest permits higher ultrasound frequencies than do transcutaneous techniques, thereby enhancing image resolution. Recent literature on the subject has further explored the role of EUS in the evaluation of chronic pancreatitis and pancreatic neoplasms. Although interval improvements have occurred with CT, MRI, and PET, endoscopic ultrasonography still offers unique information and these aspects will be discussed.

CHRONIC PANCREATITIS

Evaluation of tests to diagnose chronic pancreatitis is problematic because of the absence of a "gold standard" in this disease. Ideally, the gold standard for chronic pancreatitis would be histologic findings. However, these are seldom available, and the presence of pancreatic fibrosis and pancreatic ductal dilatation can occur in elderly patients and alcoholics without chronic pancreatitis.[1–4] Despite these limitations, EUS has consistently been demonstrated to be an extremely sensitive technique for detecting pancreatic parenchymal and ductal abnormalities. Several large studies have compared the findings on EUS with pancreatography, as well as pancreas function testing.[4–9] Summary observations of the role of EUS in chronic pancreatitis are possible: (1) all patients with a normal EUS also had normal findings on endoscopic retrograde cholangiopancreatography (ERCP)[5–7]; (2) the sensitivity and specificity of EUS varies depending on the number of threshold criteria used to establish the diagnosis; (3) patients with dyspepsia and alcoholism with no clinical evidence of pancreatic disease can have abnormalities identified on EUS[3,4,10]; and (4) interobserver agreement on EUS findings in chronic pancreatitis (Kappa 0.42 to 0.46) are similar to other accepted imaging procedures (CT localization of stroke, Kappa 0.56 to 0.62).[11]

The role of EUS in diagnosing chronic pancreatitis is evolving. Current reports would support the notion that a normal EUS examination of the pancreas confidently excludes chronic pancreatitis.

CYSTIC PANCREATIC NEOPLASMS

Although most cystic lesions of the pancreas are pseudocysts, identifying and then planning therapy in a patient with a suspected cystic neoplasm can be difficult. Serous cystadenomas and simple cysts are typically thought of as benign lesions that may not require resection. However, mucinous lesions may have a more aggressive clinical course, and surgical resection is desirable.[12] Problems persist in differentiating cystic neoplasms, as well as in understanding the natural history and malignant potential of the different cystic neoplasms.

The utility of EUS in differentiating pancreatic cystic neoplasms is unclear. Koito et al.[13] reported 92% accuracy for EUS when differentiating benign from malignant lesions. We have explored the role of EUS, EUS-guided aspiration with cytologic examination, and intracystic carcinoembryonic antigen (CEA) in a series of 34 patients with pancreatic cystic lesions who underwent surgical treatment.[14] Overall accuracy of EUS was 87%, cytologic examination 55%, and CEA analysis (>50 mg/ml abnormal) only 27% in distinguishing benign from malignant/potentially malignant lesions. Brugge et al.[15] evaluated results of similar tests in 64 patients presenting with cystic pancreatic lesions who had a pathologic correlation. For distinguishing benign (n = 44) from malignant (n = 20) cystic lesions, the sensitivity for EUS (65%) and CEA (90%) was higher than for cytologic examination (20%). However, the specificity of cytology (95%; $P = 0.03$) was greater than that of CEA (45%), and similar to EUS (79%). The authors also analyzed their results by distinguishing nonmucinous (n = 23) from mucinous (n = 18) lesions, with CEA having the highest sensitivity (83%; $P = 0.001$) vs. EUS (32%) and cytologic examination (32%). Although not significantly different, the specificity of cytology (96%) was higher than that of

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EUS (74%) and CEA (71%). The conflicting results from these three studies may in part arise from the subjective nature of EUS interpretation. In neither study did complications arise from EUS-guided cyst aspiration. Nonetheless, cyst fluid analysis may be of help in those patients with indeterminate pancreatic cystic lesions in whom the need for surgery is uncertain or the risk of surgery is high.

DISTINGUISHING INFLAMMATORY FROM NEOPLASTIC PANCREATIC MASS LESIONS

Patients presenting with neoplastic pancreatic mass lesions can often be distinguished by their presenting symptom and signs, and by imaging studies. A problematic patient is one in whom a mass lesion in the head of the pancreas develops in the background of chronic pancreatitis, or the initial presentation of the mass lesion is associated with an episode of pancreatitis. Surgical planning may be affected in that patients with symptomatic inflammatory masses might be considered for a duodenum-preserving resection of the head of the pancreas, whereas patients with neoplastic masses are typically treated by pancreaticoduodenectomy. Several studies have evaluated the performance of EUS in distinguishing benign from malignant pancreas masses (Fig. 1). Glasbrenner et al.[16] prospectively evaluated 95 patients with masses in the pancreatic head using both EUS and ERCP. The overall results for EUS and ERCP, respectively, were as follows: sensitivity for malignancy 78% and 81%; specificity for benign disease 93% and 88%; positive predictive value 93% and 89%; negative predictive value 78% and 80%; and diagnostic accuracy 85% and 84%. When EUS and ERCP were combined, the sensitivity was 92% (specificity 85%), but the diagnostic accuracy (89%) was not substantially improved. EUS provided additional information regarding lymph node stage (accuracy 81%). Baron et al.[17] looked exclusively at EUS for differentiating benign from malignant pancreatic masses in 105 patients, and identified a sensitivity of 95% and a specificity of 88% when EUS-guided fine-needle aspiration was applied in selected patients. Four patients classified as without malignancy by EUS were subsequently diagnosed with cancer. In three of these patients, chronic pancreatitis was present, making identification of the cancer difficult. In the fourth patient, the tumor was in the tail (a region that can be difficult to completely image using EUS). Mertz et al.[18] compared EUS, CT, and PET in identifying adenocarcinoma of the pancreas in 35 patients, where 31 had an adenocarcinoma and the remaining four had chronic pancreatitis. For tumor identification, EUS was more sensitive (93%) and specific (75%) than CT (53% and 25%, respectively) or PET (87% and 50%, respectively).

Gress et al.[19] evaluated results of EUS fine-needle aspiration in 102 patients with suspected pancreatic cancer and previously negative CT-guided or ERCP-guided cytologic examinations. In these patients who had failed these other biopsy procedures, the sensitivity for EUS fine-needle aspiration cytology was 93% (57/61) and specificity was 83% (34/41), with an overall complication rate of 3%. These complications included two episodes of self-limited bleeding and one patient with mild pancreatitis. Of note, the posterior probability of harboring a pancreatic tumor after a positive cytologic examination was 94%, and after a negative examination it was 6.9%. These results clearly demonstrate that EUS fine-needle aspiration may assist in those patients with a pancreatic mass when results of other biopsy methods are normal but pancreatic cancer is suspected.

DETERMINING RESECTABILITY OF PANCREATIC CARCINOMA

In those patients with pancreatic carcinoma, accurate preoperative staging is essential to determine which patients may benefit from surgery. The introduction of helical CT has permitted multiple scans to be obtained through the abdomen during different phases of contrast enhancement. The dual-phase technique permits images to be obtained when arterial and pancreatic parenchymal features are optimally visible and then later when hepatic abnormalities may be better detected. Employing this CT technique plus EUS, Legmann et al.[20] studied 30 patients with suspected pancreatic carcinoma. The results obtained with both techniques did not differ significantly. For prediction of resectability, CT and EUS both had 90% accuracy. In another comparative study, Ahmad et al.[21] followed 63 patients with pancreatic adenocarcinoma who underwent preoperative EUS and MRI. Neither EUS nor MRI alone was highly accurate in predicting resectability (62% and 73%, respectively). At the current time, in a patient with a resectable tumor based on a dual-phase helical CT (or multidetector three-dimensional CT), the added diagnostic value of EUS may be limited to enhanced lymph node detection.

SCREENING FOR PANCREATIC CARCINOMA

Pancreatic carcinoma carries a poor prognosis. Although hereditary predisposition may account for up

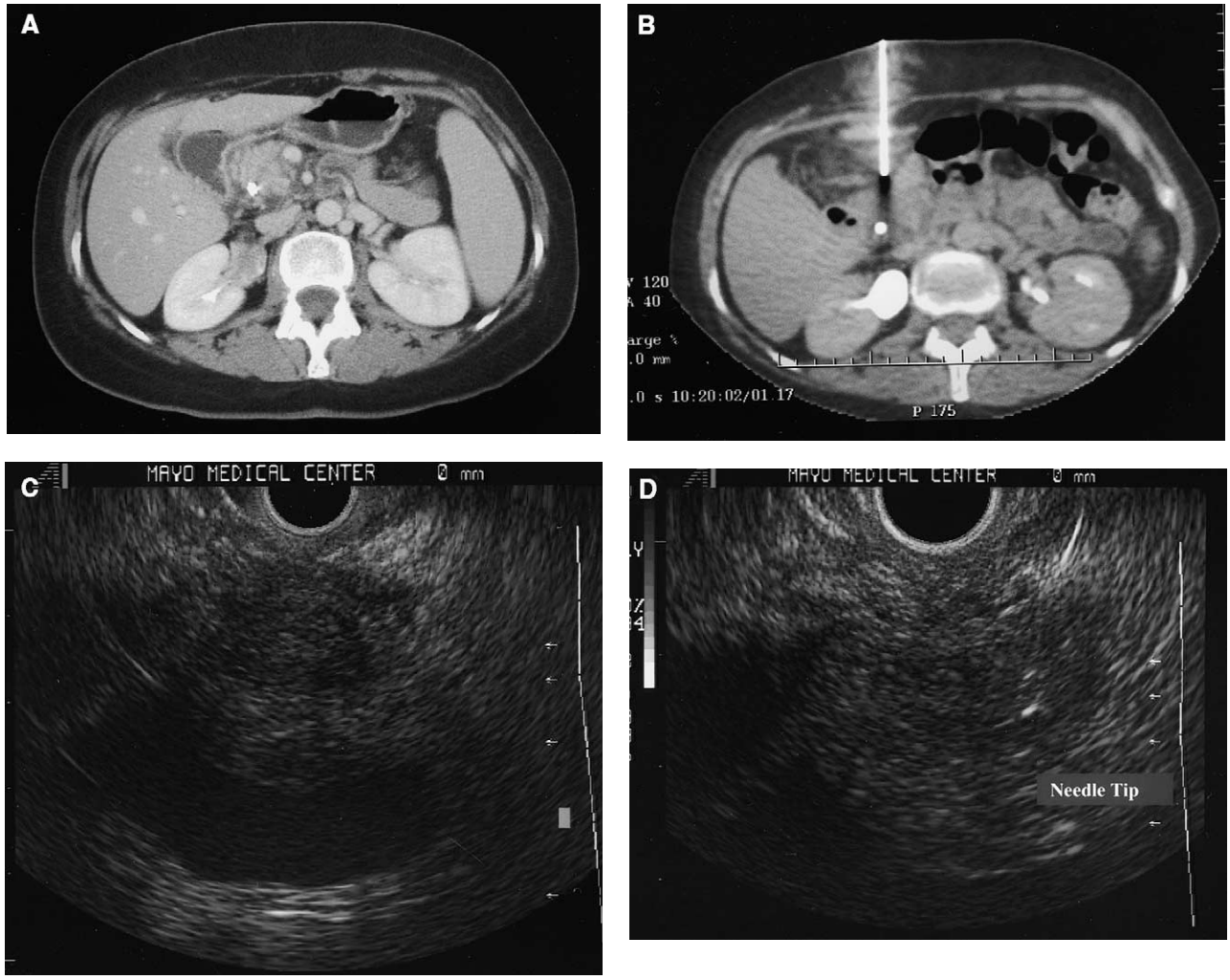

Fig. 1. A, A 57-year-old woman with painless jaundice had biliary dilatation on abdominal ultrasound without a mass. ERCP demonstrated a stricture of the pancreas and bile ducts, with the latter treated by stent placement. Triple-phase helical CT during the pancreas parenchyma enhancement phase shows slight fullness of the head of the pancreas but not a focal mass. **B,** CT-guided biopsy was performed using the biliary stent as a landmark for needle entry. Cytologic and histologic examinations revealed chronic pancreatitis and no malignancy. **C,** At EUS, a 25 mm echo-poor mass was identified in the pancreatic head, with invasion of the portal vein. **D,** EUS fine-needle aspiration of the mass was performed and demonstrated adenocarcinoma. Because of the young age and fitness of the patient, exploratory laparotomy was undertaken and the patient was deemed unresectable.

to 10% of cases of pancreatic cancer, the value of screening at-risk patients is uncertain.[22, 23] Brentnall et al.[23] evaluated 14 patients from three kindreds with a history of pancreatic cancer. Seven of the 14 patients were thought to have dysplasia based on abnormal ERCP and EUS examinations. All seven patients underwent pancreatectomy and had histologic evidence of dysplasia but no malignancy. Although EUS was able to demonstrate abnormalities similar to those of chronic pancreatitis, the specificity of these abnormalities cannot be determined because three patients who were not operated on also had abnormalities. Further investigation is needed to clarify the optimal strategy for screening high-risk individuals.

SUMMARY

Patients with suspected chronic pancreatitis who have a normal pancreatic EUS examination have a very low probability (<5%) of having the disease. EUS is a sensitive and specific method for evaluating pancreatic mass lesions. The addition of EUS fine-needle aspiration is particularly valuable in those pa-

tients with pancreatic masses and prior nondiagnostic biopsies. Whether EUS will be helpful in screening patients or families at risk for pancreatic cancer is uncertain.

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Magnetic Resonance Imaging of the Pancreas in 2001

Caroline Reinhold, M.D., M.Sc.

Recent technical innovations in hardware and software have allowed magnetic resonance (MR) imaging to play a routine role in evaluating patients with suspected disease of the pancreas. These include the advent of high-resolution imaging, fast imaging, volume acquisitions, magnetic resonance cholangiopancreatography (MRCP), and functional imaging. Optimal imaging of the pancreas requires high field strength magnets with high gradient and slew rates. The addition of a torso multicoil array provides the signal-to-noise ratio needed to acquire images with a high spatial and temporal resolution, as well as three-dimensional volume acquisitions.

Sequences with a high temporal resolution are needed for both the detection and staging of pancreatic neoplasms. An arterial and portal venous phase are needed for accurate staging and evaluation of arterial and venous patency. Small pancreatic adenocarcinomas are best imaged during the arterial phase of a dynamic contrast-enhanced scan. Three-dimensional volume acquisitions with slice reconstruction at 2.5 mm avoid misregistration artifacts typically associated with two-dimensional imaging. Three-dimensional volume acquisitions with various reconstruction algorithms allow the depiction of vessels and the relative position of the mass to surrounding structures.

MRCP images are heavily T_2-weighted images that result in solid tissues and flowing blood having a low signal, whereas stationary fluids such as bile in the biliary tree and pancreatic juice having a high signal. This results in a cholangio-pancreatographic effect without the need to administer a contrast medium.

This review will discuss the relative role of MR imaging in evaluating patients with benign and malignant diseases of the pancreas.

ANATOMIC VARIANTS

MRCP is accurate at diagnosing the presence of a pancreas divisum (Fig. 1). In a study of 268 consecutive patients undergoing MRCP, of whom 106 had ERCP correlation, MRCP correctly diagnosed pancreas divisum in all six patients.[1] There were no false positive and no false negative findings in this subgroup of patients. The administration of secretin can improve the visualization of the atrophic duct in the ventral pancreas.[2]

CHRONIC PANCREATITIS

Imaging features of chronic pancreatitis include the following (Fig. 2): (1) dilatation/strictures of the main pancreatic duct and its side branches; (2) parenchymal atrophy; (3) parenchymal calcification and intraductal calculi; (4) pseudocysts; (5) focal pancreatic enlargement; (6) biliary duct dilatation; and (7) changes of the peripancreatic fat.[3,4] In severe cases, the marked dilatation of the side branches has a "chain of lakes" appearance. Morphologic changes of the main pancreatic duct and its side branches are best demonstrated by endoscopic retrograde cholangiopancreatography (ERCP) or MRCP. ERCP has a higher accuracy than MRCP for detecting early changes of chronic pancreatitis involving the main pancreatic duct or side branches. This is due to the higher spatial resolution of ERCP, as well as the distention of the ducts during the procedure, allowing for better depiction of subtle areas of narrowing. The addition of secretin allows MRCP to better detect the more subtle changes in the main pancreatic duct and side branches associated with chronic pancreatitis.[5] MRCP is highly accurate at detecting calculi within the main pancreatic duct. Stones as small as 2 mm have been identified at MRCP. However, stones lying within normal-caliber or minimally dilated side branches of the main pancreatic duct are frequently not identified by MRCP. CT is highly accurate for demonstrating pancreatic calcification. Because of the underlying fibrosis in chronic pancreatitis, the common bile duct may be narrowed as it traverses the pancreatic head. Typically,

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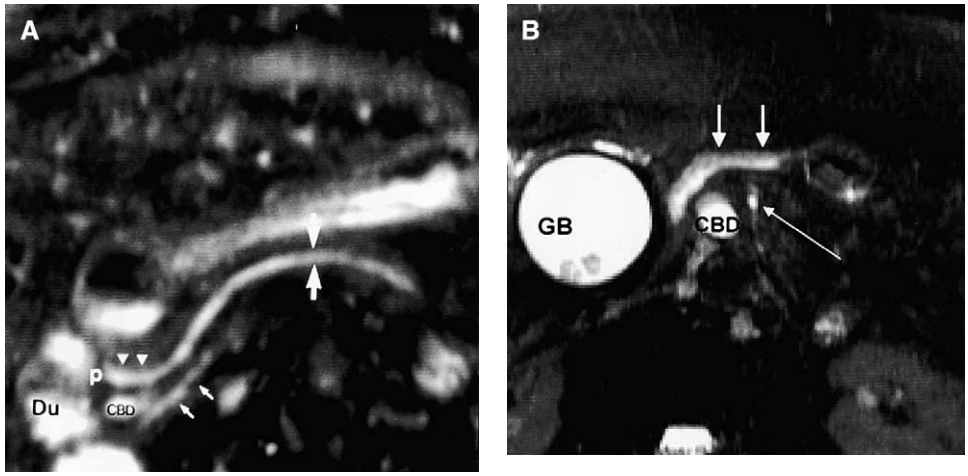

Fig. 1. Pancreas divisum. **A,** Axial reformatted high-resolution MRCP demonstrates the main pancreatic duct (*large arrows*) to drain into the minor papilla (*p*) via the duct of Santorini (*arrowheads*). Note the atrophic ventral duct (*small arrows*) that does not communicate with the main pancreatic duct. **B,** Axial reformatted high-resolution MRCP shows a dilated duct of Santorini (*vertical arrows*). The ventral duct (*long oblique arrow*) is atrophic and does not communicate with the main pancreatic duct. CBD = common bile duct; Du = duodenum; GB = gallbladder.

bile duct strictures associated with chronic pancreatitis are smooth and tapered and are well depicted on MRCP. Conventional MR imaging (T_1-weighted fat-suppressed sequences) show decreased signal of the pancreatic parenchyma in patients with moderate-to severe-chronic pancreatitis.

Fewer than 50% of pseudocysts fill with contrast material when it is injected into the pancreatic duct during ERCP. In some instances the ductal communication can be demonstrated with MRCP (accuracy 61% to 74%).[3,4] Although ERCP has a high specificity in the diagnosis of pseudocysts, it has a low sensitivity because of the variable communication with the duct. Therefore MRCP and other cross-sectional imaging techniques (ultrasound and CT) have a higher sensitivity than ERCP for the detection of noncommunicating pseudocysts. CT for the most part is the modality of choice for the initial evaluation and follow-up of pancreatic pseudocysts. MR is better at depicting necrotic debris and large blood clots that may explain failure of percutaneous drainage with conventional small-bore catheters.

Additionally, MRCP is highly accurate for depicting the ductal anatomy for preoperative planning in patients with chronic pancreatitis.

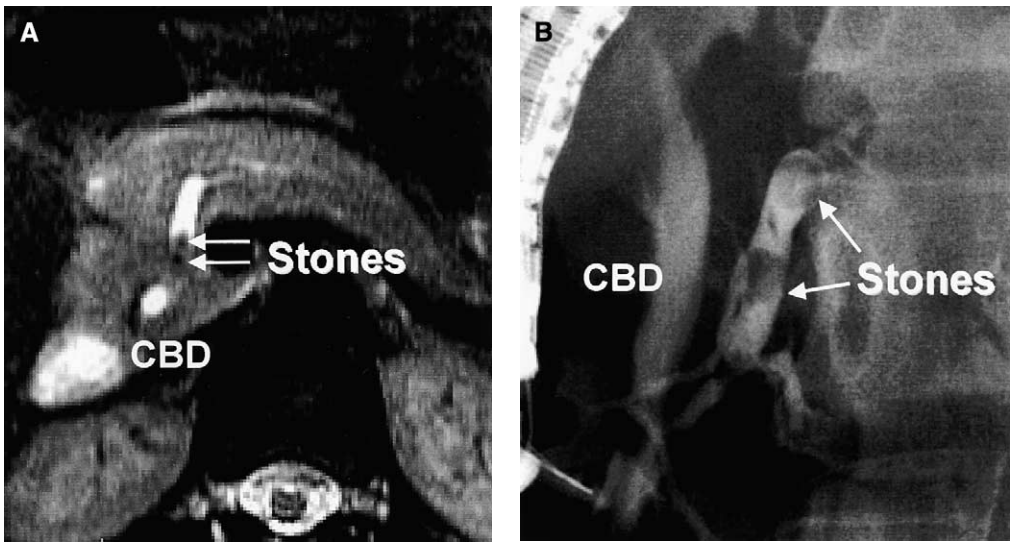

Fig. 2. Chronic pancreatitis. **A,** Axial high-resolution source MRCP shows a dilated pancreatic duct with two pancreatic stones (*arrows*). **B,** ERCP confirms the presence of the stones (*arrows*). CBD = common bile duct.

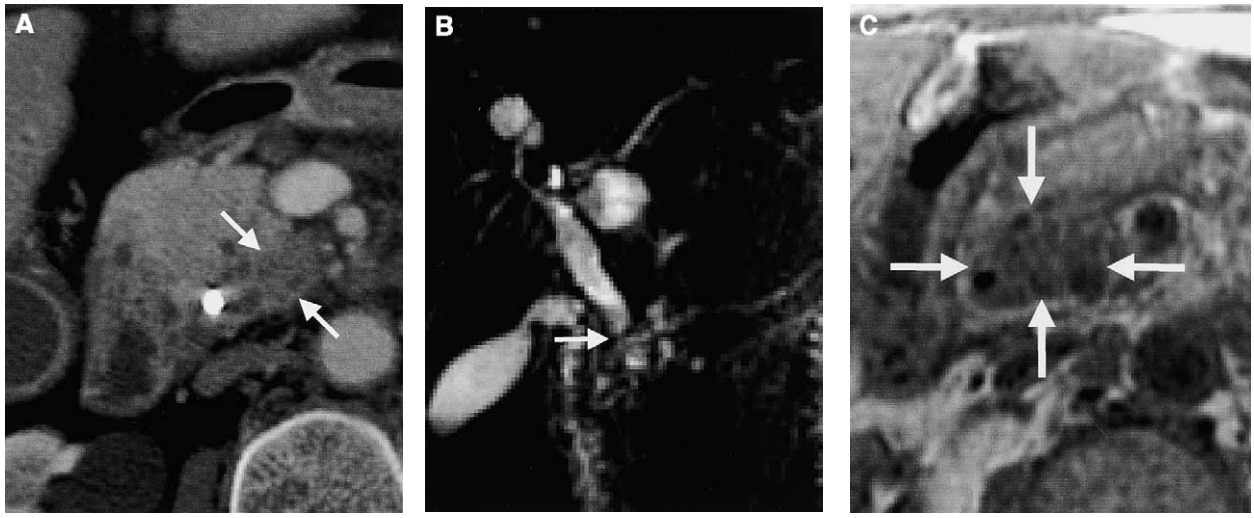

Fig. 3. Pancreatic adenocarcinoma. **A,** Axial image through the head and uncinate process of the pancreas on contrast-enhanced helical CT shows the presence of an endobiliary stent within the common bile duct (*dense opacity*), and a vague area of hypodensity (*arrows*) in the uncinate process medial to the stent. **B,** Coronal breath-hold MRCP shows a stricture (*arrow*) of the distal common bile duct without dilatation of the main pancreatic duct. **C,** Fat-suppressed T_1-weighted MR image through the head and uncinate process of the pancreas shows a hypointense mass (*arrows*) in the head and uncinate process of the pancreas. Note that the mass does not exactly correspond to the abnormality seen on CT.

PANCREATIC NEOPLASMS

Histologically, pancreatic cancer has a dense cellularity and sparse vascularity, which accounts for the low signal intensity of the tumor on T_1-weighted fat-suppressed MR images and diminished enhancement on dynamic contrast-enhanced images (Fig. 3). MR imaging possesses high inherent contrast resolution for these tumors and is particularly well suited for the detection of small non–organ-deforming tumors, which may at times be difficult to identify on CT images.[6] Pancreatic cancer frequently manifests at an advanced stage. At initial presentation, 65% of patients have advanced local disease or distant metastases, 20% have localized disease with spread to regional lymph nodes, and only 15% have tumor confined to the pancreas. These numbers reflect the lack of a distant capsule around the gland, which allows rapid infiltration of tumor into the peripancreatic tissues. MR imaging and helical CT scanning are comparable for the detection of lymphadenopathy and liver metastases.[7] For vascular invasion and encasement, optimally performed MR imaging and helical CT scanning provide similar results. Both imaging modalities are highly accurate (approaching 100%) at predicting unresectability.

Cystic tumors of the pancreas are accurately diagnosed with MR imaging. MR imaging is superior to CT for evaluating cyst contents. Although ultrasound can accurately diagnose the presence of septations or solid components in cystic neoplasms of the pancreas, it is often technically limited for lesions in the tail. MRCP is accurate in diagnosing mucin ductectatic tumors (intraductal pancreatic mucinous neoplasms) and is less invasive than ERCP.

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Imaging of Pancreatic Cancer Using Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography

Naomi Alazraki, M.D.

Recent interest in fluorodeoxyglucose (FDG) tumor imaging has been fueled by several factors including the following: (1) increasing availability of fluorine-18 FDG; (2) commercial commitment to PET and the development of coincidence camera instrumentation; (3) workload increases in oncology; and (4) Health Care Financing Administration approval of current Medicare reimbursement for PET/coincident FDG imaging for several cancers. Medicare reimburses for PET imaging in patients with lung cancer, colorectal cancer, lymphoma, melanoma, esophageal cancer, and head and neck cancers. FDG imaging is clearly effective in localizing pancreatic cancers, but sufficient documentation in the scientific literature is just now accumulating. Using positron-emitting tracers such as fluorine-18, radiopharmaceuticals that closely mimic endogenous molecules (key in many basic physiologic and metabolic processes) can be synthesized. Glucose is the prime example of such a metabolic substrate and FDG, labeled with fluorine-18, behaves as a glucose analog in vivo.

Fluorine-18 has a 2-hour physical half-life; labeled to FDG, it is rapidly taken up by malignant tumor cells. Hexose kinase is upregulated in malignant cells, resulting in more FDG uptake in malignant cells than in benign cells. Thus FDG imaging is very effective for localizing tumor cells wherever they are in the body.

There are two good methods for imaging fluorine-18 FDG: (1) conventional PET scanner (approximate cost $2 million) and (2) coincidence modification of the dual- or triple-head (single photon emission computed tomography (SPECT) camera (approximately $100,000 to $200,000 more than a conventional SPECT camera; approximately $500,000 to $600,000). Conventional PET images are capable of higher count rate acquisitions than coincidence cameras. The result is that the images are superior and patients spend less time on the imaging table than is necessary for coincidence studies.

FDG is very sensitive and specific for detecting malignancy, and is superior to CT: sensitivity in pancreatic cancer 92% (PET) vs. 65% (CT), and specificity 85% (PET) vs. 62% (CT) according to one report.[1] Of note, FDG localizes at sites of infection and inflammation, as well as in tumors. Thus pancreatic cancer may not always be distinguishable from active pancreatitis. Nonetheless, FDG is effective and has high value for cancer staging. If remote sites of focal increased uptake (likely metastases) are found on PET images, it is far more likely that the pancreatic lesion will be diagnosed as cancer rather than pancreatitis. Frequently PET has been used to assess recurrence of pancreatic cancer, extent or presence of metastases,[2] and/or response to therapy (Figs. 1 and 2).[3]

A semiquantitative index called the "standard uptake value" (SUV), which is used in PET imaging, helps to distinguish malignant from benign lesions. The SUV is an index derived from the decay-corrected dose (corrected for the patient's body weight). As a general guide, lesions with an SUV greater than 2.5 correlate with malignancy, whereas lesions with an SUV less than 2.5 are more likely to be benign.[4] Investigators have observed that tumor FDG metabolism decreases as early as 45 minutes after a dose of adriamycin.[5] Eary et al.[6] reported that SUVs for sarcoma tumors fell in patients treated with adriamycin after 1 or 2 days. Furthermore, changes in tumor FDG uptake after chemotherapy may predict patient outcome.[7] Prognosis of patients with pancreatic cancer has been documented. In one report,[8] median survival in 26 patients with a low SUV (<6.1) was 9 months vs. 5 months in 26 patients with a higher SUV (≥ 6.1).

Data collected on differentiating benign from malignant disease in 37 patients with possible pancreatic cancer showed that FDG uptake was increased in pancreatic lesions in 24 patients and that adenocarcinoma was found in 22 (92%).[8] Others[9] have reported that PET is an independent functional assay that adds to the diagnostic accuracy of ERCP and CT, and can detect hepatic, peritoneal, or other distant metastases.

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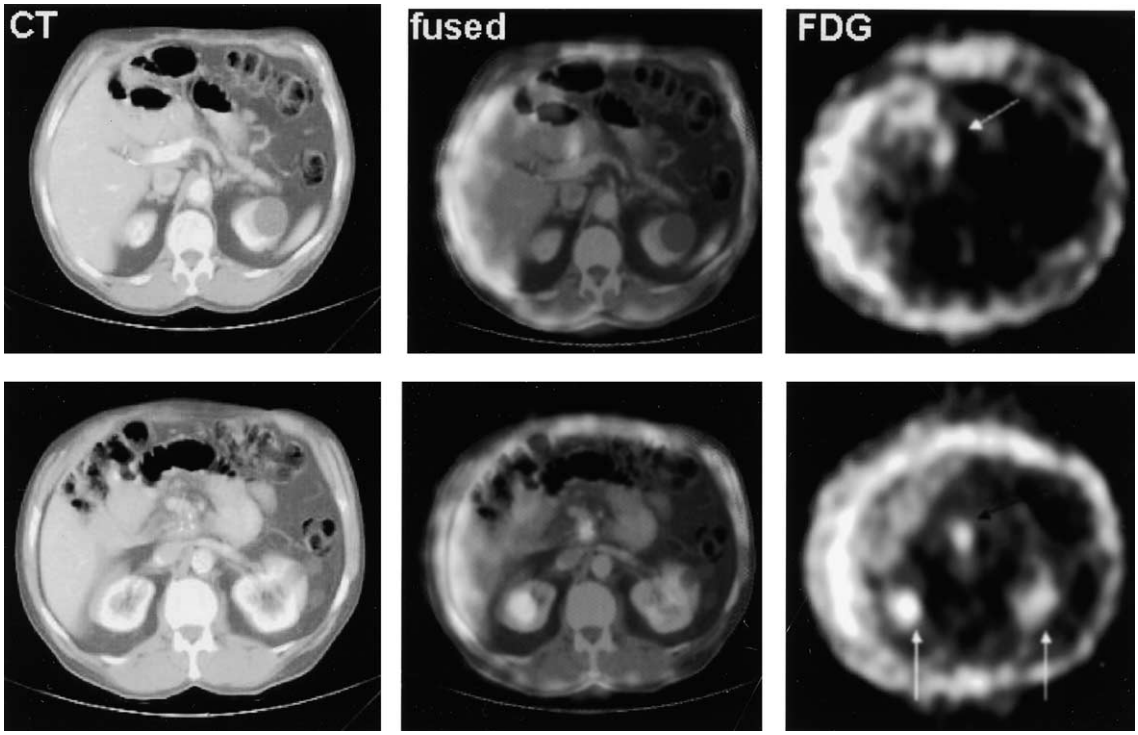

Fig. 1. Pancreatic CA with porta hepatis node. A 56-year-old man with recurrent pancreatic cancer identified on the FDG coincidence images (*far right*), which show abnormal uptake. The abnormal white focus on the lower image (*black arrow*) at the prior pancreatic surgical site is the site of recurrent tumor. The upper far right image (*white arrow*) is the site of a porta hepatitis metastatic node. The fused CT/FDG images (*middle panel*) help to anatomically localize the abnormal FDG activity. Also evident on the FDG images is normal renal activity (*lower right; white arrows*).

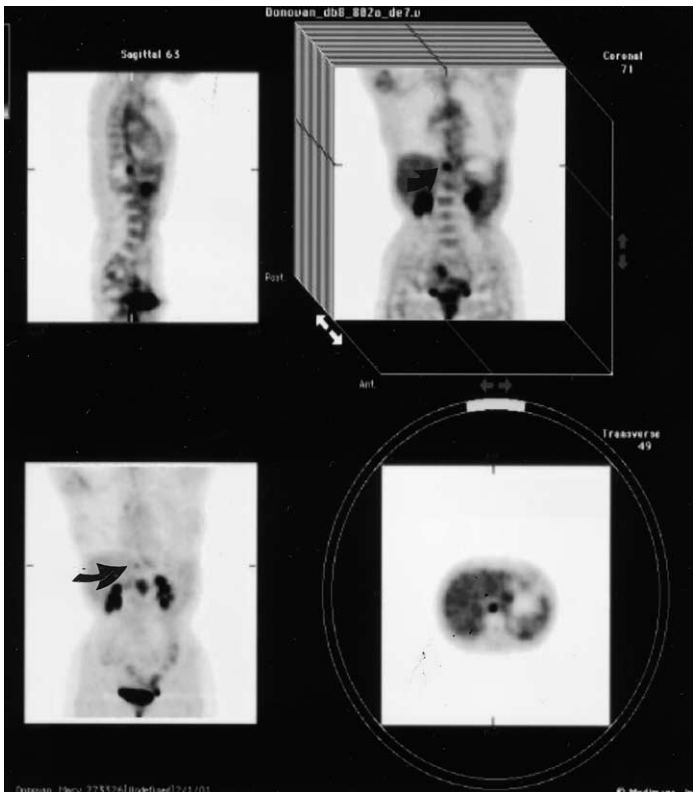

Fig. 2. Conventional PET FDG scan from a 49-year-old woman (pancreatic adenocarcinoma, stage IVa, S/p 2 cycles chemotherapy, upper abdominal mass [lymph node], SUV 2.2). Images show recurrence of pancreatic cancer (*abnormal black activity in midline between normal renal activity on lower left projection image*). Also seen is a metastatic node (*arrow*), which is superior and posterior to the mass lesion on the lower left image. Coronal and sagittal images (*left and right above*) and transaxial image (*lower right*) show the upper abdominal midline lymph node metastasis (*arrow in upper right image*).

Fusion of FDG images (coincidence or conventional PET) to CT is a very powerful tool that adds anatomic precision to strong metabolic imaging information. In addition to offering strikingly pretty pictures, fusion has been shown to add accuracy in staging cancer.[10] Achieving precise fusion of CT and FDG images requires technically demanding computer interactions, although commercial software is becoming more available. New instrumentation capable of acquiring sequential CT and PET scans at one sitting can make fusion imaging easier to achieve. Fusion can be applied to any combination of imaging modalities, for example, CT to FDG, MRI to FDG, SPECT to PET, SPECT to CT, or MRI to SPECT. SPECT studies that use indium-111–labeled octreotide (Octreoscan) or iodine-123 (or I-131)–labeled metaiodobenzylguanidine (MIBG) are effective for imaging neuroendocrine tumors. Because these tumors are well differentiated, they are often better imaged with SPECT Octreoscan or MIBG than with PET FDG. They can also be fused to CT for more precise localization of abnormal uptake on scans.

In summary, FDG PET is highly sensitive for pancreatic cancer, particularly for staging patients and monitoring for recurrence.

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The Value of Laparoscopy in the Management of Ampullary, Duodenal, and Distal Bile Duct Tumors

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Laparoscopy identifies radiologically occult advanced disease in patients with pancreatic adenocarcinoma. The value of laparoscopy in the management of peri-ampullary tumors was determined. One hundred forty-four patients with radiologically resectable nonpancreatic adenocarcinoma, periampullary tumors were identified from a prospective database between August 1993 and December 2000. Criteria for laparoscopic unresectability included histologically proved peritoneal or hepatic metastases, distant nodal involvement, arterial involvement, and local extension outside the resection field. Median age at operation was 70 years (range 31 to 87 years) and 56% of the patients were men. An adequate laparoscopy was performed in 134 cases (93%). Laparoscopy identified 13 patients (10%) with unresectable disease. Of 121 patients with laparoscopic resectable disease, 111 (92%) went on to subsequent resection; CT correctly predicted resectability in 82%. Laparoscopy spared 36% of unresectable patients a nontherapeutic laparotomy. Patients with resectable disease were treated by pancreaticoduodenectomy (n = 91, 76%), ampullectomy (n = 12, 10%), duodenal resection (n = 10, 9%), or bile duct excision (n = 6, 5%). The addition of diagnostic laparoscopy to dynamic CT scanning in this selected patient population identifies an additional 10% of patients with unresectable disease. We believe that laparoscopy should be used in a selective manner for preoperative staging of patients suspected of having nonpancreatic periampullary tumors. (J GASTROINTEST SURG 2002;6:139–146.)

KEY WORDS: Laparoscopy, upper GI malignancy, staging, pancreatic cancer

Several series have demonstrated that the routine use of diagnostic laparoscopy for patients with pancreatic adenocarcinoma identifies occult metastatic disease in up to 50% of patients deemed resectable by preoperative radiologic studies.[1–10] Although the yield of positive laparoscopy that avoids unnecessary laparotomy is highly dependent on the quality of the radiologic studies and the site of the primary tumors, these patients may be spared a nontherapeutic laparotomy and may quickly move on to other types of treatment without fear of complications. However, for resected patients, critics have argued that the procedure results in an increase in total operative expenses suggesting that a more selective approach would be more cost-efficient.

In an attempt to better define the role of diagnostic laparoscopy in the management of peripancreatic malignancies, we have evaluated several subsets of these patients on the basis of histologic findings or tumor location.[11,12] For the current study, we sought to evaluate the value of routine diagnostic laparoscopy in a cohort of patients with a presumptive diagnosis of periampullary tumors that were not pancreatic adenocarcinomas. These patients would be expected to have a high resectability rate if radiologically not contraindicated.

METHODS

Patients

Patients with radiologically resectable nonpancreatic adenocarcinoma, periampullary tumors were identified from our prospective endosurgical database of procedures performed between August 1993 and December 2000. Patients were excluded if they were diagnosed with adenocarcinoma of the pancreas or

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islet cell tumors of the pancreas. All patients were brought to the operating room and underwent extended multiport laparoscopic staging immediately before laparotomy, and resection if indicated. Our approach to laparoscopic staging has been described previously.[2] In brief, a 10 mm Hasson trocar is placed at the umbilicus, and up to three working ports are placed along the line of the intended bilateral subcostal incision. All peritoneal surfaces are inspected, and the liver is palpated and inspected on all sides after mobilization. The transverse colon is elevated, the omentum is swept cranially, and the root of the mesentery and ligament of Treitz are examined. The left lobe of the liver is elevated and the lesser omentum is incised. The stomach is elevated and the lesser sac is evaluated with special attention to the common hepatic artery and nodes. The laparoscopic ultrasound probe is introduced from the right side of the patient and is used to examine the liver for occult metastases. The probe is placed on the stomach to provide a window into the head of the pancreas where the portal vessels can be evaluated.

Definitions

All patients had contrast-enhanced thin-cut CT scans of the abdomen. In general, patients were considered resectable if they had disease that would be encompassed by a standard surgical resection. Criteria for radiologic unresectability were major arterial involvement, long segment portal vein/mesenteric vein involvement, or metastatic disease. Patients were deemed unresectable at diagnostic laparoscopy or laparotomy if they were found to have histologically proved peritoneal or hepatic metastases, distant nodal involvement, arterial involvement, or local extension outside the resection field.

Diagnostic laparoscopy was considered adequate if histologic confirmation of metastatic disease was obtained. Criteria for inadequate negative laparoscopy included inability to obtain pneumoperitoneum, operative time less than 10 minutes, or failure to complete the standard staging algorithm.

Resection was defined as removal of an organ or organs with curative intent. Biliary or gastric bypass, common duct exploration, and exploratory laparotomy were not considered resections.

Survival data are reported only for patients with a confirmed tissue diagnosis of malignancy.

Data

Clinical, pathologic, and perioperative data were obtained. Surgical variables included adequacy of diagnostic laparoscopy, results of laparoscopy, findings at open exploration, and type of open operation. Survival was calculated from date of operation to death or last follow-up.

Statistics

Positive predictive value (PPV) was calculated using the formula:

$$\text{PPV} = \frac{\text{Number resected}}{\text{Number resectable by test}}$$

to determine the ability of a test to predict curative resection. Univariate analysis was conducted using chi-square analysis for measures of association and log-rank analysis for survival. Binary logistic regression was used for multivariate analysis of variables associated with resectability and the need for biliary and/or gastric bypass.

RESULTS

Demographics

The prospective peripancreatic laparoscopy database contained more than 1000 patients for the 7-year period examined. Review of the database identified 144 patients whose final diagnosis was not pancreatic adenocarcinoma and who were brought to operation with radiologically resectable disease in the periampullary region. All patients had at least one dynamic CT scan as part of their evaluation, and 122 patients (85%) underwent endoscopic retrograde cholangiopancreatography. The median age at operation was 70 years (range 31 to 87 years), and 80 (56%) patients were men.

Laparoscopy

Fig. 1 displays the surgical findings and outcomes for the entire cohort. Median length of the laparoscopic procedure was 39 minutes (±32 minutes), and laparoscopic ultrasound was used in 55% of all procedures. Diagnostic laparoscopy was adequate in 134 patients (93%). Of these patients, laparoscopy identified 13 (10%) with unresectable disease, including six patients with liver metastases, five with peritoneal spread, and two with other metastatic disease. Four of these patients went on to palliative bypass surgery. An inadequate laparoscopy was performed in 10 patients.

Open Exploration

Of the 121 patients with laparoscopic resectable disease, 111 (92%) went on to resection. Laparoscopy had failed to diagnose three patients with liver

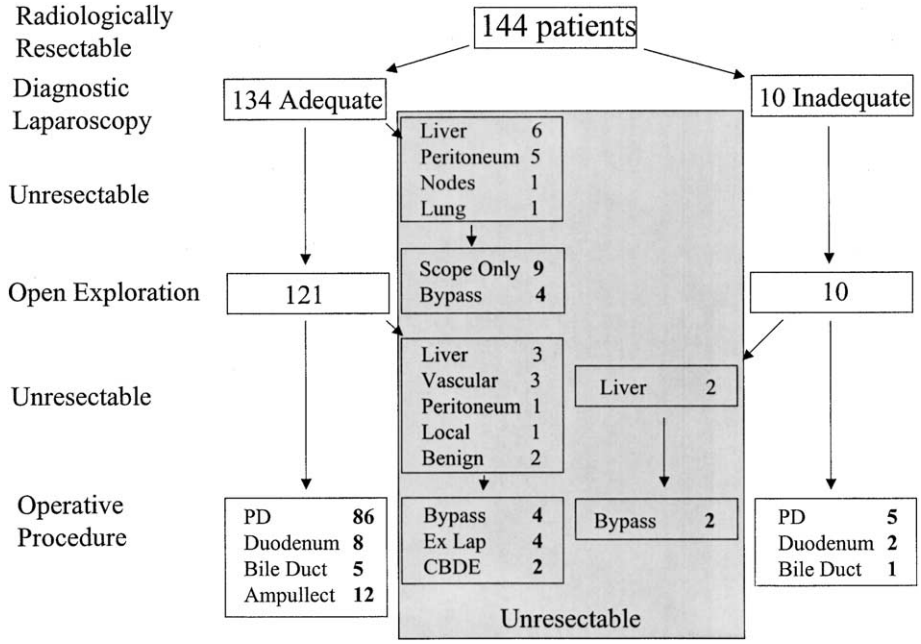

Fig. 1. Flow diagram listing surgical outcomes for 144 patients with nonpancreatic adenocarcinoma periampullary tumors deemed resectable on CT scan. The left two columns display the outcomes of the 134 patients who had adequate laparoscopy. The right two columns indicate the findings and procedures done after an inadequate laparoscopy. Displayed in gray are the reasons why patients were found to be unresectable at laparoscopy or laparotomy. PD = pancreaticoduodenectomy; Duodenum = segmental resection of the duodenum; Bile Duct = bile duct excision and drainage; Ampullect = transduodenal ampullectomy; Bypass = includes gastric and/or biliary bypass procedures; Ex Lap = nontherapeutic laparotomy; and CBDE = common bile duct exploration and drainage.

metastases, three with vascular invasion, one with peritoneal spread, one with local extension, and two with choledocholithiasis. Of these patients, four underwent palliative bypass, four had a nontherapeutic laparotomy, and in the two patients with common duct stones, common duct exploration and drainage was performed. In the group of 10 patients having inadequate staging laparoscopy, all underwent surgical exploration, eight (80%) were resected, and two patients (20%) were found to have liver metastases. Both of these patients underwent palliative bypass surgery.

Operative Treatment

Patients with resectable disease were treated by pancreaticoduodenectomy (n = 91, 75%), ampullectomy (n = 12, 10%), duodenal resection (n = 10, 9%), or bile duct excision (n = 7, 6%). Table 1 details the reasons for unresectability in the 23 patients with unresesctable disease (excluding choledocholithiasis) by tumor site. Ten patients (43% of unresectable patients) had an operative biliary and/or gastric bypass performed, and 13 did not. Of the 13 patients who did not undergo bypass, nine (39% of unresectable patients) were spared nontherapeutic laparotomy and in four (17%) the lesions were missed at laparoscopy.

Impact of Adequate Laparoscopy by Site and Histology

Analysis by site (Fig. 2) reveals that for ampullary lesions, all 59 patients (100%) with laparoscopically resectable disease underwent resection; four of six unresectable patients had a bypass, and nontherapeu-

Table 1. Distribution of metastatic lesions precluding resection by site

	Ampulla (n = 67)	Duodenum (n = 38)	Bile duct (n = 37)
Liver	3	3	5
Peritoneum	1	1	4
Local extension	1	0	1
Vascular invasion	0	2	1
Other metastasis	1	0	0
TOTAL	6 (9%)	6 (16%)	11 (30%)

Does not include two patients with benign cholelithiasis, initially read as a bile duct lesion, in whom a resection was not indicated.

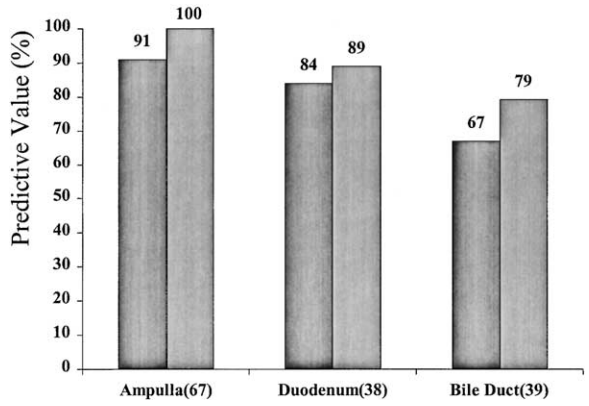

Fig. 2. Bar graph demonstrating predictive value for resectability by tumor location. Predictive value of CT (*shaded bars*) indicates percentage of patients undergoing resection after being deemed resectable by CT scan (all patients in this cohort). Predictive value of laparoscopy (*hatched bars*) indicates percentage of patients undergoing resection after being deemed resectable by CT and an adequate laparoscopy.

tic laparotomy was avoided in two patients (33%). For duodenal primary lesions, 30 (88%) of 34 patients with laparoscopically resectable disease were resected, two of six unresectable patients had bypasses, two patients underwent nontherapeutic laparotomy, and laparotomy was avoided in two patients (33%). For bile duct primary tumors, 22 (79%) of 28 patients with laparoscopically resectable disease were resected, four of 13 unresectable patients had bypasses, two underwent common bile duct exploration, two underwent nontherapeutic laparotomy, and five (38%) avoided nontherapeutic laparotomy. Predictive values for resectability for CT scanning by site were 91% for ampulla, 84% for duodenum, and 65% for bile duct.

Resectability was not related to preoperative symptoms of nausea, vomiting, or back pain; only 3 of 20 patients with these symptoms were unresectable (P = NS). A bypass was done in 5 of 15 patients with indwelling stents and five of 10 patients without stents (P = NS).

A multivariate analysis was performed using binary logistic regression to identify factors associated with resectability. Age, sex, tumor location, size, symptoms, and stent placement were included in the analysis. The only independent predictor of resectability was a bile duct tumor. None of these same factors were independently associated with the need for a biliary or gastric bypass in unresectable patients.

Pathology

Final pathologic examination revealed invasive malignancy in 124 patients. Thirty-eight patients (26%) were diagnosed with duodenal tumors, including 31 carcinomas, three carcinoid tumors, one gastrointestinal stromal tumor, one metastatic tumor, one adenoma, and one lipoma. Sixty-six patients (46%) were found to have ampullary lesions, including 53 adenocarcinomas, one carcinoid tumor, one intraductal papillary mucinous neoplasm, one carcinoma in situ, and 10 adenomas. Thirty-four patients (24%) were diagnosed with bile duct tumors including 33 cholangiocarcinomas, one cystadenoma, and one carcinoid tumor. Two patients were found to have choledocholithiasis and one patient had focal pancreatitis. In three patients a resection or bypass was performed for a benign biliary stricture, one patient was later found to have a synchronous lung cancer, and one of these patients developed a primary pancreatic ductal adenocarcinoma 1 year later.

Survival

With a median follow-up of 14 months (range 1 to 83 months), 67 patients had no evidence of disease, 21 patients were alive with disease, 49 patients were dead of disease, and seven patients died of other causes. In patients with invasive malignancies, median postoperative overall survival was 48 months (range 1 to 82 months) in resected patients and 10 months (range 1 to 27 months) in nonresected patients. Survival curves for resected patients by site of primary tumor are displayed in Fig. 3. There was no significant difference in survival by tumor site.

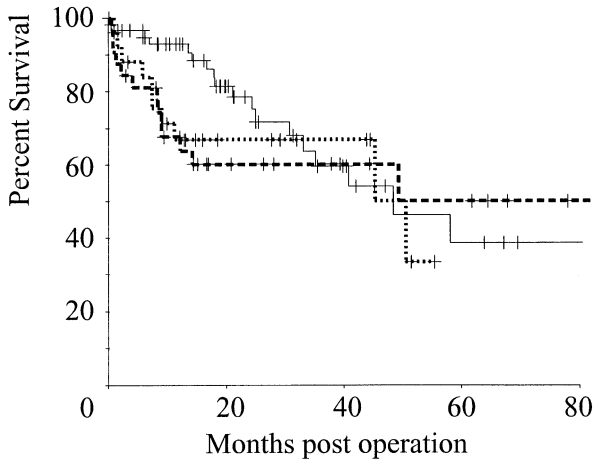

Fig. 3. Kaplan-Meier plot of disease-specific survival for resected patients by primary tumor site. Ampullary is represented by a solid line, duodenal is represented by a dashed line, and distal bile duct by a dotted line. There was no significant difference in survival by primary tumor site using log-rank analysis.

DISCUSSION

The aim of laparoscopic staging is simply to mimic the open surgical exploration and thereby avoid unnecessary laparotomy in the subset of patients who would not benefit from such a procedure. The initial report of our experience with staging laparoscopy for pancreatic neoplasms cited an improvement in resectability rates from 50% based on CT scanning alone to over 90% when staging laparoscopy was added.[2] Others have reported varying degrees of improvement in predictive value for resectability when laparoscopy is added (Table 2).[3–7,9,10] In one study, laparoscopy combined with CT scanning failed to diagnose four patients with liver metastases and three patients with vascular invasion.[3] It is likely that the liver metastases would have been identified if an adequate multiport laparoscopy (per our definition) had been performed. Bemelman et al.[4] reported their initial experience with laparoscopic ultrasound in pancreatic staging with an improvement in predictive value from 41% with CT alone to 95% when combined with laparoscopy and laparoscopic ultrasound.

Evaluation of pancreatic neoplasms by site and histology has elucidated some subsets where laparoscopy is strongly indicated. In patients with tumors in the body or tail of the pancreas, the incidence of occult metastases was 30%, and the routine use of diagnostic laparoscopy was recommended.[12] We have also reviewed our experience with nonfunctioning islet cell tumors and found a high incidence of occult metastases at laparoscopy, which precluded curative resection.[11]

Newer abdominal imaging techniques have demonstrated increased ability to visualize local extension and vascular invasion preoperatively.[4,13–16] However, these techniques have not been shown to detect small-volume liver disease or peritoneal carcinomatosis with any greater efficacy. The addition of diagnostic staging laparoscopy to spiral CT has been shown to avoid nontherapeutic laparotomy in 20% to 50% of patients deemed resectable on CT scanning.[1,3–10,17,18] These patients do not require a lengthy hospital stay, they avoid the morbidity associated with a laparotomy, and there is no treatment delay if chemotherapy and/or radiation therapy are to be offered.

In the present study, laparoscopy increased the resectability rate from 83% based on CT scan alone to 92% when an adequate laparoscopy revealed no evidence of unresectability. In the subset of patients in whom adequate laparoscopy was not performed, 80% of patients were subsequently resected. An adequate diagnostic laparoscopy spared nine patients (36%) a nontherapeutic laparotomy. Of the 25 patients deemed unresectable, 10 had a bypass, two had a common duct exploration, and four (3%) underwent a nontherapeutic laparotomy. If laparoscopy had not been done, then 13 patients (52%) would have undergone a nontherapeutic laparotomy.

Examining the surgical outcomes by primary site reveals that ampullary and duodenal lesions have a low unresectability rate, whereas cholangiocarcinomas have a higher rate of occult metastatic disease. The natural history of the duodenal and ampullary tumors is that they present earlier and are not often found to be metastatic at exploration. This is reflected in the predictive value of modern CT scanning, which is highest in ampullary and duodenal tumors. The added yield of laparoscopy for predicting resectability in duodenal and ampullary tumors is on the order of 5% to 9%. More important, the increase in predictive value when laparoscopy is used is 12% for distal bile duct tumors. In this series, as in our pancreatic adenocarcinoma dataset,[19] the majority of unresectable patients (67% to 69%) were found to have peritoneal or occult liver metastases, making unresectable patients easier to identify using a laparoscopic exploration.

Unresectable cholangiocarcinomas in this series had a high bypass rate. In this retrospective review it is difficult to determine the reasons for the increased bypass rate. In essence, these results reflect our management approach to this aggressive disease. Many of these surgical bypasses were performed early in our experience with laparoscopic staging. As our experience with managing patients without surgical bypass grows,[19] the minimally invasive options available for the management of patients with advanced disease have increased.[20,21] Early experience with stenting for cholangiocarcinomas had been unsatisfactory, making a surgical biliary bypass a more attractive op-

Table 2. Added value of laparoscopy

Reference	Year	N	Predictive value of CT	Predictive value of laparoscopy
Andren-Sandberg et al.[3]	1999	60	33%	53%
Bemelman et al.[4]	1995	70	41%	95% (LUS)
Conlon et al.[2]	1996	115	53%	91%
Callery et al.[5]	1997	50	52%	93% (LUS)
Durup Scheel-Hincke et al.[6]	1997	35	43%	88 % (LUS)
Fernandez-Del Castillo et al.[7]	1995	114	26%	93% (angiography)
John et al.[9]	1999	50	38%	68% (LUS)
Reddy et al.[10]	1999	99	35%	69%
Current Study	2001	145	83%	92% (LUS in 55%)

LUS = laparoscopic ultrasound.

tion. However, currently available endoscopic and percutaneous techniques have proved more reliable in our experience. In patients with unresectable duodenal tumors (although rare in this cohort), a gastric bypass may be indicated in symptomatic patients because of the anatomic location and size of the lesion.

This study illustrates that the routine use of diagnostic laparoscopy in patients with known duodenal or ampullary primary lesions has a limited utility with only a minimal increase in predictive value and reduction in nontherapeutic laparotomies. This appears to be consistent with a report by Saldinger et al.[22] who demonstrated a zero yield of metastatic disease in their subset of patients with nonpancreatic adenocarcinoma periampullary tumors. However, the data for distal bile duct tumors indicate that there is a decreased predictive value for a negative laparoscopy in this location, although there is an increased yield of CT occult metastatic lesions. Our present recommendation is that diagnostic staging laparoscopy be added to spiral CT scanning in the management of patients with distal bile duct tumors in whom a biliary or gastric bypass is not contemplated preperatively. In addition, laparoscopy is indicated in patients with very large duodenal or ampullary tumors, where distant metastatic spread is more likely. This approach is supported by the decision analysis completed by Obertop and Gouma,[23] where diagnostic laparoscopy has a higher utility than exploratory laparotomy in a situation where laparoscopy has a higher yield of unresectable disease.

Laparoscopic staging is less likely to be helpful in patients with ampullary tumors when the histologic diagnosis is known or strongly suspected preoperatively, as was the case in the current cohort. In patients with radiologic resectable distal bile duct tumors, laparoscopy can help avoid a significant number of nontherapeutic laparotomies, especially if unresectable patients are then managed by endoscopic or percutaneous metallic stent placement.

CONCLUSION

This study suggests that patients with distal bile duct tumors benefit from laparoscopic staging both in terms of determining resectability and avoiding unnecessary surgery. In contrast, those with known duodenal and ampullary tumors gain little added value from laparoscopy. However, we continue to advocate diagnostic staging laparoscopy for all patients with pancreatic adenocarcinoma, as well as those patients in whom the histologic diagnosis of a periampullary tumor is not known preoperatively, as this modality has been demonstrated to be safe, accurate, and cost-efficient.

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Discussion

Dr. Henry A. Pitt (Milwaukee, WI)*:* I would use your data to conclude that there is definitely no role for laparoscopy in ampullary tumors or in duodenal tumors, and I wonder about your distal bile duct tumors—whether you can really be sure that is what they were versus pancreatic tumors. When we examined our patients with distal bile duct tumors in Baltimore a few years ago, 92% of them were resectable, and I think it can be sometimes quite difficult, especially if these tumors are not explored or resected, to know where they are coming from. Occasionally, the ERCP will provide a clue if the pancreatic duct is open. How do you know these were all distal bile duct tumors?

Dr. A. Brooks: That is an excellent question. The point you are making is that whether these tumors are unresectable is not known for sure, and we have to use a combination of imaging, ERCP, and pathology data. The best way to know is once the final pathology report on the resected specimens is available, and of course, if the tumors have all been resected, then the yield for the unresected patients cannot be calculated. The main point is that patients with tumors in that location were spared a laparotomy.

Dr. Jeffrey Matthews (Cincinnati, OH)*:* Several years ago we presented data showing that for nonpancreatic cancer the unresectability rate as predicted by high-quality CT angiograms was really quite low. So I am wondering if you could expand on the quality of your CT scans over the period of the study and how those were uniformly or nonuniformly used to predict the ability to resect.

Dr. Brooks: The study period goes from 1993 to 2000. After 1995, we performed helical CT scans in all patients, and more recently we have been using the double- and even triple-phase CT. As you can see from the data, the reasons that were found for unresectability at laparoscopy still mostly involved the peritoneum and liver surface, and we have not found even triple-phase CT to help in those patients. I believe that there will be a certain percentage yield, maybe 5% in the duodenum, but up to 20% for the other tumors in those locations.

Dr. Keith Lillemoe (Baltimore, MD)*:* In the example you presented, was that the sole liver metastasis?

Dr. Brooks: No. That patient actually had another metastasis on the right that was actually smaller. I could not get a good picture of it.

Dr. Lillemoe: With a duodenal or ampullary carcinoma that is otherwise resectable, in the presence of one or two small liver metastases that are amenable to local resection, would your group perform a Whipple procedure? I think probably at our institution that a patient with duodenal cancer with isolated liver metastasis confirmed on ultrasound in the operating room might still be resected. What would the treatment be at Memorial Sloan–Kettering in that situation?

Dr. Brooks: I think that the tumor could technically be resected. That patient's tumor would not be resected by our group because we are not aware of the benefit. Really, biologically, I do not think that patient would have been helped much.

Dr. Lillemoe: But there is a 60% 5-year survival for duodenal cancers that are resected. I think you can almost apply the same logic with duodenal cancer that you would with colon cancer.

Dr. Brooks: That may need to be proved a bit more conclusively before that becomes the standard of care.

Dr. W. Scott Helton (Chicago, IL)*:* The break-even point for cost or the cutoff cost versus additional diagnostic yield, whatever you want to call it, for diagnostic laparoscopy in this setting is usually determined by the average length of stay for patients who undergo a nontherapeutic laparotomy. With that in mind, I would like to ask what is the average length of stay for a nontherapeutic laparotomy at your institution when metastatic cancer is diagnosed? Many people have determined at their hospitals that the

break-even rate is one of 10 patients. So sparing only 10% of patients a nontherapeutic laparotomy is cost-effective.

Dr. Brooks: I am familiar with that analysis. The 10% number is helpful for our data, but I could not really give you any figures on length of stay because I had only four patients who underwent a nontherapeutic laparotomy. But overall, including pancreatic adenocarcinomas in the patients who underwent surgical exploration, our median length of stay for nontherapeutic laparotomy is 5 days. It may be getting shorter now, but I have had so few patients in the recent few years that I cannot answer that. But I agree with the 10% point. I think that the earlier decision analysis by Obertop et al.[23] showed that that number was actually applicable to pancreatic cancer.

Dr. John Hoffman (Philadelphia, PA)*:* I would like you to expand on the laparoscopic failures. Why didn't you put them into your denominator of laparoscopically determined resectability? Second, aren't you concerned about a large tumor of the duodenum or ampulla bleeding or creating an obstruction, thus benefitting from removal or bypass?

Dr. Brooks: Regarding your first question, we had 11 patients who were deemed to have had inadequate laparoscopy, and for technical reasons, either severe adhesions or other problems, the patients did not receive a complete evaluation with laparoscopy, and that was the reason why we left them out of the denominator. If you take a lot of extra time and apply the principles of staging to a complete laparoscopy, then we feel that you have done an adequate job.

The second question relates to prophylactic bypass in these patients. That was not in the scope of this presentation, but there was a prior presentation from our institution regarding that. As I mentioned, the patient with the metastatic lesion had a small duodenal tumor, and it has been our experience with pancreatic carcinomas that they do not require prophylactic bypass. In that particular study, we showed that only 3% to 3.5% went on over the entire remaining survival to require a gastric bypass in a therapeutic fashion. So prophylactic bypass has not been the standard of care at our institution.

Dr. Carlos Fernandez-del Castillo (Boston, MA)*:* Is that the case for duodenal carcinomas or for pancreatic cancers?

Dr. Brooks: The group was pancreatic adenocarcinoma in the particular abstract presented here, but in general that has been our approach unless there is an indication because of symptoms. If the patient was truly bleeding or obstructed, we would proceed to a palliative procedure.

En Bloc Resection for Locally Advanced Cancer of the Pancreas: Is It Worthwhile?

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The benefit of radical surgical resection of contiguously involved structures for locally advanced pancreatic cancer is unclear. The aim of this study was to examine patient outcome after extended pancreatic resection for locally advanced tumors and to determine if any subset of extended resection affected outcome. We retrospectively reviewed the records of 116 patients with adenocarcinoma of the pancreas, who underwent extirpative pancreatic surgery between 1987 and 2000. Of the 116 patients, 37 (32%) required resection of surrounding structures (group I), and 79 patients (68%) underwent standard pancreatic resections (group II). In all cases, all macroscopic disease was excised. In group I a total of 46 contiguously involved structures were resected: vascular in 25 patients (54%), mesocolon in 16 (35%) (colic vessels in 3, colon in 13), adrenal in three (7%), liver in one (2%), stomach in one (2%) (for a tumor in the tail of the pancreas), and multiple structures in four. Excision of regional blood vessels included the superior mesenteric vein and/or portal vein in 16, hepatic artery in five, and celiac axis in four. No differences between groups I and II were detected for any of the following parameters: age, sex, history of previous operation, estimated blood loss, or hospital stay. For the entire cohort the morbidity and mortality were 38% and 1.7%, respectively, and these rates were similar in the two groups. Adjuvant therapy was administered to more than 90% of patients in both groups. However, patients in group I were more likely to have received neoadjuvant therapy (76% vs. 42%, $P = 0.001$). Total pancreatectomy and distal pancreatectomy were more often performed in group I ($P = 0.005$). Additionally, the median operative time was longer (8.5 hours compared to 6.9 hours ($P = 0.0004$)). Both groups had similar rates of microscopically positive margins and involved lymph nodes, as well as total number of lymph nodes removed. The median survival was 26 months for patients in group I and 16 months for patients in group II ($P = 0.08$). The median disease-free survival for groups I and II was 16 months and 14 months, respectively ($P = 0.88$). In comparing patients in group I, who underwent vascular resection vs. mesocolon (colon or middle colic vessels) resection, the median survival was 26 months and 19 months, respectively ($P = 0.12$). We were unable to detect a difference in outcome for patients with locally advanced cancers requiring extended pancreatic resections compared to patients with standard resections. En bloc resection of involved surrounding structures, to completely extirpate all macroscopic disease, may be of benefit in selected patients with locally advanced disease, particularly when combined with preoperative chemoradiation therapy. (J GASTROINTEST SURG 2002;6:147–158.)

KEY WORDS: Extended resection, advanced pancreatic cancer

The prognosis of adenocarcinoma of the pancreas is poor, with a 5-year survival rate of less than 5%.[1] The only potentially curative treatment for pancreatic carcinoma is surgical resection. However, because the disease is so advanced at the time of presentation, only a minority of patients are resectable for cure, with nearly a third of patients presenting with locally advanced disease.[2] The median survival for patients with locally advanced, unresectable tumors is 9 months,[3] and survival is not improved with the use of palliative chemoradiation therapy.[4] In contrast, after resection, 5-year actuarial survival rates of 19% to 24% have been reported.[5,6]

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In 1973 Fortner,[6] in an effort to increase resectability, proposed en bloc resection of the pancreas along with the superior mesenteric vein/portal vein (SMV/PV) confluence and surrounding lymphatic vessels.[7] Furthermore, arterial involvement was managed with resection and subsequent arterial reconstruction. However, this was associated with significant morbidity.[7,8] Recent series have reported low morbidity and no detrimental effect on oncologic outcome after resection of the SMV/PV confluence.[2,9,10] Contemporary reports of venous and arterial (hepatic artery, superior mesenteric artery, and celiac axis) resections have observed increased morbidity and decreased long-term survival.[10,11]

Along with involvement of the regional blood vessels, locally advanced cancer of pancreas may infiltrate surrounding visceral organs. When this occurs, complete surgical extirpation requires resection of the involved structures. However, data regarding multivisceral resections for adenocarcinoma of the pancreas are lacking. The objective of this study was to examine our experience with extended pancreatic resections for locally advanced adenocarcinoma of the pancreas.

MATERIAL AND METHODS

We retrospectively reviewed the records of 116 patients who underwent surgical resection for adenocarcinoma of the pancreas at Fox Chase Cancer Center from 1987 to 2000. Patients undergoing palliative procedures were excluded, and only patients treated with curative intent were included. Data regarding clinicopathologic characteristics were recorded. Overall, the median age was 68 years (range 41 to 85 years) and 64 patients were women (55%). The location of the tumor was in the pancreatic head in 101 patients (87%), the body in nine (8%), and the tail in six (5%). Preoperative imaging studies included computed tomography and visceral arteriography, and encroachment of the tumor to the SMV/PV confluence, as described by Ishikawa et al.,[12] was recorded (Table 1). Twenty-two patients (19%) had undergone prior exploratory operations (at outside institutions) and were considered unresectable at those institutions. Neoadjuvant chemoradiation therapy was administered in 61 patients (53%) and consisted of 5-fluorouracil–based[13,14] or gemcitabine regimens.[15] This was delivered concurrently with external beam radiation therapy in 180 cGy fractions to a dosage of 3960 cGy and to a target area 3 cm around the tumor bed. An additional dose of 1080 cGy, for a total 5040 cGy, was targeted to an area 2 cm around the tumor.

Table 1. Radiographic classification of superior mesenteric vein and portal vein involvement

Ishikawa score	Findings
Type I	Normal
Type II	Lateral displacement
Type III	Unilateral narrowing
Type IV	Bilateral narrowing
Type V	Bilateral narrowing with collateral veins

Operative Details

Extended resection was defined as the en bloc resection of contiguously involved structures required to completely extirpate all gross disease. Standard pancreaticoduodenectomy included resection of the head of the pancreas, duodenum, gallbladder, and antrum of the stomach. Extended pancreaticoduodenectomy included, in addition to the standard procedure, resection of surrounding structures not routinely excised, that is, major blood vessels (SMV, PV, hepatic artery, and celiac axis) and adjacent organs (adrenal, colon, and liver). The same methodology was applied in total and distal pancreatectomies. When possible, splenic conservation was attempted in patients undergoing total or distal pancreatectomy. However, en bloc splenectomy was not considered as an extended resection. Only planned excision of vascular structures was considered as an extended resection. Unintentional vascular injury requiring repair was not included. Resections of adjacent structures and organs were performed with the supposition that these structures were clinically involved and with the intent of extirpating all gross tumor.

Among the 116 patients undergoing resections, there were 98 (84%) pancreaticoduodenectomies (Whipple procedures, one of which was pylorus preserving), 11 total pancreatectomies, six distal pancreatectomies, and one central pancreatectomy. A total of 37 patients (32%) required resection of surrounding structures (group I) and 79 patients (68%) had standard pancreatic resections (group II). In all cases, all macroscopic disease was excised. In group I a total of 46 contiguously involved structures were resected: vascular in 25 patients (54%), mesocolon in 16 (colic vessels in 3, colon in 13) (35%), adrenal in three (7%), liver in one (2%), stomach in one (2%), and in four patients multiple structures were excised. Resection of major regional blood vessels included SMV and/or PV in 16, hepatic artery in five, and celiac axis in four patients. Management of tumor adherence to the SMV or PV included tangential excision in 11 patients and segmental excision in four patients. Reconstruction included patch venoplasty

utilizing the greater saphenous vein in three patients with tangential excision. Eight patients had primary closure of the defect, with care being taken not to narrow the lumen by more than 50%. Adequacy of primary closure was occasionally evaluated by intraoperative Doppler ultrasound imaging and measurement of pressure gradients. Three segmental resections were reconstructed primarily, with one patient requiring a superficial femoral vein interposition graft.

In three patients the tumor extended to the hepatic artery at the origin of the gastroduodenal artery, necessitating excision of a portion of the hepatic artery. Reconstruction was performed via a patch angioplasty and interposition graft utilizing the saphenous vein in two patients, whereas the third patient had primary repair in an end-to-end fashion. Tumor involvement of a replaced right hepatic artery required excision of the arterial segment with transposition of the distal segment to the proper hepatic artery at the origin of the gastroduodenal artery. One patient had excision of a long segment of the common hepatic artery (along with the celiac trunk) and revascularization was performed with a saphenous vein graft from an aberrant left gastric artery to the proper hepatic artery.

Four patients with tumors located in the head (n = 1) and body (n = 3) of the pancreas underwent resection of the celiac axis because of local extension to the celiac axis. One of these resections was performed in conjunction with a central (segmental) pancreatectomy. Hepatic arterial revascularization was accomplished using saphenous vein grafts from the superior mesenteric artery to the proper hepatic artery (2 patients), and a saphenous vein graft from the aberrant left gastric artery to the proper hepatic artery (1 patient). No revascularization was required for the patient who underwent central pancreatectomy because the gastroduodenal artery was spared, providing collateral blood flow to the liver. Combined pancreaticoduodenectomy and celiac trunk resection results in sacrificing the gastroduodenal, left gastric, and splenic arteries, thereby greatly reducing gastric vascularity and compromising the integrity of the gastroenterostomy. Of the four patients with celiac axis resections, two required revascularization of the left gastric artery, and this was accomplished with a saphenous vein graft from the hepatic artery graft to the stump of the left gastric artery. In two patients this was not required because of an aberrant origin of the left gastric artery from the aorta in one patient and sparing of collateral flow from the gastroduodenal artery in the other patient.

Tumor infiltration of the mesocolon in 16 patients mandated resection of the middle colic vessels (n = 3) and colon (n = 13). Resection of involved middle colic vessels in three patients was performed without concomitant colectomy. However, in 13 patients extensive involvement of the transverse and right colon mesentery resulted in significant foreshortening of the mesentery requiring partial colectomy to completely extirpate the lesion. In one patient the tumor appeared to be adherent to the liver and an en bloc wedge resection was performed, along with resection of the hepatic artery and right colon. The need for multivisceral resection in this patient is attributable to dense adhesion formation from a previous attempt at resection at an outside institution. Four adrenalectomies and one partial gastrectomy were performed for locally advanced lesions originating from the tail of the pancreas.

Pathologic Findings

Pathology reports were reviewed and only patients with histologic findings indicative of adenocarcinoma were included. Routine examination of the specimens included detailed analysis of the resection margins, tumor size, differentiation, and lymph node involvement. Tumor infiltration of surrounding structures and organs was not routinely recorded and these data are not available for analysis. The margins of pancreaticoduodenectomy specimens were thoroughly examined microscopically and included the SMV, superior mesenteric artery (SMA), pancreas (at transection edge), common bile duct (CBD), and retroperitoneum (defined as tissue on the posterior aspect of the pancreas between the duodenal sweep and SMV).[16] Assessment of margins for total pancreatectomy specimens included the above-mentioned margins with the exception of the cut edge of the pancreas, whereas for distal pancreatectomy the margins evaluated were the retroperitoneum and pancreas (at the transection edge). Neoplastic cells present 1 mm or less from the margin were considered positive. The size of the tumor was determined by serial sectioning of the specimen and gross measurement of the lesion in the largest dimension, reported in centimeters. In three patients the tumor size was unavailable, and in 10 patients no tumor was grossly visible but disease was detected microscopically. The pathologic characteristics of the two groups are shown in Table 2.

Statistical Analysis

Patient follow-up included regularly scheduled physical examinations and imaging tests. Median follow-up of surviving patients was 19 months (range 4 to 150 months). Comparisons of categorical and

Table 2. Clinicopathologic features of patients

	Group I (extended resection)		Group II (standard resection)		
	n = 37	32%	n = 79	68%	*P* value
Age (yr)					
Median	65		69		0.14
Range	41–85		41–83		
Sex					
Male	15	41%	37	47%	0.55
Female	22	59%	42	53%	
Prior attempts at resection	9	24%	13	16%	0.32
Tumor location					
Head	26	70%	75	95%	0.001
Body	6	16%	3	4%	
Tail	5	14%	1	1%	
Procedure					
Whipple	26	70%	72	91%	0.005
Total pancreatectomy	5	14%	6	8%	
Distal pancreatectomy	5	14%	1	1%	
Central pancreatectomy	1	3%	0	0	
Ishikawa score[12]					
1	9	30%	31	44%	0.12
2	4	13%	14	20%	
3	5	17%	10	14%	
4	7	23%	13	19%	
5	5	17%	2	3%	
Neoadjuvant therapy					
None	9	24%	46	58%	0.001
5-Fluorouracil*	13	35%	22	28%	
Gemcitabine*	15	41%	11	14%	
Mean tumor size (cm)	3.2		2.9		0.23
Microscopically positive margins	22	59%	49	62%	0.84
No. of positive margins					
0	15	42%	31	39%	0.93
1	12	33%	27	34%	
2	7	19%	14	18%	
3	2	6%	4	5%	
4	0	0	3	4%	
Malignant lymph nodes	16	43%	44	56%	0.24
No. of positive lymph nodes					
Median	3		3		0.28
Range	1–7		1–12		
Lymph nodes removed					
Median	17		17		0.74
Range	2–43		2–55		
Pathology (differentiation)					
Well differentiated	4	13%	10	14%	0.69
Moderate	15	48%	40	56%	
Poor	12	39%	21	30%	
Median operative time (hr)	8.5		6.9		0.004
Estimated blood loss (ml)					
Median	1200		950		0.4
Range	150–8500		200–6000		
Length of hospital stay (days)					
Median	16		17		0.79
Range	8–61		8–144		
Complications	13	35%	31	39%	0.84
Operative mortality	1	2.7%	1	1.3%	0.6

*Chemotherapy was administered concurrently with external beam radiation therapy (5040 cGy).

continuous values were performed using Fisher's exact test or Wilcoxon tests, where appropriate. Overall survival was recorded from the time of tissue biopsy until death or last follow-up. Disease-free survival was defined as the length of time from diagnosis (histologically confirmed) until recurrence (local or distant) was detected. Failure was defined as the first site of tumor recurrence and was characterized as local, arising from the resection area, or distant (metastatic). Failure was documented when tumor recurrence was detected by imaging modalities; histopathologic confirmation of recurrence was not required. Overall survival and disease-free survival curves were estimated using the method of Kaplan and Meier. Log-rank tests and Cox proportional hazards methods were used to conduct between-group comparisons in overall survival and disease-free survival distributions. Patients lost to follow-up were censored from the analysis at the time of their last visit. A multivariable model was fit to the survival data using Cox proportional hazards methods. A stepwise variable selection procedure was used to find a parsimonious model. All tests were two sided with a type I error of 5%.

RESULTS

Comparative Analysis Between Groups

A comparison of the clinicopathologic characteristics is shown in Table 2. No difference was detected between the two groups for the following parameters: age, sex, and prior attempt at resection. There were more body and tail tumors in group I than in group II ($P = 0.001$) and, accordingly, more total and distal pancreatectomies were performed in group I ($P = 0.005$). Preoperative imaging demonstrated a trend toward more vascular involvement (as determined by the Ishikawa score) in group I than in group II, although the difference did not reach statistical significance ($P = 0.12$). Patients in group I were more likely to have received preoperative chemoradiation therapy than in group II ($P = 0.001$). Operative and postoperative parameters such as median estimated blood loss and length of hospital stay were similar in both groups. However, the median operative time was significantly longer in patients with extended resections (8.5 hours) than in those with standard resections (6.9 hours) ($P = 0.004$).

For resections that included the head of the pancreas, the specimen was subjected to a comprehensive inspection of multiple margins including the SMV, SMA, retroperitoneum, CBD, and pancreatic cut edge. The rate of microscopically positive margins for group I and group II was 59% and 62%, respectively ($P = 0.84$). Also, the number of involved margins was similar in each group ($P = 0.93$). The administration of preoperative chemoradiation therapy has been associated with a decrease in the incidence of microscopically positive margins.[16] Comparing patients who only received preoperative therapy in groups I and II, the rate of positive margins tended to be higher in group I (61%) vs. group II (45%) ($P = 0.31$).

The median number of lymph nodes identified was 17 for both groups. It should be noted that the specific aim in performing extended resections was to completely extirpate all gross disease and not to perform a wide-field lymphadenectomy. Malignant lymphadenopathy was present in 43% of patients in group I compared to 56% of patients in group II ($P = 0.24$). In addition, the median number of involved lymph nodes was three in each group ($P = 0.28$).

Morbidity and Mortality

Complications occurred in 13 patients (35%) in the extended group and 31 patients (39%) in the standard group ($P = 0.84$). The complications are listed in Table 3. The postoperative mortality rate, defined as death during the initial hospitalization or within 30 days postoperatively, for the entire cohort was 1.7%. One death occurred in each group for a

Table 3. Postoperative complications

Complications	Group I (extended resection)	Group II (standard resection)
Wound infection	3	
Intra-abdominal abscess	3	3
Adult respiratory distress syndrome	1	
Cholangitis	2	1
Sepsis	1	1
Ascites	3	1
Upper gastrointestinal bleeding	2	2
Delayed gastric emptying/gastric outlet obstruction	1	3
Pancreatic fistula	2	
Liver abscess	3	1
Gastrocutaneous fistula	1	
Deep venous thrombosis	3	1
Stroke	1	
Chylous ascites	2	2
Sepsis	1	1
Atrial fibrillation (requiring cardioversion)	2	
Clostridium difficile colitis	3	1
Intra-abdominal hemorrhage	1	
Death	1	1

Some patients had more than one complication.

mortality rate of 2.7% in group I and 1.3% in group II ($P = 0.6$).

Survival Analysis

The 5-year actuarial overall survival rate of 16% for group I (median 26 months) was higher than the 9.5% rate for group II (median 16 months), but this did not reach statistical significance ($P = 0.08$) (Table 4; Fig. 1). The median disease-free survival was 16 months and 14 months, respectively ($P = 0.88$) (Fig. 2). For group I, factors that significantly affected overall survival (as determined by univariate analysis) include positive SMV and CBD margins, and postoperative complications (Table 5). For disease-free survival the significant factor was the development of presence of postoperative complications. Although disease-free survival was decreased in patients with positive margins and positive SMV and CBD margins, this did not reach statistical significance ($P = 0.07$, $P = 0.08$ and $P = 0.1$, respectively). In patients with standard resections (group II), the following parameters for overall survival were significant: positive margins, number of positive margins, SMA margin, SMV margin, metastatic lymphadenopathy, number of positive lymph nodes, increasing Ishikawa score, and age. Significant factors for disease-free survival were positive margins, number of positive margins, SMA margin, metastatic lymphadenopathy, number of positive lymph nodes, and increasing Ishikawa score.

Multivariate analysis, performed by stepwise regression, identified microscopically positive margins as a predictor of overall survival for patients in group II. The presence of metastatic lymphadenopathy was an independent prognostic factor of decreased disease-free survival in group II. For group I no significant variable was identified for either overall or disease-free survival, probably because of the smaller number of patients (Table 6).

To determine the impact of tumor infiltration of the mesocolon necessitating pancreatic resection along with colon or middle colic vessel resection for locally advanced tumors, a separate survival analysis of patients in group I comparing resection of vascular structures vs. mesocolon involvement was performed. Patients with resection of both structures (n = 4) were grouped with patients requiring resection of the mesocolon. The median overall survival of patients requiring excision of regional blood vessels was 26 months compared to 19 months for patients with colectomies, and the 5-year actuarial overall survival was 21% vs. 7%, respectively ($P = 0.12$) (Fig. 3). Median disease-free survival was 17 months and 14 months, respectively ($P = 0.29$).

Table 4. Results of survival analysis

	Group I (extended resections)	Group II (standard resections)	*P* value
Overall survival			
5-yr actuarial	16%	9.5%	0.08
Median (mo)	26	16	
Disease-free survival			
5-yr actuarial	22%	15%	0.88
Median (mo)	16	14	

DISCUSSION

Nearly 35% of patients present with locally advanced pancreatic cancers with involvement of surrounding structures and organs.[2] The role of extended pancreatic resection in these circumstances is controversial. Increased morbidity and limited survival benefit have been cited in favor of nonresectional behavior.[8,17]

This series demonstrates that en bloc resection of contiguously involved structures and organs can be performed safely. No differences in morbidity and mortality were detected in patients requiring extended vs. standard resection. A complication rate of 35% and a mortality rate of less than 3% are in accordance with rates in other recent series.[18,19] Although operative time was significantly longer in extended resections compared with standard resections (8.5 hours vs. 6.9 hours, $P = 0.004$), the estimated blood loss and length of hospital stay were similar between the two groups.

In this series the median overall survival for patients with locally advanced adenocarcinoma of the pancreas requiring extended resection was 26 months compared to 16 months for standard resection ($P = 0.08$). However, this was not a randomized trial and meaningful comparison of survival data may be unreliable. The trend toward increased survival in the extended group is unlikely to persist with a larger number of cases and also is probably the result of selection bias. Nonetheless, it does appear that at the very least, survival after extended resection is no worse than after standard resection. This is in agreement with other recent reports.[2,10,11,18] Moreover, it has been suggested that venous involvement is not an indicator of aggressive biological behavior of the tumor but rather a function of tumor location in the pancreas.[18]

For patients undergoing extended resection, pathologic examination of the specimen to detect tu-

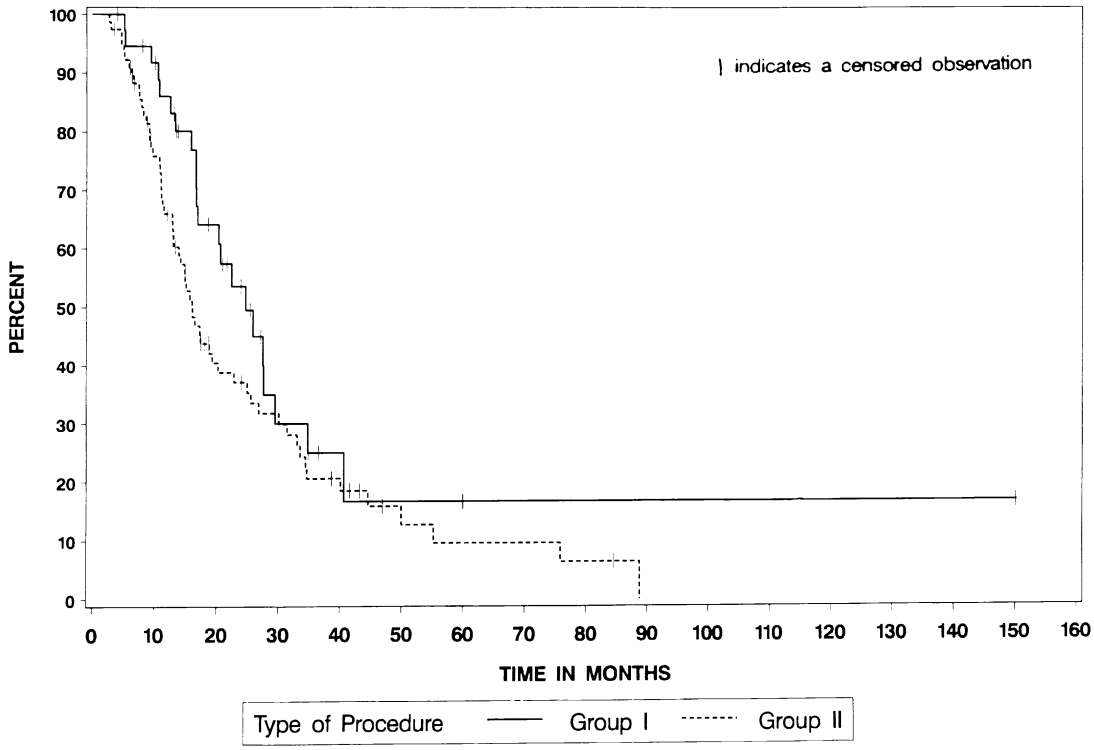

Fig. 1. Comparison of overall survival in patients with extended vs. standard resections.

mor infiltration of surrounding structures was not routinely performed and sufficient data for analysis are not available. When specifically examined, the reported incidence of tumor invasion of the SMV and PV ranges from 3% to 72%.[9–11,19] However, the ability to differentiate between tumor adherence and infiltration is often not possible intraoperatively. Hence the decision to resect surrounding adherent structures must be made clinically, and en bloc resection permits extirpation with potentially negative margins and may reduce the risk of local recurrence. The determination of resectability when performing a pancreaticoduodenectomy is often based on suspected involvement of nearby major vascular structures. We report that resection of surrounding structures adherent to the pancreatic neoplasm can be performed safely and survival is not adversely affected in patients requiring extended resections.

We observed that patients undergoing extended resections (group I) were much more likely to have received preoperative chemoradiation therapy than patients undergoing standard resections (group II), $P = 0.001$. However, of the 61 patients who received preoperative therapy, nearly 55% of them did not require an extended resection. Furthermore, the increased use of preoperative therapy in group I most likely represents an institutional bias for using neoadjuvant therapy when locally advanced pancreatic cancer is suspected based on diagnostic imaging tests. This is supported by the trend toward increasing Ishikawa scores in patients in the extended group compared to the standard group ($P = 0.12$). Nonetheless, inflammatory changes induced by preoperative chemoradiation therapy may mimic tumor adherence and clinically distinguishing between the two may not be possible. The relationship between neoadjuvant therapy and the need for resection of surrounding structures needs to be further investigated. No doubt, careful pathologic examination of the specimen to determine the extent of tumor invasion of surrounding structures in patients undergoing preoperative chemoradiation therapy would be instrumental in clarifying this issue.

Of the 16 patients requiring SMV and PV resection in this series, 11 (69%) underwent tangential excision with primary repair or vein patch repair. Several investigators have advocated excision of the SMV/PV confluence, when clinically indicated, with either a primary or interposition graft repair.[10,18] A recent retrospective series comparing tangential excision and segmental resection observed no significant difference in survival between the two groups.[19]

Large tumors of the head and body of the pancreas may extend superiorly to the celiac trunk, often considered a criterion of unresectability. In this series four patients had en bloc resection of the celiac

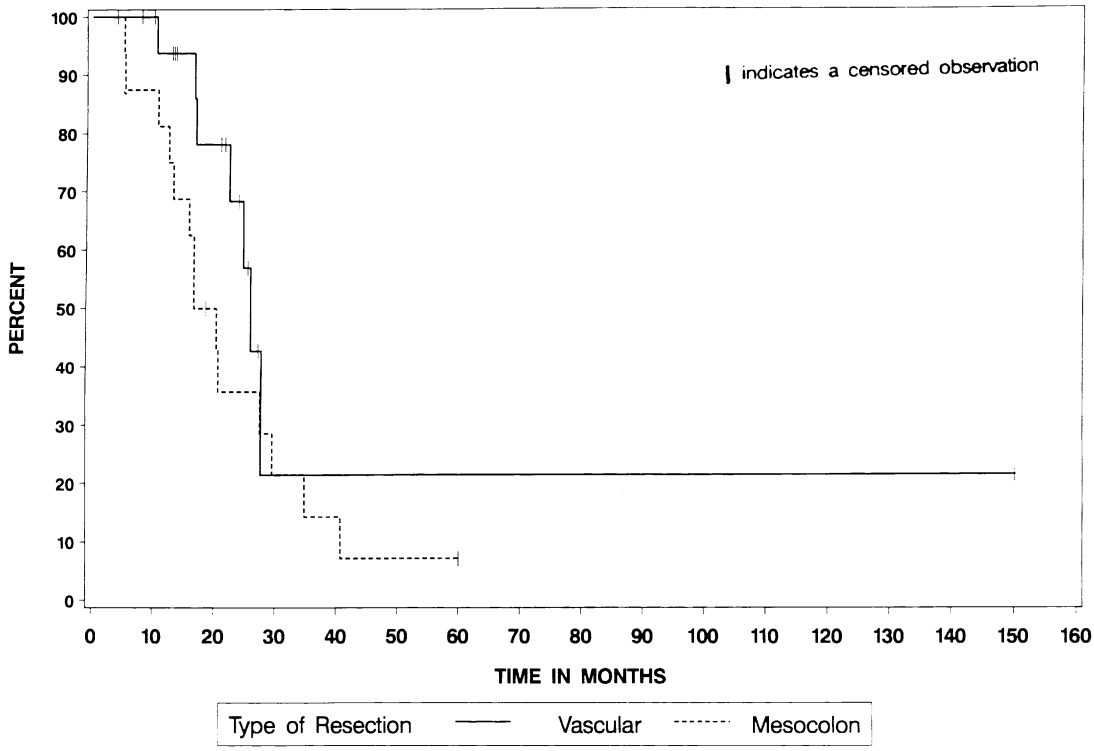

Fig. 2. Disease-specific survival in patients with extended and standard resections.

trunk, and currently two patients are alive with disease at 27 and 22 months, respectively, and two patients are alive without evidence of disease at 13 and 10 months, respectively. Although long-term survival is unlikely in these patients, a significantly prolonged survival compared to no resection has been reported.[20]

Table 5. Significant factors influencing overall survival as determined by univariate analysis

Group	*P* value
Group I (extended resection)	
Positive SMV margin	0.03
Positive CBD margin	0.02
Postoperative complications	0.002
Group II (standard resection)	
Positive margins	0.004
No. of positive margins	0.001
Positive margins	0.001
Positive SMA margin	0.01
Positive SMV margin	0.004
Metastatic lymphadenopathy	0.04
No. of positive lymph nodes	0.02
Increasing Ishikawa score	0.0003
Increasing age	0.03

In the group requiring extended resections, en bloc colectomy and resection of middle colic vessels because of infiltration of the mesocolon was performed in 16 patients. We were unable to detect a statistically significant difference in overall survival for patients undergoing resection of the middle colic vessels or colon vs. vascular resection, although there was a trend toward increased survival in the group undergoing vascular resection ($P = 0.12$). It has been suggested by Klempnauer et al.[11] that concomitant colon resection is associated with a poor prognosis. They reported a median survival of 6.2 months and a postoperative mortality rate of 35% for patients requiring colectomy. In contrast, we observed a me-

Table 6. Independent prognostic factors for survival as determined by multivariate analysis

Group	*P* value
Group I (extended resection)	
No factors identified	
Group II (standard resection)	
Overall survival	
Positive margins	0.02
Disease-free survival	
Metastatic lymphadenopathy	0.01

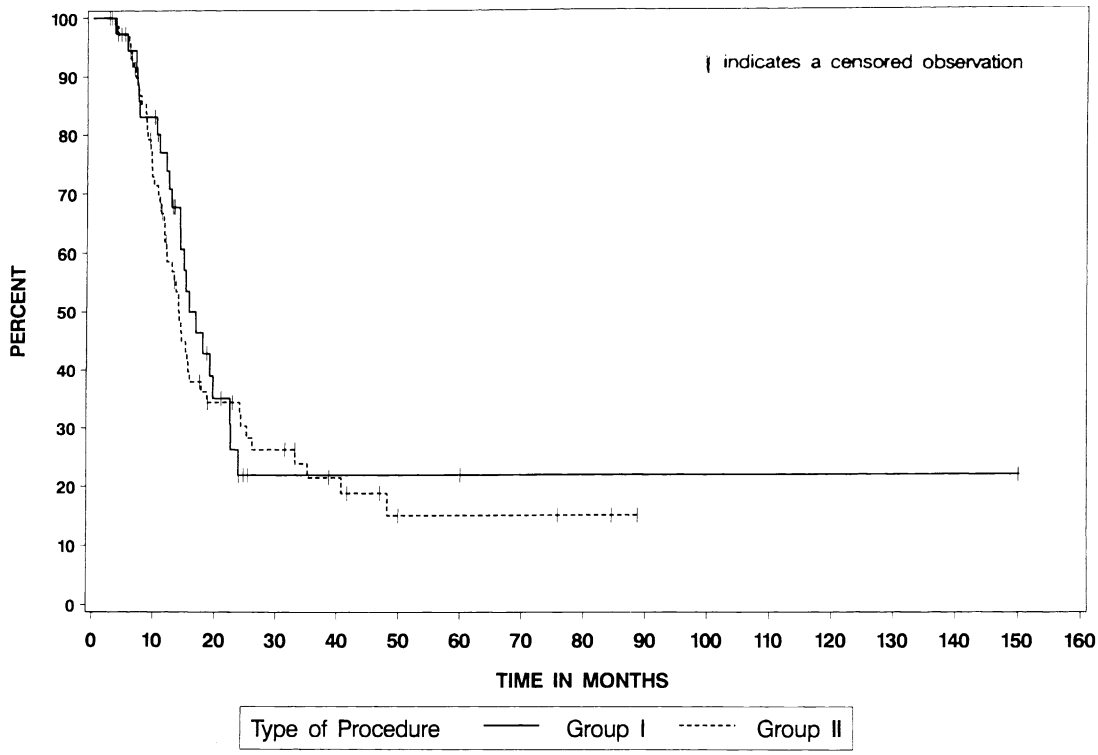

Fig. 3. Comparison of overall survival in patients with resection of major vascular structures vs. resection of middle colic vessels or colectomy.

dian survival of 19 months and had only one postoperative death (6%). Because of the infrequent resection of liver, stomach, and adrenal gland, no meaningful comparison was possible.

Multivariate analysis identified positive resection margins and metastatic lymphadenopathy as independent prognostic factors for overall survival and disease-free survival for patients with standard resections. The negative survival impact of positive margins and lymph node involvement has been observed in many recent studies and is associated with high rates of local and regional recurrences.[21–25] Interestingly, neither of these two pathologic features proved to be prognostic in patients undergoing extended resections. A possible explanation for this could be the increased use of preoperative chemoradiation therapy in the extended resection group as opposed to those undergoing standard resection. Neoadjuvant therapy has been associated with a decrease in microscopically positive resection margins as well as a decrease in the number of involved lymph nodes.[16,26–29] Furthermore, the significance of positive margins in the setting of preoperative radiation therapy is uncertain. Ishikawa et al.[30] have suggested that preoperative radiation therapy results in degenerative neoplastic cells, particularly at the periphery of the lesion. In the setting of preoperative therapy, the presence of nonviable tumor cells at the margins of resection may have little oncologic impact and further prospective studies of neoadjuvant therapy are needed to investigate this possibility.

The presence of positive resection margins is associated with high rate of recurrence and low overall survival. Pragmatically, resections that result in positive margins are often viewed as palliative in nature and not worthy of performing.[31] However, despite the presence of microscopically positive margins, a survival benefit has been shown in comparison to no resection (bypass procedure).[3] Additionally, patients with one positive margin and preoperative chemoradiation therapy had a median survival of 18 months in this series. The incidence of positive margins and overall survival in patients receiving neoadjuvant therapy in group I vs. group II were similar ($P = 0.31$ and $P = 0.21$, respectively).

Although the 5-year actuarial survival rate for patients with extended resections was 16%, there are currently only two patients who are alive and have been disease free for more than 5 years; both of them had negative margins. Despite the lack of many long-term survivors, the median survival for patients with extended resection was 26 months, which is better than the 9 months reported for patients who were unresectable.[3] At present, there are four patients who

are alive without evidence of disease at 19 to 26 months of follow-up. Thus, if the surgery can be accomplished with low mortality and low serious morbidity, it would seem worthy of consideration.

CONCLUSION

We have demonstrated that resection of surrounding structures and organs can be performed en bloc for locally advanced adenocarcinoma of the pancreas with acceptable perioperative morbidity and mortality. This report is nonrandomized and the results of survival analysis should be interpreted with caution, although it does appear that outcome after extended resection is no worse than that after standard resection. Furthermore, infiltration of the mesocolon requiring colectomy or resection of colic vessels along with pancreatectomy was not associated with a significant decrease in survival. Although there were few long-term survivors among patients undergoing extended resections, the median survival in these patients is markedly longer than that in patients who did not have resections. En bloc resection of involved surrounding structures to completely extirpate all macroscopic disease may be of benefit in selected patients with locally advanced disease, particularly when combined with preoperative chemoradiation therapy.

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Discussion

Dr. W.S. Helton (Chicago, IL): I am curious to know, because many of your patients received neoadjuvant therapy, whether there was evidence of tumor on the resected specimen. For example, in the portal vein resections, was there cancer or just scarring on the vein?

Dr. Sasson: Unfortunately the documentation of invasion of the surrounding structures was recorded very sporadically, so we were unable to make any generalizations relating to the group. With regard to margins, we do know from previous work that patients who have received preoperative chemoradiation therapy are more likely to have a greater amount of fibrosis in the tumor specimens, and as we demonstrated at last year's SSAT meeting, these patients were less likely to have positive margins.

Dr. Conlon (New York, NY)***:*** This was an excellent presentation. I have a question about the arterial involvement. Although the numbers were small, did you get some sense of survival among patients who had documented arterial resections?

Dr. Sasson: I will comment on the patients who had celiac axis resections, since they were the ones whom we analyzed in detail, and they were also the most recent. There are four patients who are currently alive at 27, 20, 10, and 11 months. However, two former patients have had recurrences.

Dr. Newman (New York, NY): I assume this was contiguous disease, not discontiguous disease.

Dr. Sasson: That is correct; this is all contiguous disease.

Dr. Newman: In your group I patients, the median survival was 26 months. That is probably, on average, 10 months longer than most other investigators report. To what do you attribute this?

Dr. Sasson: The patients in group I were more likely to receive neoadjuvant therapy, and we suspect that the improvement in survival is probably the result of a selection bias. There was an interval of time from the initiation of therapy until resection. So those patients who were more likely to develop early metastatic disease after resection would have been excluded because we waited an interval of about 8 weeks to 3 months before performing resection after the initiation of chemotherapy.

Dr. R. H. Bell, Jr (Chicago, IL): When you conduct a study that shows no difference between the two groups, one wonders about the power of the study to detect differences between the groups, and I think that is a particular problem here because the literature suggests that venous resections behave like standard resections. You are left with a very small number of patients who had nonstandard resections. I am nervous about advocating resection of the major arteries. I believe others have evaluated this approach and ultimately found it to be nonproductive.

In that regard, I wonder if you could tell us about the power analysis of this study. In other words, if you wanted to show that the patients with extended resection had a 50% poorer survival, for example, than the patients who had a conventional resection,

how many patients would you have had to enroll in the study to achieve statistical significance?

Dr. Sasson: We did not do a power analysis, particularly for those patients who did not undergo just a standard venous resection and those who required the arterial resection. But I suspect that if we analyzed only those patients with vascular resections, the number of patients would be very small, and probably the number required for a randomized study to determine any significance would be quite large.

Specific Targeting of Tumor Vasculature by Diphtheria Toxin-Vascular Endothelial Growth Factor Fusion Protein Reduces Angiogenesis and Growth of Pancreatic Cancer

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Tumor vessels abundantly express receptors for vascular endothelial growth factor (VEGF), a mediator of neoangiogenesis. The aim of this study was to specifically target and damage the vasculature of pancreatic cancer (PaCa) by fusing VEGF to diphtheria toxin (DT), which inhibits protein synthesis of target cells. DT-VEGF fusion protein was produced in vector pGEX-KG and expressed in *E. coli* SG12036. Human PaCa cell lines (HPAF-2 and AsPC-1) and human endothelial cells (HUVEC) were exposed to DT-VEGF (10 ng/ml – 10,000 ng/ml). Proliferation was assessed after 3 days. One mm^3 fragments of subcutaneous PaCa donor tumors were implanted into the pancreas of nude mice that received either DT-VEGF (200 μg/kg, every other day) or phosphate-buffered saline intraperitoneally for 14 weeks. Tumor volume, metastatic spread, and animal weight were determined at autopsy. Microvessel density was analyzed in CD31-stained tumor sections. Proliferation of PaCa cells was inhibited at high concentrations of DT-VEGF (>1000 ng/ml). DT-VEGF decreased the growth of HUVEC at 10 ng/ml. In vivo, DT-VEGF reduced tumor volume (HPAF-2, 76%; AsPC-1, 53%), microvessel density (HPAF-2, 54%; AsPC-1, 62%), and tumor spread (HPAF-2, 89%; AsPC-1, 50%). Survival was increased (HPAF-2, 7/8 vs. 4/8 animals; AsPC-1, 6/8 vs. 1/8 animals). Weight was not influenced by DT-VEGF. The DT-VEGF effect is due to its toxic action on the tumor vasculature rather than to direct inhibition of PaCa cell growth. DT-VEGF therapy was not associated with systemic side effects. (J GASTROINTEST SURG 2002; 6:159–166.)

KEY WORDS: Pancreatic cancer, vascular endothelial growth factor, diphtheria toxin, angiogenesis

Exocrine pancreatic cancer is the fifth leading cause of cancer death in the United States, Japan, and the European Union, with an overall 5-year survival rate of less than 5%.[1,2] Even after curative resection, the 5-year survival rates achieved in specialized centers are less than 20%, and the majority of patients surviving 5 years will still die of metastatic cancer recurrence.[3,4] Unfortunately, the conventional adjuvant treatments such as radiation therapy and chemotherapy in various combinations have not improved long-term survival after resection.[5,6]

As in all solid tumors, pancreatic cancer growth beyond the size of few cubic millimeters and metastasis are dependent on the induction of neoangiogenesis[7] mediated by the release of proangiogenic factors such as vascular endothelial growth factor (VEGF).[8] VEGF stimulates endothelial cell proliferation and migration by binding to two distinct cell surface receptor tyrosine kinases: VEGFR-1 (flt-1) and VEGFR-2 (KDR/flk-1).[8,9] VEGF receptors are expressed most abundantly in the tumor vasculature and less abundantly in the endothelium of resting, mature blood vessels.[10] High levels of VEGFR expression in the tumor vessels thus provide a unique opportunity to target tumors with cytotoxic agents. VEGF fused with the translocation and enzymatic domains of bacterial toxins may cause selective toxicity to the tumor vasculature.[11,12]

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In this study, diphtheria toxin (DT) was chosen for fusion with VEGF165, the most abundantly expressed VEGF isoform.[11] Mature DT protein with 535 residues is cleaved into two fragments, DTA (residues 1 to 193) and DTB (residues 194 to 535), by the action of proteolytic enzymes.[13] The DTB fragment is responsible for binding to the cell surface and the translocation of DTA into the cytosol. The DTA fragment ADP ribosylates a unique amino acid, diphtheramide, which is present in elongation factor 2, and thereby inhibits protein synthesis in mammalian cells.[14,15] DT truncated at residue 389 lacks the eukaryotic cell–binding domain and is nontoxic to human cells. Substitution of the binding domain with ligands such as VEGF is able to direct the toxin to endothelial cells.

The aims of the present study were to assess the effect of DT-VEGF fusion protein on the proliferation of human pancreatic cancer cell lines and endothelial cells in vitro, and to evaluate the therapeutic potential of DT-VEGF in a clinically relevant orthotopic nude mouse model of human pancreatic cancer.

MATERIAL AND METHODS

Antiangiogenic Drug

The construction of DT-VEGF fusion protein was described previously.[11,16] In brief, VEGF165 fusion protein containing 390 amino acids of DT with the enzymatic and translocation domains was produced with glutathione-S-transferase (GST) in vector pGEX-KG. The fusion protein was expressed from the pGEX-KG vector in *E. coli* SG12036. Bacterial cells were disrupted by sonication, and extracts were centrifuged to remove unbroken cells and debris. The supernates were loaded onto glutathione–Sepharose 4B columns, and the fusion protein was eluted with 10 mmol/L gluta-thione. The fusion protein was cleaved with thrombin and passed again on the glutathione column to remove the GST domain. Purified proteins were analyzed on sodium dodecyl sulfate (SDS) gels, and Western blot analysis was also performed for the recombinant proteins to show the reactivity with specific antibodies.

Cell Lines and Culture Conditions

The following human pancreatic adenocarcinoma cell lines were obtained from American Type Culture Collection (Rockville, Md.): AsPC-1 (poorly differentiated[17]) and HPAF-2 (moderately differentiated[18]). AsPC-1 cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY), and HPAF-2 cells in minimum essential medium (MEM; Gibco). Human umbilical vein endothelial cells (HUVEC) were also purchased from American Type Culture Collection and maintained in RPMI-1640. 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.

In Vitro Assessment of Cell Proliferation and Viability

To examine the effect of DT-VEGF fusion protein on in vitro cell proliferation, 2×10^5 cells of each cell line were seeded in six-well culture plates in 2 ml of the respective cell culture medium. The medium was changed on the next day (day 1) and DT-VEGF was added in the following concentrations: 10 ng/ml, 100 ng/ml, 1,000 ng/ml, and 10,000 ng/ml. After 72 hours (day 4), the cells were trypsinized and counted in a standard hemocytometer. Cell viability was assessed by a monotetrazolium (MTT)–based colorimetric assay (Boehringer, Mannheim, Germany) according to the manufacturer's instructions.[19] Briefly, 5×10^3 cells of each cell line were seeded in 96-well culture plates in 200 μl of the respective cell culture media. The medium was changed the next day (day 1) and DT-VEGF was added as described earlier. After 72 hours (day 4), 10 μl of the MTT solution (5 mg/ml) was added to each well. After an additional 4 hours of incubation, 100 μl of 10% SDS solution was added. The plates were allowed to stand overnight in an incubator (37° C, 5% CO_2). Absorbance at 570 nm, which has been shown to strongly correlate with the number of viable cells, was then determined using a microplate enzyme-linked immunosorbent assay (ELISA) reader (Biotek Instruments Inc., Burlington, VT).

Laboratory Animals and Orthotopic Implantation Technique

Four-week-old male nude mice (Crl:NU/NU-nuBR) 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 main-tained on a daily 12-hour light/12-hour 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.[20,21] Five $\times 10^6$

cells of 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 with a scalpel (No. 11) into fragments of 1 mm^3 in size. The abdomen of anesthetized tumor recipient nude mice was 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 in 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 With DT-VEGF Fusion Protein

Thirty-two animals (16 per pancreatic cancer cell line) were randomly allocated into either the treatment or control group. Treatment with DT-VEGF (200 $\mu g/kg$[11] intraperitoneally every other day) or the vehicle (phosphate-buffered saline solution) was started 3 days after orthotopic tumor implantation. The mice were monitored daily to assess their clinical condition; they were weighed weekly and killed by a lethal dose of sodium pentobarbital (0.5 mg/g body weight) 14 weeks after 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 >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.

Autopsies were performed in all animals at the end of the observation period. The perpendicular diameters of the primary orthotopic tumor were 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.[20] 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 as distant metastases. The sites of evaluation included 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 diaphragm, mesentery, and retroperitoneum), and metastatic nodules >50 mm^3. Clinical consequences of the tumor growth were incorporated into this scoring system: formation of ascites (2 points if volume >5 ml) and 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 an 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 done 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 a 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 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.[22] 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 ± SEM. Continuous, normally distributed variables were analyzed by Stu-

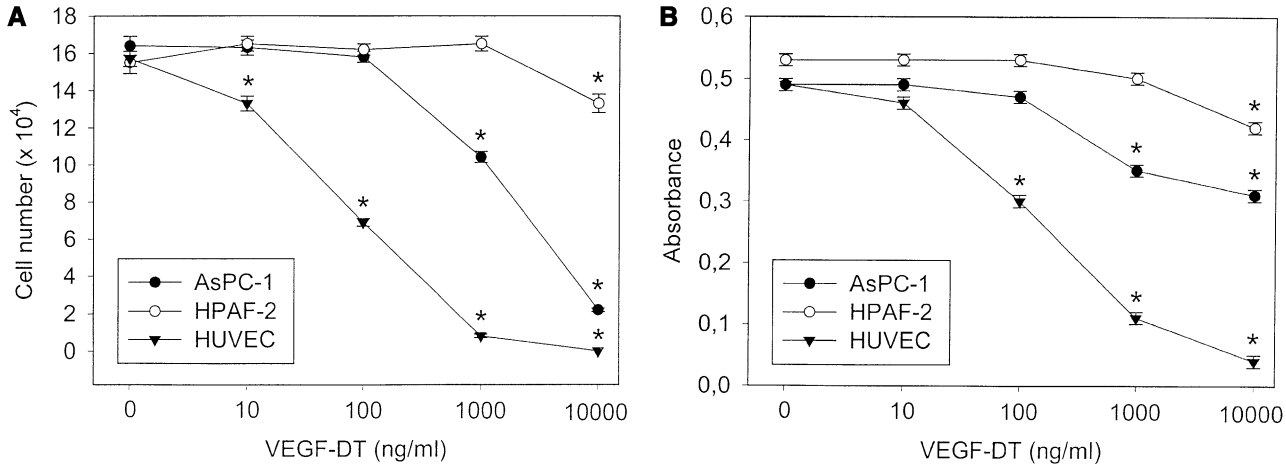

Fig. 1. Effect of DT-VEGF on cell proliferation (**A**, cell count) and viability (**B,** MTT assay).

dent's *t* test. Discontinuous variables (dissemination score, microvessel density) were analyzed by the Mann-Whitney rank-sum test. Differences in survival were analyzed by the chi-square test; $P < 0.05$ was considered statistically significant.

RESULTS

In Vitro Effects of DT-VEGF

DT-VEGF fusion protein significantly decreased cell proliferation (Fig. 1, *A*) and viability (Fig. 1, *B*) of human umbilical vein endothelial cells (HUVEC). In contrast, significantly higher concentrations (1000 ng/ml) of the protein were necessary to effectively inhibit cell proliferation and viability of AsPC-1 pancreatic cancer cells. Proliferation and viability of HPAF-2 cells were slightly decreased only at a toxic concentration (10,000 ng/ml) of DT-VEGF.

Volumes of Primary Tumors

In vivo treatment with DT-VEGF fusion protein significantly decreased the volumes of orthotopic pancreatic tumors in both groups. The size of tumors derived from the moderately differentiated HPAF-2 cell line was reduced by approximately 75% (3920 ± 495 vs. 927 ± 122 mm^3; $P < 0.001$; Fig. 2, *A*). Treated animals in the AsPC-1 group developed tumors with half of the average volume of the control group (1386 ± 155 vs. 652 ± 73 mm^3; $P < 0.001$; Fig. 2, *B*).

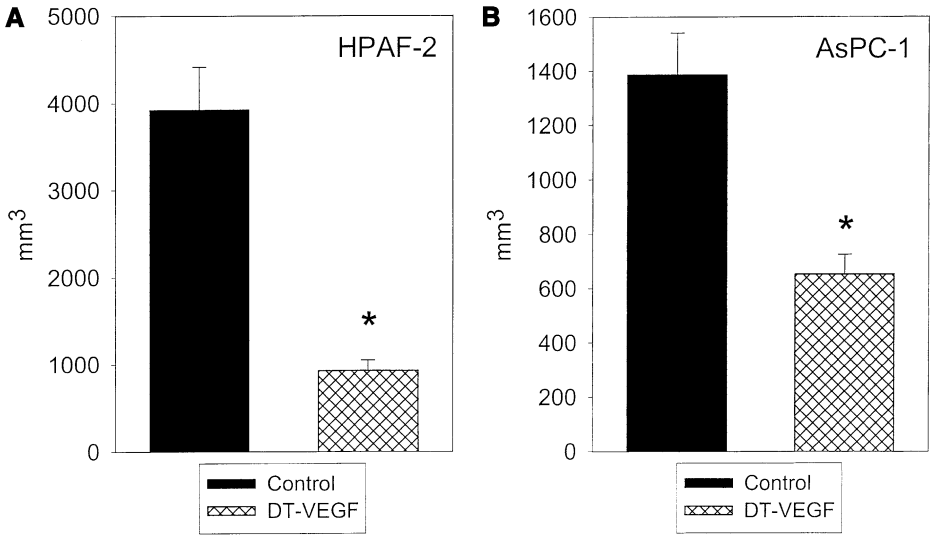

Fig. 2. Volume of the primary tumor in controls and animals treated with DT-VEGF. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**).

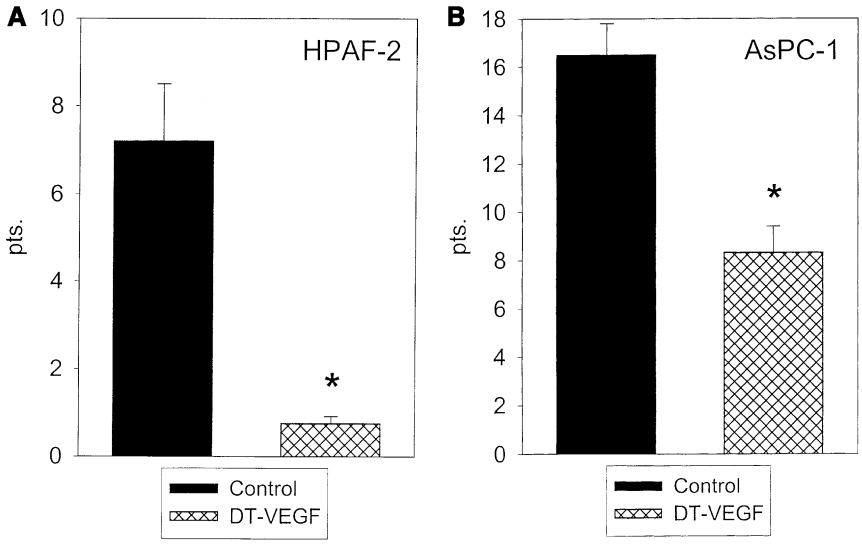

Fig. 3. Dissemination scores quantifying local and distant tumor spread, in controls and animals treated with DT-VEGF. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**).

Dissemination Score

Local infiltration and distant metastasis were summarized by a dissemination score. Treatment with DT-VEGF resulted in a statistically significant reduction of tumor spread. The score was diminished from 7.2 ± 1.3 to 0.75 ± 0.16 points in the HPAF-2 group ($P < 0.001$; Fig. 3, *A*). Control animals with tumors derived from the poorly differentiated AsPC-1 cell line reached the highest score (16.5 ± 1.3 points) and this was reduced to 8.3 ± 1.1 points in treated mice ($P < 0.001$; Fig. 3, *B*).

Survival

Half of the animals in the HPAF-2 control group survived the 14-week observation period. In contrast, seven of eight animals in the HPAF-2 group treated with DT-VEGF 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 aggressive in vivo behavior of AsPC-1 tumors was reflected by a low 14-week survival in the control group (12.5%). Treatment with the antiangiogenic protein resulted in a significantly increased survival (75%, $P < 0.05$; Fig. 4, *B*).

Animal Weight

Animal weight was recorded weekly after tumor induction and at autopsy. The average animal weight at autopsy in the HPAF-2 groups was comparable (controls = 30.0 ± 0.7 g; DT-VEGF treatment = 29.6 ± 1.3 g). Similarly, weight differences between AsPC-1 controls and treated animals were not statistically significant (28.2 ± 0.8 g vs. 27.3 ± 0.9 g).

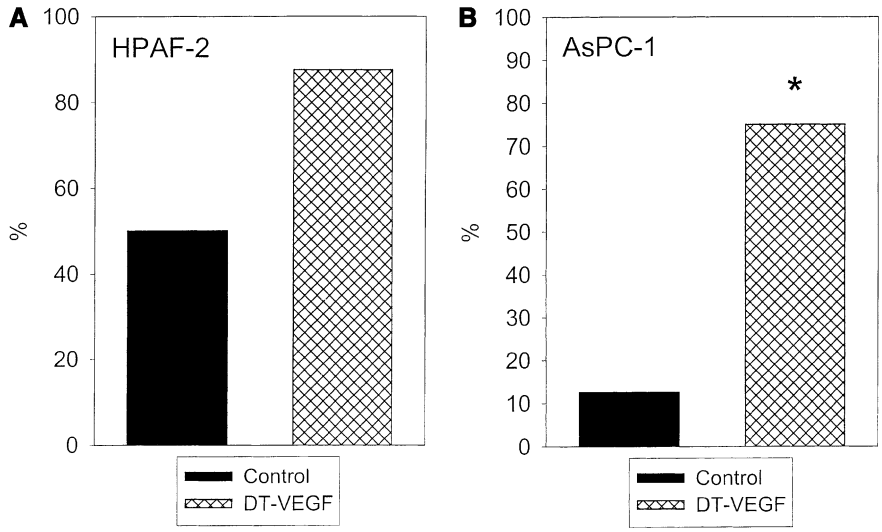

Fig. 4. Fourteen-week survival in controls and animals treated with DT-VEGF. Tumors were derived from HPAF-2 cells (**A**) and AsPC-1 cells (**B**).

Microvessel Density in Primary Tumors

Microvessel density as a parameter of angiogenic activity was significantly enhanced in the untreated primary tumors of both pancreatic cancer cell lines tested, compared to normal exocrine pancreas (HPAF-2 = 85.7 ± 7.2/0.74 mm^2; AsPC-1 = 69.8 ± 5.1/0.74 mm^2; native pancreas: 15.6 ± 1.5/0.74 mm^2; $P < 0.001$). Treatment with DT-VEGF fusion protein significantly reduced neoangiogenesis in tumors of both human pancreatic cancer cell lines: HPAF-2 = 39.6 ± 4.3/0.74 mm^2; AsPC-1 = 26.6 ± 3.8/0.74 mm^2; $P < 0.001$ vs. controls.

DISCUSSION

Angiogenesis is a key factor in the growth of solid tumors.[7] The inhibition of angiogenesis is an attractive and promising new treatment strategy,[23, 24] especially for devastating malignancies such as exocrine pancreatic cancer, which are virtually noncurable by surgical resection and/or chemoradiation.[2–6] One rationale for using antiangiogenic treatment in pancreatic cancer is the fact that the angiogenic activity in pancreatic cancer tissue is significantly enhanced in comparison to the normal pancreas. Kuehn et al.[25] demonstrated that microvessel density and expression of proangiogenic factors such as VEGF were significantly higher in pancreatic cancer than in normal exocrine parenchyma. Up to 93% of resected human pancreatic cancer specimens were found to be positive for VEGF.[26–29] Moreover, tumor VEGF correlated with local tumor progression[26] and survival in patients with pancreatic cancer.[27–29]

The VEGF receptors 1 and 2 are expressed at high levels in the activated endothelium of tumor vasculature in general[10] and in pancreatic cancer,[30] whereas normal mature blood vessels show negligible levels of VEGFR-1 and VEGFR-2.[31] Coupling of VEGF to toxins therefore allows us to specifically target and damage tumor blood vessels. We used the coding region for the most abundant VEGF165-amino acid isoform to direct the delivery of a 390 amino acid DT fragment, which lacks its natural binding domain to mammalian cells. This truncated DT is nontoxic to cells but retains the functions of translocation and protein synthesis inhibition when delivered with the targeting VEGF molecule. The DT-VEGF construct has been shown to be highly toxic to proliferating endothelial cells and to completely inhibit the growth of new blood vessels in the chick chorioallantoic assay.[11] Specificity of the mode of action of DT390-VEGF165 was previously demonstrated by antibodies to VEGF and VEGFR-2, which inhibited the activity of the fusion protein.[11]

Our in vitro results confirmed the inhibitory action of DT-VEGF on VEGFR-positive endothelial cells. The fusion protein dose dependently decreased cell proliferation and viability (see Fig. 1) of HUVEC. In contrast, DT-VEGF exerted almost no inhibition on HPAF-2 pancreatic cancer cells, which are negative for both VEGFR types. Proliferation and viability of AsPC-1 pancreatic cancer cells—positive for VEGFR-1 but negative for VEGFR-2[32, 33]—was inhibited at higher doses of DT-VEGF. This is consistent with the finding that DT-VEGF targets not only endothelial cells but also other (cancer) cell types that are positive for VEGFRs.[11] The limited effect of DT-VEGF on AsPC-1 cells as compared to HUVEC is consistent with the hypothesis that the effect of the fusion protein is mainly mediated via VEGFR-2.[11, 12]

To further investigate the effect of DT-VEGF on human pancreatic cancer in vivo, we applied the fusion protein in an orthotopic nude mouse model. This model mirrors the development of clinical disease and allows not only the assessment of primary tumor growth, but also of metastasis and survival.[20, 21] Moderately differentiated HPAF-2 cells grew to large, partly cystic tumors in control mice and displayed a moderate local and systemic pattern of spread. DT-VEGF therapy significantly reduced both primary tumor volume (see Fig. 2, *A*) and tumor dissemination (see Fig. 3, *A*) in animals bearing HPAF-2 tumors. Whereas half of the animals in the HPAF-2 control group had to be killed before the end of the observation period, seven of eight survived for 14-weeks (Fig. 4, *A*). Poorly differentiated AsPC-1 tumors displayed a more aggressive growth pattern, killing seven out of eight control animals (Fig. 4, *B*). Treatment with DT-VEGF significantly improved survival—only two of eight animals had to be killed before the end of the 14-week observation period (see Fig. 4, *B*). This positive effect on survival was mainly due to a significant reduction of local infiltration and metastasis (see Fig. 3, *B*), which are usually the cause of death in control animals.

Reduced angiogenic activity in the primary tumors of treated animals was illustrated by a significant reduction in microvessel density, both in HPAF-2 and AsPC-1 tumors. Because the in vitro proliferation of HPAF-2 cells was not influenced at physiologic concentrations of DT-VEGF, the in vivo effect of DT-VEGF in the HPAF-2 model system seems to be due to a mere antiangiogenic mechanism. In contrast, direct toxicity of DT-VEGF on VEGFR-1 positive AsPC-1 cells may contribute another antitumor effect in addition to the antiangiogenic action of the fusion protein in AsPC-1 tumors.

Despite the fact that VEGFRs are predominantly expressed on activated tumor endothelial cells, there

is a potential risk of dose-dependent toxic side effects of DT-VEGF on normal endothelium. Recent data also suggest that VEGF165—the isoform used in DT-VEGF—binds not only to VEGFRs but to the newly discovered neuropilin-1 receptor.[31] Neuropilin-1 is widely expressed in normal tissue and therefore presents a potential target for unwanted toxicity. In our study, all mice treated with 200 μg/kg DT-VEGF every other day did not exhibit any clinical evidence of toxicity, such as a change in food intake or activity. Body weight, which was determined as a surrogate marker for toxic side effects throughout the experiment, was not found to be different at autopsy, in either the HPAF-2 or the AsPC-1 group. Tissue sections from kidney, liver, and lung of DT-VEGF–treated animals did not reveal microscopic evidence of organ toxicity in comparison to healthy mice (results not shown). This is consistent with previous reports that DT-VEGF constructs did not cause toxic side effects during short-term treatment in rodents.[11,31,34] However, the present study was the first to evaluate DT-VEGF for a treatment period as long as 14 weeks in mice.

In summary, this study adds further evidence that angiogenesis is a critical component of pancreatic cancer growth and metastasis. Targeting the VEGF receptors on tumor vessels by a DT-VEGF fusion protein resulted in a significant biological improvement in a relevant orthotopic animal model and raises the possibility that this strategy may prove useful as an adjuvant clinical treatment for this deadly malignancy.

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Discussion

Dr. C. Fernandez del-Castillo (Boston, MA): Has this type of therapy been employed for other tumors?

Dr. H. Hotz: This type of fusion protein has been constructed by our cooperating partner, Dr. Parkash Gill, from the University of Southern California. He is involved in AIDS research, and he has applied this protein to Kaposi's sarcoma experimentally; this is of particular interest because in Kaposi's sarcoma not only are the endothelial cells positive for the receptors but also the cancer cells themselves. So you have a dual effect on endothelial cells and on cancer cells, and this was very effective in that type of treatment.

Dr. Fernandez del-Castillo: Has it been used for any gastrointestinal tumors?

Dr. Hotz: There is one other group that has also applied a construct of VEGF in diphtheria toxin; this one is not linked by recombinant methods but is chemically linked, and I believe it was applied to ovarian cancer.

Dr. J. Neoptolemos (Liverpool, UK): Can you tell us how the agent was delivered?

Dr. Hotz: It was delivered by intraperitoneal injection.

Dr. Neoptolemos: You saw no toxicity anywhere else in the animals?

Dr. Hotz: We did not see any toxicity. As I mentioned, we used weight as a general marker of animal well-being. We did not find differences in food intake in the animals.

Dr. Neoptolemos: Did you look for toxicity in the brain or other organs?

Dr. Hotz: We used conventional histologic examination to check the lungs, liver, and kidneys, and with this dosage we did not find any differences between normal healthy mice and these treated mice.

Dr. Neoptolemos: You did confirm the presence or absence of receptors to VEGF in your cells lines?

Dr. Hotz: Yes, we had done that previously, and we found different results for pancreatic cancer cells. There are some pancreatic cancer cells that are negative.

Dr. Neoptolemos: In the cell lines you used for these experiments, did you confirm the presence or absence of receptors?

Dr. Hotz: As I demonstrated, the HPAF-2 cells are negative for VEGF receptors. So in the HPAF-2 group, this seems to be a mere antiangiogenic effect on endothelial cells. AsPC-1 cells, however, are positive for one type of receptor, and there seems to be a combined effect on the cancer cells themselves and on the endothelial cells.

Dr. V. Fink (Chicago, IL): This is very interesting, very important work that you are doing. As you know, Dr. Judah Folkman has some compounds that he is studying in an attempt to block angiogenesis. How far away are you from pilot studies in humans?

Dr. Hotz: To be honest, with particular regard to pancreatic cancer, we are still a long way from human studies. As you have seen, this was a prophylactic treatment; it was begun just 3 days after tumor induction. So the next step will be to experiment with therapeutic treatment in this model, for example, starting 5 or 6 weeks after the tumors are already established. I truly believe that monotherapy with these substances is not capable of eradicating these tumors. So it is necessary to combine it with additional treatment modalities, for example, chemotherapy.

Dr. Fink: How long will it be before you are ready to conduct human trials?

Dr. Hotz: I cannot really answer that question.

Human Heparanase-1 Gene Expression in Pancreatic Adenocarcinoma

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Extracellular matrix degradation is an essential step that allows tumor cells to penetrate a tissue barrier and become metastatic. Heparanase-1 (HPR1) is an endoglycosidase that specifically degrades heparan sulfate proteoglycans, a chief component of the extracellular matrix. HPR1 is not expressed in normal epithelial cells but can be detected in a variety of malignancies. In the present study, we examined HPR1 expression in pancreatic cancer by using in situ hybridization and tested whether HPR1 expression correlated with any clinicopathlogic parameters. HPR1 was not detected in the ductal cells of normal pancreas samples obtained from 10 patients at autopsy. However, HPR1 was detected in 77 (78%) of 99 pancreatic adenocarcinomas. Among them, 69 (78%) of 89 primary pancreatic adenocarcinomas and 8 (80%) of the 10 metastases were HPR1 positive. Age, sex, tumor stage, and lymph node status were not predictive of HPR1 expression. Log-rank test of the Kaplan-Meier survival curves revealed that HPR1 expression in early-stage tumors was associated with decreased survival. HPR1 expression was frequent in pancreatic adenocarcinomas and was associated with decreased survival in early-stage tumors. This suggests that HPR1 may contribute to the highly invasive and early metastatic behavior of pancreatic cancer. (J GASTROINTEST SURG 2002;6:167–172.)

KEY WORDS: Heparanase-1, pancreatic cancer, metastis, survival

Heparanase (HPR) is an endoglycosidase responsible for the degradation of the heparan sulfate proteoglycan, a major component of the extracellular matrix (ECM) and cell surface. Two heparanase genes, HPR1 and HPR2, have been recently cloned and characterized.[1–3] HPR1 cleaves heparan sulfate at the β-D-glucuronosyl-N-acetylglucosaminyl intrachain site and is therefore known as an endo-β-D-glucuronidase.[4] HPR1 seems to play a major role in malignant disease and has been identified in various human carcinomas including colon cancer and melanoma.[1,5] HPR levels were elevated in the sera of rats with metastatic adenocarcinoma and patients with melanoma.[3,4] Specific inhibitors of HPR1 have been shown to control tumor metastases and angiogenesis in vitro and in animal models.[6,7] These observations collectively suggest that HPR1 has a role in tumor metastases.

HPR1 mediates tumor invasion primarily through the direct cleavage of the heparan sulfate proteoglycan in the ECM. This breaks down the ECM barrier that normally prevents spread of tumor cells. Degradation of the ECM also leads to the release of growth factors and cytokines such as basic fibroblastic growth factor, which is sequestered in depot form within the heparan sulfate glycosaminoglycan. These growth factors are potent mitogens and chemotactic agents for endothelial cells that promote tumor angiogenesis. Other mitogenic and proteolytic factors that are sequestered by the heparan sulfate proteoglycan and released when it is cleaved include urokinase and tissue plasminogen activator. Their release further potentiates tumor metastases.[8]

Pancreatic adenocarcinoma is the fourth most common cancer in the United States and the second

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most lethal cancer of the gastrointestinal tract. At presentation, 40% of patients have locally advanced disease and another 50% have distant metastases precluding surgical therapy. Despite multiple modalities of treatment, the overall 5-year survival from this malignancy is only 4%. Because HPR1 may play a critical role in tumor metastases, we hypothesized that HPR1 may be highly expressed in primary and metastatic pancreatic adenocarcinomas. To test this hypothesis, HPR1 expression was measured in pancreatic adenocarcinoma tissue samples and the results were correlated to the clinicopathologic features of the patients evaluated.

MATERIAL AND METHODS

Tumor Samples and Clinicopathologic Data

Paraffin-embedded tissue blocks from patients with either primary or metastatic pancreatic adenocarcinomas were obtained from the Department of Pathology. The stage of the pancreatic adenocarcinomas was classified according to the TNM scheme used by the American Joint Committee on Cancer. The use of specimens from human subjects in the present study was approved by the institutional review board of Rush Presbyterian–St. Luke's Medical Center in Chicago.

The mean age of the 99 patients (48 women and 51 men) from whom the paraffin-embedded specimens were derived was 63 ± 10 years (range 34 to 81 years). Eighty-five specimens were primary pancreatic adenocarcinomas obtained from pancreatic resections. Four specimens were primary pancreatic adenocarcinomas obtained from pancreatic biopsies. An additional 10 specimens were obtained from metastatic implants. These implants included three on the small bowel, three on the peritoneum, two on the liver, one on the gallbladder, and one on the thigh. Normal pancreas specimens were obtained at autopsy in 10 patients with no evidence of pancreatic disease.

In Situ Hybridization

Total cellular RNA was extracted from platelets of a healthy donor by using TRIzol (Life Technologies, Rockville, MD) according to the manufacturer's instructions and quantified by ultraviolet absorption. The first chain of the cDNA was synthesized by reverse transcriptase with 2 μg of total RNA as template and oligo(dT) as primer. Human heparanase cDNA was polymerase chain reaction (PCR) amplified with pfu of DNA polymerase and two primers. The forward primer flanking the sequence from position 87 to 110 (TATCGGTACCAGGTGAGCCAAGATGCTGC) contained a Kpn I site. The reverse primer complementary to the heparanase cDNA sequence from position 1707 to 1731 (ACGGTCG AATTCTCAGATGCAAGAGCAACTTTGGC) contained an EcoRI cleavage site. PCR product was digested with KpnI and EcoRI and subsequently cloned into pcDNA 3 expression vector. The resultant plasmid was designated as pcDNA/HPR. The digoxigenin-labeled heparanase sense probe was also prepared by in vitro transcription with T7 RNA polymerase and a PCR fragment as template (amplified from pcDNA vector containing a Bam HI-EcoRI fragment of heparanase cDNA, T7 primer, and a primer complementary to the heparanase cDNA from position 965 to 993). Paraffin-embedded tissue sections were dewaxed with xylene and rehydrated by serial concentrations of ethanol. They were then pretreated with 0.2 N HCl for 30 minutes followed by proteinase K digestion (15 μg/ml) or sense probe as a negative control. Slides were washed with prewarmed 2×, 1×, 0.1× SSC solution. Tissue sections were then blocked in blocking solution containing 2% normal sheep serum followed by incubation with 1:500 diluted sheep antidigoxigenin for 2 hours at room temperature.

Statistical Analysis

Student's *t* test and chi-square regression analysis were used to compare differences between groups. Kaplan-Meier analysis with log-rank test was used to examine differences in survival between groups. $P < 0.05$ was considered statistically significant.

RESULTS

In Situ Hybridization Analysis of HPR1 Expression in Pancreatic Adenocarcinoma

Previous studies have demonstrated that HPR1 is expressed in several human malignancies such as breast and colon cancer.[1,3,4,9] Here we tested whether HPR1 was expressed in pancreatic adenocarcinomas. We conducted in situ hybridization by using a digoxigenin-labeled HPR1 antisense probe followed by an alkaline phosphatase–conjugated antidigoxigenin antibody and its specific substrates BCIP and NBT. As shown in Fig. 1, deep brown to purple cytoplasmic staining (Fig. 1, *A* and *B*) in both primary and metastatic pancreatic adenocarcinomas indicated HPR1 expression. The results summarized in Table 1 show that 77 of 99 pancreatic adenocarcinomas were HPR1-positive. HPR1 expression was found in 69 (78%) of 89 primary pancreatic adenocarcinomas and 8 (80%) of 10 metastatic pancreatic adenocarcinomas. In contrast, the ductal epithelium of all 10

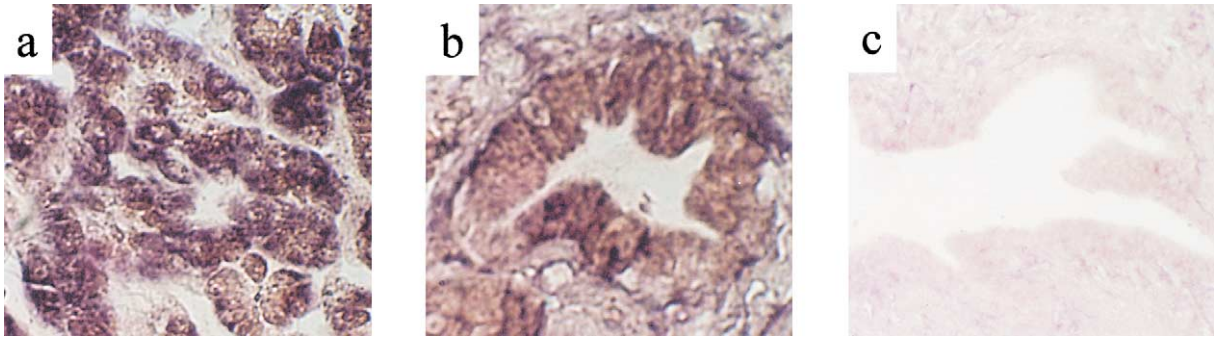

Fig. 1. In situ hybridization analysis of HPR1 gene expression in primary and metastatic pancreatic adenocarcinoma. Preparation of digoxigenin-labeled HPR1 antisense RNA probes as well as in situ hybridization was conducted as described in Material and Methods. Paraffin-embedded human specimens were from (**A**) primary pancreatic adenocarcinomas, (**B**) metastatic pancreatic adenocarcinomas, and (**C**) normal ductal epithelium. Positive HPR1 expression by in situ hybridization was indicated by the deep brown to purple cytoplasmic staining seen in **A** and **B** but not in **C**.

normal pancreas specimens obtained at autopsy were HPR1 negative (see Fig. 1, *C*).

Lack of Correlation Between HPR1 Expression and Clinicopathologic Features

HPR1 expression was evaluated to identify correlations between patient demographic data and clinicopathologic features. As shown in Table 2, HPR1 by in situ hybridization was expressed in 41 (80%) of 51 adenocarcinomas obtained from male patients and 36 (75%) of 48 adenocarcinomas from female patients. The mean age of the patients with HPR1-positive and HPR1-negative adenocarcinomas was 64 ± 10 years and 60 ± 10 years, respectively. Statistical analysis revealed no correlation between HPR1 expression and patient sex or age.

A recent study by Friedmann et al.[5] showed that HPR expression correlates with the progression of colon adenocarcinomas. We also tested whether there was a correlation between HPR1 expression and the stage of pancreatic adenocarcinoma. As shown in Table 3, HPR1 was expressed in pancreatic adenocarcinomas at various stages with a positive rate ranging from approximately 70% to 100% by in situ hybridization. Statistical analysis indicated that HPR1 expression did not correlate with any particular stage of the tumor.

HPR1 Expression Is Predictive of Patient Survival in Low Tumor Stages

Kaplan-Meier survival curves were used to analyze survival based on different variables. Patients who died within 30 days of surgery (perioperative period) and those who underwent a pancreatic biopsy alone were excluded from this analysis, leaving a total of 56 patients for survival analysis. Overall survival between HPR1-positive and HPR1-negative patients as determined was not significantly different (Fig. 2). Subgroup analysis was performed comparing survival between HPR1-positive and HPR1-negative patients at different tumor stages, with different nodal status, and for the presence and absence of metastases. Early-stage primary pancreatic adenocarcinomas, or tumors that were categorized as pT1 through pT2, were associated with a lower survival when they were HPR1 positive (median survival 10.7 months) compared to

Table 1. HPR1 is expressed in primary and metastatic pancreatic adenocarcinomas detected, but not in normal ductal epithelium

Specimen	Total	HPR1+	%
Adenocarcinoma			
Primary	89	69	78
Metastasis	10	8	80
Normal duct epithelium			
Autopsy	10	0	0

Table 2. HPR1 expression does not correlate with patient age and sex

	Total	Mean age (yr)	Median age (yr)	Range
HPR1+	77	64 ± 10	66	34–81
Male	41	64 ± 10	65	39–81
Female	36	64 ± 11	68	34–80
HPR1−	22	60 ± 10	60	37–79
Male	10	62 ± 4	61	56–69
Female	12	60 ± 13	58	37–79

Differences between positive and negative groups were not significant.

Table 3. HPR1 expression does not correlate with tumor stage

TNM	Total	HPR1+	%
Tumor			
1	22	18	82
2	46	32	70
3	17	15	88
Nodes			
0	37	30	81
1	48	35	73
Metastases			
0	85	65	76
1	10	8	80
Biopsies	4	4	100

Differences between primary tumor, nodal status, and metastases groups were not significant.

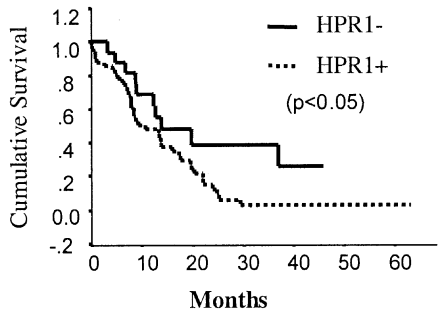

Fig. 3. Survival for HPR1-positive early-stage tumors (pT1 and pT2). Kaplan-Meier survival curves show that HPR1 expression in early-stage pancreatic adenocarcinoma is associated with a significantly decreased survival ($P < 0.05$). The median survival for patients with HPR1-positive pancreatic adenocarcinomas was 10.7 months; the median survival for patients with HPR1-negative pancreatic adenocarcinomas was 13.8 months.

when they were HPR1-negative (median survival 13.8 months) ($P < 0.05$) (Fig. 3). Significant differences in survival between HPR1-positive and HPR1-negative patients were not observed in any other subgroup analysis. Patients with metastatic pancreatic adenocarcinomas who were HPR1 positive had a decreased survival (median survival 1.5 months) when compared to patients who were HPR1 negative (median survival 2.4 months), but this did not reach statistical significance.

DISCUSSION

It is well documented that HPR1 is expressed in the cells of the hematologic and immunologic systems, as well as in the placenta, but not in normal epithelial cells.[1,10] However, HPR1 is detected in a variety of tumors such as colon, breast, and hepatocellular carcinomas. Friedmann et al.[5] recently reported that all 12 primary and eight metastatic colon adenocarcinomas in their study expressed HPR1, whereas tissue from normal colon did not express HPR1. Consistent with these observations, our present study shows that HPR1 expression was not detected by in situ hybridization in the ductal epithelium of the normal pancreas specimens obtained from 10 patients at autopsy, but was expressed in 78% of 89 primary pancreatic adenocarcinoma samples studied by in situ hybridization. These observations support a recent report showing that HPR1 was expressed at high levels in a pancreatic cancer xenograft and two pancreatic cancer cell lines, but not in normal pancreatic tissue.[2]

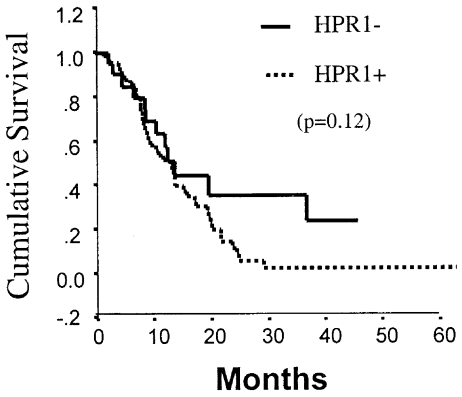

Fig. 2. Comparison of survival in patients with HPR1-positive and HPR1-negative pancreatic adenocarcinomas. Kaplan-Meier survival curves for patients with HPR1-positive and HPR1-negative tumors show no difference in survival. The median survival for patients with HPR1-positive pancreatic adenocarcinomas was 13.3 months; the median survival for patients with HPR1-negative pancreatic adenocarcinomas was 13.8 months.

Although the molecular mechanisms by which HPR1 is dysregulated in pancreatic cancer and in other malignancies remain unknown, we hypothesize that at least two genetic factors in pancreatic cancer may contribute to increased HPR1 expression. First, the frequent mutation of the K-*ras* oncogene in pancreatic adenocarcinomas[11,12] may result in elevated HPR1 gene expression. This is based on previous observations showing that transformation of murine fibroblasts by the H-*ras* oncogene leads to the increased heparanase activity and subsequently confers metastatic potential to these cells.[13] Second, increased HPR1 expression in pancreatic cancer may be due to increased expression of nerve growth factor (NGF) and its receptor. Marchetti et al.[14] demonstrated that NGF and neurotrophin-3 could induce HPR1 expression and promote tumor metastases in malignant

melanoma cell lines. A recent study demonstrated that several pancreatic adenocarcinoma cell lines express NGF and NGF receptors at high levels.[15] Therefore it is possible that a NGF-rich environment resulting from increased expression of NGF and its receptors in pancreatic adenocarcinomas can stimulate HPR1 expression in pancreatic tumor tissue. Other unidentified environmental factors such as pH, glucose, oxygen, growth factors, and cytokines[14–19] may also contribute to increased HPR1 gene expression and HPR1 enzymatic activity in pancreatic malignancy. It appears that HPR1 dysregulation in pancreatic cancer is mediated by multiple genetic and environmental factors.

A recent study by Friedmann et al.[5] demonstrated that HPR1 expression increases as the stage of colon adenocarcinomas progresses, suggesting that HPR1 plays a role not only in tumor metastases but also in tumorigenesis. We found that approximately 80% of pancreatic adenocarcinomas in all stages were HPR1 positive, indicating that there was no correlation between HPR1 expression and tumor progression. Several possibilities may account for this discrepancy. First, the onset and progression of pancreatic adenocarcinoma may differ from that proposed for colon cancer. Second, the sample number examined by Friedmann et al.[5] is relatively small. Larger studies may be required to demonstrate this correlation between HPR1 expression and tumor stage. Third, pancreatic cancer is often metastatic, even in its early clinical course, whereas colon cancer becomes metastatic during later tumor evolution. In our study, HPR1 expression was increased regardless of the tumor stage or other clinicopathologic features that were evaluated. This observation may explain why an aggressive pancreatic adenocarcinoma is capable of metastasis at any stage. In support of this, HPR1 expression was associated with a decreased survival rate in patients with early-stage tumors. This further suggests that HPR1 may play a critical role in promoting tumor angiogenesis and metastasis that lead to a shorter survival.

Pancreatic adenocarcinoma is often associated with both locally advanced and distant metastatic disease at the time of diagnosis, precluding operative resection. Adjuvant therapy may prolong survival; however, no adequate adjuvant therapy regimen exists. Our present study demonstrates that HPR1 is highly expressed in pancreatic adenocarcinomas. Given that HPR1 plays a critical role in tumor angiogenesis and metastases, HPR1-specific inhibitors may be used as novel therapeutic agents in the treatment of pancreatic adenocarcinoma. In support of this notion, recent studies of animal tumor models show that polysaccharide HPR1 inhibitors are capable of suppressing tumor metastases.[20,21] For example, Parish et al.[22] showed that sulfated polysaccharides could inhibit ECM breakdown by rat mammary adenocarcinoma cells in vitro and subsequently control tumor pulmonary metastases in vivo. In another study Miao et al.[21] reported that laminarin sulfate, a specific inhibitor of HPR1, could control tumor metastases of a breast cancer cell line in nude mice. These findings together with our observation that HPR1 was frequently expressed in pancreatic adenocarcinomas suggest that inhibition of the HPR1 enzymatic activity may open a new avenue to control the progression and metastases of pancreatic cancer.

After we submitted our manuscript, Koliopanos et al.[23] reported the following findings: (1) HPR1 expression is increased in pancreatic cancer by more than sixfold in approximately 75% of patients with this malignancy and is increased by less than sixfold in the remaining samples; (2) HPR1 expression does not correlate with tumor stage; and (3) patients with HPR1-positive pancreatic cancer appear to survive for a shorter period than those with HPR1-negative cancer. These observations are consistent with our findings that HPR1 is expressed in approximately 80% of pancreactic adenocarcinomas and that HPR1 expression in early-stage tumors is associated with a shorter survival.

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Quality of Life in Chronic Pancreatitis: A Prospective Trial Comparing Classical Whipple Procedure and Duodenum-Preserving Pancreatic Head Resection

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Few data are available with respect to quality of life after pancreatic head resection in patients with chronic pancreatitis. The aim of this study was to compare the classical Whipple pancreatoduodenectomy (PD) with the Beger duodenum-preserving pancreatic head resection (DPPHR), in terms of quality of life, using standardized, valid, and reliable questionnaires. Sixty-five consecutive patients were included in this study. The PD procedure was chosen when pancreatic cancer could not be ruled out (n = 30); otherwise DPPHR was performed (n = 35). Quality of life was measured prospectively three times with the European Organization for Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire (QLQ-C30) and the Gastrointestinal Quality-of-Life Index (GIQLI). Both procedures led to a significant improvement in quality of life, especially with regard to pain status. However, at the second follow-up examination (18 to 24 months postoperatively), all functional scales and the most important symptom scales of the EORTC QLQ-C30 revealed a better quality of life in the DPPHR group compared to the PD group. After classical PD, more patients seem to develop diabetes mellitus. The EORTC QLQ-C30 was found to be a better tool for quality-of-life assessment than the GIQLI in patients with chronic pancreatitis. (J GASTROINTEST SURG 2002;6:173–180.)

KEY WORDS: Chronic pancreatitis, pancreatoduodenectomy, duodenum-preserving pancreatic head resection, quality of life

Surgical options for chronic pancreatitis include decompression/drainage operations and resection procedures. Longitudinal pancreaticojejunostomy should only be performed in patients with a large-duct disease without other complications. Because of its ineffectiveness and the high rate of new-onset diabetes mellitus associated with distal resections,[1,2] and the complications associated with total pancreatectomy,[3] resections targeting the head of the pancreas are the primary operation for patients with chronic pancreatitis. Four main operations are presently available to these patients: classical Whipple pancreatoduodenectomy (PD) (with antrectomy);[4] pylorus-preserving pancreatoduodenectomy (PPPD) (so-called Traverso-Longmire procedure);[5] and two duodenum-preserving techniques—that is, the Beger duodenum-preserving pancreatic head resection (DPPHR)[6] and the Frey procedure (a combination of resection and drainage with longitudinal pancreaticojejunostomy and local pancreatic head excision).[7] There is no consensus regarding the optimal type of pancreatic head resection.

The results of studies comparing short- and long-term findings for the classical Whipple procedure and PPPD are controversial. The largest published series systematically comparing standard Whipple resection and PPPD in the treatment of chronic pancreatitis has shown similar long-term results with regard to pain relief, new-onset diabetes, and weight maintenance.[8] However, postoperatively delayed gastric emptying was found to be twice as common after PPPD than after the Whipple procedure in this study.

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Three prospective, randomized controlled trials comparing classical Whipple operation or PPPD with a duodenum-preserving procedure have demonstrated the superiority of the duodenum-preserving technique, especially with regard to nutritional status, glucose metabolism, and delayed gastric emptying.[9–11]

Concerning assessment of quality of life, there is only one prospective investigation comparing a duodenum-preserving procedure with a non–duodenum-preserving procedure (PPPD vs. Frey procedure).[11] Furthermore, there is no study comparing quality of life in the classical Whipple procedure with that in the Beger DPPHR in patients with chronic pancreatitis using a standardized, valid, and reliable questionnaire. In a prospective trial, we compared the classical Whipple procedure with the Beger DPPHR with regard to quality of life, pain status, and endocrine function.

MATERIAL AND METHODS

Patients and Inclusion Criteria

Sixty-eight consecutive patients with chronic pancreatitis underwent a pancreatic head resection. The diagnosis of chronic pancreatitis was verified by history, endoscopic retrograde cholangiopancreaticography, ultrasonography, contrast-enhanced CT, and endocrine and exocrine function tests. Patients were included when they presented with an inflammatory mass in the head of the pancreas (>4 cm) in combination with intractable pain, obstruction of the common bile duct or duodenum, or entrapment of the superior mesenteric/portal vein, and when cancer could not be ruled out. Moreover, clinical and quality-of-life data had to be available at each point of assessment. Informed consent was obtained from all patients. Three of these 68 patients were excluded because a pancreatic carcinoma was found during surgery on frozen-section analysis.

Surgical Procedures

The DPPHR as described by Beger et al.[6] was performed in 35 patients and classical PD[4] was carried out in 30 patients. The PD procedure was chosen if pancreatic cancer could not be ruled out despite a preoperative and intraoperative diagnostic workup and in four patients after Billroth II resection.

Prospective Assessment of Quality of Life

For measurement of quality of life, the European Organization for Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire (QLQ-C30, version 3, EORTC Study Group on Quality of Life, Brussels, Belgium)[12] and the Gastrointestinal Quality-of-Life Index (GIQLI)[13] were used. Both questionnaires were self-assessed by the patients. The EORTC questionnaire comprises 30 items relating to symptoms, physical status, working ability, and emotional, cognitive, and social functioning, as well as a global quality-of-life scale. Its validity is excellent,[12,14–17] and it has previously been validated for patients suffering from chronic pancreatitis.[18] The GIQLI consists of 36 items pertaining to gastrointestinal function. In contrast to the EORTC QLQ-C30, it is poorly validated.[13] These two questionnaires were prospectively assessed at three time points during the study: (1) in the hospital during the week before surgery, (2) 9 to 12 months after surgery, and (3) 18 to 24 months after surgery. The eligible patients were informed of the goals of the study and the methods of data collection. They were assured that refusal to participate in the study would not jeopardize their treatment. The second and third assessments were carried out in the outpatient department when the patients received routine follow-up care. They were given quality-of-life questionnaires so they could complete them on-site or fill them out at home and return them to the hospital. At each assessment, relevant clinical data were obtained by the treating physician.

Pain Score

Pain intensity was estimated on the basis of three parameters: frequency of pain, use of analgesic medications, and the pain scale from the EORTC QLQ-C30 questionnaire.

Endocrine Function

Endocrine pancreatic function was assessed on the basis of the need to treat diabetes mellitus with diet modification, oral hypoglycemic agents, or insulin. In all patients who were not insulin dependent, an oral glucose tolerance test was performed, and results were classified as normal, impaired oral glucose tolerance test, or diabetes mellitus, according to criteria set forth by the expert committee on the diagnosis and classification of diabetes mellitus in 1997.[19] Complete prospective data for the oral glucose tolerance test were obtained from 31 patients in the DPPHR group and all patients in the PD group.

Statistical Analysis

The results of the questionnaire scales are expressed as means ± standard deviation. Differences between the two groups were estimated using the Mann-Whitney U test for the questionnaires and the

chi-square test for the clinical data. The effects of the two procedures on quality of life over time were calculated by means of the Wilcoxon rank test. For evaluation of the reliability of the GIQLI, Cronbach's alpha coefficient was calculated for all scales. The domains of the GIQLI were correlated to parallel scales of the EORTC QLQ-C30 by the two-sided Spearman test. Statistical significance was set at $P < 0.05$.

RESULTS

Characteristics of the Study Population

There was no significant difference between the two groups with regard to sex, etiology, duration of symptoms, pain for at least 1 year, cholestasis, morphologic findings, pathologic details of the surgical specimen, body mass index, regular daily routine, and mean follow-up time. The average age for patients in the PD and DPPHR groups was 48 and 43 years, respectively ($P < 0.05$). The study groups were also similar with regard to the indications for surgery. Histopathologic examination of the resected specimens confirmed the diagnosis of chronic pancreatitis in all patients.

Table 1. Effect of PD (n = 30) and DPPHR (n = 35) on quality of life over time based on EORTC Quality-of-Life questionnaire

	Effect over time (A0, A1, A2)	
Scales	**PD**	**DPPHR**
Functional scales		
Physical status	A0 < A1 = A2	A0 < A1 = A2
Working ability	A0 < A1 = A2	A0 < A1 = A2
Cognitive	A0 < A1 = A2	A0 < A1 = A2
Emotional	A0 < A1 = A2	A0 < A1 = A2
Social	A0 < A1 < A2	A0 = A1 = A2
Global quality of life	A0 < A1 = A2	A0 < A1 < A2
Symptom scales		
Pain	A0 < A1 = A2	A0 < A1 = A2
Diarrhea	A0 = A1 = A2	A0 < A1 = A2
Nausea/vomiting	A0 = A1 = A2	A0 < A1 = A2
Constipation	A0 = A1 = A2	A0 < A1 = A2
Appetite loss	A0 < A1 = A2	A0 < A1 = A2
Fatigue	A0 < A1 = A2	A0 < A1 = A2
Insomnia	A0 < A1 = A2	A0 < A1 = A2
Dyspnea	A0 < A1 < A2	A0 < A1 = A2
Financial strain	A0 < A1 = A2	A0 = A1 < A2

A0 = preoperative; A1 = follow-up 1 (9 to 12 months after surgery); A2 = follow-up 2 (18 to 24 months after surgery); < significant improvement; = no change.
Differences were estimated using the Wilcoxon rank test.

EORTC Quality-of-Life Questionnaire

Development of Quality of Life Over Time in the PD and DPPHR Groups (Table 1). At the first follow-up examination, both groups showed a significant increase compared to the preoperative status in all functional scales except that for social function in the DPPHR group. With respect to the symptom variables, almost all scales were significantly less pronounced after both surgical procedures. However, the two important symptoms—diarrhea and nausea/vomiting—did not improve in the PD group. From the second to the third assessment, significantly better scores were again found for the variables social function and dyspnea in the PD group and for global quality of life and financial strain in the DPPHR group. All other scales remained stable at the second follow-up.

Comparison of the PD and DPPHR Groups

Functional Scales (Table 2). The patients in the two groups were comparable preoperatively with respect to all functional parameters. A significantly better quality of life was found in the DPPHR group compared to the PD group at the first follow-up examination with respect to physical status, emotional function, and global quality of life and at the second follow-up with respect to all parameters except for cognitive function.

Symptom Scales (Table 3). The two therapy groups also showed comparable findings preoperatively with regard to all symptom parameters except for financial strain. The most important symptoms, that is, pain, diarrhea, fatigue, nausea, and vomiting, were significantly less pronounced after DPPHR as compared to classical PD at both the first and second follow-up examinations.

To evaluate the impact of the differences in mean age in the two groups, the items on the EORTC index were computed as percentiles standardized for age and sex in reference to data from the general German population.[17] In this setting the differences in functional and symptom scales between the two groups are still the same.

Gastrointestinal Quality-of-Life Index

Using the GIQLI questionnaire (Table 4), the DPPHR revealed a better quality of life with respect to physical health (symptoms and physical status) but not mental health (emotional and social functioning).

Table 2. Functional scales of the EORTC Quality-of-Life questionnaire in PD (n = 30) as compared to DPPHR (n = 35)

	Preoperative			Follow-up 1 (9–12 mo)			Follow-up 2 (18–24 mo)		
	PD	DPPHR	P*	PD	DPPHR	P*	PD	DPPHR	P*
Functional scales									
Physical status	55 ± 23	53 ± 23	NS	79 ± 15	86 ± 23	<0.001	84 ± 11	89 ± 22	<0.001
Working ability	36 ± 31	37 ± 30	NS	73 ± 19	74 ± 36	NS	69 ± 13	73 ± 35	<0.05
Cognitive	76 ± 23	73 ± 25	NS	95 ± 8	91 ± 16	NS	97 ± 8	89 ± 19	<0.05
Emotional	45 ± 23	48 ± 23	NS	69 ± 25	83 ± 24	<0.05	68 ± 19	81 ± 26	<0.001
Social	50 ± 34	53 ± 37	NS	73 ± 22	75 ± 38	NS	60 ± 19	73 ± 34	<0.001
Global quality of life	28 ± 17	30 ± 19	NS	60 ± 15	68 ± 26	<0.05	64 ± 13	72 ± 26	<0.001

Scores range from 0 to 100; higher scores represent a higher level of functioning (values are mean ± SD).
*Test of difference between PD and DPPHR (Mann-Whitney U test).

Validity of the GIQLI vs. EORTC QLQ-C30 Questionnaire

Even if the GIQLI provides a good internal consistency (range of Cronbach's alpha coefficients for different scales: 0.789 to 0.891), content validity is very low. Furthermore, the criteria-based validity is reduced because the correlation with parallel scales of the well-validated EORTC QLQ-C30 is low (range of Spearman rank correlation coefficient: 0.323 to 0.640).

Pain Intensity

Preoperative and postoperative pain status is presented in Table 5. At the second follow-up examination, there was a marked improvement in pain in both groups with regard to the frequency of pain, the need for pain medication, and the pain scale of the EORTC QLQ-C30. Comparing the two groups, a significant difference was seen only in the use of analgesic medication and the EORTC QLQ-C30 responses in favor of the DPPHR group.

Diabetes Mellitus

Preoperatively normal endocrine function was found in 63% of the patients in the PD group and in 71% in the DPPHR group ($P > 0.05$). At the second follow-up examination, normal glucose tolerance dropped to 47% in the PD group and 61% in the DPPHR group ($P < 0.05$). Three patients in the PD group and none in the DPPHR group developed insulin-dependent diabetes mellitus after surgery.

Table 3. Symptom scales of the EORTC Quality-of-Life questionnaire in PD (n = 30) as compared to DPPHR (n = 35)

	Preoperative			Follow-up 1 (9–12 mo)			Follow-up 2 (18–24 mo)		
	PD	DPPHR	P*	PD	DPPHR	P*	PD	DPPHR	P*
Symptom scales									
Fatigue	71 ± 18	72 ± 26	NS	37 ± 18	23 ± 32	<0.001	36 ± 16	22 ± 30	<0.001
Nausea, vomiting	43 ± 37	44 ± 33	NS	32 ± 18	11 ± 17	<0.001	27 ± 21	8 ± 18	<0.001
Pain	82 ± 25	79 ± 24	NS	39 ± 21	21 ± 30	<0.001	33 ± 21	20 ± 30	<0.001
Loss of appetite	79 ± 23	72 ± 30	NS	41 ± 17	26 ± 31	<0.001	29 ± 22	24 ± 29	NS
Dyspnea	29 ± 33	24 ± 28	NS	11 ± 21	9 ± 17	NS	4 ± 14	6 ± 13	NS
Sleep disturbance	70 ± 23	64 ± 35	NS	37 ± 35	33 ± 36	NS	32 ± 20	31 ± 29	NS
Constipation	15 ± 29	11 ± 25	NS	31 ± 23	8 ± 16	<0.001	27 ± 23	7 ± 16	<0.001
Diarrhea	40 ± 36	44 ± 37	NS	37 ± 18	25 ± 32	<0.05	36 ± 20	20 ± 30	<0.001
Financial strain	53 ± 39	35 ± 36	<0.05	32 ± 27	20 ± 32	<0.05	25 ± 17	30 ± 31	NS

Scores range from 0 to 100; higher scores represent a higher degree of symptoms (values are mean ± SD).
*Test of difference between PD and DPPHR (Mann-Whitney U test).

Table 4. Dimensions of the Gastrointestinal Quality-of-Life Index in PD (n = 30) as compared to DPPHR (n = 35)

	Preoperative			Follow-up 1 (9–12 mo)			Follow-up 2 (18–24 mo)		
GIQLI-dimension	PD	DPPHR	*P**	PD	DPPHR	*P**	PD	DPPHR	*P**
Symptoms	36 ± 10	38 ± 12	NS	47 ± 13	56 ± 14	<0.05	46 ± 14	57 ± 14	<0.05
Emotional functioning	7 ± 4	9 ± 4	<0.05	15 ± 5	15 ± 5	NS	14 ± 4	15 ± 5	NS
Physical status	10 ± 4	8 ± 4	NS	18 ± 7	21 ± 8	<0.05	18 ± 7	22 ± 8	<0.05
Social functioning	7 ± 4	7 ± 4	NS	12 ± 4	11 ± 5	NS	12 ± 4	12 ± 4	NS
Medical treatment	2.1 ± 0.8	2.6 ± 0.9	<0.05	3.2 ± 0.7	3.1 ± 1	NS	3.1 ± 0.8	2.9 ± 0.8	NS

Values are mean ± SD; higher scores indicate better health.
*Significance: test of difference between PD and DPPHR (Mann-Whitney U test).

DISCUSSION

Health-related quality of life seeks to measure the impact of the disease process on the physical, psychological, and social aspects of the person's life and feeling of well-being.[20] Outcome related to quality of life should guide the surgeon in selecting the most appropriate operation, especially with respect to palliative surgery for chronic diseases such as chronic pancreatitis. The aim of this study was to compare a duodenum-preserving with a non–duodenum-preserving procedure in terms of quality of life.

We chose the EORTC QLQ-C30, a disease-specific instrument, because it is a valid and reliable instrument for assessing overall quality of life and it had been validated in patients with chronic pancreatitis.[18] Izbicki et al.[11, 21] applied the EORTC QLQ-C30 to compare the Beger and Frey procedures and the Beger technique with DPPHR. The GIQLI was used because it has not been previously applied to patients with chronic pancreatitis except in the study by McLeod et al.[22] According to the recommendation in the literature, we performed three assessments to detect relevant changes in quality of life over time.[23] The importance of this assessment protocol was confirmed by our observations. The follow-up period of at least 18 months in this study is relatively short in view of the fact that we are dealing with a chronic, dynamically changing disease. However, one would expect that the medium-term benefit of surgery could be evaluated in a reliable manner after 2 years.

The fact that the patients are not randomly allocated to the PD and DPPHR groups might introduce a bias. However, on the basis of clinical and pathomorphologic data, particularly sex distribution, etiology, duration of symptoms, pain status, cholestasis, and incidence of calcareous chronic pancreatitis, the two groups are comparable with no apparent evidence of bias. The younger age in the DPPHR group could be controlled for by comparison with standard values derived from the general German population, matched for age and sex.[17] Another bias might be different preoperative expectations in the two groups. The patients in the PD group may be relieved not to be suffering from the cancer that had been suspected preoperatively; this feeling of relief has a positive effect on quality of life.

PPPHR is considered to be superior to classical PD with respect to nutritional status and quality of life, but the data in the literature are controversial. There are three comprehensive studies comparing quality of life after both classical PD and PPPD. Patel et al.[24] found no differences between the groups with little deficit in function. In the retrospective study by Melvin et al.,[25] only three of eight quality-of-life domains (SF-36, United States version 1.0) revealed significantly better results for patients after PPPD vs. classical Whipple procedure. In addition, pancreatic endocrine and exocrine function and nutritional parameters were similar in the two groups.

Table 5. Preoperative and postoperative (18 to 24 months) pain status after PD (n = 30) and DPPHR (n = 35)

	Preoperative			Postoperative		
	PD	DPPHR	*P**	PD	DPPHR	*P**
Frequency of pain attacks						
0–2	21	24	NS	27	34	NS
>2	9	11	NS	3	1	NS
Analgesic medication						
None	0	0	NS	12	23	<0.05
Occasional	10	18	<0.05	16	11	<0.05
Regular	20	17	NS	2	1	NS

*Test of difference between PD and DPPHR (Mann-Whitney U test).

Further, a recent retrospective study on quality of life after PD, using a standard quality-of-life survey instrument comprising 30 items, found no significant differences between PPPD and classical PD.[26] However, the studies by Melvin et al.[25] and Huang et al.[26] included patients with malignant and benign pancreatic diseases and the study by Patel et al.[23] comprised different types of periampullary cancers. Additionally, all three studies applied different questionnaires for quality-of-life assessment. On the basis of these data and in spite of the important limitations of the studies (retrospective assessment, different questionnaires, small study populations), the better quality of life after DPPHR compared to that after classical PD found in this study cannot be explained only by the type of PD.

In this study, effective pain relief was seen after both the PD and DPPHR procedures in terms of the EORTC pain score, frequency of pain attacks, and the need for analgesic medication. In a comparison of the two procedures, improvement of pain was more pronounced after DPPHR only with respect to the need for analgesic medication and the EORTC pain score. In a comparison of duodenum-preserving and non–duodenum-preserving methods, comparable improvements in pain were found in the study by Izbicki et al.[11] and a significantly higher rate of freedom from pain was found after DPPHR in the investigation by Büchler et al.[9] Both surgical procedures led to improvements in both the physical condition and global quality of life with significantly higher levels in the DPPHR group. These results are in accordance with the data by Izbicki et al.[11] The smaller difference in the effect on social function between the two methods can be explained by the higher rate of unemployment in the DPPHR group (45% for the DPPHR group vs. 28% for the PD group). The better results after DPPHR as compared to PD obtained with respect to the most important symptoms, physical status and global quality of life, are also reflected in the better emotional state after DPPHR.

In a prospective cohort study by Malka et al.,[27] it has been shown that with the exception of distal pancreatectomy, elective surgery does not seem to influence the risk of diabetes mellitus up to 25 years after the onset of the disease. There is no long-term prospective study comparing PD vs. DPPHR targeted to the development of diabetes mellitus. The prevalence of diabetes mellitus depends on the diagnostic criteria for disturbances in glucose metabolism. In this study, the percentage of patients with preoperatively normal oral glucose tolerance tests was clearly higher (71% in the DPPHR group vs. 63% in the PD group) than in the studies by Izbicki et al.[11] (32%) and Beger et al. (49%).[28] One explanation for these different rates of normal oral glucose tolerance tests might be different stages of disease. After DPPHR, in our study population the percentage of patients with normal oral glucose tolerance tests dropped to 61% in the DPPHR group and to 47% in the PD group. In the series by Izbicki et al.,[11] the rate of normal oral glucose tolerance tests dropped from 32% to 26% after the Frey procedure and from 33% to 23% after PPPHR after a medium follow-up of 2 years. In the report by Beger et al.[28] on long-term follow-up of DPPHR, the rate of normal oral glucose tolerance tests remained stable at 48% after a median follow-up of 3.6 years. None of the DPPHR patients developed de novo insulin-dependent diabetes mellitus after surgery; this is similar to the results reported by Beger et al.[30] after a comparable follow-up period. However, after PD, 10% developed insulin-dependent diabetes mellitus. This rate is a little higher than that reported by Jimenez et al.[8] (6%). The follow-up periods in our study and in the literature are too short to allow a conclusion to be drawn as to whether there is a real difference in the new onset of diabetes mellitus after the two procedures.

In a comparison of the two questionnaires, the validity of the EORTC QLQ-C30 is superior to that of the GIQLI. The good reliability of the GIQLI for all scales is irrelevant because of its limited validity. In this study the EORTC QLQ-C30 was found to be a better tool for quality-of-life assessment than the GIQLI for patients with chronic pancreatitis.

CONCLUSION

Based on these findings, the following conclusions may be drawn:

1. Both the classical Whipple procedure and the Beger duodenum-preserving pancreatic head resection lead to a significant improvement in quality of life, especially in terms of pain status.
2. Patients undergoing duodenum-preserving pancreatic head resection have consistently more favorable quality of life scores than those undergoing a classical Whipple procedure.
3. After classical Whipple procedure, more patients seem to develop diabetes mellitus.
4. The validity of the EORTC QLQ-C30 questionnaire is superior to that of the GIQLI for quality of life measured in patients with chronic pancreatitis.

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Discussion

Dr. M. Zenilman (Brooklyn, NY): You used the patients as their own controls. What would have happened with the quality-of-life indices if you had just followed a control set of patients who did not have surgery? Did the quality of life diminish with your parameters using your tests?

Dr. H. Witzigmann: We used the tests only in patients with chronic pancreatitis who were operated on, but not in healthy patients and not in patients with chronic pancreatitis who were not operated on.

Dr. Zenilman: But in the patients with chronic pancreatitis whom you did not operate on, what happens to their quality of life over time? Does it diminish?

Dr. Witzigmann: Yes, one can assume that in these patients, quality of life diminishes.

Dr. M. Sarr (Rochester, MN): I do not understand how the patients were treated with either a Whipple procedure or a duodenum-preserving resection, because in your presentation you stated that if there was a question of malignancy, either preoperatively or intraoperatively, a Kausch-Whipple procedure was used. Is that correct?

Dr. Witzigmann: That's correct.

Dr. Sarr: Isn't there an up-front bias then in these two groups? That is, in one group cancer was suspected and in the other group no cancer was involved.

Dr. Witzigmann: That might be a bias because the patients who had a Whipple procedure are significantly older than the patients who had a duodenum-preserving pancreatic head resesection.

Dr. Sarr: I noticed how you tried to quantitate their quality of life preoperatively, and apparently the scores were similar preoperatively. Is that correct?

Dr. Witzigmann: That is correct. Moreover, the clinical and morphologic characteristics are also compatible preoperatively between the two groups.

Dr. Sarr: Then why were some patients thought to have a malignancy preoperatively, whereas others were not? Was it related to the CT scan or to weight loss or some other parameter such as that?

Dr. Witzigmann: It was related to the CT scan, the ERCP, and the experience of the surgeon.

Dr. Sarr: Did you look into trying to control for the CT scan, size of the mass, or something similar?

Dr. Witzigmann: No, we did not look at these parameters.

Dr. L. Traverso (Seattle, WA): I am concerned about the high incidence of diabetes after all of your operations. Can you tell me what the average time was when diabetes occurred after these operations in both groups? We have found that no patients are diabetic after a pancreatic head resection using the Whipple procedure if they are properly selected, and if they do develop diabetes it will be more than a year after their Whipple procedure, because the indications for the pancreatic head resection were based on whether there was true exocrine and endocrine ablation in the first place. If you take out a pancreatic head that was totally replaced by scar tissue, there is no change in the endocrine function. That is why these studies are so important for establishing the pathomorphologic criteria that was displayed in more detail in your second or third slides as demonstrated by the more severe stages of the Cambridge classification. It would be important to look at your patients that way.

Dr. Witzigmann: In the group who had the Whipple procedure, we had a high rate of calcifications. I believe approximately 40% to 50% of patients had calcifications, and it is known that patients with calcifications have a high incidence of diabetes mellitus. We really do not know why we have such a high rate of diabetes mellitus in both of our groups.

Dr. Traverso: How long after the operation did the diabetes occur? Was it immediately after the resection or much later in the course of the disease?

Dr. Witzigmann: Most patients developed diabetes mellitus later than 1 year after the resection; thus we think that the development of diabetes mellitus was independent of our operations.

Dr. R. Bell, Jr. (Chicago, IL): I have two questions. Which type of duodenum-preserving pancreatic head resection was used, the Fry or the Beger procedure?

Dr. Witzigmann: The Beger procedure.

Dr. Bell: My second question concerns Dr. Izbicki's randomized trial of pylorus preserving pancreatoduodenectomy vs. duodenum-preserving pancreatic head resection.[11,21] My recollection is that both procedures were approximately equally effective in relieving pain, but the quality of life in the pancreatoduodenectomy patients was worse for other reasons related to their having undergone a pancreatic head resection. As I viewed your slides, it occurred to me that you have reached a somewhat different conclusion—that the duodenum-preserving pancreatic head resection was more effective in relieving pain. Am I correct in my understanding that the better quality-of-life scores in your study had more to do with superior relief of pain than with other complications of the surgery? Is that a correct interpretation?

Dr. Witzigmann: We do not think that the duodenum-preserving pancreatic head resection is superior with respect to pain relief. In our results, we were able to show that with regard to pain medication and in the EORTC questionnaire, there is a significant difference between these two groups, but there is no significant difference concerning frequency of pain attacks. We think that over a longer time there is no significant difference between these two procedures.

Dr. Bell: So the quality-of-life issues relate to other aspects of the operation, not to failure of efficacy and relief of pain?

Dr. Witzigmann: Yes. We think that the difference in quality of life relates more to other aspects of surgery than the relief of pain.

Dr. Bell: So is your conclusion the same as that reached by Izbicki's group?

Dr. Witzigmann: Yes, our conclusion equals that of Izbicki et al.

Laparoscopic Management of Giant Type III Hiatal Hernia and Short Esophagus: Objective Follow-Up at Three Years

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We wished to evaluate the long-term effectiveness of the laparoscopic Hill repair in the treatment of type III hiatal hernia. Fifty-two patients underwent laparoscopic repair of a type III hiatal hernia. No esophageal lengthening procedures were performed. Short esophagus was determined from the operative record. Late symptomatic follow-up and a satisfaction questionnaire were completed in 71% (37/52) of patients at a mean of 39 months (range 6 to 84 months). Esophagrams were completed in 65% (34/52) of patients at a mean of 37 months (range to 84 months) after repair. Eighty-one percent were without any adverse symptoms, and 86% rated outcome as excellent or good at 39 months. Symptoms requiring treatment were present in 19% (7/37). Esophagrams revealed a recurrent hernia in 32% (11/34) of patients of whom 36% (4/11) were asymptomatic. Six patients with short esophagus underwent esophagram with one recurrence identified (17%). This was compared with 28 patients without short esophagus, of whom 10 had a recurrence (35%) ($P = 0.70$). The laparoscopic Hill repair provides long-term satisfaction and relief of symptoms. The incidence of anatomic recurrence on video esophagram is high and does not always correlate with symptoms. The presence of short esophagus does not play a role in recurrence when the Hill repair is used. (J GASTROINTEST SURG 2002;6:181–188.)

KEY WORDS: Paraesophageal hernia, hiatal hernia, Hill repair, laparoscopy, short esophagus

Although the laparoscopic approach to giant hiatal hernia repair has been proved to be safe in experienced hands, the early success of this procedure has been based almost entirely on symptomatic follow-up.[1–4] When compared with the open approach, recent reports have documented a disturbingly high rate of recurrence on objective testing with a poor correlation to symptoms. Although the clinical significance of asymptomatic, small anatomic recurrences is unclear, these data question the durability of the laparoscopic repair. Consequently, some centers have returned to transabdominal or transthoracic approaches. Regardless of the approach for repair of paraesophageal hernias, recurrence may be related to one or a combination of the following: (1) an unrecognized short esophagus; (2) failure of the crural repair; and (3) herniation of the gastric fundus through the hiatus. Although the Hill repair does not employ an esophageal lengthening procedure, two critical aspects of the procedure are fixation of the gastroesophageal junction (GEJ) to the preaortic fascia and suturing the fundus to the anterior hiatus and both diaphragmatic crura.[6] Placement of repair sutures is located precisely at the level of the GEJ rather than out on the fundus, as is performed with fundoplications (Fig. 1). These tenets are essential to the Hill repair and could significantly influence the rate of recurrence of paraesophageal hernias.

Because several aspects of the Hill repair for paraesophageal hernia appealed to us, we wished to examine the efficacy of this procedure in an objective manner (i.e., with follow-up radiographic studies) and to compare subjective results (i.e., patient satisfaction and symptoms) with those results obtained by radiography.

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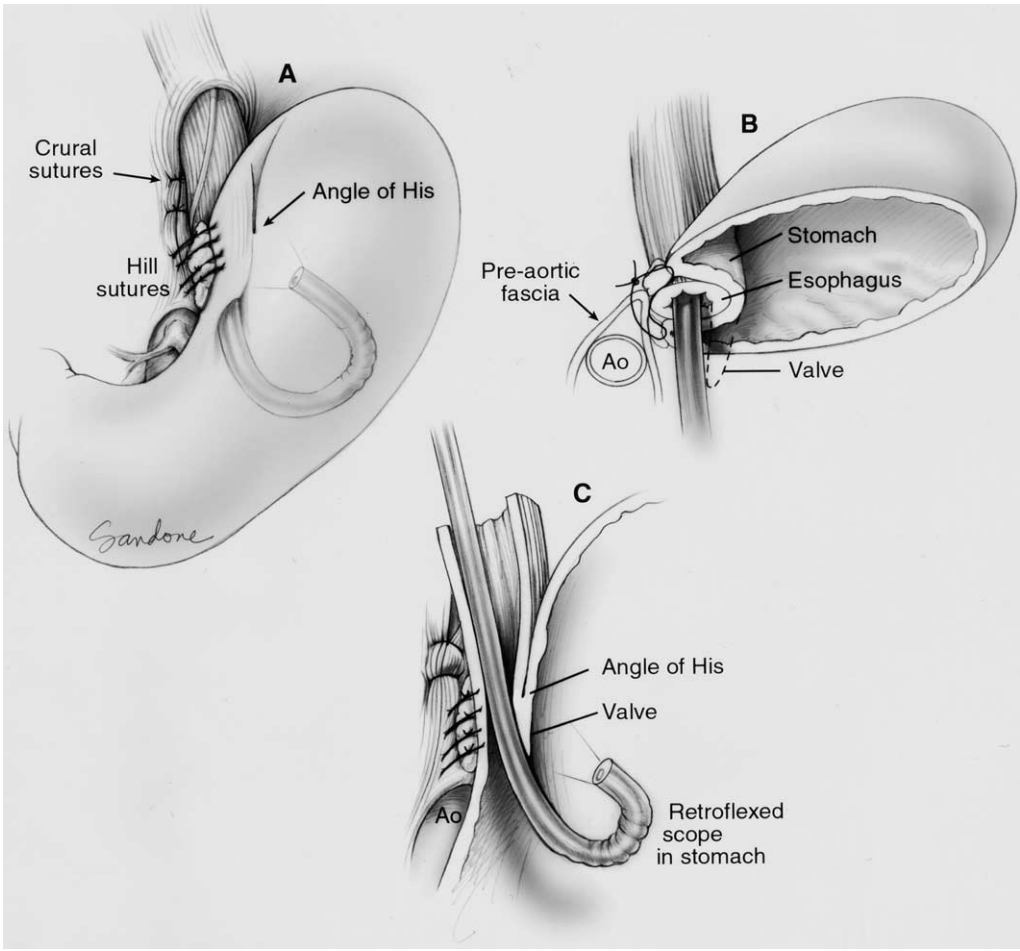

Fig. 1. Anatomy of the completed laparoscopic Hill repair. The GEJ is sutured to the preaortic fascia, thereby recreating the acute angle of His and tightening the collar sling musculature. This serves to reconstruct the one-way gastroesophageal flap valve and distal high-pressure zone (**A**). Oblique (**B**) and coronal (**C**) sections are also shown. Ao = aorta.

MATERIAL AND METHODS

Study Design and Patient Selection

This is a retrospective cohort analysis of 52 patients who underwent laparoscopic type III hiatal hernia repair in conjunction with a Hill procedure between April 1993 and August 2000. All hernias were primary. Patients with type I or type II hiatal hernias were excluded. Four patients converted from a laparoscopic to an open approach were not included in this study. Data were gathered prospectively under a standardized outcome protocol.

Preoperative Evaluation

Symptom assessment was carried out during the initial patient contact. Routine preoperative objective testing included a video esophagram (52/52), esophagogastroduodenoscopy (52/52), and esophageal manometry (39/52). Ambulatory 24-hour pH monitoring was not routinely employed.

Surgical Procedure

Two surgeons (L.D.H. and R.W.A.) performed all procedures during the defined interval. Hiatal hernia repair consisted of mediastinal sac reduction and excision, primary posterior crural closure without mesh, and a Hill antireflux procedure. Pledgets were used with the hiatal closure when the opening was larger than 4 cm or if the crura were severely attenuated. The key feature of the Hill repair is intraabdominal fixation of the anterior and posterior phrenoesophageal bundles to the preaortic fascia with four interrupted sutures spanning a total distance of 3.5 cm. Intraoperative manometry is performed for the purpose of calibrating the tightness of

the repair. Finally, the gastric fundus is sutured to the diaphragmatic hiatus apically and along both crura in order to increase valve length and cover the anterior hiatal opening. The short gastric vessels are spared and a gastropexy is not performed.

The presence of a short esophagus was determined before the Hill repair when, despite extensive mediastinal dissection, there was difficulty delivering the GEJ subdiaphragmatically in a tension-free manner. These data were obtained from the operative record. There were no esophageal lengthening procedures performed and the GEJ was successfully delivered into the abdominal cavity in all cases.

Symptomatic Follow-Up

Complications were recorded as they occurred. Patient contact was made in person or by telephone at a mean of 3 months (range 1 to 8) and 39 months (range 6 to 84), respectively, postoperatively. A structured symptom assessment and satisfaction questionnaire was completed in 88% (46/52) and 71% (37/52) of patients at early and late follow-up, respectively. Four patients had died of unrelated causes and 11 patients were lost to follow-up. Outcome grading was based on the presence or absence of a particular symptom, as listed in Table 1. A detailed medication history was obtained. Patients rated their overall outcome as excellent, good, fair, or poor at early and late follow-up.

Table 1. Symptom assessment and satisfaction questionnaire

Symptoms and satisfaction	
Symptoms failure	
Heartburn	Yes/No
Regurgitation	Yes/No
Chest pain	Yes/No
Dysphagia	Yes/No
Medications	Yes/No
Symptoms related to Hill repair	
Hyperflatulence	Yes/No
Bloating	Yes/No
Dysphagia	Yes/No
Ability to belch	Yes/No
Dumping	Yes/No
Satisfaction	
Excellent	Yes/No
Good	Yes/No
Fair	Yes/No
Poor	Yes/No

A list of symptoms and level of patient satisfaction queried at early and late follow-up.

Objective Follow-Up

Sixty-five percent (34/52) of patients underwent barium esophagram at a mean of 37 months (range 5 to 84 months) after surgery. The study was performed with the patient taking several swallows of liquid barium while in the upright and supine positions. This examination was not controlled for the interpreting radiologist, nor were they blinded to the operation performed. The hernia recurrences were categorized under the following headings: type I, GEJ above the diaphragm without a paraesophageal component; type II, properly positioned GEJ with a paraesophageal component; and type III, GEJ above the diaphragm with a paraesophageal component.

Statistical Method

The statistical method was comparison of proportions using binomial distribution.

RESULTS

The patients were 54% female with a mean age of 70 years (range 50 to 89 years). The mean duration of disease before the operation was 11 years (range 3 to 30 years). All patients were symptomatic, and preoperative primary symptoms included chest pain (27%), regurgitation (22%), respiratory compromise (20%), dysphagia (16%), and heartburn (16%). Three patients required emergent repair for signs of strangulation or hemorrhage. Seventeen percent had Barrett's esophagus, 21% had erosive esophagitis, 13% had distal esophageal stricture, and 5% had Cameron's ulcer.

Operations averaged 3.1 hours in duration (range 2 to 6 hours), and the length of hospital stay was 2.6 days (range 1 to 7 days). Overall, the complication rate was 19% (10/52), and there were no procedure-related deaths (Table 2). Esophageal perforation occurred in two patients during passage of the esophageal dilator, and one patient sustained a small bowel

Table 2. Complications

Intraoperative	Perioperative	Late
Esophageal perforation (n = 2)	Atrial fibrillation	Readmission with N/V, dehydration
Bowel injury	Pneumonia (n = 2) ETOH withdrawal Wound infection	Pleural effusion

trocar injury. All injuries were repaired laparoscopically. Overall, the incidence of short esophagus was 19% (10/52).

Ninety-one percent (42/46) of patients had no adverse symptoms, and patient satisfaction at 3-month follow-up examination was rated as follows: excellent in 93% (43/46), good in 5% (2/46), and poor in 2% (1/46). The four patients with symptoms had heartburn only, and two were taking daily proton pump inhibitors.

At 39 months, 81% (30/37) of patients were without any adverse symptom, and 72% (27/37) rated outcome satisfaction as excellent; 14% (5/37) rated it as good, 8% (3/37) rated it as fair, and 6% (2/37) rated it as poor (Figs. 2 and 3). Seven patients (19%) had symptoms consisting primarily of heartburn, regurgitation, or chest pain (Table 3); five patients are currently being maintained on daily proton pump inhibitors, and two have required reoperation for severe symptoms. All seven patients had evidence of an anatomic recurrence on follow-up esophagram.

Thirty percent (14/46) had early dysphagia after Hill repair that resolved within 6 weeks. There were no dilations required for late dysphagia. There were no admissions or interventions for gas bloat syndrome. The primary late symptom related to the Hill repair was hyperflatulence in 27% (10/37) of patients at 39 months. This was life limiting in two patients.

On follow-up video esophagram at 37 months, recurrent hernia had occurred in 32% (11/34) of patients. Thirty-six percent (4/11) of these patients were asymptomatic. Of the 11 recurrences, three were type I, four were type II, and four were type III. None of the patients with a type I recurrence were symptomatic, but seven of eight patients with a paraesophageal recurrence (types II and III) were symptomatic (0/3 vs. 7/8, $P < 0.05$) (Table 4).

To date there have been two patients who underwent reoperation, and four patients may require repair in the future. At surgery there was disruption of the hiatal repair with a type III recurrence in both patients. All of the patients with type I recurrences and two patients with very small type II recurrences are being treated nonoperatively and observed with serial esophagrams (Table 5). If distributed over time from the date of surgery to the discovery of anatomic recurrence, the recurrence increases from 24% (4/13) at 0 to 2 years to 57% (4/3) at 4 to 7 years (Fig 4). Two patients had very small type I recurrences at 4 to 7 years.

Six patients with short esophagus underwent esophagrams, and a recurrence was identified in one of them (17%). This was compared with 28 patients without short esophagus, of whom 10 (35%) had a recurrence on esophagram ($P = 0.70$).

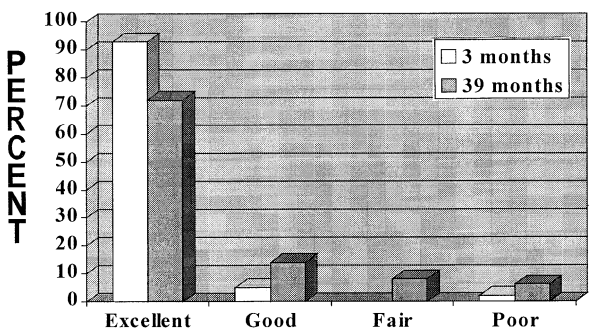

Fig. 2. Patient satisfaction at early (3 months) and late (39 months) follow-up.

DISCUSSION

We have retrospectively reviewed our experience with the laparoscopic approach to giant hiatal hernia repair in combination with the Hill procedure. The impetus for this review stemmed from the findings of other investigators, who discovered an inordinately high rate of recurrence (documented radiographically) associated with the laparoscopic approach[5,7] (Mattar et al., unpublished data) when compared to the transabdominal or transthoracic approach.[5] Hashemi et al.[5] discovered a 42% recurrence rate associated with laparoscopic repair at 17 months' follow-up with a video esophagram. More than half of these patients were asymptomatic. This was compared to the 15% recurrence at 35 months associated with open approaches. Wu et al.[7] described a 23% recurrence associated with laparoscopic repair of type II and type III hernias at 3 months. These findings are inconsistent with the results of several studies that reported excellent long-term symptomatic outcomes with the laparoscopic approach.[1–4] This suggests a poor correlation between anatomic recurrence and symptoms. Our investigation stringently evaluated the effectiveness of the laparoscopic Hill repair in relieving all symptoms at 39 months' follow-up. Eighty-one percent of patients were without any adverse symptoms, and 86% found their outcome to be excellent or good. These excellent subjective outcomes were contrasted by a high recurrence rate on esophagram (32%). Similarly, more than 30% of these patients were asymptomatic.

Because objective follow-up is missing in 35% of our cohort, the possibility of selection bias exists. It has been our experience that patients with continued symptoms are more likely to return for objective follow-up testing. However, if we were to assume that the 18 unstudied patients had intact repairs, the

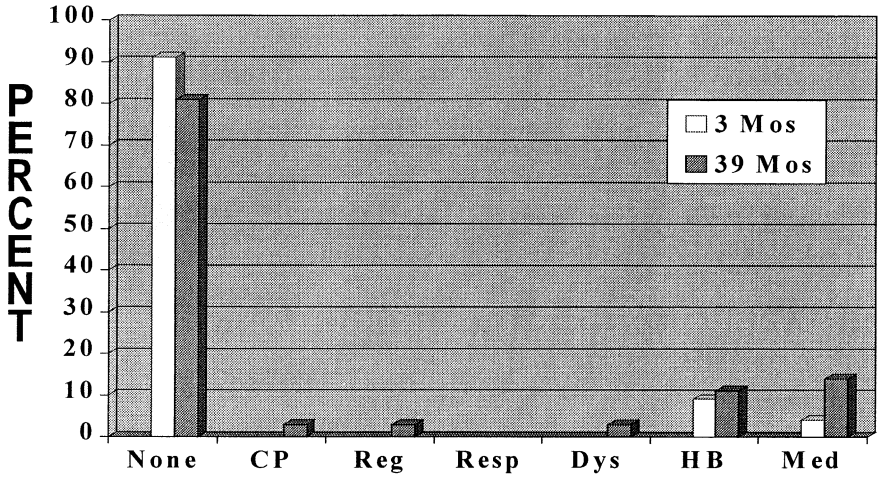

Fig. 3. Patient symptoms at early (3 months) and late (39 months) follow-up. CP = chest pain; Dys = dysphagia; HB = heartburn; Med = return to proton pump inhibitors; None = no adverse symptoms; Reg = regurgitation; Resp = respiratory compromise.

prevalence of anatomic recurrences would remain high at 21% (11/52). Additionally, the level of recurrence observed within this study correlates with three recent investigations that objectively evaluated the outcome of laparoscopic giant hiatal hernia repair[5,7] (Mattar et al., unpublished data).

The precise implications of these recurrences are unclear. As such, we have stratified recurrences into three categories based on their anatomy. Type I recurrences represent an axial transgression of the GEJ into the chest without a paraesophageal component. None of the patients with a type I recurrence were symptomatic, but seven of eight patients with a paraesophageal recurrence (types II and III) were symptomatic. Although the sample size is small, it appears more likely that hernia recurrence with a paraesophageal component will produce symptoms and should raise the index of suspicion for a type II or III recurrence. Our group currently obtains follow-up esophagrams after all paraesophageal hernia repairs (open or laparoscopic) at 1 and 3 years and observes asymptomatic type I recurrences with serial studies. Unless the recurrence is very small, we repair type II and type III recurrences, because they represent the potential for torsion and strangulation. The natural history of type I recurrence is unknown, and thus it is difficult to gauge clinical significance.

Interestingly, if type I recurrences are discounted, the overall rate of recurrence would decrease from 32% (11/34) to 24% (8/34) at 37 months' follow-up. Because we did not examine our cohort with serial esophagrams from the time of surgery to the present, we are unable to determine the precise time of recurrence. However, if anatomic recurrences are distributed over time from surgery to discovery on esophagram, the failure rate at 4 to 7 years is 57% (4/7). Although this may reflect our early learning curve or a gradual failure of the repair, two patients with type I recurrences fell within this interval. Again, if this group is subtracted, the recurrence at 4 to 7 years de-

Table 3. Symptoms and satisfaction at late follow-up (mean 39 months)

Primary symptoms (n = 37)	
None	81% (30/37)
Heartburn	11% (4/37)
Regurgitation	3% (1/37)
Chest pain	3% (1/37)
Dysphagia	3% (1/37)
Satisfaction (n = 37)	
Excellent	72% (27/37)
Good	14% (5/37)
Fair	8% (3/37)
Poor	6% (2/37)

Table 4. Correlation between types of hernia recurrence and symptoms

Recurrence type	Symptoms	No symptoms
I (n = 3)	0	3
II (n = 4)*	3	1
III (n = 4)*	4	0

Distribution of symptoms by type recurrence.
*$P < 0.05$ when type I is compared to types II and III combined.

Table 5. Patients with a documented recurrence on esophagram

Patient	Primary symptom	Secondary symptom	Identification of recurrence (months postoperative)	Type and size of recurrence	Course of action
1	Asymp	Asymp	84	I (small)	Obs
2	Asymp	Asymp	25	I (small)	Obs
3	Asymp	Asymp	72	I (small)	Obs
4	Asymp	Asymp	12	II (moderate)	Obs
5	HB	N/A	24	II (small)	PPI/Obs
6	HB	N/A	20	II (small)	PPI/Obs
7	Dysphagia	HB	71	II (moderate)	PPI/POR
8	HB	Regurg	12	III (large)	ROP
9	HB	Regurg	4	III (large)	ROP
10	Chest pain	Resp	65	III (moderate)	PPI/POR
11	Regurg	HB	15	III (moderate)	PPI/POR

Asymp = asymptomatic; HB = heartburn; large = hiatal hernia >4 cm; moderate = hiatal hernia 2 to 4 cm; N/A = no secondary symptom; Obs = observation with serial esophagram; PPI = proton pump inhibitor; POR = planned reoperation; Regurg = regurgitation; Resp = pulmonary symptoms; ROP = reoperation; small = hiatal hernia < 2 cm.

creases to 29%. These "adjusted" recurrence rates may be similar to those identified with open approaches at late follow-up. Because this study represents one of the latest objective examinations of the laparoscopic approach to giant paraesophageal hernia, and because of the paucity of data regarding open anatomic recurrences, it becomes difficult to make an accurate comparison between the two groups. Because the minimally invasive approach greatly minimizes the effect of surgical trauma in this frail group of patients, we believe that further investigation is warranted before considering a categorical return to the open methods.

The tenets of proper paraesophageal hernia repair include sac reduction and excision, sound crural closure, restoration of an intra-abdominal length of esophagus, and an antireflux procedure. Because the actual recurrence rate of the open approach to paraesophageal hernia repair is unknown, it is difficult to discern whether or not laparoscopic methods are actually deficient in one or more of the preceding principles of repair. Irrespective of the approach employed, a major source of failure appears to be related to disruption of the hiatal closure.[8–10] In this study there were two patients who required reoperation for hiatal failure with resultant type III recurrence. Both were repaired via celiotomy. One patient had a portion of the posterior sac remaining within the mediastinum, which was thought to have contributed to recurrence. Prosthetic mesh has been used to aid in hiatal closure, but several reports suggest that its use may lead to increased complications such as esophageal erosion, perforation, or stricture.[9–11] No patients in our series underwent mesh repair of the hiatus. Others advocate a tension-relieving incision within the diaphragm and subsequent primary closure of the hiatus. In turn, the remaining diaphragmatic incision is closed with mesh, and the left hepatic lobe serves to prevent contact between the mesh and the abdominal viscera. Because the Hill repair requires fixation of the GEJ to the preaortic fascia inferior to the union of the crura, there is a risk of esophageal angulation and resultant dysphagia associated with the extensive posterior hiatal closure required for paraesophageal hernia repair. Because of this, we occasionally close a portion of the hiatus anteriorly, after mobilizing the left side of the diaphragm away from the pericardium. The gastric fundus is then sutured to the diaphragmatic hiatus apically and along both crura in order to increase valve length and cover the anterior hiatal closure.

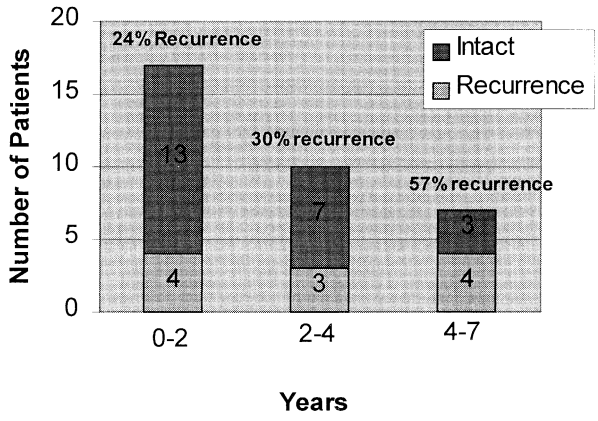

Fig. 4. Distribution of anatomic recurrences over time of discovery with esophagram. Two patients had small, asymptomatic type I recurrences at 4 and 7 years, respectively. To date, type I recurrences have not required reoperation and are currently followed with serial esophagrams.

The recognition and treatment of short esophagus is controversial. Categorically it has been defined by the need for further lengthening after near-complete intrathoracic esophageal mobilization.[12,13] The Hill repair does not ever employ a gastroplasty, which has resulted in some debate as to the existence of short esophagus altogether. The incidence of short esophagus (requiring a gastroplasty) associated with giant paraesophageal hernia is reported to range from 2% to 80%.[5,14] This wide range reflects the lack of consensus as to what exactly defines this entity. This study suggests that the presence of a short esophagus does not play a role in recurrence when the Hill repair is used, and thereby avoids the need for a Collis gastroplasty. This is a distinct advantage, as it avoids the greater than 50% risk of placing functional parietal cells within the neoesophagus proximal to the fundoplication.[15] Subsequently the need for long-term endoscopic surveillance and proton pump inhibitors is obviated. Also, by avoiding a Collis gastroplasty, distal esophageal body function and thus clearance are preserved at the level of the antireflux barrier. With the Hill repair, a short esophagus is addressed by laparoscopic transhiatal mobilization of the thoracic esophagus up to the level of the aortic arch and fixation of the GEJ to the preaortic fascia. Although there may be an element of axial tension placed on the esophagus during this portion of the procedure, it is thought that less intra-abdominal esophageal length is required for repair. This is because repair sutures are placed precisely at the level of the GEJ, as opposed to a fundoplication, which is performed around the distal 3 cm of the esophagus. As a result, the Hill procedure has proved to be quite useful in patients who have undergone prior gastric resection and have subsequently required antireflux surgery. Additionally, having the preaortic fascia as the primary point of fixation for the repair is thought to "unweight" the crural closure and thereby prevent subsequent dehiscence. Conceptually this is quite different from other repairs, which rely, in part, on the fundoplication to anchor the GEJ below the diaphragm.[16,17] This may result in undue axial stress at the hiatus closure. It is because of this that the liberal use of Collis gastroplasty should be considered when one suspects a short esophagus (after near-complete transhiatal esophageal mobilization) and employs Nissen fundoplication as the primary antireflux surgery.

CONCLUSION

The laparoscopic Hill repair provides long-term patient satisfaction and relief from symptoms in patients with type III hiatal hernias. This approach is associated with a high rate of anatomic recurrence, and more than 30% of these patients are asymptomatic. The natural history and clinical significance of small asymptomatic recurrences are unknown, and further investigation is warranted. Serial postoperative esophagrams in all patients undergoing type III hiatal hernia repair (open or laparoscopic) are recommended. The effectiveness of laparoscopic repair of paraesophageal hernia compared to the open approach is unknown. A randomized controlled trial comparing approaches is needed.

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Discussion

Dr. J.H. Peters (Los Angeles, CA): Several papers have now documented a 30% to 40% recurrence rate in patients with paraesophageal hernia. I would also agree with your premise that recurrence is largely related to problems with the hiatal hernia rather than problems with fundoplication. It is interesting that you even show it in the presence of a Hill repair where the cardia is actually sewn to the crura. Could you comment on why you think this is occurring? Are we not suturing well enough laparoscopically, or are there other reasons that can account for these observations?

Dr. B.A. Jobe: Why the hiatus is breaking down?

Dr. Peters: Correct.

Dr. Jobe: My feeling is that these patients have a defect in the make-up of their collagen. Because the hiatus is closed under tension, the repair sutures ultimately pull through. This is seen in patients who have had an abdominal aortic aneurysm repair. A large percentage of those patients end up with incisional hernias, compared to those who do not have an abdominal aortic aneurysm. I think there is some fundamental problem with their collagen, and therefore we must figure out a better way to close the hiatus. The Hill repair takes the weight off of the hiatal closure by sewing it to the preaortic fascia.

One of the premises for using a wrap to repair a paraesophageal hernia is to "anchor" the GEJ subdiaphragmatically. Each time the patient breathes, it creates upward tension on the hiatal closure and that is why it blows out.

Dr. F. Serafini (Tampa, FL): I have two questions. The first concerns esophageal motility. You stated that you assess this by means of manometry; however, occasionally you were unable to complete the manometry. So how did you assess motility in those patients in whom you were not able to perform manometry? Second, you stated that you encounter a short esophagus in a fair number of patients, and you did not perform a Collis gastroplasty. I agree with that, but how did you manage the short esophagus?

Dr. Jobe: With respect to the motility, we did try to determine esophageal body function in all patients; however, sometimes this was not possible. One of the aspects of the Hill repair is to tailor the antireflux surgery or the tightness of the repair based on intraoperative manometry, but in some patients this was not possible, so we just performed our standard repair.

With respect to short esophagus, the premise of the Hill repair is to suture this GEJ down to the preaortic fascia, and that is how it is taken care of. This is done after an intrathoracic mobilization of the esophagus in an attempt to achieve as much length as possible.

Dr. L.L. Swanstrom (Portland, OR): My question is, in those patients who do fail after a Hill procedure, what do you do next? Is there an indication for using a Collis procedure at that time? In how many of these patients can you do a repair? Do you only perform a repair in the patients with symptoms or do you also do re-repairs to those who are asymptomatic?

Dr. Jobe: All reoperations are performed with an open Hill procedure. The procedure is done in an open fashion. In this cohort of patients, there were two who had a reoperation, and both of them had a type III recurrence. We performed an open pledgeted closure of the hiatus and then performed another Hill procedure.

Laryngoscopy and Pharyngeal pH Are Complementary in the Diagnosis of Gastroesophageal-Laryngeal Reflux

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Pharyngeal pH monitoring and laryngoscopy are routinely used to diagnose gastroesophageal-laryngeal reflux as a cause of respiratory symptoms. Although their use seems intuitive, their ultimate diagnostic value is yet to be defined. We studied 10 asymptomatic (control) subjects and 76 patients with respiratory symptoms. Both patients and control subjects were given a symptom questionnaire. Each underwent direct laryngoscopy using the reflux finding score (RFS) to grade laryngeal injury, esophageal manometry, and 24-hour esophagopharyngeal pH monitoring. The patients were then classified as RFS+, if the score was greater than 7, and pharyngeal reflux (PR)+, if they had more than one episode of PR detected during pH monitoring. The most common symptoms reported by patients were hoarseness (87%), cough (53%), and heartburn (50%). Control subjects had a significantly lower RFS (2.1 vs. 9.6, $P < 0.01$) and fewer episodes of PR (0.2 vs. 3.4, $P < 0.01$), than patients. None of the control subjects had more than one episode of PR during a 24-hour period. Fifty patients (66%) were RFS+ and 26 (34%) were RFS−. Thirty-two patients (42%) were PR+ and 44 (58%) were PR−. Fifteen patients had a normal RFS and no PR (group I = RFS−/PR−). Forty patients had discordance between the laryngoscopic findings and the pH monitoring (group II = RFS−/PR+ or RFS+/PR−). Twenty-one patients had both an abnormal RFS and PR (group III = RFS+/PR+). Patients in group III had significantly higher heartburn scores and distal esophageal acid exposure. Eighty-three percent of patients in group III but only 44% in group I improved their respiratory symptoms as a result of antireflux therapy. An abnormal PR or RFS differentiates patients with laryngeal symptoms from control subjects. Agreement between PR and RFS helps establish or refute the diagnosis of gastroesophageal reflux as a cause of laryngeal symptoms. Patients who are RFS+ and PR− may have laryngeal injury from another source, whereas patients who are RFS− and PR+ may not have acid entering the larynx, despite the presence of PR. Patients who are RFS+ and PR+ have more severe gastroesophageal reflux disease and their reflux causes laryngeal damage. Laryngoscopy and pharyngeal pH monitoring should be considered complementary studies in establishing the diagnosis of laryngeal injury induced by gastroesophageal reflux. (J GASTROINTEST SURG 2002;6:189–194.)

KEY WORDS: Extraesophageal reflux, GERD, pharyngeal reflux, laryngoscopy, respiratory symptoms, pH

Gastroesophageal reflux disease (GERD) has long been recognized as a potential cause of many laryngeal and respiratory symptoms. Although cough, hoarseness, wheezing, and other airway symptoms may be caused by several etiologic factors, recent emphasis has been placed on the role of gastroesophageal reflux as the culprit. Although several tests have been reported to help determine whether reflux in a given patient is the cause of respiratory symptoms, none is perfect and a "gold standard" for this diagnosis remains elusive.

Patti et al.[1] showed that patients with respiratory symptoms caused by GERD may be identified by the amount of acid exposure into the proximal esophagus. Because direct monitoring of laryngeal pH is impractical,[2] the placement of a probe in the pharynx, just above the upper esophageal sphincter, has been employed to detect extraesophageal acid reflux.

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Pharyngeal pH monitoring detects reflux in approximately 40% of patients with laryngeal symptoms of unknown etiology.[3] Detection of abnormal pharyngeal reflux (PR) predicts response to acid suppression therapy,[4] but some patients who do not have abnormal PR also respond to this type of therapy.

Laryngoscopy is a common screening tool for patients with symptoms such as hoarseness, cough, and laryngitis. This often reveals erythema, nodularity, ulceration, granuloma, or leukoplakia, but to date no single finding seems to be pathognomonic of reflux-induced laryngeal reflux.[5] Recently, Belfasky et al.[6] developed a scoring system based on laryngoscopic findings, named the reflux finding score (RFS), to identify those patients with laryngeal injury due to gastroesophageal-laryngeal reflux. They showed that patients with confirmed PR experienced improvement in the RFS with acid suppression.

We wished to determine if there was a relationship between laryngoscopic findings (specifically the RFS) and pharyngeal pH monitoring. Furthermore, because it appeared that clinically neither was perfect to accurately define whether in a given patient reflux caused laryngeal symptoms, we wanted to determine whether and at what level of agreement these tests would become clinically relevant. Last, we wanted to explore the incidence of PR and the total score of laryngeal damage as defined by Belfasky et al.[6] in a group of control subjects to define its "normal" values. It was our aim to be able to improve our ability to positively identify the cause-effect relationship in patients with laryngeal symptoms and abnormal reflux, as this would have substantial impact when planning therapy.

METHODS

Our study population consisted of 11 control subjects who had no esophageal or extraesophageal symptoms of reflux and 76 patients with respiratory symptoms thought to be due to reflux. Our procedure was the same for both patients and control subjects, and consisted of the following: (1) symptom frequency questionnaire; (2) direct laryngoscopy; (3) manometry; and (4) 24-hour esophagopharyngeal pH monitoring. The investigators scoring the laryngoscopic findings (N.M.) and interpreting the pharyngeal pH studies (B.K.O.) were blinded to the findings of each other's studies.

Symptom Score

Symptoms were rated on a frequency scale ranging from 0 to 4: 0 = never, 1 = once per month, 2 = once per week, 3 = once per day, and 4 = several times per day. Any frequency that fell between two numbers was upgraded to the higher number. We posed 22 symptom questions: 11 gastrointestinal (heartburn, regurgitation, abdominal pain, belching, dysphagia for liquids and solids, bloating, nausea, chest pain, odynophagia, and globus) and 11 extraesophageal (coughing, hoarseness, wheezing, laryngitis, aspiration, choking, dyspnea, sore throat, asthma, bronchitis, and pneumonia).

Laryngoscopy and Reflux Finding Score

Laryngoscopy with videostroboscopy was performed with a flexible endoscope and topical anesthetic by one of two laryngologists (A.H. or N.M.). The larynx was examined during both quiet respiration and free phonation. The RFS was determined based on the presence and severity of eight different laryngoscopic findings using the criteria of Belfasky et al.[6] (Table 1). The RFS was considered positive, or likely to be related to reflux, if greater than 7. If the RFS was equal to or less than 7, it was considered negative.[6]

Pharyngeal pH Monitoring

Acid suppression therapy was discontinued 5 to 7 days before testing. A four-sensor solid-state pH catheter was placed with the proximal sensor 1.5 to 2.0 cm above the upper esophageal sphincter, as de-

Table 1. Components of the reflux finding score

Subglottic edema	2 = present
	0 = absent
Ventricular obliteration	2 = partial
	4 = complete
Erythema/hyperemia	2 = arytenoids only
	4 = diffuse
Vocal cord edema	1 = mild
	2 = moderate
	3 = severe
	4 = polypoid
Diffuse laryngeal edema	1 = mild
	2 = moderate
	3 = severe
	4 = obstructing
Posterior commissure hypertrophy	1 = mild
	2 = moderate
	3 = severe
	4 = obstructing
Granuloma/granulation	2 = present
	0 = absent
Thick endolaryngeal mucus/other	2 = present
	0 = absent
	TOTAL

termined by stationary esophageal manometry. The remaining three sensors are spaced at 5 cm intervals along the catheter, the most distal sensor being located approximately 13 cm below the upper esophageal sphincter. Measurements of pH were sampled in a recorder (Medtronic, Shoreview, MN) worn by the patient for a 24-hour period. The patients kept a diary of their symptoms. The recorder and diary information were entered into a software program, which reported events (number and duration of reflux episodes) and calculated acid exposure times over the course of the study. All tracings were individually reviewed, rather than relying on the computer interpretation, to determine episodes of PR.

To be considered an episode of PR, the pH in the proximal sensor had to fall below 4, had to drop more than one point from its previous baseline, and had to be accompanied by a simultaneous drop in esophageal pH to below 4 in all distal sensors. Episodes of PR that occurred during a meal were excluded to control for the effect of eating on pH.[7]

Distal acid exposure times with a pH <4 (normal <4% in the standard position) were generated from the caudad sensor. Although standard sensor placement in the distal esophagus would be 5 cm above the lower esophageal sphincter in our center, the distal sensor placement in this study varied from 5 to 14 cm above the lower esophageal sphincter because of the fixed position of the pharyngeal sensor. Therefore the distal sensor was, in all cases, either at or above the standard position and thus, by necessity, the percentage of acid exposure was less than it would have been 5 cm above the lower esophageal sphincter.

Statistical Analysis

Data are expressed in means and standard deviation (SD) unless otherwise stated. Esophageal acid exposure, PR, and RFS scores were compared using an unpaired Student's *t* test. Differences in nonparametric data, such as symptom scores, were analyzed using a Mann-Whitney U test. All statistical calculations were performed using commercially available software (SPSS for Windows).

RESULTS

Control Subjects

The mean percentage of time that the pH was <4 in our 11 subjects for the distal channel was 1.33% (SD ± 0.24). Only two subjects experienced a single short-lived episode each of PR. None of the other nine subjects had any episodes of PR detected during the 24-hour monitoring period. Based on these findings (confidence interval = 0 to 1), we considered any patient with more than one episode of PR in a 24-hour period to have abnormal PR score (PR+). The RFS among our control subjects ranged from 0 to 4, with a mean of 2.09 (confidence interval = 0 to 4.9).

Patients

All patients were evaluated because of laryngeal or respiratory symptoms thought to be possibly due to reflux. The most common symptoms reported by these patients were hoarseness (87%), cough (53%), laryngitis (53%), heartburn (50%), dyspnea (34%), regurgitation (28%), and wheezing (24%). Of the symptomatic patients, 50 patients (66%) were RFS+ and 26 patients (34%) were RFS−. Thirty-two patients (42%) were PR+ and 44 patients (58%) were PR−. There was no significant correlation between PR and the RFS ($r = 0.02$, $P = 0.85$). Furthermore, there was no correlation between PR and individual components of the RFS. The mean number of episodes of PR was 3.4 (±4.7), but the mean number for those who were PR+ was 7.8 (±1.4, median = 6).

Control Subjects vs. Patients

Asymptomatic subjects had significantly less esophageal acid exposure (1.3% vs. 4.0%, $P < 0.01$), fewer episodes of PR (0.2 vs. 3.4 episodes, $P < 0.01$), and lower RFS (2.1 vs. 9.9, $P < 0.01$) than did the symptomatic patients (see Table 1). Patients and subjects showed similar results in esophageal motility, upper esophageal sphincter pressure, and characteristics of peristalsis (Table 2). Eighteen patients had abnormal distal esophageal acid exposure; of these 12 (67%) had a positive RFS and 13 (72%) were PR+.

Patient Groups

As shown in Table 3, we divided the patients into three groups based on their RFS and PR results. Group I (n = 15) had no laryngeal evidence of reflux and no PR (RFS−/PR−). Group II (n = 40) had discordance between laryngoscopic findings and pH monitoring (RFS+/PR− or RFS−/PR+). Group III (n = 21) had both an abnormal RFS and PR (RFS+/PR+).

The symptom scores for these groups are shown in Table 3. Among the three groups, there was no significant difference in the frequency of extraesophageal symptoms (i.e., hoarseness, cough, laryngitis, dyspnea, or wheezing). There was significantly more severe heartburn in group III (1.9 ± 1.6) compared to group II (0.9 ± 1.3, $P < 0.05$). There was also more distal esophageal acid exposure in group III (5.9% ± 6.4) than in group II (3.3% ± 4.8, $P <$

Table 2. Comparison of 76 symptomatic patients and 11 asymptomatic volunteers

	Patients	Controls	*P* value
% Acid exposure	4.0 ± 6.0	1.3 ± 0.8	<0.01
PR	3.4 ± 6.4	0.2 ± 0.4	<0.01
RFS	9.6 ± 4.7	2.1 ± 1.4	<0.01
LES	17.2 ± 10.1	18.6 ± 6.2	0.65
UES	86 ± 56	71 ± 31	0.65
Peristalsis	96%	100%	0.27
Distal esophageal amplitude	98 ± 40	86 ± 37	0.41

% Acid exposure = the percentage of time the distal probe detected a pH <4 in 24-hours; PR = pharyngeal reflux episodes; RFS = reflux finding score; LES = resting lower esophageal sphincter pressure (mm Hg); UES = resting upper esophageal sphincter pressure (mm Hg); peristalsis = percentage of propagated peristalsis in response to wet swallows; distal esophageal amplitude = amplitude in mm Hg of peristaltic contractions in the smooth muscle portion of the esophagus.
Values expressed as mean (± SD).

0.05). The difference in acid exposure between groups I and III was not statistically significant ($P = 0.13$). In 83% of the patients in group III, but in only 44% of those in group I, respiratory symptoms disappeared after institution of medical or surgical antireflux therapy.

DISCUSSION

The goal of our study was to determine the value of laryngoscopy using a predetermined score and the value of pharyngeal pH monitoring using criteria previously described by us,[3,4] in the identification of reflux as a cause of symptoms in patients with laryngeal manifestations. The use of a laryngoscopic scoring system and PR monitoring are promising modalities for identifying patients with gastroesophageal-laryngeal reflux, but they are pretty much in an embryonic stage of their development and their usefulness must still be validated. Furthermore, whether normal subjects do reflux gastric contents to their pharynx and to what extent is unclear. Although Belfasky et al.[6] have shown that the RFS is sensitive to medical therapy (i.e., gets lower as the patient gets better), no one has previously used the score in normal asymptomatic subjects. Thus we determined the RFS in normal subjects and in a large group of patients with laryngeal symptoms. We also evaluated them all with pharyngeal pH monitoring.

Table 3. Patient symptom frequency scores and esophageal acid exposure for the most common symptoms

	Group I (n = 15)	Group II (n = 40)	Group III (n = 21)
Heartburn	1.3 ±1.4	0.9 ±1.3*	1.8 ±1.6*
Hoarseness	2.3 ±1.3	2.9 ±1.5	2.6 ±1.6
Cough	1.5 ±1.9	2.2 ±1.7	2.1 ±1.9
Laryngitis	1.1 ±1.4	1.1 ±1.6	1.0 ±1.4
Wheezing	1.1 ±1.8	1.0 ±1.6	0.4 ±1.2
% Acid exposure (distal channel)	3.1 (±7.9)	3.3 (±4.8)*	5.9 (±6.4)*

Values expressed as mean frequency score (± SD). Group I = RFS−/PR−; group II = RFS−/PR+ or RFS+/PR−; and group III = RFS+/PR−. Frequency scores: 0 = never; 1 = once/mo; 2 = once/wk; 3 = once/day; 4 = more than once/day.
*$P < 0.05$ group II vs. group III.

Normal (Control) Subjects

There is disagreement about how much, if any, PR is normal.[8–10] We attempted to define "normal" by performing laryngoscopy and pH studies on 11 asymptomatic volunteers. Our results suggest that one episode in a 24-hour period may be in the normal range.

Although Belfasky et al.[6] have shown a response of the RFS to medical therapy, the normal range of the RFS has not been validated in asymptomatic subjects. Based on the original definition of Belfasky et al.,[6] we consider an RFS above 7 as abnormal. Our evaluation of asymptomatic subjects would support the notion that the upper limit of normal is no higher than this, as no one had a score greater than 4. It may be that the true upper limit of normal is less than 7, but the threshold for attributing the laryngoscopic findings to reflux may be different, and therefore higher, than the true upper limit of normal.

As a group, the measurement of PR differentiated the symptomatic patients from the asymptomatic volunteers, because they had significantly more episodes of PR. Furthermore, our study suggests that PR+ patients and PR−patients are quite different. Indeed, whereas PR+ patients had an average of seven episodes (median of 6), PR− patients had a

median of zero. The large spread in the amount of PR episodes in the PR+ and PR− patients makes the differentiation easy, and perhaps more meaningful from a clinical standpoint. It is also evidence that the PR− and PR+ groups are different patients, and not just separated by an arbitrary definition of normal reflux.

In a similar way laryngoscopy, using the RFS, differentiated control subjects from patients. The definition of RFS− and RFS+ is more vague than for pharyngeal pH monitoring, because most control subjects have some finding on laryngoscopy. The cutoff of an RFS of 7 seemed reasonable to us, since as in PR, there was a large gap between the values obtained in normal individuals and in patients, and furthermore there was a significant gap between RFS− and RFS+ patients. If this method of laryngeal evaluation could reliably differentiate these patients, it should add to the diagnostic yield of pharyngeal pH monitoring. For this reason we sought to compare PR with laryngoscopic findings using the RFS.

Laryngoscopy and Pharyngeal pH in Patients With Respiratory Symptoms

When laryngoscopy and pharyngeal pH are used in clinical practice, three scenarios arise from their results. These scenarios were the basis for our three groups. The first possible scenario occurs when there is no evidence of reflux-induced injury by laryngoscopy and no PR (group I). This scenario represents a low likelihood of gastroesophageal-laryngeal reflux, and another cause for the patient's symptoms should be sought. The second scenario occurs when there is discordance between laryngoscopic and pharyngeal pH findings (group II). In our patients this was the most common scenario, raising the question of which test should we believe? The third possible scenario arises when both the RFS and PR are positive (group III). This group is likely to have gastroesophageal-laryngeal reflux and is the most likely one to respond to treatment. In fact, in our study, these patients responded to treatment with the same frequency as we see in patients with typical symptoms of reflux.

Without a gold standard for confirmation, the evidence for validating PR and RFS must be indirect. We know that the measurement of distal esophageal pH exposure identifies patients with GERD.[11] The pathophysiology of gastroesophageal reflux and gastroesophageal-laryngeal reflux are related. Therefore we used "classic" GERD characteristics to compare these groups. Heartburn in group III was significantly more severe than in group II, as was the average distal acid exposure. Thus group III had two pieces of indirect evidence that strongly suggested a higher likelihood of these patients having reflux-induced respiratory symptoms. It should be kept in mind that distal acid exposure in these patients is determined by a catheter that is based on the upper esophageal sphincter, so that the distal sensor's relation to the lower esophageal sphincter is variable. Thus, by design, the distal acid exposure under-represents the amount of reflux into the esophagus. Further evidence for the effectiveness of laryngoscopy and pharyngeal pH monitoring, when used together, is that nearly twice as many patients improved with antireflux therapy among those with abnormal PR and RFS. This therapy was almost exclusively medical in the form of a proton pump inhibitor administered twice a day (brand was at the discretion of the treating physician). Two patients in each group underwent antireflux surgery.

An explanation for the complementary nature of laryngoscopy and pharyngeal pH monitoring in these patients is that each is measuring a different component of the disease process. The strength of laryngoscopy may be its ability to detect injury. Because the etiology of laryngeal injury can be multifactorial, laryngoscopy has traditionally been relatively nonspecific in linking reflux and airway symptoms. The RFS quantifies the injury, thus identifying patients whose respiratory symptoms are more likely due to a caustic agent such as acid. Pharyngeal pH monitoring, by detecting acid exposure near the airway, gives supportive evidence that the injury detected on laryngoscopy is caused by reflux. By themselves PR and RFS support or help refute the relationship between reflux and the patient's respiratory symptoms. Together, however, they make a stronger argument for causation.

Our study did not allow us to answer the question of what is the meaning of one positive test (RFS or PR). In other words, do these patients have gastroesophageal-laryngeal reflux that warrants treatment? We postulate that RFS+ and PR− patients have laryngeal injury from a source other than reflux. Of course it is possible that pharyngeal pH monitoring, as we practice it, may not be able to detect some patients who have reflux into the pharynx. Indeed, we discard prandial times and we discard isolated episodes of drop of pH in the pharynx without concomitant drops in the rest of the esophagus. Thus our way of measuring PR may be underestimating the problem. Our previous study on the subject suggested that including all episodes was not practical because almost all subjects have drops in pharyngeal pH during and immediately after meals.[3] Thus this may be a true limitation in the diagnostic ability of pharyngeal pH measurements to assess the problem. Another theory is that distal esophageal reflux may stimulate laryngeal spasm or cough through vagally

mediated reflexes, leading to laryngeal lesions and symptoms.[12] This may also explain why some of the patients in group I responded to therapy. These studies increase the diagnostic yield, but are not 100% sensitive or specific. Moreover, the treatment of these patients was not controlled, and therefore most patients received other treatments such as voice training and education, which are often effective and may explain the improvement in many of these patients. We postulate that patients who are PR+ and RFS− have acid that reaches the pharynx but does not spill over into the larynx, so as to cause laryngeal injury.

CONCLUSION

The RFS and pharyngeal pH monitoring can distinguish normal subjects from patients with significant gastroesophageal-laryngeal reflux. Therefore, laryngoscopy and pharyngeal pH monitoring appear to be able to accurately rule out gastroesophageal-laryngeal reflux when both studies are normal. There is evidence that when both studies are abnormal, gastroesophageal-laryngeal reflux is the likely etiology of a patient's respiratory symptoms. Laryngoscopy and pharyngeal pH monitoring should be considered complementary studies in establishing the diagnosis of gastroesophageal-laryngeal reflux.

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Malabsorptive Gastric Bypass in Patients With Superobesity

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Weight loss in superobese patients has been problematic after conventional gastric restrictive operations including conventional Roux-en-Y gastric bypass (RYGB). The goal of the present study was to compare weight loss in patients with superobesity (body mass index ≥50 kg/m^2) using a distal RYGB (D-RY) in which the Roux-en-Y anastomosis was performed 75 cm proximal to the ileocecal junction (N = 47) vs. patients who had Roux limbs of 150 cm (N = 152) and 50 to 75 cm (N = 99). All operations incorporated the same gastric restrictive parameters. Minimum follow-up was 3 years and ranged to 16 years. Weight loss and reduction in body mass index were significantly greater after D-RY vs. both RYGB-150 cm and short RYGB and in RYGB-150 cm vs. short RYGB through 5 years. Mean percentage of excess weight loss peaked at 64% after DRY, at 61% after RYGB-150 cm, and at 56% after short RYGB. Weight loss maintenance through 5 years was correlated with Roux limb length with D-RY greater than RYGB-150 cm greater than short RYGB. More than 95% of obesity-related comorbid conditions improved or resolved with weight loss. There was no difference in the early postoperative morbidity rates: 9% after D-RY; 8% after RYGB-150 cm; and 2% after short RYGB with one death (0.3%). All D-RY patients had at least one postoperative metabolic abnormality. Anemia was significantly more common after D-RY vs. the shorter RYGB with no difference in the incidence of metabolic sequelae between RYGB-150 cm and short RYGB. No operations were reversed or modified for nutritional complications. Two D-RY patients required total parenteral nutrition for protein malnutrition. These results show that Roux limb length is correlated with weight loss in superobese patients. However, the greater incidence of metabolic sequelae after D-RY vs. RYGB-150 cm calls into question its routine use in superobese patients undergoing bariatric surgery. We conclude that some degree of malabsorption should be incorporated into bariatric operations performed in superobese patients to achieve satisfactory long-term weight loss. (J GASTROINTEST SURG 2002;6:195–205.)

KEY WORDS: Obesity, gastrointestinal surgery, nutrition, vitamins, weight loss

Mason et al.[1] introduced the concept of superobesity in 1987 when they recognized that the heaviest patients often failed to achieve satisfactory weight loss after vertical banded gastroplasty (VBG). In that report, Mason et al.[2] arbitrarily defined superobesity as ≥225% above ideal weight. At about the same time we arbitrarily defined superobesity as ≥200 pounds above ideal weight. Our definition was chosen to reflect a minimum weight limit that corresponded to twice the minimum weight criterion (≥100 pounds overweight) that was used to define morbid obesity. Subsequent reports of weight loss after gastric bariatric operations have defined superobesity as a body mass index (BMI) ≥50 kg/m^2.[3] However, superobesity is not "officially" recognized as a weight category by classification systems that are designed to stratify various treatment regimens.[4,5]

There are several justifications for making a distinction between morbid obesity and superobesity. First, the incidence of coexisting medical problems is substantially greater in superobese patients, which implies a greater overall health risk.[6] Second, a number of bariatric surgeons have independently reported that the likelihood of successful weight loss in

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superobese patients is substantially lower than in less obese patients after conventional gastric restrictive operations.[1,6–9] It seems obvious that the heaviest patients must lose more weight to achieve a level that would represent a valid reduction in their actuarial mortality risk.

In a 1992 prospective randomized study, we reported that Roux limb length had a significant impact on weight loss in superobese patients after Roux-en-Y gastric bypass (RYGB).[6] In that report we showed that a 150 cm Roux limb improved weight loss without increasing the postoperative complication rate. However, recidivism was common after 4 or 5 years in the longer-limb group. This observation led us to study a more malabsorptive modification of RYGB in which the enteroenterostomy was performed 75 cm proximal to the ileocecal junction. This modification was prospectively evaluated over a 10-year period. The results of this malabsorptive modification of RYGB were then retrospectively compared with findings in superobese patients who underwent RYGB using two shorter Roux limb lengths.

MATERIAL AND METHODS

RYGB has been the preferred operation for treatment of morbid obesity at our medical center since June 1983. During the ensuing 16 years, 662 RYGB operations were performed at Robert Wood Johnson University Hospital including 298 in superobese patients. Three Roux limb lengths were evaluated during this interval. The short-limb procedures were generally performed before 1990, whereas most of the 150 cm operations were performed after the conclusion of our prospective randomized study. The first distal malabsorptive RYGB (D-RY) was performed in 1988. All patients in the present report were at least 200 pounds or more overweight preoperatively, and all but one had a BMI of $\geq$50 kg/m^2. This report includes patients from two sequential clinical studies. The first (previously reported) prospectively compared a 150 cm Roux limb with a 75 cm Roux limb.[6] Evaluation of each of the two longer-limb modifications was approved by the institutional review board at our medical center. The RYGB-150 cm protocol was active between 1984 and 1990. The D-RY protocol was conducted from 1988 through 1997. The present report is focused on the distal malabsorptive procedure (D-RY), which was not randomized with another procedure. Twelve patients declined to undergo D-RY during the study interval and received RYGB using a 150 cm Roux limb.

All patients were evaluated preoperatively by the surgeon and a clinical dietitian (H.A.K. or L.B.L). Weight, blood pressure, and pulse rate were recorded at each visit. A brief medical history was obtained by the surgeon, who then reviewed the clinical protocol for the longer Roux limb modifications with prospective patients. Patients who were interested in having the D-RY modification were mailed a consent form, which was reviewed with the patient a second time before hospital admission.

All operations were performed by one surgeon using a technique described in detail elsewhere.[10] The gastric restrictive parameters were the same for all patients. All measurements of the small bowel were performed on a stretch along the antimesenteric border. The length of the afferent (bypassed) jejunum ranged from 15 to 25 cm in all patients. The length of the Roux limb ranged from 50 cm to 75 cm in the short-limb group. In the 150 cm group Roux limb length was constant, whereas the length of bowel below the jejunojejunostomy was not measured. Conversely, in D-RY patients the common channel measurement below the jejunoileostomy was constant at 75 cm proximal to the ileocecal junction with Roux limb measurements ranging from 265 cm to 570 cm. The D-RY technique is illustrated in Fig. 1.

Preoperative patient characteristics are shown in Table 1. There was no difference in mean age or sex distribution among the three groups. However, the mean weight and BMI of patients undergoing D-RY were significantly greater than that of patients in the two shorter Roux limb groups. The only change in protocol during the 15-year study period was directed at decreasing the total length of stay for uncomplicated hospitalizations. During the past 6 years, the nasogastric tube was removed in the morning of postoperative day 1, which was 24 hours sooner than in the previous 9 years. This resulted in patients beginning a liquid diet sooner and typically decreased the hospitalization by 1 day.

Patients followed a pureed diet for 4 weeks postoperatively and then were advanced to a solid diet consisting of a variety of soft foods.[11] Chewier solids were subsequently introduced as tolerated. A multivitamin supplement containing minerals was recommended for all patients postoperatively. After 1994, oral iron supplements containing at least 50 mg of elemental iron were recommended to all menstruating women. Follow-up after the 4-week visit was scheduled at 3-month intervals during the first year, 6-month intervals in the second year, and then annually for patients who were doing well and were free of major metabolic complications. Weight and blood pressure were recorded at each visit. Laboratory studies were performed at 6-month intervals during

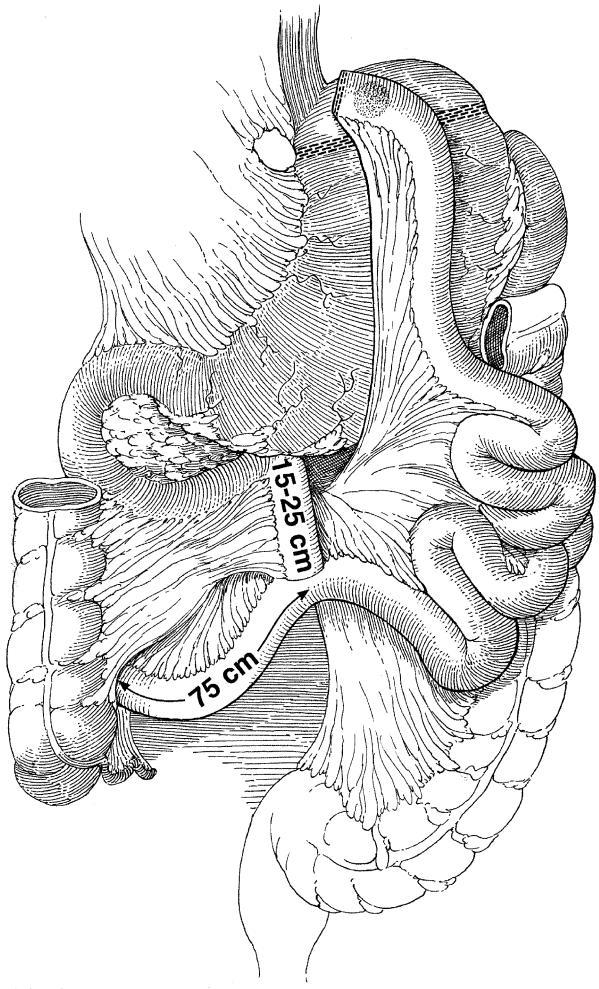

Fig. 1. Roux-en-Y gastric bypass in which the TA 90-B stapler (U.S. Surgical, Norwalk, CT) is fired across the cardia of the stomach creating a 20 ± 5 ml upper pouch. The jejunum is divided approximately 15 to 25 cm distal to the ligament of Treitz with the distal end anastomosed to the upper stomach using a circular stapler to create a 1.1 cm diameter anastomosis. The proximal end of the jejunum is then anastomosed 75 cm above the ileocecal junction.

the first 2 years, and annually thereafter. Hemoglobin and hematocrit and serum levels of iron/iron-binding capacity, vitamin B_{12}, and folate were measured in all patients. Serum electrolytes, albumin, total protein, calcium, bilirubin, SGOT, alkaline phosphatase, and vitamin A and vitamin D 25-OH levels were measured in patients who had undergone D-RY. Patients who developed metabolic deficiencies had their laboratory tests repeated at 4- to 6-month intervals until the deficiency was corrected.

Statistical analysis of data was performed using the chi-square test, Fisher's exact test, unpaired Student's *t* test, and analysis of variance (ANOVA) with Student–Newman-Keuls test.

RESULTS

The minimum follow-up evaluation period for this study was 3 years. Actual follow-up ranged from 6 months to 16 years. Mean follow-up was 60 ± 46 months in the short-limb group, 37 ± 34 months in the RYGB-150 cm patients, and 46 ± 25 months in the D-RY group. The follow-up rate at 3 years was 80% in the short-limb group, 68% in the RYGB-150 cm patients, and 85% in the D-RY patients. At 5 years the follow-up rate was 66% of 88 available patients in the short-limb group, 64% of 56 available patients in the RYGB-150 cm group, and 78% of 22 available D-RY patients.

Weight Loss

Fig. 2 shows postoperative weight loss expressed in pounds and as a reduction in BMI. Weight loss usually stabilized between 12 and 18 months after the shorter Roux limb length procedures. Weight loss after D-RY tended to last longer, with many patients reaching their nadir between 24 and 36 months postoperatively. There were significant differences in weight loss among the three groups favoring the longer Roux limb lengths. These differences were apparent as early as 6 months postoperatively between D-RY and the shorter Roux limb lengths. The significantly greater weight loss provided by D-RY persisted through 5 years. Significant differences in weight loss between the short-limb RYGB and RYGB-150 cm groups were apparent at 12 months postoperatively and persisted beyond that point. Some recidivism after 3 years was noted in all groups with the greatest weight regain observed in the short Roux limb group.

Reductions in BMI were generally parallel with the weight loss results, with significantly greater reductions observed after D-RY compared to the shorter Roux limb lengths, as shown in Fig. 3. Significant differences in BMI between the short-limb RYGB and RYGB-150 cm groups were noted at 12 months and persisted through 3 years postoperatively. Differences in BMI units lost between D-RY and the shorter Roux limb lengths were significant at 6 months postoperatively and persisted through 5 years. The lowest mean BMI in the short Roux limb group was noted at 18 months postoperatively, whereas the lowest mean BMI observed after the RYGB-150 cm and D-RY procedures was observed at 2 and 3 years, respectively.

The greatest mean percentage of excess weight loss in the short-limb patients was 56% at 18 months postoperatively as compared with 61% at 24 months postoperatively in the 150 cm group and 64% at 3

Table 1. Preoperative patient characteristics

Operative group	Weight (pounds)	BMI (kg/m^2)	Age (yr)	Sex (M/F)
Short limb	331 ± 54	56.9 ± 7	38.4 ± 10	17:82
150 cm	356 ± 57	55.3 ± 7	39.4 ± 9	35:117
D-RY	*448 ± 76	*67.5 ± 8	37.6 ± 9	20:27

Data expressed as mean ± SD.
*Indicates significant difference between D-RY patients vs. the other two groups ($P < 0.05$ by ANOVA with Student–Neuman-Keuls test).

years in the D-RY patients. The number of patients who achieved ≥50% loss of excess weight at some point during the study was 65 (65%) in the short-limb group, 114 (76%) in the RYGB-150 cm group, and 36 (80%) in the D-RY group. However, mean percentage of excess weight loss declined to 45% by 5 years after short-limb RYGB and to 51% at 5 years after RYGB-150 cm. There was a lesser decline in mean excess weight loss after D-RY with maintenance in the range of 60% through 5 years postoperatively. However, the difference in mean excess weight loss at 5 years between the RYGB-150 cm and D-RY groups was not significant.

Improvement of Comorbidity

Tables 2 and 3 show the comorbid conditions related to severe obesity in the study cohort. Only 35 patients (12%) in this study did not have at least one comorbid condition; all but two of these subjects were under age 40. There were significant differences in the incidence of comorbidity among the three operative groups, with sleep apnea, cardiac disease, and venous stasis more prevalent in D-RY patients. Objective improvement in blood pressure, diabetes, and serum lipids is easily measured. Conversely, improvement in cardiac symptoms (angina, congestive failure) and weight-bearing joint pain was largely subjective. Improvement in asthma, sleep apnea, and venous stasis was assessed by a combination of improved symptoms and objective parameters including reductions in medication, positive-pressure breathing aids/polysomnography, and ankle swelling. Although improvement of comorbidity was generally consistent with weight loss results, many patients who did not lose 50% of their excess weight had notable improvement in comorbidity.

Complications

Perioperative complications for the three groups are shown in Table 4. There were minimal differences in the incidence of early complications among the three procedures. The overall incidence of early complications was 6.4%. The leaks included three acute disruptions of the stapled gastric partition caused by violent retching within the first several days postoperatively and one esophageal perforation. The two cases of bowel obstruction were treated successfully by tube decompression. The dehiscences included two in the midline fascial closure and one major skin level disruption. There were two small bowel fistulas related to erosion of polypropylene mesh used in prior hernia repairs and one jejunal perforation, which occurred at a stay suture site at 3 weeks postoperatively. The one perioperative death occurred in a 50-year-old woman who weighed 354 pounds and had a BMI of 65.0 kg/m^2; this patient had an uncomplicated 5-day postoperative course. She was readmitted 12 days later with pulmonary embolism and died 2 days after that admission of cardiac arrest secondary to another embolus.

Late complications (after 30 days postoperatively) are shown in Table 5. There was a similar incidence of specific complications among the three groups except for marginal ulcers, which was more common in D-RY patients. However, the difference in the incidence of marginal ulcers was not significant. All patients with stomal stenosis were hospitalized for intractable postprandial emesis. Patients with

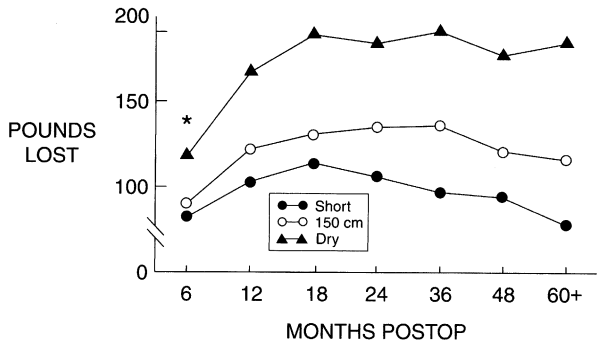

Fig. 2. Weight loss in pounds through 5 years postoperatively. There were significant differences between each of the three groups at ≥1 year postoperatively. *Significant difference between the short-limb groups vs. D-RY patients at 6 months postoperatively ($P < 0.05$ by analysis of variance with Student–Newman-Keuls test.)

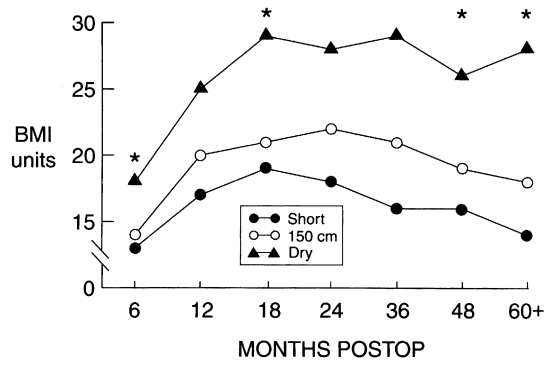

Fig. 3. Change in body mass index (BMI) through 5 years postoperatively. There were significant differences between each of the groups at 12, 24, and 36 months postoperatively. *Significant difference between the D-RY patients compared to the shorter-limb groups noted at 6, 18, 48, and 60 months ($P < 0.05$ by analysis of variance with Student–Newman-Keuls test).

stomal stenosis who subsequently were found to have ulcers were included in the ulcer category. Diarrhea, defined as three or more loose stools per day, was noted in 17 patients (36%) after D-RY and in one patient in the 150 cm group, and did not occur in any of the short-limb patients. The quality of loose stools in the D-RY patients was consistent with steatorrhea.

There were five late deaths including three in the short-limb group; a woman, aged 67 years, died 15 years after surgery of complications following open heart surgery; a second woman, aged 57 years, died 8 years after surgery of complications of lupus, and a 49-year-old man died 5 years after surgery of cirrhosis and congestive heart failure. The one death in the RYGB-150 cm group occurred in a 63-year-old man at 15 years postoperatively as a result of a stroke. A 33-year-old man with preexisting cirrhosis died of hepatic failure 9 months after D-RY.

There were 25 revision operations in this series of 298 patients (8.7%), including 12 in the short-limb group (12%), 11 of which were performed for unsatisfactory weight loss and one for a marginal ulcer. The 13 revision procedures in the RYGB-150 cm group (8.5%) included seven for unsatisfactory weight loss and six for nonhealing marginal ulcers. There were no reoperations for unsatisfactory weight loss or nutritional complications in D-RY patients. However, one D-RY patient (2.1%) required reoperation for an intractable marginal ulcer. Nine of the patients who required reoperation (35%) had disruption of the stapled gastric partition, including six of nine patients who had intractable marginal ulcers. In addition, 35 patients in this series (12%) required repair of incisional hernias and 14 subsequently had cholecystectomy for symptomatic gallstones.

The incidence of metabolic sequelae in patients followed for 2 years or more is shown in Table 6. All D-RY patients followed for at least 2 years (N = 39) had at least one metabolic abnormality. The incidence of anemia in D-RY patients was significantly greater than in the short-limb groups. Surprisingly, the incidence of vitamin B_{12} deficiency in D-RY patients was significantly lower in comparison with the shorter-limb groups. Two of the five patients with low protein/albumin levels were hospitalized so they could receive parenteral nutritional support. Both had troublesome postprandial vomiting in addition to rapid weight loss. One of these two patients had stigmata of zinc deficiency. Measurements of serum albumin, total protein, and fat-soluble vitamins A and D were not routinely performed in short-limb and 150 cm limb patients. However, protein/albumin levels were routinely measured before other subsequent operations, for example, hernia repair or abdominoplasty, and occasionally by the primary care physician. No deficiencies in protein- or fat-soluble vitamins were noted in the shorter-limb groups.

Table 2. Medical comorbidity

	Short (N = 99)	150 cm (N = 152)	D-RY (N = 47)
Hypertension	61 (62%)	78 (51%)	32 (68%)
Arthritis	45 (45%)	53 (35%)	20 (43%)
Asthmia	22 (22%)	25 (16%)	6 (13%)
Hyperlipidemia	18 (18%)	29 (19%)	6 (13%)
Diabetes	9 (9%)	31 (21%)	8 (17%)
Cardiac disease*	13 (13%)	15 (10%)	12 (26%)
Sleep apnea*	5 (5%)	30 (20%)	22 (47%)
Venous stasis*	12 (12%)	12 (8%)	15 (32%)

The number and percentage of patients with each comorbid condition are listed for each group.
*Indicates significantly greater prevalence of a comorbidity in distal Roux patients compared to the other groups ($P < 0.02$ by chi-square test).

Table 3. Improvement of comorbidity

	Resolved	Improved	Unchanged
Hypertension	74	56	5
Arthritis	4	81	4
Asthma	6	31	2
Hyperlipidemia	22	15	3
Diabetes	32	6	—
Cardiac disease	7	26	1
Sleep apnea	21	26	1
Venous stasis	5	20	3
TOTAL	167 (38%)	261 (58%)	19 (4%)

Improvement in comorbid conditions analyzed at 3 years postoperatively. Comorbidity was considered resolved after normalization without the need for medication and improved when controlled by lower dosages of medication than required preoperatively.

DISCUSSION

Combining Gastric Restriction with Malabsorption

Because of the high rate of recidivism with gastric restrictive operations, the pendulum in American bariatric surgery has clearly swung away from pure restriction toward malabsorption. Malabsorptive modifications of RYGB, Scopinaro's biliopancreatic bypass (BPB), and the so-called duodenal switch are now being performed in large numbers in the United States. Weight loss after BPB and the duodenal switch is primarily due to intestinal malabsorption, Both techniques include transection of the small intestine near its midpoint and exclusion of nearly 50% of the small bowel from the functional digestive tract.[12,13] In BPB the proximal ileum is joined to a 200 to 500 cm capacity gastric remnant after resection of the distal stomach. In the duodenal switch, the duodenum is transected approximately 5 cm beyond the pylorus, and approximately two thirds of the stomach is excised along the greater curvature.

Because conventional RYGB does not provide sufficient long-term weight loss in superobese patients, it seemed logical to add malabsorption to an operation that was primarily oriented toward restricting caloric intake. It also seemed reasonable that the "passive" malabsorptive component might limit later weight regain and improve weight loss maintenance. We were enamored by the superlative weight maintenance in Scopinaro's series of BPB patients who were followed for 17 years.[14] The modifications of RYGB used in the present study differ substantially from the BPB and the duodenal switch in that only 15 to 25 cm of small bowel is totally excluded from digestive continuity. The technique used in D-RY was designed to induce greater malabsorption of dietary fat without producing the manifestations of protein malabsorption or clinically overt malnutrition that have been associated with BPB.

Sugerman et al.[15] were the first to prospectively compare a small 30 ml pouch BPB with a conventional RYGB in superobese patients. Sugerman's small-pouch BPB patients had a greater than 50% incidence of serious postoperative complications and metabolic sequelae, including two deaths resulting from hepatic failure. Although weight loss at 1 year was significantly greater after the small-pouch BPB, in comparison with conventional RYGB, Sugerman et al.[15] concluded that the incidence and severity of metabolic complications after their modification BPB, which included a 50 cm common channel, was too great to justify its use as a primary operation for treatment of patients with superobesity. In the same report Sugerman et al.[15] described a series of 22 superobese patients who, after failing conventional RYGB, had a distal modification of gastric bypass, which incorporated a 150 cm common channel and a 30 ml capacity gastric pouch. This "distal" RYGB more closely resembles BPB and the duodenal switch than our D-RY in that at least 50% of the small bowel is totally excluded from the functional digestive tract. Four (18%) of these 22 patients had subsequent surgical procedures (3 reductions of bypassed

Table 4. Early complications

Complication	Short (N = 99)	150 cm (N = 152)	D-RY (N = 47)
Wound infection	—	1	2
Leaks	1	2	1
Pulmonary embolism	—	3*	—
Small bowel obstruction	—	2	—
Gastrointestinal bleeding	—	1	—
Wound dehiscence	1	1	1
Bowel fistula	—	3	—
TOTAL	2 (2%)	13 (8%)	4 (9%)

*The one death in this series was caused by pulmonary embolism.

Table 5. Late complications

Complication	Short (N = 99)	150 cm (N = 152)	D-RY (N = 47)
Incisional hernia	14 (14%)	20 (13%)	7 (15%)
Marginal ulcer	4 (4%)	8 (5%)	5 (11%)
Staple disruption	2 (2%)	6 (4%)	2 (4%)
Small bowel obstruction	1 (2%)	6 (4%)	—
Stomal stenosis	2 (2%)	2 (1%)	1 (2%)
Liver failure	—	—	1 (2%)
TOTAL*	22 (22%)	41 (27%)	15 (32%)

*The ulcers associated with staple disruption were combined as one rather than two separate complications in calculating the overall incidence of late complications in each group.

bowel, 1 tube gastrostomy) to correct protein-calorie malnutrition. Mean percentage of excess weight loss in these patients was 67% at 3 years and 69% at 5 years. There was a high incidence of metabolic sequelae despite aggressive prophylaxis with multivitamins, calcium, and additional supplements of vitamins A, D, E, and B_{12}.

The Mayo clinic group was the first to publish a series using a very, very long Roux limb in 26 superobese patients.[16] This procedure was compared with the results in 11 patients who underwent the Scopinaro BPB. Weight loss in the very, very long–limb group was less than with BPB at 2 years postoperatively (57% vs. 68% mean excess weight loss) with a comparable early morbidity rate. Unfortunately, the incidence of late metabolic sequelae was not stated in the Mayo series, although one patient who underwent BPB subsequently died of liver failure.

Further support for the notion that a combination of restriction and malabsorption is more potent than either mechanism alone was provided by Scopinaro et al.,[12] when they modified their original BPB by reducing gastric capacity from 500 ml to 200 ml in superobese patients (the so-called very little stomach modification) to improve weight loss. Yet the precise mechanisms by which malabsorptive bariatric gastric bypass procedures produce weight loss and, more important, the metabolic side effects are grossly understudied. Remarkably, there are no clinical studies that have carefully measured absorption of macronutrients after any technique of RYGB. We previously showed that weight loss maintenance after RYGB is related to postoperative caloric intake in that recidivism generally occurred when calorie intake exceeded 1500 calories per day in women and 1800 calories per day in men.[6] Patients who restricted their intake of high-calorie liquids and soft junk food invariably maintained their weight loss. Because mean calorie intake was consistently greater in RYGB-150 cm compared to the short-limb patients, the superior weight loss in the 150 cm group could be attributed to greater fat malabsorption resulting from more distal diversion of bile and pancreatic secretions in the functional digestive tract. The role of inadequate oral intake in development of severe nutritional complications after combined restrictive-malabsorptive bariatric operations is acknowledged but poorly documented. Clearly, adding malabsorption to gastric restriction carries an increased metabolic price.

Risk-Benefit Analysis

To account for recidivism, a valid analysis of any obesity operation probably requires postoperative follow-up of at least 5 years. Postoperative follow-up becomes an increasingly time-consuming endeavor with steady growth of a bariatric surgical practice. In the United States, long-term follow-up seems to be more problematic than in Canada and some Euro-

Table 6. Postoperative metabolic deficiencies

Operation	Iron	B_{12}	Anemia	Vitamin A	Vitamin D	Calcium	Albumin
Short (N = 80)	42 (52%)	30 (37%)	33 (41%)	—	—	—	—
150 cm (N = 102)	46 (45%)	34 (33%)	36 (35%)	—	—	—	—
D-RY (N = 39)	19 (49%)	3 (8%)*	29 (74%)	4 (10%)	20 (51%)	4 (10%)	5 (13%)

N = number of patients followed with numbers/percentages corresponding to the incidence of deficiency below each nutrient. Dashes indicate that nutrients were not measured in the shorter limb groups.
*Significant difference between D-RY patients and the other two groups ($P < 0.003$ by χ^2 test).

pean centers, which have nationalized registries of patient names and addresses. Patients move out of the area without informing their surgeons or other physicians. At our medical center, cost cannot be considered a primary reason for lack of follow-up because there has never been a professional fee for follow-up visits, and the cost of postoperative blood tests is virtually always covered by third-party payors. In the present study the great majority of "lost" patients flatly refused further follow-up, ignoring repeated phone calls and postcard requests. Although patients who regain weight may be embarrassed to return, there are no objective data that show weight loss failure as the primary reason for lack of follow-up. We believe that the 78% and 70% follow-up rates at 3 and 5 years, respectively, in the present study are probably better than follow-up rates at those intervals in most busy bariatric surgical practices. Maintaining adequate long-term follow-up is the most vexing issue in bariatric surgery today.

Although the 6.4% early complication rate in the present study is similar to the incidence of perioperative complications reported by other surgeons in unselected series of gastric bypass patients,[2,3,7] the early complication rate in the present series of superobese patients is more than twice the morbidity rate previously reported in our lighter bariatric surgical patients.[11] The 30% to 40% incidence of postoperative vitamin and mineral deficiencies after the two shorter limb operations in this study is also similar to the overall incidence of these deficiencies previously reported in patients after conventional RYGB with similar lengths of follow-up.[17–19] There was no difference in the incidence of postoperative metabolic deficiencies between short-limb bypass and RYGB-150 cm patients. All of the postoperative vitamin and mineral deficiencies in these two groups of patients were managed on an outpatient basis. The great majority of metabolic sequelae in D-RY patients were also managed in the outpatient setting. However, two D-RY patients required several weeks of inpatient parenteral nutritional support. Patient noncompliance in regularly taking multivitamin supplements and in returning for follow-up blood tests contributed to the development and progression of many of these deficiencies. Although the incidence of most metabolic sequelae after our D-RY is similar to that reported after BPB and the duodenal switch, the incidence and severity of protein-calorie malnutrition appear to be lower after D-RY.

Isolated cases of fatal hepatic failure are reported after various malabsorptive bariatric procedures. Marceau et al.[20] reported 11 cases of subsequently *improved* hepatic histopathology in patients with cirrhosis who had the duodenal switch. Hence the death from hepatic failure after D-RY was unexpected, even in the setting of preexisting cirrhosis. On the basis of our negative experience, we will not perform D-RY in a patient with cirrhosis. The virtual certainty of developing at least one deficiency coupled with the potential for developing diarrhea and protein-calorie malnutrition strongly suggest that D-RY should not be offered to patients who are not committed to long-term follow-up.

Defining "successful" outcome after weight loss surgery is a complex issue. Loss of 50% of the excess weight has been used for many years as a minimum criterion to define successful weight loss after bariatric surgery.[7,21] The 1991 National Institutes of Health Consensus Development Panel on Gastrointestinal Surgery for Severe Obesity recommended use of the BMI rather than a quantity of percentage of overweight in both preoperative patient selection and in reporting postoperative results.[22] Analysis of weight loss in the present study is complicated by the significant difference in preoperative weight/BMI between the D-RY group and the shorter-limb patients. This difference somewhat nullifies the greater weight loss observed after D-RY. However, the significant difference in both weight and BMI units lost between the short-limb and RYGB-150 cm patients provides solid evidence that adding malabsorption to restriction in the form of a longer Roux limb length results in superior long-term weight loss in superobese patients. The rate of recidivism in both the short-limb and 150 cm limb patients was similar after 3 years. Conversely, recidivism was less after D-RY, suggesting that more malabsorption provides better weight loss maintenance in the long term.

The mean BMI at the point of maximum weight loss among the superobese patients in the present study was 37.3 kg/m^2 in short-limb patients, 35.8 kg/m^2 in RYGB-150 cm patients, and 38.6 kg/m^2 in D-RY patients. Calculated BMI for patients who are approximately 50% overweight falls in the range of 34 to 36.5kg/m^2. The mean BMI of 42.0 kg/m^2 recorded in short-limb patients at 5 years postoperatively corresponds to approximately 100% overweight and exceeds the BMI limit of 40kg/m^2 that was established by the 1991 NIH Consensus Development Panel as the minimum definition of morbid obesity. Only 51 (17%) of the 298 patients in the present series stabilized at a BMI $\leq$30 kg/m^2 (approximately 20% overweight) and only 15 (6%) reached a BMI of $\leq$25 kg/m^2 (normal weight) at the nadir of weight loss. These BMI data provoke the question of what are realistic and worthwhile weight loss goals for superobese patients after gastric bariatric operations. It is probably unrealistic to expect that most patients with a preoperative BMI exceed-

ing 50 kg/m^2 should reach a BMI of less than 30 kg/m^2 after weight stabilization.

Improvement of both coexisting medical problems and general life-style were dramatic among patients who lost substantial amounts of weight. The 96% incidence of improvement or resolution of medical problems in these patients was substantially greater than the incidence of successful weight loss outcome in that 28%, patients in this study did not lose at least 50% of their excess weight. Moreover, there was virtually no regression of improved or resolved medical problems in patients who maintained satisfactory weight loss for the duration of the study. Conversely, improvement or resolution of medical problems was limited or transient in some patients who either did not lose 50% of their excess weight or regained a substantial portion of their lost weight.

In summary, this study demonstrates that increasing the length of the Roux limb in RYGB produces significantly greater postoperative weight loss in superobese patients than conventional short-limb RYGB. The 150 cm Roux limb produced significantly better weight loss in comparison to short limb procedures, with a similar incidence of late metabolic sequelae. Protein-calorie malnutrition was not observed in the 150 cm group. Although the malabsorptive D-RY produced greater weight loss and better weight loss maintenance than the two shorter limb length procedures, the higher incidence of metabolic complications in D-RY patients coupled with the unpredictability in postoperative follow-up mitigate against its routine use for all superobese patients. We continue to offer D-RY in selected patients with a BMI $\geq$60 kg/m^2 who are committed to long-term follow-up.

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Discussion

Dr. John G. Kral (Brooklyn, NY): I want to make some comments rather than ask questions. The reason for these comments is that there are many newcomers who are doing laparoscopic procedures in the field of bariatric surgery who do not have the perspective that Dr. Brolin's group has. This represents extremely careful work done under adverse conditions by a single, dedicated, very competent group of investigators who followed a very difficult group of patients. The critical comments I have to make pertain to the field as such, and I think our Society can actually take a lead here.

The American College of Surgeons formulated requirements for performing this surgery in hospitals and by groups in a bulletin last year, but there is another level of standards that should be introduced. We are repeating the mistakes that were made back in the 1970s with the intestinal bypass. The biggest mistake then was to not recognize how important it was to provide competent, dedicated follow-up. The standards for how that follow-up must be performed need to be enunciated extremely clearly.

The dilemma with this entire field is that obesity is a disease of the poor; not only are more of the obese patients poor, but the poor are also more obese. It would seem logical that those persons who are most obese, as we learned today about the superobese, are the ones who should have the more aggressive, more effective operation. On the other hand, the poor, because of socioeconomic and educational factors, are the ones who are least able to afford the proper education and follow-up that is necessary to avoid the past mistakes with intestinal bypass. Vomiting and diarrhea, for example, are not necessary sequelae of any type of bariatric surgery. These patients need to be aggressively pursued to avoid many of the attendant complications.

Who is going to pay? It is my suggestion that public funding be made available to carry out appropriate studies of experimental surgery of this drastic nature in a group that really needs it. The cost-benefit analysis is fairly clear on one level, and that is reduction of comorbidity, as we have seen from Dr. Brolin's presentation. But it will require so much more in controlled series before we can reach a standard that we can all live with.

I must commend Dr. Brolin and make it clear to everyone here that this is about as good as it gets for a dedicated clinical investigator in this field.

Dr. R. Brolin: Thank you for your comments.

Dr. H. Sugerman (Richmond, VA): You suggest that when we convert from a proximal to a distal bypass for patients who have failed a proximal bypass, that we bypassed more of the intestine than we really did. When we moved the biliary limb down, it was a short biliary limb. We moved it down to various distances from the ileocecal valve, as close as 150 cm, which is double what you did, and yet we had a number of patients who developed profound protein-calorie malnutrition and required further revision to move the limb back up; our most recent patient was operated on a week ago. There is a surgeon in Richmond who is doing this routinely in all obese patients, including those who are not superobese, and he is seeing profound malnutrition in these patients referred to us.

So there is concern about the need, as Dr. Kral has pointed out, for careful follow-up of all these patients. You have a follow-up of only 60%. Our rate is even worse than that, and we do our very best to achieve follow-up. There has to be some form of insurance coverage to ensure that these patients return for follow-up. The malnutrition is a serious problem with these malabsorptive distal bypasses. Perhaps the duodenal switch procedure will produce less of it.

Dr. Brolin: Your operation and the one that I presented here are different. Your biliopancreatic limb is much longer and, consequently, I think that may explain some of the differences in terms of reoperation rates that have been observed between your experience and ours.

A more general comment has to do with the fact that the pendulum in bariatric surgery has swung back toward malabsorption because of the very poor long-term results observed with gastric restriction that Dr. Sarr's group presented to this Society last year. Unfortunately there are no controlled, head-to-head, randomized studies comparing malabsorptive operations, and that is what is desparately needed in this field.

Dr. H. Sax (Rochester, NY): Your BMI units were approximately 12 higher in your largest group, and one issue that could explain both your higher marginal ulcer rate and your lower B_{12} deficiency rate is higher acid secretion in patients with the proximal pouch. Sometimes in these really, really large patients, it is difficult to create a true 20 ml pouch, and I am wondering if you saw a correlation between those patients who had marginal ulceration and those who also were not B_{12} deficient, because the higher acid levels allowed them to absorb free B_{12} from their food?

Dr. Brolin: That is a good question. No correlation was observed. Again, the numbers are small, and I think that to address this question properly, a larger group of patients is needed. The higher incidence of marginal ulcers in the distal group I believe is of some concern, although it was not significant.

Dr. B. Wolfe (Sacramento, CA): There are two mechanisms whereby nutrient intake may be diminished in the patients with a distal Roux-en-Y bypass: first, nutrients passing more distally in the intestinal lumen are a satiety signal, and second, patients who are malabsorbing fat are stimulated to dramatically reduce their fat intake to control diarrhea. Do you have any data on the role of diminished nutrient intake in achieving greater weight loss in the patients with a distal Roux? In particular, was diminished nutrient intake a factor in the causation of the protein malnutrition that you observed in some of your patients, as Dr. Sugerman and others have observed in some of their patients?

Dr. Brolin: We do not have any good objective data across the board regarding nutrient intake in the distal bypass group. In the two patients who were hospitalized and given total parenteral nutrition, there is no doubt that inadequate oral intake played a role in the development of overt manifestations of malnutrition. This is a subject that has been debated in the field, and there are no good quantitative data available that address your questions. I think this is another area that requires serious investigation.

Dr. J. Starling (Madison, WI): Virtually all of my superobese patients at 2 years postoperatively request, and most receive, plastic surgery to remove a massive pannus and other redundant tissue. How does that weight loss, sometimes up to 20 to 40 pounds in my experience, factor into your weight reduction statistics?

Dr. Brolin: It did not in this group of patients. My experience is that a far smaller percentage of patients request skin reduction procedures, and even a smaller percentage than that actually go through with it. You would be surprised by the weight of these specimens relative to the appearance and size of the pannus that is removed and when they are put on a scale. I always win the "weight-guessing" bets in the operating room because I pick the lowest weight. Even though a lot of skin and fat is taken off, the amount that is removed usually is no more than 7 or 8 kg, as a rule.

Roux-en-Y Gastric Bypass After Previous Unsuccessful Gastric Restrictive Surgery

Agneta Westling, M.D., Ph.D., Màrgàretà Öhrvall, M.D., Ph.D., Sven Gustavsson, M.D., Ph.D.

In the treatment of morbid obesity, simple gastric restrictive methods such as silicone adjustable gastric banding, vertical banded gastroplasty, and nonadjustable gastric banding often fail to control weight in the long run or give rise to intolerable side effects. Here we review our results from conversion of such failures to Roux-en-Y gastric bypass. The study comprised 44 patients (median age 42 years, range 24 to 60 years) who underwent revision surgery in 1996 and 1997. Body mass index at revision was 35 kg/m^2 (range 21 to 49 kg/m^2). Previous bariatric procedures included silicone adjustable gastric banding (n = 26), vertical banded gastroplasty (n = 13), and gastric banding (n = 5). The most common reasons for conversion after silicone adjustable gastric banding and nonadjustable gastric banding were band erosion (n = 12) and esophagitis (n = 11). Staple line disruption (n = 12) with subsequent weight loss failure was the primary cause after vertical banded gastroplasty. There were no postoperative deaths or anastomotic leaks. One patient underwent reexploration because of an infected hematoma. Reflux symptoms and vomiting resolved promptly. At global assessment 2 years later, 70% of the patients were very satisfied. Median body mass index had decreased to 28 kg/m^2 (range 18 to 42 kg/m^2). No patient was lost to follow-up. As reported previously, failure after vertical gastric banding can be treated by conversion to Roux-en-Y gastric bypass with good results. In this study we found that failure after silicone adjustable gastric banding can be treated successfully with Roux-en-Y gastric bypass as well. (J GASTROINTEST SURG 2002;6:206–211.)

KEY WORDS: Band erosion, staple line disruption, revision bariatric surgery, Roux-en-Y gastric bypass

Simple gastric restrictive procedures such as silicone adjustable gastric banding (SAGB) and vertical banded gastroplasty (VBG) are very common operations in the treatment of morbid obesity.[1–4] Gastric banding (GB) by means of Dacron, Marlex mesh, or Gore-Tex was mostly used in the 1980s[5,6] and has in recent years been largely abandoned in favor of SAGB. All of these procedures belong to the "non-complex" group of bariatric methods because they involve surgical manipulation of one organ only and are considered rather straightforward from a technical point of view. Moreover, the risk of developing postoperative malnutrition is small.

However, simple gastric restriction has several drawbacks. Thus VBG often fails to control weight in the long run because of a high incidence of staple line disruption.[7] GB and SAGB give rise to intolerable side effects and complications such as esophagitis in 50% to 75%, band erosion in 10%, and severe reflux symptoms with frequent vomiting.[8–10]

When faced with insufficient weight control or severe side effects after simple gastric restrictive procedures, patients generally have two options. According to the first option, patients will be forced to accept the lack of weight control and/or learn to live with unpleasant symptoms such as frequent vomiting and heartburn. Symptoms of esophagitis can be treated with proton pump inhibitors, and an eroded band can be excised laparoscopically or endoscopically.[11]

The other option is to abandon the principle of simple gastric restriction and convert to an operation with established long-term safety and efficacy such as Roux-en-Y gastric bypass (RYGBP). The procedure is technically demanding with transection and anastomosis both of the stomach and the jejunum followed by a higher risk of postoperative complications, especially when performed as a revision procedure. After the procedure, the stomach and duodenum are bypassed and hence the patient runs a

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risk of postoperative metabolic disturbances. Therefore gastric bypass is considered to belong to the "complex" group of bariatric operations. However, many believe that the drawbacks are outweighed by superior weight control and a high degree of quality of life postoperatively. RYGBP is therefore considered to be the method of choice for morbid obesity.[12,13] We review our experience with RYGBP as a secondary bariatric operation after failed SAGB, VBG, and GB.

MATERIAL AND METHODS

Patients and Previous Bariatric Procedures

Prospectively collected data from all patients who underwent a second (or third) bariatric operation at our institution in 1996 and 1997 were analyzed. A total of 44 patients (41 women and 3 men) with a median age of 42 years (range 24 to 60 years) and a body mass index (BMI) at the time of reoperation of 35 kg/m^2 (range 21 to 49 kg/m^2) were analyzed. BMI at the time of the original surgery was 42 kg/m^2 (range 35 to 54 kg/m^2). Three patients had undergone more than one previous bariatric procedure. The original (or latest) obesity operations were SAGB (n = 26), VBG (n = 13), and GB (n = 5). Two VBG patients with staple line disruption had been converted to SAGB in the past, a policy that we abandoned in 1996. One patient had had a jejunoileal bypass that was taken down because of small bowel obstruction. Because of weight gain 1 year later, the patient underwent SAGB, but because of patient dissatisfaction with frequent vomiting a conversion to RYGBP was performed. The time between the original (or latest) operation and revision was 23 months for SAGB, 52 months for VBG, and 106 months for GB.

Indications for Revision Surgery

The reasons for conversion to Roux-en-Y gastric bypass are listed in Table 1. The main reason for conversion in the VGB group was staple line disruption (n = 12) resulting in insufficient weight control (BMI 41 kg/m^2). None of these patients had symptoms of gastroesophageal reflux or functional stenoses. After SAGB and GB, the principal reasons were band erosion (n = 12) and esophagitis (n = 11) (BMI 33 and 39 kg/m^2, respectively).

Revision procedures after VBG, GB, and SAGB without known band erosion were elective. In cases of SAGB with band erosion, the operations were all performed semiurgently, that is, within a week or two. One SAGB patient came to the emergency room with peritonitis secondary to band erosion. In this patient we excised the banding system and drained the abdomen at the first session. After the patient recovered, RYGBP was performed electively. Another patient was admitted urgently with a hemoglobin level of 48 g/L. Gastroscopy revealed band erosion. This patient was given a blood transfusion and the revision was performed a few weeks later. In all patients (n = 43) except the one with peritonitis, conversion to RYGBP was accomplished in a single operation.

Table 1. Reasons for conversion to Roux-en-Y gastric bypass

	SAGB (n = 26)	VBG (n = 13)	GB (n = 5)
Esophagitis	9	—	2
Band erosion	10	—	2
Staple line disruption	—	12	
Pouch dilatation	2	—	—
Vomiting/reflux	3	—	—
Allergy (lopamiro*)	1	—	—
Weight loss failure	1	1	1

*Contrast for filling the injection port.

Technical Aspects

All operations were performed openly through a short upper midline incision. Usually, dense adhesions between the stomach and the left lobe of the liver had to be dissected. The stomach was always divided with staples, and the proximal pouch (4 × 3 cm) was constructed along the lesser curvature. The Roux limb was made >50 cm long and was brought to the gastric pouch behind the colon and excluded the stomach. In 20 patients the gastrojejunostomy was stapled with a linear stapler (endoscopic GIA stapler with 4.5 mm staples) and defects were hand sewn. In 24 patients a purely manual suturing technique was used.

In SAGB patients the silicone band was found to be surrounded by a fibrous sheet that had to be opened sharply with scissors. The entire banding system was always removed. Because of an inflammatory reaction in the gastric wall, especially in band erosion, special attention had to be focused on managing the thick gastric wall. Transection of the gastric wall with staples had to be done above or below this area. To obtain a small proximal pouch, the stapling was preferably done above the level of the band.

In revision from VBG to RYGBP, we reduced the size of the original proximal gastric pouch by horizontal transection above the Marlex band. The pouch construction was completed with cutting linear staples (TLC 55 stapler with 4.5 mm staples) toward the an-

gle of His. No gastrostomy tube was left in the excluded stomach. The abdomen was closed without drainage with the use of a running PDS loop.

Postoperative Care

The nasogastric tube was removed the day after surgery, and patients were allowed fluids by mouth on postoperative day 2. Barium upper gastrointestinal radiographic examinations were not routinely performed. Intake of regular food was begun before patients were discharged from the hospital.

Follow-Up

The surgeon at the outpatient clinic checked all patients after 4 to 6 weeks. Thereafter patients were followed in the Metabolic Unit; they were contacted by a dietitian 3 months postoperatively and were examined by an internist after 6 months and then yearly. In this study all patients except four have been seen by one of us after a minimum of 2 years. Four patients were interviewed by telephone for geographic reasons. Patients answered questions from a standardized questionnaire regarding epigastric pain, dumping (palpitations, fatigue after eating), dysphagia, diarrhea, vomiting, and other problems possibly associated with the RYGBP procedure. Patient satisfaction was evaluated using a visual analog scale, where 1 is very dissatisfied and 5 is very satisfied. Clinical examination included a search for incisional hernias. All patients with symptoms of dysphagia and/or epigastric pain were scheduled for gastroscopy.

Analysis of Data

All data are reported as median and range.

RESULTS

Operative Data

The median duration of surgery was 155 minutes (range 85 to 240 minutes). The perioperative blood loss was 1000 ml (range 200 to 2700 ml), and 14 patients needed a blood transfusion of 3 units (range 1 to 8). The only serious perioperative complication was hemorrhage from a splenic injury necessitating splenectomy. The median length of hospital stay after operation was 6 days (range 4 to 15 days).

Complications During the Primary Hospital Stay

There were no postoperative deaths. There were no obvious anastomotic leaks, but one patient (2%) underwent reexploration after 4 days because of an infected hematoma. Minor early complications occurred in five patients (11%) and included fever of unknown origin in two, wound infection in one, pleural effusion in one, and a postoperative decrease in hemoglobin necessitating blood transfusion in one.

Late Complications

One patient (2%) was readmitted 3 weeks postoperatively because of septicemia and underwent reoperation. An intra-abdominal abscess was found and drained. Two patients (4.5%) developed stricture in the gastroenteroanastomosis (one hand sewn, one stapled) that did not respond to repeated endoscopic dilatation and required reoperation 1.5 and 4 months, respectively, after the RYGBP operation. One of these patients had to undergo two reoperations. Four patients (9%) were operated on for bowel obstruction after 3 weeks, 6 months, 2 years, and 3 years, respectively. One patient was found to have severe adhesions after multiple previous abdominal procedures. The other three cases were caused by partial internal herniation of the small bowel behind the Roux limb. This complication could perhaps have been avoided by closure of all mesenteric defects, a practice we did not routinely employ. Two patients (4.5%) developed deep venous thrombosis after discharge from the hospital and were treated with anticoagulants.

Follow-Up Data

The median BMI after 2 years was 28 kg/m^2 (range 18 to 42 kg/m^2) (Fig. 1). Reflux, vomiting, and symptoms of esophagitis resolved promptly after conversion to RYGBP. In the global assessment, 70% of the patients were "very satisfied" with their improved weight control and absence of the symptoms associated with their previous gastric restrictions; 28% of the patients were "satisfied" at follow-up. One patient (2%) was very dissatisfied because of an inadequate weight loss, reaching a BMI of only 42 kg/m^2. Three patients (7%) described dumping (palpitations, fatigue after eating) daily, three patients (7%) weekly, and four patients (9%) had dumping only when overeating. Two patients (4.5%) had between three and five loose stools per day. The minimum duration of follow-up in this group was 24 months. No patient was lost to follow-up. At examination after 2 years, one patient (2%) was found to have an incisional hernia. During follow-up, seven patients complained of epigastric pain. Gastroscopy revealed stomal ulcers in five patients (11%), whereas no cause for the pain could be found in the

other two. Another patient was hospitalized for melena requiring blood transfusion. Results of gastroscopy and colonoscopy were normal. The excluded stomach was examined by means of gastroscopy through a gastrostomy established by ultrasound-guided percutaneous gastrostomy.[14] However, a bleeding source could not be detected. The bleeding did not recur in those being treated with protein pump inhibitors, so the bleeding could have possibly been related to an overlooked stomal ulcer. One patient died 3.5 years postoperatively as a result of advanced lung cancer. Two patients have given birth to healthy children during follow-up.

DISCUSSION

We found that conversion to RYGBP in patients with a failed simple gastric restrictive procedure achieved several goals. First, severe symptoms such as esophagitis and vomiting were promptly relieved. Second, weight control was improved after conversion to RYGBP. Third, but not the least important, a high degree of satisfaction was expressed spontaneously by the patients after the conversion. This high degree of quality of life after RYGBP gave us the impression that the results of RYGBP are superior to those of simple gastric restriction, as has been reported by Delin and Anderson[15] and Hell et al.[16]

We now use RYGBP not only as a standard revision procedure after previous failure with other methods but also as a primary bariatric procedure in the surgical management of morbid obesity. Also, at other institutions there has been a switch from simple gastric restriction to RYGBP. Thus, at the Mayo Clinic, RYGBP replaced VBG as the routine procedure in 1989 because of unsatisfactory weight control and a high prevalence of postoperative heartburn and vomiting after VBG.[17]

Other investigators have also reported that failure after VBG and various other gastroplasties can be effectively managed by conversion to RYGBP.[18–20] Balsiger et al.[21] recently reported that symptoms of gastroesophageal reflux after VBG were promptly relieved by conversion to RYGBP. There seems to be consensus in the literature that restapling of a staple line disruption is not worthwhile.[22,23] Many authors have expressed the view that a failure of one restrictive method should not be treated with another restrictive method. It is likely that such a policy will be followed by the same side effects all over again.

Recently there has been widespread use of SAGB in Europe and elsewhere, with a large number of bands implanted in recent years. The cumulative abdominal reoperation rate has been estimated at

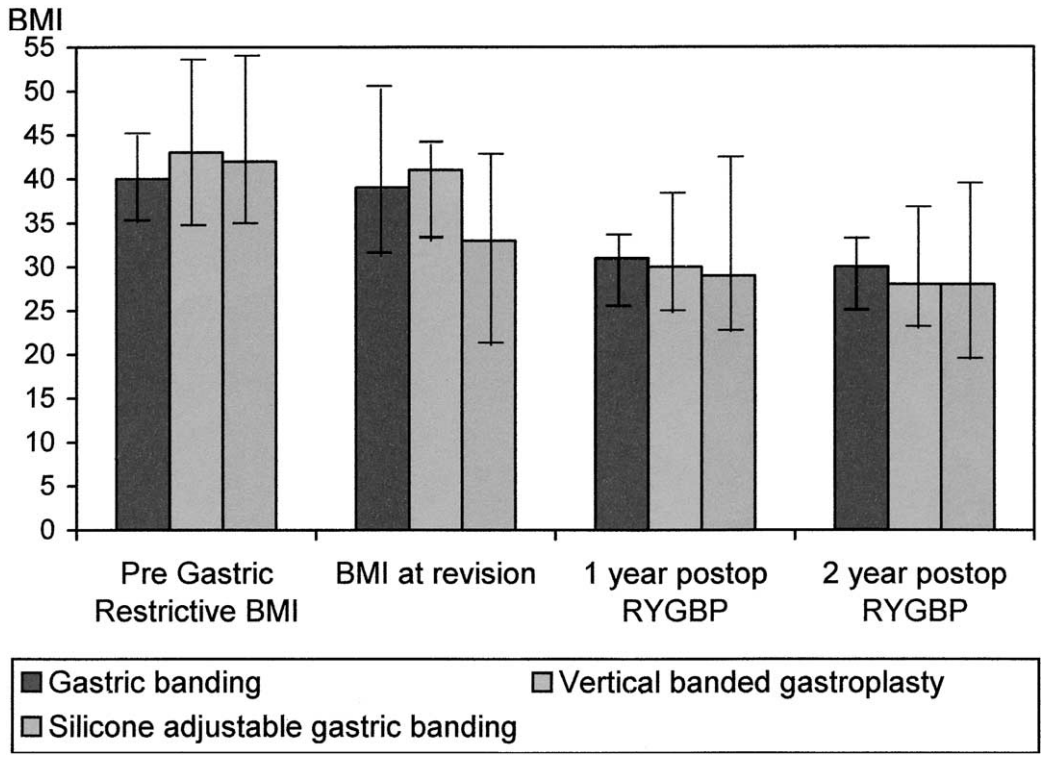

Fig. 1. Median BMI at revision to Roux-en-Y gastric bypass (*RYGBP*) 1 and 2 years postoperatively.

10.5%.[24,25] Thus a large number of patients will require a revision procedure in the near future.

The general opinion among laparoscopic bariatric surgeons is that the preferred method of treating a failed SAGB is repositioning of the old band or insertion of a new band after laparoscopic or endoscopic removal of the old band.[26,27] Excision of the SAGB only, as has been suggested,[11] will inevitably lead to weight regain.

In the present study we have shown that a failed SAGB can be effectively treated with conversion to RYGBP. We believe this policy is more reliable than treating failed SAGB failures with continued restriction.

In our opinion the surgical correction of failed SAGB is the most technically demanding of all revision procedures. This is especially the case after band erosion when there is a severe inflammatory reaction in the gastric wall and an inevitable communication between the surgical field and the contaminated gastric lumen. Because of the technical complexity of this revision procedure, we cannot see a place for laparoscopy in conversion to RYGBP, as has been suggested by Gagner et al.[28] Clearly, revision surgery has a higher frequency of complications in comparison to primary procedures.[22,29,30] However, our frequency of serious complications is within the lower limit of what has been reported after revision bariatric surgery.[19,29,31,32]

The present study included only patients operated on in 1996 and 1997 in order to provide a reasonable duration of follow-up. However, we have continued the same policy and converted an additional 45 patients between 1998 and 2000, with early results similar to those presented here.

CONCLUSION

We propose RYGBP as the method of choice when simple gastric restrictive procedures fail to control weight or produce intolerable side effects and complications. RYGBP achieves superior weight control and relieves symptoms of reflux and vomiting. Our impression is that conversion to RYGBP gives the patients an improved quality of life.

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Neoadjuvant Chemotherapy with CPT-11 and Cisplatin Downstages Locally Advanced Gastric Cancer

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We examined the role of neoadjuvant therapy in downstaging locally advanced gastric cancer. Preoperative staging was performed with a combination of CT scans, endoscopic ultrasonography and/or laparoscopy, and laparoscopic ultrasonography. Patients with T ≥3 tumors and/or node-positive disease by preoperative clinical staging were eligible for entry. Neoadjuvant therapy consisted of two cycles of CPT-11 (75 mg/m^2) with cisplatin (25 mg/m^2) weekly four times every 6 weeks. This was followed by resection with D2 lymph node dissection and two cycles of intraperitoneal chemotherapy with floxuridine and cisplatin. Twenty-two patients were entered into the study (4 with T3N0 disease and 18 with T3N1 disease). Induction chemotherapy was well tolerated with major toxicities being neutropenia and diarrhea. A median of 78%/75% of the planned dosage of CPT-11/cisplatin was delivered. Two patients withdrew consent during the first cycle and were lost to follow-up. One patient progressed to stage IV disease during induction chemotherapy and did not undergo surgery. Nineteen patients underwent surgery. One patient had undetected stage IV disease (liver) and underwent a palliative R2 resection. Of the 18 remaining patients, 17 had curative R0 resections and one had a palliative R1 resection. A median of 21 lymph nodes (range 1 to 121) were examined histologically. There was one postoperative death. Surgical morbidity did not appear to increase after the neoadjuvant regimen. The median postoperative length of hospital stay was 9 days (range 3 to 75 days). Postoperative pathologic staging yielded 16% T3 lesions compared to 85% before treatment based on clinical staging; postoperative American Joint Committee on Cancer staging yielded 37% stage IIIA disease compared to 70% stage IIIA before treatment. With a median follow-up of 15 months, median survival has not yet been reached. We conclude that CPT-11–based neoadjuvant therapy downstages locally advanced gastric cancer. Further follow-up is necessary to determine the ultimate impact of this combination therapy on recurrence and survival. (J GASTROINTEST SURG 2002;6:212–223.)

KEY WORDS: Neoadjuvant therapy, gastric cancer, downstaging

Patients with gastric cancer represent a major global problem, with almost 800,000 newly diagnosed cases being reported yearly worldwide and more than 600,000 deaths.[1] Here, in the United States, it is the third most common gastrointestinal cancer. A total of 21,000 new cases of gastric cancer are expected in the year 2001, with 13,000 deaths.[2] Although there has been an overall decrease in the number of reported cases in the United States since the 1930s, there has been a significant increase in the incidence of proximal cardia and gastroesophageal cancer in the past two decades.[3] The overall dismal outcome associated with this disease is directly related to the stage at presentation. Approximately 20% of patients with invasive cancers first present with early-stage localized disease, with 5-year survival rates approximating 60% to 70%. The vast majority of patients present with regional or distant dis-

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ease, with 5-year survival for these stages ranging from 35% for stage II disease to less than 5% for stage IV disease.[2]

The mainstay of treatment for gastric adenocarcinoma is surgery. However, despite apparently curative resections, high recurrence rates are the rule, as stated earlier. Attempts to improve the poor survival rates with adjuvant therapy have generally been unsuccessful,[4] although modest benefits are occasionally reported in some European trials.[5,6] Recently MacDonald et al.[7] reported the results of Intergroup Trial 0116, which found a significant improvement in disease-free and overall survival in patients with gastric adenocarcinoma stages IB to IV (M0) treated with adjuvant 5-fluorouracil/leucovorin with concurrent radiation compared to a surgery-only arm. This is the first randomized controlled trial to show a benefit to combination chemoradiation after surgery in gastric adenocarcinoma.

Because of the general lack of success with adjuvant therapy, a number of investigators have employed a neoadjuvant approach to the treatment of gastric cancer.[8–11] Neoadjuvant therapy has a number of potential benefits including the following: (1) its potential role in downstaging and improving the "R0" resectability rate; (2) the greater likelihood of successfully delivering the chemotherapy, inasmuch as the patients are not recovering from major abdominal surgery; (3) the ability to assess responses at the time that patients have measurable disease; and (4) those patients with biologically aggressive disease that progresses during neoadjuvant therapy will be identified and spared an unnecessary exploratory operation.

We initiated a phase II trial of neoadjuvant chemotherapy in patients with locally advanced gastric adenocarcinoma, defined as T $\geq$3 and/or node-positive disease, with a combination of CPT-11 and cisplatin. These drugs were selected based on their antitumor activities as single agents, synergism from this combination, and obviating some tolerance issues when these drugs are given postoperatively (e.g., severe gastrointestinal toxicity).

METHODS

Between October 1998 and October 2000, 22 patients were registered in a phase II trial of neoadjuvant CPT-11/cisplatin chemotherapy for gastric cancer. All patients had histologically confirmed gastric adenocarcinoma of the gastroesophageal junction or stomach. Pretreatment staging was by history and physical examination, chest x-ray examination, upper endoscopy, endoscopic ultrasonography (EUS) and/or laparoscopic ultrasonography (LUS), abdominal and pelvic CT scans, and chest CT scans for lesions at the gastroesophageal junction. Laparoscopic staging was encouraged but not mandated. Only potentially curable patients with T3 or T4 tumors as shown by EUS/LUS or any tumor with node-positive disease as shown by EUS/LUS or CT scan were eligible. Patients had to be at least 18 years of age to be eligible, with Southwestern Oncology Group performance status ranging from 0 to 2. No prior chemotherapy or radiation therapy was allowed. An adequate bone marrow reserve (neutrophil count $\geq$4000/μL, thrombocytes $\geq$100,000 μL, hemoglobin $\geq$9 g/dl). Preserved liver and renal function (total serum bilirubin $<$2 mg/dl, SGOT/SGPT $\leq$3 times the upper limit of normal, alkaline phosphatase $\leq$3 times the upper limit of normal, BUN $\leq$30 mg/dl, creatinine concentration $\leq$1.5 mg/dl, or creatinine clearance $>$60 ml/min). All patients were informed of the investigational nature of the protocol and were required to sign informed consent as approved by the Institutional Review Board of New York University Medical Center. Patients were not offered the protocol if they were in need of urgent surgery for indications such as gastrointestinal obstruction, perforation, or hemorrhage. Other exclusion criteria included the following: (1) patients with another invasive malignancy (adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or other cancers where the patient had been free of disease for a minimum of 5 years were acceptable); (2) patients with active or uncontrolled infection including HIV; (3) patients with psychiatric disorders that would interfere with their consent and/or follow-up of the protocol; and (4) pregnant and nursing patients.

Induction Chemotherapy

Induction chemotherapy with cisplatin and CPT-11 was given weekly on days 1, 8, 15, and 22, with 2 weeks of no treatment on weeks 5 and 6. Two 6-week cycles were administered before patients were evaluated for surgery. Placement of a central venous access device for delivery of chemotherapy was recommended (Fig. 1).

At the beginning of each treatment, hydration with 1 L of normal saline solution, mixed with 2 g $MgSO_4$ and 20 mEq KCl, was given over 1 to 2 hours. This was followed by cisplatin, 25mg/m^2/wk intravenously, given in 500 ml of normal saline, injected over approximately 90 to 120 minutes. Then CPT-11, 75 mg/m^2/wk (formulated in 500 ml of 5% dextrose/water), was administered over 90 minutes intravenously. Post-therapy hydration consisted of

0.5 to 1.0 L of normal saline solution given over 1 to 2 hours, depending on the patient's ability to tolerate the fluid load. Standard antiemetic therapy with 5-HT3 antagonists and dexamethasone was given to all patients. Atropine, 0.25–1.0 mg intravenously, was administered to subjects with the cholinergic side effects of CPT-11. Patients who had recurrent cholinergic side effects were given prophylactic atropine. Patients were instructed to take loperamide at the earliest sign of diarrhea, loose stools, or abdominal cramping. The loperamide dosage was 4 mg by mouth at the onset of symptoms, then 2 mg every 2 hours for 12 hours, or until the patient was symptom free for 12 hours. Patients were allowed 4 mg loperamide every 4 hours overnight. Additional antidiarrheal medication and intravenous hydration were given at the discretion of the treating physician. Routine prophylactic use of granulocyte colony-stimulating factor (G-CSF) was not recommended. Prophylactic administration of G-CSF for recurrent grade 3 and 4 neutropenia, or therapeutic use of G-CSF in patients with sepsis or febrile neutropenia was recommended, in accordance with the American Society of Clinical Oncology guidelines.[12] Epoietin-alpha use was allowed for anemia due to malignancy or treatment.

Toxicity and Dosage Modifications

Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria, version 2. Hematologic toxicities were dealt with in the following manner: In the event of grade 3 neutropenia, the CPT-11 dosage was reduced by 20 mg/m^2/wk, and cisplatin was omitted. When the neutropenia was grade $\leq$2, CPT-11 was reduced by 10 mg/m^2/wk and the cisplatin dosage was reduced by 25%. For grade 4 neutropenia, both CPT-11 and cisplatin were omitted until neutropenia was grade $\leq$2, and then the dosage was decreased by 10 mg/m^2/wk (CPT-11) and by 25% (cisplatin). For febrile neutropenia, CPT-11 and cisplatin were omitted until the neutropenia resolved to grade 0, and then the dosage was reduced by 20 mg/m^2/wk (CPT-11) and by 50% (cisplatin). Dosage modifications for thrombocytopenia or anemia were the same as those recommended for neutropenia.

Nonhematologic toxicities were dealt with in the following manner: For grade 2 diarrhea, the CPT-11 dosage was decreased by 10 mg/m^2/wk. For grade 3 diarrhea, CPT-11 was omitted until toxicity was grade $\leq$2; then the dosage was reduced by 10 mg/m^2/wk. For grade 4 diarrhea, CPT-11 was withheld until toxicity was reduced to grade $\leq$2; then the dosage was decreased by 20 mg/m^2/wk. With renal toxicity (creatinine 1.5 to 2.0 mg/dl), cisplatin was reduced by 50%. In the event of neurotoxicity (any grade) or a creatinine value greater than 2 mg/dl, cisplatin was omitted until both toxicities had resolved to grade 0.

For all other grade 2 nonhematologic toxicities, the dosage of CPT-11 was reduced by 10 mg/m^2/wk. For other grade 3 and 4 toxicities, the patient was taken off of the protocol. In the event of a 1-week delay in therapy because of toxicity, makeup treatment was allowed over a 7-week cycle instead of a 6-week cycle. If a 2-week delay occurred because of toxicity, the remainder of that cycle was omitted and the second cycle was begun once toxicity was grade $\leq$2.

Surgery

Gastric resection was performed approximately 4 weeks after the patient had completed induction chemotherapy (10 weeks after beginning chemotherapy treatment) among those who did not have evidence of progression of disease in the induction phase (Fig. 1). Patients were reevaluated with CT scans of the abdomen and pelvis. Repeat endoscopy and EUS/LUS were not routinely performed, because a number of investigators have demostrated the lack of accuracy of postchemotherapy EUS and final pathologic analysis.[11,13] A curative (R0) resection is defined as en bloc removal of a gastric or gastroesophageal junction tumor with draining lymph nodes and the omentum, leaving disease-free proximal and distal margins. D2 lymph node dissections were encouraged as described by the Japanese Gastric Cancer Research Group.[14] A minimum of 15 lymph nodes were necessary for node (N) staging.[15] At the completion of resection, an intraperitoneal catheter (Mediport, Bard Access Systems, Salt Lake City, UT) was implanted subcutaneously over the costal margin, with the catheter tip tunneled to lie preferably in the intraperitoneal pelvis for the delivery of postoperative intraperitoneal chemotherapy.

Intraperitoneal Chemotherapy

Intraperitoneal chemotherapy was begun from 7 to 28 days postoperatively in the outpatient setting (Fig. 1). CT scans with intraperitoneal contrast[16] were performed in all of the patients before intraperitoneal therapy was instituted, and all studies demonstrated good fluid distribution throughout the abdominal cavity. These studies were performed by infusing 50 ml of nonionic contrast medium diluted in 1.5 L of normal saline solution through the intraperitoneal catheter. No oral or intravenous contrast medium was used.

Two cycles of floxuridine (FUDR) and cisplatin were administered intraperitoneally as previously described[17] on a 3-week cycle, and if carboplatin was administered instead of cisplatin (because of neuropathy or renal dysfunction), the treatment interval was every 4 weeks. Intraperitoneal catheters were accessed using sterile technique with 19- or 20-gauge noncoring (Huber) needles. As long as the dressings remained dry and intact, intraperitoneal catheters remained accessible over the 3-day course of therapy, minimizing the risk of infection. Patients received treatment with FUDR, 3 g daily, for three successive days. The first 2 days in 1.5 L normal saline solution with 1000 units of heparin/L and 5 mEq KCl/L, and on the third day FUDR was administered in 750 ml of normal saline solution followed by cisplatin, 60 mg/m^2 (using actual body weight), or carboplatin (area under the curve = 5), in an additional 250 to 750 ml of normal saline solution. Heparin, 1000 U/L, and KCl, 5 mEq/L, were added to the intraperitoneal fluid. The volume of fluid delivered was dependent on patient tolerance. Before removal of the Huber needle, the catheters were flushed with 1000 units of heparin in 9 ml of normal saline solution to reduce the risk of fibrin sheath formation.

Patients receiving cisplatin were given intravenous hydration with 1 to 2 L of normal saline solution with 20 mEq/L KCl and $NaHCO_3$, 25 g/L at 250 ml/hr, and were premedicated as described earlier for the neoadjuvant intravenous cisplatin. They were given additional intravenous hydration and parenteral antiemetic therapy with 5HT3 antagonists on day 4 if they were unable to drink fluids because of nausea and/or vomiting. Patients required antiemetic therapy for 5 days or less after treatment for nausea and vomiting. Patients who developed "spasm-like" abdominal pain on days 4 or 5 were given oxycodone or Tylenol with codeine every 4 hours as needed for 1 to 2 days with good pain relief. Laboratory tests including complete blood count/differential and BUN/creatinine with electrolytes were conducted weekly.

Follow-Up Evaluations

Patients had follow-up examinations every 3 to 4 months after the completion of therapy by both the medical and surgical oncology staff. CT scans of the abdomen and pelvis were obtained routinely every 3 to 4 months. Additional studies were obtained as clinically relevant on an individual basis. Site of first recurrence was documented on the basis of appropriate imaging. Histologic verification was performed only in questionable circumstances and when feasible. Additional therapy after recurrence was at the discretion of the treating physicians.

RESULTS

Patient demographics are outlined in Table 1. Twenty-two patients were registered, of whom 15 were men and seven were women; median age was 60 years. The majority of tumors were proximally located. Tumor staging was by EUS/LUS assessment. All tumors in the gastroesophageal junction had the bulk of disease extending into the cardia, and there were no patients with Barretts disease. The clinical American Joint Committee on Cancer (AJCC) staging was by means of EUS/LUS in combination with CT scans.

Drug Delivery

During the induction phase of chemotherapy, 10 patients completed two cycles of treatment, whereas in 12 patients treatment was incomplete. Reasons for not completing therapy were as follows: two patients withdrew their consent (and were lost to follow-up), one patient had progressive disease, one had emergency surgery for gastrointestinal bleeding, one had emergency surgery for gastric outlet obstruction, and seven had treatment-related complications.

For CPT-11 and cisplatin therapy, a total of 40 out of 44 planned cycles were delivered, at least in part. The planned total dosage per patient of CPT-11 was 600 mg/m^2, with a median of 78% being delivered (range 13% to 100%). The planned total dosage

Table 1. Patient characteristics (N = 22)

M/F		15/7
Median age		60 (range 37–77)
Race		
White		11
Black		2
Hispanic		2
Asian		7
Tumor location		
Gastroesophageal junction/cardia		11
Body		5
Antrum		6
Clinical T stage (by EUS/LUS)		
T2		3
T3		19
Clinical AJCC stage (by EUS/LUS)		
IB	T2N0M0*	1
II	T2N1M0	2
	T3N0M0	4
IIIA	T3N1M0	15

*Originally staged as T3N0M0 and entered into the protocol; however, on later review of EUS, thought to be T2/T3, which according to AJCC staging criteria is classified as a lower (T2) stage.

per patient of cisplatin was 200 mg/m^2, with a median of 75% being delivered (range 13% to 100%).

Induction Chemotherapy Toxicity

The common toxicities encountered during induction chemotherapy were neutropenia (including febrile neutropenia), diarrhea, dehydration, and fatigue. Specific hematologic and nonhematologic toxicities are outlined in Table 2. A total of five patients required hospitalization during the induction period. Four patients were admitted for febrile neutropenia and one for intractable diarrhea. The average length of stay for these admissions was 3 days. One patient developed a superior vena cava syndrome secondary to placement of the Mediport for delivery of chemotherapy. This occurred after the completion of induction chemotherapy and before the planned surgery. The patient was admitted for removal of the port and anticoagulation therapy, and the syndrome resolved. There were no deaths related to the induction chemotherapy.

Surgical Outcomes

Table 3 outlines the surgical outcomes. Of the 22 patients entered into the study, two withdrew from the protocol during the first cycle and were lost to follow-up. One had tumor progression in the liver during induction chemotherapy and did not undergo surgery. Of the 19 patients who underwent surgical exploration, all had resections. One patient was found to have small-volume disease in the liver that had not been seen on preoperative imaging. This patient underwent a palliative R2 resection. A second patient had microscopically positive distal margins after a distal gastrectomy (at the distal duodenal margin). All other patients had a curative R0 resection. One operative death occurred in a patient who developed unexplained acidosis and hypotension on postoperative day 8. He underwent reexploration, but no intra-abdominal source could be identified as a cause for the problem. An autopsy was refused. Of note, this was the same patient described earlier who had had the superior vena cava syndrome. The presumption of a pulmonary embolus as a cause of death therefore has been entertained. Two additional patients required reoperation. One was for an anastamotic leak, and one was for an atonic stomach after a transabdominal proximal gastrectomy requiring conversion to a total gastrectomy with Roux-en-Y esophagojejunostomy. Other relatively minor morbidities are as listed.

Pathologic Findings

For the 19 patients who underwent resections, pathologic data are summarized in Table 4. There was an equal distribution of diffuse, intestinal, and mixed types of tumors. The majority of tumors were moderately to poorly differentiated. Response of the primary tumor was characterized by a dense lymphocytic infiltrate and fibrosis (Fig. 2). When the lymph nodes were assessed, three pathologic findings were seen. The majority of lymph nodes (21 per patient) were architecturally normal lymph nodes without evidence of tumor or chemotherapy effect. Approximately four lymph nodes per patient demonstrated significant histiocytic infiltration and stromal fibrous

Table 2. Induction chemotherapy toxicity in 22 evaluable patients

	Grade 2	Grade 3	Grade 4
Hematologic			
Neutropenia	4 (20%)	8 (40%)	2 (10%)
Febrile neutropenia	—	4 (20%)	0
Anemia	2 (10%)	1 (5%)	0
Thrombocytopenia	0	0	0
Nonhematologic			
Diarrhea	9 (45%)	6 (30%)	0
Nausea/vomiting	5 (25%)	2 (10%)	0
Dehydration	5 (25%)	0	0
Hepatic transaminitis	1 (5%)	1 (5%)	0
Anorexia	0	1 (5%)	0
Fatigue	4 (20%)	1 (5%)	0
Abdominal pain	3 (15%)	0	0
Neuropathy	1 (5%)	0	0

Table 3. Surgery results

No. of patients explored	19
No. of patients resected	19
Curative (R0)	17
Palliative (R1/R2)	2
Total gastrectomy	2
Proximal gastrectomy*	9
Distal gastrectomy	8
Median No. of lymph nodes	21 (range 1–121)
Intraperitoneal catheter placed	17
Operative (30-day) mortality	1
Morbidity	
Reoperation	3
Anastamotic leak	1
Cardiac/respiratory	3
Wound infection	4
Median postoperative length of stay (days)	9 (range 3–75)

*Three true proximal gastrectomies by transabdominal approach and six esophagogastrectomies by left thoracoabdominal approach.

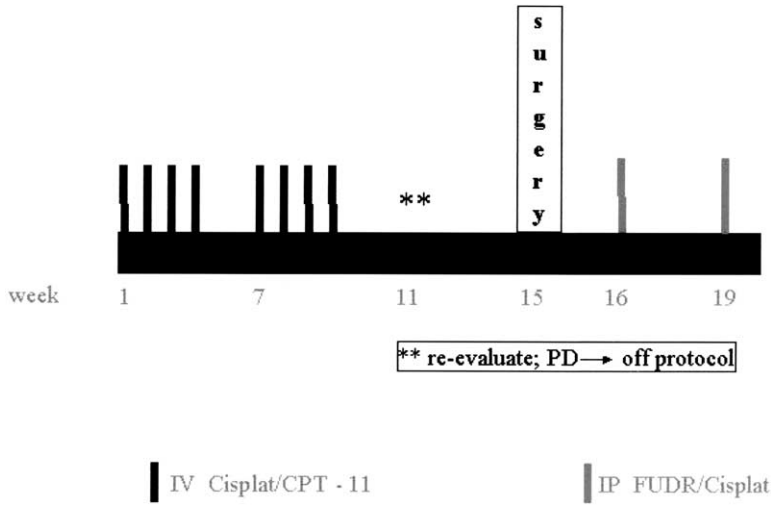

Fig. 1. Overview of protocol schema. Dark black bar indicates intravenous systemic (*IV*) cisplatin/CPT-11 treatment. Light gray bar indicates intraperitoneal (*IP*) FUDR/Cisplatin treatment. PD = progression of disease.

replacement consistent with a chemotherapy effect (Fig. 3). An average of five lymph nodes per patient had tumor metastases or a combination of fibrosis with tumor metastases consistent with no effect or a partial chemotherapy effect.

Table 4. Pathologic findings

	N = 19	%
Histology		
Diffuse	7	37
Intestinal	6	32
Mixed	6	32
Differentiation		
Well	0	
Well to moderate	1	5
Moderate	4	21
Moderate to poor	6	32
Poor	7	37
Undifferentiated	1	5
Mean no. of lymph nodes	29 (range 1–121)	
Normal	21 (range 0–96)	
Fibrosis only	4 (range 0–24)	
Tumor	5 (range 0–20)	
AJCC classification (1997)		
T1NXM0	1	
T2NXM0	1	
TISN0M0	1	
IA T1N0M0	2	
IB T2N0M0	3	
II T2N1M0	4	
IIIA T2N2M0	4	
IIIB T3N2M0	1	
IV T2N3M0	1	
IV M1	1	

Downstaging Effect

There were no complete pathologic responses. A partial pathologic response was evident by the shift to a lower tumor stage and AJCC stage on final pathologic analysis as compared to the pretreatment clinical stage (Table 5).

Intraperitoneal Chemotherapy

For the intraperitoneal treatment, 17 patients had an intraperitoneal catheter inserted, and 12 of them received intraperitoneal chemotherapy. Five of the original 22 patients did not have a catheter placed for the following reasons: three did not undergo surgery (two because of withdrawal from the protocol and one because of progressive disease during induction chemotherapy); one was found to have unexpected liver metastases at surgery and therefore only had a palliative resection; and one patient refused. Five patients did not receive therapy after port placement for the following reasons: two withdrew consent, one died postoperatively, one had an infected catheter, and one had technical difficulties with the catheter. Eleven patients received two cycles of adjuvant intraperitoneal chemotherapy, whereas one patient received one cycle (Table 6).

Carboplatin was substituted for intraperitoneal cisplatin in five patients for the following reasons: one case each of: anaphylactoid reaction to cisplatin (grade 4); nausea and vomiting (grade 2); neuropathy, (grade 2); and two cases of renal impairment (grade 2). Five cycles of carboplatin were delivered with an area under the curve dose of 4 to 5. Toxicities during the intraperitoneal chemotherapy were mild to moderate in nature, mostly related to fatigue and abdominal pain (see Table 6).

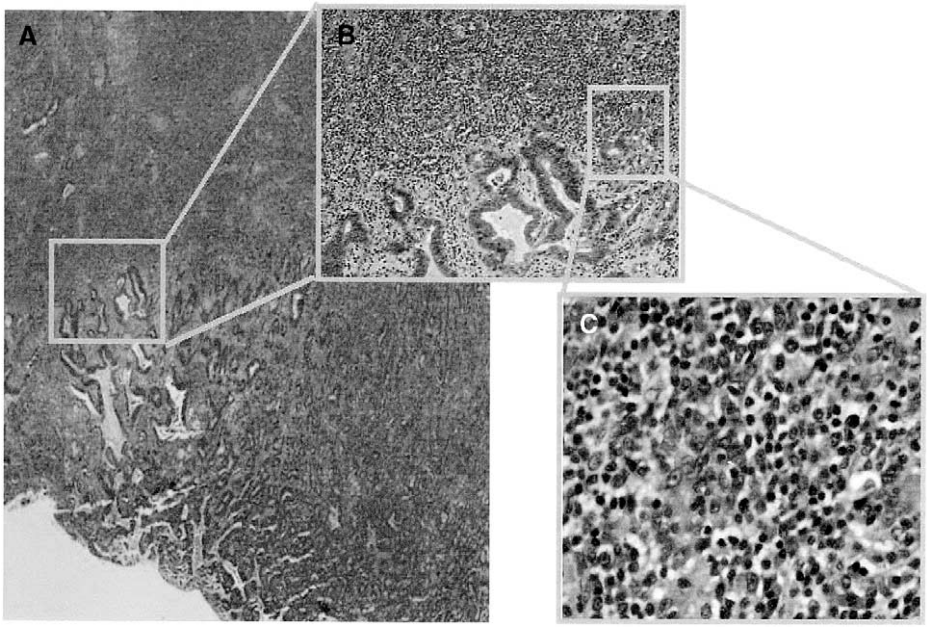

Fig. 2. Effect of chemotherapy on the primary tumor. Medium-power magnification of mucosal surface (**A**) shows infiltration by carcinoma, which progressively becomes more difficult to identify at higher magnification as one looks deeper (**B**, *inset*). **C,** *Inset* demonstrates higher magnification of the dense lymphocytic infiltrate surrounding isolated tumor cells often only identifiable with immunohistochemical staining.

Follow-Up and Recurrence

At a median follow-up of 15.5 months, there have been five recurrences: two intra-abdominal (liver and gastric bed) and three distant (two retroperitoneum and one bone). Four patients have died of disease. The mean disease-free survival from the beginning of induction chemotherapy was 19.5 months and the mean overall survival from the beginning of induction chemotherapy was 23 months. Median disease-free or overall survival has not yet been reached (Fig 4).

DISCUSSION

The poor outcome associated with surgery alone for locally advanced gastric cancer has prompted many investigators to look for additional therapeutic modalities in this disease. Adjuvant therapy trials for gastric cancer date back to the mid-1970s with generally no significant impact on survival.[4] Most recently MacDonald et al.[7] reported the results of Intergroup Trial 0116, which found a significant improvement in disease-free and overall survival in patients with gastric cancer treated with adjuvant chemoradiation. Although this is a large, properly powered, prospective randomized trial, there are a number of concerns with this study. First, this study required an R0 resection (microscopically margin-free curative resection) in order for a patient to be eligible for entry. However, data from the American College of Surgeons patient care study indicate that only about 30% of patients with gastric adenocarcinoma who undergo surgical exploration will have an R0 resection.[18] As such, there is a significant need to improve curative resection rates in gastric cancer. Second, because this therapy was delivered in the adjuvant postoperative setting, concerns about delivering the intended therapy to a group of patients recovering from a major abdominal procedure are raised. In Intergroup Trial 0116, 35% of patients did not complete the intended therapy: 17% because of toxicity and 8% because they refused treatment.[7] This concern over the ability to deliver such therapy in the postoperative setting has been expressed in other clinical settings such as pancreatic cancer and esophageal cancer.[19,20] Third, the majority of recurrences were regional recurrences, defined as intra-abdominal recurrences, and these were found in approximately 70% of patients in both arms of the study,[7] indicating that additional regional therapy might improve outcome in these patients.

The use of a neoadjuvant approach with preoperative chemotherapy is attractive for a number of reasons. First, because the therapy is being delivered before surgery, there are fewer chances of treatment delay or difficulties in delivering therapy, which can result when adjuvant chemotherapy is delivered in

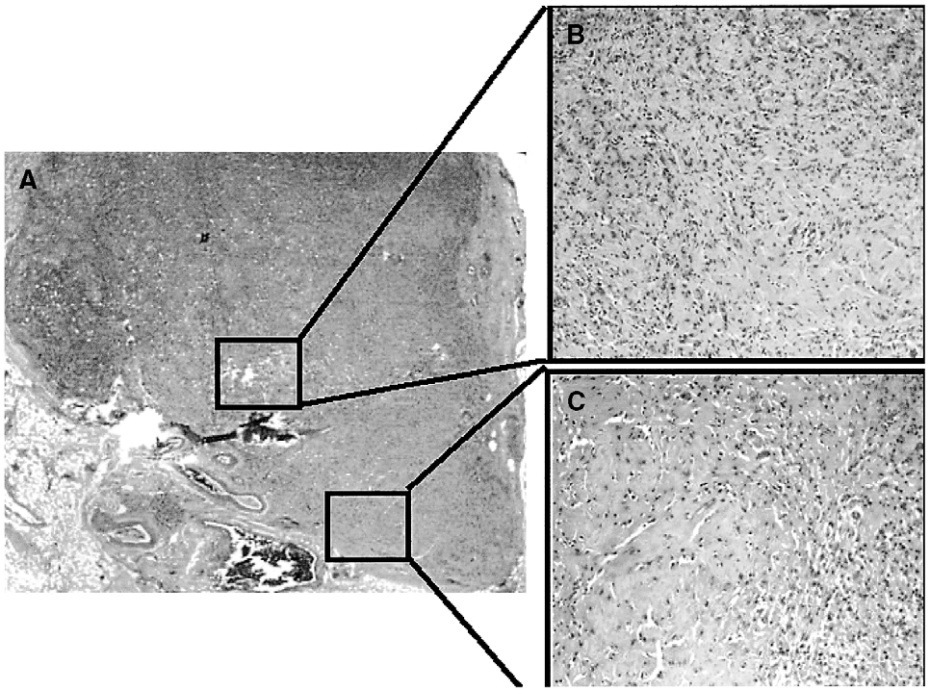

Fig. 3. Effect of chemotherapy on lymph nodes. Medium-power magnification of lymph node (**A**). **B**, demonstrates significant histiocytic infiltration, presumably in response to the cellular damage and inflammatory response from chemotherapy effect on lymph nodes that were involved with metastatic disease before treatment. As the inflammatory process continues, histiocytes are replaced by significant areas of fibrosis (**C**).

the setting of recovery from major abdominal surgery. Second, the neoadjuvant approach can downstage disease leading to a potentially higher curative resection rate and potentially better survival outcome. Finally, in those patients with biologically aggressive disease, the preoperative time interval may identify this subset of patients as subclinical metastatic disease becomes evident, thereby sparing these patients an unnecessary exploratory operation. Of course, one of the main concerns for surgeons when they are delivering neoadjuvant treatment is the impact that therapy has on postoperative outcome, because a toxic regimen that leads to significant morbidity and mortality will clearly negate any potential benefit described earlier.

The early findings in our trial suggest the following with regard to neoadjuvant CPT-11/cisplatin chemotherapy: (1) it can be delivered with acceptable toxicities, allowing for more than 70% of the planned dosage to be administered; (2) it can lead to a high curative resection rate, as 85% of evaluable patients were able to undergo an R0 resection; (3) it does not have a significant impact on operative morbidity and mortality; and (4) it is associated with significant downstaging, as evidenced by the higher percentage of stage I and II tumors on final pathologic analysis. However, longer follow-up is necessary to determine whether this combination therapy, in association with the adjuvant intraperitoneal therapy, will ultimately lead to improved local control and survival.

The use of CPT-11 in gastric cancer was initially reported in Japan[21] with a 23% response rate. The response rate was nearly doubled when CPT-11 was

Table 5. Pretreatment clinical stage vs. postoperative pathologic stage

	Pretreatment clinical	Postoperative pathologic
T stage*	n = 20	n = 19
TIS	0	1
T1	0	3
T2	3	13
T3	17	2
AJCC stage	n = 20	n = 17†
IA	—	2
IB	1	4
II	5	4
IIIA	14	4
IIIB	—	2
IV	—	1

*Pretreatment T stage by EUS and/or LUS.
†Two patients with inadequate node retrieval to allow for postoperative AJCC staging and one patient who did not have surgery.

Table 6. Adjuvant intraperitoneal chemotherapy

No. of patients receiving intraperitoneal catheter	17
No. of patients treated (at least one treatment)	12
% Cisplatin delivered	60 (range 2–100)
% FUDR delivered	90 (range 50–100)
Catheter complications (among 17 patients with catheter placement)	
Peritonitis	2
Mediport infection	1
Intraperitoneal toxicity (among 12 patients treated)	
Fatigue (grade 2/3)	7/1
Abdominal pain (grade 2/3)	1/3
Nausea/vomiting (grade 2/3)	4/2
Renal impairment (grade 2)	2
Anorexia (grade 2)	1
Anemia (grade 2)	1
Allergic reaction (grade 4)	1
Dehydration (grade 2)	1
Deep venous thrombosis (grade 2)	1
Neuropathy (grade 2)	1
Rash (grade 2)	1

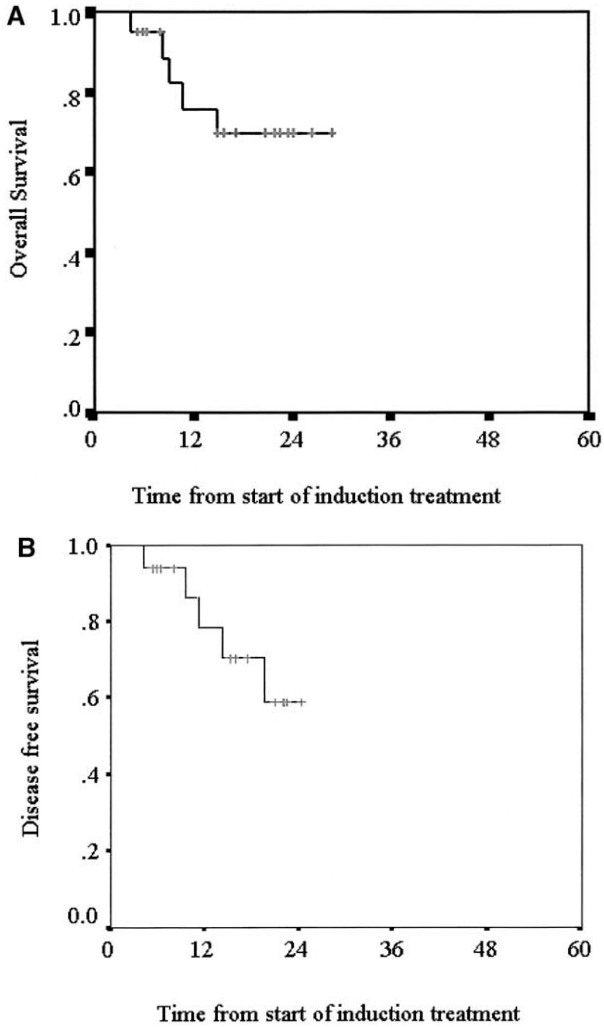

Fig. 4. Overall (**A**) and disease-free (**B**) survival from the beginning of induction treatment for 20 evaluable patients. Median follow-up was 15.5 months.

combined with cisplatin.[22] Toxicity of this regimen, which consisted of CPT-11 given every 2 weeks and cisplatin given in a cycle of once every 4 weeks, was mainly related to neutropenia. Our regimen of once-weekly treatment four times for both CPT-11 and cisplatin followed by a 2-week break is similar to that of other investigators in the United States.[9,23] Similar to these reports, our main toxicities were related to neutropenia and diarrhea. Although a high percentage of the drugs could be administered with this regimen, this regimen was not easily tolerated, as approximately 85% of patients required delays or a dosage reduction, or did not complete induction chemotherapy. In addition, 25% required hospital admission. The possibility of improving drug delivery and decreasing toxicity by modifying the 6-week cycle to two periods of 2 weeks of treatment followed by 1 week of rest is being investigated.[9]

As stated earlier, one of the potential benefits of neoadjuvant therapy is increasing resectability rates and, more important, R0 curative resection rates. A number of reports in Europe and the United States have indicated that with current practice patterns, curative resection is only possible in approximately 30% of patients with locally advanced disease.[18,24,25] In addition, entry into the adjuvant Intergroup trial showed that a significant survival benefit with chemoradiation required an R0 resection.[7] Our ability to achieve an 85% R0 resection rate with our neoadjuvant therapy is certainly very encouraging. Other investigators have also reported high resectability rates after neoadjuvant therapy. These included 5-fluorouracil (5-FU) with adriamycin and methotrexate, 5-FU with cisplatin, and 5-FU with cisplatin and interferon-α.[10,11,26] Clearly, the high resectability rates in our study, as well as in these in other phase II studies, are in part due to careful preoperative staging and patient selection. Nevertheless, our ability to more than double the curative resection rate from the general community experience[18] suggests that this strategy is helpful in better identifying those patients who can benefit from surgery and spares others an unnecessary exploratory operation. Part of this benefit comes from the ability of neoadjuvant therapy to select out those with subclinical metastatic disease, as suggested earlier. In our study, one patient was found to have progressive disease during induction therapy, thereby avoiding a nontherapeutic exploratory laparotomy.

Whenever preoperative therapy is considered, one of the most important concerns is its effect on postoperative outcome. The potential survival benefits of neoadjuvant therapy can easily be negated by increased postoperative morbidity and mortality.[27] The use of chemotherapy alone before surgery has generally been well tolerated, with no reports of increased postoperative complications.[10,11,28] Our findings are consistent with these findings, as evidenced by the low postoperative morbidity and mortality in our study patients. Our median postoperative length of stay of 9 days compares favorably with our median length of stay for nonprotocol patients treated during the same period (data not shown) and with other reports on gastrectomy patients in the postchemotherapy setting.[10,11]

The most difficult end point to assess in any study that examines the effect of neoadjuvant therapy is that of response and downstaging. The ability to clinically stage gastric cancer preoperatively, and then assess downstaging by comparing this to postoperative pathologic staging, is limited by the accuracy of preoperative staging modalities. We employed a combination of pretreatment endoscopy and EUS,[29,30] CT scans,[31] laparoscopy, and LUS[32–34] to assess the clinical TNM stage before entry into the protocol. We recognize that even with these state-of-the-art imaging modalities, the accuracy of our preoperative staging is an important limitation in this study. In addition, in any situation where pretreatment staging did not clearly identify T3 or node-positive disease, we opted to exclude those patients to provide us with a population of clearly locally advanced but potentially resectable patients. Despite our best efforts, in one case, on review of the EUS images, we could not confirm T3 disease and as such had one patient with a pretreatment T2N0M0 stage on the protocol.

Assessment for progression of disease and exclusion from surgery was carried out 4 weeks after completion of the second cycle with repeat high-quality CT scanning. We elected not to repeat routine endoscopy, EUS, or LUS because a number of investigators have reported the lack of concordance between postchemotherapy imaging and the final pathology report,[11,14,26] and this would therefore be an inaccurate measure of response. Neither would it have any impact on our clinical management, as we believe that the best indicator for progression of disease, which would contraindicate exploration and resection, would be distant disease seen on CT scan. Our findings support this contention in that among the 20 evaluable patients, only one case (5%) of metastatic disease was missed on post-treatment/preoperative CT reevaluation. This patient underwent a palliative R2 gastrectomy, because small liver implants were not seen on the preoperative study. Among 18 patients (90%) who had no evidence of disease progression on repeat imaging, all underwent resection with only one palliative R1 resection.

Despite the inaccuracies in clinical staging, as discussed earlier, our findings on final pathologic analysis clearly point to a downstaging effect with neoadjuvant therapy. Before treatment, 85% of the patients were staged as T3 by EUS or LUS, whereas on postoperative pathologic analysis only 3 (16%) of 19 patients were staged as T3, and 4 (21%) of 19 patients had T1 or TIS tumors. In addition, pretreatment clinical staging showed 70% to be stage IIIA, whereas postoperatively only 37% were stage IIIA or greater. Even with the inaccuracy rates cited earlier for the pretreatment imaging staging modalities that we employed, there is still a clear shift toward lower tumor and AJCC stage on final pathologic analysis.

Although demonstrating a downstaging effect is certainly suggestive of the efficacy of this regimen in locally advanced gastric cancer, the ultimate end point is still clinical recurrence and survival. We did not observe any complete pathologic responses in our study. The suggestion that the response to neoadjuvant therapy relates to ultimate survival[35] would imply that whatever our outcome, there is room for improvement if we could induce a complete pathologic response. We are currently assessing our need to add additional therapy in the form of more drugs and/or radiation therapy as a way to improve pathologic responses and downstaging. In addition, our use of adjuvant intraperitoneal therapy in an attempt to control local recurrence will hopefully also improve our survival outcomes. It is too early in this study to make any definitive statements about the long-term effects of this combination therapy. We are encouraged that at a median follow-up of 15 months, we have yet to reach median survival, and we have had only five recurrences: two intra-abdominal and three distant.

CONCLUSION

We have demonstrated that neoadjuvant CPT-11/cisplatin therapy downstages patients with locally advanced gastric cancer. This therapy can be delivered with acceptable toxicity and surgical morbidity. Longer follow-up is needed to determine the ultimate impact of this combination therapy on local control and survival.

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Discussion

Dr. V. Velanovich (Detroit, MI): I have a question about the preoperative or pretreatment laparoscopy. How many patients were excluded because of the laparoscopic findings, and what were the reasons for the exclusion?

Dr. E. Newman: In terms of numbers, I am not sure I can give you an exact number. In general, patients were excluded if they had small metastatic lesions that were not appreciated on preoperative imaging, or if they had earlier lesions that were T1 or T2 on ultrasound. I do not know the exact number out of the total. Because this is a phase II trial, we are evaluating everyone.

Dr. K. Kelly (Scottsdale, AZ): How did you choose the interval between the chemotherapy and the operation? I notice that you had a few postoperative complications. If you had waited longer after the chemotherapy, would the patients have been able to heal better after the operation?

Dr. Newman: That is a good question. It was after discussions concerning our own experiences, and with others, and reviewing reports in the literature on how long patients usually wait after neoadjuvant approaches, that we decided on somewhere in the range of 4 to 6 weeks. We believed that the postoperative morbidity and mortality were comparable to that in our nonprotocol patients. I am not sure we harmed them with a 4-week wait and that a longer wait would have made any difference.

Dr. M. Zenilman (Brooklyn, NY): Is it really fair to compare your staging preoperatively with radiologic and postoperative pathologic findings?

Dr. Newman: That is, by far, the biggest problem.

Dr. Zenilman: Toward that end, I think it may pay to perform a physiologic test of the extent of the cancer, such as positron emission tomography (PET), to determine whether size and nodal status actually do change.

Dr. Newman: PET scanning would be a good idea. That is not something that is readily available to us at the present time, but it is something we have thought about. The point that you raise is by far the major weakness in all of these types of studies, which is the pretreatment to postoperative comparisons.

Dr. V. Fink (Chicago, IL): Do you have any control subjects or did you use historical controls, or what are you controlling this against?

Dr. Newman: Because this is a phase II trial, it is really more an evaluation of our efficacy, and basically the best that we did was compare our preoperative staging to postoperative pathologic findings. We could go back, I suppose, and look at our comparable patients and see whether from a pathologic perspective we had a different group when we were finished. We have not done that.

Dr. C. Pellegrini (Seattle, WA): Do you know if any of these patients had Barrett's esophagus? Is it possible that any of these tumors were esophageal tumors?

Dr. Newman: No, none of our patients had Barrett's esophagus. We excluded anyone who had Barrett's disease. If a patient had a lesion of the gastroesophageal junction, it was not Barrett's disease.

Predictors of Microvascular Invasion in Patients with Hepatocellular Carcinoma Who Are Candidates for Orthotopic Liver Transplantation

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Microvascular invasion affects survival after orthotopic liver transplantation (OLT) for hepatocellular carcinoma (HCC). We sought to identify preoperative predictors of microvascular invasion in patients with HCC who were candidates for OLT. A cohort of 245 patients who underwent resection for HCC and fulfilled the criteria for OLT (i.e., single tumors ≤5 cm or no more than three tumors ≤3 cm) were identified from a multi-institutional database. Thirty-three percent of the patients had pathologic evidence of microvascular invasion. Thirty percent of patients with single tumors and 47% with multiple tumors had microvascular invasion ($P = 0.04$). Only 25% of patients with tumors smaller than ≤2 cm had microvascular invasion, compared to 31% and 50% with tumors greater than 2 to 4 cm or larger than 4 cm, respectively ($P = 0.01$). Tumor grade was highly correlated with microvascular invasion: 12% of patients with well-differentiated tumors had microvascular invasion, compared to 29% and 50% with moderately or poorly differentiated tumors, respectively ($P < 0.001$). The independent predictors of microvascular invasion were tumor size greater than 4 cm (odds ratio [OR], 3.0, 95% confidence interval [CI], 1.2 to 7.1), and high tumor grade (OR, 6.3; 95% CI, 2.0 to 19.9). Tumor size and grade are strong predictors of microvascular invasion. A tumor biopsy with pathologic grading at the time of pretransplantation ablative therapy could improve selection of patients with HCC for OLT. (J GASTROINTEST SURG 2002;6:224–232.)

KEY WORDS: Hepatocellular carcinoma, liver transplantation, vascular invasion

Recent advances in the diagnosis of hepatocellular carcinoma (HCC) and perioperative management after resection have resulted in 5-year survival rates ranging from 17% to 53%.[1,2] The presence of cirrhosis in the adjacent liver is often associated with diminished functional reserve, reduced resectability rates, and decreased overall and disease-free survival.[3–6] In a recent analysis of 159 patients who survived 5 or more years after resection for HCC, the presence of moderate-to-severe fibrosis or cirrhosis was the most powerful predictor of death, overshadowing all other clinicopathologic factors.[7] Recent studies suggest that orthotopic liver transplantation (OLT) is a viable alternative in patients with cirrhosis and HCC, particularly in patients with early-stage malignant disease. In a study by Bismuth et al.,[8] overall survival rates after resection and OLT were similar, but disease-free survival rates at 3 years were higher after OLT (46% vs. 27%), particularly in patients with tumors measuring less than 3 cm (83% vs. 18%). In a recent study by Mazzaferro et al.,[9] the 4-year overall and dis-

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ease-free survival rates after OLT in patients with cirrhosis/HCC and early-stage disease (defined as one tumor ≤5 cm or no more than three tumors, none >3 cm) were 85% and 92%, respectively.[9]

Vascular invasion is a major determinant of outcome after resection or transplantation for HCC.[10–14] None of the patients in the report by Mazzafero et al.[9] had evidence of microvascular or major vascular invasion. In contrast, a recent study by Otto et al.[15] showed that the 3-year survival rate after resection or transplantation for HCC was reduced by more than half in patients with vascular invasion. In another series of 71 patients with HCC treated with OLT, none of the patients who had microvascular invasion were alive at 3 years.[16] Although magnetic resonance imaging and ultrasonography can detect tumor invasion of the major branches of the portal or hepatic veins 81% to 95% of the time,[17–19] the presence of microvascular invasion cannot be established prior to resection or transplantation. Recent figures from the United Network of Organ Sharing indicate that although as many as 17,000 patients are currently awaiting liver transplants, fewer than 5000 patients received transplants in 1999.[20] Given the severe shortage of donor organs, methods to improve the preoperative selection of patients prior to transplantation are needed. The purpose of this study was to identify preoperative predictors of microvascular invasion in patients with HCC who are candidates for OLT.

METHODS

Patients and Clinicopathologic Variables

We identified 591 patients who underwent complete resection for HCC between 1980 and 1998 at four major hepatobiliary centers: The University of Texas M.D. Anderson Cancer Center (Houston, TX), Mayo Clinic (Rochester, MN), Hôpital Beaujon (Paris, France), and Kyoto University Graduate School of Medicine (Kyoto, Japan). Clinical data were reviewed on site by two of us (J.N.V. and D.M.N.). Because we were interested in identifying the clinicopathologic predictors of microvascular invasion in patients with HCC who fulfilled criteria commonly used for transplantation, we limited our analysis to patients with single tumors measuring 5 cm or less or multiple tumors (3 or less), none larger than 3 cm, with information on microvascular invasion (n = 252). In addition, we omitted patients with evidence of major vascular invasion (n = 7). None of the 245 patients in the final cohort had fibrolamellar tumors or positive lymph nodes.

Serologic presence of any hepatitis B antigen or antibody was considered positive evidence of hepatitis B exposure. Serologic presence of hepatitis C antibody was considered positive evidence of hepatitis C exposure. The pathologic material of all patients was reviewed on site by one of us (G.Y.L.). In all centers, hematoxylin and eosin was the stain of choice. A mean of 4.3 sections per tumor were reviewed (range 1 to 21). Tumor size was based on the largest dimension of the tumor specimen. Patients with more than one tumor involving more than one lobe were classified as having multiple bilobar tumors. Minor vascular invasion was defined as either gross or microscopic involvement of the lobar or segmental branches of the portal or hepatic veins. Microscopic vascular invasion was defined by the presence of tumor emboli within the central veins, or the portal or large capsular vessels. Major vascular invasion was defined as gross invasion of the right or left main branches of the portal or hepatic veins.[21] Histopathologic tumor grade was assessed using the scheme outlined by Edmonson and Steiner,[22] and was based on the area showing the highest grade. The hepatitis activity and fibrosis stage of the surrounding parenchyma was scored according to the classification of Ishak et al.[23] It was performed on trichrome-stained slides, available in most cases on sections distant from the tumor to avoid secondary changes due to mass effect.

Statistical Analysis

Age, serum alpha-fetoprotein (AFP) level, and tumor size were treated as both continuous and dichotomous variables, using their respective medians as the breakpoints for statistical analyses. Because of sample size limitations, patients with poorly differentiated and undifferentiated tumors were combined into a single group. The associations between the continuous and the categorical clinicopathologic variables and microvascular invasion were analyzed using Mann-Whitney U tests and chi-square tests, respectively. Univariate prognostic factors ($P \leq 0.1$) were entered into a stepwise logistic regression model to identify the independent predictors of microvascular invasion. Statistical significance was defined as a P value ≤ 0.05. SPSS 10.0 software package (SPSS Inc., Chicago, IL) was used for the statistical analyses.

RESULTS

Patient and Tumor Characteristics

The demographic and clinicopathologic characteristics of the 245 patients in the study cohort are shown in Table 1. The median age was 62 years, and the ma-

jority of the patients were male. Data regarding hepatitis B and C serology were available for 239 and 143 patients, respectively. Among 159 patients with severe fibrosis/cirrhosis (fibrosis score 5 to 6) and Child classification information, 74% of the patients were classified as Child class A, 25% were Child class B, and only one patient was classified as Child class C, reflecting the fact that subjects were drawn from a population of patients referred for resection. The median preoperative AFP level was 40 μg/L. The median tumor size was 3.0 cm, and the majority of patients had single, unilobar tumors. Among the 36 patients with multiple tumors, 30 patients had two tumors and six patients had three tumors. Approximately 15% of the patients had well-differentiated tumors; of the remaining 85%, approximately half had either moderately differentiated or poorly differentiated/undifferentiated tumors. More than half of the patients had a hepatitis activity

Table 1. Patient and tumor characteristics

Characteristic	No microvascular invasion	Microvascular invasion	*P* value‡
Median age*	62 yr (range 28–81)	62 yr (range 43–79)	0.8
Sex*			0.3
Males	120 (70%)	53 (30%)	
Females	44 (63%)	26 (37%)	
Hepatitis B serology*			0.4
Negative	67 (71%)	28 (29%)	
Positive	94 (65%)	50 (35%)	
Hepatitis C serology*			0.3
Negative	65 (70%)	28 (30%)	
Positive	31 (62%)	19 (38%)	
Child class*†			0.4
A	76 (64%)	42 (36%)	
B	28 (70%)	12 (30%)	
C	0	1 (100%)	
Median preoperative alpha-fetoprotein level*	39 μg/L (range 0–19,700)	69 μg/L (range 0–23,700)	0.2
Median tumor size	2.8 cm (range 0.8–5.0)	3.0 cm (range 1.0–5.0)	0.01
0–2	52 (75)	17 (25)	0.01
>2–4	90 (69)	40 (31)	
>4	23 (50)	23 (50)	
Number of tumors			0.04
Single	146 (70)	63 (30)	
Multiple	19 (53)	17 (47)	
Tumor location			0.1
Unilobar	160 (68)	74 (32)	
Bilobar	5 (45)	6 (55)	
Tumor grade*			<0.001
Well-differentiated	28 (88)	4 (12)	
Moderately differentiated	72 (71)	30 (29)	
Poorly differentiated/undifferentiated	43 (50)	43 (50)	
Hepatitis activity score*			0.7
0–4	66 (63)	39 (37)	
5–8	63 (69)	29 (32)	
9–13	23 (68)	11 (32)	
Fibrosis/cirrhosis score*			0.8
0–2 (None-mild fibrosis)	17 (68)	8 (32)	
3–4 (Moderate fibrosis)	33 (70)	14 (30)	
5–6 (Severe fibrosis/cirrhosis)	111 (66)	58 (34)	
Site of institution/treatment			0.3
France	41 (60)	27 (40)	
Japan	104 (71)	43 (29)	
United States	20 (67)	10 (33)	

*Information available for less than 245 patients.
†In patients with severe fibrosis or cirrhosis.
‡*P* value for chi-square or Mann-Whitney U test, as appropriate.

score of 5 or higher, and 70% had evidence of severe fibrosis or cirrhosis (fibrosis score 5 to 6) in the adjacent liver. Approximately 60% of the patients were treated in Japan, whereas 28% and 12% were treated in France and the United States, respectively.

Univariate Predictors of Microvascular Invasion

The univariate predictors of microvascular invasion and their corresponding *P* values are shown in Table 1. Eighty patients in our series (33%) had evidence of microvascular invasion. There was no association between age or sex and microvascular invasion. The percentage of patients with microvascular invasion was not statistically different among patients with positive and negative hepatitis B and C serologies In addition, there was no association between Child classification and microvascular invasion. The preoperative AFP level of patients who had microvascular invasion was slightly higher than that of patients without microvascular invasion (69 μg/L vs. 33 μg/L); however; this difference was not statistically significant ($P = 0.2$).

There was an association between tumor number and microvascular invasion: 47% of patients with two or more tumors had evidence of microvascular invasion compared to 30% of patients with single tumors (Fig. 1; $P = 0.04$). Because of the small number of patients with multiple tumors, we were unable to analyze the association between the actual number of tumors and microvascular invasion. Increasing tumor size was also associated with higher rates of microvascular invasion. Approximately 25% of patients with tumors measuring 2 cm or less had evidence of microvascular invasion, compared to 31% and 50% of patients with tumors measuring >2 to 4 cm, and more than 4 cm, respectively (Fig. 2; $P = 0.01$). The effect of tumor location was also analyzed. Although patients with multiple bilobar tumors were more likely to have evidence of microvascular invasion than patients with unilobar tumors (55% vs. 32%, respectively), this difference was not statistically significant ($P = 0.1$). Because none of the patients in this study had evidence of adjacent organ invasion, we were unable to analyze its relationship with microvascular invasion.

There was no association between hepatitis activity or fibrosis scores and microvascular invasion ($P = 0.7$ and $P = 0.8$, respectively). In contrast, there was a highly significant association between histopathologic tumor grade and microvascular invasion. Only 12% of patients with well-differentiated tumors had microvascular invasion, compared to 29% and 50% of patients with moderately and poorly differentiated tumors, respectively (Fig. 3; $P < 0.001$).

Although 40% of patients treated in France had microvascular invasion, compared to 29% of patients treated in Japan and 33% of patients treated in the United States, this difference was not statistically significant ($P = 0.3$).

Independent Predictors of Microvascular Invasion

The univariate predictors were entered into a stepwise logistic regression model to identify the independent predictors of microvascular invasion (Table 2). Patients with tumors larger than 4 cm were three times more likely to have microvascular invasion than patients with tumors measuring 4 cm or less. Patients with poorly differentiated/undifferentiated tumors were six times more likely to have microvascular invasion than patients with well-differentiated tumors. Patients with multiple tumors or moderately differentiated tumors were 2 and 2.6 times more likely to have microvascular invasion than patients with single or well-differentiated tumors; however, the confidence intervals for these factors failed to reach statistical significance.

The frequency distribution of microvascular invasion according to tumor size and histopathologic grade is shown in Fig. 4. Microvascular invasion was present in 21% of patients with low- or moderate-grade tumors measuring ≤ 4 cm. The percentage of patients with microvascular invasion rose to 40% in patients who had either larger tumors (>4 cm) or high-grade tumors, and to 61% in patients with larger, high-grade tumors ($P < 0.001$).

DISCUSSION

It is estimated that approximately 15% to 45% of patients with hepatitis C will develop HCC within the next 15 years.[24,25] Only 10% to 15% of patients with HCC are candidates for resection at the time of presentation, making OLT the sole treatment option for the remaining patients with disease confined to the liver and limited hepatic reserve.[26] Early transplantation series for HCC often included patients with advanced tumors, which resulted in poor overall and disease-free survival rates.[27] The recognition during the last decade that patients with HCC and cirrhosis stand to benefit the most from transplantation if they have smaller, technically resectable tumors has led to a renewed interest in OLT for HCC.[8,28]

In light of the severe shortage of donor organs, several investigators have attempted to identify clinicopathologic predictors of recurrence and death after transplantation in order to improve the selection

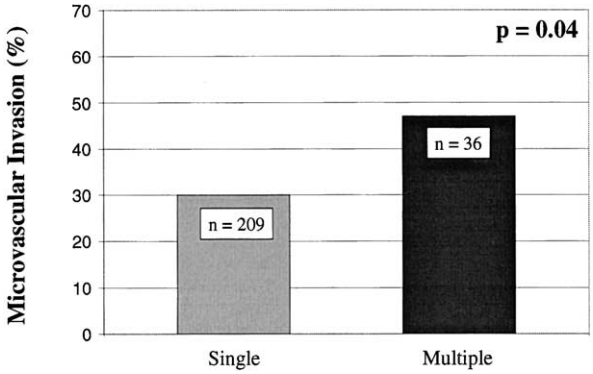

Fig. 1. Frequency distribution of microvascular invasion according to number of tumors.

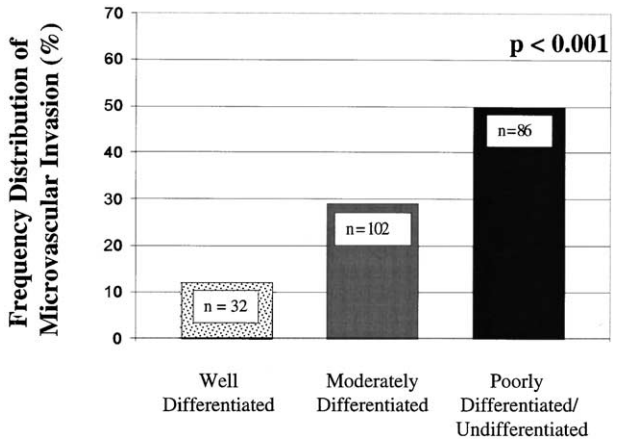

Fig. 3. Frequency distribution of microvascular invasion according to histopathologic tumor grade. *P* value represents the difference among the groups.

of candidates for OLT. Numerous studies have shown that tumor size and tumor number are strong predictors of overall and disease-free survival,[8,9,28] leading many transplant centers to narrow their selection criteria to patients with cirrhosis and single tumors measuring 5 cm or less or fewer than three multiple tumors, none larger than 3 cm. Even with such stringent selection criteria, however, recurrence rates of 8% to 15% and 5-year survival rates of 63% to 71% are still reported.[9,28–32]

Recent studies suggest that vascular invasion is a strong predictor of outcome in patients with HCC treated with OLT. In a recent report by Hemming et al.,[33] the 5-year survival rate of patients without vascular invasion was 68%, compared to 33% in patients with vascular invasion. Tumor size greater than 5 cm, poorly differentiated tumor grade, and vascular invasion were predictors of tumor recurrence on univariate analysis, whereas only vascular invasion was significant on multivariate analysis. In another report of 307 HCC patients receiving transplants, vascular invasion was the most powerful independent predictor of tumor recurrence, followed by tumor size, lymph node status, and lobar distribution.[14] In that study, microvascular and major vascular invasions were associated with a three- to fourfold increased risk of recurrence by Cox multivariate analysis. Recently Iwatsuki et al.[13] reviewed their experience with 344 patients with HCC treated with OLT. Microvascular and major vascular invasions were associated with a 4.4- and 15-fold increased risk of recurrence, respectively.

Because microvascular invasion is a histopathologic diagnosis, it cannot be made prior to removal of the explant liver. Given the fact that microvascular invasion has a significant impact on recurrence and survival after transplantation, preoperative means of

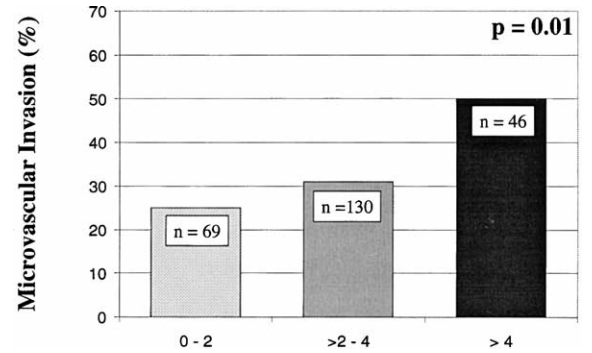

Fig. 2. Frequency distribution of microvascular invasion according to size of largest tumor. *P* value represents the difference among the groups.

Table 2. Independent predictors of microvascular invasion

Prognostic factor	Odds ratio	95% Confidence interval
Tumor size		
0–2 cm	1.0	—
>2–4 cm	1.2	0.6–2.4
>4 cm	3.0	1.2–7.1
Tumor grade		
Well-differentiated	1.0	—
Moderately differentiated	2.6	0.8–8.2
Poorly differentiated/ undifferentiated	6.3	2.0–19.9

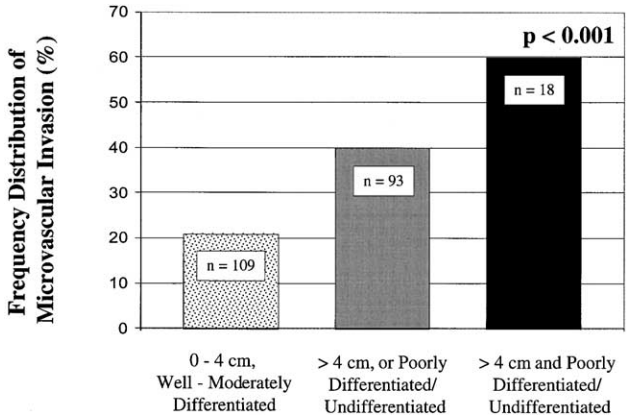

Fig. 4. Frequency distribution of microvascular invasion according to tumor size and histopathologic grade. *P* value represents the difference among the groups.

assessing the probability of microvascular invasion are needed. In our study we attempted to identify the preoperative predictors of microvascular invasion in patients who were potential candidates for OLT. We found that multiple tumors, larger tumors, and higher grade tumors were more likely to be associated with microvascular invasion. In particular, tumor size greater than 4 cm and poorly differentiated/undifferentiated histopathologic tumor grade increased the odds of microvascular invasion by 3- and 6.3-fold, respectively. Although tumor number was a significant predictor of microvascular invasion on univariate analysis, its confidence interval failed to reach statistical significance on multivariate analysis, likely because of confounding by tumor size and sample size limitations. In addition, the fact that only 11 patients had bilobar tumors in our study may have precluded us from finding an association between tumor location and microvascular invasion.

Although only 69% of the patients in this series had severe fibrosis or cirrhosis, and almost all of these patients were classified as either Child class A or B, the prevalence of microvascular invasion in our study was similar to that reported in previous transplantation series.[13–15,34] In addition, there was no association between the degree of fibrosis and microvascular invasion. Therefore we believe that our findings would be equally applicable to patients with more advanced cirrhosis and HCC who are potential candidates for OLT.

Smaller tumor size is associated with superior rates of overall and disease-free survival after transplantation for HCC. Our results suggest that the effect of tumor size on outcome may be partly due to its association with microvascular invasion, particularly in patients with tumors larger than 4 cm. The fact that tumor size was an independent risk factor for recurrence in studies that also controlled for vascular invasion further suggests that size may affect recurrence rates after OLT by other means.[13,14]

Tumor grade is associated with an increased risk of recurrence after OLT for HCC. In a report by Tamura et al.,[34] the disease-free survival after OLT in patients with well-differentiated or moderately differentiated tumors was 68.3%, compared to 40.0% in patients with poorly differentiated tumors. In that study, high tumor grade was independently associated with a threefold increased risk of tumor recurrence. Our data suggest that there is a strong association between poorly differentiated tumor grade and microvascular invasion, which may explain why vascular invasion is often eliminated from multivariate models that also include tumor grade.[34,35]

Given the fact that up to 28% of transplanted patients may have poorly differentiated tumors,[34] and that poorly differentiated tumor grade is associated with microvascular invasion and worse survival after OLT, it would be valuable to assess tumor grade before transplantation. Tumor grade can be determined preoperatively by means of a percutaneous fine-needle biopsy. Histopathologic tumor grade based on needle biopsy has been shown to be an important prognostic parameter in patients with Okuda's stage I tumors.[36] Needle tract recurrence after biopsy for HCC has been reported to occur in 2% to 3.4% of patients treated with resection or OLT.[37–42] In a recent report of 137 patients with HCC treated with resection or OLT at a single institution, ultrasound-guided biopsy using an 18-gauge needle was associated with a needle tract seeding rate of only 1.6%.[42] The authors concluded that in patients with a small nodule and well-compensated cirrhosis, the 1.6% risk of needle tract seeding compared favorably with the 10% risk of death associated with OLT, particularly since many needle tract recurrences were amenable to local excision.

Hepatocellular tumors secrete serum proteins that could serve as surrogate markers of vascular invasion. In a study by Chen et al.,[43] patients with preoperative AFP levels greater than 400 μg/L had an 85% recurrence rate after resection, compared to 55% in patients with levels less than 10 μg/L. Nomura et al.[44] reported an association between increasing tumor size and higher AFP levels, particularly in patients with tumors larger than 5 cm. In that study 24.1% to 40.4% of patients with tumors measuring 5 cm or less had normal AFP levels, whereas 53.2% to 68.5% had only mildly elevated AFP levels (>20 to 1000 μg/L). The current study was limited to patients with tumors measuring 5 cm or less, which may ex-

plain why there was no association between vascular invasion and AFP level.

Although a recent study suggests that serum AFP levels are associated with high tumor grade,[45] previous reports are conflicting. In a series of 683 cases of HCC reported by Okuda et al.,[46] patients with well-differentiated tumors and patients with undifferentiated tumors had lower AFP levels, compared to patients with moderately to poorly differentiated tumors. In our study the median serum AFP level in patients with low-grade tumors was 23 μg/L, compared to 203 μg/L in patients with high-grade tumors ($P < 0.001$). Despite the significant association between serum AFP levels and tumor grade, however, there was no association between AFP levels and microvascular invasion. Other investigators have found no relationship between AFP production by tumors and the degree of vascular invasion.[47]

In a report by Jinno et al.,[48] elevated plasma vascular endothelial growth factor (VEGF) levels were detected in patients with HCC, particularly in patients with metastatic disease. More recently, Poon et al.[49] noted a significant correlation between high serum VEGF levels and microvascular invasion after resection for HCC. Tumor size greater than 5 cm and serum VEGF level greater than 500 pg/ml were associated with 5.8- and 3-fold increased risks of microvascular invasion by multivariate analysis, respectively. In that study, high-grade tumors had slightly higher serum VEGF levels, although the difference was not statistically significant. Unfortunately, tumor grade was not incorporated into the final multivariate model and, therefore, its ability to predict microvascular invasion relative to that of serum VEGF level was not evaluated. Quantitative measurement of the serum VEGF level requires the use of an enzyme-linked immunosorbent assay, and is complicated by the fact that many patients with HCC have chronic hepatitis and cirrhosis, which are also associated with elevated VEGF levels.[48] Future studies should attempt to determine the relative value of tumor size, histopathologic grade, serum VEGF level, and novel molecular markers in predicting the presence of microvascular invasion in patients with HCC treated with resection or OLT.

CONCLUSION

Our study suggests that tumor size greater than 4 cm and high tumor grade could be used as preoperative predictors of microvascular invasion in patients with HCC who are potential candidates for transplantation. Tumor size can be easily and reliably obtained from preoperative imaging studies, whereas a tumor biopsy with histopathologic grading could be easily obtained at the time of diagnosis or pretransplantation ablative therapy (e.g., percutaneous ethanol injection, radiofrequency ablation). This information can be used to identify patients at high-risk for microvascular invasion, and may improve the selection and survival of patients undergoing transplantation for HCC.

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Discussion

Dr. W. Scott Helton (Chicago, IL): That is an excellent cohort of patients and the long-term outcome will be very important with respect to providing useful prognostic information. I am wondering if the presence of microvascular invasion in your patient cohort makes any difference in their long-term outcome, because the published literature up until now has shown that microvascular invasion has no impact on long-term survival after transplantation.

Dr. N. F. Esnaola: This population, as I stated, includes patients who underwent complete resection, not transplantation, but in this population the difference in survival was approximately half of what is achieved in patients without microvascular invasion.

Dr. Steven M. Strasberg (St. Louis, MI): Congratulations. I think this is a very important study with a large number of patients. To be used clinically, what one would like is a tool that can predict microvascular invasion in individual patients. If detected in individual patients with confidence, one might employ neoadjuvant therapy or perhaps not operate at all if indeed it was an overwhelming prognostic factor. So my question is, did you use some form of discriminate analysis or some other test to determine how well the factors that you have identified predicted microvascular invasion in individual patients?

Dr. Esnaola: Yes we did. If you assume that patients with tumors larger than 4 cm or high-grade tumors have microvascular invasion, the sensitivity of this rule would be 70% and the negative predictive value would be 79%. So you would be correct in about 80% of the patients if you decided to perform a transplant in patients with those criteria.

It is a little dangerous to make prediction rules based on two factors, and I think it is more important to look at other factors. Recently there have been some data showing that VEGF levels predict microvascular invasion. Unfortunately, in that study, tumor grade was not included in the multivariate analysis. So it is difficult to know whether VEGF would add information to the factors presented here.

Ninety-Five Cases of Intestinal Transplantation at the University of Miami

Seigo Nishida, M.D., Ph.D., David Levi, M.D., Tomoaki Kato, M.D., Jose R. Nery, M.D., Naveen Mittal, M.D., Nicholas Hadjis, M.D., Juan Madariaga, M.D., Andreas G. Tzakis, M.D., Ph.D.

Intestinal failure requiring total parenteral nutrition (TPN) is associated with significant morbidity and mortality. Intestinal transplantation can be a lifesaving option for patients with intestinal failure who develop serious TPN-related complications. The aim of this study was to evaluate survival, surgical technique, and patient care in patients treated with intestinal transplantation. We reviewed data collected from 95 consecutive intestinal transplants performed between December 1994 and November 2000 at the University of Miami. Fifty-four of the patients undergoing intestinal transplantation were children and 41 were adults. The series includes 49 male and 46 female patients. The causes of intestinal failure included mesenteric venous thrombosis (n = 12), necrotizing enterocolitis (n = 11), gastroschisis (n = 11), midgut volvulus (n = 9), desmoid tumor (n = 8), intestinal atresia (n = 6), trauma (n = 5), Hirschsprung's disease (n = 5), Crohn's disease (n = 5), intestinal pseudoobstruction (n = 4), and others (n = 19). The procedures performed included 27 isolated intestine transplants, 28 combined liver and intestine transplants, and 40 multivisceral transplants. Since 1998, we have been using daclizumab (Zenepax) for induction of immunosuppression and zoom videoendoscopy for graft surveillance. We began to use intense cytomegalovirus prophylaxis and systemic drainage of the portal vein. The 1-year patient survival rates for isolated intestinal, liver and intestinal, and multivisceral transplantations were 75%, 40%, and 48%, respectively. Since 1998, the 1-year patient and graft survival rates for isolated intestinal transplants have been 84% and 72%, respectively. The causes of death were as follows: sepsis after rejection (n = 14), respiratory failure (n = 8), sepsis (n = 6), multiple organ failure (n = 4), arterial graft infection (n = 3), aspergillosis (n = 2), post-transplantation lymphoproliferative disease (n = 2), intracranial hemorrhage (n = 2), and fungemia, chronic rejection, graft vs. host disease, necrotizing enterocolitis, pancreatitis, pulmonary embolism, and viral encephalitis (n = 1 case of each). Intestinal transplantation can be a lifesaving alternative for patients with intestinal failure. The prognosis after intestinal transplantation is better when it is performed before the onset of liver failure. Rejection monitoring with zoom videoendoscopy and new immunosuppressive therapy with sirolimus, daclizumab, and campath-1H have contributed to the improvement in patient survival. (J GASTROINTEST SURG 2002;6:233–239.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Intestinal transplantation, intestinal failure, short bowel syndrome

Intestinal transplantation has evolved as a therapeutic option for selected patients with intestinal failure who have developed serious complications from total parenteral nutrition (TPN). The most common complications are cholestatic liver failure and complications related to vascular access. The earliest experimental intestinal transplants were performed in 1902.[1–3] The first clinical intestinal transplants were performed in the 1960s. However, rejection of the graft and sepsis posed what seemed to be insurmountable barriers to success. The introduction of cyclosporine led to the first successful intestinal transplants in pigs in 1988.[4] Also that year, the first successful human isolated intestinal transplant was reported.[5,6] In 1990 the first successful combined liver-intestinal transplant plus cyclosporine was reported.[7] Intestinal transplantation with the addition of tacrolimus was initiated at the University of Pittsburgh in the early 1990s.[8] Immunosuppression, graft surveillance for rejection, and surgical techniques have continued to evolve with promising results. This report describes our experience with intestinal transplantation in 95 consecutive patients.

Presented at the Forty-Second Annual Meeting of The Society for Surgery of the Alimentary Tract, Atlanta, Georgia, May 20–23, 2001 (oral presentation).

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MATERIAL AND METHODS

Patients

The indications for intestinal transplantation include intestinal failure with serious TPN-related complications including liver dysfunction and threatened central vein access. Many patients with intestinal failure can do well on TPN. However, some develop irreversible cholestatic liver disease. Others suffer from repeated complications with the central line, including thrombosis and sepsis. The causes of the intestinal failure are presented in Table 1. All patients had TPN-related complications including 67 patients with liver failure. Candidates for intestinal transplantation require careful attention. Early referral, detailed TPN history, detailed medical and surgical histories, and radiographic studies documenting gastrointestinal and vascular anatomy are essential. Results of liver function tests need to be evaluated carefully. A liver biopsy may be necessary to determine the degree of injury. If irreversible liver injury exists, the graft should include the liver. An evaluation of renal function is also important. In cases of poor renal function, the kidney can be included as part of the graft.

Surgical Technique

Donor Operation. At the time of organ procurement, the surgeon recovering the intestinal graft should discuss the procedure with any organ procurement surgeons present. If the intestinal graft does not include the liver, the portal vein is cut above the splenic vein confluence and the superior mesenteric artery is cut with an aortic cuff. If a right replaced hepatic artery exists, the superior mesenteric artery is cut distal to the replaced hepatic artery. It is possible to separate the small intestine graft from the pancreas in the cadaver if both are to be used for different recipients. For the multivisceral graft, both the celiac and superior mesenteric arteries are taken with the descending thoracic aorta and the abdominal aorta. The graft is flushed with cold University of Wisconsin solution through a cannula placed in the distal aorta. Cold ischemic time (CIT) should be minimized to prevent graft injury.

Table 1. Causes of intestinal failure in 95 patients requiring intestinal transplantation

Causes of intestinal failure	No. of patients
Mesenteric thrombosis	12
Necrotizing enterocolitis	11
Gastroschisis	11
Volivulus	9
Desmoid tumor	8
Intestinal atresia	6
Trauma	5
Hirschsprung's disease	5
Crohn's disease	5
Pseudoobstruction	4
Others	19

Recipient Operation. We performed three distinct recipient operations in this series. The isolated intestinal transplant includes only the small intestine. The arterial anastomosis is usually performed between the superior mesenteric artery of the donor and the superior mesenteric artery of the recipient with or without an interposition graft. The arterial graft can be placed to the supraceliac or infrarenal aorta of the recipient. The venous outflow of the isolated intestinal graft can be to the recipient portal vein (portal drainage) or to the inferior vena cava (systemic drainage).

Combined liver and intestine transplantation includes the liver and the small intestine as a composite graft. Arterial inflow is usually achieved by anastomosing the donor descending thoracic aorta and the recipient aorta in an end-to-side fashion. The caval anastomosis is usually performed in an end-to-side fashion, joining the donor suprahepatic cava with the confluence of the recipient hepatic veins ("piggyback" technique).

The multivisceral graft includes the small intestine, stomach, and pancreas, usually with the liver, with or without the kidney. The arterial and caval anastomoses are the same as for the combined liver and intestinal transplant. The proximal end of the donor intestine in the isolated intestinal and combined liver and intestine transplant are anastomosed side to side to the distal end of the native small intestine. In the multivisceral transplant, the proximal enteric anastomosis is performed between the donor stomach and the recipient esophagus.

A catheter for enteral feeding access is placed in the jejunum or stomach. The distal end of the small intestine is exteriorized as a stoma to decompress the bowel and to allow access for monitoring. If the native rectosigmoid colon is present, we prefer to perform an anastomosis between the colon and the distal small intestine just proximal to the terminal ileostomy.

If the graft is larger than the recipient abdominal cavity, a portion of the graft liver and/or intestine can be resected. Abdominal closure may require mesh and/or staged approximation. Skin flaps, muscle flaps, and synthetic mesh can be used in cases where a discrepancy exists.

Postoperative Care

After intestinal transplantation, patients require monitoring in the intensive care unit. Fluid resuscitation and replacement are important in the early postoperative period because of interstitial fluid loss and stomal output. Baseline immunosuppression is initiated with tacrolimus and corticosteroids. Tacrolimus trough levels are maintained at 15 to 20 ng/ml with intravenous and/or enteral administration. Daclizumab is used for induction therapy. Sirolimus has been used for patients who have experienced complications from tacrolimus. Some patients are placed on a combined low-dose tacrolimus and rapamycin regimen. Campath-1H without steroids for induction therapy is an ongoing protocol. Prophylaxis against cytomegalovirus (CMV) is mandatory. We employ a regimen consisting of anti-CMV immune globulin (Cytogam) and intravenous ganciclovir. Since 1997, we have been using an intense CMV prophylaxis protocol. Cytogam (100 mg/kg) is administered every other day for 1 month and every 2 weeks for 3 months. Antifungal and antibacterial prophylactic antibiotics and gut decontamination are administered.

Enteral feeding is begun approximately 4 to 7 days after the transplant to maintain mucosal integrity and function. An elemental diet is given initially. Tube feedings are increased slowly up to the calculated goal. Oral intake is begun at approximately 2 weeks for the isolated intestinal recipient and at approximately 4 weeks for the multivisceral recipient. TPN is slowly tapered off. The stoma is closed after 6 to 12 months.

Monitoring for signs of rejection is begun on postoperative day 3. We perform frequent endoscopic assessments and directed mucosal biopsies using a magnifying, flexible zoom videoendoscope.[9] Clinical signs of rejection, such as low stomal output, diarrhea, and fever, are also very important. Before 1998, indications for endoscopy were dependent on the clinical sign of acute rejection. Since 1998, we have used a protocol that includes endoscopy to monitor for signs of rejection. After transplantation, endoscopy is performed on postoperative day 3. Then it is performed twice a week for 1 month and once a week for 3 months. If ongoing rejection exists, endoscopy is performed every other day until the rejection resolves. Rejection of the graft is treated without delay.

Statistical Analysis

Data were collected prospectively from 95 consecutive patients undergoing intestinal transplantation performed between December 1994 and November 2000 at the University of Miami. This database is the basis for this review. Patient and graft survival estimates were generated using the Kaplan-Meier product limit method. The log-rank test was performed for survival analysis. Graft failure was defined as having occurred upon graft removal or patient death. For numeric data, results are expressed as mean ± SD. Nonparametric data are compared using Fisher's direct test and chi-square analysis. $P < 0.05$ was considered significant.

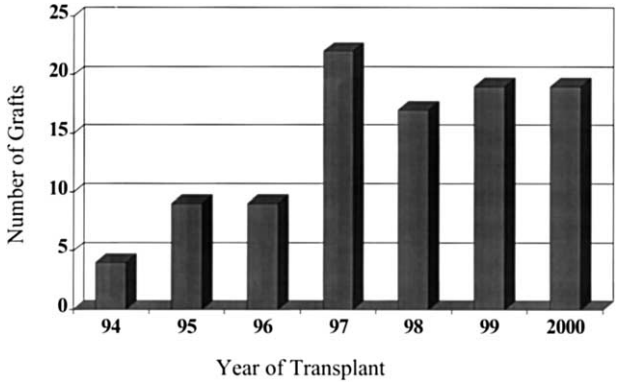

Fig. 1. Number of intestinal transplants per year from 1994 to 2000.

RESULTS

A total of 95 transplants were performed in 87 patients. The causes for intestinal failure were as follows (see Table 1): mesenteric thrombosis (n = 12), necrotizing enterocolitis (n = 11), gastroschisis (n = 11), volvulus (n = 9), desmoid tumor (n = 8), intestinal atresia (n = 6), trauma (n = 5), Hirschsprung's disease (n = 5), Crohn's disease (n = 5), intestinal pseudoobstruction, (n = 4), and various other causes (n = 19). All patients experienced TPN-related complications including 67 patients with liver failure. The

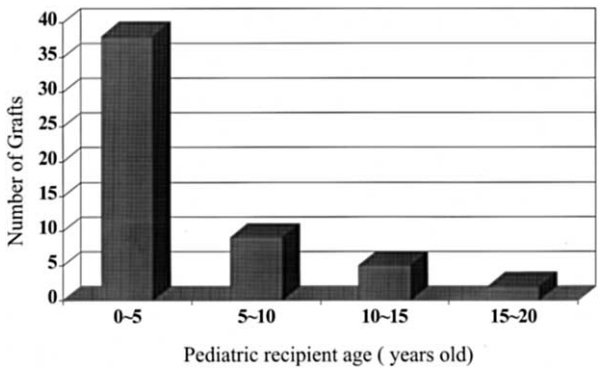

Fig. 2. Number of pediatric intestinal grafts vs. age of patients.

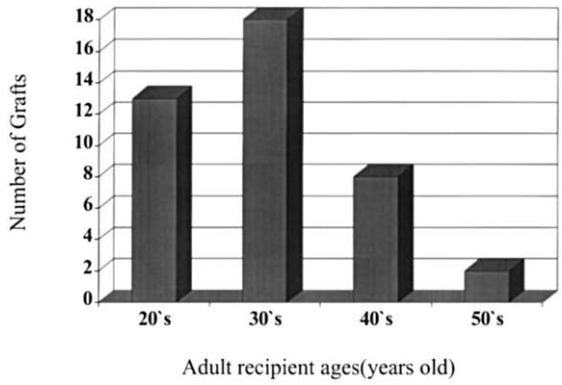

Fig. 3. Number of adult intestinal grafts vs. age of patients.

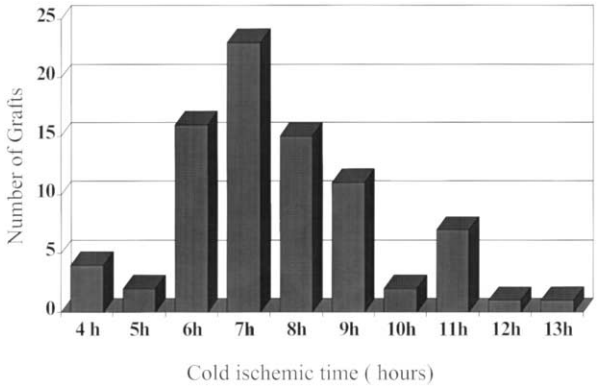

Fig. 5. Number of grafts vs. cold ischemic time of the transplants.

number of cases per year has increased since 1994 (Fig 1). Sex distribution was approximately equal with 49 male patients (52%) and 46 female patients (48%). There were 54 pediatric patients (57%) and 41 adult patients (43%). The age distribution for pediatric and adult recipients is shown in Figs. 2 and 3. Most of the pediatric patients were less than 5 years of age. The oldest recipient was 53 years of age.

All of the grafts were obtained from cadaveric donors. The sex distribution of the donors was 56 males (59%) and 39 females (41%). The donor age distribution is presented in Fig 4. Donor selection criteria included an ABO identical blood type, an appropriate graft size, and negative serology for human immunodeficiency virus (HIV) and hepatitis viruses. We employ a regimen consisting of anti-CMV immune globulin (Cytogam) and intravenous ganciclovir. Cytogam was given every 2 weeks for 4 months (group A; n = 39, nine episodes of CMV infection). Since 1997, Cytogam has been given every other day for 1 month and every 2 weeks for 3 months (group B; n = 56, four episodes of CMV infection). There is a significant difference in the occurrence of CMV infection between the groups ($P = 0.0317$). Since 1997 we have routinely used grafts from CMV-positive donors. The CMV status of the donors and recipients are as follows: donor positive/recipient positive (n = 21), donor positive/recipient negative (n = 27), donor negative/recipient positive (n = 21), and donor negative/recipient negative (n = 26). There is no difference in the occurrence of CMV infection between the groups ($P = 0.86138$). All grafts were preserved with cold University of Wisconsin solution. Our mean CIT was 480 ± 12.3 minutes. The longest CIT was 13 hours. The CIT distribution is presented in Fig. 5.

The types of grafts transplanted were isolated intestine in 27 cases (28%), combined liver and intestine in 28 cases (29%), and multivisceral grafts including the stomach, pancreas, intestine, and/or liver in 40 cases (43%). Four mutivisceral grafts did not include the liver.

The 1-year patient and graft survival rates for isolated intestine transplantation performed between 1994 and 1997 (group 1, n = 10) were 64% and 63%, respectively. The 1-year patient and graft survival rates for isolated intestinal transplantation since 1998 (group 2, n = 16) were 84% and 72%, respectively (Fig. 6). There is not a statistically significant difference between the two groups (patient survival $P = 0.26156$; graft survival $P = 0.20895$). However, the patient and graft survival rates for group 2 showed a tendency to improve. Since 1998, we have been using zoom videoendoscopy and daclizumab induction. The incidence of severe rejection has decreased since 1998. The 1-year patient and graft survival rates for combined liver and intestine transplantation were 40% and 37%, respectively (Fig. 7). For multivisceral recipients, the 1-year patient and graft survival

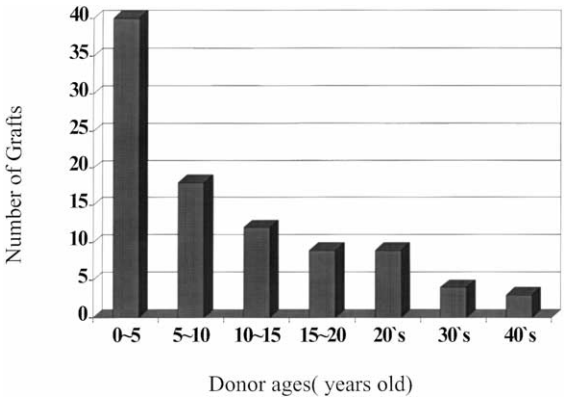

Fig. 4. Number of intestinal transplants vs. donor age.

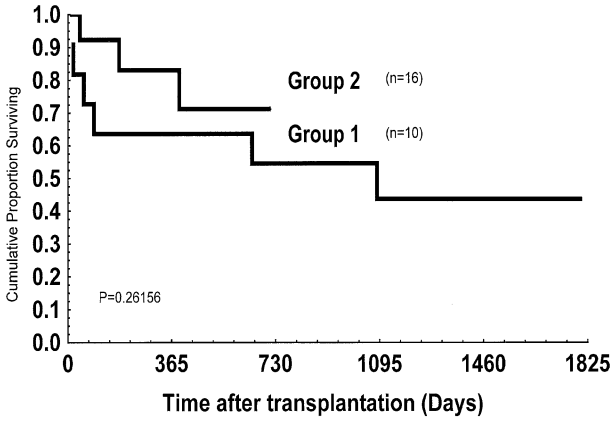

Fig. 6. Kaplan-Meier patient survival for isolated intestinal transplants before and after 1998 (group 1 = 1994–1997; group 2 = 1998–2000).

rates were 48% and 40%, respectively. Preliminary results with the use of Campath-1H are excellent. We have used it for 10 patients. All 10 patients are alive and doing well with no major signs of rejection or episodes of infection (follow-up of 14 to 270 days).

Causes of death included the following: sepsis after rejection (n = 14), respiratory failure (n = 8), sepsis (n = 6), multiple organ failure (n = 4), arterial graft infection (n = 3), aspergillosis (n = 2), post-transplantation lymphoproliferative disease (n = 2), intracranial hemorrhage (n = 2), and fungemia, chronic rejection, graft vs. host disease, necrotizing enterocolitis, pancreatitis, pulmonary embolism, and viral encephalitis (n = 1 case of each).

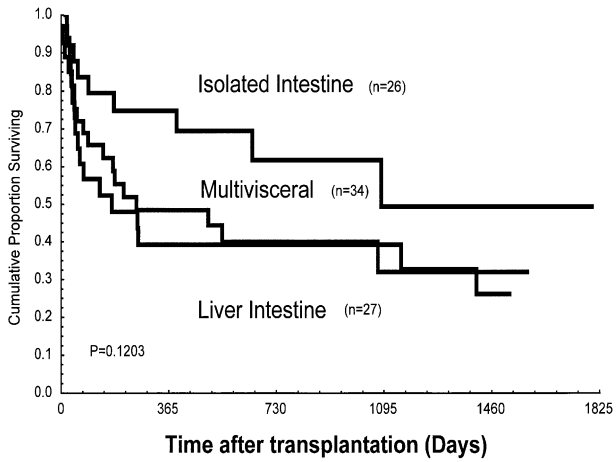

Fig. 7. Kaplan-Meier patient survival for three different types of intestinal transplants.

DISCUSSION

Intestinal transplantation can be a lifesaving alternative for patients with intestinal failure. Moreover, it may be the only option for patients with exhausted central venous access or for patients with TPN-induced liver failure. On the other hand, at this stage in the development of intestinal transplantation, a very fine balancing act must be exercised to ensure that a patient with chronic intestinal failure does not become debilitated, with no quality of life, as against the risk of embarking too early on a procedure that is still associated with considerable morbidity and carries survival rates inferior to those of other organ transplants. A detailed TPN history should be obtained from all prospective transplant patients, as well as medical and surgical histories. Radiographic studies documenting gastrointestinal and vascular anatomy are essential. Liver function should be evaluated carefully. A liver biopsy may be necessary to determine the degree of TPN-induced injury. If the injury is irreversible, the graft should include the liver. Evaluation of renal function is also important. Severe renal impairment qualifies the patient to have a kidney included as part of the graft.

The quality of the harvested organs determines substantially the outcome of the entire transplant endeavor. To that extent, close cooperation among the teams harvesting different organs and a well-defined plan of action are important steps. Anatomic mastery ensures the integrity of the grafts and their vascular channels, a prerequisite for a successful outcome. The liver, pancreas, and small intestine can be removed and transplanted separately.[10] The anatomy of the artery and portal vein are the most important issues. The liver graft needs all of the hepatic artery and portal vein. The pancreas graft needs the inferior pancreatoduodenal artery, splenic artery, splenic vein, portal vein, and inferior pancreaticoduodenal vein. The intestinal graft needs the superior mesenteric artery and superior mesenteric vein.

We performed three distinct recipient operations in this series. Although the differences in survival are not significant ($P = 0.1203$), there is a trend toward improved survival in the isolated intestine group. There is no definite explanation for the differences in survival rates among the types of operations. When the other organs were transplanted with the liver, immunologic protection of them by the liver is known.[11,12] However, our clinical results showed a tendency toward better survival without the liver graft. Chronic rejection may be the issue for patients undergoing isolated intestinal transplantation. Long-term follow-up will be necessary to clarify this issue. We believe that with improved immunosuppression and re-

jection monitoring, the prognosis is better when the transplant is performed before the onset of liver failure.

The venous outflow of the isolated intestinal graft can be to the recipient portal vein (portal drainage) or to the inferior vena cava (systemic drainage). Although there are sound physiologic reasons in favor of restoring anatomic vascular inflow, and venous outflow in particular, this is not always possible. On the other hand, in terms of the venous outflow for recipients of isolated intestine and liver-intestine grafts, systemic drainage to the host vena cava is technically easier and safer than undertaking portal drainage. Indeed, our ongoing study comparing patients with systemic drainage (n = 11) vs. portal drainage (n = 14) has thus far shown no difference in terms of metabolic and immunologic parameters. However, long-term follow-up is needed to ascertain any effects on growth in pediatric patients, cognitive function, and the incidence of infection and graft rejection.

Baseline immunosuppression is initiated with tacrolimus and corticosteroids. Tacrolimus trough levels are maintained at 15 to 20 ng/ml with intravenous and/or enteral administration. Sirolimus has been used in patients with complications from tacrolimus. However, until the issue of a sirolimus-induced thrombotic state is resolved, we would avoid its use early in the postoperative period. Some patients are on a combined low-dose regimen of tacrolimus and sirolimus. Daclizumab has been used for induction therapy. Recently we started to use campath-1H with low-dose tacrolimus for induction therapy, without steroids. Campath-1H is an antibody that is specific for the common lymphocyte and monocyte antigen CD52. It temporarily depletes all mature lymphocytes and monocytes without altering neutrophils or hematopoietic stem cells.[13] Ten patients have been enrolled, and the preliminary results, both in terms of the frequency and severity of rejection as well as the incidence of septic complications, have thus far been very encouraging (follow-up 14–270 days).

Rejection remains the "Damocles sword" of intestinal transplantation. Clinical signs are often absent during the early stage. Therefore we have addressed this problem by utilizing endoscopic surveillance with a zoom scope. A semiquantitative scoring system has been devised that factors in the height of the villi, the shape of their tips, any bleeding in the villi, the friability of the mucosa, and the presence of background erythema. Endoscopic graft assessment is quick, repeatable, and can be done at the bedside without sedation or monitoring. Indeed, in our experience, this method has allowed us to not only detect and treat rejection earlier but also prevents "overimmunosuppression" with its well-documented deleterious effects. An ongoing study is attempting to correlate rejection, as predicted by this system, with histologically confirmed rejection. More recently, a study of citrulline as a serum marker for early acute cellular rejection has been undertaken in our program, with promising preliminary data.[14] Bolus intravenous steroids and a cycle of steroids followed by a taper off over several days are the standard treatment for rejection. OKT3 is used in cases of steroid-resistant rejection. Sirolimus is added for persistent rejection. In our experience, intestinal grafts with mild or moderate acute rejection can be rescued if they are treated immediately.

Enterocytes have been studied at our institution. Our study demonstrated the presence of host-derived (male) enterocytes in the intestinal allografts (female).[15] This might assist in the development of novel strategies to increase intestinal absorptive surface and repair, and to engineer neointestine for patients with short bowel syndrome.

Infections are another major problem encountered after intestinal transplantation. The spectrum of infection includes line sepsis, wound infection, intra-abdominal abscess, pneumonia, urinary tract infection, sepsis during rejection, fungal infection, and viral infection. During an episode of rejection, bacterial translocation and increased immunosupression can lead to sepsis. Gram-negative organisms are frequently involved. Overimmunosuppression, in general, is an important risk factor for septic complications. Indwelling catheters and lines need to be monitored, kept clean, and should be removed as soon as they are not essential. Common bacteria isolated in catheter-related infections include *E. coli*, *E. facium*, *Staphylococcus*, *Klebsiella*, *Proteus*, *P. aeruginosa*, and *E. cloacae*.[16] Fungal infections due to *Aspergillus* and *Candida* are also common. The risk of viral infection is ever present. Prophylaxis with ganciclovir and Cytogam has drastically reduced the incidence of CMV infection. Under our CMV prophylaxis protocol, there was no difference in the incidence of CMV infection between recipients of grafts from CMV-positive donors and those from CMV-negative donors. Based on these data, we have continued to use donors irrespective of their CMV status. Epstein-Barr virus infection is often associated with post-transplant lymphoproliferative disease. In keeping with our recent experience, the incidence of post-transplant lymphoproliferative disease in our patient population has decreased substantially in recent years. In pediatric patients, adenovirus may be the cause of severe pneumonia and enteritis. On the other hand, infection with the respiratory syncytial virus should always be considered in the differential diagnosis of pneumonias in this population. We encountered three cases of arterial graft

infection with anastomotic dehiscence and hemorrhage. Careful attention to aseptic technique during the donor and recipient operations is critical. Intestinal anastomotic leaks were observed in two patients, at the esophagogastric and ileocolic anastomosis, respectively. Both patients recovered very well. Postoperative bleeding was encountered in some patients, usually in the face of liver dysfunction and thrombocytopenia. Postoperative bleeding always required an exploratory operation.

Many patients with chronic intestinal failure have renal dysfunction before the transplant. Nephrotoxic drugs and a variety of renal insults may lead to renal failure postoperatively, which requires hemodialysis and in some cases kidney transplantation. Careful selection and judicious use of antibiotics and immunosuppressive agents helps to preserve renal function. In our experience, reducing stoma losses by means of a "chimney-type" ileostomy with primary anastomosis of the small bowel to the colon, whenever possible, considerably lessens the risk of dehydration and helps preserve renal function.

Survival after intestinal transplantation is slowly but steadily improving. Although there are no statistically significant differences in survival rates among the three types of intestinal transplants, the 1-year patient and graft survival rates for isolated intestinal transplants performed in our program since 1998 were 84% and 72%, respectively. In 1998 we began using the zoom videoendoscope and daclizumab induction. Patient survival rates for each type of transplant are presented.

Recently we have noted many changes in intestinal transplantation. Improvement in surgical techniques, CMV prophylaxis, graft surveillance, and immunosuppression have all contributed to improved patient and graft survival. Undoubtedly, continued efforts will contribute to future success.

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Role of Extrinsic Innervation in Jejunal Absorptive Adaptation to Subtotal Small Bowel Resection: A Model of Segmental Small Bowel Transplantation

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Segmental small bowel transplantation offers theoretic advantages over total jejunoileal transplantation, but the regional ability of the transplanted segment to adapt is unknown. Absorption was measured in an 80 cm jejunal segment via a triple-lumen perfusion technique. Separate experiments measuring absorption of four nutrients (glucose, glutamine, oleic acid, and taurocholic acid) were performed before and 2 and 12 weeks after operative intervention. Control dogs (CON, n = 6) underwent distal 50% enterectomy. Experimental dogs (EXT DEN, n = 6), in addition to resection, underwent complete extrinsic denervation of the remaining jejunum. All dogs developed diarrhea, which rhesolved in all CON dogs but persisted in all EXT DEN dogs. Maximal weight loss was greater in the EXT DEN group. Glucose and oleate absorption was decreased 2 weeks after ileal resection in both the CON and EXT DEN dogs; glutamine absorption was decreased at 2 weeks in EXT DEN dogs only. Taurocholate and water absorption remained unchanged in both groups. Absorption of all solutes returned to baseline at 12 weeks in both groups. Despite greater weight loss and persistent diarrhea in EXT DEN dogs, at 12 weeks there were no differences in net absorptive fluxes between the EXT DEN and the CON group after extrinsic denervation. The extrinsic denervation necessitated by small bowel transplantation does not appear to blunt the net jejunal adaptive response to total ileal resection, but may temporarily alter glutamine absorption. (J GASTROINTEST SURG 2002;6:240–247.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Intestinal adaptation, extrinsic denervation, small bowel transplantation, glucose absorption, fat absorption, glutamine absorption, bile acid absorption

Small bowel transplantation (SBT) has now become a clinical reality;[1,2] however, multiple factors limit the success and widespread use of SBT. Limitations include surgical complications, acute and chronic rejection, graft-vs.-host disease, and serious post-transplant infections and lymphoproliferative disorders. The concept of segmental SBT offers several advantages over the current standard of whole small bowel grafts obtained from cadaveric donors.[3] A segmental transplant offers decreased total antigen load, while potentially considerably increasing organ availability, as has happened with living-related or living-unrelated kidney and split-liver transplants. Furthermore, this concept offers the potential for a living-related donor, which may lead to an increased role for human leukoctye antigen matching, preoperative immunomodulation, more thorough preoperative evaluation of both recipient and donor, decreased waiting time to receive the transplant (with less malnutrition) and, finally, the opportunity for an elective, staged operative procedure, with the attendant benefit of decreased warm and cold ischemic times.[3] Experimental studies in rats have shown the feasibility of segmental SBT in maintaining nutrition and supporting growth.[4] However, although it is well established that under normal conditions a previously healthy patient can maintain an adequate nutritional state with greater than 50% intestinal resection, the functional ability of a trans-

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planted (and obligately denervated) segment of intestine and the minimum length needed in humans remains poorly defined. In addition, concerns remain also for the living donor.

The aim of this study was to determine the role of extrinsic innervation in the functional adaptive response of the jejunum in a large animal model of moderate short bowel syndrome. Our goals were both of physiologic interest, extending our laboratory's investigations into the role of the extrinsic nervous system in controlling and/or modulating enteric function, as well as of clinical relevance in that we investigated absorptive function in a large animal model of segmental intestinal (auto)transplantation devoid of immune considerations and ischemia/reperfusion injury. Previous work in our laboratory examining absorptive function showed that extrinsic denervation of the entire jejunoileum induced an early diarrhea that resolved 6 to 8 weeks postoperatively,[5–11] suggesting an adaptive response. Based on these previous studies, we hypothesized that early net absorptive function of the denervated jejunal remnant would be less efficient than a neurally intact remnant of similar length. We further hypothesized that the jejunal adaptive response to subtotal distal small bowel resection would be blunted in the extrinsically denervated bowel. We used our previously validated canine model of intestinal autotransplantation, because it is devoid of confounding factors of immune phenomena and ischemia/reperfusion injury, and permits study of absorptive function of the extrinsically denervated gut.

MATERIAL AND METHODS

Overall Design

Two groups of six dogs each underwent baseline in vivo experiments of jejunal absorption of four simple nutrients (glucose, glutamine, taurocholate, and oleic acid). After completing baseline studies, both groups underwent resection of the distal 50% of the small bowel; one group (CON) remained neurally intact, whereas the other group (EXT DEN) underwent complete extrinsic denervation of the remaining jejunum. Both groups were restudied 2 and 12 weeks postoperatively with identical experiments of absorption in vivo.

Animal Preparation

Surgical procedures, postoperative care, and subsequent conduct of experiments were performed with

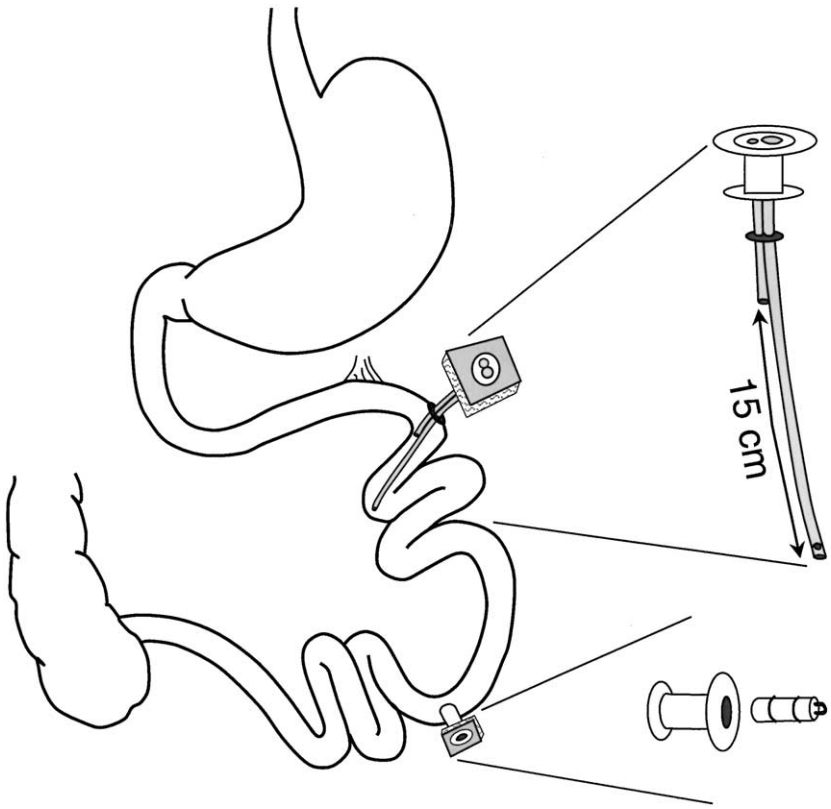

Fig. 1. Insertion site of perfusion catheter system in the jejunum. Inserts detail the proximal infusion and proximal aspiration site and the distal cannula.

approval from the Animal Care and Use Committee of the Mayo Foundation in accordance with the guidelines of the National Institutes of Health and the Public Health Service policy on the humane use and care of laboratory animals. All dogs were fasted for 12 hours before the surgical procedures were begun.

Catheter and Cannula Insertion

Twelve healthy female mongrel dogs (weight 14 to 22 kg) were anesthetized with intravenous sodium methohexital induction (12.5 mg/kg intravenously) and maintained on inhaled halothane (Ayerst Laboratories, New York, NY). After randomization to one of two groups, dogs underwent a midline celiotomy and placement of a jejunal infusion catheter (internal diameter 1.5 mm), a proximal jejunal aspiration catheter (internal diameter 3.0 mm), and a modified Thomas cannula (internal diameter 1 cm) at 25 cm, 40 cm, and 120 cm from the ligament of Treitz, respectively. The proximal ends of the catheters were cemented in a metal cannula; this cannula and the modified Thomas cannula were exteriorized (Fig. 1). This configuration allows for intrajejunal infusion, a 15 cm mixing segment, and an 80 cm study segment similar in principle to our previous studies.[7–11] For the first 3 days, dogs were given intramuscular butorphanol for pain control and maintained on parenteral fluids and electrolytes before ad libitum feeding was allowed. Baseline absorptive experiments (see below) were performed after allowing 2 weeks for recovery. At this stage, both groups were comparable—that is, all dogs had a complete length of neurally intact small intestine.

Small Bowel Resection. After the baseline experiments were completed, a second celiotomy was performed, and all dogs underwent resection of the distal 50% of the jejunoileum, maintaining the last 3 cm of ileum (preserving the ileocecal junction) (Fig. 2, *A*). Intestinal continuity was restored with an end-to-end, hand-sewn jejunoileostomy. This procedure creates a moderate short bowel syndrome with weight loss and diarrhea but without death.[12–14] To control for the effects of disrupting proximal continuity of the enteric nervous system in our model of complete extrinsic denervation (see below), the CON dogs also underwent transection and reanastomosis of the proximal jejunum, just distal to the ligament of Treitz (Fig. 2, *B*). All extrinsic neural innervation to the remaining jejunum was carefully maintained.

Previously randomized EXT DEN dogs, in addition to undergoing distal 50% enterectomy, also underwent our model of in situ neural isolation of the remaining jejunoileum, as described previously.[7–11] This preparation involves complete extrinsic denervation as well as disruption of intrinsic neural and

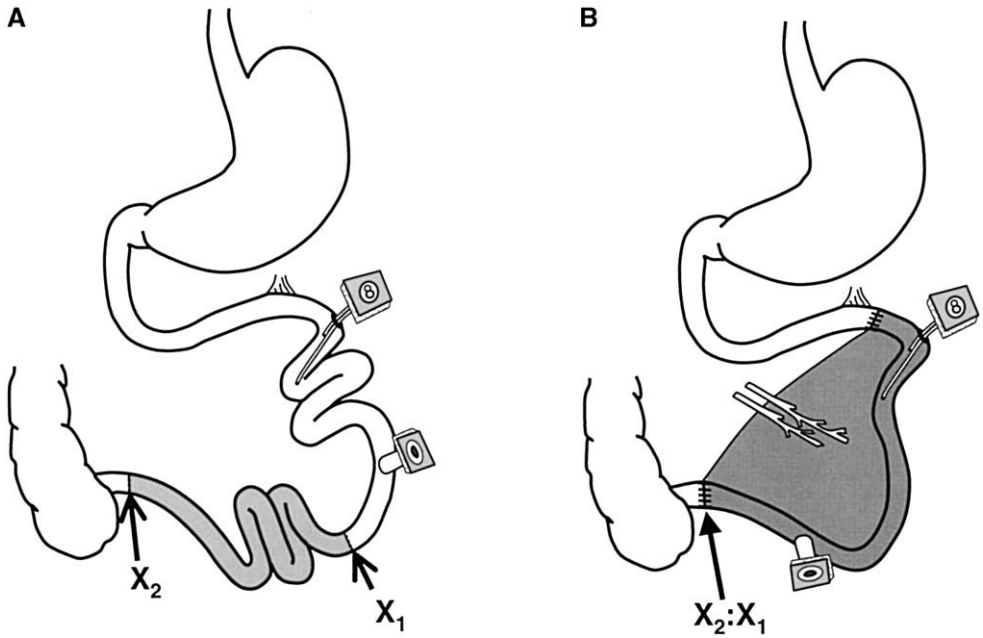

Fig. 2. Experimental model. **A,** The bowel between X_1 and X_2 is resected. **B,** After resection of the ileum, the remnant jejunum (*shaded area*) is extrinsically denervated jejunum in the EXT DEN group and remains extrinsically innervated in the control group. Note that both groups of dogs underwent transection/reanastomosis of the proximal jejunum: in EXT DEN dogs, to complete the extrinsic denervation of the bowel; in CON dogs, to control for proximal disruption of the enteric nervous system.

lymphatic continuity to the jejunoileum without interruption or occlusion of the primary blood supply during the operation. In brief, all mesenteric, lymphatic, and neural tissues at the base of the small bowel mesentery were transected except for the superior mesenteric artery and vein. The artery and vein were carefully skeletonized, with the aid of optical magnification, by stripping the investing adventitia and associated neural elements from the vessel walls. The proximal jejunum, just distal to the ligament of Treitz, was then transected to complete intrinsic neural disruption at that site. Intestinal continuity was restored by end-to-end jejunojejunostomy (Fig. 2, *B*). After recovery, absorption experiments in both groups of dogs were performed 2 and 12 weeks postoperatively.

Absorption Experiments

After an overnight fast, fully conscious dogs resting in a Pavlov sling were studied before (at baseline, 0 weeks) and at 2 and 12 weeks after distal enterectomy using a modification of the triple-lumen perfusion system. Each experiment began with gentle flushing of the jejunal segment with infusate to remove particulate debris. The infusate, designed to reproduce the electrolytic milieu of the jejunum, was a warmed (39° C), isosmolar electrolyte solution (sodium, 140 mmol/L; potassium, 5 mmol/L; chloride, 110 mmol/L; bicarbonate, 35 mmol/L) containing 5 g/L of the nonabsorbable volume marker polyethylene glycol (PEG; molecular weight 3350 Daltons) labeled with ^{14}C-PEG (5 μCi/L), and one of four solutes labeled with ^{3}H (discussed next). After flushing, the test solutions were infused continuously via the proximal jejunal catheter at a continuous rate of 5 ml/min. Intestinal samples were aspirated from the second catheter (15 cm distally) at a constant rate of 1 ml/min using a withdrawal pump (Harvard Apparatus Co., Dover, MA). The effluent from the end of the 80 cm test segment was collected by gravity flow via the distal jejunal cannula. A 1-hour equilibration period was allowed to establish steady state dynamics, based on previous experiments.[7–11] Thereafter samples from the proximal catheter and distal cannula were collected during six subsequent 30-minute intervals for analysis. These experiments were conducted using four different test solutions composed of the infusate containing the following: (1) 2.5 mmol glucose labeled with ^{3}H-glucose (10 μCi/L); (2) 2.5 mmol glutamine labeled with ^{3}H-glutamine (10 μCi/L); (3) 5 mmol taurocholic acid (the primary bile acid in the dog) labeled with ^{3}H-taurocholic acid (10 μCi/L); or (4) 5 mmol oleic acid labeled with ^{3}H-oleic acid (10 μCi/L); the latter solute was delivered as a bile salt emulsion by adding desiccated, unfractionated bovine bile (11.8 mmol bile salts) to control for the expected depletion of the bile salt pool in these dogs with an ileectomy. The concentrations of the solutes glucose and glutamine were chosen to evaluate primarily carrier-mediated active transport (as opposed to diffusion) based on their coefficients of absorption. Each test solution was evaluated twice at each time point (baseline, and 2 weeks and 12 weeks after resection).

Analytic Methodology

All samples were analyzed in duplicate and run within days of the experiment. Concentrations of the nonabsorbable marker, ^{14}C-PEG, and the solutes of interest, ^{3}H-glucose, ^{3}H-glutamine, ^{3}H-taurocholic acid, and ^{3}H-oleic acid, were measured by dual-label scintillation techniques.

Analysis of Data

Net absorption of glucose, glutamine, oleic acid, taurocholic acid, and water was determined using principles of the triple-lumen perfusion technique, as described previously.[7–11] In brief, after allowing the first hour for establishment of steady state conditions (i.e., the amount of nonabsorbable marker entering the study segment equaling the amount of marker leaving the distal end of the segment), net absorption of water for each experimental interval was calculated from the difference in volume entering and leaving the perfused 80 cm jejunal test segment, as calculated by changes in concentrations of ^{14}C-PEG between the proximal jejunal aspiration site and the distal jejunal diverting cannula. Absorption was expressed as net absorptive flux (μl) per centimeter of intestine per minute. Positive flux values represent net absorption, while negative flux values represent net secretion. Net absorptive fluxes for glucose, glutamine, taurocholic acid, oleic acid, and water were determined for each experimental time interval using corrections based on the changes in concentrations of ^{14}C-PEG, ^{3}H-solute, and standard formulas for the 80 cm test segment.[7-11]

The means were calculated for individual values of net absorption for each of the six separate 30-minute intervals per experiment, and these mean values for the two duplicate experiments were also determined. Results of absorption were grouped into 3-hour long periods. Grand means across dogs were calculated for the basal, 2-week, and 12-week absorptive experiments.

Statistical Analysis

Experiments were evaluated by comparing data within groups to baseline values (paired analysis) and values between the CON and EXT DEN groups at each time point (unpaired analysis). Mean net absorptive fluxes within groups were compared for all dogs across the time points (baseline, 2 weeks, and 12 weeks) using analysis of variance (ANOVA) and a subsequent Student's *t*-test for paired data with probability adjusted according to the Bonferroni correction for multiple related comparisons when appropriate. Data in the text are presented as mean values ± standard error of the mean.

RESULTS

Health/General Characteristics of Dogs

All dogs tolerated catheter and cannula placement and subsequent ileal resection well, maintained a good appetite, and remained active and healthy. After the ileal resection, all dogs developed a watery diarrhea, which returned to a soft, partially formed stool in all CON dogs at a mean of 10 weeks; in contrast, watery diarrhea persisted in all EXT DEN dogs throughout the study. Preoperative body weights were similar (16.3 ± 0.7 kg vs. 19.5 ± 1.0 kg); all dogs lost weight after distal 50% small bowel resection, but the maximum weight loss was greater in EXT DEN dogs (2.3 ± 0.5 kg vs. 3.8 ± 0.3 kg, $P < 0.05$). These differences occurred despite subjectively similar appetites, activity levels, and overall health in the two groups.

Absorptive Function

Table 1 shows net absorptive fluxes for glucose, glutamine, oleic acid, and taurocholate. Absorptive flux of glucose (mmol/cm/min) was decreased at 2 weeks when compared to baseline values (0 weeks) in both groups (CON 4.2×10^{-5} to 2.7×10^{-5}; EXT DEN 4.2×10^{-5} to 2.3×10^{-5}, $P<0.05$ for each); net absorptive fluxes returned to baseline at 12 weeks. Net absorptive flux of oleic acid followed very similar trends to glucose in CON and EXT DEN dogs with a decrease at the 2-week time point. In addition, when CON and EXT DEN were compared at each time point, there were no differences in net absorptive fluxes of glucose or oleic acid between groups.

In contrast, the net absorptive flux of glutamine (mmol/cm/min) in CON dogs did not differ from baseline at 2 and 12 weeks after distal 50% small bowel resection, whereas in the EXT DEN dogs, net absorptive flux of glutamine was decreased from baseline at 2 weeks but returned to baseline at 12 weeks (baseline, 4.1×10^{-5}; 2 weeks, 2.5×10^{-5}; 12 weeks, 3.5×10^{-5}, $P<0.05$). Net absorptive fluxes of taurocholate were extremely low (<2 μmol/cm/min) (compared to values in ileum[7,11]), remained unchanged throughout the study, and did not differ between groups.

Table 2 shows the net absorptive flux of water for each nutrient solution. Net absorptive fluxes of water did not change from baseline values for either CON or EXT DEN dogs at 2 or 12 weeks after distal 50% small bowel resection. Net absorptive fluxes for water in the oleic acid and taurocholate solutions were less than for the glucose and glutamine solutions ($P<0.05$). No consistent differences were noted between groups at any time point.

DISCUSSION

In this study we demonstrated that extrinsic denervation does not globally blunt the jejunal absorptive adaptive response to ileectomy. Distal 50% enterectomy induces a transient short gut syndrome with diarrhea and weight loss in the dog, which is worsened by extrinsic denervation. Net absorptive fluxes of glucose and oleic acid were decreased at 2 weeks, but jejunal function adapted such that net absorptive fluxes returned to baseline values at

Table 1. Net absorptive fluxes of nutrients

	Net absorptive fluxes ($\times 10^{-5}$ mmol/cm/min)*							
	Glucose		Glutamine		Oleic acid		Taurocholate	
Period	CON	EXT DEN	CON	EXT DEN	CON	EXT DEN	CON	EXT DEN
Baseline	4.2 ± 0.2	4.2 ± 0.5	4.0 ± 0.2	4.1 ± 0.3	9.7 ± 1.0	9.1 ± 0.6	0.5 ± 0.2	0.6 ± 0.2
2 wk	2.7 ± 0.3†	2.3 ± 0.3†	3.5 ± 0.3	2.5 ± 0.5†‡	6.3 ± 0.5†	5.2 ± 0.9†	1.4 ± 0.4	0.7 ± 0.6
12 wk	3.6 ± 0.1	3.7 ± 0.5	4.1 ± 0.4	3.5 ± 0.3	8.3 ± 0.5	7.1 ± 1.0	2.1 ± 0.9	1.2 ± 0.3

*mean ± SEM; n = 6 dogs/group.
†Differs from baseline values within the same group, $P < 0.05$.
‡Differs from same time point in CON group, $P < 0.05$.

Table 2. Net absorptive flux of water with each nutrient solution

	Net absorptive flux (μl/cm/min)*							
	Glucose		Glutamine		Oleic acid†		Taurocholate†	
Period	CON	EXT DEN	CON	EXT DEN	CON	EXT DEN	CON	EXT DEN
Baseline	11.2 ± 1.2	12.3 ± 2.0	12.2 ± 1.4	11.6 ± 1.1	6.6 ± 1.7	5.1 ± 0.7	8.4 ± 1.2	8.2 ± 0.7
2 wk	10.8 ± 1.9	9.6 ± 2.2	11.6 ± 1.2	8.7 ± 2.0	5.6 ± 0.7	5.1 ± 2.0	6.6 ± 1.7	5.8 ± 1.9
12 wk	12.0 ± 0.7	9.4 ± 0.5	14.1 ± 2.7	11.2 ± 0.6	9.1 ± 1.5	6.4 ± 1.2	11.0 ± 2.1	5.5 ± 1.2

*Values are mean ± SEM; n = 6 dogs per group. No statistical differences in net absorptive flux of water noted within or between dog groups for any nutrient solution.
†Differs from values in glucose and glutamine solutions at each time point, $P < 0.05$.

12 weeks. In contrast, absorptive flux of glutamine was decreased at 2 weeks in the EXT DEN dogs, an effect not seen in the neurally intact dogs. Jejunal absorption of taurocholate was low (as expected) and did not change in either group throughout the study. Thus, although extrinsic innervation appears to play a role in the early postresectional adaptive response to glutamine absorption, the global net adaptive response was not blunted by extrinsic denervation. These observations may have potentially important implications for the feasibility of segmental SBT, which has several theoretic advantages over whole small bowel transplant.[3]

For segmental SBT to be a feasible alternative to whole-bowel transplant, some degree of adaptation must occur for the graft to provide enough absorptive capacity for the recipient to maintain a satisfactory nutritional state with oral intake. Adaptation in morphology and enteric function occurs in the small bowel mucosa after small bowel resection or bypass. Factors important in this response include luminal nutrients, enteric or plasma glutamine, pancreaticobiliary secretions, circulating humoral agents such as hormones, growth factors, and putative regulatory peptides, and the anatomic region of the remaining gut.[15–20] The role of extrinsic innervation to the gut (sympathetic and vagal/parasympathetic), which modulates absorptive function, may be another important factor in the adaptive response that has not been well investigated.

In our laboratory we previously developed a canine model of small intestinal (auto)transplantation devoid of confounding pathophysiologic factors of immune rejection/immunosuppression, ischemia/reperfusion injury, and systemic venous drainage of the gut, each of which may alter absorptive function, to study the isolated effects of extrinsic denervation on post-transplantation enteric physiology. We showed that net jejunal[8] and net ileal[7] absorption of water, electrolytes, and bile salts decreased early after this model of extrinsic denervation but returned toward normal 8 weeks later, suggesting an adaptation by the extrinsically denervated jejunoileum. Jejunal absorption of glutamine also decreased at 2 weeks returning to normal levels 8 weeks after denervation, findings similar to our current study at 2 weeks after denervation.[9] In contrast, jejunal absorption of the simple solutes arginine, leucine, and glucose in vivo, in concentrations in which absorption was mediated primarily by active transport, were decreased both early (2 weeks) and late (8 weeks) after this model;[9] ileal absorption of glutamine was decreased in vivo both 2 weeks and 8 weeks after extrinsic denervation.[10] Transport kinetics from brush-border membrane vesicles showed that active, carrier-mediated transport of glutamine in the extrinsically denervated small bowel was decreased in the jejunum at 2 weeks and in the ileum at 2 weeks and 8 weeks after denervation; this decrease was secondary to a decrease in V_{max}, a measure of the number of functional carrier molecules expressed per gram tissue, and not by a change in K_m, a function of carrier affinity.[9,10] These experiments demonstrated that extrinsic innervation plays a role in intestinal absorptive function, reinforcing the importance of extrinsic denervation in SBT.

In an attempt to examine the role of extrinsic innervation in adaptation, we extended our previous model of total jejunoileal extrinsic denervation to a model of extrinsic denervation of anatomic segments after subtotal small bowel resection. Our previous work showed no differences in net absorption of water, electrolytes, or simple nutrients (glucose, glutamine, oleic acid, bile salts) early or late after complete extrinsic denervation of the ileum.[11] Our current study of the extrinsically denervated jejunum differs in that a definite decrease in net absorption of glucose, oleic acid, and glutamine was measured early on; yet some form of adaptation occurred because net absorption returned toward normal by 12 weeks after ileectomy. Our findings in the canine ileum,[11] however, differ from those of Lauronen et al.[21] in the porcine ileum. These investigators found a blunted functional and morphometric adaptation of a fully autotransplanted 100 cm segment of distal ileum after total proximal small bowel resection when compared to innervated, nontransplanted ileum. The differences in their find-

ings and ours may reflect use of a different species (dog vs. pig), different anatomic regions (jejunum vs. ileum), or differences in models of transplantation—that is, our canine model is devoid of ischemia/reperfusion injury, maintains portal venous drainage of the study segment, and specifically isolates the effects of extrinsic denervation; in contrast, the porcine model of Lauronen et al.[21] involves a full autotransplantation with its other confounding factors.

In this study, distal 50% enterectomy induced a short bowel syndrome, with weight loss and diarrhea, as well as early (2 weeks) decreases in absorption of several simple nutrients in vivo. Decreases in absorption of glucose and oleic acid are consistent with previous studies evaluating adaptation to massive resection.[22] Structural adaptation begins within hours of injury with hyperproliferation of crypt cells and an increased rate of enterocyte migration, which results in deeper crypts and increased villus height; there is also a decrease in enterocyte function for several weeks after resection, which is attributed to immature enterocytes in the villus.[23] In contrast, maintained or even increased absorption of glutamine, the primary fuel for the enterocyte, early (2 weeks) after resection also occurs after significant resection.[24] Because glutamine absorption early (2 weeks) after resection was not maintained in the EXT DEN dogs as in the CON group, our study suggests that extrinsic innervation may play a role in early postresection absorption of glutamine consistent with our previous studies.[9,10] Recovery of glutamine absorption at 12 weeks after resection suggests that there is little long-term effect of this early decrease in glutamine absorption and that a jejunal adaptation to glutamine absorption must occur. On the other hand, despite similar absorptive capacities of simple nutrients at 12 weeks, EXT DEN dogs had persistent diarrhea and weight loss. The reason(s) for this clinical difference remains unclear; however, our experiments were performed in the fasting state. We hypothesize that there may be some difference in postprandial augmentation of absorption and are currently conducting studies to examine the role of extrinsic innervation in the postprandial augmentation of absorption. It is also not clear why net absorptive flux returns to preoperative levels instead of an increased level of uptake. The jejunum alone must be of sufficient length to maintain adequate absorption of water, electrolytes, and simple nutrients after adaptation to the ileectomy occurs.

CONCLUSION

In contrast to our previous study examining the role of extrinsic innervation in adaptation of an ileal segment after total jejunectomy, a distal 50% bowel resection resulted in a decrease in absorptive function in both control and extrinsically denervated dogs to glucose and oleic acid, but to glutamine only after extrinsic denervation. Full recovery to baseline values by 12 weeks after resection suggests that extrinsic denervation does not prevent absorptive adaptation of the canine jejunum.

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Donor Hepatic Function: A Factor in Postreperfusion Syndrome

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Reperfusion of support livers after cold preservation produces hemodynamic instability (i.e., postreperfusion syndrome) in the recipient during both orthotopic liver transplantation and extracorporeal liver perfusion. We evaluated the effect of the normal porcine cold-preserved support liver on healthy recipient hemodynamics and in situ liver function during extracorporeal liver perfusion. Support livers were harvested from Yorkshire pigs and reperfused in an extracorporeal circuit with a healthy, anesthetized recipient pig. Correlation analyses were performed between support liver variables of function (oxygen consumption, bile flow, and biliary phospholipid and cholesterol output) and both recipient hemodynamic stability (heart rate, blood pressure, urine output, and vasopressor use) and hepatic function (bile flow and biliary phospholipid secretion). The data indicate that optimally functioning support livers are associated with improved recipient hemodynamic stability manifested by decreased recipient heart rate and vasopressor use and increased recipient urine output. Support livers exhibiting poor biliary secretory function (i.e., bile flow and phospholipid output) were associated with similarly diminished recipient liver biliary secretory function. These data indicate that the functional condition of the support liver after harvest and cold preservation may influence both recipient hemodynamic parameters and the endogenous function of the recipient liver. (J GASTROINTEST SURG 2002;6:248–254.)

KEY WORDS: Postreperfusion, graft, function, bile, ischemia

Despite the multiple advances made in orthotopic liver transplantation throughout the years, the intraoperative management of the recipient during liver transplantation remains a difficult challenge. It is well recognized[1,2] that reperfusion of a harvested, cold-preserved support liver during transplantation frequently results in recipient hemodynamic changes requiring vigorous fluid resuscitation and/or vasopressor support. This hemodynamic instability has been termed the "postreperfusion syndrome." Although not clearly understood, postulated mechanisms for postreperfusion syndrome have included release of endotoxins,[3] cytokines,[4,5] and arachidonic acid metabolites[6] during graft reperfusion, as well as the generation of free radicals during graft reperfusion. Another potential mechanism is the induction of recipient ventricular dysfunction secondary to volume overload.[7]

Hepatic support systems employing hepatocytes, either as cell cartridges[8] or as whole livers in extracorporeal whole-liver perfusion (ELP),[9] have been employed to sustain critically ill patients until transplantation. These patients in liver failure are usually hemodynamically quite unstable, so the acute influences of hepatic support systems may go unrecognized. Few studies have evaluated the effect of hepatic support systems on either recipient hemodynamic status or endogenous hepatic function. The purpose of our study was to assess the role of the quality of the ex vivo support liver graft (after cold preservation) on both recipient hemodynamic stability and recipient liver function during ELP. The data demonstrate that the functional condition of the ex vivo support liver graft may influence both recipient hemodynamic stability and in situ hepatic function.

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METHODS

Support Liver Harvest

The Institutional Animal Care and Use Committee approved all studies at the University of Massachusetts. Healthy donor Yorkshire pigs (n = 13), weighing between 30 and 40 kg, were selected for the procurement of the support livers. The pigs were sedated with a mixture (10 ml/kg) of telazol, 150 mg/ml (Fort Dodge Animal Health, Fort Dodge, IA); ketamine, 50 mg/ml (Fort Dodge Animal Health; and xylazine, 10 mg/ml (Phoenix Pharmaceuticals, Inc., St. Joseph, MO). Pigs underwent endotracheal intubation and were anesthetized during the procedure with inhalation isoflurane (1.5%). The femoral vein and artery were cannulated for intravenous infusions and continuous blood pressure monitoring, respectively.

Through a midline laparotomy, the cystic duct was ligated without removing the gallbladder, and a 14 F cannula (Sherwood Medical, St. Louis, MO) was inserted into the common bile duct. The infrarenal aorta and portal vein were isolated. At that time, the chest was opened through a median sternotomy and the suprahepatic inferior vena cava was isolated. After intravenous heparin administration (20,000 units), perfusion cannulas (Medtronic Biomedicus, Eden Prairie, MN) were placed in the infrarenal aorta (15 F) and the portal vein (17 F). The liver was perfused in situ with 3 L of Euro-Collins solution (Fresenius USA, Ogden, UT) at 4° C (1 L in the portal vein, 2 L in the aorta). The liver was cooled to 4° C with ice-cold saline solution for 2 hours and the vena cava was vented in the chest. The infrahepatic vena cava was ligated and the liver was removed with cannulas in place. Additional cannulas were placed in the suprahepatic vena cava (32 F) and hepatic artery (8 F), while the liver remained on ice during the preparation of the recipient pig.

Preparation of the Recipient and Venovenous Circuit

Healthy recipient Yorkshire pigs (n = 13) were sedated, intubated, and anesthetized in the same manner described for the donor. Femoral vein and artery catheters were placed for intravenous infusions and continuous blood pressure monitoring. The common bile duct was catheterized as in the donor harvest and externalized through the abdominal wall to allow for continuous bile collection. After heparin administration (15,000 units), perfusion cannulas (Medtronic Biomedicus) were placed in the right femoral vein (15 F) and right external jugular vein (17 F), and the perfusion circuit consisting of pumps, filters, an oxygenator, and a reservoir was connected as previously described.[10] The external perfusion circuit had a total volume of 900 to 1000 ml and was primed with lactated Ringer's solution. The temperature of the recipient pigs was maintained between 36° and 38° C with a heating blanket.

Reperfusion of the Support Organ

The support organ (n = 13) was removed from the ice bath after 120 minutes and placed in the venovenous circuit at room temperature in a basin with the porta hepatis facing upward.[10] The liver was connected via the portal vein and flushed with 1000 ml of lactated Ringer's solution at room temperature. After connection of the vena cava cannula, portal vein perfusion was slowly begun and gradually increased to 550 to 750 ml/min (0.50 to 0.75 ml/g liver/min). Portal vein flow was monitored via an in-line bypass flowmeter (Transonic Systems Inc., Ithaca, NY). The hepatic artery was perfused with additional nonpulsatile flow at rates between 160 and 200 ml/min (0.16 to 0.2 ml/g liver/min) via a separate roller pump (Cardiovascular Instrument Corp., Wakefield, MA). These rates are similar to those that we have observed in the normal in situ pig liver (data not shown). The ex vivo graft was maintained at 37° C by the circuit warmer.

Markers of Support Organ Function

The effects of ex vivo support liver function on recipient hemodynamic parameters and in situ hepatic function were evaluated after graft reperfusion. The following parameters of support organ function were evaluated during reperfusion: (1) biliary production, (2) biliary phospholipid output, (3) biliary cholesterol output, and (4) oxygen consumption and CO_2 transport.

Bile flow is a sensitive indicator of hepatic graft function. Bile was collected from the support liver every 15 minutes, protected from light, and stored at −20° C before assay. Total bile production was measured in standard graduated cylinders. Analysis of bile components was performed with commercially available kits, phospholipid (Wako Chemicals, Osaka, Japan) and cholesterol (Sigma Chemical, St. Louis, MO), as previously described.[10] After 2 hours of perfusion, all support livers received incremental doses of taurocholate (Sigma Chemical) (186 to 5952 μmol/15 min) to augment biliary secretion and replicate portal bile acid transport in the in situ pig liver. This pattern of delivering incremental doses of bile acid to the liver (to maximize hepatic biliary production) has been previously described.[10]

Arterial blood gas samples were obtained from the serum of the ex vivo graft (portal vein and vena cava) and

the recipient systemic circulation every 30 minutes and analyzed by means of a model 1640 blood gas analyzer (Instrumentation Laboratory, Lexington, Mass). Oxygen consumption was calculated using the standard equation: [inflow O_2 saturation − outflow O_2 saturation) * (1.34 * hemoglobin * portal vein flow/min + hepatic artery flow/min)/liver weight (g)]. The CO_2 gradient was calculated with the equation: [P_{CO_2} (outflow) − P_{CO_2} (inflow)]. Blood gas analysis allowed for simultaneous evaluation of hemoglobin content of the perfusate. These determinations were standardized to the hospital laboratory's complete blood count determinations.

Markers of Recipient Hemodynamic and Bile Secretory Parameters

Recipient hemodynamic parameters and in situ hepatic functional variables were evaluated after reperfusion of the support organ. The following recipient hemodynamic variables were evaluated: (1) vasopressor use, (2) blood pressure and heart rate, and (3) urine output. Also, recipient in situ hepatic function was evaluated through (1) biliary production, (2) biliary phospholipid output, and (3) biliary cholesterol output.

Blood pressure and heart rate were monitored continuously after reperfusion. Mean values were determined over the course of the experiment. On reperfusion of the support liver, norepinephrine was often required to prevent prolonged hypotension. A systolic blood pressure below 75 mm Hg was defined as severe hypotension requiring pressor support. Once the pressure rose above 75 mm Hg, the norepinephrine was gradually discontinued. Norepinephrine requirements were graded based on the length of time the pressor was required during a 6-hour perfusion period. A score of 0 was given to animals that required no pressors. Those animals requiring up to 20 minutes of pressors immediately after insertion of the support organ onto the venovenous circuit received a score of 0.33. Animals with pressor requirements lasting more than 20 minutes but less than 2 hours receive a score of 0.66. If pressors were used throughout the entire perfusion period, the animal received a score of 1.0.

As with the support organ, bile was collected from the in situ liver every 15 minutes, protected from light, and stored at −20° C before assay. Biliary phospholipid and cholesterol output was also evaluated as described earlier.

Statistical Analysis

Correlation analyses were used to determine significance between the data. A P value <0.05 was considered significant.

RESULTS

Recipient Hemodynamics and Hepatic Function on Venovenous Bypass

Recipient animals (n = 13) were obtained from a healthy pool of animals and demonstrated no evidence of illness before experimentation. After intubation and preparation of the animals, the recipients were placed onto the venovenous circuit without the support organ. Recipient animal blood pressure and heart rate did not change significantly after initiation of the circuit. None of the animals required pressor support after initiation of the circuit and before placement of the support organ onto the circuit. No difference in recipient animal bile production was noted in the in situ state as compared to the interval when the recipient was on the venovenous circuit (Table 1).

Recipient Hemodynamics and In Situ Function With the Support Organ

The introduction of a donor support liver into the venovenous circuit of a healthy recipient pig with normal blood pressure and heart rate resulted in significant hypotension and either tachycardia or bradycardia. After placement of the support organ into the venovenous circuit, recipient heart rates ranged from 75 to 130 beats/min and averaged 107 ± 4.6 beats/min. Also, recipient hypotension was at time severe (systolic blood pressure <70 mm Hg) and prolonged (longer than 5 minutes) requiring pressors (norepinephrine) to maintain hemodynamic stability. Pressor usage was graded from 0 to 1.0 depending on the duration of usage and averaged 0.6 ± 0.1 for all perfusions. Urine output for recipient animals ranged from 122 to 1200 ml/hr and averaged 490 ± 102 ml/hr. The temperature of the recipient animals was maintained between 36° and 38° C.

After reperfusion of the cold-preserved support organ, the recipient in situ liver demonstrated decreased bile production and diminished bile lipid

Table 1. Values for recipient animals

	Before bypass	On bypass (without support organ)	On bypass (with support organ)
Blood pressure	105 ± 6	101 ± 5	89 ± 1†
Heart rate	88 ± 5	90 ± 5	107 ± 5*
Pressors	0	0	0.6 ± 0.1

Values represent mean ± SEM for recipient animals before placement onto the bypass circuit, after placement onto the circuit without the support organ, and after placement onto the bypass circuit with the support organ.

*$P < 0.02$ as compared to on bypass with the support organ.

†$P < 0.05$ as compared to on bypass with the support organ.

output. In situ hepatic bile production ranged from 9.5 to 24.6 ml/15 min for individual recipient livers and averaged 14.3 ± 1.3 ml/15 min. During reperfusion, in situ recipient hepatic biliary cholesterol output ranged from 0.01 to 0.33 μmol/15 min/kg and averaged 0.23 ± 0.02 μmol/15 min/kg. Phospholipid output ranged from 0.6 to 2.7 μmol/15 min/kg and averaged 1.4 ± 0.2 μmol/15 min/kg.

Support Liver Function

After reperfusion, the cold-preserved support organ (n = 13) demonstrated low levels of biliary output and bile solute output. In the first 2 hours of reperfusion, bile production ranged from 0.0 to 1.6 ml/15 min for individual support organs and averaged 0.84 ± 0.1 ml/15 min. In the first 2 hours of reperfusion, biliary cholesterol and phospholipid concentrations were significantly lower than in the in situ liver, as previously described.[10] Support organ biliary cholesterol output ranged from 0.0 to 0.18 μmol/15 min/kg and averaged 0.06 ± 0.013 μmol/15 min/kg. Phospholipid output ranged from 0.0 to 0.37 μmol/15 min/kg and averaged 0.21 ± 0.07 μmol/15 min/kg in the first 2 hours of reperfusion.

At 2 hours of reperfusion, all support organs received taurocholate infusions to simulate the enterohepatic return of bile acids. Biliary production after taurocholate infusion ranged from 7.0 to 20.0 ml/15 min for individual support organs and averaged 12.8 ± 1.2 ml/15 min. After taurocholate infusion, support organ biliary cholesterol output ranged from 0.27 to 0.75 μmol/15 min/kg and averaged 0.53 ± 0.04 μmol/15 min/kg. After taurocholate infusion, phospholipid output ranged from 0.6 to 5.2 μmol/15 min/kg and averaged 2.3 ± 0.4 μmol/15 min/kg for all 13 support organs.

Oxygen consumption of the support organ remained relatively stable during a 6-hour perfusion experiment. Oxygen consumption for individual support organs ranged from 1.2 to 2.4 ml 100 g/min and averaged 1.8 ± 0.1 ml/100 g/min. P_{CO_2} gradients ranged from 3 to 11 mm Hg and averaged 6.1 ± 0.7 mm Hg.

Correlation Between Support Liver Function and Recipient Hemodynamics

Markers of support organ function (i.e., bile secretory function) were compared with recipient hemodynamics. Support organ biliary output after taurocholate infusion correlated inversely with recipient heart rate (n = 13, $r = 0.76$, $P < 0.005$) (Fig. 1, *A*) and pressor requirements (n = 13, $r = 0.60$, $P < 0.05$) (Fig. 1, *B*). Support organ biliary output after taurocholate infusion correlated directly with recipient urine output (n = 13, $r = 0.83$, $P < 0.001$) (Fig. 2). Support organ oxygen consumption correlated inversely with recipient heart rate (n = 13, $r = 0.48$, $P < 0.05$) (Fig. 3). Other support liver functional parameters (i.e., bile phospholipid or cholesterol outputs) were not associated with recipient urine output, tachycardia, or pressor usage.

Correlation Between Support Liver Function and Recipient In Situ Liver Function

Not only did the functional status of the support livers influence the hemodynamic stability of the recipient, but correlations were also observed between the biliary secretory function of the support liver and

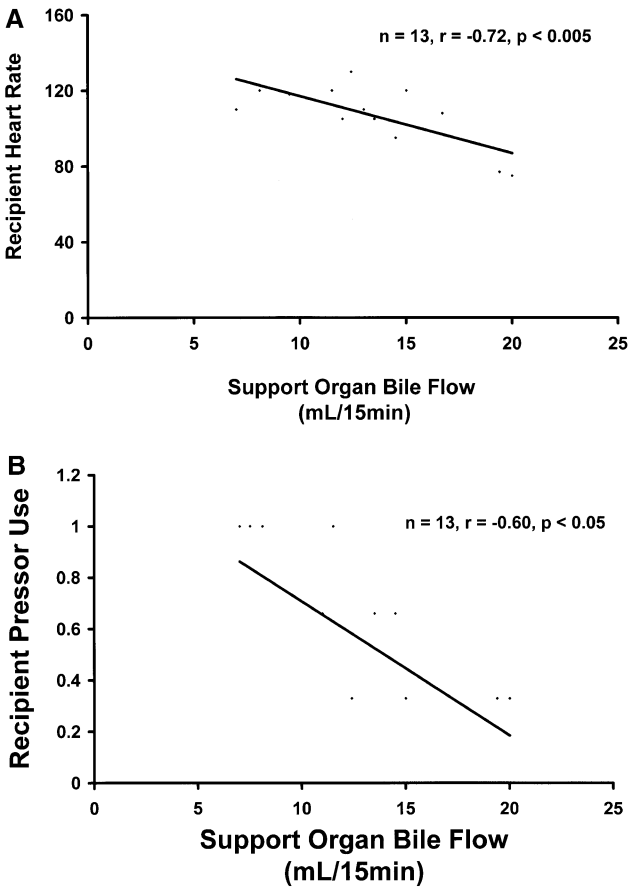

Fig. 1. A, Effect of support organ biliary production on recipient heart rate. The trend line represents the correlation analysis for mean support organ biliary production in 13 dual-perfused livers and the respective mean recipient heart rates. The negative association between support organ biliary production and recipient animal heart rates is significant. **B**, Effect of support organ biliary production on recipient vasopressor use. The trend line represents the correlation analysis for mean support organ biliary production in 13 dual-perfused livers and the respective recipient pressor utilization. The negative association between support organ biliary production and recipient animal vasopressor use is significant.

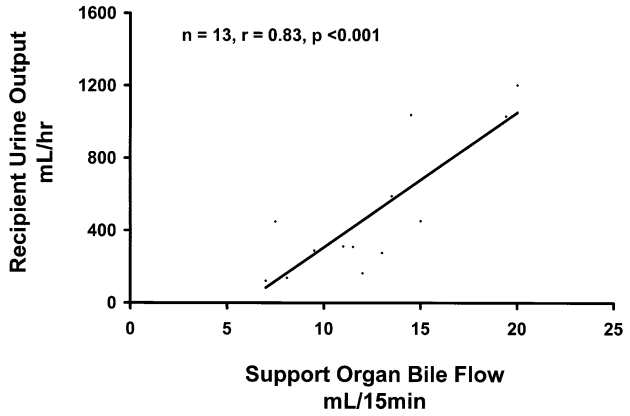

Fig. 2. Effect of support organ biliary production on recipient urine output. The trend line represents the correlation analysis for mean support organ biliary production in 13 dual-perfused livers and the respective recipient urine output during reperfusion. The linear association between support organ biliary production and recipient animal urine output is significant.

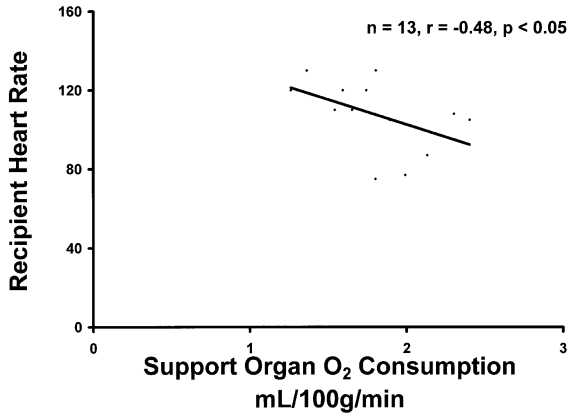

Fig. 3. Effect of support organ oxygen (O_2) consumption on recipient heart rate. The trend line represents the correlation analysis for mean support organ biliary production in 13 dual-perfused livers and the respective mean recipient heart rates. The negative association between support organ oxygen consumption and recipient animal heart rates is significant.

that of the recipient liver. Support livers with the largest bile output after taurocholate infusion were associated with the largest bile output in the recipient livers (n = 13, $r = 0.60$, $P < 0.05$) (Fig. 4). Similarly, those support livers with the greatest bile phospholipid output correlated with the largest bile phospholipid output in recipient livers (n = 13, $r = 0.72$, $P < 0.005$) (Fig. 5).

DISCUSSION

The present study evaluated the influence of the functional status of a support liver after cold preservation on the hemodynamic and hepatic status of a healthy recipient animal. The data demonstrated that poorly functioning support organs are associated with deleterious changes in the hemodynamic status of recipient pigs. Poorly functioning support livers following cold preservation were also associated with poor recipient in situ liver function during ELP. It is apparent that the functional status of an ex vivo support liver may contribute to both aberrations in recipient hemodynamic and in situ liver function.

Hepatic support systems with exogenous hepatocytes, either as cell cartridges[8] or as whole livers,[9] have been employed to sustain critically ill patients until transplantation. These patients are often hemodynamically unstable, so the acute influences of these support systems on the recipients often go unrecognized. After hepatic transplantation of hemodynamically stable patients, reports have detailed episodes of transient but appreciable decreases in mean arterial pressures of those patients.[1,2,7] These occurrences have been classified as "reperfusion syndrome." This is often observed when a cold-preserved porcine liver is inserted into a venovenous circuit of a hemodynamically stable pig with the same blood type as the hepatic support organ.

In these studies we characterized the functional status of the donor support liver and compared it to recipient hemodynamic parameters and in situ recipient hepatic function. A healthy recipient was used to ensure normal in situ recipient hemodynamic parameters and hepatic function. It is through the use of healthy recipient animals that we attempted to demonstrate that the

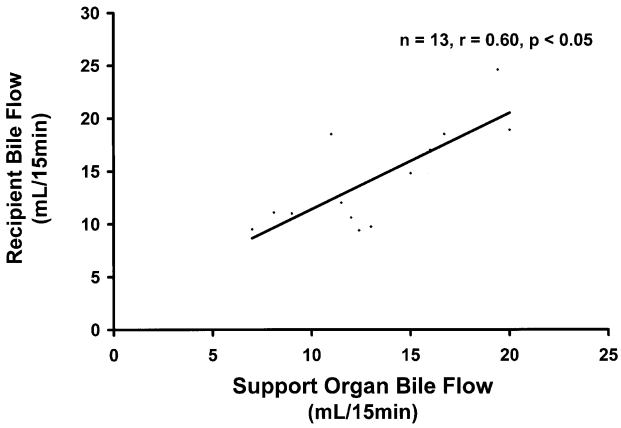

Fig. 4. Effect of support organ biliary production on recipient biliary production. The trend line represents the correlation analysis for mean support organ biliary production in 13 dual-perfused livers and the respective mean recipient biliary production. The linear association between support organ biliary production and recipient animal biliary production is significant.

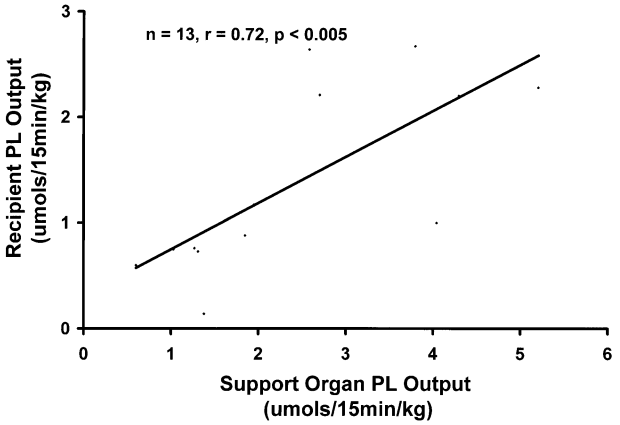

Fig. 5. Effect of support organ phospholipid (PL) output on recipient PL output. The trend line represents the correlation analysis for mean support organ PL output in 13 dual-perfused livers and the respective mean recipient PL output. The linear association between support organ PL production and recipient animal PL output is significant.

support organ itself might affect recipient hemodynamic stability and in situ liver function. This concept is particularly consequential when a poorly functioning graft is harvested for support in ELP.

Poorly functioning support livers were associated with longer and more frequent episodes of recipient hypotension and tachycardia, as well as diminished urine output. Postreperfusion hypotension is not an unusual occurrence after liver transplantation[1] but is quite unusual after renal transplantation. This may suggest that it is the nature of the support organ inserted into the recipient circulation that provokes this hypotension. It has been proposed[5] that the venovenous shunt in place before incorporation of the support liver activates complement and neutrophils and produces this hypotension. The lack of hypotension in the porcine recipients in the study who were connected to the venovenous circuit alone indicates that the polymer surfaces of the circuit[5] are most likely not an etiology for the diminished vascular resistance. The observation of hypotension, even in the absence of the polymer surfaces during hepatic transplantation, reemphasizes the important role of the liver in this phenomenon. However, a third yet to be identified factor (not a recipient or donor animal) cannot be ruled out as the cause of the recipient dysfunction.

The occurrence of postreperfusion hypotension in our studies, where a normal recipient liver is part of the circulation, indicates that it is not a metabolic or detoxification defect of the cold ischemia–reperfused liver, but possibly various compounds lost from the inserted liver, that provokes the syndrome. The progressive hypotension accompanying acute or chronic hepatic deterioration[11,12] may also have a similar etiology. Many compounds with a molecular spectrum from saturated ferritin or hemosiderin to nitric oxide can be released from hepatocytes and have the potential to produce hypotension and replicate the postreperfusion syndrome.[13] Although this syndrome has been extensively studied with numerous proposed etiologies,[12] there is no single pathogenic mechanism identified at this time.

The status of the support liver is not only associated with the acute hemodynamic status of the recipient, but with the biliary secretory potential of the recipient as well. The direct correlation between support and recipient bile aqueous and lipid output after taurocholate infusion suggests a possible influence of the support organ on recipient function. It is not unreasonable that a support organ with significant bile secretory dysfunction after cold ischemia and reperfusion would extend this process to the endogenous recipient liver possibly by induced hypotension, or another unknown mechanism. If such a functional interrelation occurs, it could have important implications if the support objective was to assist in the cellular regeneration and functional restoration of the damaged recipient liver. If the ex vivo hepatic support system were employed only to "bridge" the patient to hepatic transplantation, this would be of little consequence.

These three data indicate that the introduction of a cold-preserved organ onto a venovenous circuit with a healthy recipient produces hemodynamic instability in the recipient. Although the exact mechanisms for this hemodynamic instability are unknown, the data in the present study do associate the functional status of the support organ with the severity of the postreperfusion syndrome. Furthermore, these studies emphasize the importance of recognizing a failing ex vivo hepatic support system during reperfusion so that it can be removed, thereby avoiding further injury to the recipient. At this time, additional data are needed to delineate the causative factors of postreperfusion syndrome.

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Local, Intrahepatic, and Systemic Recurrence Patterns After Radiofrequency Ablation of Hepatic Malignancies

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The objective of this study was to describe the recurrence patterns in patients with unresectable hepatic malignancies treated with radiofrequency ablation (RFA). As RFA is applied more widely to patients with hepatic tumors, a better understanding of the biologic behavior of these tumors and the risk of recurrence, both in the liver and systemically, is needed. A multidisciplinary team evaluated patients referred for RFA and followed them prospectively to assess local, intrahepatic, and extrahepatic disease recurrence and complication rates. Forty-five patients with 143 lesions and a minimum follow-up of 6 months (median 19.5 months) were treated. Overall, 7.7% of treated lesions had local recurrence. New intrahepatic disease was seen in 49% of patients, and 24% had evidence of new systemic tumor progression. Patients with colorectal metastatic lesions ≥4 cm at the time of the first RFA were more likely to present with local recurrence ($P = 0.048$). Complications occurred in 27% of patients. Although RFA has a satisfactory local failure rate and safety profile, the patient population being treated is at high risk of developing new disease. Multimodality adjuvant therapy will be necessary to realize the full potential of hepatic malignancy control with RFA. (J GASTROINTEST SURG 2002;6:255–263.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Liver neoplasms, radiofrequency catheter ablation, neoplasm recurrence local, catheter ablation percutaneous, hepatic cancer

Liver resection remains the standard therapy for hepatic malignancy,[1–6] but the advent of new minimally invasive therapies has led to more patients being treated with nonresectional therapy. On the leading edge of these new therapies is radiofrequency ablation (RFA).[7–11] This technique uses heat to destroy liver tumors by inducing necrosis and thrombosis. The great appeal of RFA is that it is minimally invasive.[7,8,12] As it becomes more widely available, physicians and their patients are pushing for its use outside of the established indications for liver-directed therapy. The natural history of common diseases, such as hepatic metastases of colorectal cancer, is well known and extending the indications for liver-directed therapy could potentially result in poor outcomes. It is therefore critical that in evaluating this new treatment modality, the established tenets of surgical management of liver tumors be followed, or that management be conducted under protocol.

Before initiating the use of RFA at our institution, the Hepatobiliary Tumor Service gathered a multidisciplinary group of surgeons, interventional radiologists, and oncologists to review all potential patients and determine the indications for and method of delivery of RFA. All patients were reviewed and the treatment plan was formulated. Data were collected prospectively. It was hypothesized that disease progression, rather than local technical failure in treated lesions, would prove to be the limiting factor in patient outcome.

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MATERIALS AND METHODS

This study was approved by and conducted in accordance with the guidelines set forth by the University of Minnesota institutional human research review committee. A total of 45 patients with liver tumors underwent RFA at our institution from November 1, 1998 through February 29, 2001. The RITA (RITA Medical, Mountain View, CA) model 70 (3 cm array) was used through April 30, 2000; the RITA Star Burst XL (5 cm array) was used thereafter. The Hepatobiliary Tumor Service reviewed the records of all potential RFA candidates before treatment in order to confirm adherence to selection criteria and ensure that the least invasive technical approach was used. Percutaneous RFA was used for lesions that were not on the surface of the liver and not adjacent to a hollow viscus or the diaphragm/lung base; otherwise, laparoscopic RFA was used. If other intra-abdominal procedures were planned, then open RFA was used.

Patients with the following findings were excluded from RFA therapy:

1. Unresectable extrahepatic disease.
   Except patients with symptomatic carcinoid syndrome refractory to medical therapy.
2. Patients with technically resectable disease (determined by our hepatobiliary surgeon).
   Patients with colorectal cancer who had less than 6 lesions; patients with hepatocellular carcinoma (HCC) awaiting liver transplant who underwent RFA as a bridge to transplantation
3. Inability to achieve a completely ablated margin of at least 0.5 cm.
4. All lesions larger than 6 cm; for percutaneous therapy, lesions larger than 5 cm.
5. HCC with vascular invasion or thrombosis.
6. Tumors adjacent to a sectoral, or larger, bile duct.

Patients scheduled for percutaneous RFA received conscious sedation and local anesthesia. In a few instances, general anesthesia was used. The RFA probe was introduced and advanced under CT fluoroscopy (Fig. 1) or ultrasound guidance. Once the tumor was reached, the tines of the RFA probe were deployed and positioning was confirmed in three dimensions. Current was applied using generators capable of producing 100 to 150 watts and 350 to 500 MHz. Probes at the tip of the tines monitored the temperature in the surrounding tissue. Once the temperature reached 80° to 110° C, the lesion was ablated, from 12 minutes (3 cm lesions) up to 20 minutes (5 cm lesions), while being monitored by means of CT or ultrasound imaging. Contrast enhancement or color Doppler ultrasound was used in most cases to confirm the absence of blood flow in vascular tumors. The needle tract was ablated by the probe tip during probe removal. Patients were monitored up to 4 to 6 hours after the procedure before being discharged home. The same thermal protocol was used for laparoscopic and open RFA, with the monitoring done by means of ultrasonography. Needle tract hemostasis was achieved with conventional electrocautery in laparoscopic and open cases. Lesions larger than 3 cm treated with the 3 cm probe were ablated with overlapping thermal cycles. All lesions were accessed through normal liver substance to avoid the risk of needle-related free peritoneal tumor seeding (Fig. 2). Needle tract hemostasis was achieved with conventional electrocautery in laparoscopic and open cases.

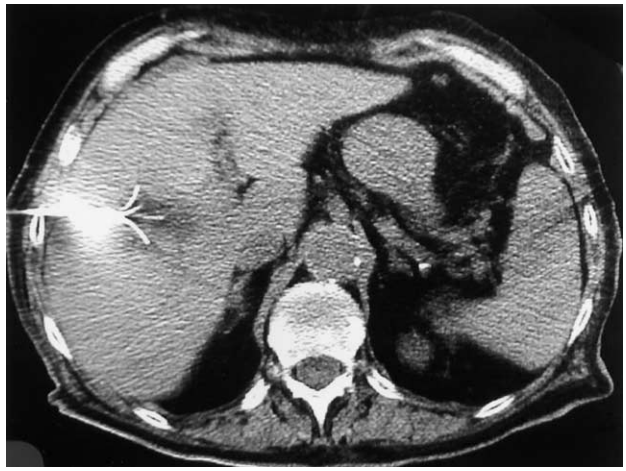

Fig. 1. CT-guided RFA of a carcinoid tumor. This lesion was adjacent to the right portal bifurcation and was treated partially. Immediate relief of refractory carcinoid syndrome was obtained, and repeat completion percutaneous ablation was performed at a later time.

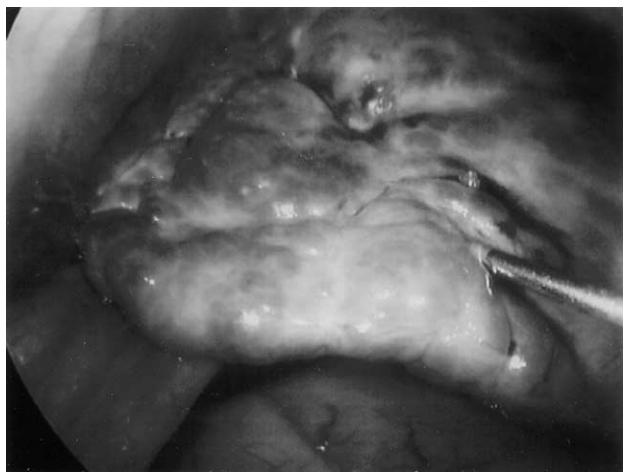

Fig. 2. Laparoscopic RFA for a superficial HCC. Note that this superficial lesion was not punctured directly.

Collected data included the primary diagnosis, patient age at the time of the initial RFA, sex, tumor location, size and number of lesions, primary approach to the lesions, and length of follow-up. Outcome variables included local recurrence, new intrahepatic disease, and new systemic disease. The number and the nature of complications were recorded, as well as overall survival. Complications were considered major if they required subsequent intervention (e.g., surgery, medical intervention, or use of consultants) and/or directly increased the length of hospital stay (LOS). Minor complications constituted those instances where minimal intervention alleviated the problem without prolonging the LOS. Local recurrence was defined as growth of a treated lesion after the 1-month postprocedure scan, development of an enhancing hyperemic rim more than 2 to 3 mm thick, or a new nodule (Fig. 3). The follow-up protocol included contrast-enhanced CT or MRI of the liver at 1 month and every 3 months thereafter. Many of these patients were managed by referring oncologists, but all films were reviewed by one of the surgeons and interventional radiologists.

Statistical Analysis

Data were analyzed on a computer (StatView-V 5.0.1., SAS Institute, Cary, NC). Student's *t* test was used to perform pairwise comparisons between continuous variables, whereas analysis of variance was applied to multiple comparisons. Logistic regression was used to test the correlation between the independent variables (see preceding risk factors) to the dichotomous primary outcome of the study (recurrence). A multivariate approach was employed to control for potential confounding effects of all other measured independent variables. The level of statistical significance was set at $P < 0.05$.

RESULTS

Of the 45 patients, 27 were men and 18 were women; the median age was 58 years (range 30 to 81 years) (Table 1). Median follow-up was 19.5 months (range 6 to 34 months). The median number of tumors per patient at the time of the initial RFA was two (range 1 to 11).

A total of 52 RFAs—11 percutaneous, 21 laparoscopic, and 20 open—were performed to ablate 143 lesions (Tables 2 and 3). Only two open procedures were performed without a secondary procedure; those were done during the early phase of RFA. All other open RFAs were associated with other procedures such as liver resection, intra-arterial pump place-

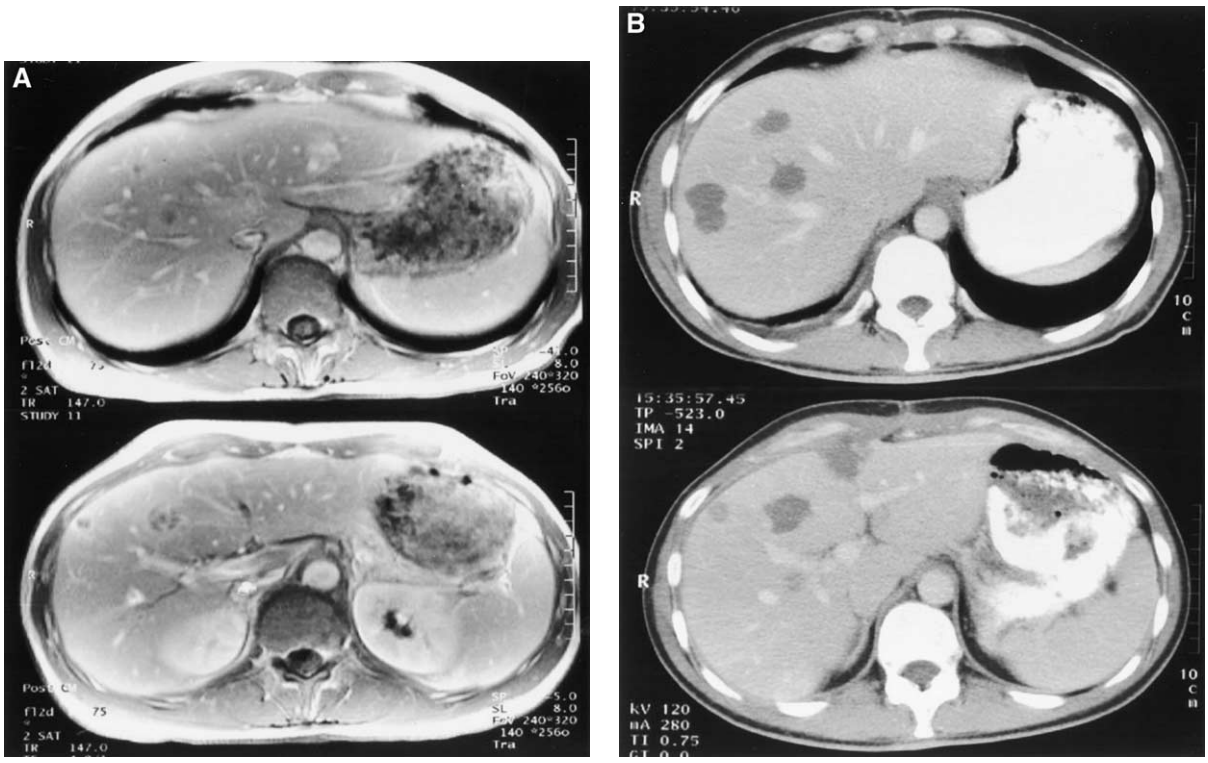

Fig. 3. MRI before (**A**) and CT 3 months after (**B**) ablation. Note that all of the treated lesions grew after ablation but were without vascular enhancement. This patient had 11 discrete metastases from colorectal cancer and was also treated with hepatic artery infusion and systemic chemotherapy.

Table 1. Patient characteristics

Males:females	27:18
Median age (yr)	58 (range 30–81)
Median follow-up (mo)	19.5 (range 6–34)
Median no. of tumors/patient*	2 (range 1–11)

Total patients = 45. Numbers in parentheses refer to range.
*At time of initial RFA.

ment, and bowel resection. The overall median LOS was 2 days (range 0 to 46 days). By RFA method, median LOS was as follows: open = 5 days (range 1 to 46 days); laparoscopic = 1 day (range 1 to 18 days); and percutaneous = 0 days (range 0 to 9 days). The patient whose LOS was 46 days had a concomitant abdominoperineal resection and subsequent complications not related to the RFA (discussed below). Similarly, the 9-day LOS after a percutaneous RFA was related to medical factors other than the RFA procedure itself. Of those patients who underwent a secondary procedure along with the RFA, the longest LOS was associated with bowel resection (n = 3; median 27 days; range 7 to 46 days) followed by liver resection (n = 10; median 6 days; range 4 to 7 days).

Of the 143 treated tumors, 76 (53%) were metastases from malignancies of the colon and rectum, 29 (20%) were carcinoid cancer, 16 (11%) were HCC, and 13 (9%) were leiomyosarcomas (see Table 3). Other metastatic tumors included four renal cell carcinomas, two melanomas, two ovarian cancers, and one breast cancer. Mean size of the four most common tumor types was as follows: HCC = 3.2 cm; carcinoid cancer = 2.2 cm; sarcoma = 2.0 cm; and colon and rectal cancer = 1.7 cm. Within this subset, HCC lesions were significantly larger than the other lesions ($P = 0.0167$). The difference in size among the other three types did not reach statistical significance ($P > 0.05$).

Patients with colon and rectal tumors comprised 40% of the patient pool (18 of 45), all of whom met the inclusion criteria for the study. Five patients qualified for RFA because they had six or more liver metastases. Of the remaining 13 patients who underwent RFA, two had a medical contraindication to major surgery and six underwent RFA in association with another procedure such as liver or bowel resection (Table 4).

The local recurrence rate for treated tumors was 7.7% (11 of 143) (Table 5). Mean size of those original tumors was 3.3 cm (range 1 to 4.5 cm), compared to 1.9 cm (range 0.38 to 7 cm) for original tumors without recurrence ($P < 0.0001$). The cause of recurrence in one patient treated for carcinoid cancer was most likely growth of residual tumor that was left behind after a planned subtotal ablation (subtotal because the tumor was near the sectoral ducts in the right lobe). Of the 10 patients who underwent resection along with RFA, four had a local recurrence at the site of RFA. Two of the four patients had multiple metastases of colon and rectal cancer; three of these lesions recurred locally (sizes 4 cm, 4 cm, and 1 cm). One patient had recurrence of one HCC tumor (size 4 cm), and one patient with renal cell carcinoma had recurrence of three tumors (sizes 4.5 cm, 2.7 cm, and 2.5 cm). Local recurrence was noted in only one of five patients in whom an intra-arterial pump was placed.

Multivariate logistic regression was employed to analyze potential independent variables that could have an impact on local recurrence (i.e., the dependent variable). First, all lesions were treated as a single population (n = 143), and the following were used as independent variables: age at the time of the

Table 2. RFA method and length of stay

Method	Number of RFAs (%)	Length of stay (days)	
		Median	Range
Percutaneous	11 (21)	0	0–9
Laparoscopic	21 (40)	1	1–18
Open	20 (38)	5	1–46
RFA with liver resection	10	6	4–7
RFA with hepatic artery pump	6*	6	3–7
RFA with bowel resection	3	27	7–46
RFA with tumor resection	3	4	3–4
RFA with ventral hernia repair	1	5	NA
TOTAL	52†	2	0–46

*One patient underwent placement, and later removal, of an intra-arterial pump.
†Seven patients underwent a second RFA.

Table 3. Tumor type

Tumor type	No. of patients (%)	No. of tumors treated (%)	Mean size (cm)
Colon and rectal cancer	18 (40)	76 (53)	1.7
Carcinoid cancer	7 (16)	29 (20)	2.2
Hepatocellular carcinoma	12 (27)	16 (11)	3.2*
Sarcoma	4 (9)	13 (9)	2.0
Renal cell carcinoma	1 (2)	4 (3)	3.1
Melanoma	1 (2)	2 (1)	2.8
Ovarian cancer	1 (2)	2 (1)	0.8
Breast cancer	1 (2)	1 (0.7)	3.0
TOTAL	45	143	2.0

*Significantly larger than other tumor types ($P = 0.0167$).

first RFA, sex, lesion size, probe size (3 cm vs. 5 cm), approach, main diagnosis, and number of lesions at the time of the first RFA (Table 6). With all other independent variables controlled for, size was the only variable that was statistically significantly associated with local recurrence ($P = 0.038$; odds ratio 1.821; 95% confidence interval [CI] = 1.03 to 3.21). On further breakdown, original tumor size as small as 3 cm was significantly associated with local recurrence ($P = 0.038$; odds ratio 6.82; 95% CI = 1.11 to 41.94), as well as lesion size ≥4 cm ($P = 0.007$; odds ratio 25.24; 95% CI = 2.46 to 258.72). Size significance was lost when local recurrence was evaluated using the patients (n = 45) as the study population.

To further delineate the relationship between original tumor size and local recurrence, the subset of patients with liver metastasis from colon and rectal cancer was analyzed (Table 7). This subset had 76 original tumors (mean size 1.7 cm; range 0.38 to 4.5 cm) and five instances of local recurrence (mean size 3.1 cm; range 1 to 4 cm). Multivariate logistic regression analysis of lesions (n = 76) as the study population demonstrated that, after controlling for the other previously mentioned independent risk factors, lesion size ≥4 cm was significantly associated with local recurrence ($P = 0.048$; odds ratio 648.43; 95% CI = 1.049 to 400847). This small significance disappeared when the analysis was repeated using the patients (n = 18) as the study population.

Of the 45 patients, 22 (49%) developed new hepatic disease and 11 (24%) had new systemic disease (Table 8). Of the 22 patients with new hepatic disease, eight originally had colon and rectal cancer; similarly, of the 11 with new systemic disease, six originally had colon and rectal cancer. Of this total of 33 cases (22 plus 11), 10 patients (22%) had concurrent progression of systemic disease and new hepatic tumors, 12 (27%) had new hepatic disease only, and one (2%) had progression of systemic disease but no documented new intrahepatic disease. Therefore 51% (23 of 45) of the patients in the study demonstrated some form of progression of disease outside of the treated lesion. Interestingly, only two of these 23 patients had *local* recurrence of their ablated lesions. The first had local recurrence of three of his liver metastases from renal cell origin, the largest of which was 4.5 cm, as well as new hepatic and new systemic progression. The second patient had local recurrence of HCC plus new hepatic disease. None of the 45 patients had probe needle tract recurrences.

Among the 52 patients who underwent RFA procedures, complications occurred in 12 (23%) (Table

Table 4. Management of patients with colon and rectal cancer

Indication	No. of patients
Six or more lesions	5
Medical contraindication to major surgery	3
Anatomic contraindication	4
RFA in conjunction with another procedure	
Liver resection	3
Bowel resection	2
Hepatic artery pump	1
TOTAL	18

Table 5. Original tumor type with local recurrence

Tumor type	No. of tumors	Size (cm)	
		Mean	Range
Colon and rectal cancer	5	3.1	1–4
Hepatocellular carcinoma	1	4	NA
Carcinoid cancer*	2	3.5	1–3.5
Renal cell carcinoma	3	3.2	2.5–4.5
TOTAL	11/143 (7.7%)	3.3	1–4.5

*Nonendocrine origin.

Table 6. Impact of variables on local recurrence, by multivariate logistic regression analysis—all lesions

Variable	*P* value	Odds ratio	95% CI
Patient age at time of first RFA	0.861		
Patient sex	0.476		
Lesion size	0.038*†	1.821	1.033–3.21
Probe size (3 vs. 5 cm)	0.965		
RFA approach	0.699‡		
Main diagnosis	All >0.99‡		
No. of lesions at first RFA	0.380		

*On further breakdown, original tumor size as small as 3 cm was significantly associated with local recurrence ($P = 0.038$; odds ratio 6.82; 95% CI = 1.11 to 41.94), as well as lesion size ≥4 cm ($P = 0.007$; odds ratio 25.24; 95% CI = 2.46 to 258.72).
†Size significance was lost when local recurrence was evaluated using the patients (n = 45) as the study population.
‡Individual data not shown.

9). Major complications occurred in nine patients. One patient with Child's class C cirrhosis died of retrograde portal vein thrombosis after laparoscopic RFA of a 4 cm lesion adjacent to the ascending branch of the left portal vein. Four patients (3 after open RFA and 1 after laparoscopic RFA) suffered a hemorrhage. All four responded to transfusion of 2 units of packed red blood cells and required no other intervention. Two patients had a postoperative myocardial infarction after open RFA: one received heparin and underwent stent placement; the other was discharged home on aspirin. One patient who underwent abdominoperineal resection along with RFA had a threatened limb secondary to occlusion of the iliac vessels. Finally, one patient had a pneumothorax after percutaneous RFA; it was successfully treated with chest tube placement.

Three patients suffered minor complications. One patient had transient ascitic leakage through a laparoscopic port, which resolved without intervention. One patient had a superficial wound infection after a combination of RFA and liver resection for colon cancer metastasis. The infection was treated with local measures. Last, one patient had pleural effusion, which was observed without intervention.

In addition to the one perioperative death, five other patients have now died, all with recurrent disease. Three patients died of cirrhosis, with evidence of new disease other than the treated lesion. The fourth patient died of the antecedent complications of uncontrolled carcinoid syndrome. The fifth patient died of new hepatic and systemic metastases of colorectal cancer 2.5 years after initial treatment.

Table 7. Impact of variables on local recurrence by multivariate logistic regression analysis—all lesions from colon and rectal metastatic source

Variable	*P* value	Odds ratio	95% CI
Patient age at time of first RFA	0.279		
Patient sex	0.415		
Lesion size ≥4 cm	0.048*	648	1.049–400800
Probe size (3 vs. 5 cm)	0.360		
RFA approach	0.136		
No. of lesions at first RFA	0.379		

*This small significance disappeared when the analysis was repeated using the patients (n = 18) as the study population.

DISCUSSION

Our multidisciplinary Hepatobiliary Tumor Service reviewed all patients and employed strict selection criteria for the application of RFA in patients with advanced hepatic malignancies. It was hypothesized that the disease progression, rather than local technical failures, would prove to be the limiting factor in patient outcomes. Our data confirm that recurrence after RFA treatment is uncommon, but a high rate of new hepatic disease and new systemic disease was found.

Our data show that 92.3% of tumors treated by RFA remained free of local recurrence—a finding that is consistent with the findings of other investigators (Table 10). The RFA approach did not have a statistically significant impact on local recurrence; the only factor that did was original tumor size ≥4 cm (see Table 4). We did not see any needle tract recurrences. This is attributed to the strict avoidance of direct puncture of superficial lesions (see Fig. 2) and ablation of the needle tract. From a practical qualitative standpoint, the more difficult lesions to treat were sclerotic in character (renal cell and colorectal metastases specifically). The complexity of full ablation of sclerotic, larger lesions increases rapidly with size. In the past, overlapping spheres were used to ablate tumors that were larger than the ablation capabilities of the earlier probes. Of 12 large tumors that were treated with this overlapping technique in the early phase of the study, 10 did not show local recurrence. However, from a technical standpoint, the availability of larger probes may facilitate the ablation of larger tumors and eliminate the need for less accurate methods of eliminating lesions such as overlapping spheres. Nevertheless, just as is expected with margin-positive liver resection, failure to completely ablate all edges of the tumor will be associated with local recurrence.

Table 8. Patients with new hepatic or systemic disease

Original tumor type	No. of new tumors	Total patients with tumor
A. New hepatic disease*		
Colon and rectal cancer	8	18
Hepatocellular carcinoma	5	12
Carcinoid cancer	4	7
Sarcoma	3	4
Melanoma	1	1
Renal cell carcinoma	1	1
B. New systemic disease†		
Colon and rectal cancer	6	18
Hepatocellular carcinoma	1	12
Carcinoid cancer	2	7
Sarcoma	2	4
Melanoma	1	1
Renal cell carcinoma	1	1

*22/45 (49%).
†11/45 (24%)

It is too early to determine either the significance of local recurrence or the survival benefit associated with successful treatment in overall patient survival. Early in our experience we salvaged locally recurrent tumors with repeated thermal ablation or cryoablation. One patient who underwent a combination of resection and ablation could not be offered salvage therapy because of the advanced nature of the local failure. With the recent increases in probe size and concomitant ability to thermally ablate larger volumes, the issues related to local recurrence may shift and the local success with lesions up to 5 cm may be improved. Furthermore, the route of application did not influence the rate of local recurrence. We believe that careful review of all films by both surgeons and interventional radiologists maximized the likelihood of technical success with the percutaneous and laparoscopic approaches. Patients are best served by both the least invasive and most successful approach to RFA application.

Despite effective local treatment, the rate of new hepatic disease was a disappointing 49%, and the rate of new systemic disease was 24%. It must be emphasized that uniform selection criteria were applied in this study. Only patients with carcinoid tumors who had medically refractory disease were treated with "debulking" of liver tumors in the presence of unresectable extrahepatic disease. Colorectal metastasis was the most common disease treated in this time frame. Preoperative evaluation was thorough but included positron emission tomographic scans for only the latter third of the study. These patients, therefore, met the standard criteria for liver resection, except for the number of lesions or anatomic issues. Although multivariate analyses have shown a survival benefit for liver resection in patients with up to six resectable lesions,[1,13–15] we are providing liver-directed therapy beyond that already liberal cutoff. Despite careful evaluation for other disease prior to RFA, the postoperative recurrence of new hepatic and/or systemic disease was 51% (23 of 45). A larger study will be necessary to compare RFA-treated patients to those treated with resection, but a general comparison with resected historical control subjects would indicate that a disease-free survival of 49% at a median of 23.5 months is suboptimal.

Because these patients are at high risk for failure, both in the liver and systemically, we have attempted to enroll all patients in adjuvant therapy protocols whenever possible. Of great interest is the combined use of hepatic artery infusion chemotherapy with systemic chemotherapy and tumor ablation for the treatment of colorectal metastasis. The small studies[16,17] (Ravikumar) and anecdotal experiences must be fol-

Table 9. Complications

Complications	Number of patients	RFA method	Intervention
Major complications			
Bleeding	4	Laparoscopic (n = 1) Open (n = 3)	2 U packed red blood cell transfusion (n = 4)
Myocardial infarction	2	Open	Heparin stent (n = 1) Aspirin (n = 1)
Death	1	Laparoscopic	
Threatened limb	1	Open	
Pneumothorax	1	Percutaneous	Catheter suction
Minor complications			
Transient ascitic leak	1	Laparoscopic	Observation
Wound infection	1	Open	Incision and drainage
Right pleural effusion	1	Percutaneous	Observation

Table 10. Clinical studies on RFA with minimum of 6 months of follow-up

Reference	Mo/Year	Number of patients	Number of lesions	Follow-up	Local recurrence	New hepatic disease	New systemic disease	Notes
Curley[7]	9/2000	110	149	Median 19 mo (minimum 12 mo)	4 pts (3.6%)	(see Notes)	(see Notes)	*50 patients (45.5%) with new intrahepatic or systemic disease
Izumi[9]	3/2001	92		Median 22 mo (12 to 44 mo)	?6	26%	?	Includes MCT or RFA
Kosari	2001	45	143	Median 19.5 mo (range 6–34 mo)	7.7% lesions (11/143)	49% of patients	24% of patients	Current study

MCT = microwave coagulation therapy.

lowed with multicenter group protocols to determine the utility of this approach in colorectal cancer. No doubt the high recurrence rates found in this study were due to undetectable micrometastases. Adjuvant therapies as part of our RFA treatments were employed whenever possible (e.g., RFA along with hepatic artery infusion pump therapy and systemic chemotherapy for colorectal metastases; liver transplantation for HCC). The RFA treatment of patients with multiple hepatic metastases may be considered similar to the treatment of patients undergoing tumor volume reduction therapy. The survival benefit seen in the report by Kemeny et al.[18] on the use of adjuvant hepatic artery infusion chemotherapy, especially in those patients with positive margins, may offer a glimpse at a patient population similar to our RFA-treated group. Multi-institutional protocols have been initiated or are undergoing careful consideration by cooperative groups. When available, RFA practitioners should be encouraged to participate. We feel strongly that despite the positive performance of RFA as local therapy, effective adjuvant therapy will be vital for overall success.

The availability of percutaneous and laparoscopic techniques makes RFA an attractive option for minimally invasive treatment of liver tumors. For the 30% to 50% of patients who undergo curative liver resection for colon and rectal carcinoma but who then develop isolated hepatic recurrences, RFA may have a significant role.[15] However, patients who are candidates for liver resection, either for primary metastatic disease or recurrence, should not undergo RFA. We believe that a multidisciplinary team helps ensure the most appropriate selection of all forms of treatment for patients with complex liver cancer.

The method of RFA delivery does alter efficacy. Of interest, in the report by Curley et al.[7] of their experience in 76 patients, six of those treated with percutaneous RFA (7.9%) required a second ablation because of incomplete ablation during the first procedure. In our series we observed that a similar, but slightly smaller, proportion of tumors required repeat ablation. We attribute the cause of incomplete ablation to the amount of pain that patients have during RFA, despite sedation. With the increasing power of RF generators, we have found that patients require more intensive intravenous sedation during ablation of larger lesions.

RFA appears to be a safe procedure. Bleeding was the most common complication, occurring in four patients. One of our patients had an episode of bleeding from a laparoscopic trocar site. The three other patients experienced bleeding related to the associated operation. None of these patients required reoperation. The postoperative death was in a patient with Child's class C cirrhosis who was being considered for liver transplantation and had a superficial 4 cm lesion in proximity to the ascending branch of the left portal vein. The patient developed hepatic encephalopathy postoperatively, which was attributed to retrograde portal vein thrombosis. Kim et al.[19] have reported on the laparoscopic treatment of HCC in patients awaiting liver transplantation. Of the nine patients there was one death, two patients developed worsening liver insufficiency, and one patient required a blood transfusion. Clearly, patients with Child's class C cirrhosis who require general anesthesia are at notable risk for complications, and we strive to treat these patients percutaneously whenever technically possible. We did not observe any major bile duct injuries, primarily because we subselected out those patients with tumor proximity issues.

For local treatment of unresectable liver tumors, RFA is 92% effective. Local recurrence rates are acceptable and, with careful monitoring, repeated RFA can be successful. The complication profile has been

acceptable. In our experience, tumors ≥4 cm posed a greater risk for local recurrence, independent of probe size (up to 5 cm lesions). Still, RFA alone is not likely to be truly effective in the group of patients with unresectable hepatic malignancies. Intense effort must be placed in the identification of efficacious adjuvant therapies (hepatic artery infusion chemotherapy, systemic chemotherapy for metastatic lesions, and liver transplantation for HCC). We believe that a multidisciplinary approach for patient selection, using strict criteria to rule out synchronous extrahepatic disease, will enhance the final outcome and determine the proper role of RFA in the management of hepatic malignancies.

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Hepatic Cryoablation–Induced Multisystem Injury: Bioluminescent Detection of NF-κB Activation in a Transgenic Mouse Model

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Hepatic injury from cryoablation has been associated with multisystem injury, including adult respiratory distress syndrome, renal insufficiency, and coagulopathy; but the responsible mechanisms have not been well defined. In the present study we investigated the role of the transcription factor NF-κB in the multiorgan inflammatory response to hepatic cryoablation utilizing a novel in vivo system for determining NF-κB activity. Using transgenic mice expressing *photinus* luciferase under the control of the 5′ HIV-LTR (an NF-κB–dependent promoter), we measured luciferase activity in the liver, lungs, and kidneys as a marker for NF-κB activity. Luciferase production was determined by in vivo bioluminescence and by luciferase assays of tissue homogenates. After measurement of basal luciferase activity, mice were treated with 35% hepatic cryoablation or sham laparotomy and injected with luciferin (0.75 mg/mouse). Photon emission from the liver, lungs, and kidneys was measured at multiple time points. Hepatic cryoablation induced a significant increase in photon emission by the liver, lungs, and kidneys, which correlated with markedly increased luciferase activity measured from each organ after death. Lung lavage 4 hours after cryoablation showed neutrophilic lung inflammation with increased MIP-2 levels compared with sham surgery. These findings demonstrate that 35% hepatic cryoablation is associated with NF-κB activation in the remnant liver and multiple distant sites, and may be causally related to the multisystem injury that is seen after direct liver injury. (J GASTROINTEST SURG 2002;6:264–270.)

KEY WORDS: Hepatic cryoablation, multisystem injury, NF-κB, liver injury

Primary liver malignancy and liver metastases from gastrointestinal primary lesions result in significant cancer-related mortality worldwide. Surgical resection of primary tumors, and in some cases metastatic tumors, continues to be the mainstay of treatment. Hepatic cryoablation is a treatment option for the management of unresectable liver tumors.[1–6] It is a unique method of tumor destruction that kills tumors by physicochemical changes, as well as by obliteration of small blood vessels, with resultant microcirculation failure and hypoxic cell death. This technique involves circulation of liquid nitrogen through metallic probes placed on the surface or into the center of the tumor. Cryosurgery results in tumor ablation and patient survival comparable to standard hepatic resection.[7]

Although cryoablation of small areas of the liver is usually well tolerated with few adverse effects, significant complications have been noted when more than 35% of the liver volume has been treated with cryoablation.[1,8,9] Reported clinical complications include thrombocytopenia, disseminated intravascular coagulopathy, renal failure, hepatic failure, and adult respiratory distress syndrome. These complications are now well recognized and have been described as "cryoshock phenomenon,"[10] although the mecha-

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nisms of multisystem injury are less well defined. We have shown previously that hepatic cryosurgery results in systemic inflammation with activation of NF-κB in the liver and lung with increased serum concentrations of NF-κB–dependent cytokines in rat and sheep models.[11,12]

To develop a convenient quantitative method for examining NF-κB activation in vivo, we have engineered a line of transgenic mice that possess the proximal 5′ human immunodeficiency virus (HIV-1) long terminal repeat (LTR) driving the expression of *photinus* luciferase cDNA (referred to as HLL mice [HIV-LTR/Luciferase]).[13] The proximal HIV-LTR is a well-characterized NF-κB responsive promoter, containing a TATA box, an enhancer region between −82 and −103 with two NF-κB motifs, and three Sp1 boxes from −46 to −78.[14–16] Luciferase production in these transgenic reporter mice reflects NF-κB activation over time and can be assessed in multiple organs.[13,17] We have developed bioluminescence technology as a noninvasive means of measuring luciferase production in the lungs of our NF-κB reporter transgenic (HLL) mice.[17]

In the presence of its substrate (luciferin), ATP, and oxygen, firefly luciferase emits photons with a wavelength of 560 nm. These photons can penetrate several centimeters of tissue and be detected externally using an intensified charge-coupled device (ICCD) camera.[18] This technique provides the ability to assess multiple time points in each animal, helps minimize the effects of biological variation, reduces the number of animals used for each experiment, and allows each animal to be used as its own control.

Mouse macrophage inflammatory protein-2 (MIP-2) was originally identified as a heparin-binding protein secreted by a lipopolysaccharide-stimulated mouse macrophage cell line.[19] Mouse MIP-2 cDNA encodes for a 100 amino acid residue precursor protein from which the amino–terminal 27 amino acid residues are cleaved to generate the mature mouse MIP-2. The protein sequence of mouse MIP-2 shows an approximate 63% identity to human GRO-Alpha, Beta, and Gamma chemokines. Similar to human interleukin-8, mouse MIP-2 exhibits potential neutrophil chemotactic activity and may be a key mediator of neutrophil recruitment in response to tissue injury and infection.[20,21] Increased MIP-2 expression has been found to be associated with neutrophil influx in various inflammatory conditions.[22–26]

In the present study we used bioluminescent detection of NF-κB activation in intact, living HLL mice after 35% hepatic cryoablation. We also characterized lung inflammation induced by cryosurgery by measuring total and differential cell counts and MIP-2 levels in lung lavage fluid 4 hours after hepatic cryoablation.

MATERIAL AND METHODS

Animal Model and Surgical Procedures

Transgenic mice expressing *photinus* luciferase cDNA under the control of an NF-κB–dependent promoter 5′HIV-LTR mouse (C57B6/DBA background), weighing 20 to 30 g, were used for all experiments. After anesthesia was induced with intramuscular ketamine hydrochloride (87 mg/kg) and xylazine (13 mg/kg), mice were placed on a small heated operating table, the abdomen was prepared with povidone-iodine (Betadine), and a midline laparotomy was performed. Aliquots of blood were aspirated directly from the inferior vena cava and stored for later analysis. Avascular attachments to the liver (falciform and gastrohepatic ligaments) were divided, and the liver was mobilized. Cryoablation was performed with a 3 mm surface probe (CryoTech LC System 2000; Cryogenic Technology Ltd, Derbyshire, U.K.) with careful isolation of adjacent structures, including the gastrointestinal tract, to avoid inadvertent organ injury. A freeze-thaw cycle of the left lobe was induced, encompassing approximately 35% (by weight), with careful maintenance of normothermia during the freeze-thaw period. In separate studies, temperature testing demonstrated that there was a transient decrease in temperature during the actual cryoablation period, averaging 6° C in the perfused liver temperature, occurring at 5 to 9 mm from the edge of the cryoablated segment. However, there was no change in core body temperature during or after the cryosurgery. After completion of the freeze-thaw cycle, 3 ml of warmed saline solution was instilled into the abdominal cavity, and the fascia and skin were closed in layers.

Mice were then imaged as described below. After imaging was completed, animals were killed with CO_2 asphyxiation at 4 hours after surgery, and lung lavage was performed with a total of 3 ml normal saline solution in 0.8 to 1 ml aliquots.

Animals undergoing sham operation were anesthetized by the above-mentioned method, placed on a small heated operating table, the abdomen was prepared with Betadine, and a midline laparotomy was performed. Three milliliters of warm saline solution was instilled into the abdominal cavity, and the fascia and skin were then closed in layers.

In Vivo Bioluminescence

Anesthetized mice were immobilized for the duration of the integration time of photon counting (approximately 3 minutes). Luciferin (0.75 mg/mouse in 200 μl isotonic saline) was injected by intraperitoneal injection, and mice were imaged with an ICCD

camera (Hamamatsu C2400-32, Photonics K.K., Japan) housed inside of a light-tight box. Basal bioluminescence was measured 30 minutes after intraperitoneal injection of luciferin. Subsequently mice underwent cryoablation of 35% of their livers (as described previously), intraperitoneal injection of luciferin was performed immediately after surgery, and mice were imaged 30 minutes later. Additionally, mice were imaged at 1, 2, and 3 hours after cryosurgery. Thirty minutes before imaging at each time point, luciferin (0.75 mg) was given by intraperitoneal injection.

Light emission was detected as photon counts over a standardized area of the organs of interest using the ICCD camera, and image processing was accomplished with the use of proprietary hardware and software (Hamamatsu). A digital false-color photon-counting image of the mouse was generated. Photon counts were measured over a standard area that corresponded to the region of the thorax overlying the midlung zone or over the right lobe of the liver or kidneys.

Organ Luciferase Measurement

Lungs, liver, and kidneys were harvested, and luciferase activity was measured in organ homogenates by standard luciferase assays. Luciferase measurements on postmortem tissue samples were performed by adding 100 μl of freshly reconstituted luciferase assay buffer to 20 μl of the tissue homogenate ground in reporter lysis buffer (buffers from Promega, Madison, WI). Luciferase activity was then quantified as relative light units using a standard luminometer. Luciferase activity was normalized for protein content, which was measured by the Bradford assay.[27]

Lung Lavage Total and Differential Cell Counts

Lung lavage fluid was centrifuged at 400 × g for 10 minutes to separate cells from supernate. Supernate was separated and frozen for subsequent cytokine analysis, and pelleted cells were suspended in a small amount of serum-free RPMI culture medium. Total cell counts were determined on a grid hemocytometer. Differential cell counts were determined by staining cytocentrifuged slides with a modified Wright stain (Diff–Quick; Baxter, McGraw Park, IL) and counting 400 to 600 cells in cross section.

MIP-2 Enzyme-Linked Immunosorbent Assay

Mouse macrophage inflammatory protein–2 (MIP-2) levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Ninety-six-well microtiter plates were coated with 100 μl of mouse monoclonal MIP-2 antibodies (R&D, Minneapolis, MN) at a concentration of 2.5 μg/ml and incubated overnight at 4° C. Plates were washed with phosphate-buffered saline solution containing 0.05% volume/volume Tween 20 and immediately blocked with bovine serum albumin, 1% weight/volume, then incubated for 2 hours at 37° C. Plates were decanted and MIP-2 standards (containing 0.005% dithiothreitol) and bronchoalveolar lavage supernatant samples were added in duplicate and incubated at 37° C for 1 hour. Plates were washed and incubated at 37° C for 1 hour with 100 μl goat anti–MIP-2 polyclonal biotinilated antibody (R&D). Streptavidin–horseradish peroxidase diluted in 0.05% PBS-Tween 20 with 1% bovine serum albumin was added and incubated for 15 minutes at 37° C. After an additional wash, 100 ml tetramethylbenzidine was added and the plates were left for 20 minutes at room temperature. The reaction was stopped with 2N sulfuric acid. Optical density of the wells was read at 450 nm and quantified by interpolation from a standard curve constructed from known concentrations of mouse MIP-2.

Statistical Analysis

For comparison between groups, a one-way analysis of variance (ANOVA) was used with the Tukey-Kramer multiple-comparisons test (P values <0.05 were considered significant). Graphic results are expressed as mean ± standard error of the mean (SEM).

RESULTS

After cryosurgery, there was a significant increase in photon emission from each organ compared to basal luciferase activity (Fig. 1). Time-course experiments showed that photon emission peaked over the lungs, liver, and kidneys within 1 hour after cryoablation, but the timing of peak activity was different for the liver than for the other organs. Bioluminescence peaked at 30 minutes in the remnant liver, whereas the lungs and kidneys showed peak photon emission at 1 hour. One hour after cryosurgery, photon emission was increased in the lungs, liver, and kidneys compared with sham laparotomy and baseline values (Fig. 2). The increase in bioluminescence after cryosurgery can be seen easily on a computer-enhanced image using an artificial color scale to identify the intensity of detected photon emission (Fig. 3). A representative baseline image is shown, as well as images of mice 1 hour after sham laparotomy and cryosurgery. Sham laparotomy did upregulate luciferase activity above baseline in the various or-

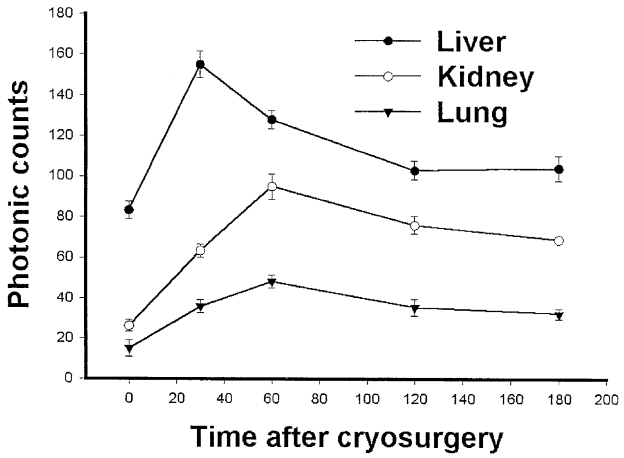

Fig. 1. Graph showing bioluminescence from HLL transgenic reporter mice in lung, liver, and kidney after cryosurgery. Photon emission was quantified by computerized counting of detected photon emission in a 3-minute scan over a standardized area of the thorax, liver, and kidneys. Baseline scans were obtained before hepatic cryoablation. After cryosurgery, scans were obtained at 30 minutes, 1 hour, 2 hours, and 3 hours. Intraperitoneal luciferase (75 mg) was given 30 minutes before each scan to ensure that adequate substrate was available for light generation by luciferase (N = 5).

gans; however, the photon counts of the cryoablated animals were statistically higher than counts in the sham laparotomy group and baseline counts in both groups ($P < 0.01$).

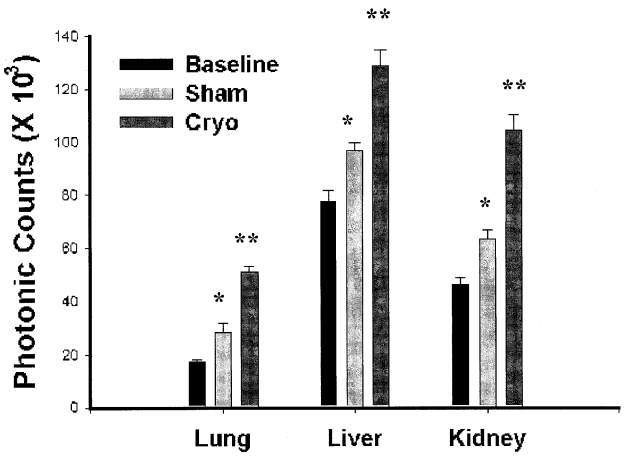

Fig. 2. Bioluminescent activity in mice 1 hour after cryosurgery or sham laparotomy. Photon emission was quantified by computerized counting of detected photon emission in a 3-minute scan over a standardized area of the thorax, liver, and kidneys. Baseline scans were obtained before surgery. Intraperitoneal luciferase (75 mg) was given 30 minutes before each scan. One hour after surgery, mice treated with cryosurgery had significantly higher photonic counts over lung, liver, and kidney compared to baseline values or counts in sham-operated mice (N = 5 mice per group). *$P < 0.05$, Sham vs. Baseline; **$P < 0.001$, Cryo vs. Baseline.

The finding of increased luciferase activity by bioluminescent detection in multiple organs after cryosurgery was corroborated by measuring luciferase activity in various organs from mice killed 4 hours after cryosurgery or sham laparotomy. Fig. 4 shows the luciferase activity measurements in organ homogenates. In each organ, luciferase activity (corrected for protein concentration) was higher in the cryosurgery group compared with the sham-operated controls. Peak luciferase activity in each organ, as measured by bioluminescence, correlated closely with the luciferase activity in organ homogenates, as measured in a standard luminometer ($R^2 = 0.816$) (Fig. 5). Together these findings show that in a mouse model of hepatic cryoablation, early NF-κB–dependent gene transcription can be detected in multiple organs, similar to our previous report of increased NF-κB activation by gel mobility shift assay in lungs and livers of rats undergoing cryosurgery.[28] Because luciferase activity in the noncyroablated remnant livers peaks before the luciferase activity in other organs, these studies support the notion that cryosurgery initially causes NF-κB activation in the remaining liver tissue and products of NF-κB activation in the liver result in subsequent downstream activation of NF-κB in the lungs and other organs, mediating end-organ inflammation and injury.

We found that lung inflammation was present at 4 hours after hepatic cryoablation but not after sham laparotomy. Hepatic cryoablation resulted in a significant increase in total white blood cell counts and neutrophil counts in lung lavage fluid compared with the sham operation (Fig. 6). We measured levels of the NF-κB–dependent chemokine MIP-2 in lung lavage fluid to determine whether levels of this chemokine correlated with neutrophil influx. MIP-2 levels in lung lavage, like neutrophil counts, were significantly increased in mice that underwent cryosurgery compared with the sham-operated group (Fig. 7). These findings provide evidence for lung inflammation induced by the systemic inflammatory response to hepatic cryoablation.

DISCUSSION

Our clinical experience has demonstrated that hepatic injury after cryoablation can lead to pulmonary dysfunction.[9] Previous studies in our laboratory focused on establishment of suitable animal models to assess the effects of liver injury on pulmonary pathophysiology. In a rat model of hepatic cryoablation, we have previously shown that a systemic inflammatory response is induced with elevation of serum TNF-α and MIP-2 levels and resultant lung injury at

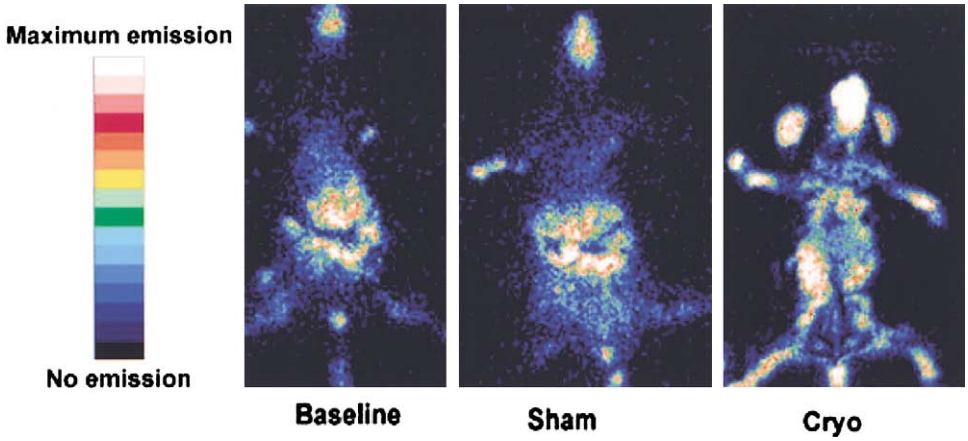

Fig. 3. Bioluminescence of representative mice at baseline and 1 hour after sham laparotomy or hepatic cryoablation. Luciferin (75 mg) was given by intraperitoneal injection 30 minutes before imaging. Increased bioluminescence can be seen in the area of the thorax and right upper quadrant of the abdomen in the mouse treated with cryosurgery (Cryo; *right panel*). Substantial basal level luciferase activity is identified in the brains of these reporter mice, which is not altered by intervention.

24 hours.[28,29] We have also shown that NF-κB activation occurs in the liver and lungs using electrophoretic mobility shift assays after hepatic cryoablation. In a sheep model of hepatic cryoablation, we characterized the pulmonary hemodynamic response and showed that an increase in pulmonary artery pressure occurs, which may be mediated by thromboxane.[11] We further compared the effects of partial cryoablation to those of radiofrequency ablation and were able to demonstrate that hepatic cryosurgery, but not radiofrequency ablation, induces NF-κB activation in the nonablated lung and liver and is associated with acute lung injury.[12] We hypothesized that liver injury induced by hepatic cyroablation stimulates activation of the transcription factor NF-κB in remaining viable liver, resulting in production and release of proximal inflammatory cytokines, including TNF-α into the systemic circulation. These cytokines then trigger NF-κB activation in the lungs, local generation of proinflammatory cytokines, and chemokines, and thus neutrophilic inflammation and injury.

In the current studies we used a line of transgenic reporter mice that were constructed for use as an in vivo indicator of NF-κB transcriptional activity.[13] In prior studies we have shown that luciferase activity correlates with NF-κB activation measured by conventional means and can be blocked by the use of

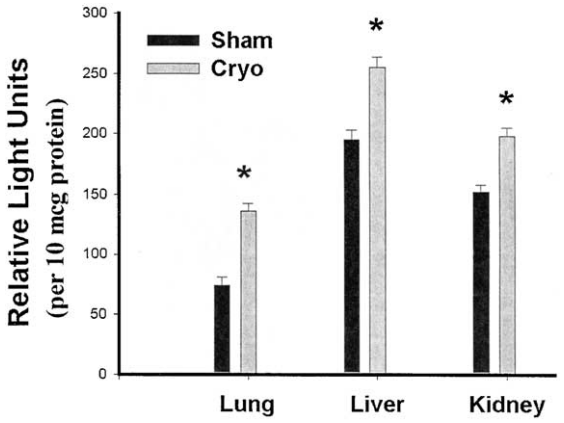

Fig. 4. Luciferase activity from the lungs, liver, and kidneys in organ homogenates from HLL mice treated with cryosurgery or sham laparotomy. Organs were harvested 4 hours after surgery. Mice undergoing cryosurgery showed a statistically significant increase in luciferase activity in lung, remnant liver, and kidney as compared to mice treated with sham laparotomy (N = 5). *$P < 0.05$.

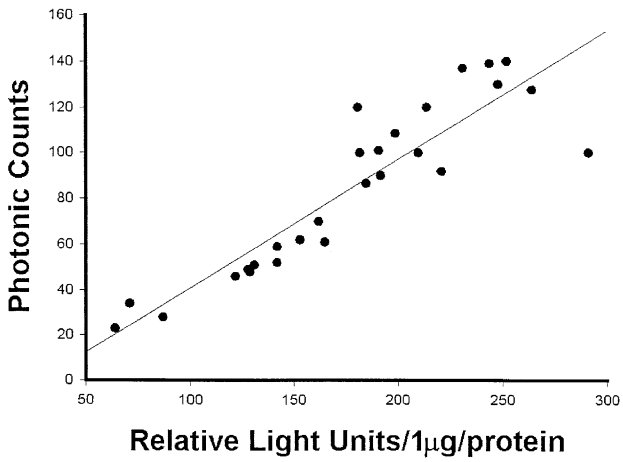

Fig. 5. Correlation between peak photon counts over the lungs, liver, and kidneys as detected by bioluminescence and luciferase activity in the lungs, liver, and kidneys ($R^2 = 0.816$).

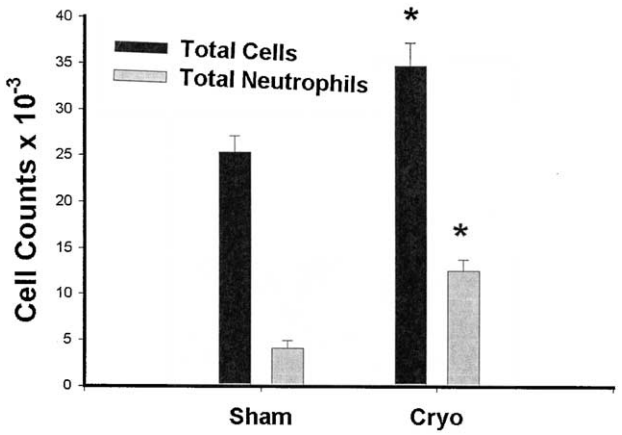

Fig. 6. Total cell counts and neutrophil counts in lung lavage of mice 4 hours after cryosurgery or sham laparotomy. After cryosurgery, mice had increased neutrophils in lung lavage fluid compared to mice treated with sham operation (N=5). *$P < 0.001$.

NF-κB inhibitors in vitro and in vivo.[17,30] Here we assessed luciferase activity in these reporter transgenic mice as an indicator of NF-κB activation after direct hepatic injury with cryoablation. We demonstrated that this liver injury induces NF-κB–dependent luciferase production in multiple distant organs and is associated with a corresponding neutrophilic lung inflammatory response. This real-time imaging has shown that NF-κB–dependent luciferase production occurs in multiple organs as early as 30 minutes after 35% hepatic cryoablation. NF-κB transcriptional activity was demonstrated in multiple organs including the liver, lungs, and kidneys. In these reporter mice, bioluminescence was correlated with an increase in luciferase activity measured after death from the organs of interest (liver, lungs, and kidneys). Sham laparotomy resulted in an increase in luciferase activity in the organs of interest; therefore the laparotomy procedure itself appears to cause some increase in NF-κB–dependent transcriptional events that can be detected by this sensitive indicator. We further characterized the lung inflammation by performing lung lavage and demonstrated that cryosurgery was associated with a neutrophilc alveolitis with increased levels of the NF-κB–dependent chemokine MIP-2 compared to sham-operated control mice. In addition, we found differential kinetics for NF-κB–dependent gene expression in the remnant liver, lungs, and kidneys, with an earlier peak in the liver compared to other organs. These findings support our hypothesis that the systemic inflammatory response seen after 35% hepatic cryoablation is mediated, at least in part, by cytokines that are NF-κB dependent.[28]

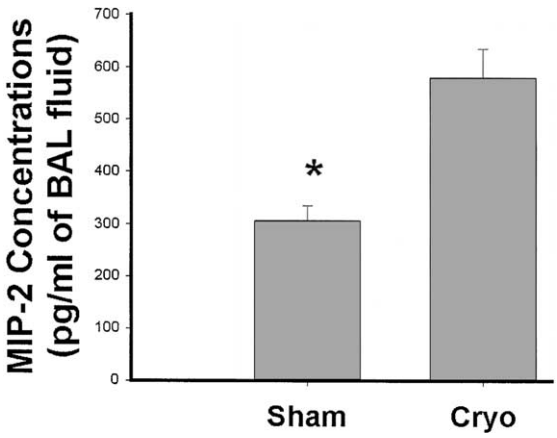

Fig. 7. MIP-2 levels in lung lavage of mice 4 hours after cryosurgery or sham laparotomy. Mice treated with cryosurgery showed significantly increased MIP-2 levels as compared to mice treated with sham laparotomy (N = 5). *$P < 0.01$.

We have provided further evidence that hepatic cryoablation may be a clinical situation where liver injury induces a systemic response that is mediated, at least in part, by NF-κB–dependent production of inflammatory mediators. Enhanced understanding of cryoablation-induced multisystem injury will provide useful insights for the many patients undergoing this procedure, and possibly others with hepatic injury, and may facilitate development of effective preventive strategies.

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The American Hepato-Pancreato-Biliary Association (AHPBA)

Steven M. Strasberg, M.D., AHPBA President

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AHPBA AND JOGS

Effective this year, JOGS (JOURNAL OF GASTROINTESTINAL SURGERY) becomes the official journal of the AHPBA, as it has been for The Society for Surgery of the Alimentary Tract (SSAT) since its inception. All members of AHPBA will receive the Journal as part of their membership package. At least one issue per annum will feature AHPBA papers. A number of AHPBA members are also on the Editorial Board of JOGS. AHPBA encourages submission of manuscripts by its members to this prestigious journal.

AHPBA MEMBERSHIP

AHPBA is a young organization of surgeons, interventional radiologists, and interventional endoscopists with a major interest in liver, pancreatic, and biliary diseases. We welcome membership of academically- and community-based specialists with an interest in these areas. Membership application forms are available on our website at AHPBA.org. Membership benefits include reduced fees for meetings, participation in committees of the Association, early notification of meetings and events, the AHPBA newsletter, and JOGS. AHPBA is closely affiliated with the International Hepato-Pancreato-Biliary Association (IHPBA) and, as a member of AHPBA, you will receive all notifications regarding IHPBA meetings and their association news. *AHPBA es una organización de las Américas y damos la bienvenida determinado a nuevos miembros de la central y de Suramérica.*

AHPBA MEETINGS

Two very successful meetings were conducted in 2001. In February, the third biannual "Americas" meeting was held at the Eden Roc Hotel in Miami, Florida. It included a hands-on postgraduate course on radiofrequency ablation and two and one-half days of papers, lectures, and symposia. In November, the annual Surgical Forum was held in conjunction with the American Association for the Study of Liver Diseases (AASLD). Topics discussed included intraductal papillary mucinous tumor and mucinous cystic neoplasm (M. Sarr), biliary injuries (W. Chapman), and hepatocellular carcinoma in cirrhosis (S. Helton).

FUTURE MEETINGS

April 2002: 5th World Congress IHPBA Biannual Meeting, Tokyo, Japan, April 25–29, 2002. For information contact: ihpba@convention.co.jp

November 2002: AASLD/AHPBA Surgical Forum, Boston, MA, November 1–5, 2002. This will be a liver-based program. Visit AHPBA.org for more information.

February 2003: 5th AASLD Biannual "Americas" Meeting, February 27–March 2, 2003. This will again be held at the Eden Roc Hotel in Miami, FL. The first day will be a postgraduate course chaired by C. Wright Pinson. Make this one with the family! Visit AHPBA.org for more information.

May 2004: 6th World Congress IHPBA Biannual Meeting, Washington, DC, May 27–June 2, 2004. The AHPBA hosts the IHPBA.

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Number and Size of Stones in Patients With Asymptomatic and Symptomatic Gallstones and Gallbladder Carcinoma

To the Editors:

An interesting study on the correlation between size and number of gallstones and gallbladder cancer in Chile appeared recently in the JOURNAL OF GASTROINTESTINAL SURGERY.[1] The study confirms previous reports of a positive association between gallstones of large diameter and gallbladder cancer,[2–4] although such a relationship has not always been found.[5,6] The paper by Csendes et al. appears to be based on a selected surgical population in Chile. To assess the validity of the study, it is important to know how study subjects were selected from what was almost certainly a larger group of patients undergoing cholecystectomy.

It is unfortunate that the analysis did not include the calculation of odds ratios that measure cancer risk according to categories of gallstone diameter. Patients with cancer were older than those with nonmalignant gallbladder disease. Because gallstone diameter may increase as stones grow with time, the findings should be adjusted for patient age.

We believe that the association between gallstones and gallbladder cancer is mediated by the length of time that the stones remain in the gallbladder. A long duration provides time for chronic trauma to the mucosa, and eventually additional risk factors, which initiate a sequence of pathologic changes that can result in cancer. In that context, the decrease in rates of cholecystectomy in Chile during the 1970s and 1980s has been associated with an increasing incidence and mortality from gallbladder cancer, with the frequency of older and perhaps larger gallstones likely to be a key factor. This inverse correlation was first observed in Sweden[7,8] and was subsequently noted in other countries including Chile.[9–11] The logical consequence of a decrease in the cholecystectomy rate is an increase in the number of gallstone carriers in the population, associated with an increasing diameter of gallstones. A Chilean series of 95 consecutive patients with gallbladder cancer in the 1980s found that 31.6% of the gallstones in these patients were larger than 2 cm.[12] Did the authors record the time of the biliary colic before surgery, or the timing of the sonographic diagnosis of gallstones in patients with gallbladder cancer compared to patients with symptomatic gallstones who did not have cancer?

Chile has the highest mortality rate for gallbladder cancer in the world both for men and women.[13] Two case-control studies have suggested that gallstone age is a strong risk factor for gallbladder cancer.[14,15] This observation provides a basis for the development of prevention programs for this type of cancer, a lethal disease the treatment of which has shown little progress. Since 1986, gallbladder cancer has been the leading cause of cancer deaths among Chilean women, exceeding gastric, breast, and cervical cancers.[16] Gallbladder cancer mortality rates have shown a constant and sharp increase in both men and women not only during 20 years[1] but over the past 30 years as well.[17]

The precise ratio between men and women for the incidence and associated mortality of gallbladder cancer in Chile is approximately 1:3, not 1:6-8. Exact ratios are as follows: in 1970, 1:3.6; in 1980, 1:2.7; in 1990, 1:2.9; and in 1999, 1:2.8. It is important to distinguish results from selected populations from the national experience as a whole. Gallstone disease has a smaller sex ratio in the Chilean population, approximately 1:2.5, suggesting that both women and men have additional, ill-defined risk factors for developing neoplasia in the gallbladder.

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Reply

I read with great interest the comments of Drs. Serra and Diehl concerning our study. The following are my responses to some of their comments:

1. The patients included in the present study corresponded to patients who were seen by our surgical group during a certain period of time when this study was performed. There was no special selection of cases.
2. We strongly believe that the long duration of gallstones is the main cause of gallbladder cancer, as was stated in our discussion. We have seen a sequence of pathologic changes as age increases as well as the length of time the stones remain in the gallbladder.[1]
3. We did not record the time of biliary cholic prior to surgery in patients with gallbladder cancer, but in a previous study we noted that 60% of the 373 cases with gallbladder cancer had no symptoms at all.[2]
4. We obtained our data stating that gallbladder cancer is eight times more frequent among women than men from a careful study of 10,000 cholecystectomies performed during a 7-year period, with a complete histopathologic study in all of them.[3] This does not represent the national statistics.
5. The policy of Chilean surgeons is to perform cholecystectomy, even when patients are asymptomatic, because of this high frequency of gallbladder cancer in our patients with gallstones.

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Nonsurgical Therapies for the Gut and Abdominal Cavity

Edited by Jeffrey C. Brandon and Steven K. Teplick. New York, NY: Thieme, 2001. Pages: 124. Illustrations: 74. Price: $99.00.

The aim of this book is to familiarize readers with established or emerging interventional techniques used in the gastrointestinal system. The goal of the editors, both of whom are radiologists, is to provide enough detail to radiologists so that these readers can successfully perform the many procedures described. The intended audience obviously includes interventional radiologists, as well as diagnostic radiologists, radiology residents, and nonradiology clinicians interested in minimally invasive gastrointestinal therapies.

The book is organized into five chapters: "Gastrointestinal Bleeding: A Multidisciplinary Approach"; "Gastrointestinal Stenting: Indications and Techniques"; "Percutaneous Gastrostomy, Gastroenterostomy, and Jejunostomy"; "Percutaneous Colostomy"; and "Peritoneal/Retroperitoneal Anatomy: Relevance to Performance of Interventional Procedures." All chapters are written by radiologists with the exception of the first, which is authored by a gastroenterologist, radiologist, and surgeon.

The text is written clearly and succinctly by some of the leading authorities in the field of interventional radiology. The literature has been very well reviewed, and an abundance of references are given. Disease entities are well reviewed, and various diagnostic and therapeutic techniques are exhaustively described.

The chapters reviewing percutaneous enterostomies are the most comprehensive. Although the chapter on gastrointestinal bleeding is a good overview bolstered by 13 color endoscopic images, it is a missed opportunity by the authors to fully realize an integrated, multidisciplinary review of the treatment options and a nonbiased evaluation of the results. The inclusion of a chapter on peritoneal and retroperitoneal anatomy would be more logical if presented in the context of a discussion of image-guided abscess drainage techniques.

The book is of excellent quality with high-grade paper and printing and superior illustrations. Very few typographic errors are present. The cost of the book is reasonable. The book fulfills its intended purpose of presenting state-of-the-art nonsurgical interventional radiology options applicable to the esophagus, stomach, and intestine.

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Live Advanced Endoscopic Techniques: American Society for Gastrointestinal Endoscopy 18th Interim Postgraduate Course, March 22–23, 2002, Fairmont Copley Plaza Hotel, Boston, Massachusetts. Course Directors: William R. Brugge, M.D., Massachusetts General Hospital; and David L. Carr-Locke, M.D., Brigham & Women's Hospital. For further information contact: American Society for Gastrointestinal Endoscopy. Phone: 978-526-8330; fax: 978-526-7521; e-mail: asge@shore.net

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Bariatric Surgery Mini Fellowship Program, March 24–29, 2002; August 25–30, 2002; November 3–8, 2002; The University of Texas Southwestern Medical Center at Dallas. Fees: $10,000 (team of 2 physicians and 1 nurse); $5000 (physician); $1000 (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

American Society for Gastrointestinal Endoscopy Hands-On Endoscopy Series: 2002 EUS Training Course, April 5–6, 2002, Endo-Surgery Institute, Cincinnati, Ohio. Course Directors: Irving Waxman, M.D., University of Chicago; and Maurits J. Wiersema, M.D., Mayo Medical School and Clinic. For further information contact: Endo-Surgery Institute. Toll free: 1-877-477-6333; e-mail: contactesi@ eesus.jnj.com

Society for Surgery of the Alimentary Tract 35th Annual Meeting & Postgraduate Course, May 19–22, 2002, San Francisco, California. Includes the postgraduate course, guest speakers, Poster Session, SSAT/ASCR Symposium, SSAT/SAGES Symposium, Consensus Conference, SSAT Public Policy Committee Panel, and plenary sessions. For more information, contact: SSAT Meetings Department, 13 Elm Street, Manchester, Massachusetts 01944. Phone: 978-526-8330; fax: 978-526-7521; e-mail: ssat @prri.com

American Society for Gastrointestinal Endoscopy (ASGE) Annual Postgraduate Course Endoscopic Oncology: Gastrointestinal Endoscopy and Cancer Management, May 22–23, 2002, San Francisco, California. Course Directors: Gary W. Falk, M.D., and Douglas O. Faigel, M.D. Fees: $450, ASGE members; $550, nonmembers; reduced fees for fellows and assistants. For further information contact: American Society for Gastrointestinal Endoscopy. Phone: 978-526-8330; fax: 978-526-7521; e-mail: asge@shore.net

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Bariatric Surgery, June 21–22, 2002; September 27–28, 2002; The University of Texas Southwestern Medical Center at Dallas. Fees: 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

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