SSAT/AHPBA Joint Symposium on Advances in Liver Resection for Metastasis

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The results of liver resection for colorectal metastases in North America were collected by James Foster in the late 1960s.[1] His seminal reports critically analyzed the topic, set the stage for further clinical outcome analyses, and, importantly, raised interest in the topic. Liver resection for metastatic disease has become increasingly common, with the majority of reports focusing on colorectal liver metastasis. The results in terms of operative mortality and long-term outcome have become remarkably better with operative mortality reported in the best of circumstances at 1–2% in patients without underlying liver disease and 5–10% in patients with preexisting cirrhosis, and long term survival over 50% at 5 years. These improvements have been fueled by advances in imaging and preoperative assessment of the patient including the cardiopulmonary status and liver function. There are now many effective hepatic transection techniques. Intraoperative management of the patient with good team communication and close attention to patient volume status have contributed. While chemotherapy and other regional therapies are important in improving results in these patient populations overall, liver resection in an appropriately selected subset of patients has proven to provide a dramatic contribution.

These good results with colorectal metastases have stimulated the application of liver resection to other types of metastases, reported mostly in case reports or very small series with encouraging but inconclusive data. After performing a MEDLINE search of the English language literature and focusing on a series of 10 or more patients, Dr. N. Joseph Espat from the University of Illinois at Chicago performed a type of meta-analysis. He studied results after liver resection for tumor types that relatively frequently metastasize to the liver. He presents his findings in the first article in which Tables 1–3 summarize results for metastases from breast carcinoma, melanoma, gynecologic cancers, sarcoma, gastric carcinoma, and neuro-endcorine tumors. He notes that liver resection is appropriate in a very small percentage of patients with these diagnoses, perhaps around 1% overall. And even in these patients, liver resection constitutes only one component of the total oncologic treatment. The interval from initial diagnoses is an important prognostic factor as is a complete R0 resection. However, for neuroendocrine metastases, liver resection provides long-term benefits in terms of symptom relief and survival despite rarely achieving R0 status. This summary of the limited available level 3 evidence helps advance our thinking on appropriate application of liver resection for noncolorectal metastases in appropriately selected patients.

There have been many advances in radiologic techniques over the past few decades that have contributed to improved selection and outcomes of liver resection for metastases, including magnetic resonance imaging (MRI), positron emission tomography (PET), and multiphase computed tomography (CT). More recently, three-dimensional (3-D) image analysis techniques have become available, applied initially in living donor liver transplantation. These techniques for estimating liver volumes, perfusion territory, and detailed intra-hepatic anatomy have now been applied to liver resection for metastases. Dr. Christoph Wald, M.D., Ph.D., from the Department of Radiology at the Lahey Clinic Medical Center in Burlington, Massachusetts, in the second paper of this symposium, points out that this 3-D modeling of patient anatomy very closely resembles the actual pathologic findings found intraoperatively. To apply this technology, surgeons must work in concert with radiologists, as the process is still operator dependent. For institutions that do not have the necessary local expertise or numbers of cases to support this investment in technology, remote analysis is available. Once this analysis is complete, the surgeon has an accurate 3-D map of the portal structures and hepatic

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veins. This can generate an appropriate tactical approach in cases with difficult anatomy and advanced disease to provide adequate margins of resection, either avoidance of large structures or calculation of the volume affected by the watershed as well as the expected volume of the liver remnant. The 3-D image analysis will advance liver resection to a higher level of sophistication with better results, much as CT, MRI, and PET scanning have contributed before.

The goals of techniques of liver resection for metastasis are removal of the lesion with adequate surgical margin, limit of blood loss, and avoidance of unintended parenchymal trauma. Historically, finger fracture or crushing clamp technique has been widely applied. In the past 20 years, new technology has become available and applied to liver division in the form of bipolar forceps, ultrasonic scissors, the monopolar floating ball, the cavatron ultrasonic aspirator (CUSA), and water hydrojet. The application of these devices has been useful in many hands, but no single surgical technique has proved superior. In the third paper, Dr. Pierre-Alain Clavian from the Department of Surgery at the University of Zurich, Switzerland, summarizes the results of prospective randomized trial in 100 noncirrhotic, noncholestatic patients undergoing liver resection, comparing four different techniques: the standard clamp-crushing technique under inflow occlusion, the CUSA, the hydrojet, and the monopolar floating ball. This report adds to only two prior randomized controlled trials comparing clamp-crushing technique versus CUSA[2] and CUSA versus hydrojet.[3] Their study demonstrates that the clamp-crushing technique had the highest transection velocity, lower blood loss, and, of course, lower cost. Postoperative reperfusion injury and complications were the same among the four methods. Surgeon preference and experience remain important factors in choice of technique. These other methods become more important in patients with underlying liver disease such as cholestasis, cirrhosis, fibrosis, and steatosis.

Important to the results of liver resection is the intraoperative management of the patient. Dr. Ann Walia, Chief of the Section of Anesthesia for Liver Surgery and Transplantation, Vanderbilt University Medical Center, summarizes, in the fourth symposium paper, some of the important aspects of managing the patient in the perioperative period. Preoperative evaluation of the extent of liver disease, including coagulation status, and of the pulmonary, cardiac, and renal function is routine. Intraoperatively, invasive monitoring and adequate venous access are critical. Transesophageal echocardiography is beneficial in patients with limited cardiac reserve. Blood transfusion is an independent predictor of operative mortality, length of hospital stay, and complications. Significantly, then, intraoperative techniques that have limited blood loss include initial low central venous pressure and application of vasopressors prior to the application of the Pringle maneuver or total vascular isolation. Other blood conservation techniques include the potential use of the erythropoietin, acute normovolemic hemodilution, antifibrinolytics, recombinant factor VIIa, and hemoglobin-based oxygen-carrying solutions.

In conclusion, this SSAT/AHPBA joint symposium highlighted certain advances in liver resection for metastasis. This included selection of patients for resection with noncolorectal disease, novel image analysis techniques unraveling liver anatomy and volume prior to resection, evaluation of surgical transection techniques, and perioperative management to obtain best outcomes.

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Reported Outcome Factors for Hepatic Metastasectomy

N. Joseph Espat, M.D., M.S., F.A.C.S.

The following SSAT/AHAPBA symposium summary is based on a more comprehensive review of the topic by Alseidi A, Helton WS, Espat NJ. Does the literature support an indication for hepatic metastasectomy other than for colorectal primary? J GASTROINTEST SURG in press. (J GASTROINTEST SURG 2006;10:155–160)

Over the past decade, advances in anesthesia techniques, operative instrumentation, and improved imaging have in combination resulted in better patient selection and reduced operative blood loss, time, and operation-related morbidity for patients undergoing liver resection. Colorectal cancer (CRC) hepatic metastases have become the paradigm upon which hepatic metastasectomy is based, given that liver resection is potentially curative for CRC hepatic metastases. With these factors in mind, can hepatic metastasectomy for malignancy other than CRC be supported? And what are the patient selection factors potentially favoring a survival advantage?

Approximately 13,000 liver resection procedures are performed annually in the United States. Although no specific data are available to define the underlying diseases being resected, it is a fair assumption that the majority of procedures are CRC given the annual incidence of the disease. Using the American Cancer Society annual incidence data, the 11 most prevalent malignancies were considered for inclusion into this analysis. Of these, prostate,[1] bladder,[2] uterus/cervix,[3] and kidney[4] only rarely metastasize to the liver; similarly, the treatment for non-Hodgkin's lymphoma[5] is medical, not surgical, and as such, these diagnoses were not included in the present report.

Of the remaining six malignancies, hepatic metastasis from lung[6] is considered to represent significant metastatic disease and resection is contraindicated. Pancreatic adenocarcinoma[7] frequently metastasizes to the liver; however, hepatic resection for this disease has never been shown to result in a survival benefit. As defined, CRC[8] has been excluded from the focus of this discussion.

After excluding malignancies with biologic behavior that does not commonly cause metastasis to the liver or because liver metastasis is a harbinger of extremely advanced disease, the list of potential hepatic metastasectomies with potential *indication* for resection was narrowed to breast,[9] melanoma,[10] and ovary.[11] In an effort to be more complete, several other uncommon malignancies with an annual incidence of less than 20,000 cases for which data are available for analyses were included: neuroendocrine tumors, sarcoma (specific subtypes), and gastric cancer.

In order to define the parameters of survival outcome and patient selection, a MEDLINE search of the English-language literature was performed using the "disease" as key word and search terms *hepatic resection* and *survival benefit*. Anecdotal case reports, single-digit series, and "ablative" (radiofrequency ablation, cryoablation, ethanol injection, etc.) therapies were not included.

Breast, melanoma, gynecologic (ovarian), sarcoma (GIST), and gastric constitute the tumor types where potential patient benefit was defined as an overall improved survival. Based on the prognostic factors from the series reviewed, potential patient selection criteria are summarized (Tables 1 and 2).

BREAST

Several single-institution studies are available. Recognizing the incidence of this disease, hepatic resection for breast cancer constitutes less than 1.0% of patients afflicted. Survival benefit is attainable for selected patients undergoing resection, albeit for

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Table 1. Patient selection/outcome factors for breast, melanoma, and gynecologic hepatic metastases, as reported in the literature

	Ref	Design	n	Median follow-up	OS	DFS	Prognostic indicators (+/−)	Resection indicated	Comments/other findings
Breast	Elias et al.[1] (2003)	Retrospective (collected prospectively)	44	32 mo	3 yr 50% 5 yr 34%	3 yr 42% 5 yr 22%	Negative hormonal status (−)	OS +	• Sole metastasis to liver. Stable Dis. • No impact: R status, Hilar node, number of liver metastases • HAIC ↓ liver recurrence, with no change in OS
	Maksan et al.[9] (2000)	Retrospective	9	29 mo	51%		Long DFI (+)		
							Node neg 1° (primary tumor) Ca(+)	+	
	Pocard et al.[6] (2000)	Retrospective	52	23 mo	49%		• DFI: >48 mo 82% 3 yr OS <48 mo 45% 3 yr OS	+	• Allowed for discontinuation of chemotherapy in 46% of patients
-									
Melanoma	Rose et al.[8] (2001)	Retrospective (collected prospectively)	24		Median OS 28 mo 5-yr OS 29%	Median DFS 12 mo 5-yr OS 12%	R0 status significantly and improved OS DFS (+)	+	• Metastases limited to liver only • Median OS for LM resection group versus exploration only group 28 versus 4 mo, respectively.
Gynecologic	Chi et al.[5] (1997)	Retrospective	12	25 mo	Median OS 27 mo 5-yr OS 50%	Median DFS 12 mo	Metachronous metastases (+)	+	• Median DFI 32 mo • Solitary lesions • 1° Ovary (7), cervix (2), endometrial (2), fallopian (1)
	Merideth et al.[2] (2003)	Retrospective	26		Median OS 26.3 mo 61% 35%		DFI > 12 mo (+) Optimal cytoreduction (+)	+	• 1° Ovarian • Eight (31%) alive at 33 mo

OS = overall survival; DFS = disease-free survival; DFI = disease-free interval (from primary cancer to discovery of metastasis); QOL = quality of life; HAIC = hepatic artery infusion chemotherapy.

Table 2. Patient selection/outcome factors for sarcoma and gastric cancer hepatic metastases, as repted in the literature

	Ref	Design	n	OS (yr)			Prognostic indicators (+/−)	Resection indicated	Comments/other findings
Sarcoma	DeMatteo et al.[3] (2001)	Retrospective	56	Median OS 39 mo 1-, 3-, 5-yr OS, 88%, 50%, 30%			• DFI >2 yr (+)	+	• All R0 • All GIST tumors • Nonresected patients: 4%, 5 yr OS
Gastric	Okano et al.[4] (2002)	Retrospective	19	1-yr 77%	3-yr 34%	5-yr 34%	• Metachronous presentation (+) • Solitary metastases (+)	+	• Three patients lived >3 yr
	Sakamoto et al.[10] (2003)	Retrospective	22	73%	38%	38%	• Solitary metastases (+) • LM <5 cm in size (+)	+	• Five patients lived >3 yr
	Zacherl et al.[11] (2002)	Retrospective	15	Median OS 8.8 mo			• Metachronous presentation (+) • Unilobar LM (+) • 1° (primary tumor) Distal/middle third of stomach (+)	+	• Two patients lived >3 yr

OS = overall survival; DFI = disease-free interval (from primary cancer to discovery of metastases); HAIC = hepatic artery infusion chemotherapy; LM = liver metastases; GIST = gastrointestinal stromal tumor; 1° = site of primary cancer.

a very limited subset, and the series are in agreement at an approximately 50% 3-year overall survival rate for resected patients. A consistent selection factor for a survival advantage was the interval from primary resection to the development of metastases, the completeness of resection (R0); however, the issue of ER receptor status remains unclear. Resection constitutes a component of total oncologic treatment.[1,6,9]

MELANOMA

Data from the two largest melanoma prospectively collected databases (John Wayne Cancer Center and Sydney Melanoma Unit) included 1750 combined total patients; 24 patients underwent resection (1.4%). Eighteen had complete with curative intent and six with palliative intent. Median overall survival was improved in resected patients, 28 months versus 4 months. Prognostic factors are limited achievable (R0) staus post resection. Resection constitutes a component of total oncologic treatment.[8]

OVARIAN/GYNECOLOGIC CANCER

Data are limited to two series of 12 and 26 patients, respectively, from two specialty centers, with mixed-disease populations. Median survival following resection was similar at approximately 27 months. Selection factor for survival advantage was the interval from primary resection to the development of metastases. Resection constitutes a component of total oncologic treatment.[2,5]

SARCOMA

A large single-center experience of 331 metastases, of which 56 patients were resected, has been reported. GIST and gastrointestinal leiomyosarcoma compose the majority of disease subtypes. The 1-, 3-, and 5-year survivals (median, 39 months) were significantly improved compared with incompletely resected patients. A separate study specifically addressing leiomyosarcoma metastases of 26 patients revealed a similar survival advantage. Factors significant for positive outcome benefit include complete resection (R0) and the interval from primary resection to the development of metastases.[3]

GASTRIC

Opposing patient selection criteria exist from Japanese and Western center studies. The discordance is focused on synchronous versus metachronous hepatic disease, predictive value of tumor size, and location of the primary tumor. Interestingly, the Japanese report represents the 17-year experience of a specialty center, suggesting that metastasectomy for gastric cancer is potentially possible only in a very select patient population and that resection constitutes a component of total oncologic treatement.[4,10,11]

NEUROENDOCRINE

Neuroendocrine metastases are unique among the malignancies reviewed in that potential patient benefit can be defined as an overall improved survival or as a quality of life improvement from symptom relief. Based on the prognostic factors from the series reviewed, potential patient selection criteria are summarized (Table 3). The largest body of data in the literature concerns this disease. The data are not clearly defined, as carcinoid, pancreatic neuroendocrine, and nonpancreatic neuroendocrine are included in the series. What is consistent is the approximately 70% or greater 5-year survival for patients undergoing resection. Unfortunately, the series are also in accordance with the high frequency ($>70\%$) of recurrence after resection. Differing observations have been made concerning the biologic behavior of carcinoid versus islet cell neuroendocrine disease. Symptom relief is reported following resection, although recurrence of symptoms is commonplace across the series. All reports have individual long-term survivors, but no specific selection criteria can be discerned.[7,12] Interestingly, there is a correlation between resection and survival with symptom relief; which would be expected given that biologically most would agree that resection for neuroendocrine tumors is a "debulking operation," given the underestimated volume disease present and the frequency of relapse.

In summary, the data in support of hepatic metastasectomy for non-CRC malignancies are derived from level 3 evidence, retrospective reports, and most series are single-institution–specific experiences. It is clear that the operations can be performed safely, but limited agreement has been attained on the optimal factors for patient selection. The reported series suggests that hepatic resection is a component of total oncologic therapy rather than a "stand-alone" procedure. In the specific instance of neuroendocrine disease, the data suggest that symptom relief is attainable, but complete and long-term cure is uncommon.

Table 3. Patient selection/outcome factors for neuroendocrine hepatic metastases, as reported in the literature

	Reference	Design	n	Median follow-up	OS (yr)		DFS (yr)	Prognostic indicators (+/−)	Resection indicated		Comments/other findings
Neuroendocrine	Elias et al.[7] (2003)	Retrospective (collected prospective)	47	62 mo	5 yr 71%	10 yr 35%	75% 10 yr LRR	• None for OS • ↑ DFS: R0 status, <10 LM, pancreatic origin	+	+	• Well-differentiated endocrine tumors; 23 pancreatic • 53% R0 status • 77% also had extrahepatic tumor resected
	Sarmiento et al.[12] (2003)	Retrospective	170		61%	31%			+	+	• 1° (primary tumor) (120) carcinoid, (52) pancreatic, (85) ileum • 104 of 108 patients with symptom relief; 59% 5-yr recurrence of symptoms

OS = over all survival; DFS = disease-free survival; LM = liver metastases; 1° = site of primary cancer.

The role of surgical resection for noncolorectal hepatic metastases cannot be generalized and at present should be individualized based on the patient's clinical course and by specific malignancy.

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Role of New Three-Dimensional Image Analysis Techniques in Planning of Live Donor Liver Transplantation, Liver Resection, and Intervention

Christoph Wald, M.D., Ph.D., Holger Bourquain, M.D.

The ever-increasing complexity of hepatobiliary surgical and percutaneous interventions, including various forms of live donor liver transplantation, liver resection, embolization, and ablation, has resulted in a growing demand for sophisticated preprocedural diagnostic imaging. Fortunately, this trend coincides with dramatic advances in technology and knowledge in diagnostic radiology that allow modern-day radiologists to keep up with surgeons to meet this demand.

Contemporary scanner hardware and software allow for acquisition of high-resolution volumetric images, especially in computed tomography (CT) and magnetic resonance imaging (MRI). Scanners are coupled and coordinated with high-volume fast automatic injection devices, often resulting in repeat scans of the same anatomic area of interest several times during multiple phases of contrast enhancement. Resulting digital datasets contain a wealth of anatomic detail and also physiologic information; images are increasingly made up of isotropic or near isotropic data elements that are ideal for postprocessing on suitable workstations. Many advanced postprocessing software tools have been developed that not only allow high-resolution three-dimensional modeling of patient anatomy closely resembling the intraoperative appearance but also move image analysis into a quantitative realm allowing for total and partial organ volume estimates, perfusion as well as drainage territory analysis, and quantification, etc. This represents a departure from traditional diagnostic imaging, which was dominated by the depiction of anatomy for the purpose of rendering a diagnosis. The combination of qualitative and quantitative computer analysis of image datasets lends itself to surgical planning, with simulation of operative and percutaneous interventions.

MULTIDISCIPLINARY TEAMS

Multidisciplinary teams of computer scientists, mathematicians, radiologists, and surgeons have been the key to development of meaningful computer based solutions to real clinical problems. There is an incredible wealth of computer-programming know how available today, such as in the gaming industry, yet these software experts do not have the necessary background to recognize how their incredibly powerful visualization tools can be adapted to and made useful in the medical field. Radiologists optimize scanning protocols to obtain high-quality datasets tailored to the clinical question at hand. They are in an important intermediary position between the computer scientists and the surgeon, as they understand the possibilities of imaging and image postprocessing while they have the necessary medical training to be able to comprehend the clinical situation of the patient and the surgeons' needs for operative planning. Close collaboration and good communication between these groups of professionals are crucial to yield good results. Surgeons need to spend time with interested subspecialty radiologists to convey specific technical issues that may arise from certain anatomic configurations in the operating room. Radiologists should spend time in the operating room to get a first-hand understanding of the intraoperative findings and surgical strategies before they are able to embark on meaningful computer simulation of surgical procedures.

POSTPROCESSING

Postprocessing of modern image datasets requires not only suitable software but also a significant degree of specialized training and skill. The sequential postprocessing steps necessary to arrive at the desired results are currently at best semiautomatic and still quite operator dependent. Complex automatic segmentation algorithms, that is, computer programs that can automatically isolate a particular piece of pertinent anatomy or several anatomic

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subsystems such as a solid organ and its vasculature, are being developed but are not yet ready for prime time.

REMOTE IMAGE ANALYSIS

The ever-growing availability of high-bandwidth networks within hospitals and the ever-increasing Internet bandwidth allow for distributed solutions. Datasets may be acquired in a given institution and then transferred in a secure fashion to a remote site that has the appropriate personnel and know-how to perform complex image analysis, subsequently providing the results of this work in a clinically useful format to the treating physician. Such remote services have been tested and are available today. This represents a viable solution for institutions that do not have the necessary local expertise or the numbers or resources to support an advanced image analysis infrastructure. Thankfully, even under such circumstances, collaboration with the right remote service partner can bring the same high level of care to smaller institutions and their patients.

Examples are provided in the following text of state-of-the-art imaging of the liver and its vasculature, as well as the biliary tree, including complex three-dimensional visualization and quantification algorithms in the context of live donor liver transplantation planning and liver resection planning. All images shown are based on real clinical datasets, acquired with modern multidetector-row spiral CT scanners; however, the postprocessing is not limited to CT datasets, as high resolution MRI datasets are becoming increasingly available.

LIVE DONOR LIVER TRANSPLANTATION

Commercially available software such as the Advantage Windows Workstation (GE Medical Systems, Milwaukee, WI) provides functionality, which allows performance of basic pretransplant workup.[1] More sophisticated software has been developed to meet to special needs of liver surgery planning, such as MeVis LiverAnalyzer (formerly "HepaVision 2," MeVis, Bremen, Germany).[2] The goal is to provide the surgeon with a detailed map of the hepatic vasculature, including hepatic arteries, hepatic veins, and portal veins as well as the biliary tree (Figs. 1 to 3). These maps can be viewed separately or in combined three-dimensional views that illustrate their relationships to each other, of particular importance along the anticipated resection line and in the hepatic hilum (Fig. 4). The anatomy of branching structures can be shown in relationship

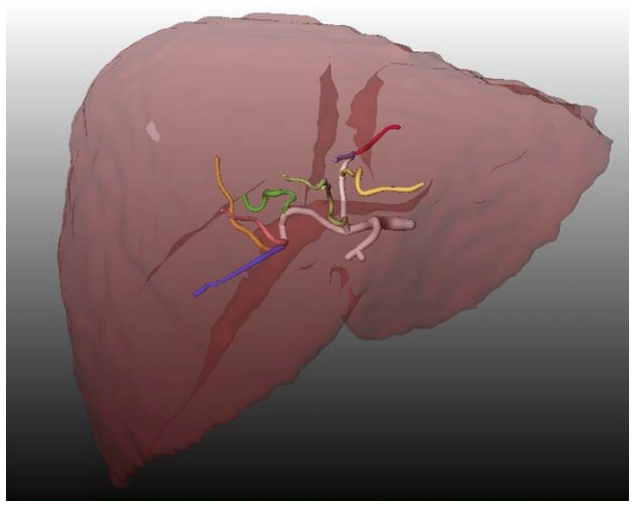

Fig. 1. Three-dimensional arterial cast within liver parenchyma; colors code branches to individual segments after Couinaud's classification (salmon=segment 5; blue=segment 6; green=segment 7; gold=segment 8; light green=segment 4b; light blue=segment 4a; red=segment 2; yellow=segment 3).

to the whole organ outline. Recently, noninvasive preoperative biliary imaging has been added to the workup, which saves the patient a more invasive and time-consuming intraoperative cholangiography.[3] Important biliary variants can thus be recognized preoperatively (Figs. 5 and 6). Furthermore, the volume of the entire liver can be calculated. A virtual hepatectomy is performed on the computer preoperatively, closely resembling the intraoperative

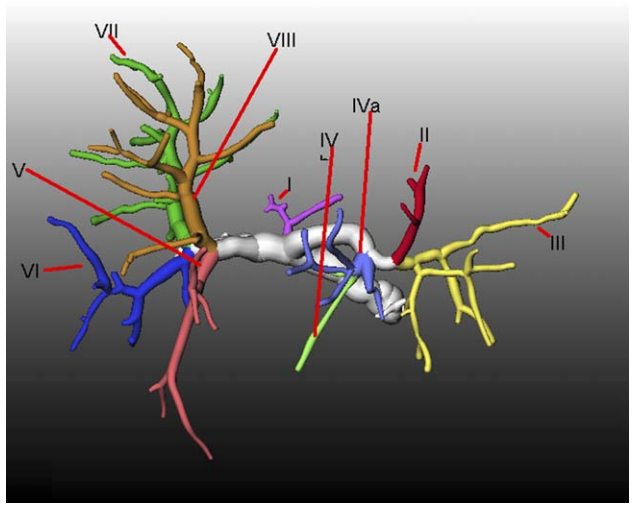

Fig. 2. Three-dimensional portal vein cast, colors code branches to individual segments in analogy to the arterial tree colors in Figure 1. Roman numbers correspond to the Couinaud segments.

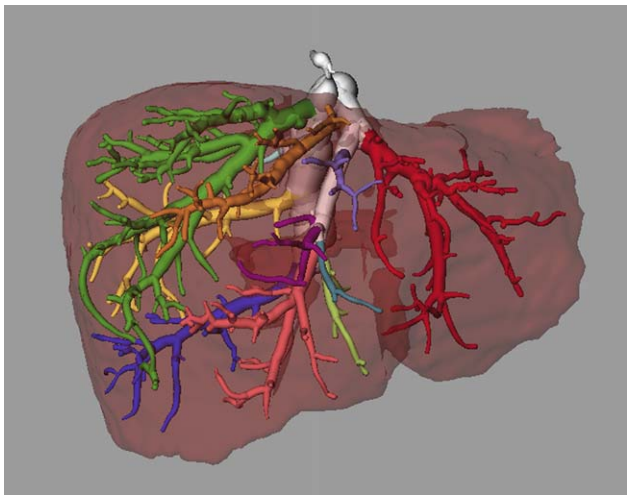

Fig. 3. Three-dimensional hepatic vein cast in liver model, coloring codes segments drained.

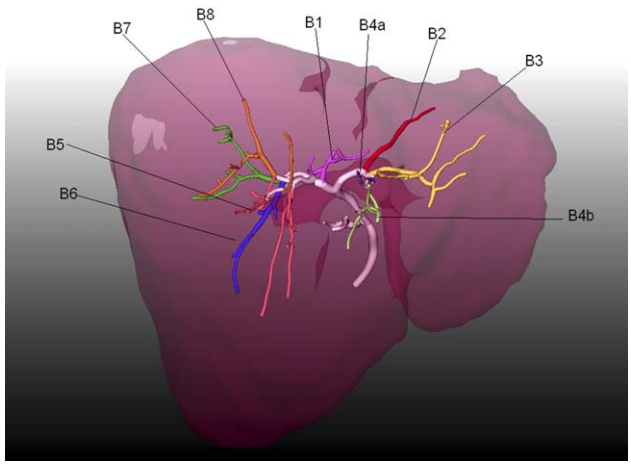

Fig. 5. Three-dimensional CT cholangiogram in a liver model, biliary radicles are coded and numbered according to the respective Couinaud segment they drain.

resection strategy. This can be done interactively at the workstation. The resulting partial organ volumes can be calculated, providing the surgeon with important information about expected liver remnant and graft volume (Fig. 7). Sophisticated software can estimate the volume of portal venous, hepatic arterial, biliary, or hepatic venous territories. These are portions of liver parenchyma depending on specific vascular branches for blood supply or drainage. Combining this territorial analysis with the resection planning, it is possible to estimate what portion of graft and remnant liver may be at risk for venous congestion after resection (Fig. 8). This information may provide clues when to perform reconstructions especially of the anterior right lobe graft venous drainage in the live donor liver transplantation situation.

ONCOLOGIC RESECTON PLANNING

Modern postprocessing methods allow depiction of the tumor(s) in relation to the organ borders and the pertinent anatomy of vascular and biliary anatomy in the individual patient's liver. The tumor volume can be calculated, which represents important information when deciding whether a patient can be listed for transplantation. Resection simulation allows the visualization of computer-generated safety margins around a lesion or several lesions while at the same time highlighting structures that would have to be resected when applying a particular safety margin to a given lesion. Should resection of

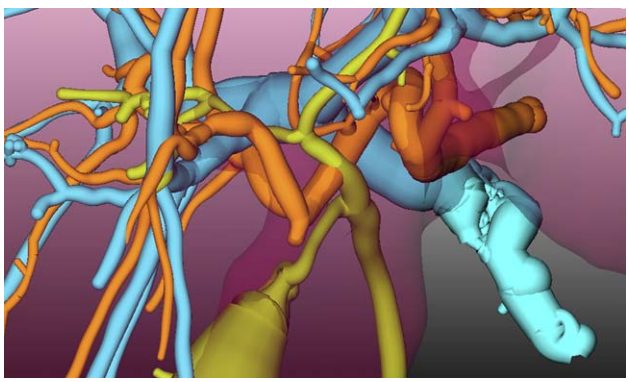

Fig. 4. Detailed composite view of the hepatic hilum demonstrating hilar branching of biliary ducts (yellow), hepatic arterial branches (red), and portal venous branches (blue). This powerful view displays important relationships of these arborizing systems to each other.

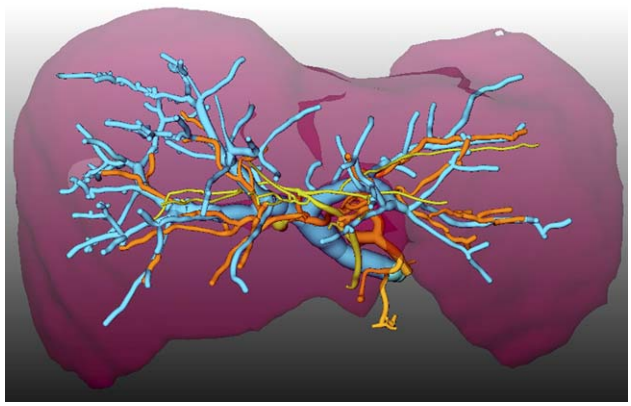

Fig. 6. Composite view of arterial, portal venous, and biliary tree in a patient with aberrant biliary branching who was ruled out from right lobe live liver donation based on this finding: three biliointestinal anastomoses would have had to be fashioned during surgery, almost impossible considering the small size of the radicles to segments 5 and 8.

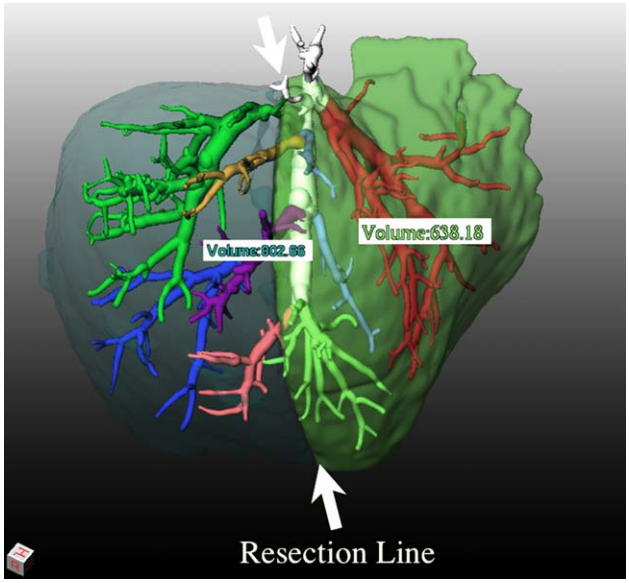

Fig. 7. Three-dimensional model of donor liver after virtual hepatectomy has been performed, resulting graft volume (dark green, 802 ml) and remnant volume (light green, 638 ml) are displayed. Hepatic vein cast inside model was used to orient the resection line appropriately.

a lesion with a particular desired safety margin result in removal of a crucial vessel, the simulation can quantify the amount of hepatic parenchyma at risk of losing its blood supply or drainage during such an operation (Figs. 9 and 10). This provides important adjunct information when assessing the

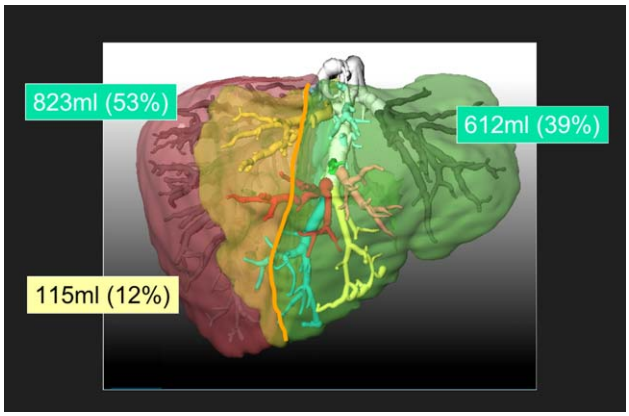

Fig. 8. Three-dimensional liver model and hepatic vein cast after virtual hepatectomy has been performed. Yellow wedge shows volume of anterior right lobe (115 ml, 12% of R lobe graft), which drains blood into the middle hepatic vein (MHV) via side branches. This parenchyma is at risk for venous congestion during and after right hepatectomy when the MHV side branches get disrupted. Red wedge shows portion of right lobe not at risk (functional graft volume, 823 ml, 53% of total) and green wedge demonstrates liver remnant volume (612 ml, 39% of total liver volume).

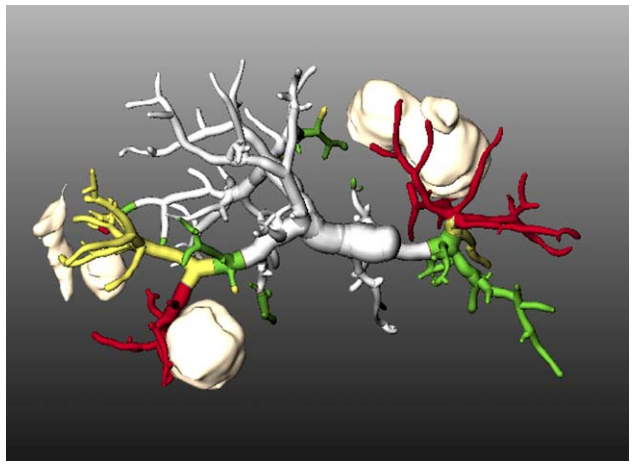

Fig. 9. Portal vein cast and four segmented tumors (metastases). Depending on the desired safety margin, an increasing number and extent of portal veins (and dependent parenchyma) would have to be sacrificed (red=5 mm; yellow=10 mm; green=15 mm). (Data courtesy of Prof. Oudkerk, Groningen, the Netherlands.)

feasibility of a resection in an individual patient preoperatively.

INTERVENTION PLANNING

Three-dimensional modeling of liver anatomy may be very useful prior to surgical or radiologic percutaneous intervention. Three-dimensional CT cholangiography prior to percutaneous cholangiography and intervention may decrease the procedure time and allow combined visualization of skeletal landmarks (ribs, etc.) and underlying liver anatomy,

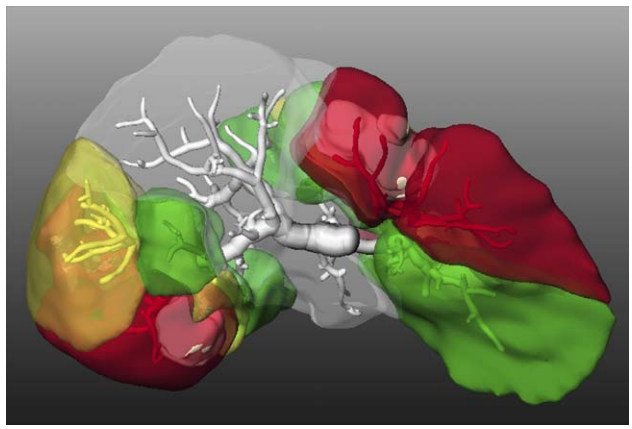

Fig. 10. Colored wedges show amount of hepatic parenchyma at risk when metastasectomy is performed with various safety margins (see Fig. 9). This view and corresponding volume numbers aid preoperative feasibility assessment. (Data courtesy of Prof. Oudkerk, Groningen, the Netherlands.)

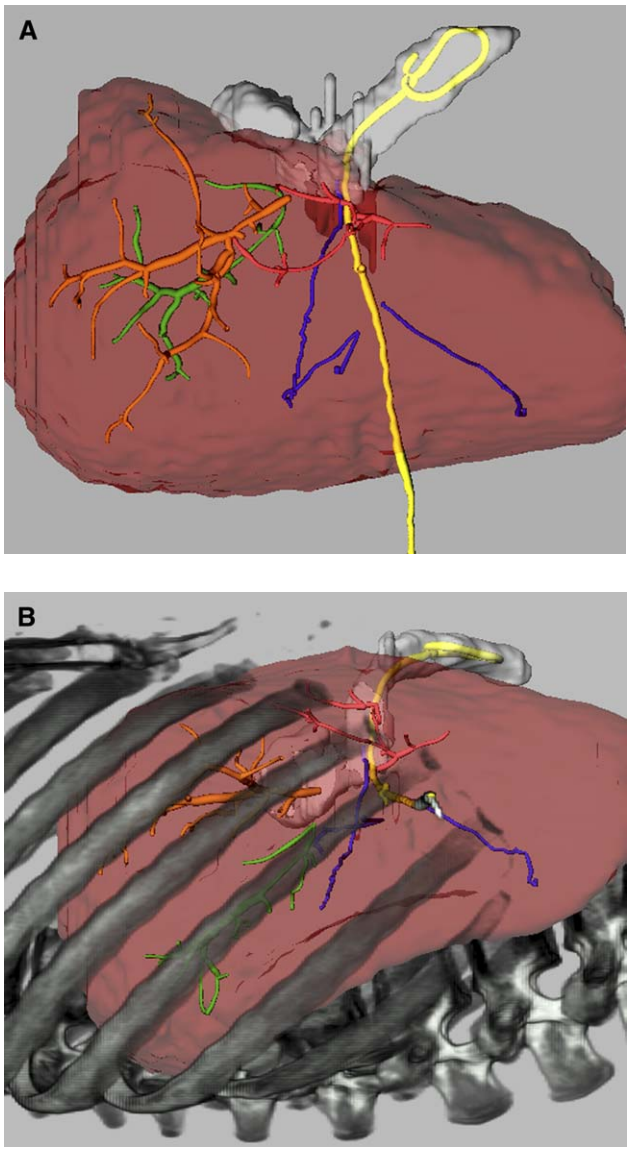

Fig. 11. (**A**) Frontal three-dimensional CT cholangiogram of right lobe graft, percutaneous transhepatic cholangiocatheter (PTC, yellow) in intestinal loop (gray). Segmental ducts to segments 7 and 8 (green and gold) are dilated and not draining into the loop properly. (**B**) Lateral view of the same patient, now with rib cage overlay; this aids in percutaneous access to the upper right lobe ducts and decreases the number of passes through the graft parenchyma required for successful introduction of a second PTC catheter.

which helps with targeting of otherwise invisible structures (Fig. 11, *A* and *B*).

Best routes of intervention, such as for radiofrequency ablation, can be planned at the computer simulation system. In the future, incorporation of experience from radiofrequency ablations may allow simulation of the heat-sink effect (unwanted cooling effect due to blood flow in vessels adjacent to a targeted lesion). This may eventually become a powerful tool to determine the number of electrodes and energy needed to adequately treat a liver lesion. Real-time imaging during the ablation, preferably using imaging modalities not dependent on ionizing radiation, is under investigation.

SUMMARY

The combination of modern scanner technology with advanced image postprocessing allows for high-fidelity three-dimensional imaging of pertinent patient anatomy. Currently available solutions provide not only precise qualitative but also advanced quantitative imaging, resulting in precise estimates of whole or partial organ or tumor volumes. Simulations of open surgical or percutaneous interventions are an important application of advanced image analysis. Image postprocessing may be performed locally or remotely using readily available Internet connectivity. Results can be offered to the treating physician in a very realistic format and can be made available in an interactive fashion, such as in the operating room. Clinically appropriate use of this powerful technology can alter the surgical approach to individual patients' problems, help with preoperative feasibility and risk assessment, and ultimately may result in shorter and safer procedures.

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Surgical Techniques for Liver Resection

Pierre-Alain Clavien, M.D., Ph.D., F.A.C.S.

Major goals during liver resection are the reduction of intraoperative blood loss and avoidance of parenchymal trauma. Despite refinements in many techniques of liver resection over the past 20 years, intraoperative hemorrhage has remained an important issue. For many years, liver transections have been done using finger-fracture or other crushing techniques using a Kelly (or similar) clamp.[1] Over the past two decades, several novel devices have been developed aiming at more bloodless and accurate parenchymal transection, including the bipolar forceps, ultrasonically activated scissors, argon beam coagulator, monopolar floating ball, and dissecting sealer (TissueLink Medical, Inc.; Dover, NH). However, these techniques may cause deep tissue damage and do not have the ability to discriminate vascular or biliary structures from the surrounding parenchyma. Other devices, which do not generate heat and thereby do not cause thermal damage to the surrounding healthy liver tissue, have been proposed, including the cavitron ultrasonic surgical aspirator (CUSA; Tyco Healthcare, Mansfield, MA) and the Hydrojet (Hydro-Jet; Erbe, Tubingen, Germany).

Inflow occlusion (Pringle maneuver) has been used for many years to prevent bleeding during parenchyma transection. The concomitant use of low central venous pressure (CVP) anesthesia further minimizes blood loss by preventing retrograde bleeding from the hepatic veins. Assuming that inflow occlusion and low CVP anesthesia cause significant damage through ischemia and reperfusion, there has been a growing interest in using new devices that facilitate bloodless transection, obviating the need for inflow occlusion.

However, none of these devices or techniques have gained unanimous acceptance among liver surgeons. It is also unknown how to adapt these techniques for specific diseases or underlying liver diseases. For example, we recently reported some advantages to the use of the Hydrojet for the radical treatment of hydatid disease in patients with bilobar diseases.[2] However, no consensus exists regarding the best surgical techniques or devices to be used in patients with cystic or solid lesions or in patients with malignant or benignant disease.

So far, liver resection devices have been tested in only two randomized controlled trials comparing clamp-crushing technique versus CUSA[3] and CUSA versus Hydrojet,[4] using inflow occlusion in all cases. Both randomized trials had critical limitations, as Takayama et al.[3] included normal and cirrhotic livers, and the study of Rau et al.[4] was not based on a sample size and power calculation. There are no randomized controlled trails that compare the most commonly used devices to each other such as the clamp-crushing technique, CUSA, Hydrojet, and dissecting sealer. Further experience with transection devices has been reported by only lower evidence retrospective studies.

Therefore, in view of the lack of available convincing data, we designed a prospective randomized trial in 100 noncirrhotic and noncholestatic patients undergoing liver resection, comparing four different techniques of parenchyma transection: clamp-crushing technique under inflow occlusion, CUSA, Hydrojet, and dissecting sealer. The results have been recently reported in the *Annals of Surgery*.[5] Inflow occlusion was used in the three latter groups only when needed. End points such as intraoperative blood loss, transection time, degree of reperfusion injury, and postoperative complications were determined to identify the most efficient device for liver parenchyma transection in terms of safety and costs. In our study, the clamp-crushing technique had the highest transection velocity and the lowest blood loss and proved to be most cost-efficient device compared with CUSA, Hydrojet, and dissecting sealer.[5] However, the degree of postoperative reperfusion injury and complications did not differ significantly among the groups.

In conclusion, there is evidence that the clamp-crushing technique associated with inflow occlusion is the most effective and cost-effective surgical technique for liver transection in patients with normal liver parenchyma. However, which technique should be preferred in patients with an injured liver (e.g., cholestasis, cirrhosis, fibrosis, fatty liver) or in some

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patients with specific diseases remains unknown. Further properly designed trials are necessary to address these issues.

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Anesthetic Management for Liver Resection

Ann Walia, M.D.

PREOPERATIVE ASSESSMENT

As a result of advanced improved diagnostic surveillance, better surgical techniques, and improved anesthetic management and perioperative care, hepatic resections have become more common and more complex but with strikingly better outcomes. For nontumorous hepatic masses, only routine preanesthetic assessment is required. Preexisting liver disease warrants careful assessment of coagulation status, pulmonary and renal function, and cardia status. This may include obtaining a room air arterial blood gas sample and an echocardiogram in addition to the routine preoperative tests.

The site and volume of planned resection must be carefully assessed in the preoperative period. This, in addition to the patient's comorbidities, will determine the extent of invasive monitoring that is required. Previous history of abdominal surgery may increase intraoperative blood loss. Recent data suggest that there has been a marked decrease in transfusion rate in patients undergoing liver resections. More than 60% of the patients do not require blood transfusion, 80% of the patients transfused receive less than 6 units of packed red cells, and only 2% of the patients require greater than 10 units of blood.[1]

Blood transfusion has been cited as an independent predictor of operative mortality, major complications, and length of hospital stay. Patients requiring no transfusions had a 1% to 2% mortality, those receiving 1 to 2 units of packed red blood cells had a similar mortality of 2.5% but those who received greater than 2 units of blood had a mortality rate of 11%.[2] Most intraoperative and postoperative complications have been reported during resection of large tumors, particularly in the right lobe or tumors located near or invading the inferior vena cava, the portal veins, or the cavoportal junction. In addition, total vascular occlusion and combined organ surgery and repeat abdominal surgery can lead to sudden or protracted bleeding with intraoperative hemodynamic compromise and increased incidence of air embolism.

ANESTHETIC MANAGEMENT

No one anesthetic technique has been proved to be superior to others. General anesthesia can be induced using standard induction agents, keeping in mind that hepatic clearance of drugs may be depressed, especially after parenchymal resection. A peripheral arterial catheter is useful in providing beat-to-beat blood pressure monitoring and allows for easy blood sampling. More invasive hemodynamic monitoring should be dictated by the patient's comorbidities and the site and extent of planned resection. Adequate venous access must be secured in order to allow rapid fluid or blood administration. In some series, outcomes in liver resection have been directly related to the ability of the anesthesiologist to provide rapid, warm resuscitation, to maintain perfusion and euthermia, and to avoid metabolic acidosis. This should not be considered as a "senseless pumping of blood." Overresuscitation will lead to increased central venous pressure, more blood loss, bowel and liver edema, smaller operating field with limited surgical exposure, and difficult closure.[3] Hypovolemia in the event of an air embolism, can be life threatening. Infusion of fluids should be restricted until after the parenchymal resection. Central venous pressure of less than 5 cm H_2O has been correlated with decreased blood loss. Vasopressors like phenylephrine or norepinephrine should be considered early during vascular clamping to maintain mean arterial blood pressure at 50 to 75 mm Hg. Additionally, intervention with volume expansion and diuretics should be initiated early to prevent kidney failure.

Total vascular occlusion of the liver is associated with a 30% to 60% decrease in cardiac output, thus requiring preparation with volume expansion and vasopressor support. Portal triad clamping is much better tolerated with a resultant 5% to 10% decrease in cardiac output and 20% to 30% increase in left ventricular afterload. Intraoperative transesophageal echocardiography would be the monitor of choice in patients with limited cardiac reserve as it would

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allow an accurate assessment of left ventricular preload, wall stress, and function.

BLOOD CONSERVATION TECHNIQUES

Erythropoietin. Enhanced erythropoisis must be considered preoperatively in patients with rare blood groups, renal failure, anemia, and in a Jehovah's Witness. A 3- to 4- week preoperative course of erythropoietin will increase hemoglobin by 3 to 5 g/dl. It is expensive and requires preoperative planning and has not yet been approved for elective liver resection.

Acute normovolemic hemodilution. This is an option in American Society of Anesthesiologists (ASA) Class I and II patients only, and the benefits are still unclear. Intraoperative cell salvage is contraindicated in patients with cancer.

Antifibrinolytics. Aprotinin has been shown to decrease blood loss and the number of units transfused during hepatic surgery. Serious concerns remain regarding the increased risk of thrombosis with these drugs. Severe allergic reactions have also reported.

Recombinant factor VIIa. Novo-7 is very expensive. Recent literature shows no decrease in blood loss or the number of units of transfused blood during liver resection, compared with placebo.[4]

Hemoglobin-Based Oxygen-Carrying Solutions

HBOC solutions may be used as a bridge to transfusion during acute hemodynamic decompensation. Long-term effects, risks, and cost remain undermined.[5]

POSTOPERATIVE PAIN MANAGEMENT

Pain management post liver resection remains a challenge. Complicating issues include intraoperative blood loss, loss of liver parenchyma, capacity of remaining liver to produce clotting factors, thrombocytopenia, and limited therapeutic index of conventional IV opioids. Continuous epidural analgesia would provide the best form of pain relief, but use is limited due to the risk of epidural hematoma from continuous trauma caused by an indwelling catheter and again at the time for catheter removal in the presence of coagulopathy. Another option would be a preoperative single-shot epidural injection with a combination of long-acting drugs like morphine and ketamine. This could provide up to 24 hrs of pain relief.

Intravenous patient-controlled analgesia remains the best option. A small trial with intravenous patient-controlled analgesia in addition to continuous intramuscular bupivacaine (CIB) appears promising.[6]

POSTOPERATIVE COURSE

Following liver resection, the mortality rate is about 1% in patients without preexisting underlying liver disease and about 10% in patients with preexisting underlying liver disease. In patients with healthy livers, ascites occurs in about 50% of the patients after resection and resolves in 3 to 5 days. The ascitic fluid accumulates at the expense of intravascular volume. This will require volume expansion, preferably with colloid substitutes. Fluid and sodium restriction and diuretics will only lead to further hypovolemia and compromise organ perfusion. Hepatocellular insufficiency can occur in 1% to 3% of the patients and will require close monitoring in an intensive care unit setting.

NEW TECHNIQUES

Laparoscopic liver resection avoids a large abdominal incision and postoperative pain associated with it. Hemodynamic and respiratory concerns are no different than for any other laparoscopic surgery. Surgical learning period may prolong the duration of surgery.

Radiofrequency ablation of liver masses allows intervention in otherwise unresectable tumors. It is minimally invasive and spares liver parenchyma. Standard general anesthesia should be all that is required. The energy released from the release of radiofrequency waves can cause a transient 1° to 2°C increase in body temperature. Other complications can include hemorrhage, cholecystitis, intrahepatic abscess formation, and hemolysis associated with prolonged procedures.

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Does the Intraoperative Peritoneal Lavage Cytology Add Prognostic Information in Patients With Potentially Curative Gastric Resection?

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Peritoneal recurrence is the foremost pattern of failure after potentially curative resection for gastric cancer. Our aim was to evaluate the prognostic value of intraperitoneal free cancer cells (IFCCs) in peritoneal lavage of patients who underwent potentially curative resection for gastric carcinoma. Two hundred twenty patients with gastric cancer stage I, II, or III were prospectively evaluated with peritoneal lavage and cytologic examination. Aspirated fluid from the abdominal cavity was centrifuged and subjected to Papanicolaou staining. The mean age was 60.9 years (range, 21–89 years), and 63.6% were men. IFCCs were detected in 6.8% of the patients; suspicious in 2.7%, and negative in 84.5%. No judgment could be given in 5.9% of the cases. Invasion of the gastric serosa (pT3) was observed in all positive cytology patients. Patients with IFCCs had a mean survival time of 10.5 months, while those with negative IFCC had a mean survival time of 61 months ($P = 0.00001$). There was no correlation between the presence of IFCCs and tumor size, histology, pN, or tumor site. Our conclusions are that (1) positive cytology indicates a poor prognosis in patients who underwent potentially curative gastric resection and (2) peritoneal lavage cytology improves staging in assessing these patients and may alter their therapeutic approach. (J GASTROINTEST SURG 2006;10:170–177)

KEY WORDS: Peritoneal lavage, cytology, prognosis, gastric cancer, curative gastric resection

Gastric carcinoma is still one of the leading causes of cancer death worldwide,[1] regardless of decreasing incidence in developed countries.[2] It is the most common malignancy among men and the second most common among women in many countries of Latin America and Asia.[1] This disease carries an overall poor prognosis, and even after curative resections (R0), approximately 50% of the patients succumb to recurrent disease during the first 2 years of follow-up.[3,4]

Peritoneal dissemination is the most frequent pattern of recurrence in gastric cancer after potentially curative resection, and no efficient method of treatment is available.[5–10] Such recurrences may be attributable to possible intraperitoneal dissemination of malignant cells at the microscopic level, already present at the time of surgery, or due to surgical manipulations.[5–7,9,11]

Peritoneal lavage as a diagnostic/staging method has not yet been definitely included in the management of gastrointestinal carcinomas, although pancreas, colon, and gastric cancers are quite often associated with ascites and/or intraperitoneal free tumor cells at the time of the initial diagnosis.[5–12] The

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search for intraperitoneal free cancer cells (IFCCs) seems to be appropriate in staging gastric carcinomas, inasmuch as accurate preoperative and intraoperative staging is crucial to determine suitable therapeutic management of patients with gastric cancer.

We previously reported that the prevalence of IFCCs detected by cytologic study during preoperative laparoscopic peritoneal lavage is increased in proportion to the extent of the area of serosal invasion.[11] IFCCs were seen when invasion of the gastric serosa was greater than 3 cm^2 (8 of 21 [38%]) or when adjacent structures were involved by tumor (12 of 21 [57.1%]). There was a parallel correlation between the incidence of IFCCs and the morphology of the tumor, as well as International Union Against Cancer (UICC) classification of tumor stage.[11,12] IFCCs detected by laparoscopic peritoneal lavage during staging procedure was also associated with worsening prognosis.[12] The prognostic value of positive cytology has been seldom investigated in the Western patients.[12–15] Therefore, in patients estimated to have a poorer prognosis after potentially curative resection, for instance, IFCC-positive patients, gastrectomy with aggressive multimodal therapies combining neoadjuvant or adjuvant strategies may offer better outcomes.[15,16]

The purposes of this study were (1) to determine the prevalence of positive peritoneal lavage cytology in patients who underwent potentially curative surgical treatment (R0) for gastric carcinoma and (2) to analyze the clinical significance of IFCCs, specifically in regard to prognosis.

PATIENTS AND METHODS

Two hundred twenty consecutive patients with gastric cancer stage I, II, or III according to UICC classification were evaluated with peritoneal lavage and cytologic examination at the time of gastric resection from 1993 to 2002. Preoperative evaluation included a thorough medical history and physical examination, complete blood count, and serum chemistries. Tumor characteristics were assessed by upper digestive endoscopy, computed tomography (CT) scanning, and endoscopic ultrasonography in some patients. Distant metastases were evaluated by chest radiography and abdominal CT scanning. Patients were selected based on the following inclusion criteria: (1) potentially curative resection with complete macroscopic removal of the tumor and histologically negative margins (R0 resection); (2) tumor invasion restricted to the gastric wall, including the serosa (pT1–pT3); (3) lymph node involvement restricted to pN0–pN2; (4) no macroscopic evidence of peritoneal seeding or other distant metastasis; (5) no other forms of oncologic treatments, including immunotherapy, chemotherapy, or radiotherapy, preoperatively or postoperatively; and (6) patients who had undergone peritoneal lavage cytology.

Peritoneal lavage cytology was collected immediately after laparotomy, as soon as the peritoneal cavity was opened, prior to any surgical manipulations. Gastric resection was then immediately performed, independent of the cytologic results.

Peritoneal lavage cytology was performed as described elsewhere.[5–12] Briefly, the peritoneal cavity was washed with 100 ml of physiologic saline kept at 37° C instilled into the upper abdomen and allowed to collect. After dispersion in the peritoneal cavity, 10–20 ml of fluid was aspirated under direct vision from the subhepatic and/or subdiaphragmatic space. The fluid was immediately centrifuged at 2000 rpm for 5 minutes. The nucleated cell layer was smeared on to a glass slide and stained by Papanicolaou methods.

The slides were interpreted by experienced cytologists who were blinded to the preoperative studies and/or the operative findings. The results were classified as positive, negative, suspicious, or no judgment could be given for IFCCs. The following cellular characteristics were used to determine the presence of malignant cells: number, size, shape, type of cytoplasm, cytoplasmic vacuoli, nuclear abnormalities, nuclear chromatin, nuclear-cytoplasmic ratio, mitotic figures, and nucleolar prominence. The surgical treatment was based on preoperative and intraoperative staging and operative findings and was carried out without previous knowledge of the cytologic status.

The prognostic value of IFCCs detected by peritoneal lavage was evaluated with respect to the gender, histologic type of tumor, extent of surgical treatment, UICC classification of tumor stage, lymph nodes, and serosal invasion, all confirmed by histopathologic examination.

We defined potentially curative resections as those in which the patient showed no distant or peritoneal metastases, and all gross tumor was removed at operation. We included patients with clinically enlarged nodes provided that all such nodes were removed by D2 lymphadenectomy. Patients underwent a radical lymphadenectomy with en bloc resection of at least two echelons of regional lymph nodes (designated $R > 2$). We removed perigastric lymph nodes (N1), as well as lymph nodes located more than 3 cm from the tumor, including the left gastric, common hepatic, and celiac lymph nodes (Japanese N2). All patients were followed to the present time or until death. There was a 17% loss

to follow-up, and the patients were excluded from the study. Peritoneal recurrence was diagnosed on the basis of clinical symptoms, physical examination, or CT scanning. Clinical findings of bowel obstruction and ascites were confirmed by barium swallow or barium enema studies when appropriate, along with paracentesis and laparotomy in some patients according to the surgeon's judgment.

The χ^2 test, Fisher's exact test, and/or Student's *t* test was used to analyze the data, with statistically significant two-tailed $P < 0.05$. The Kaplan-Meier method was used to calculate the survival curves, while log-rank test was used to compare the curves. Multivariate analysis using Cox's proportional hazards model was performed to identify the independent prognostic factors.

RESULTS

The mean age was 60.9 years (range, 21–89 years), and 63.6% were men. All patients underwent a potentially curative gastric resection with D2 lymph node dissection. Total gastrectomy was performed in 98 patients, and partial gastrectomy, in 122. Distal esophagectomy was required in 12, splenectomy in 19, pancreatectomy in 1, and cholecystectomy in 18 patients.

Cytology of the peritoneal fluid obtained during laparotomy was positive for IFCCs in 6.8% of the patients (Fig. 1), suspicious in 2.7%, and negative in 84.5%. No judgment could be given in 5.9% of the cases. The relationship between cytologic results and various clinicopathologic parameters is presented in Table 1. Invasion of the gastric serosa (pT3) was observed in all IFCC-positive patients. Lymph node involvement was also detected in all but one of the positive cytology patients; 46.7% of the positive tumors were pN1 and 46.7% were pN2. Although a higher incidence of IFCCs was observed in patients with lymph nodes metastases, no statistically significant association was found ($P = 0.13$) (Table 1). The incidence of IFCC positivity increased significantly as the stage category progressed ($P = 0.01$). There was no association between the presence of IFCCs and gender, tumor size, type of resection, tumor site, or histology.

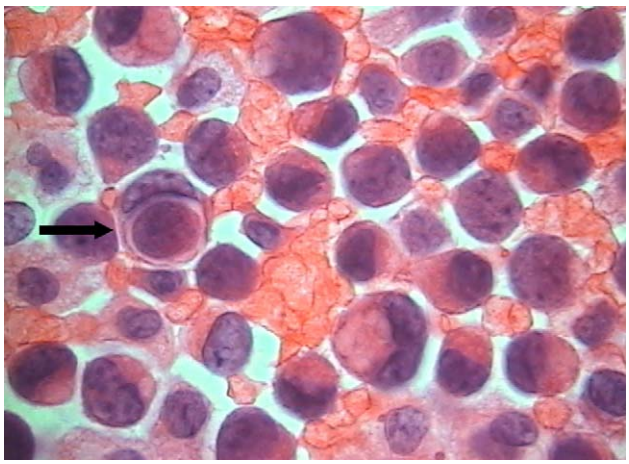

Fig. 1. Papanicolaou staining showing signet ring cell carcinoma in peritoneal lavage (original magnification ×400).

The distribution of the various clinicopathologic parameters from 15 IFCC-positive patients is shown in Table 2. There was no statistically significant difference between the frequencies of each parameter. All IFCC-positive patients had peritoneal recurrences; four patients had additional local and lymph node failures, and two patients had liver metastases.

The mean follow-up period of the patients was 64 months (SE = 5 months; 95% confidence interval [CI], 55–73 months) for the entire group, while it

Table 1. Distribution of 201 potentially curative resected patients according to the cytologic results*

	Cytology		
	Negative, n (%)	**Positive, n (%)**	***P* value**
No. of Patients	186 (92.5)	15 (7.5)	
Mean age (yr)	61.0, SD = 13.3	64, SD = 10.2	0.39
Gender			
Men	114 (61.3)	12 (80)	0.33
Women	72 (38.7)	3 (20)	
Tumor size (cm)	5.33, SD = 1.8	5.5, SD = 3.0	0.86
Type of resection			
Total gastrectomy	82 (44.1)	7 (46.7)	0.93
Partial gastrectomy	104 (55.9)	8 (53.3)	
Tumor site			
Upper third	18 (9.7)	1 (6.7)	0.92
Medium third	48 (25.8)	4 (26.6)	
Lower third	120 (64.5)	10 (66.7)	
Histology (Lauren)			
Intestinal	85 (45.7)	6 (40)	0.87
Diffuse	101 (54.3)	9 (60)	
Depth of invasion			
pT1	21 (11.3)	0	0.01
pT2	45 (24.2)	0	
pT3	120 (64.5)	15 (100)	
No. of positive lymph nodes			
pN0	55 (29.6)	1 (6.7)	0.13
pN1	75 (40.3)	7 (46.7)	
pN2	56 (30.1)	7 (46.7)	
Clinical TNM stage			
Ia	16 (8.6)	0	0.01
Ib	18 (9.7)	0	
II	46 (24.7)	1 (6.7)	
IIIa	55 (29.6)	7 (46.7)	
IIIb	51 (27.4)	7 (46.7)	

*Suspicious cytology and/or without judgment was excluded from the analysis.

Table 2. Distribution of 15 positive IFCC patients according to clinicopathologic parameters

Patient	Age (yr)	Gender	Type of resection	Tumor site (third)	Tumor size*	Positive lymph node	Total lymph node	Lauren	Histology	pT	pN	TNM staging	Status	Follow-up (mo)
1	66	M	Total	Medium	8	6	30	Diffuse	Mucinous	3	1	IIIa	Dead with disease	13
2	64	W	Total	Lower	4.5	6	29	Diffuse	Mucinous	3	1	IIIa	Dead with disease	12
3	63	M	Total	Lower	4	8	27	Intestinal	Tubular	3	2	IIIb	Dead with disease	8
4	54	M	Partial	Lower	3	3	22	Intestinal	Tubular	3	1	IIIa	Dead with disease	10
5	53	M	Total	Upper	5.5	3	38	Intestinal	Papillary	3	1	IIIa	Dead with disease	10
6	67	M	Partial	Lower	7	8	23	Diffuse	Mucinous	3	2	IIIb	Dead with disease	8
7	72	M	Total	Lower	5	0	65	Intestinal	Undifferentiated	3	0	II	Dead with disease	7
8	67	M	Partial	Lower	7	10	31	Intestinal	Mucinous	3	2	IIIb	Dead with disease	10
9	54	W	Total	Medium	7	14	37	Diffuse	Microtubular	3	2	IIIb	Dead with disease	17
10	64	M	Partial	Lower	6.5	5	30	Diffuse	Mucinous	3	1	IIIa	Dead with disease	4
11	63	M	Total	Medium	3.5	10	29	Diffuse	Signet ring cell	3	2	IIIb	Dead with disease	9
12	46	M	Partial	Medium	4	9	39	Diffuse	Microtubular	3	2	IIIb	Dead with disease	16
13	58	M	Partial	Lower	3	4	21	Diffuse	Signet ring cell	3	2	IIIb	Dead with disease	8
14	67	M	Partial	Lower	6	1	36	Diffuse	Signet ring cell	3	1	IIIa	Dead with disease	13
15	82	W	Partial	Lower	4.5	6	27	Intestinal	Tubular	3	1	IIIa	Dead with disease	9

M = man; W = woman.
*Maximum diameter measurement.

was 60 months (SE = 7 months; 95% CI, 11–79 months) for the alive patients. Seventy (31.8%) patients died of recurrent disease, and the plot for overall survival is shown in Figure 2. Survival curves stratified for the presence of IFCCs revealed a significant reduction in overall survival for patients positive for IFCCs (Fig. 3). Patients with IFCC had a mean survival time of 10.5 months (95% CI, 9–12 months), while those with negative IFCC had a mean survival time of 61 months (95% CI, 60–79 months) (P = 0.00001). All IFCC-positive patients have died, and none of the positive patients survived longer than 17 months.

Univariate analysis identified IFCC (P = 0.009), pN (P = 0.0002), pT (P = 0.04), and total gastrectomy (P = 0.02) as significant predictors of survival (Table 3). Multivariate analysis with these positive factors as variables identified pN, positive cytology, and pT as independent prognostic factors of significance (Table 4).

DISCUSSION

Accurate staging of gastric cancer is decisive for planning adequate therapy and minimizing both unnecessary patient discomfort and useless laparotomy.[11,12,17] Peritoneal cytology may add another unique element to the intraoperative staging of these patients. IFCCs are assumed to play an important role in the development of peritoneal metastases, which is the foremost pattern of failure after potentially curative resection for gastric cancer.[5–10] The peritoneal cavity can be a route for dissemination of malignant cells by direct continuity with the lesion or act as a receptacle for lymphatic spread.[7,10,12] The majority of patients with IFCCs do not escape postoperative peritoneal recurrence.[12,13,15]

Peritoneal lavage at the time of laparotomy for evaluation of patients with gastric cancer has been performed in some centers, mainly in Japan.[5–10,18,19] The rate of detection of IFCC in the literature ranges from 14% to 47% and is dependent of the cohort of studied patients.[5–12] When only potentially curative resected patients are included, the rate of IFCC positivity varies from 4.4% to 11%.[14,15,20] However, references to this method in the Western literature is scarce.[14,15] The Dutch Gastric Cancer Group has reported their cytologic results in 457 patients undergoing curative resections who had randomized D1 or D2 dissections. Positive cytologic findings were found in 4.4% of the patients and were indicative of a poor prognosis, with a median survival of 13 months.[14] Bentrem et al.[15] studied 371 patients with gastric carcinoma

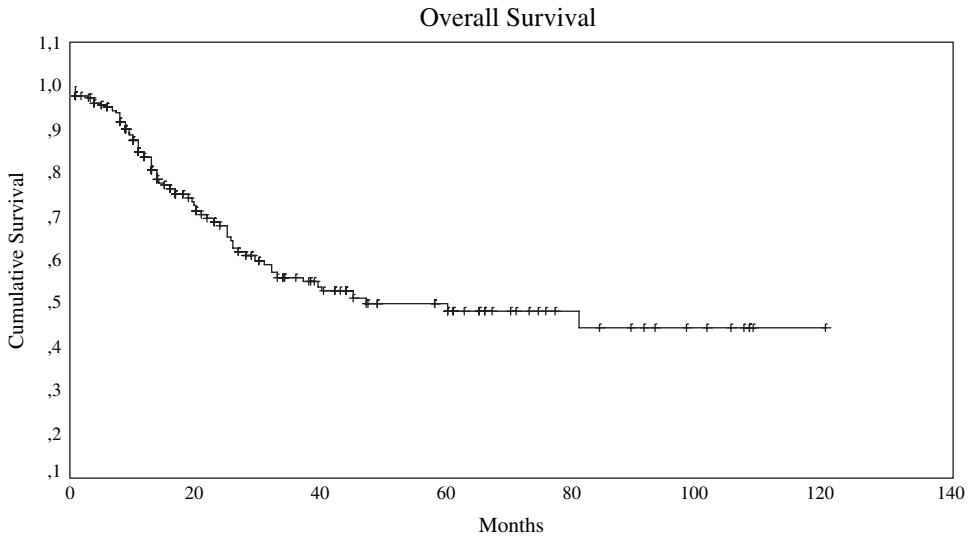

Fig. 2. Cumulative curve of overall survival for 220 potentially curative resected patients; 5-year survival rate is around 53%.

who underwent diagnostic laparoscopy and peritoneal washing cytology before R0 resection. IFCCs were detected in 24 patients (6.5%), and the positive rate was comparable to that of this investigation. Positive cytology was an independent prognostic factor, correlating with a median survival of 14.8 months versus 98.5 months for patients with negative cytology ($P < 0.001$).

The peritoneal fluid cytology has a sensitivity of 90% to 96.7% and nearly 100% specificity in the diagnosis of IFCCs.[21] False-positive peritoneal lavage cytology has been recognized by some authors, with a rate of 4.5% to 5%, probably secondary to reactive mesothelial cells.[7,9–11] In order to diminish the false-positive and -negative rates, several authors have used immunocytochemistry[22] and molecular biology techniques, including reverse transcriptase–polymerase chain reaction for carcinoembryonic antigen messenger RNA.[23]

According to our data, histologic type of tumor and lymph node invasion were not associated with the presence of IFCCs. Similar results have been found by other authors.[5,10–12,15]

Peritoneal cytology might be used as a predictor of the intent or success of surgical treatment in gastric cancer patients since it was demonstrated that

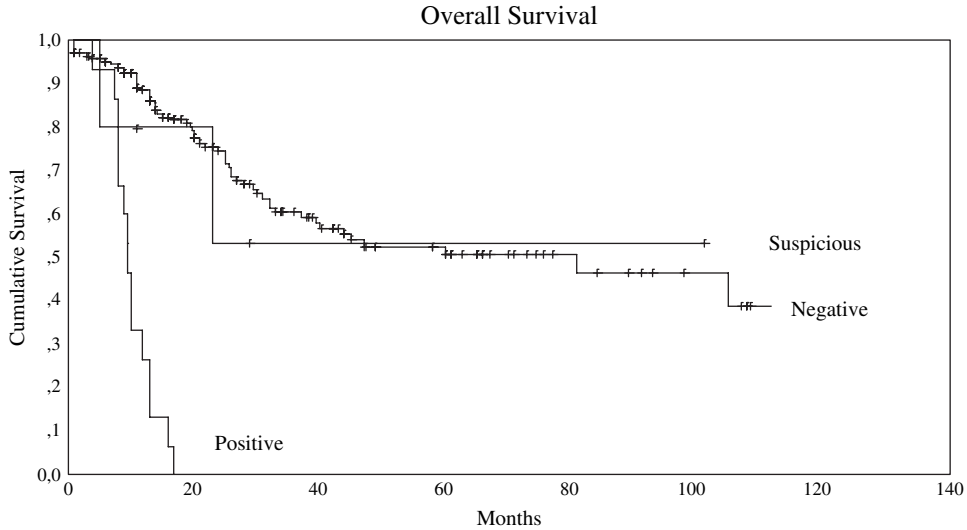

Fig. 3. Cumulative curves of overall survival stratified for cytological results (log-rank $P = 0.00001$).

Table 3. Univariate analysis with several prognostic factors

Variable	Relative risk (5% confidence interval)	*P* Value
Positive cytology	6.68 (1.74–5.05)	0.0097
Surgical resection (total gastrectomy)	2.33 (0.75–2.00)	0.029
Histology (Lauren)	2.51 (0.78–2.52)	0.11
Tumor site	0.31 (0.71–1.32)	0.57
Gender	0.13 (0.67–2.12)	0.70
pT	3.87 (0.65–1.71)	0.049
pN	13.52 (0.25–1.71)	0.0002

Table 4. Multivariate analysis using positive prognostic factors from the univariate analysis

Variable	Relative risk (5% confidence interval)	*P* Value
Positive cytology	22.32 (1.83–4.31)	0.00001
Surgical resection (total gastrectomy)	3.13 (0.96–2.15)	0.076
pT (pT3 versus pT1 e pT2)	7.7 (1.10–1.77)	0.0053
pN (pN1–pN2 versus pN0)	38.5 (1.97–3.70)	0.00001

unresectable tumors had higher positivity of IFCCs than those who underwent palliative or curative gastrectomy.[11] We previously studied 49 patients with advanced gastric cancer through the use of laparoscopic peritoneal lavage cytology for staging.[11] Only 45% of the patients (66.7% palliative and 33.3% curative) with gastric cancer associated with positive peritoneal cytology could undergo resection, whereas 84.6% of the patients (36.4% palliative and 63.6% curative) with negative cytology successfully underwent resection. Therefore, peritoneal cytology may improve the selection of patients suitable for curative or palliative resection.[11]

Peritoneal cytology, in addition to serosal and nodal status, may have prognostic implications, emphasized in this study by the correlation with tumor stage. Previous reports have stated that the prognosis in surgically treated patients with gastric carcinoma is significantly affected by the presence of IFCCs at the time of gastrectomy.[6,8–10,12–15,18,19] The survival rate also appears to be related to the number and the arrangement of IFCCs, that is, clustered, isolated, or clustered-plus-isolated type.[10]

None of our patients who had IFCCs survived longer than 17 months after surgical treatment. The follow-up of these patients confirmed the prognostic value of IFCCs. Our data demonstrated that peritoneal lavage cytology is an independent predictor of poor outcome in patients with gastric adenocarcinoma and should be included as an integral part of the staging evaluation and classification. Patients with positive lavage cytology are essentially stage IV, even in the absence of macroscopic peritoneal disease, because of the poor overall survival. The behavior of the patients could be divided into two groups, depending on the IFCC status. Survival curves showed a significant difference between the two groups. Therefore, patients with IFCCs should be considered stage IV, because the overall survival correlates with stage IV outcome.

It is noteworthy that non–clinically evident malignant peritoneal disease manifested by IFCCs was observed in 15 of 220 (6.8%) patients who were treated with radical, potentially curative surgery (R0). Similar results have been obtained by other investigators.[14,15,20] These subclinical micrometastases may have the potential to develop into recurrent disease and influence both treatment and survival. Adjuvant chemotherapy would theoretically be beneficial in this subgroup of patients, because micrometastases are more susceptible to chemotherapy than macroscopic disease.[6]

Abnormal cytology possibly may serve as a guide to continuing chemotherapy or changing the mode of therapy.[15,16,24] Preoperative chemotherapy protocols may select patients more likely to benefit from resection. Patients who have progressive disease due to a more biologically aggressive cancer may be spared laparotomy,[15,16] particularly in those patients who do not have outlet obstruction or bleeding.

Additionally, these tumors could be treated by local administration of cytotoxic, immunotherapeutic, or targeted agents injected directly via an intraperitoneal catheter, which might lead to a higher concentration of the agent in the peritoneal cavity than with systemic administration, improving therapeutic results.[24,25] Hyperthermic intraperitoneal chemotherapy may also be used to treat these patients.[26]

In conclusion, if there is a suspicion of the invasion of the gastric serosa (pT3 or pT4), a peritoneal fluid cytology examination becomes obligatory in order to verify the presence of IFCCs. Positive cytology indicates a poor prognosis in patients who underwent potentially curative gastric resection. Intraoperative cytologic evaluation of peritoneal lavage improves staging in assessing patients with potentially curative gastric carcinoma (R0) and may alter the therapeutic approach.

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Discussion

Dr. Mitchell Posner (Chicago, Illinois): I want to congratulate Dr. Ribeiro for an excellent presentation and thank him for providing me with the manuscript well in advance of this meeting.

The work presented here today reflects the intense interest of the group from the University of San Paulo in gastric cancer, a malignancy that is so highly prevalent in Brazil and most of Latin America. As in the past, they continue to contribute to our understanding and provide recommendations to improve our management of this disease. In this prospective study, they describe the potential value of intraoperative peritoneal lavage and cytologic examination of collected cells in over 200 patients with curatively resected gastric carcinoma. They conclude that positive cytology is a useful staging tool and predicts poor outcome, equivalent to patients who are initially deemed stage IV. I have a number of questions.

Can you describe for us the patterns of failure for patients with positive cytology?

Second, can you suggest how we should incorporate the staging method into our intraoperative

management of gastric cancer patients? Since only 7% of patients were upstaged by this procedure, do you and should we perform this routinely or only in select patients with documented serosal involvement or, since it is such a small number of patients, not at all?

Third, in patients with positive cytology, should we alter our therapeutic approach? Should we perform lavage laparoscopically or even in the outpatient setting, and if positive, not offer gastrectomy? Should patients receive in the same scenario preoperative neoadjuvant chemotherapy, or should they be resected and then treated with postoperative adjuvant therapy via a systemic or an intraperitoneal route?

And finally, could you describe your plans for the future? Will you be using other potentially more sensitive methods to detect epithelial cells, such as immunocytology, or molecular techniques like RT-PCR, to improve your accuracy in identifying patients who are destined to fail?

Dr. Ribeiro: Thank you very much, Dr. Posner, for having the time to read the manuscript and for your relevant questions and comments. All patients with positive cytology had peritoneal recurrence; four of them had local and lymph node failures and two of them had liver metastases at the time of the diagnosis.

Regarding the second question, we believe and postulate that all patients with serosal involvement should have routine laparoscopic cytology or just intraoperative cytology if the laparoscopy has not been done before surgical resection. It would be useful if we could have the results right after the laparotomy so we could change the treatment plans at that time. So we are convincing our pathologists to do the staining right after the collection of the fluid.

I also think that laparoscopy can be used to select a subset of patients who are more likely to respond to chemotherapy or neoadjuvant chemotherapy. Neoadjuvant chemotherapy may be beneficial in terms of survival for patients with positive cytology. A more aggressive approach with intraperitoneal chemotherapy might be another option as prophylaxis for peritoneal dissemination. We have published in the *Journal of Gastrointestinal Surgery* our results of laparoscopy in advanced cancer patients. Poor prognosis was correlated with positive cytology. I do believe the best way to palliate patients without carcinomatosis or multiple liver metastases is still palliative gastric resection because these patients have bleeding and obstruction of the upper digestive tract. Even with positive cytology, I still think that we should do a palliative gastrectomy for them, but I would avoid extended lymphadenectomy in these patients, because this may increase morbidity.

For the future we are collecting peritoneal lavage so we can do RT-PCR, reverse-transcriptase polymerase chain reaction. We intend to do carcinoembryonic mRNA in these patients. Another possibility, and we did this in colon cancer, is immunohistochemistry on this fluid, and several antibodies can be used, including anti-cytokeratins and CEA. Additionally, this data must be utilized to compare the clinical results with all this new methodology that we have now.

The Split-Stomach Fundoplication After Esophagogastrectomy

Vic Velanovich, M.D., Nathan Mohlberg, B.S.

Two complications associated with esophagogastrectomy are anastomotic leak and gastroesophageal reflux. We describe here a modification of an intrathoracic esophagogastrostomy using the gastric fundus to address these issues. After completion of the esophagogastrectomy, the fundus is divided to produce "wings." After the esophagogastrostomy is performed, the wings are used to form a wrap around the anastomosis. This wrap is secured to the esophagus and to the stomach. All patients undergoing the split-stomach fundoplication were compared with all patients undergoing standard esophagogastrectomies. End points were in-hospital mortality, anastomotic leak, and postoperative endoscopic dilation. All living patients were contacted and questioned about refluxlike symptoms and completed the Gastroesophageal Reflux Disease–Health Related Quality of Life (GERD-HRQL) symptom severity questionnaire. Twenty-six patients underwent the split-stomach fundoplication (wrap group), compared to 54 patients undergoing standard resection (no wrap group). Occurrence of end points in the wrap vs. no wrap groups were, respectively, in-hospital mortality, 3.8% vs. 7.4% (P = NS); anastomotic leak, 0% vs. 17% ($P = 0.03$); reflux symptoms 20% vs. 60% ($P < 0.001$); postoperative dilation, 40% vs. 30% (P = NS). The median total GERD-HRQL score was 5 for the wrap group vs. 14 for the no wrap group ($P = 0.03$). The addition of the split-stomach fundoplication to esophagogastrectomy may decrease the incidence of anastomotic leak and postoperative refluxlike symptoms. (J GASTROINTEST SURG 2006;10:178–185)

KEY WORDS: Anastomotic leaks, anastomotic stricture, esophagectomy, fundoplication, gastroesophageal reflux

Esophagogastrectomy is the surgical treatment of choice for a wide variety of benign and malignant diseases involving the esophagus, gastroesophageal junction, and gastric cardia. These are major surgeries that carry with them significant morbidity and mortality.[1] Although a variety of complications can occur, anastomotic leak and postoperative gastroesophageal reflux have led to technical modifications to decrease their occurrence. Nevertheless, anastomotic leak rates are still reported in the 3% to 16% range,[2–11] whereas gastroesophageal reflux has been reported in 60% to 80% of patients with both intrathoracic and cervical anastomoses.[12–16] This has lead some authorities to claim that a cervical anastomosis is less "refluxogenic,"[17,18] whereas others have developed antireflux modifications after esophagectomy.[19–21] Whereas a cervical anastomosis has gained in popularity and may be the most common type of anastomosis performed, antireflux modifications have not become common place.

Fundoplications have become standard surgical techniques for the management of gastroesophageal reflux disease. Although they work in a number of ways, the wrap itself augments the lower esophageal sphincter (LES). Because the LES is removed in its entirety during esophagectomy, it is logical to think that replacing this lost LES with a wrap may help provide an antireflux effect. In addition, vascularized tissue is a mainstay in the treatment of esophageal perforation. As the wrap is stomach, once again it is logical to think that placement of this well-vascularized stomach around the anastomosis in the form of a wrap will reduce leaks.

This study describes a new technique to create a wrap around an intrathoracic esophagogastrostomy using the stomach, with the goal of decreasing the

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anastomotic leak rate and decreasing the incidence and severity of postoperative reflux symptoms.

MATERIAL AND METHODS

Description of the Surgery

Patients selected for this surgery included all patients for whom a transabdominal or Ivor-Lewis type of esophagectomy was planned. Specifically, patients who required a total esophagectomy with cervical anastomosis due to tumor location or stricture length were excluded. The surgery can be performed in the standard Ivor-Lewis technique, or in an entirely transabdominal approach for short-segment esophagogastric resections. In the Ivor-Lewis approach, the abdominal portion is performed first with mobilization of the stomach, with care taken to preserve the gastroepiploic vessels and right gastric artery. A Kocher maneuver is performed to mobilize the duodenum. If the surgery is being done for a lower esophageal, gastroesophageal junction, or gastric cardia malignancy, then a celiac, hepatic artery, and splenic artery lymph node dissection is performed. The esophagus is mobilized into the mediastinum. If an Ivor-Lewis approach is being performed, the abdomen is closed after placement of a feeding jejunostomy. The chest is entered through a right posterolateral thoracotomy, and the remainder of the esophageal and en bloc lymph node dissections are completed. At this point with either the transthoracic or the transabdominal approach, the stomach with lesser curvature lymph nodes is divided with an adequate margin with a stapling device. The esophagus proximal is divided, with care taken to preserve the segmental arteries to the esophagus. A pyloroplasty is not done.[22]

After completion of the esophagogastrectomy, the stapled cut end of the stomach is oversewn with interrupted silk suture. Using a linear stapler, the fundus of the stomach is divided in the long axis of the organ for approximately 5 cm (Fig. 1) to produce two "wings" (Fig. 2). A gastrotomy is made 2 cm caudad to the apex of this split (Fig. 3). An end-esophagus to side-stomach esophagogastrostomy is completed with interrupted silk suture (Fig. 4). The wings of the split stomach are brought around the anastomosis and sutured together to form a wrap (Fig. 5). This wrap is secured to the esophagus superior to the anastomosis and to the stomach inferior to the anastomosis.

This surgery can also be performed as a vagus-sparing esophagectomy for high-grade Barrett's metaplasia, using the same dissection as with a highly selective vagotomy.

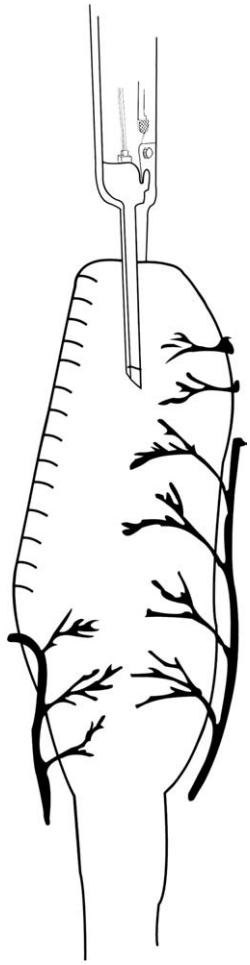

Fig. 1. A linear stapler is used to divide the stomach in the axis of the organ for approximately 5 cm.

As the use of a wrap after esophagogastrectomy has been previously described and is in practice, it was felt that animal model testing of this modification was not necessary.

Comparison to Standard Resection

The first author had performed 54 transhiatal, Ivor-Lewis, or transabdominal esophagogastrectomies prior and subsequent to the split-stomach fundoplication. These patients are the no wrap group. The records of these patients were reviewed for in-hospital mortality, anastomotic leak, and stricture development requiring endoscopic dilation. Asymptomatic patients routinely had esophagograms performed on postoperative day number 5. For patients who had symptoms or signs suggestive of an anastomotic leak, esophagography or computed tomography was performed when the question of this complication arose. An anastomotic leak was defined as any contrast extravagating from the area of the anastomosis, whether contained or not. Only

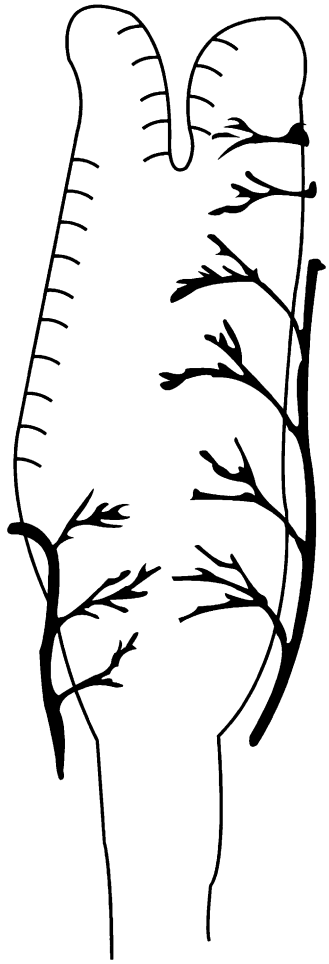

Fig. 2. This creates two "wings" of fundus which will be used for the fundoplication.

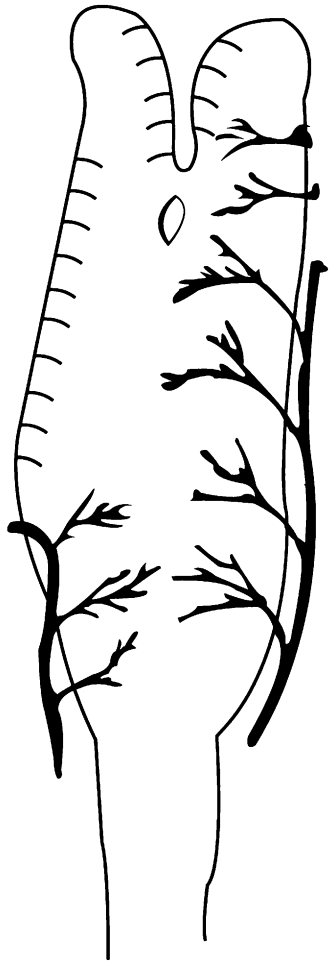

Fig. 3. A gastrotomy is created 2 cm caudad to the apex of the split for the anastomosis.

extravasation of contrast was considered a leak. Patients were continuously followed by the first author, either until death or if the patients choose not to return for follow-up visits. Part of the normal follow-up included questioning as to symptoms of dysphagia or reflux. For this study, all living patients were contacted either by telephone or during follow-up clinic visit and asked to complete the Gastroesophageal Reflux Disease–Health Related Quality of Life (GERD-HRQL) symptom severity questionnaire.[23] The GERD-HRQL is a 10 item Likert-type scale based on descriptive anchors with the best possible score being 0 (asymptomatic in all items) and the worst possible score 50 (incapacitated in all items).

Similarly, patients who underwent the split-stomach fundoplication also had their records reviewed for in-hospital mortality, anastomotic leak, and stricture development. These patients are the wrap group. At least 2 months postoperatively, patients were interviewed with respect to the presence or absence of refluxlike symptoms and completed the GERD-HRQL questionnaire.

As this modification was not considered experimental, it was not submitted for review to the Henry Ford Health System Institutional Review Board. However, the IRB did approve this retrospective review.

Statistical Analysis

Nominal data was analyzed using the Fisher exact test. Ordinal data were analyzed using the Mann-Whitney *U* test. A *P* value of 0.05 was considered statistically significant.

RESULTS

Demographics

Of the patients in the wrap group, 21 of 26 (81%) were male with an average age of 65 ± 9 years, compared to 42 of 54 (78%) males in the no wrap group with an average age of 65 ± 14 years. The wrap

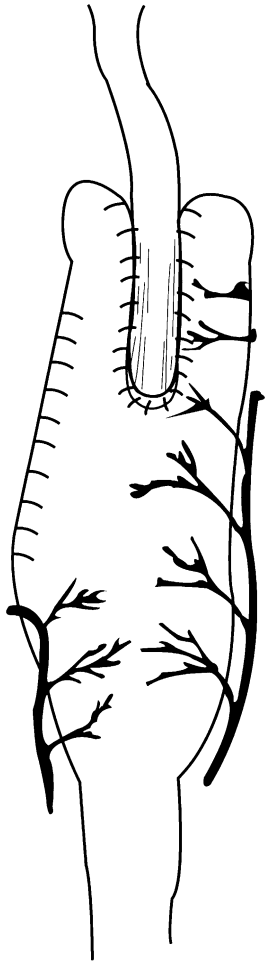

Fig. 4. An end-esophagus to side-stomach esophagogastrostomy is completed using interrupted silk suture.

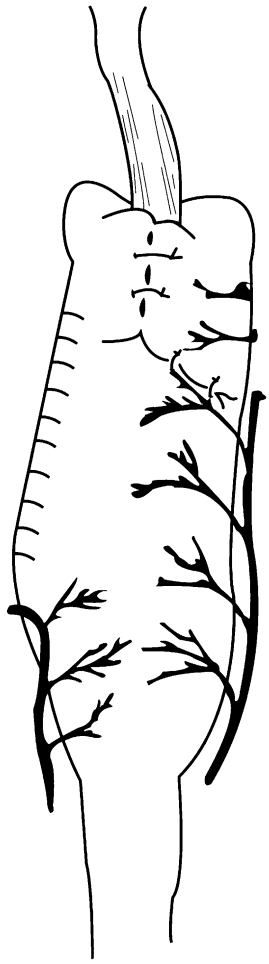

Fig. 5. The wings of the wrap are brought around the anastomosis to create the fundoplication.

group had the following distribution of pathology: squamous or adenocarcinoma, 21 (81%); Barrett's metaplasia with high-grade dysplasia, 4 (15%); and benign stricture, 1 (4%). The distribution of pathology for the no wrap group was carcinoma, 46 (86%); Barrett's metaplasia with high-grade dysplasia, 4 (7%); and benign stricture/end-stage achalasia/end-stage GERD, 4 (7%). For the wrap group, median follow-up was 10 months, with a range of 2 to 35 months. Six of these patients (23%) either died of their disease or were lost to follow-up. For the no wrap group, median follow-up was 16 months, with a range of 2 to 86 months. Thirty-four of these patients (63%) either died of their disease or were lost to follow-up. One patient in the wrap group could not be located; therefore, we had complete follow-up in 96% of patients. Four patients could not be located in the no wrap group; therefore, we had complete follow-up in 93% of patients.

Outcomes

In-hospital mortality was 3.8% in the wrap group, compared to 7.4% in the no wrap group (P = NS). The single death in the wrap group was due to pulmonary failure secondary to pneumonia in a patient with chronic obstructive pulmonary disease, whereas the 4 deaths in the no wrap group were due to anoxic brain injury secondary to cardiac arrest, sepsis secondary to postoperative pneumonia, sepsis secondary to an anastomotic leak, and seizure secondary to an unsuspected brain metastasis. No anastomotic leaks occurred in the wrap group, compared to nine (17%) in the no wrap group (P = 0.03). Of these leaks, three required reoperation, whereas six were treated conservatively with drainage, antibiotics, and subsequent resolution. In the wrap group, 20% (5 of 25) of patients reported heartburn or regurgitation consistent with gastroesophageal reflux, compared to 60% (30 of 50) of no wrap patients

($P < 0.0001$). In the wrap group, 40% (10 of 25) of patients required endoscopic dilation for postoperative benign anastomotic stricture, compared to 30% (15 of 50) of patients in the no wrap group (P = NS).

Nineteen patients in the wrap group were available at the time of the study to complete the GERD-HRQL, compared to 20 no wrap patients. For the wrap group, the median total GERD-HRQL score was 5 (range 0 to 13), compared to 14 (range 1 to 28) for the no wrap patients ($P = 0.03$).

DISCUSSION

This study demonstrates that the split-stomach fundoplication after esophagogastrectomy may reduce the incidence of anastomotic leaks and reflux-like symptoms. Although there are shortcomings to this study (which will be addressed below), there are both theoretical and practical reasons why the fundoplication may work. Firstly, the concept of a second layer around an esophageal anastomosis has been previously proposed.[19,20] In fact, the Peiper-Siewert modification of an esophagojejunostomy after a total gastrectomy[24] is in the same vein as the split-stomach fundoplication. Other intrathoracic fundoplications such as the Belsy Mark IV repair[25] and the Nissen fundoplication[26] have provided symptomatic relief of reflux symptoms. Therefore, the concept of a functional intrathoracic fundoplication is successful. More recently, Aly et al.[21] have developed a fundoplication for intrathoracic esophagogastrostomy using the apex of the gastric tube wrap around the anastomosis. They reported severe reflux in 12% of their fundoplication patients compared to 63% of their standard anastomosis patients. They also report that about one half of their patients in each group required dilation for postoperative dysphagia. In addition, the concept of using well-vascularized tissue to treat esophageal perforation is well accepted. Therefore, it is easy to conceptualize that using well-vascularized tissue (in the form of the stomach not on tension) would help prevent or ameliorate anastomotic leaks.

Nevertheless, this study clearly suffers from several shortcomings. First and foremost, it is not a randomized trial and comparisons are made to historical controls; hence, the groups may not be truly comparable. Secondly, although all the surgeries were done by the first author; therefore, this may dampen the variation caused by use of multiple surgeons and may well be a source of bias. The fundoplication patients had the benefit of the surgeon's increased experience, and this may account for some of the improved results. Certainly, a leak rate of 17% is at the higher end of the published literature,[2–11] and this may reflect poor technique in these patients. Hence, the difference in the general population of esophagectomy patients may not be as great. Lastly, although a well-established symptom severity instrument was used to assess reflux symptoms, there is no objective evaluation using routine upper endoscopy, esophageal manometry, or 24-hour esophageal pH testing to confirm the lack of pathologic reflux. Therefore, there may be a component of reporting bias from the patients. On the other hand, the use of these physiologic tests may be limited in patients who have undergone a vagotomy with their esophagectomy. For example, Mathew et al.[27] have demonstrated that with short lengths of residual esophagus after esophagectomy, no consistent motility pattern emerged, whereas with longer lengths, early peristaltic activity was evident but diminished over the first few postoperative days. Johansson et al.[16] demonstrated, using 24-hour double pH monitoring, that the mean acid exposure ranged from 0.2% to 6.5% after esophagectomy, with most results in the normal range. Nevertheless, additional follow-up with an upper endoscopy is required to demonstrate normal-appearing esophageal mucosa.

In addition to improving symptoms, reducing gastroesophageal reflux after esophagogastrectomy may have other benefits. It has been demonstrated that, after esophagogastrectomy, up to 50% of patients will develop columnar-lined metaplasia, with over half of these patients developing specialized intestinal metaplasia.[28] This is true even if the anastomosis is in the neck,[29] which is supposedly less refluxogenic.[17,18] Once again, only endoscopic follow-up of these patients, especially those who underwent resection for Barrett's metaplasia with high grade dysplasia, will answer this question.

The rate of postoperative stricture may be somewhat higher in patients who have undergone the split-stomach fundoplication. Although early in this series, the difference was statistically significant; with the addition of additional patients, it has ceased to be so. There are two potential causes: the wrap being constructed too tight to begin with, or extrinsic scar formation of the wrap. Another cause of stricture is ischemia. However, despite the fundus being split, both wings show good vascularization with no visual evidence of ischemia. Leaks by themselves can lead to postoperative stricture, but this is not a cause in the wrap group. Nevertheless, an overall stricture rate of 40% is not significantly different from the published literature.[3–6,30,31]

In conclusion, these preliminary results demonstrated that the split-stomach fundoplication may be a promising modification of an esophagogastrostomy

to reduce anastomotic leak and postoperative reflux symptoms. Further studies are needed to assess the true physiologic consequences of the wrap and the pathologic effects on the esophageal remnant.

We thank Adriana Lagrou for creating the illustrations.

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Discussion

Dr. Jeffrey Peters (Rochester, NY): Very nicely done, Vic, and I thank the Society for the opportunity to start the discussion. The three "horsemen," if you will, of complications associated with an esophagogastric anastomosis are ischemia, leak, and stricture, part of which you addressed today. Although each of these is definably separate clinically, they are far from independent from each other, with one commonly following the other. You will see the reason why I am making this point in a minute.

The prevalence of each has decreased over the past two decades, but none have been eliminated. They occur in roughly 5% of patients for ischemia, 15% for leak, and 25% for stricture. Vic presents the second paper of the day discussing technical modifications aimed at decreasing anastomotic leak or stricture, and in this case, the long-term consequence of gastroesophageal reflux as well.

There are two caveats, important to interpreting this data, that should be mentioned. First, as you presented, this is a consecutive series of patients. The manuscript that I had time to look over reveals that the nonwrap patients represent the early experience and the wrap patients a more recent experience. This, of course, introduces the possibility that the improvements are secondary to your learning curve, as is evidenced by your improvement in mortality.

Second, all of these patients had intrathoracic anastomoses. One of the main reasons why the anastomosis has been moved from the chest to the neck, in many centers, is because a thoracic anastomosis clearly increases the likelihood of postoperative reflux. With those caveats in mind, you have shown that, in your hands, the "split" stomach modification virtually eliminated anastomotic leak but also resulted in an increased incidence of strictures and at the same time decreased gastroesophageal reflux by two thirds.

Is this a technique that should be widely adopted? The answer doesn't come easily, but when I contemplate it, I think the answer is probably no, or at best, cautiously. There are many variables at play when you prepare a gastric interposition. These variables include the width of the tube—the narrower the more likely ischemic—the degree of resection of the lesser curvature, the size of the patient, and underlying comorbidities, with diabetes and hypertension being important.

Several questions come to mind, briefly. Firstly, is the split necessary, or could a similar process be accomplished by plicating the fundus around the esophagus? I suspect you may have tried this first. In fact, the proximal stomach is almost always relatively ischemic, and most of us would prefer not to "tempt" fate by adding more suture lines than are already necessary.

Second, does the splitting of the stomach preclude a stapled anastomosis with a linear stapler as we heard this morning from the Mayo group? This is becoming an increasingly attractive option.

And finally, if you read the paper, you defined anastomotic leak as any contrast extravasation on post operative barium study. Depending on the technique of esophagogastric anastomosis, that is, whether it is end-to-end or end-to-side, and you did end-to-side it can be difficult to distinguish between a leak, a contained leak, and flow of contrast into the superior portion of the fundus that is above the anastomosis. Could such overinterpretation of these barium studies be an explanation for the difference in leak rates?

Vic, you and your team are to be congratulated for your efforts to minimize the complications of esophagectomy. It is, of course, efforts such as these that result in the continuous improvement that has been evident in reducing morbidity and mortality of esophageal resection over the last two years.

Thank you very much.

Dr. Velanovich: Thank you, Dr. Peters, for your insightful comments and questions. To go through the questions: Is the split necessary? I think it is, because of my concern of making the wrap too tight. One of the things that I do is lay my fingers underneath the wings as they are sutured into place. Nevertheless you still get some stricturing.

Does this preclude a stapled anastomosis? I think you probably still can do a stapled anastomosis with the wrap, and actually I found that to be very intriguing.

Are the barium studies overinterpreted? I look at them all myself, and having constructed the anastomosis and the wrap so that the leaks are recorded, I did concur with the radiologist's interpretation.

Dr. Steven DeMeester (Los Angeles, CA): That is very nice and an interesting technique. I just want to question you a little bit about how the stomach looked after the split. Very elegant studies by Dorthea Lieberman have looked at the vascularization of the tip of the mobilized stomach, suggesting that a fair amount of blood flow actually comes up along the lesser curvature. If you make that split, have you had circumstances where that upper portion of the fundus,

particularly the side along the greater curvature, has gotten very ischemic, and what have you done in those circumstances? Have you had to resect that at all, or have you had to modify things because of that?

Dr. Velanovich: I do worry about ischemia at the wrap. I take a great deal of care to make sure we have a negative distal margin, yet to try to leave as much of the fundus as possible away from the resection margin. So, I haven't had a problem with the wrap becoming ischemic, nor I have to resect any of the wrap.

Dr. Gerard Aranha (Maywood, ILL): Vic, nice paper. I wanted to ask you two questions, one procedural. Is the anastomosis made to the anterior wall of the stomach? And with the wrap, does that create a stricture? Would it not empty better if you made the anastomosis to the posterior wall of the stomach?

Number two, if you are doing the operation for cancer, you are going to take part of the stomach, then split the stomach 5 cm down, and place the anastomosis 2 cm away from the apex of the split. Are you skimping on your margins? Will you leave more esophagus and thus incur an inadequate margin for a cancer operation proximally?

Dr. Velanovich: The anastomosis is in the anterior wall, so you make a good point. I didn't think about that, but that is something that should be considered.

As for the margins, basically I check the margins with a frozen section to make sure that they are adequate and never compromise the margins or lymph node dissection. I would actually forego doing this modification if we required removing a great deal of stomach or lesser curvature.

Gallbladder Cancer: An Analysis of a Series of 139 Patients With Invasion Restricted to the Subserosal Layer

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The goal was to study our experience in the management of a series of patients with a potentially curative subserosal gallbladder cancer who were prospectively treated by the authors. Between April 1988 and July 2004, 139 patients were enrolled in our prospective database. Of the above, 120 were operated on with an open procedure and the rest with laparoscopic surgery. In only eight patients was the diagnosis suspected before the cholecystectomy. The majority of tumors were adenocarcinoma. Six patients had an epidermoid tumor, and one had a carcinosarcoma. Of the patients, 74 underwent reoperation, while in 55 (70.2%) it was possible to perform an extended cholecystectomy with a curative aim. Operative mortality was 0%, and operative morbidity was 16%. Lymph node metastases were found in 10 (18.8%), while in 7 (13.2%) the liver was involved. The overall survival rate was 67.7%, while in those who underwent resection, the survival rate was 77%. Through the use of a multivariate analysis, the presence of lymph node metastasis was found to be an independent factor with respect to prognosis. The feasibility of performing an extended cholecystectomy in patients with gallbladder cancer and invasion of the subserosal layer allows for a good survival rate. The presence of lymph node metastases represents the main poor prognosis factor, and some type of adjuvant therapy should be studied in this particular group. (J GASTROINTEST SURG 2006;10:186–192)

KEY WORDS: Gallbladder cancer, survival outcomes, hepatic resection, lymph nodes

Gallbladder cancer (GC) is a very common disease in countries such as Chile, Japan, and India; however, it is uncommon in the United States.[1–3] Despite its generally poor prognosis, there is a subset of cases with long-term survival. The majority of patients with a potentially curable disease are detected after the examination of the cholecystectomy specimen. Of these patients, those with a T2 tumor (invasion restricted to the subserosal layer) are a group characterized by an intermediate prognosis and hope for long-term survival.[4,5]

Cholecystectomy alone is an adequate treatment for T1 GC (invasion through to the muscular layer). Radical second resection has been advocated in T2 patients, but its real effect on survival is discussed. Despite the lack of statistical evidence, most surgeons agree that the extended cholecystectomy would be useful as treatment.[5–8] Since 1988,

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Table 1. Protocol of management

Cholecystectomy and specimen biopsy Cancer diagnosis	
Wall infiltration of the tumor	**Procedure**
Mucosa	Only cholecystectomy
Muscle	Only cholecystectomy
Subserosa	Only cholecystectomy or reoperation
Serosa	Only cholecystectomy or reoperation

a prospective protocol of pathological diagnosis and treatment has been conducted by two of the authors (X.deA., I.R.). In general terms and as a part of the protocol, the extended cholecystectomy is offered to patients with GC and invasion deeper than the muscular layer in whom diagnosis is performed after studying the cholecystectomy specimen and are able to get a curative operation (Table 1).

The purpose of this study was to evaluate the results obtained in a cohort of potentially resectable patients with invasion limited to the subserosal layer (T2).

MATERIAL AND METHODS

We analyzed a series of 139 patients harboring a potentially resectable subserosal T2 GC detected after examination of the cholecystectomy specimen. The selection of patients was restricted to those with invasion limited to the subserosal layer (peritoneal side). We did not include patients in whom invasion was located in the adipose tissue at the hepatic side of the gallbladder. These patients were obtained from a database that included 334 patients with a potentially resectable GC enrolled prospectively over a 16-year period (April 1988–July 2004). A potentially resectable tumor is defined as a tumor in a completely resected gallbladder with no macroscopic residual tumor, or, if there is a residual tumor, it is located in areas that can be extirpated during a second operation. All patients underwent a simple cholecystectomy either open or laparoscopically in the first operation. The type of surgery depended on the surgeon choice and availability. Patient survival data were obtained from personal interviews with patients, from the clinical charts, or from the Chilean death master file. Most patients underwent a cholecystectomy at the Temuco Regional Hospital by different surgeons yet all were reoperated on by one of the authors (X.deA.).

Surgical mortality was considered as death occurring within 30 days of surgery. The interval between the cholecystectomy and the reoperation ranged between 1 and 11 months, with most undergoing surgery between 3 and 5 months.

Preoperative assessment included history, physical examination, and radiographic studies (computed tomography scan of abdomen and thorax radiograph).

An extended cholecystectomy was planned for the reoperation. Para-aortic lymph node samples were obtained from all patients as a first step during the operation.

The presence of distant tumor compromise or the impossibility of performing a curative surgery (complete resection with no gross residual cancer upon completion of surgery) was an indication to finish the operation.

An extended cholecystectomy consists of a liver wedge resection that included segments V and IVb along with a lymphadenectomy of the nodes located in the hepatic pedicle. Previous laparoscopic port sites were resected if present. Liver transection was performed using a crush clamp technique. Later, parenchymal division was accomplished using an ultrasonic dissector. Central venous pressure was maintained at 5 cm H_2O or less during the transection. The common bile duct was not resected. Adjuvant and neoadjuvant therapies were not used to any great extent in this series, making them unlikely to affect the analysis.

Numerical data are expressed as the mean, median, standard deviation, and ranges. Differences were considered significant at $P < .05$. The survival curves for selected patient groups were determined using Kaplan-Meier method. Survival durations for these groups were derived from the corresponding Kaplan-Meier curves and compared using the log-rank test.

Cox proportional hazard regression modeling was used to assess the effect that independent covariates had on the dependent variable of survival. Comparisons of patient survival curves were made using the log-rank test. Statistical analysis was performed using Stata 8.0 software (Stata Corporation, College Station, TX).

RESULTS

Patients

There were 17 men and 122 women with an average age of 58 years at presentation (range, 31–88 years). Nine patients were younger than 40.

Table 2. Presenting diagnosis of patients harboring a T2 gallbladder cancer

Diagnosis	No. of patients
Cholelithiasis	48
Acute cholecystitis	67
Cholangitis	4
Empyema	2
Jaundice	4
Neoplasia or polyp	8
Other	6

Table 3. Reasons to exclude patients from resection

Reason	No. of patients
Diffuse compromise of the bile duct	8
Paraaortic compromise	4
Port site invasion along with peritoneal compromise	2
Choledocoduodenal lymph node compromise	1
Miscellaneous	4

Presenting Symptoms and Preoperative Diagnosis

Of the patients, 120 (86%) underwent an open procedure, while the rest (19 patients) underwent a laparoscopic cholecystectomy. Of the latter, three had to be converted to an open procedure because cancer was suspected at the moment of the cholecystectomy. Most patients were operated on due to a cholelithiais (48 patients) or acute cholecystitis (67 patients). Of the 139 patients, only eight diagnoses were suspected prior to the cholecystectomy. In two of these eight patients, the lesion was suspected to be a polyp, while in six it corresponded to a gallbladder mass (Table 2). With respect to the postoperative diagnosis, in only 15 patients was the diagnosis of tumor suspected during the cholecystectomy. In the rest, diagnosis of tumor was made during the analysis of the cholecystectomy specimen. Of these patients, in 61 patients the lesion was detected only after the histologic examination, being completely unsuspected during the macroscopic examination of the gallbladder mucosa performed by the pathologist.

Pathology

Most patients had an adenocarcinoma, while six patients had an epidermoid tumor and one had a carcinosarcoma. Among the patients with an adenocarcinoma, 72 (54.5%) had a moderately differentiated tumor.

Therapeutic Procedures and Pathologic Findings

Of all of the patients, 74 (53.2%) underwent reoperation with the aim of performing an extended cholecystectomy. The rest did not undergo reoperation due to their refusal or their advanced age (older than 65). Of those who underwent reoperation, 55 (70.2%) were resected. Reasons for not undergoing the resection were diffuse common bile duct extension in eight patients, para-aortic lymph node compromise in four, port site compromise along with peritoneal compromise in two, and others (Table 3). Among the last patients, it is important to draw attention to one patient with liver cirrhosis and another patient who presented with a diffuse inflammatory compromise of the upper abdominal cavity, probably secondary to an asymptomatic biliary leak. In these patients, the risk of bleeding and liver failure and the technical difficulties derived from the inflammation were considered, respectively, to avoid the resection. Furthermore, in two patients, only a lymphadenectomy was performed, the reason being thrombosis of the hepatic artery and persistent intraoperative hemodynamic instability. Among the patients who underwent a lymphadenectomy, metastases were found in 12 (18.8%). The total number of lymph nodes dissected ranged between 2 and 21 nodes, with an average of 8.6 nodes. All patients with lymph node compromise had lymph node involvement in the hepatic pedicle. No skip metastases were observed in this series. Liver infiltration was found in 7 of the 53 who underwent liver resection (13.2%). The volume of liver resection was calculated from the weight of the specimen, with the average weight being 100 g (Table 4).

Perioperative Complications

Operative mortality was 0%. Morbidity for those undergoing resection was 16.6%. Transient biliary leakage was observed in three patients, and lymphorrhea, abdominal collection, pneumonia, and fever of unknown origin were seen in one patient each,

Table 4. Pathologic findings in patients undergoing resection

	No.	Positive
Lymph nodes	55	10 (18.8%)
Liver tissue	53	7 (13.2%)

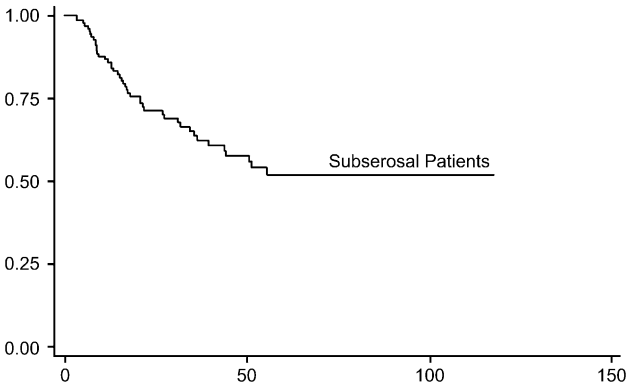

Fig. 1. Kaplan-Meier estimates of overall survival for the entire cohort.

respectively. The only patient to undergo reoperation was the one with lymphorrhea; the reason for the operation was the presence of intense abdominal pain. At reoperation, a small amount of a liquid with lymphatic characteristics was found in the minor pelvis. The abdominal abscess was drained percutaneously.

Survival

Mean follow-up of the patients in the study was 20.8 months, ranging between 2 and 116 months (SD, 27.95 months). The overall survival rate in the series was 67.7% (Fig. 1). Figure 2 shows the survival curve of patients who underwent resection versus those who underwent only cholecystectomy. Those undergoing resection had a greater 5-year survival rate but with no statistical significance ($P = 0.07$).

The presence of lymph node metastases and liver involvement were associated with a worse prognosis when survival in groups with and without invasion was studied (45% versus 70% 5-year survival rate for patients with lymph node compromise versus no lymph node involvement [$P = 0.06$] and a 42% versus 82% 5-year survival rate in those with invasion of the liver versus those without invasion [$P = 0.002$]) (Figs. 3, 4).

Prognostic Factors

To know the true value of prognostic factors, a Cox proportional hazard regression model was designed according to the following factors: (1) macroscopic type, (2) lymph node status, (3) liver infiltration, and (4) age older than 50. In this model, the absence of lymph node compromise was associated with significant improvement in overall survival. The P values and relative risks are shown in Table 5.

DISCUSSION

Among the reasons to explain the lower survival rate of patients with GC, late diagnosis has been cited as one of the most important.[9,10] In this series, the diagnosis of GC was mainly performed after the pathologic examination of the cholecystectomy specimen. This factor stresses the poor value that examinations such as ultrasonography and computed tomography scanning have in the detection of small lesions. The higher percentage of flat and

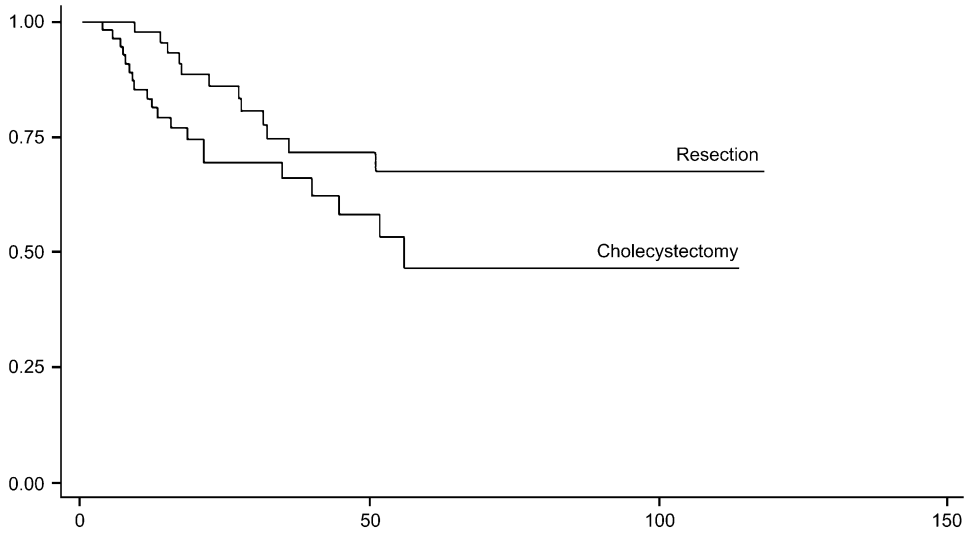

Fig. 2. Kaplan-Meier estimates of overall survival, comparing those who underwent resection with those who underwent only cholecystectomy ($P = 0.07$).

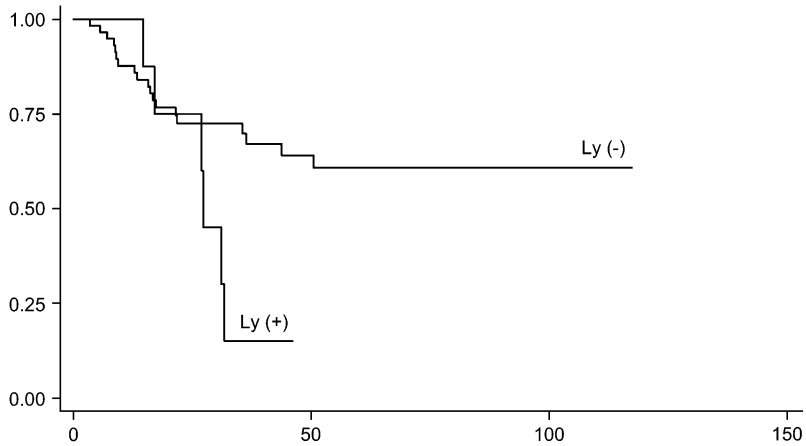

Fig. 3. Kaplan-Meier estimates of overall survival for patients who underwent resection comparing those with lymph node compromise [Ly (+)] versus those without compromise [Ly (−)] ($P = 0.06$).

nonapparent lesions, many of which were not recognized during the macroscopic examination of the gallbladder mucosa, explains the above results. Moreover, inflammation of the gallbladder wall contributes to the poor visualization.

This lower rate of preoperative suspicions makes greater the possibility of finding an unapparent tumor and increases the number of technical considerations that Chilean surgeons need to take into account when performing a cholecystectomy. Among patients older than 60 undergoing a cholecystectomy, the proportion of coincident GC is almost 10%.[11,12]

There is almost universal agreement to use an extended cholecystectomy for the management of patients with resectable GC; however, there is no scientifically proven evidence to support this management. Most series compare patients who underwent resection with a group treated only by a cholecystectomy, and we have no information about their potential resectability.[5,13,14] This study performed similar analyses and obtained similar results. However, an extended cholecystectomy is the most commonly used procedure for treatment. The definitive answer regarding its real value should come from a randomized trial comparing patients with and without reoperation.

The resection of the common bile duct associated with an extended cholecystectomy is another point of discussion: the complete excision of lymphatics around the duct would be better performed if the duct were excised. However, such a statement is based largely on theory rather than on clinical or pathologic studies. On the other hand, the addition of common bile duct resection could be associated with higher morbidity compared with the morbidity of patients without resection.

At present, the laparoscopic cholecystectomy is the "gold standard" in the treatment of gallstone disease. However, concern about its influence on the prognosis has been mentioned in a number of reports.[15–17] Because open procedures are largely used in patients undergoing emergency surgery in Chile, most patients in this series were treated in this manner. Perhaps a greater use of the laparoscopic cholecystectomy is associated with a poorer resectability and prognosis.

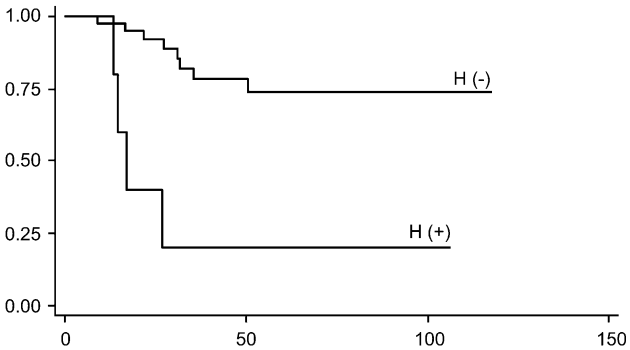

Fig. 4. Kaplan-Meier estimates of overall survival for patients who underwent resection comparing those with hepatic compromise [H (+)] versus those without compromise [H (−)] ($P = 0.002$).

Table 5. Cox's proportional hazards model

Factor	Hazards ratio	*P* Value	95% Confidence interval
Lymph node status	5.65	0.014	1.42–22.36
Liver infiltration	3.93	0.071	0.89–17.35
Age >50 y	1.03	0.960	0.26–4.02
Macroscopic type	1.24	0.800	0.23–6.56

Traditionally, the overall 5-year survival of patients with GC has been less than 10%.[9] These dismal results are mainly due to the advanced stage of the disease at the moment of diagnosis. In countries such as Chile, however, where GC is commonly detected, a higher proportion of GC cases correspond to early forms associated with longer survival.[11,12] This fact is clearly observed from the analysis of the percentage of early tumors among those with GC. At our center, mucosal and muscular tumors comprise 23% of the total number of gallbladder tumors.[18]

In this series, an overall survival rate of 67.7% was observed. This result is a consequence of the detection of patients with early forms of GC among those who underwent a cholecystectomy for a presumed benign disease.

From our results, we can also point out the importance of lymph nodes as a prognostic factor. All patients with lymph node compromise had involvement of lymph nodes located in the hepatic pedicle, mainly in the cystic and the choledocoduodenal node. The involvement of lymph nodes along the hepatic pedicle follows a constant pathway, first compromising those located near the cystic duct and then the choledocoduodenal node to reach the para-aortic nodes through the retropancreatic lymph nodes.

Despite the fact that the same surgeon operated on all the patients, there were variations in the number of dissected lymph nodes. This variation could be due to local changes in the hepatic pedicle that make dissection more difficult. The presence of fibrosis secondary to the surgical trauma or to the existence of a T-tube may be responsible for this numerical variation.

The 5-year survival rate of patients who underwent resection but had lymph node compromise was significantly worse than the survival rate in the same group without lymph node compromise. By using this factor, we can distinguish two different types of populations among those who have lymph nodes evaluated. From the analysis of our lymph node compromise rate, we realized the lower percentage of lymph node compromise observed in our patients. Only 10 (18.8%) patients undergoing dissection had lymph node metastases. This rate is lower than that published in other reports, which could be explained by the fact that all of the patients in our series had undergone a previous cholecystectomy. This first surgical approach would permit a more precise staging, meaning that only patients about whom a more precise knowledge of their disease extension is available would receive reoperation. Furthermore, our series of patients was restricted to those where invasion was located on the peritoneal side of the gallbladder. In previous studies, we observed a worse prognosis for patients with tumor invasion of the hepatic side of the gallbladder.[5] This fact is reinforced by our higher resectability.

Liver extension was also observed to be associated with a worse prognosis, although there is no statistical significance.

CONCLUSION

Despite the generally poor survival rate of patients with lymph node metastases, the study of patients with intermediate forms of the disease, such as subserosal tumors, allows us to obtain a selected group with good survival. This particular group of patients is obtained in areas where the disease is more prevalent and the cholecystectomy specimen is deeply studied.

Unfortunately, the type of treatment for the disease is mainly supported by expert opinions and analysis of dissemination routes, lacking the development of a randomized trial that challenges the true value of the extended cholecystectomy.

Given the existence of poor prognostic factors among patients undergoing curative resection, adjuvant strategies must be studied in these groups of patients.

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Nonalcoholic Fatty Gallbladder Disease: The Influence of Diet in Lean and Obese Mice

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The obesity epidemic has contributed to an increased prevalence of gallstones and a higher percentage of chronic acalculous cholecystitis. Obesity is associated with Type II diabetes and hyperlipidemia in murine models. In addition, we have previously demonstrated that serum glucose, insulin, cholesterol, and triglycerides correlated with gallbladder contractility in murine models. However, the relative role of insulin resistance and gallbladder fat infiltration in this phenomenon remain unclear. Therefore, we tested the hypothesis that gallbladder wall lipids are related to obesity and diet and are inversely correlated with gallbladder contractility. One hundred lean control (C7BL/6J) and 36 obese leptin-deficient (Lep^{ob}) 8-week-old female mice were fed either a chow diet or a 1.0% cholesterol, 15% butterfat (high-lipid) diet for four weeks. Pooled gallbladders were then analyzed for free fatty acids (FFA), phospholipids (PL), total cholesterol (TC), and triglycerides (TG). Cholesterol/phospholipid ratios were then calculated. The Lep^{ob} mice fed a chow diet had significantly higher ($P < 0.01$) gallbladder lipids than the three other groups. The lean mice that were fed a high-lipid diet had increased ($P < 0.05$) gallbladder TC compared to the lean mice on a chow diet. In addition, the cholesterol/phospholipid ratio was significantly increased ($P < 0.01$) in the lean mice fed a high-lipid diet compared to the other three groups. Finally, the high-lipid diet decreased gallbladder FFA ($P < 0.01$), PL ($P = 0.08$), and TC ($P < 0.05$) in Lep^{ob} mice. These data suggest that (1) obese mice have increased gallbladder lipids; (2) a high-cholesterol, high-fat diet increases gallbladder lipids and the cholesterol/phospholipid ratio in lean mice; but (3) decreases gallbladder fatty acids, phospholipids, and cholesterol in obese mice. Prior studies have documented similarly decreased gallbladder response to neurotransmitters in obese mice on a chow diet, as well as lean and obese mice on a high-lipid diet. Therefore, we conclude that leptin-deficient obesity and/or a high-fat diet causes nonalcoholic fatty gallbladder disease, which is manifested by diminished gallbladder contractility. (J GASTROINTEST SURG 2006;10:193–201)

KEY WORDS: Cholesterol, fatty acids, gallbladder, leptin, lipids

Obesity is a major health-care problem in the United States and the rest of the industrialized world. The number of people with a body mass index greater than 30 increases on a yearly basis.[1–3] Obese patients have a number of comorbidities, including diabetes and hyperlipidemia. The obesity epidemic has also contributed to an increased prevalence of gallstones and a higher percentage of chronic acalculous cholecystitis.[4,5] As a result, a number of groups have reported an increased rate of cholecystectomy.[6–10]

Prior research has examined the role of gallbladder wall lipids on gallbladder function, but has not evaluated the effects of obesity and diet.[11,12] A number of animal models have been used to study gallstone pathogenesis. Murine models of obesity have the advantage of having well-defined genotypic and phenotypic variations. Using these models, we have

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previously reported an inverse relationship between gallbladder contractility and serum glucose, insulin, cholesterol, and triglycerides.[13] However, the relative role of insulin resistance and gallbladder fat infiltration in this phenomenon remain unclear. Therefore, we tested the hypothesis that gallbladder wall lipids are related to obesity and diet, and are inversely correlated with gallbladder contractility.

MATERIALS AND METHODS

Animals and Diets

One hundred lean control (C57BL/6J; lean) and 36 leptin-deficient (C57BL/6J-Lep^{ob}) obese female mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 8 weeks of age. The mice were housed four to five per cage in a light (6 A.M. to 6 P.M.) and temperature (22 °C) controlled room. The animals were fed either a low-fat, trace-cholesterol chow diet (Ralston Purina, St. Louis, MO) or a high-fat (15% butterfat), high-cholesterol (1.0%) diet (XOL; Dyets, Inc., Bethlehem, PA), for 4 weeks. When animals are fed these diets for 4 weeks, their bile becomes supersaturated with cholesterol and some animals form crystals, but none have gallstones.[14] The Medical College of Wisconsin Animal Care Committee approved all protocols for these animal studies.

Tissue Collection

At 12 weeks of age, the animals were fasted overnight. In the morning, all animals were weighed, and anesthesia was achieved with xylazine (15 mg/kg) and ketamine (50 mg/kg). They then underwent a laparotomy and cholecystectomy. Gallbladders were weighed, flash frozen to −80 °C, pooled into groups of 3–10, and subsequently analyzed for free fatty acids (FFA), phospholipids (PL), total cholesterol (TC), and triglycerides (TG). Obese gallbladders were larger; and therefore, fewer were needed per pool.

Gallbladder Lipid Analysis

Gallbladder lipid analyses were conducted by the Mouse Metabolic Phenotyping Center at Vanderbilt University Medical Center. Lipids were extracted by the method of Folch-Lees.[15] Briefly, gallbladders were homogenized in chloroform (2:1 vol/vol). Extracts were then filtered, and lipids were recovered in the chloroform phase. Individual lipid classes were separated by thin layer chromatography using Silica Gel 60 A plates developed in petroleum ether, ethyl ether, and acetic acid (80:20:1) and visualized by rhodamine 6G. Phospholipids, triglycerides, and cholesteryl esters were scraped from the plates and methylated using BF_3/methanol as described by Morrison and Smith.[16] The methylated fatty acids were extracted and analyzed by gas chromatography. Gas chromatographic analyses were carried out on an HP 5890 gas chromatograph (Hewlett-Packard, Houston, TX) equipped with flame ionization detectors, an HP 3365 Chemstation, and a capillary column (SP2380, 0.25 mm × 30 m, 0.25 μm film; Supelco, Bellefonte, PA). Helium was used as a carrier gas. Fatty acid methyl esters were identified by comparing the retention times to those of known standards. Inclusion of odd chain fatty acids as standards permitted the quantitation of the amount of lipid in the sample.

Cholesterol was analyzed by the method of Rudel et al.[17] To an aliquot of the Folch extract, 5 α-cholestane was added as an internal standard. To quantitate unesterified cholesterol, an aliquot of the extract was analyzed by gas chromatography using a Hewlett Packard 5890 gas chromatograph equipped with a DB-17 column (0.53 mm id × 30 m × 1 μm film; Agilent, Palo Alto, CA) and flame ionization detector. To determine total cholesterol, a second aliquot of the extract was saponified with 1 N KOH in 90% methanol. The nonsaponifiable sterol was extracted using hexane, and total cholesterol was determined using the gas chromatograph. Cholesterol and phospholipid results were used to calculate the cholesterol/phospholipid ratio.

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using SigmaStat Statistical Software (SPSS, Inc., Chicago, IL). Different lipid levels were tested for statistical significance by one-way analysis of variance. A P value of <0.05 was considered statistically significant.

RESULTS

Free Fatty Acids

The values from the lipid assays and the cholesterol/phospholipid ratio are summarized in Table 1. Quantities of individual chains of free fatty acids for each animal group are listed in Table 2. When the total free fatty acids were compared among groups, the obese mice fed a chow diet had more free fatty acids compared to the three other groups ($P < 0.01$; Fig. 1). However, when the obese animals were fed the high-cholesterol, high-fat diet, free fatty acids decreased dramatically to lean animal levels.

Table 1. Results of the gallbladder wall lipid analysis and the cholesterol/phospholipid ratio

	Lean chow n = 5	Lean XOL n = 5	Obese chow n = 3	Obese XOL n = 3
Free fatty acids	18.5 ± 2.3	14.5 ± 2.1	76.9 ± 21.8*	16.7 ± 1.5
Phospholipids	13.8 ± 2.7	10.5 ± 0.7	38.9 ± 5.5*	18.9 ± 2.4§
Total cholesterol	3.0 ± 0.8†	10.2 ± 0.3‡	9.8 ± 1.8‡	5.9 ± 1.0
Triglycerides	2.8 ± 0.8	4.3 ± 1.0	41.9 ± 16.5‖	40.5 ± 11.0¶
Cholesterol/phospholipid ratio	0.21 ± 0.02	0.98 ± 0.06*	0.25 ± 0.03	0.32 ± 0.09

Results are mean ± SEM and are expressed as μg/mg of tissue. XOL is a high butterfat, 1% cholesterol diet.
*$P < 0.01$ vs. other 3 groups.
†$P < 0.05$ vs. other 3 groups.
‡$P < 0.05$ vs. obese XOL.
§$P < 0.08$ vs. obese chow.
‖$P < 0.05$ vs. lean XOL and obese XOL.
¶$P < 0.01$ vs. lean XOL and obese XOL.

In addition, both the high-cholesterol, high-fat diet and obesity resulted in decreased 16:00 and increased 18:01 fatty acid chains ($P < 0.01$; Table 2).

Phospholipids

As with fatty acids, the obese chow-fed mice had the highest amount of phospholipids of the four groups ($P < 0.01$; Fig. 2). In addition, the obese mice that were fed a high-cholesterol, high-fat diet had more phospholipids than the lean mice fed the same diet, which approached statistical significance ($P = 0.08$). The obese mice fed a chow diet had increased 18:01 ($P < 0.01$) and decreased 18:02 phospholipid chains ($P < 0.05$; Table 3). In addition, the lean mice on the chow diet had increased 16:00 ($P < 0.01$) and 18:00 ($P < 0.05$; Table 3).

Table 2. Individual free fatty acid chains per animal group

Free fatty acid chain	Lean chow n = 5	Lean XOL n = 5	Obese chow n = 3	Obese XOL n= 3
14:00	2.0 ± 0.2	2.4 ± 0.1§	1.3 ± 0.1	3.3 ± 0.3*
16:00	46.0 ± 1.2*	35.0 ± 1.6‡	25.3 ± 0.6	27.1 ± 3.0
16:01	6.5 ± 0.4	7.1 ± 0.2	9.8 ± 0.8	16.5 ± 1.4*
18:00	7.1 ± 0.5†	5.6 ± 0.3	4.6 ± 0.2	4.2 ± 0.5
18:01	29.4 ± 2.2‖	39.7 ± 1.8	52.4 ± 1.0*	38.4 ± 2.1
18:02	8.7 ± 2.0	8.9 ± 0.4	5.6 ± 1.0	9.9 ± 0.3
20:03	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
20:04	0.4 ± 0.4	1.3 ± 0.3	1.3 ± 0.5	0.7 ± 0.2

Values are the mean ± SEM for that animal group expressed as a percentage of the total free fatty acids. XOL is a high butterfat, 1% cholesterol diet.
*$P < 0.01$ vs. other 3 groups.
†$P < 0.05$ vs. other 3 groups.
‡$P < 0.05$ vs. obese chow and obese XOL.
§$P < 0.05$ vs. obese chow.
‖$P < 0.01$ vs. lean XOL and obese XOL.

Total Cholesterol

The lean mice fed a chow diet had the least amount of total cholesterol in the gallbladder wall compared to the other three animal groups ($P < 0.05$; Table 1 and Fig. 3). In addition, the lean mice fed the high-cholesterol, high-fat diet and the obese mice fed the chow diet had significantly more cholesterol in the gallbladder wall than the obese mice on the high-cholesterol, high-fat diet ($P < 0.05$).

Triglycerides

The leptin-deficient obese animals that were fed a chow diet had significantly more gallbladder wall triglycerides than either of the two lean mouse groups ($P < 0.05$; Fig. 4). This finding also was true for the obese mice that were fed a high-cholesterol, high-fat diet ($P < 0.01$).

Cholesterol/Phospholipid Ratio

The lean mice that were fed the high-cholesterol, high-fat diet had a cholesterol/phospholipid ratio of 0.98 ± 0.06. This ratio was greater than for all of the other animal groups tested ($P < 0.01$; Fig. 5).

DISCUSSION

In this study the gallbladders of C57BL/6J lean mice and C57BL/6J-Lep^{ob} obese mice that had been fed either a low-cholesterol, low-fat chow diet or a high-cholesterol, high-fat diet were assayed to determine the gallbladder wall lipids. The obese mice fed a chow diet had significantly increased free fatty acids, phospholipids, and triglycerides. The high-cholesterol, high-fat diet caused a significant increase in the total cholesterol within the gallbladder wall of the lean mice. This diet also led to a significant increase in the cholesterol/phospholipid ratio in

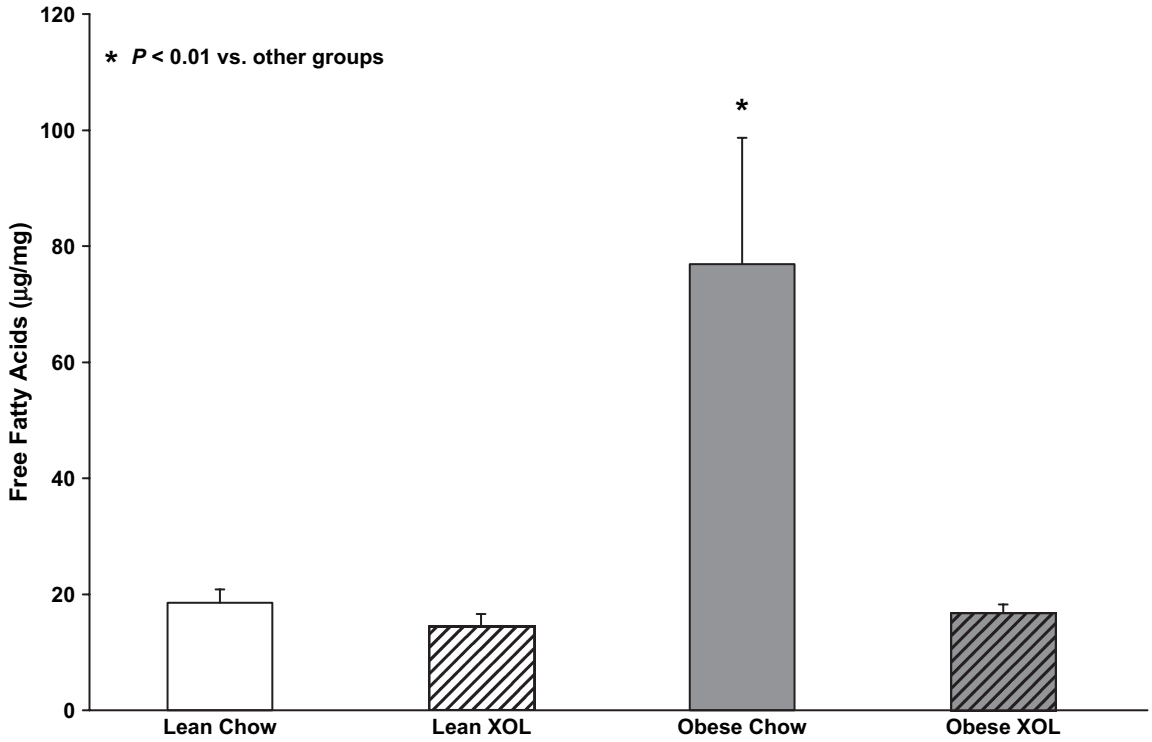

Fig. 1. Free fatty acids.

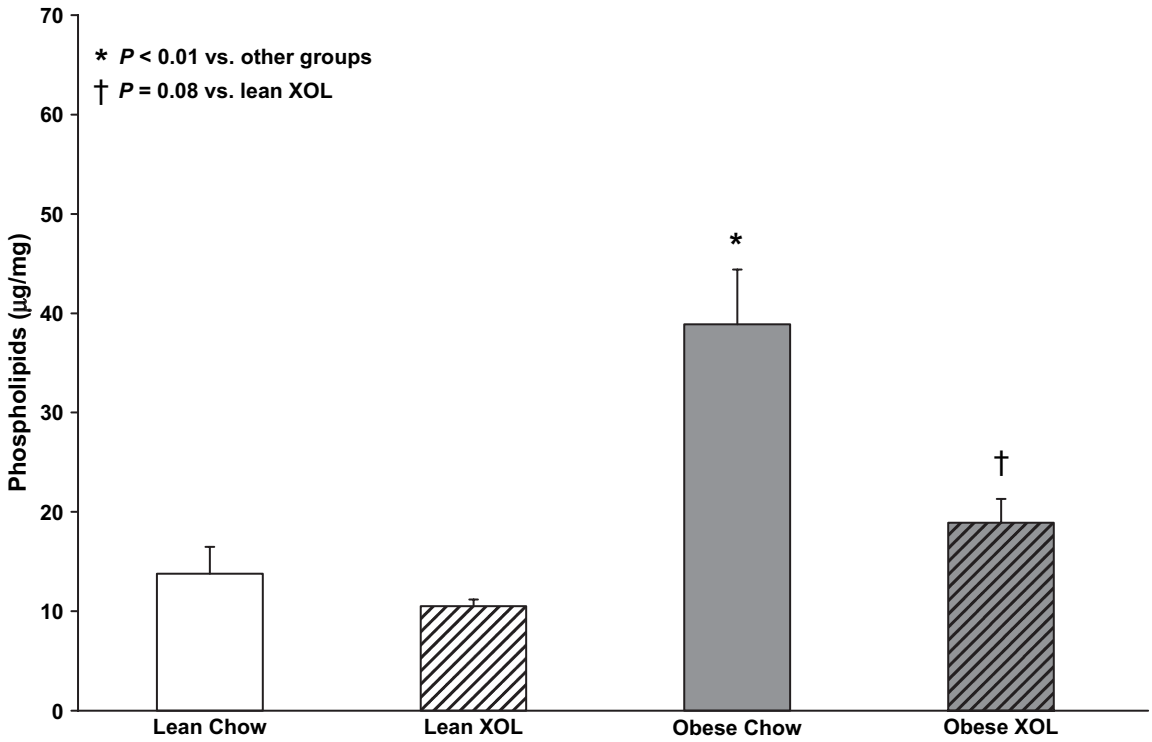

Fig. 2. Phospholipids.

Table 3. Individual phospholipid chains per animal group

Phospholipid chain	Lean chow n = 5	Lean XOL n = 5	Obese chow n = 3	Obese XOL n = 5
14:00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1
16:00	34.8 ± 2.7*	21.4 ± 1.6	20.0 ± 2.0	24.3 ± 1.1
16:01	2.9 ± 0.7	4.2 ± 0.2	5.9 ± 3.0	8.5 ± 0.9‡
18:00	10.2 ± 1.4†	4.7 ± 0.4	7.3 ± 0.7	3.9 ± 0.3
18:01	20.9 ± 2.0§	28.6 ± 1.6	43.0 ± 3.3*	29.5 ± 1.7
18:02	24.7 ± 3.6	27.3 ± 1.4	15.5 ± 1.0†	26.1 ± 1.6
18:3w3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
20:03	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	1.0 ± 0.2†
20:04	5.0 ± 1.9	9.7 ± 1.2	8.4 ± 2.6	4.3 ± 1.2
20:05	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2
22:06	1.4 ± 1.4	3.9 ± 1.0	0.0 ± 0.0	1.4 ± 0.7

Values are the mean ± SEM for that animal group expressed as a percentage of the total phospholipids. XOL is a high butterfat, 1% cholesterol diet.
*$P < 0.01$ vs. other 3 groups.
†$P < 0.05$ vs. other 3 groups.
‡$P < 0.05$ vs. lean chow and lean XOL.
§$P < 0.01$ vs. lean XOL and obese XOL.

the lean mice. Paradoxically, the same high-cholesterol, high-fat diet decreased fatty acids, phospholipids, and cholesterol in the gallbladders of the leptin-deficient obese mice. These findings correlate with prior studies that demonstrated a decrease in the gallbladder contractility of obese mice on a chow diet and in lean mice fed a high-fat, high-cholesterol diet.[13] The changes in the gallbladder lipids observed in this study may be partly responsible for the differences in gallbladder contractility.

The pathogenesis of cholesterol gallstones is multifactorial.[18] Cholesterol crystal pronucleators, supersaturation of cholesterol in bile, and bile stasis all play a role. By examining bile stasis and its

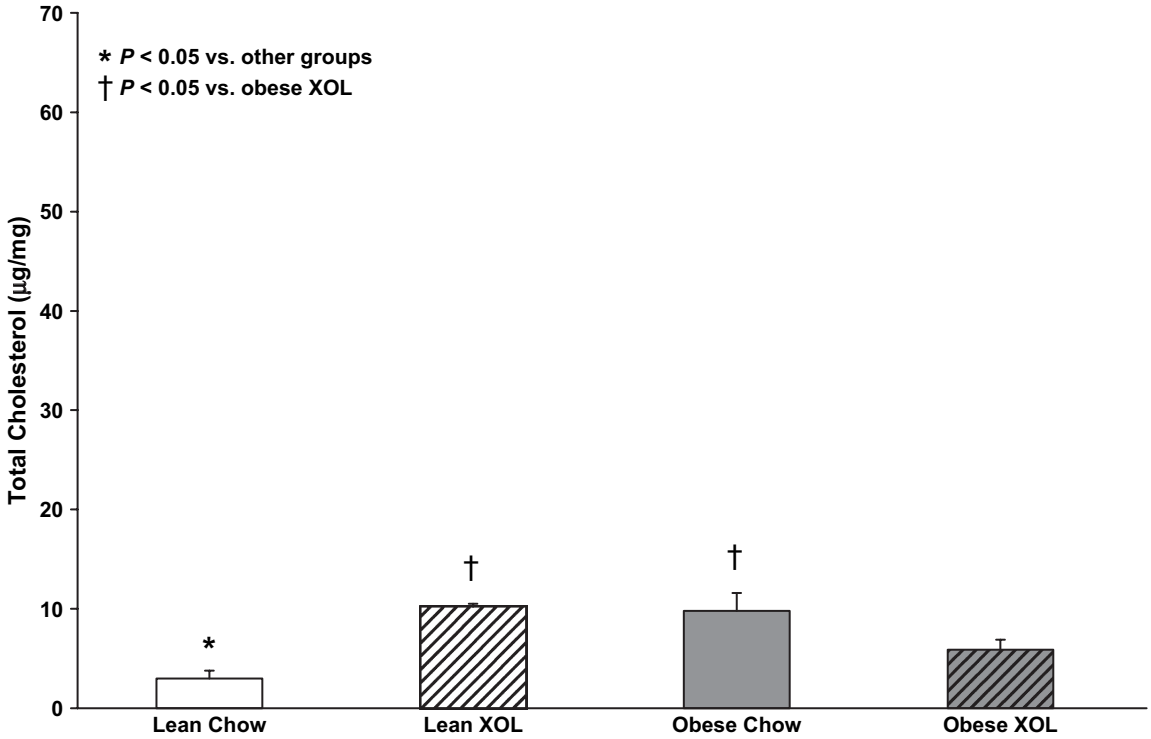

Fig. 3. Total cholesterol.

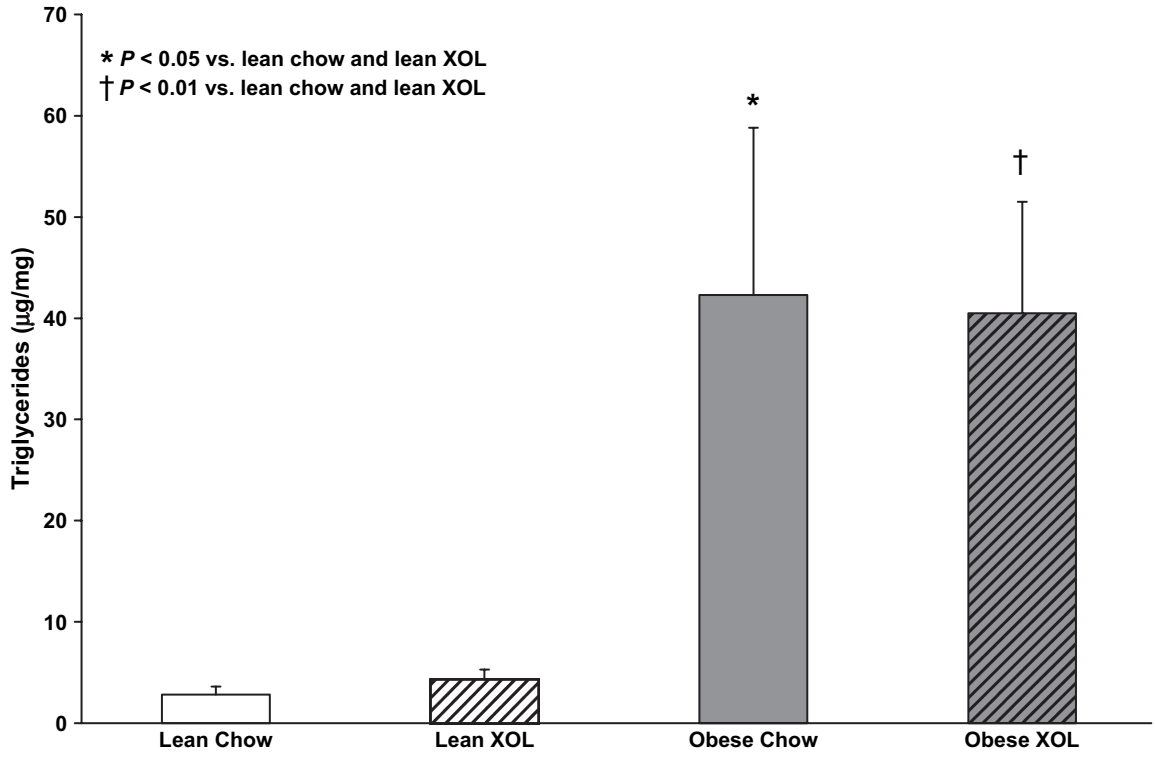

Fig. 4. Triglycerides.

relationship to obesity, our group has found that the resting gallbladder volume in leptin-deficient obese mice fed a chow diet is 2.5 times the size of the gallbladders in lean mice.[19] These gallbladders were tested for their response to various excitatory neurotransmitters, including cholecystokinin, acetylcholine, and neuropeptide Y.[20] The congenitally obese mice had decreased gallbladder responses to these neurotransmitters, which indicates gallbladder stasis in these obese animals.

In prior studies, the fasting serum glucose, insulin, cholesterol, and triglyceride levels were all significantly increased in leptin-deficient obese mice.[21] The diabetes and hyperlipidemia found in these mice correlated with their decreased in vitro gallbladder responses. These findings suggest that diabetes and hyperlipidemia may be independent risk factors for gallbladder stasis. However, serum lipids do not necessarily correlate with gallbladder wall lipids, and so the local effects of hyperlipidemia in this model needed to be examined.

The role of various lipids within the gallbladder wall has been studied before. Yu et al. looked at prairie dogs that were fed either a chow diet or a high-cholesterol diet similar to the one used in this study.[12] The gallbladders of those animals were then analyzed for lipids and were found to have more cholesterol, less phospholipids, and therefore, higher cholesterol/phospholipid ratios when fed the high-cholesterol diet. These findings mirror what is seen in this study in the lean mice. The obese mice show a similar decrease in phospholipids on the high-cholesterol diet, yet the cholesterol also decreased as did the fatty acids. Thus, the cholesterol/phospholipid ratio was unchanged in the obese animals.

Increased membrane cholesterol and the cholesterol/phospholipid ratio has been shown in a number of different cell types, including salivary gland and smooth muscle cells, to influence membrane fluidity and membrane bound protein function.[22,23] Chen et al. showed that the smooth muscle cells of human gallbladders with cholesterol stones had increased cholesterol and cholesterol/phospholipid ratios when compared with gallbladders from patients with pigment stones.[11] They also demonstrated that the membrane fluidity was decreased in the cholesterol stone group and negatively correlated with the cholesterol/phospholipid ratio, thereby validating this ratio as an indirect measure of membrane fluidity. In addition, as the cholesterol/phospholipid ratio increases, gallbladder muscle cell contraction decreases. This phenomenon seems to occur at the cell membrane level, because when the cell membranes are bypassed by second messengers such as inositol 1,4,5-triphosphate, diacylglycerol, or calmodulin, the contractility returns to normal.[24]

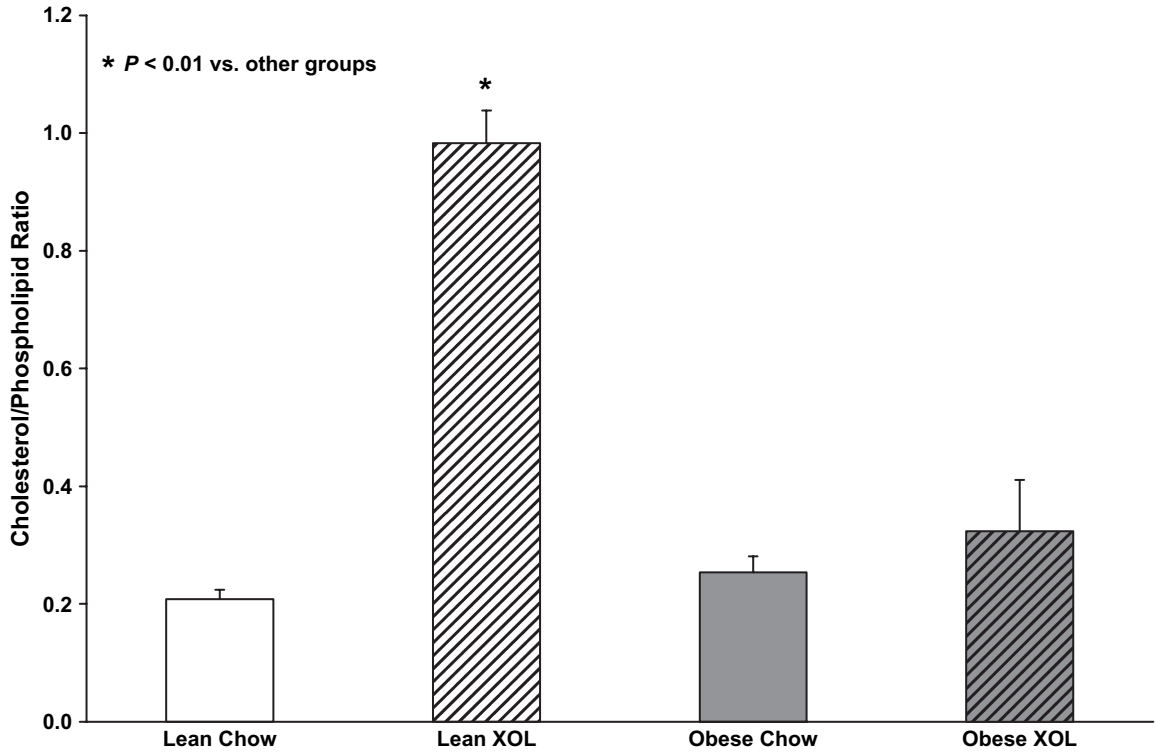

Fig. 5. Cholesterol/phospholipid ratio.

Previous work from our lab evaluated the contractility of murine gallbladder myocytes in lean and obese animals. Graewin et al. demonstrated that the gallbladder myocytes from C57BL/6J lean mice had greater contractility than their leptin-deficient (Lep^{ob}) and leptin-resistant (Lep^{db}) obese counterparts when fed a chow diet.[25] These obese animals have altered serum lipids,[21] and this current study shows that the gallbladders of the leptin-deficient obese mice have increased free fatty acids, phospholipids, triglycerides, and cholesterol. The altered gallbladder lipids are likely contributing to the biliary motility disorder found in this model of obesity.

Leptin is a 16 kDa protein, produced by adipocytes, that has influences on a number of neurotransmitters, including cholecystokinin and neuropeptide Y.[26] Leptin has also been shown to affect satiety, activity, and lipid metabolism.[27,28] Low leptin levels lead to an accumulation of lipid within all cells, including muscle cells.[29] Moreover, the administration of adenovirus-leptin to rats causes a resolution of the lipid accumulation and stimulates lipolysis.[30] This mechanism is related to stimulation of fatty acid oxidation. These observations are consistent with this experiment, where the obese leptin-deficient mice with a paucity of leptin had an increase in gallbladder lipids.

Dietary cholesterol and fat intake have been implicated in gallstone pathogenesis. Prairie dogs, when fed a high-cholesterol, high-fat diet, develop cholesterol gallstones. This phenomenon seems to be multifactorial by inducing supersaturation of cholesterol in the bile and decreasing biliary motility.[12,31] Epidemiological studies have shown that high-carbohydrate diets, particularly those diets high in refined sugars, also have a positive correlation with gallstone disease.[32,33] Because leptin and diabetes have been closely linked, the correlation of dietary sugar intake and gallstones may be a leptin-induced phenomenon.[34] Diets high in fiber have been shown to have a protective effect, which may be related to bile salt metabolism within the gut. In addition, saturated fats have a positive correlation with gallstone disease. N-3 polyunsaturated fatty acids, like those found in fish oils, have been shown to decrease gallstone formation in patients undergoing rapid weight loss.[35] This finding has been shown to be in part due to an increase in the cholesterol crystal nucleation time, and not due to a change in the cholesterol saturation index. The pro- or anti-nucleator responsible for this finding has not been established.

Another possible explanation for the increased gallbladder wall fats observed in obese mice and in lean mice fed a high-cholesterol, high-fat diet is that

biliary lipids were absorbed by the gallbladder. However, we have previously shown that the bile composition of lean and obese mice does not differ significantly when they are fed either a nonlithogenic or a high-cholesterol high-fat diet.[19] Thus, this possible explanation is unlikely. Alternately, differences in serum cholecystokinin or in gallbladder serum cholecystokinin receptors could explain the gallbladder motility differences that we have observed between strains and with different diets. Neither serum cholecystokinin nor serum cholecystokinin receptor density has been measured in our studies, so these explanations remain possible. Similarly, the accumulation of advanced glycation end products within the gallbladder wall could explain the decreased motility observed in the leptin-deficient hyperglycemic obese mice. However, when lean mice are fed a high-cholesterol, high-fat diet, they do not become hyperglycemic and would not be expected to accumulate advanced glycation end products. Interestingly, however, serum glucose levels were lower in the obese animals fed the high-fat diet, so gallbladder-advanced glycation end products may have improved and resulted in increased contractility.

In summary, these experiments demonstrate that leptin-deficient obese mice have markedly increased gallbladder lipids. When a high-cholesterol, high-fat diet is given to lean mice, they show an increase in gallbladder lipids and the cholesterol/phospholipid ratio. Paradoxically, the same high-cholesterol, high-fat diet leads to a decrease in gallbladder fatty acids, phospholipids, and cholesterol in obese mice. The pattern of gallbladder fat distribution observed in this study parallels gallbladder responses in a muscle bath with respect to both animal weight and diet.[13] Therefore, we conclude that leptin-deficient obesity and/or a high-cholesterol, high-fat diet causes nonalcoholic fatty gallbladder disease that is manifested by diminished gallbladder contractility.

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SURGISIS-Assisted Surgical Site Control in the Delayed Repair of a Complex Bile Duct Injury After Laparoscopic Cholecystectomy

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Although laparoscopic cholecystectomy has revolutionized the surgical approach to patients with gallbladder disease, it has also brought a marked increase in the incidence of complex and serious bile duct injuries. Many of these major injuries represent a major technical challenge for even the most seasoned hepatobiliary-trained surgeon. Herein, we present a case outlining the algorithmic treatment approach for delayed-presentation complex biliary injury and report on the novel use of small intestinal submucosal biomaterial for surgical site control in the staged repair of a complex biliary injury (Strasberg E_4) after laparoscopic cholecystectomy. (J GASTROINTEST SURG 2006;10:202–206)

KEY WORDS: Biliary injury, biologic prosthetic, bile duct repair, laparoscopic cholecystectomy

CASE

In early December 2002, a 74-year-old man presented to an outside hospital with signs and symptoms of gallstone pancreatitis. An abdominal ultrasound done at that time revealed cholelithiasis. The patient was taken to the operating room to undergo laparoscopic cholecystectomy. During the operation a complex biliary injury (class E_4) was created (Fig. 1). However, this adverse event was not recognized for 72 hours, after which laparotomy was performed and several Penrose drains as well as a T-tube were placed. The patient was transferred to our institution approximately 2 weeks later.

Upon presentation to our institution, the patient was noted to be jaundiced and to have frank bile draining from his abdominal drains. The patient was stabilized, resuscitated, and imaged. A CT scan of the abdomen and pelvis was obtained for treatment planning, demonstrating a moderate amount of intra-abdominal fluid in the perihepatic space, pericolic gutters, and the pelvic cul-de-sac.

The initial surgical management at our institution began with abdominal exploration for surgical site control, with intraoperative cholangiography to exactly define the anatomy of the injured biliary tree. We discovered a transected extrahepatic bile duct (at the hilar plate) that included the entire right posterior and anterior sector ducts and the medial wall of the left hepatic duct (a class E_4 injury based on the classification of bile duct injuries described by Strasberg et al.).[1] One arm of the T-tube was in the residual left hepatic duct, and the other was in the distal common hepatic duct. In addition, there was a clipped-off right hepatic artery (Fig. 1).

Given that more than 2 weeks had transpired since the biliary injury occurred, and that there was substantial inflammation in the porta hepatitis, it was our opinion that immediate reconstruction of the biliary tree was ill-advised. Inadequately drained, infected intra-abdominal fluid collections and bile required urgent operative intervention to obtain surgical site control of the injury and conversion to an externally controlled biliary fistula. Once we defined the extent of the biliary injury, we proceeded with selective cannulation of the right posterior and anterior sectors, as well as the left hepatic duct, with 8-French Foley catheters, and exteriorized them. The T-tube was left in the left system and the native distal common hepatic duct because the latter could not be dissected safely from the surrounding tissue due to inflammation. To control biliary leakage from the multiple-transected bile ducts, a C-shaped piece

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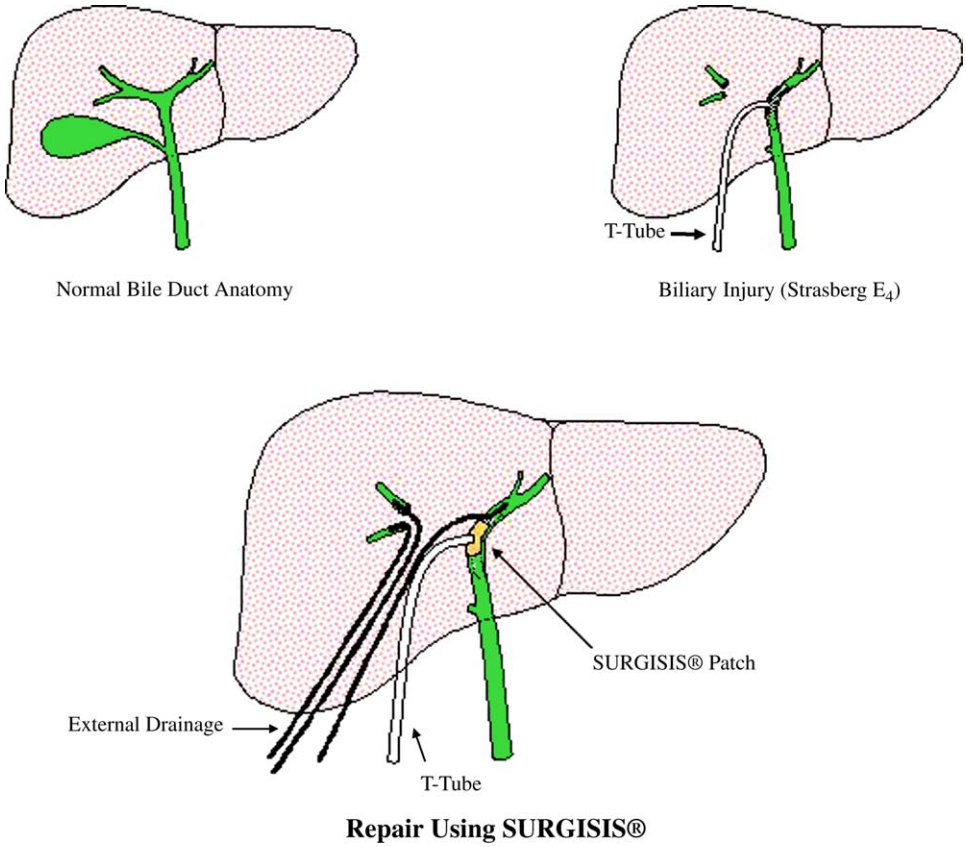

Fig. 1. Schematic drawings of the patient's biliary injury after laparoscopic cholecystectomy (Strasberg E4) and repair.

of 4-ply SURGISIS biomaterial was sewn to the medial wall from where the left system entered the hilar plate as a patch to form a new 360-degree tube over the T-tube drain (Figs. 1 and 2).

Approximately 1 month after the initial operation, the patient had two of the three operatively placed Foley catheters dislodged during routine hospital transfer. The well-formed tracts allowed for easy replacement under fluoroscopy with standard interventional radiology transhepatic drainage catheters. Two months after our initial operation, cholangiographic reassessment of the patient's biliary system demonstrated that the patient had developed competent biliary drainage of the left system into the duodenum, radiographically flowing through the SURGISIS channel (Fig. 3).

Six months after our initial operation, the patient was returned to the operating room to undergo definitive repair of his biliary injury by partial resection of segments IVB and V followed by a high hepaticojejunostomy (Hepp-Couinaud procedure). At the time of this definitive biliary reconstruction, it was noted that the SURGISIS material was completely incorporated as the lateral wall of the left biliary duct (Fig. 4). A year later, upon routine follow-up, the patient has normal liver function tests and has returned to his premorbid quality of life.

DISCUSSION

Bile duct injuries after laparoscopic cholecystectomy have been reported to have an incidence anywhere from 0.3–0.5%.[1–3] Although relatively infrequent, they are major complications of laparoscopic cholecystectomy because of the serious resulting morbidity and mortality. Operative repair of these injuries also represents a complex technical challenge for the majority of surgeons.

In a published algorithm by Strasberg et al.,[4] four separate components in the planned approach to biliary reconstruction are defined: (1) injury classification, (2) sepsis and surgical site control, (3) definition of the complete extent of the injury to the biliary tree, and (4) insertion of guide stents into each isolated branch of the biliary tree in preparation for future definitive reconstruction. In our case, we present the use of SURGISIS material as a further

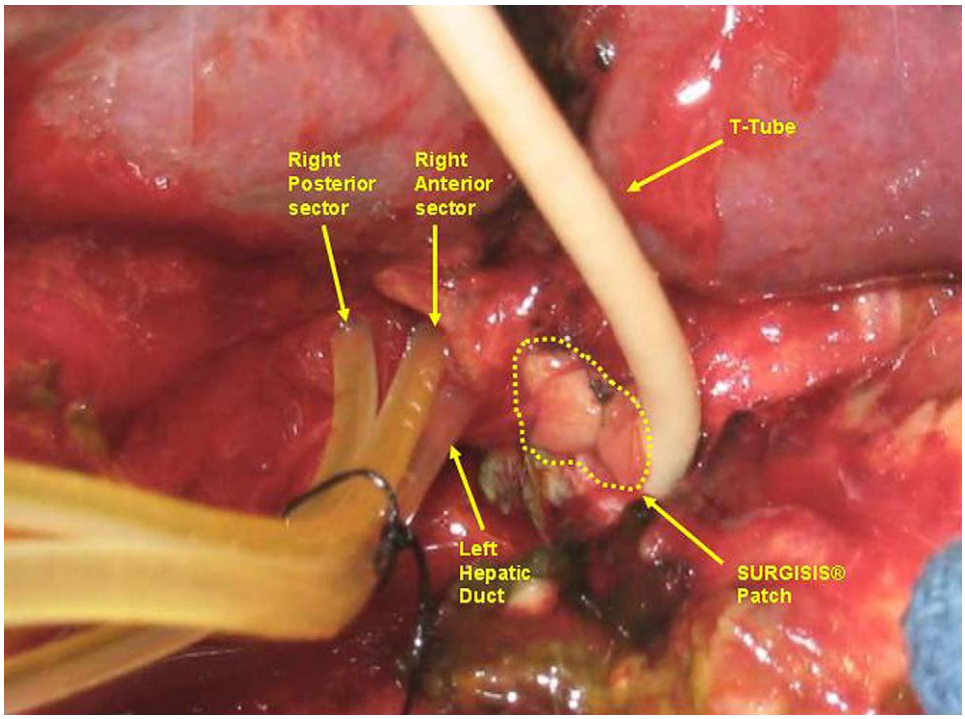

Fig. 2. Intraoperative photo at time of initial operation depicting controlled externalized biliary drainage and patching of the medial wall of the left hepatic duct with SURGISIS material (*labeled arrows*).

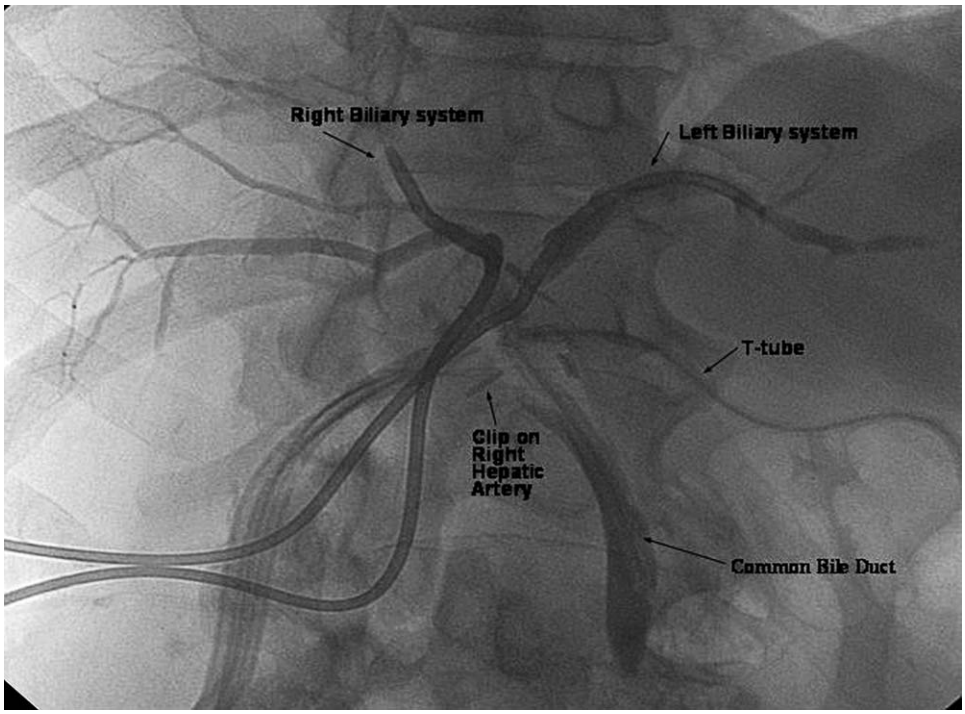

Fig. 3. Cholangiogram at 2 months after initial operative procedure demonstrating flow of contrast through the patched common hepatic duct and distal common bile duct.

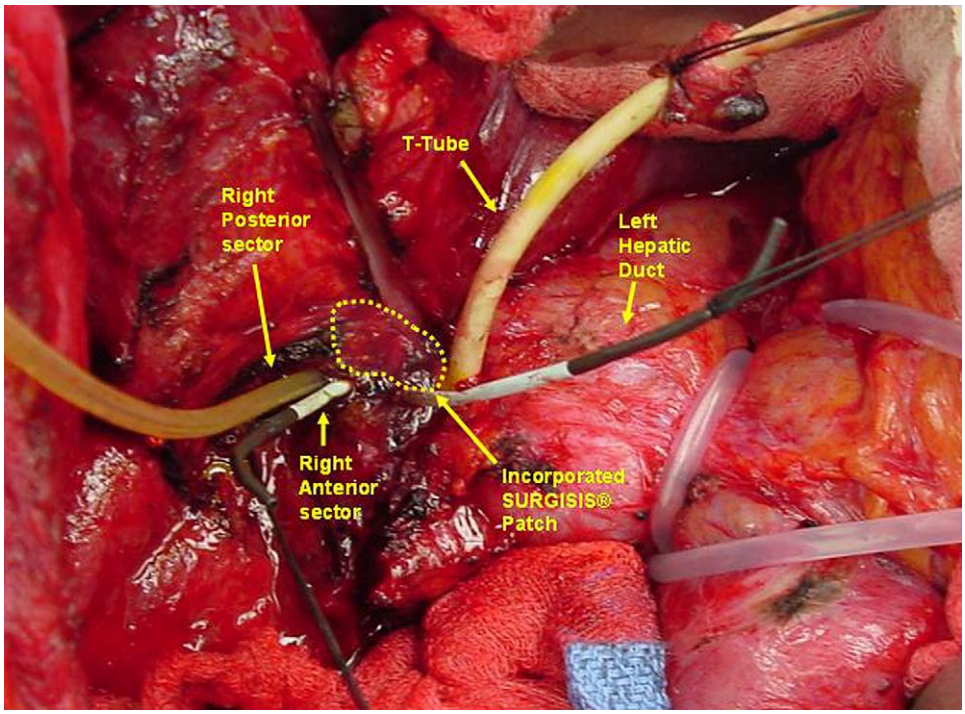

Fig. 4. Intraoperative photo at time of definitive operative repair depicting incorporation of the material as the lateral wall of the left biliary duct (*labeled arrows*).

means to attain surgical site control in preparation for a delayed definitive operative repair in keeping with the above outlined algorithm.

Intraoperative cholangiography was critical in accurately identifying the anatomy of the biliary tree. By evaluating the remaining anatomy, management could be decided based upon the classification of the injury. In this case, because the biliary injury extended high up into the hilar plate (Strasberg E_4), the definitive operative repair would eventually require a Hepp-Couinaud operation to reconstruct the biliary system. Furthermore, we achieved control of the biliary system by external drainage catheters placed into each isolated branch of the biliary tree, thus achieving biliary drainage and preparing for later definitive operative repair.

Whereas primary duct-to-intestine (mucosa-to-mucosa) repair can be performed promptly in specific cases, our patient's biliary anatomy after injury was not amenable to this. Moreover, the referral to our institution coincided with the period when there was a large amount of inflammation putting any definitive operative repair at higher risk for failure. Mercado's experience[2] supports primary biliary repair if it can be performed within 72 hours after initial injury, whereas immediate repair in a contaminated, inflamed field was associated with a high frequency of failure. From this data, it is recommended that definitive repair of biliary injuries recognized beyond 72 hours be performed 4–6 months after surgical site control.

The use of the SURGISIS material allowed us to provide control of the drainage from the left system and allowed for us to delay the dissection of the distal common bile duct, which would have been extremely difficult in the acute setting, potentially risking injury to the portal vein.

SURGISIS is a collagen-based, acellular, and nonimmunogenic material harvested from the submucosal layer of porcine intestine and has been found to have regenerative capabilities in various sites. Whereas our case represents the first clinical report of its use in the operative repair of a biliary injury, our decision to use material in this patient was supported by a recent report demonstrating the successful repair of experimentally induced defects in the bile duct in a canine model.[3] In this cited study, 15 dogs had implantation of SURGISIS material into the bile duct either as a patch or as an interposition graft. Of the 15 dogs, only one dog resulted in a large bile leak due to a technical suturing error. Moreover, in the one dog sacrificed at 5 months after implantation, the material was found to be replaced with native collagen covered with biliary epithelium. This experimental canine

study is in parallel with our clinical observation in this case that a noncircumferential defect in the biliary duct could be successfully patched with a SURGISIS interposition and that sufficient regeneration by the native bile duct occurred to provide a functional biliary channel.

CONCLUSION

The case presented highlights the algorithmic approach necessary in the treatment of delayed presentation biliary injuries. Additionally, it demonstrates the importance of controlled biliary drainage and delayed definitive repair to achieve an optimal result. Finally, this case represents the first report of the use of SURGISIS material as an adjunct in the treatment of a complex bile duct injury.

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A Structurally Optimized Celecoxib Derivative Inhibits Human Pancreatic Cancer Cell Growth

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Deregulation of the phosphatidylinositol 3-kinase (PI-3K)/PDK-l/Akt signaling cascade is associated with pancreatic cancer tumor invasion, angiogenesis, and tumor progression. As such, it has been postulated that PDK-1/Akt signaling inhibitors may hold promise as novel therapeutic agents for pancreatic cancer. Disadvantages of currently available Akt inhibitors include tumor resistance, poor specificity, potential toxicity, and poor bioavailability. Previous studies have demonstrated that OSU-03012, a celecoxib derivative, specifically inhibits PDK-1 mediated phosphorylation of Akt with IC_{50} values in the low μM range. Human pancreatic cancer cell lines AsPC-1, BxPC-3, Mia-PaCa 2, and PANC-1 were cultured in media containing varying concentrations of OSU-03012, 5-fluorouracil (5-FU), and gemcitabine, and changes in Akt phosphorylation and cell viability were evaluated using western blotting and a 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay, respectively. Treatment with OSU-03012 resulted in decreased PDK-1-mediated Akt phosphorylation and cell growth inhibition for all cell lines with IC_{50} values ranging between 1.0 and 2.5 μM. Resistance to 5-FU and gemcitabine was observed in cell lines AsPC-1 and BxPC-3. Further analyses indicate that OSU-03012 induces both proapoptotic and antiproliferative effects in these cells. Taken together, these data suggest that OSU-03012 has potential value as a novel therapy for pancreatic cancer. (J GASTROINTEST SURG 2006;10:207–214)

KEY WORDS: Akt signaling, PDK-1 specific inhibitor, celecoxib, pancreatic cancer

Pancreatic cancer is the fifth most common cancer-causing death in the United States[1] and prognosis is almost universally dismal with currently reported five-year survival rates of only 4%.[2] Surgical resection remains the only hope for cure, but most patients present with locally advanced or metastatic disease, factors that preclude attempts at resection. Chemotherapy and radiation offer only modest improvements in survival.[3,4] Novel therapeutic strategies are desperately needed, and this topic has been the subject of a recent progress review group sponsored by the National Institutes of Health.[5]

Recent advances in pancreatic cancer research have identified a number of tumorigenesis-associated genetic events including Akt activation.[6–8] As a key component in the PI3K/Akt signaling pathway, activated Akt is involved in the regulation of several important cellular events, including apoptosis, cellular proliferation, and the response to hypoxic stress (Fig. 1). Constitutive activation of Akt has been reported in 78% of pancreatic adenocarcinoma cell lines,[9] and mutations in PTEN, a negative regulator of Akt activation, have also been identified in pancreatic tumor specimens.[10–12] Moreover, PI3K/Akt-inhibiting agents have been shown to induce apoptosis in several pancreatic cancer cell lines exhibiting Akt activation.[13–17] Therefore, Akt activation appears to represent a viable target for therapeutic intervention in pancreatic cancer.

We have recently identified a series of Akt signaling inhibitors with high antiproliferative and proapoptotic activities in human prostate cancer cell lines and mouse xenografts.[18] One of these, OSU-03012, has been approved by National Institutes of Health–Rapid Access to Interventional Development (NCI RAID) for further bioavailability and pre-Investigational New Drug Application (IND) toxicology studies. As a structurally optimized celecoxib

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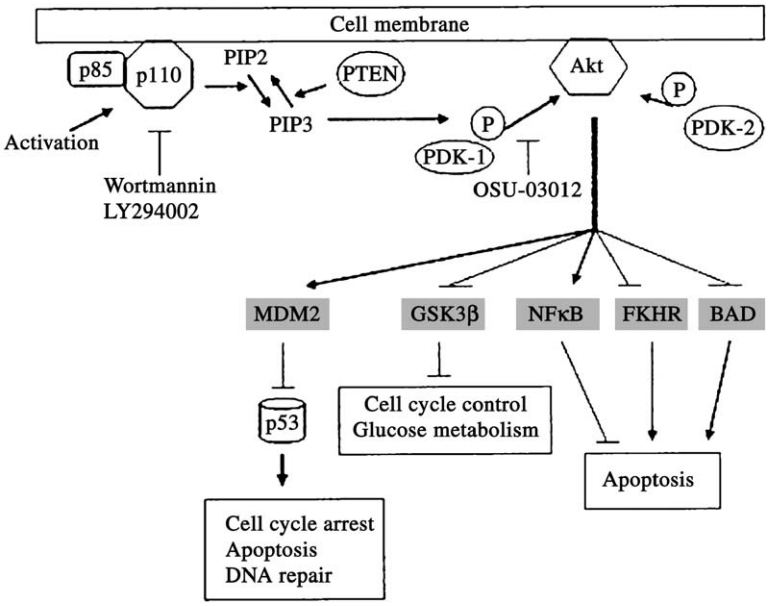

Fig. 1. The PI3K/Akt signaling pathway, modified from reference 7. *Arrows* represent positive effects, and stop bars indicate negative regulation. P-phosphorylation.

derivative[18–21] (Fig. 2), OSU-03012 specifically inhibits PDK-1 in PC-3 cells and blocks Akt and p70S6K activation, resulting in apoptotic cell death and cell growth inhibition at low μM concentrations. We hypothesize that treatment with OSU-03012 will result in enhanced apoptosis and an antiproliferative effect through modulation of Akt signaling in pancreatic cancer cells. The purpose of the present study was to evaluate the effects of OSU-03012 treatment on pancreatic cancer cell growth, Akt phosporylation, cellular proliferation, and apoptosis.

MATERIALS AND METHODS

Cell Lines

Human pancreatic cancer cell lines AsPC-1, BxPC-3, MiaPaCa-2, and Panc-1 were purchased from American Type Culture Collection (Manassas, VA). AsPC-1 and BxPC-3 cells were cultured in RPMI1640 (Invitrogen Corp., Carlsbad, CA) containing 10% fetal bovine serum (FBS; Invitrogen Corp., Carlsbad, CA) and 2 mM glutamine in a humidified 37° C incubator supplied by 5% CO_2. Dulbecco's modified Eagle medium (DMEM, Invitrogen Corp., Carlsbad, CA) was used for MiaPaCa-2 and PANC-1 cells.

Reagents and Antibodies

5-Fluorouracil, 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT), trypsin, and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO), and gemcitabine was from Eli Lilly and Company (Indianapolis, IN). M-PER mammalian

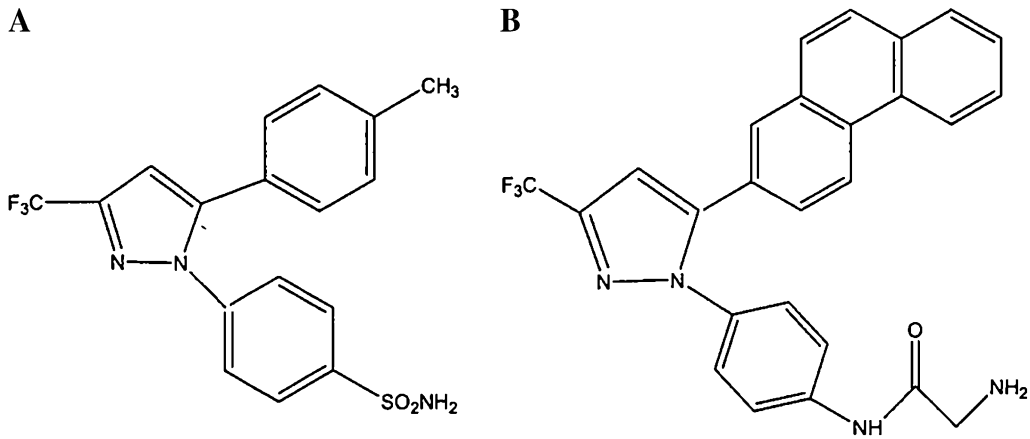

Fig. 2. The molecular structures of celecoxib (**A**) and OSU-03012 (**B**).

protein extraction reagent and BCA protein concentration determination kits were purchased from Pierce (Rockford, IL), and ECL western blotting detection reagents (RPN 2209) were purchased from Amersham Biosciences (Piscataway, NJ). Nonfat dry milk was purchased from Bio-Rad (Hercules, CA).

Rabbit antihuman Akt, rabbit antihuman phosphorylated Akt (Ser473), and rabbit antihuman poly (ADP-ribose) polymerase (PARP) antibodies were purchased from Cell Signaling Technology, Inc. (Bevery, MA). Immuno monoclonal mouse antihuman actin antibody Clone C4 was purchased from MP Biomedicals, Inc. (Livermore, CA). Peroxidase-conjugated AffiniPure rabbit antimouse IgG + IgM (H + L) and peroxidase-conjugated AffinitiPure goat antirabbit IgG (H + L) was from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA).

Cell Viability Assay

Five thousand cells per well were seeded into a 96-well plate in media containing 10% FBS. After incubation at 37° C, 5% CO_2 for 24 hours, cells were treated with 1% FBS media containing various concentrations of compounds and incubated at 37° C for another 48 hours.[18] Cell viability was evaluated using an MTT assay. Briefly, 200 uL of 10% FBS-supplemented medium containing MTT at a concentration of 75 μg/ml was added to each well, and cells were incubated in the presence of MTT for 2 hours. Mitochondrial dehydrogenate activity reduced the yellow MTT dye to a purple formazan, which was then solubilized with 200 uL of DMSO, and absorbance at 570 nm was read on an enzyme-linked immunosorbent assay (ELISA) plate reader; 0.1% of DMSO was used as a control. For AsPC-1 and BxPC-3, the medium was RPMI1640, while DMEM was used for MiaPaCa-2 and PANC-1. The value of IC_{50} was defined as the concentration at which cell viability decreased to 50%. The experiments were performed in six replicates.

Western Blotting

Pancreatic cancer cells (5×10^5) were cultured in a T-25 flask and incubated for 24 hours in the presence of 10% FBS. Cells were treated with media containing different concentrations of agents under investigation, or 0.1% DMSO (negative control) for 4–24 hours. Cells were collected by centrifugation at 6-hr intervals. Following PBS washing, the cells were resuspended in 200–400 μL of M-PER lysate buffer. After being gently vortexed at room temperature for 10 minutes, the cells were centrifuged at 14,000 rpm at room temperature for 10 minutes, and the supernatants were transferred into new Eppendorf tubes and stored at −20° C. After protein concentration determination using a BCA kit, the supernatants were mixed with 5 × SDS-PAGE loading buffer and boiled at 100° C for 5 minutes. Equal amounts (50 μg) of the supernatants were loaded onto 10% SDS-PAGE gels and separated by electrophoresis.

Proteins were then transferred to nitrocellulose membranes using a BioRad Mini-II system. The transblotted nitrocellulose membranes were washed three times with TBS buffer (15 mM Tris-Borate-0.15 M Sodium Chloride, pH 7.5) containing 0.05% Tween 20 (TBST), and then blocked with TBST containing 5% nonfat dry milk at room temperature for 2 hours. After incubation with the primary antibody at a 1:1000 dilution in TBST-5% nonfat dry milk at 4° C overnight, the membranes were washed three times with TBST and probed with peroxidase-conjugated secondary antibody at a 1:10,000 dilution in TBST-5% nonfat dry milk for 1.5 hours at room temperature. Immunoblots were visualized by enhanced chemiluminescence using an ECL kit after washing with TBST. For all western blot assays, actin was used as an internal control.

Cell Growth Inhibition Analysis

Pancreatic cancer cells were seeded into six-well plates at 50,000-75,000 cells per well in 10% FBS-containing RPMI1640 or DMEM media.[18] After incubation at 37° C in 5% CO_2 for 24 hours, cells were treated in duplicate with 1 μM or 5 μM of OSU-03012 in 10% FBS-containing media, and the control groups were treated with 0.1% DMSO.

Cells were harvested by trypsin digestion at various time intervals and numerated using a Coulter counter (model Zl D/T) (Beckman Coulter, Fullerton, CA). The relative proliferation index was defined as the ratio of OSU-03012-treated cells to DMSO-treated cells for any given time point.

RESULTS

Expression and Phosphorylation of Akt in Pancreatic Cancer Cell Lines

Because OSU-03012 acts as a specific PDK-1 inhibitor, we first investigated the expression and phosphorylation of Akt in these cell lines by Western blotting. While high concentrations of Akt were detected in the cell lysates of AsPC-1, MiaPaCa-2, and PANC-1, only basal levels were detected in the cell lysate of BxPC-3 (Fig. 3). Furthermore, increased concentrations of phosphorylated Akt (Ser473) were detected only in the cell lysate of AsPC-1, and phosphorylation of Akt in MiaPaCa-2 and PANC-1

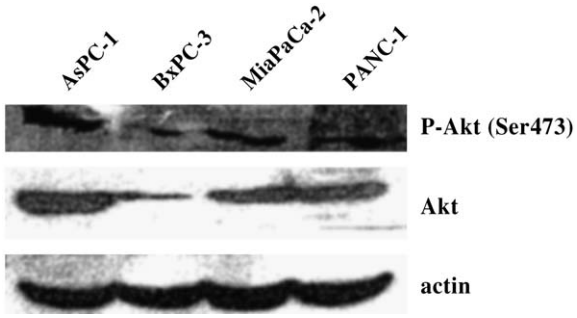

Fig. 3. Phosphorylation status of Akt in pancreatic cancer cell lines.

approximated that of BxPC-3. In regards to the expression and phosphorylation of Akt, these four cell lines can be divided into three groups: (1) AsPC-1 with high expression and phosphorylation of Akt, (2) MiaPaCa-2 and PANC-1 with high expression but low phosphorylation Akt, and (3) BxPC3 with low expression and phosphorylation of Akt.

OSU-03012 Inhibits the Growth of All Tested Pancreatic Cancer Cell Lines

Treatment with OSU-03012 resulted in a dose-dependent inhibition of cell viability for all pancreatic cancer cell lines tested (Fig. 4, *A*). In the presence of 5 μM of OSU-03012, only trace cell viability was observed. Although the four cell lines varied widely in regards to expression and phosphorylation of Akt (Fig. 3), effects on cell viability were comparable (Table 1). While the IC_{50} values of AsPC-1, BxPC-3, and MiaPaCa-2 are nearly identical (1.5 μM, 1.8 μM, and 1.3 μM, respectively), there was a moderate increase in the IC_{50} value of PANC-1 (2.3 μM). OSU-03012 appears to be an order of magnitude more potent than celecoxib (IC_{50} 20–50 μM).[22]

As controls, two drugs commonly used for human pancreatic cancer therapy, 5-fluorouracil (5-FU) and gemcitabine, were also evaluated. Results varied among the four cell lines (Fig. 4, *B*, *C*). While MiaPaCa-2 and PANC-1 are sensitive to both 5-FU and gemcitabine, AsPC-1 is resistant to both 5-FU and gemcitabine. BxPC-3 is sensitive to 5-FU but resistant to gemcitabine. OSU-03012 demonstrated more potent inhibition of cell proliferation than either 5-FU or gemcitabine for all cell lines tested (Table 1).

OSU-03012 Inhibits Akt Phosphorylation in All Tested Pancreatic Cancer Cell Lines

To explore the molecular mechanisms underlying OSU-03012-induced growth inhibition in these pancreatic cancer cell lines, we analyzed the status of Akt phosphorylation after treatment with varying concentrations of OSU-03012. Treatment with OSU-03012 resulted in a dose-dependent inhibition of Akt phosphorylation in all cell lines tested (Fig. 5). These findings indicate that OSU-03012 specifically and efficiently inhibits Akt phosphorylation in pancreatic cancer cells regardless of basal Akt phosphorylation status.[18]

OSU-03012 Induces Antiproliferative and Proapoptotic Effects in All Tested Pancreatic Cancer Cell Lines

To investigate the biological consequences resulting from OSU-03012-mediated inhibition of Akt phosphorylation, we examined the effects of OSU-03012 treatment on pancreatic cancer cell proliferation (Fig. 6). In general, OSU-03012 treatment resulted in decreased cell proliferation in a time and dose-dependent manner. The antiproliferative effect of OSU-03012 treatment was most pronounced in the MiaPaCa-2 cell line. Of note, the effects of OSU-03012 on cell growth are strikingly different in the presence of varying concentrations of FBS. As demonstrated in the cell viability assay (Fig. 4, *A*), the cell growth of all four cell lines was completely inhibited after incubation with media containing 1% FBS and 5 μM of OSU-03012 for 48 hours (2 days), while 40–60% of proliferation activity was retained after incubation with media containing 10% FBS and 5 uM of OSU-03012 for 3 days. This apparent inconsistency was also observed in previous studies using PC-3 cells and may be attributed to serum-induced growth factor/receptor tyrosine kinase-mediated activation of PI3K/Akt signaling or altered cellular uptake of OSU-03012.

Finally, OSU-03012-mediated changes in apoptosis were evaluated using a poly (ADP-ribose) polymerase (PARP) cleavage assay[18] (Fig. 7). Treatment of all pancreatic cancer cell lines with OSU-03012 resulted in a dose-dependent induction of apoptosis as measured by PARP cleavage. The proapoptotic effect of OSU-03012 did vary among cell lines and was most pronounced in the AsPC-1 and BxPC-3 cell lines. The findings indicate that OSU-03012 treatment results in both antiproliferative and proapoptotic effects in all tested pancreatic cancer cells.

DISCUSSION

The K-*ras* oncogene and multiple receptor tyrosine kinases affect intracellular signaling in malignant cells through the activation of downstream oncogenic pathways such as PI3K/Akt, MEK/Erk, and IκB/NF-κB. Downstream components result in

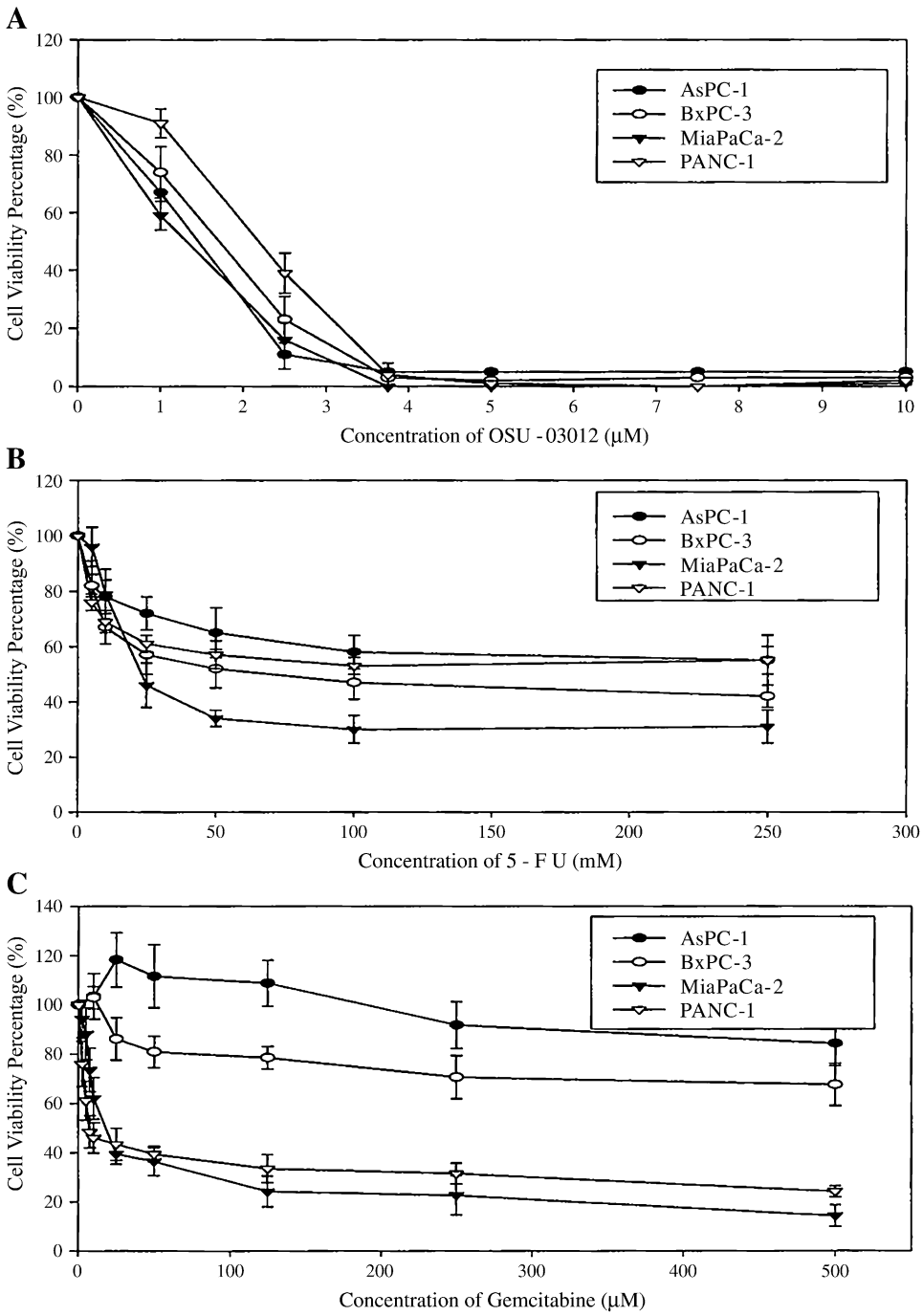

Fig. 4. Cell viability of pancreatic cancer cells in the presence of (**A**) OSU-03012, (**B**) 5-FU, and (**C**) gemcitabine.

uncontrolled cell proliferation, angiogenesis, and inhibition of apoptosis.[23] After binding of the regulatory p85 subunit of PI3K to an activated receptor tyrosine kinase, or through binding of the p110 catalytic subunit of PI3K to activated *ras*, PI3K is activated and subsequently leads to the translocation of Akt to the plasma membrane.[7,8,24] The p110 subunit of PI3K catalyzes the conversion of PIP_2 to PIP_3. PIP_3 promotes phosphorylation of Akt by PDK1 and PDK2, at Thr308 and Ser473, respectively, resulting in full activation. Activated Akt (protein kinase B, PKB) is involved in several important cellular events including apoptosis, cellular proliferation, and the response to hypoxic stress.

Table 1. IC_{50} values of tested agents

Cell line	OSU-03012 (μM)	5-FU (μM)	Gemcitabine (μM)
AsPC-1	1.5 ± 0.4	>250*	>250*
BxPC-3	1.8 ± 0.3	71.6 ± 14.2	>250*
MiaPaCa-2	1.3 ± 0.4	23.4 ± 5.3	14.5 ± 2.5
PANC-1	2.3 ± 0.8	100.0 ± 22.7	7.2 ± 3.1

*IC_{50} was out of the tested concentration range of the agent. This indicates minimal or no growth inhibition in this cell line.

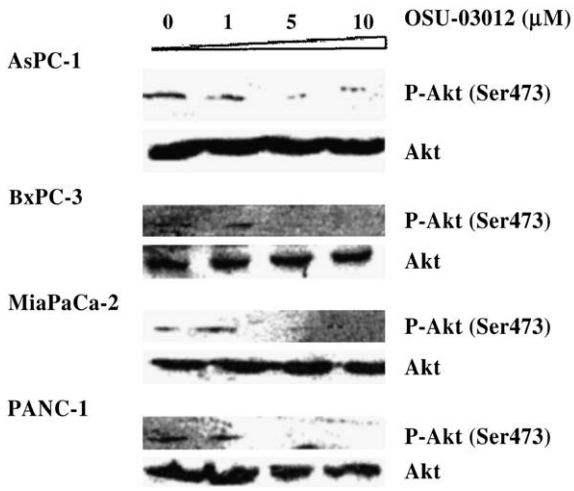

Fig. 5. OSU-03012 inhibits Akt phosphorylation in pancreatic cancer cell lines.

Antiapoptotic activity is partially mediated through Bad, IκB kinase, forkhead transcription factor, and caspase-9. Downstream regulators of cell cycle progression mediated by PI3K/Akt include p21, GSK3β, and p27. Phosphorylation of GSK3β inhibits its kinase activity and allows cyclin Dl to accumulate.[24] In addition, activated Akt enhances expression of hypoxia-inducible factor-1, resulting in increased cellular hypoxia tolerance.[25,26]

A number of recent investigations support the evalution of PI3K/Akt pathway inhibitors as potential targets for therapeutic intervention in pancreatic cancer.[7,8] The 5-lipoxygenase metabolite, 5(S)-hydroxyeicosatetraenoic acid (5(S)-HETE), is known to significantly stimulate the proliferation of pancreatic cancer cells lines and markedly increases Akt phosphorylation.[13] Treatment of these cells with the PI3K inhibitor, wortmannin, results in the blockade of 5(S)-HETE-induced Akt phosphorylation and DNA synthesis. Inhibition of the antiapoptotic regulator, NF-κB, has been reported to result in potentiation of gemcitabine-induced apoptosis in a group of gemcitabine-resistant pancreatic cancer cell lines.[14] In this study, inhibition of the PI3K/Akt pathway by LY294002 did not result in an attenuation of NF-κB mediated apoptosis. Furthermore, basal Akt activity did not correspond to gemcitabine resistance. These findings suggest that gemcitabine-induced apoptosis may not be modulated by the PI3K/Akt pathway in certain pancreatic cancers. Others have reported, however, that PI3K/Akt pathway inhibitors may induce apoptosis in a dose-dependent manner in gemcitabine-resistant pancreatic cancer cell lines.[15–17] Possible downstream mediators of apoptosis following PI3K/Akt inhibition in gemcitabine-treated pancreatic cancer cells lines include NF-κB, Bcl-2, and Bax. PI3K/Akt inhibition augments the proapoptotic and antiproliferative effects of TNF-α induced NF-κB inhibition in several pancreatic cancer cell lines. This suggests that the PI3K/Akt pathway may, in part, modulate NF-κB mediated chemoresistance in pancreatic cancer. Finally, treatment of pancreatic cancer cells with LY294002 and the cyclooxygenase inhibitor, sulindac, resulted in enhanced growth inhibition, alteration of cell cycle distribution, and decreased apoptotic threshold as compared to treatment with sulindac alone.[27]

Despite these potentially encouraging findings, early generation PI3K/Akt inhibitors exhibit a number of clinically significant disadvantages, including poor specificity, potential toxicity, and poor bioavailability.[7,8] Wortmannin is known to have unfavorable pharmacokinetic properties. Both wortmannin and LY294002 are likely to have broad inhibitory activities and may have nonspecific effects on other regulatory cellular molecules. For example, there are different isoforms of p110, the PI3K catalytic subunit. Wortmannin and LY294002 exhibit inhibitory activities against all of these isoforms, as well as distant PI3K-like kinases such as ATM and ATR. Finally, delivery issues have precluded the study of PI3K/Akt inhibitors in available animal models of pancreatic cancer.

In this study, all tested pancreatic cancer cell lines demonstrated sensitivity to OSU-03012. The IC_{50} values (1.5–2.5 μM) are an order of magnitude lower than those previously reported values for other PI3K/Akt inhibitors.[7,8,27,28] The high potency of OSU-03012 appears to be mediated through both antiproliferative and proapoptotic mechanisms.[18] It has been previously reported that inhibition of PDK-1/Akt signaling represents the underlying antitumor mechanism for OSU-03012 in PC-3 cells.[18] Considering the conserved role of PDK-1/Akt signaling in cell proliferation and survival, we believe that a similar molecular mechanism underlies the high antitumor activity of OSU-03012 in different pancreatic cancer cell lines. Our data are further

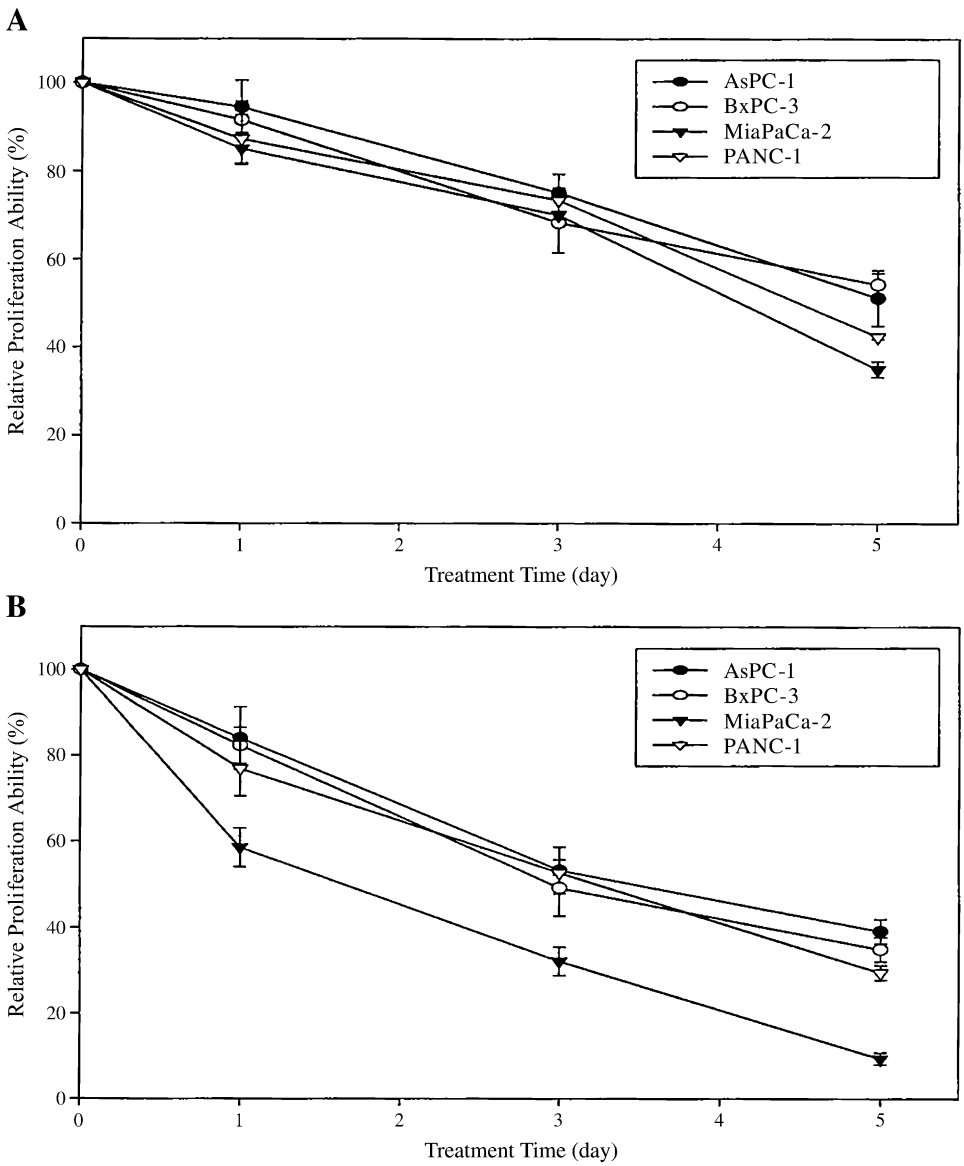

Fig. 6. The effect of OSU-03012 treatment on pancreatic cancer cell line proliferation. (**A**) 1 μM OSU-03012; (**B**) 5 μM OSU-03012.

supported by antitumor screening studies of OSU-03012 in the Developmental Therapeutic Program at the National Cancer Institute.[18] OSU-03012 demonstrated potent growth inhibition in 60 cell lines from human lung, colon, brain, ovary, breast, prostate, kidney, leukemia, and melanoma cancers with a mean IC_{50} value of 1.2 μM. While it is still unclear whether antiproliferative or proapoptotic effects are predominant in specific cancer cell lines, the bifunctionality of OSU-03012 appears to counteract cell resistance.

The data presented here indicate that OSU-03012 uniformly inhibits pancreatic cancer cell growth and suggest that this effect is mediated by inhibition of

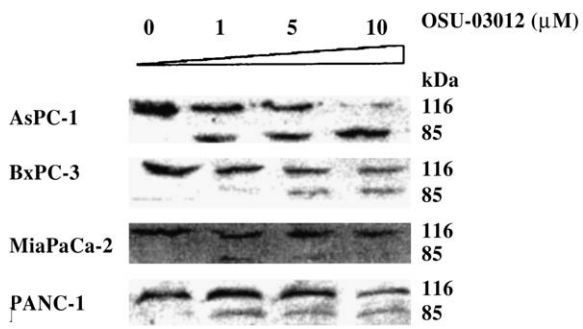

Fig. 7. The effect of OSU-03012 treatment on apoptosis.

Akt phosphorylation. The widely varying effects of 5-FU and gemcitabine on pancreatic cancer cell growth undoubtedly are a result of heterogeneity among various cell lines with regards to their mechanisms of action.[27,28] This may explain why these agents have demonstrated only limited clinical activity for the treatment of pancreatic cancer. These results support further investigation of this novel therapeutic agent for pancreatic cancer therapy in preclinical and clinical studies.

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Method of Pyloric Reconstruction and Impact Upon Delayed Gastric Emptying and Hospital Stay After Pylorus-Preserving Pancreaticoduodenectomy

Craig P. Fischer, M.D., M.P.H., Johnny C. Hong, M.D.

Preservation of the pylorus at the time of pancreaticoduodenectomy has been associated with equal oncological outcomes when compared to the classical Whipple operation. Multiple studies have demonstrated that pylorus-preserving pancreaticoduodenectomy (PPPD) has equal or superior outcomes regarding quality of life when compared with the traditional Whipple operation, but many studies have suggested a higher incidence of delayed gastric emptying (DGE). DGE prolongs hospital stay, and its association with PPPD has hampered its adoption by many pancreatic surgery centers. We describe a novel surgical technique for the prevention of delayed gastric emptying following pylorus-preserving pancreaticoduodenectomy. The technique of pyloric dilatation appears to decrease the incidence of delayed gastric emptying and facilitates earlier hospital discharge, when compared with standard pylorus preserving pancreaticoduodenectomy. (J GASTROINTEST SURG 2006;10:215–219)

KEY WORDS: Pylorus-preserving pancreaticoduodenectomy, outcomes, pancreaticoduodenectomy, pyloric dilatation, delayed gastric emptying

Pylorus-preserving pancreaticoduodenectomy (PPPD) has been adopted by many surgeons as a standard operation for periampullary disease. However, some controversy still exists regarding the incidence of delayed gastric emptying (DGE) following PPPD. Supporters of the classical Whipple operation, which requires antrectomy, point to the higher incidence of DGE following PPPD when compared to the classical Whipple.[1,2] Multiple reports have demonstrated a high incidence of DGE following preservation of the pylorus, ranging from 15%–45%[3–5] when compared with 6.4% following the classical Whipple operation.[2]

A number of randomized trials comparing PPPD to the classical Whipple operation have demonstrated shorter operative time for PPPD, decreased blood loss, and equivalent survival[6–8] when compared to the classical Whipple operation. However, the concern regarding an increased incidence of delayed gastric emptying following PPPD has prevented its adoption by some major American pancreatic surgery centers.[2]

The mechanism of delayed gastric emptying following PPPD has been postulated to relate to pylorospasm and gastrointestinal hormonal abnormalities, potentially related to devascularization of the pylorus when the right gastric artery is sacrificed.[9–11] We describe the use of a novel surgical technique of pyloric dilation performed at the time of pylorus-preserving pancreaticoduodenectomy and report its effects upon delayed gastric emptying when compared with a prospective cohort of patients undergoing standard pylorus-preserving pancreaticoduodenectomy.

MATERIAL AND METHODS

Patients

The study was submitted to and approved by the Houston Institutional Review Board of the University of Texas Health Science Center. Forty-six consecutive patients underwent traditional pylorus-preserving pancreaticoduodenectomy for periampullary disease, followed by a consecutive group of 46 patients who

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underwent mechanical dilation of the pylorus after duodenal transection and pylorus-preserving pancreaticoduodenectomy. In both groups, the right gastric artery was divided, the duodenum divided with a linear stapler 1–4.0 cm below the pylorus, and a duodenojejunostomy was performed via a hand-sewn single-layer technique. The duodenojejunostomy was formed in the retrocolic position, and the antrum was sutured to the transverse mesocolon with interrupted sutures. Pyloric dilation was performed after transection of the duodenum with sequential tapered metal sizers, beginning with 26 mm and dilating the pylorus to 29 mm. The pancreatic anastomosis was formed with a two-layered, duct to mucosa anastomosis and a second layer of interrupted sutures. All patients had a pediatric feeding tube placed as a transanastomotic stent. The biliary anastomosis was formed with interrupted absorbable sutures. Feeding jejunostomy or gastrostomy tubes were used in the early study period. Soft flexible closed suction drains were used routinely. Surgical drains were removed on postoperative day 7, or when the drain amylase was less than four times the serum amylase of the patient. Nasogastric tubes were removed when the daily drainage was less than 250 ml and the patient demonstrated return of bowel function.

Statistical Analysis

All patients had perioperative data prospectively entered into a computerized database. Delayed gastric emptying was defined as the requirement for nasogastric suction at postoperative day 10. Statistical analysis was performed using Instat statistical software (GraphPad Software, Inc., San Diego, CA). The chi-square test was used to compare categorical variables. Independent *t* tests and Mann-Whitney *U* tests were used to evaluate continuous variables. Univariate analysis was performed using the Fisher exact test. Covariates included age, sex, operative blood loss, tumor size, R resection status, and lymph node involvement; comorbid factors included diabetes, coronary disease, peripheral vascular disease, chronic obstructive pulmonary disease, and type of pyloric reconstruction (standard and pyloric dilatation); postoperative complications, including pancreatic leak, biliary leak, pneumonia, bleeding, reoperation and intra-abdominal abscess. Factors with a level of significance of $P < 0.05$ were considered to be statistically significant. The adjusted relative risk and the 95% confidence interval for each variable in the univariate model were derived.

Preoperative Evaluation

All patients considered for pancreaticoduodenectomy (PD) fulfilled criteria of resectability, namely (1) the absence of extrapancreatic metastatic disease, (2) the absence of tumor extension to the superior mesenteric artery or celiac axis, and (3) patency of the superior mesenteric vein, portal vein confluence with a suitable segment of superior mesenteric vein and portal vein to allow venous resection and reconstruction if necessary.

Operative Details

Surgical time was recorded from the anesthesia record and defined as the time from incision to the application of the final wound dressing. Intraoperative blood loss and intraoperative transfusions of red blood cells were derived from the anesthesia record.

Perioperative Complications

Major perioperative complications were defined as follows: perioperative mortality as death within the first 30 days after surgery or during the hospital admission for the operation if the stay is longer than 30 days; need for reoperation; pancreaticojejunal anastomosis leak is defined as the presence of >50 ml drain of amylase-rich fluid (>4 times the upper limit of normal serum amylase) on postoperative day 7; intra-abdominal hemorrhage; intra-abdominal fluid collection (sterile or abscess); myocardial infarction or sudden cardiac death; pulmonary complications including pneumonia; gastrointestinal bleeding; and sepsis syndrome. Prolonged intensive care unit stay greater than 7 days was defined as a complication. Length of stay was calculated by considering the day of surgery as day 1.

RESULTS

Between January 2001 and October 2003, 92 patients underwent PD for periampullary disease. Forty-six consecutive operations were performed with a standard technique of pylorus-preserving pancreaticoduodenectomy (PPPD), followed by 46 consecutive patients who underwent pylorus-preserving pancreaticoduodenectomy and pyloric dilation at the time of surgery (PPPD + PD). Among the 92 patients, 52 (56.5%) underwent PD for adenocarcinoma of the pancreas, 5 (5.4%) for cholangiocarcinoma, 4 (4.3%) for ampullary adenocarcinoma, 2 (2.2%) for duodenal adenocarcinoma, 1 (0.7%) for neuroendocrine malignancy, and 29 (31.5%) for chronic pancreatitis (Table 1). The median age, sex distribution, operative blood loss, tumor size, percent of positive lymph nodes, resection status (R_o , R_1, and R_2), and requirement for vascular resection were similar

Table 1. Patient demographics and histopathology

Variable	PPPD	PPPD+ pyloric dilatation
Gender		
Male	32	29
Female	14	17
Age (median)	65.4	64.1
Histology		
Pancreas	28	24
Bile duct	3	2
Duodenum	1	1
Ampullary	1	3
Neuroendocrine	1	0
Chronic pancreatitis	13	16
Totals	46	46

PPPD = pylorus-preserving pancreaticoduodenectomy.

between patients undergoing PPPD and PPPD + PD (Table 2). Delayed gastric emptying occurred in 12 of 46 patients who underwent PPPD (26%) and in 3 of 46 patients who underwent PPPD + PD (6.5%). The difference was statistically significant ($P < 0.05$). Average length of stay was 16.4 days in the PPPD group, 10.3 days in the PPPD + PD group, a statistically significant difference ($P < 0.001$).

A univariate analysis of 18 perioperative parameters showed that the occurrence of pancreatic fistula, intra-abdominal abscess, operative blood loss >1000 ml, and type of pyloric reconstruction (PPPD or PPPD + PD) were associated with delayed gastric emptying (Table 3).

Table 2. Perioperative outcomes

Variable	PPPD	PPPD+ pyloric dilatation	*P* value
Gender			
Male	32	29	NS
Female	14	17	NS
Age (median)	65.4	64.1	NS
Operative blood loss (ml)			
Median	1109	1052	NS
Range	3	2	
Tumor size (cm)			
Median	4.3 cm	4.9 cm	
Range	0.5–7.7 cm	1.2–5.6 cm	
R_1 resection n (%)	3/46 (6.5%)	2/46 (4.3%)	NS
Positive lymph nodes n (%)	26/33 (78.8%)	24/30 (80.0%)	NS
Vascular resection n (%)	6/46	8/46	NS
Length of stay (days)	16.4	10.3	$P < .05$
Delayed gastric emptying (DGE)	12/46 (26%)	3/46 (6.5%)	$P < .001$
Total	46	46	

PPPD = pylorus-preserving pancreaticoduodenectomy.

Table 3. Univariate analysis

Variable	No.	DGE	*P* value
Postoperative complication			
Yes	42	8	NS
No	50	7	
Pancreatic fistula			
Yes	4	4	$P < .05$
No	88	11	
Intra-abdominal abscess			$P < .0001$
Yes	8	6	
No	84	9	
Operative blood loss			
>1000 ml	42	12	$P < .05$
<1000 ml	50	3	
Type of pyloric reconstruction			
PPPD	46	12	
PPPD+ PD	46	3	$P < .05$

DGE = delayed gastric emptying; PD = pancreaticoduodenectomy; PPPD = pylorus-preserving pancreaticoduodenectomy.

DISCUSSION

The association of delayed gastric emptying (DGE) and pylorus-preserving pancreaticoduodenectomy (PPPD) has hampered its adoption by some centers.[3] DGE prolongs hospital stay, when compared with the classical Whipple operation.[2] Other studies have shown no difference in DGE,[12] and considerable debate about the incidence and prevention of this complication has continued.[11,13,14] We describe the use of a novel surgical technique that decreased the incidence of DGE and facilitated earlier discharge, when compared with standard PPPD.

The mechanism of DGE following PPPD is unknown, but a number of theories have been postulated. The first group of theories advance a mechanism related to the reconstruction technique of the duodenojejunostomy (retrocolic versus antecolic position). Some authors have demonstrated improved results with an antecolic position of the duodenojejunostomy,[15,16] while others have argued that retrocolic reconstruction prevents angulation of the duodenojejunostomy and congestion of the biliopancreatic limb, and improves gastric emptying.[17,18] The second theory focuses upon the gastrointestinal hormone motilin, with evidence that intravenous erythromycin administration increases gastric motility and improves DGE after PPPD.[19,20] Subsequent studies, however, have not shown that motilin plays an important role in gastric motility.[10] Further studies have implicated sacrifice of the right gastric artery (and duodenal

ischemia) in the development of DGE, and these authors have suggested that sparing the right gastric artery may decrease DGE.[11] It appears that there is no consensus about the pathophysiology of DGE following PPPD, only general consensus that the complication exists.

Our study suggests that mechanical dilation of the pylorus improves gastric emptying following PPPD. A number of possible mechanisms may explain this, but pyloric spasm or dysregulation seems plausible—dilation of the pylorus to 29 mm likely injures the pyloric muscle and prevents regulation and closure of the pylorus in the early postoperative period. Although our study did not investigate this, at least one provocative study implicates pylorospasm in the pathogenesis of DGE following PPPD.[10] We did not analyze the incidence of postgastrectomy syndromes in this study population, and this requires further analysis.

Recently a number of reports have shown a strong association between intra-abdominal complications, including pancreatic leak, and delayed gastric emptying following pancreaticoduodenectomy.[16,17,21,22] Clearly, a number of factors contribute to the development of DGE following PPPD, and postoperative complications certainly contribute. Our univariate analysis, however, demonstrated that the method of pyloric reconstruction (standard PPPD or PPPD + PD) had an independent effect on the development of DGE following PPPD. Pyloric dilation appears to improve gastric emptying following PPPD in our nonrandomized cohort and reduced the incidence of this complication to a rate similar to that for studies that utilized antrectomy (and reported the incidence of DGE).[2,23] If the results of this study are confirmed by other investigators, the use of antrectomy during the Whipple operation may therefore become unnecessary, except when oncological concerns require antrectomy.

CONCLUSION

Mechanical pyloric dilation reduces the incidence of delayed gastric emptying following pylorus-preserving pancreaticoduodenectomy and facilitates earlier discharge from hospital, when compared with standard pancreaticoduodenectomy.

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Combined Hepatic and Inferior Vena Cava Resection for Colorectal Metastases

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Surgical resection continues to offer the only hope for cure of colorectal cancer metastatic to the liver. Tumor involvement of the vena cava is often viewed as a contraindication to surgical resection. Whereas proven technically feasible, the survival advantages of en bloc liver and vena cava resection remain unclear. We reviewed all patients at a tertiary care center who had resection of colorectal liver metastases, including those with vena cava resections. Eleven patients had en bloc liver and vena cava resection between 1988 and 2002; during the same time period, 97 patients underwent isolated liver resection. There were no perioperative deaths in the 11 patients. All resections had negative histological margins. Mean follow-up was 33 months from the date of surgery. Median disease-free survival of the group having caval resections was 9 months, whereas median survival was 34 months. When compared to the cohort of isolated hepatic resections, the group undergoing caval resections experienced a significantly reduced disease-free survival of 18.6 vs. 9.1 months, respectively ($P = 0.03$); however, there was no difference in overall survival between the two groups at 55.2 vs. 34.3 months, respectively ($P = 0.20$). Colorectal liver metastases involving the vena cava should be considered for surgical resection. (J GASTROINTEST SURG 2006;10:220–226)

KEY WORDS: Hepatic colorectal metastasis, IVC resection

Colorectal cancer is the third most common malignancy affecting North American men and women. In 2004, there were over 145,000 new cases and over 56,000 deaths due to colorectal cancer in the United States.[1] Lymph nodes aside, the liver is often the first site for metastatic deposits from colorectal tumors. Improvements in anesthesia and perioperative care initially enabled resection of colorectal liver metastases. Advances in surgical technique and increasing segmental anatomic knowledge have facilitated more radical resections. Hepatic resection in specialized centers has an associated perioperative mortality of less than 5%.[2–5] Surgical resection of colorectal metastases offers the only hope for cure, with reported 5 year survival rates around 40%.[2–7] This contrasts sharply with chemotherapy. The best available medical treatment of metastatic disease offers virtually no chance for cure and a median survival now approaching 20 months.[8,9]

Metastases involving the inferior vena cava have traditionally been considered a contraindication to surgical resection (Fig. 1). Patients are usually offered medical management or a combination of medical and ablative therapy. Radiofrequency ablation has been reported for tumors deemed unresectable due to their location adjacent to major vascular structures.[10] Recently, the technical feasibility of resecting metastases involving the inferior vena cava (IVC) has been established.[11–14] However, the oncologic benefit of performing this en bloc resection remains uncertain. This article describes the experience of a single institution with en bloc IVC resections for colorectal tumors metastatic to the liver.

METHODS

We reviewed all patients at a tertiary care center who underwent resection of adenocarcinoma colorectal liver metastases by one of two surgeons (N.K and D.B.). From this cohort, all resections that involved the inferior vena cava (IVC) were identified. These patients were compared with a cohort of patients that underwent isolated hepatic resection for colorectal metastases. Between January 1988 and

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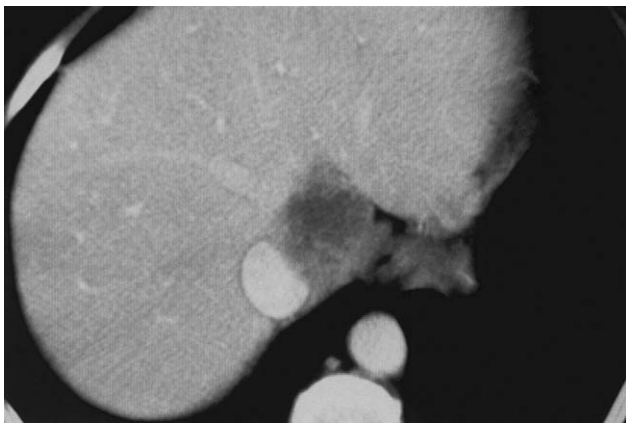

Fig. 1. CT scan demonstrating colorectal metastasis adjacent to inferior vena cava.

December 2002, 108 patients underwent surgery to remove colorectal liver metastases. Between August 1996 and September 2002, 11 (10%) of the 108 patients had en bloc resection of the IVC, leaving 97 patients with isolated hepatic resection. There was no significant difference in age between the two groups. The median age of the IVC resection cohort was 60 years (range, 41–75). Four patients were male and seven were female. Stage of the primary tumor was not different between the two groups.

Patients were considered candidates for hepatic resection if they met the following criteria: (i) absence of extra-hepatic disease, (ii) enough residual parenchyma to maintain liver function post resection, and (iii) medically fit for operation. The vast majority of tumors resected could be safely separated from the vena cava while maintaining negative margins. Suspicion of vena cava involvement was raised by preoperative imaging and intraoperative ultrasound. An intraoperative trial of dissection was undertaken to determine if the plane between the IVC and the tumor could be separated while preserving oncologic margins. En bloc liver and IVC resection was undertaken in cases where the tumor was adjacent to the IVC, and it was felt that negative margins would be compromised without vascular resection.

Comparisons between groups were carried out using the Student's t test for parametric data and the Mann-Whitney U test when the data was nonparametric. The survival differences were estimated by the log-rank test using the Kaplan-Meier method. Statistical analysis was performed using SPSS software (version 11.5, SPSS Inc., Chicago, IL). Statistical significance was set at $P < 0.05$.

RESULTS

The isolated liver resection cohort had a perioperative mortality rate of 2.1% (2/97), whereas there were no perioperative deaths in the 11 combined IVC resections. Mean operative time for isolated hepatic resection was 247 minutes (range, 130–505), which was significantly shorter than in the group that underwent

Table 1. Characteristics of patients undergoing en bloc IVC resection for colorectal metastases

Age	Procedure	Reconstruction	EBL (ml)	Transfusion (units)	Time (h:min)	LOS (ICU) (days)	F/U (mo)
74	L hepatectomy	Gore-Tex patch	700	1	3:58	9 (0)	Alive, disease free (25)
41	R trisectionectomy	Gore-Tex graft	400	0	5:20	14 (0)	Alive, hepatic recurrence (48)
67	R hepatectomy, R adrenalectomy, portion of diaphragm	Gore-Tex patch	2100	0	4:10	13 (0)	Alive, two additional resections for hepatic recurrence (24)
61	R hepatectomy	Gore-Tex patch	600	0	4:25	5 (0)	Deceased, retroperitoneal and bone metastases (22)
74	L hepatectomy	Gore-Tex patch	850	3	6:30	10 (1)	Deceased, lung recurrence (23)
53	R trisectionectomy portion of diaphragm	Gore-Tex patch	1000	4	7:10	13 (1)	Deceased, lung recurrence resected, biliary recurrence (50)
60	L trisectionectomy portion of diaphragm	Gore-Tex patch	900	4	6:32	15 (0)	Deceased, hepatic recurrence (34)
75	R trisectionectomy	Primary repair	2500	3	6:31	12 (0)	Deceased, hepatic recurrence (71)
46	R hepatectomy	Primary repair	800	0	5:05	12 (0)	Deceased, hepatic recurrence (14)
56	R hepatectomy	Gore-Tex patch	500	0	4:53	7 (0)	Deceased, hepatic recurrence (42)
59	R trisectionectomy	Gore-Tex patch	500	2	4:55	12 (0)	Deceased, bone metastasis (11)

EBL = estimated blood loss; F/U = follow-up; ICU = Intensive Care Unit; LOS = length of stay.

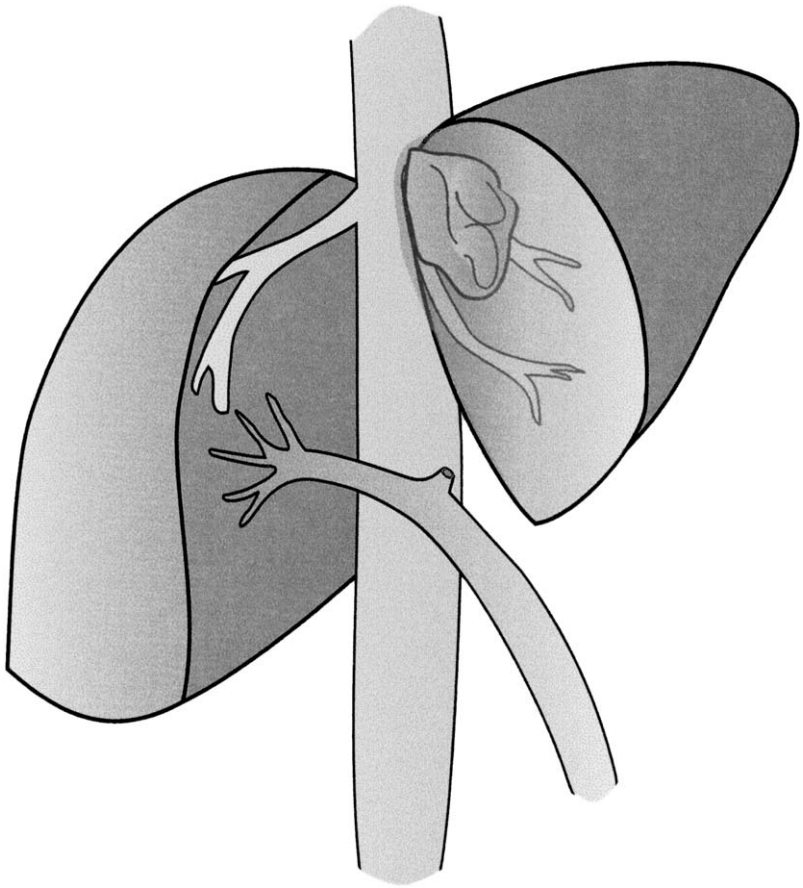

Fig. 2. Isolation of tumor in close proximity to inferior vena cava.

IVC resection with a mean operative time of 331 minutes (range, 238–446; $P = 0.003$). There was no difference in the number of resected hepatic metastases (isolated hepatic resection: median 1, range, 1–8; IVC resection: median 1, range, 1–3), nor was there any difference between the two groups in the proportion of patients receiving postoperative chemotherapy (control 54%, IVC resection 55%).

In the group undergoing IVC resection, four patients had a right hepatectomy, four had a right trisectionectomy, two had a left hepatectomy, and one had a left trisectionectomy (Table 1) . Ten patients underwent total vascular exclusion for a portion of the procedure (mean 47; range, 30–70 minutes; Figs. 2 and 3). Two patients also underwent concurrent veno-veno bypass. After resection, the vena cava was primarily repaired in two patients, replaced completely with 20 mm ringed Gore-Tex grafts in one patient, and patched with Gore-Tex in eight patients (Fig. 4). The average length of vena cava resected was 5.5 cm (range, 4–8 cm). Mean operative blood loss was 986 ml (range, 400–2500 ml). Six patients required intraoperative blood transfusions (median 1 unit). Routine subcutaneous heparin was used in both groups; no additional anticoagulation was used in the IVC resection group. All resections had negative histologic margins. No patients had direct tumor involvement of the vena cava; however, all patients had tumor directly adjacent to the IVC. Seven patients had a single lesion resected, three patients had two lesions resected, and one patient had multiple lesions resected. The mean size of the liver lesions was 6.4 cm (range, 2.5–14 cm). Complications were limited to a single pneumothorax/pleural effusion, one upper gastrointestinal bleed, and two cases of prolonged ileus. Mean length of stay was 11 days (range, 5–15). Mean follow-up was 33 months (range, 11–71 months). At last follow-up, 10 patients had a recurrence of their disease. Of the five liver recurrences, one was re-resected. Two patients recurred in the lung, one of which was also resected (Table 1). There has not been any evidence of IVC thrombosis either acutely or in follow-up.

Median disease-free survival of the group having caval resections was 9 months, whereas median survival was 34 months. When compared to the contemporary cohort of hepatic resections for colorectal cancer, the group undergoing caval resections

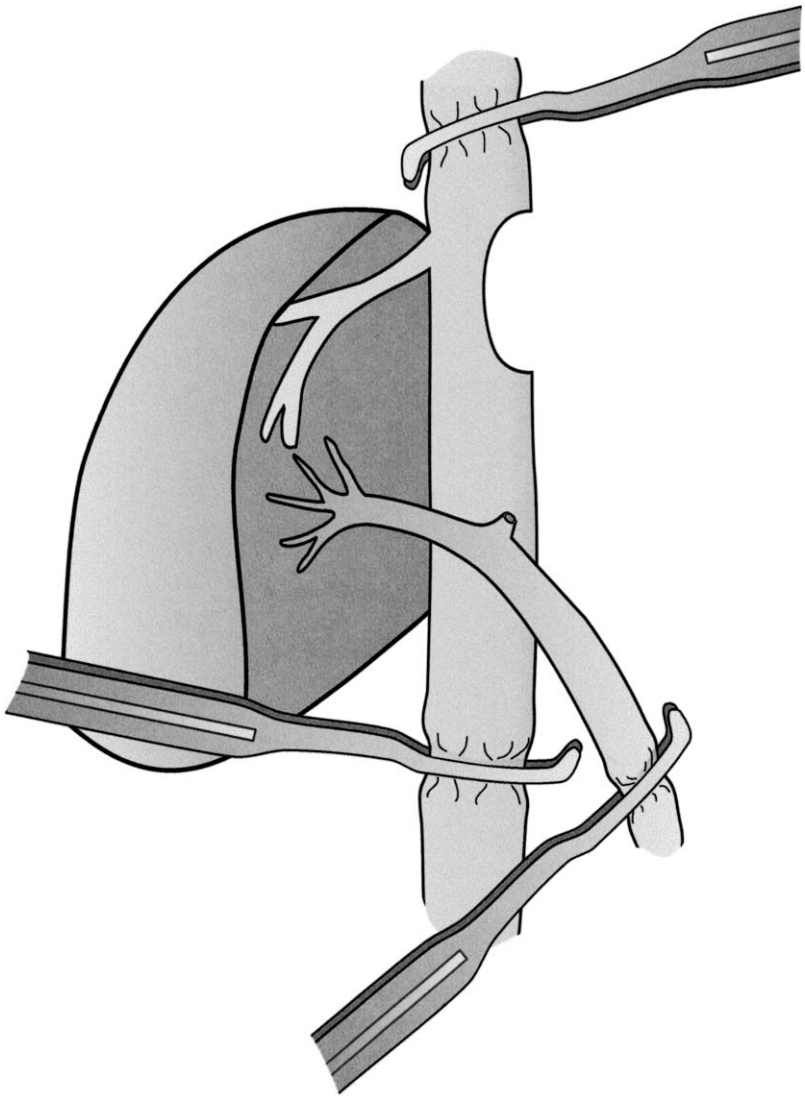

Fig. 3. Post resection with vascular isolation and defect in inferior vena cava.

experienced a significantly reduced disease-free survival of 18.6 (95% CI: 12.0–25.3) vs. 9.1 (95% CI: 6.0–12.4) months respectively, (log-rank $P = 0.03$; Fig. 5). However, there was no significant difference in overall survival between the two groups 52.2 (95% CI: 39.2–65.2) vs. 34.3 months (95% CI: 14.2–54.5) respectively, (log-rank $P = 0.20$; Fig. 6).

DISCUSSION

Resection of isolated hepatic colorectal metastases has become the treatment of choice for stage IV colorectal cancer with metastases confined to the liver. In established hepatobiliary programs, perioperative mortality is less than 5% (our series of 108 patients had a perioperative mortality of 1.85%) and 5 year survival approaches 40%.[2–6] This contrasts with medical management, which has a median survival of 20 months and offers little hope of cure.[8,9] Advances in perioperative care, anesthesia, and improved knowledge of liver anatomy have all fostered the ability to safely resect large portions of the liver.[15]

In a series from 2001, further updated in 2004, Hemming et al.[11,12] demonstrated the ability to perform combined hepatic and IVC resections for a multitude of tumors. Techniques of vascular exclusion and two ex vivo procedures facilitated the resections, whereas vascular reconstruction, when necessary, was accomplished using Gore-Tex grafts and patches. Of the 22 reported cases, six were for colorectal metastases. For the entire cohort, perioperative mortality was 0.8%; actuarial 5-year survival was 33%. At the time of publication, four of the

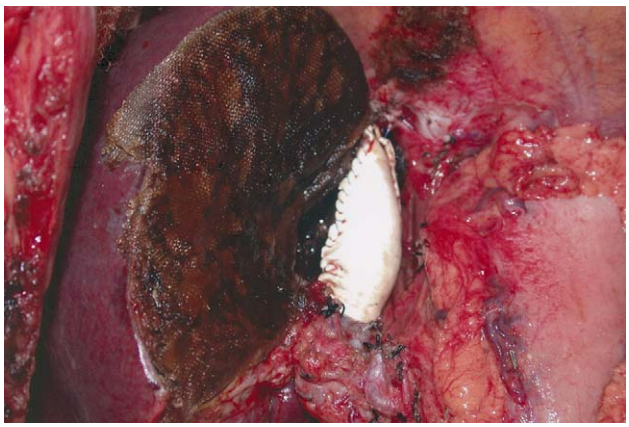

Fig. 4. Intra-operative photo showing inferior vena cava reconstruction with a Gore-Tex patch graft.

six patients with colorectal metastases were alive and free of disease with follow-up ranging between 13–36 months.

Miyazaki et al.[14] reported aggressive surgical resection for hepatic colorectal metastases in 1999. In their series of 16 patients with combined hepatic and IVC resections from Japan, 14 had surgery for colorectal metastases. All specimens showed histologic invasion of the vena cava. Mean blood loss was 2981 ml, and mean operative time was 7 hours and 58 minutes. Of the 14 en bloc IVC resections for colorectal metastases, median survival was 19 months, with a calculated 5 year survival of 22%. This survival did not significantly differ from the group of patients with colorectal metastases that did not involve the IVC (27% at 5 years). The authors concluded that aggressive surgical resection might improve prognosis in select patients with tumor involvement of the IVC.

Recently Aoki et al.[16] reported a series of nine patients with colorectal metastases involving either the IVC or hepatic venous confluence. Two of the nine patients had en bloc IVC resection; the other seven had portions of the hepatic veins removed. The group undergoing combined hepatic and vascular resection had significantly longer operating times, higher mean blood loss, and reduced median survival when compared to a cohort of 78 patients with isolated hepatic resections for colorectal metastases. Median survival of the patients with major vascular resections was 26 months, significantly shorter than the comparison group undergoing hepatic resection alone (44 months, $P < 0.01$). Interestingly, there was no statistical difference in disease-free survival between groups.

The ultimate goal in hepatic resection of colorectal metastases is to obtain negative histologic margins. Tumor involvement of the IVC can be suggested by preoperative imaging. However, the

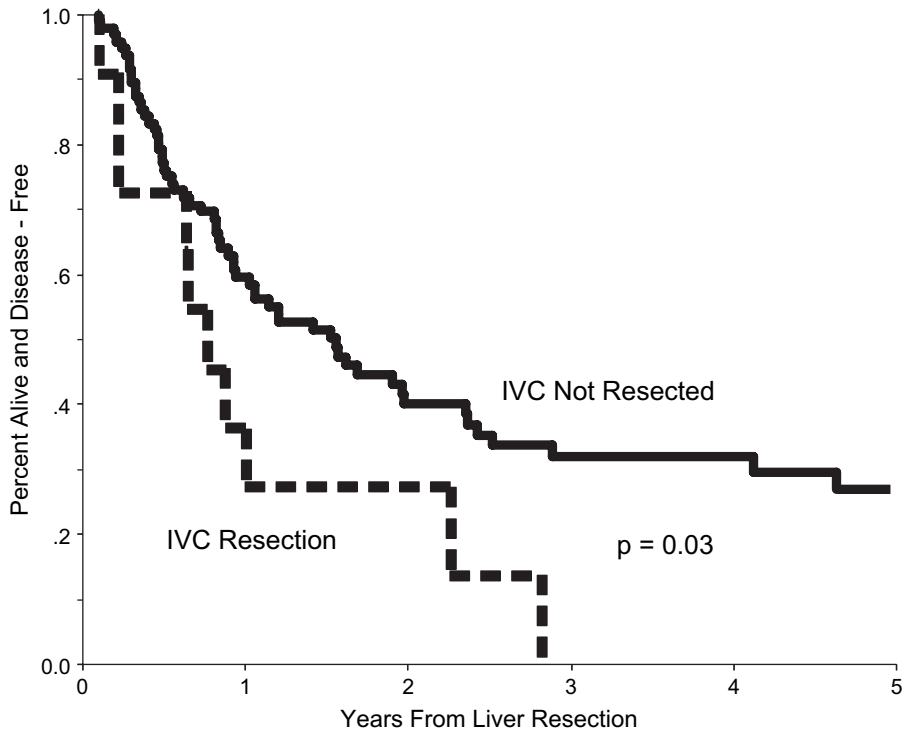

Fig. 5. Disease-free survival in patients after isolated liver resection or en bloc liver and IVC resection for colorectal liver metastases in years ($P = 0.03$).

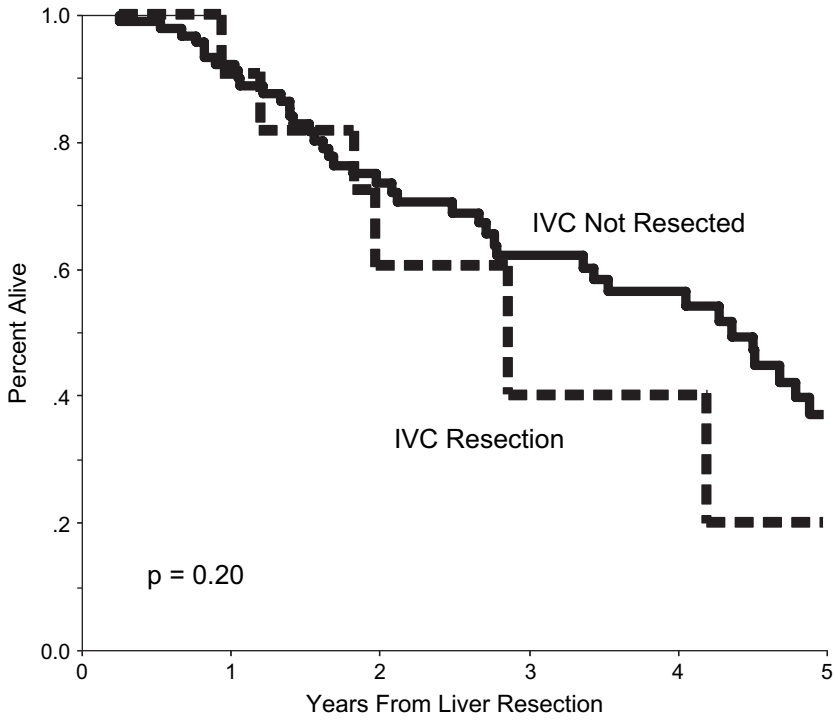

Fig. 6. Overall survival in patients after isolated liver resection or en bloc liver and IVC resection for colorectal liver metastases in years ($P = 0.20$).

ultimate decision to resect the vena cava is made intraoperatively with a combination of imaging and a trial of dissection.[12–14,16] In some patients, it may be necessary to resect the vena cava to achieve a negative histologic margin. Although the final pathology may not show direct caval invasion (but rather a negative margin of several millimeters), failure to resect the vena cava could compromise the surgical margin and leave behind microscopic disease. Positive surgical margins are predictors of poor long-term outcome.[2,5,6]

Patients presenting with colorectal metastases involving the IVC have traditionally been managed medically. However, ablative therapy has been playing an increasing role in the management of these colorectal metastases.[10,15,17] Radiofrequency ablation (RFA) is an evolving technology for treatment of colorectal metastases that uses heat to induce cellular necrosis. Published studies are limited by lack of long-term results and questionable efficacy.[17,18] There has not been a randomized trial of RFA vs. surgery for colorectal metastases. Furthermore, some papers have suggested that tumors adjacent to large vessels may not be completely amenable to RFA; a "heat sink" effect might impair the temperature-induced coagulative necrosis of perivascular tumors.[19,20]

Our cohort of combined liver and IVC resections had a median survival of 34.3 months after a mean follow-up of 33 months. Our series of en bloc liver and IVC resections was accomplished with low blood loss, moderate increases in operative time, and a few minor complications. Surgeons trained in techniques of vascular exclusion and liver transplantation performed all resections.

Our study is limited by its retrospective nature. In addition, given the small number of patients subjected to en bloc IVC resection, our data set could be underpowered and subject to type II error. Despite this, our series strongly suggests an oncologic benefit of surgery. Median survival is over 14 months longer than best available medical treatment and not significantly different from our cohort of isolated liver resections. This is a significant benefit for patients who were previously not considered candidates for surgical therapy, but rather referred for palliative chemotherapy, and more recently, radiofrequency ablation.

CONCLUSION

We believe that select patients with metastatic colorectal adenocarcinoma involving the inferior vena cava should be offered surgical resection. En bloc resection can be accomplished safely and confers an increase in survival for lesions often considered unresectable.

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Management of Pain in Small Duct Chronic Pancreatitis

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Small duct chronic pancreatitis (CP) is defined by a nondilated main pancreatic duct, and the morphological and clinical features of chronic pancreatitis with pain are the most prominent symptoms. Current treatment strategies are based on pain history and the location and extent of disease. Traditionally, radical pancreatic resectional procedures have been carried out for small duct CP, especially with an associated head mass of uncertain aetiology. Based on the information from five randomized trials, the duodenum-preserving pancreatic head resection and its modifications have proven to provide excellent long-term pain relief and to be superior to more radical operations. Therefore, these procedures can be considered the standard for small duct CP with head dominant disease. The longitudinal V-shaped excision of the ventral pancreas combines extensive drainage and a limited resection and offers good pain relief in diffuse small duct CP. However, long-term results and larger series are awaited for definite conclusions. Thoracoscopic splanchnicectomy and endosonography-guided celiac plexus blocks require controlled trials before their routine use. This article provides an overview about the current and evidence-based pain management in small duct CP. (J GASTROINTEST SURG 2006;10:227–233)

KEY WORDS: Chronic pancreatitis, pain, small duct disease, resection, drainage, neuroimmune interaction

Whereas the incidence and prevalence of chronic pancreatitis (CP) is known to be between 8 and 26.4 per 100,000 population,[1] the incidence and prevalence of small duct disease in CP remains unclear. This is partly because there is no clear definition of small duct disease of the pancreas. In many instances, the issue of whether small duct disease is in fact just a morphological step in the ultimate evolution of well-established CP, or whether it is a fully developed variant of CP, remains unresolved. As a working definition, small duct chronic pancreatitis[2] is a disease with a nondilated main pancreatic duct (4–5 mm) compared to a pancreatic duct that is normally considered dilated when it is more than 6–7 mm in diameter. Whereas there is a plethora of information about management of CP with a dilated main pancreatic duct, little information is available concerning the management of small duct CP. Because pain is the dominant symptom and principal characteristic of CP,[3] this article attempts to provide an overview about the management of pain in small duct CP.

Pathophysiology of Pain in CP

The existence of multiple hypotheses to explain the pathophysiology of pain in CP is reflected in the diverse management options that are adopted in the treatment of pain in various morphological and etiologic forms of CP.[4–6] A number of pancreatic and extrapancreatic causes have been identified. However, modern molecular biologic techniques have enabled researchers to develop a better understanding of pain mechanisms that are operational in CP. Increased intraductal pressure, one of the possible causes of pain in dilated duct CP, is obviously not the only pathway responsible for pain generation, even in dilated duct CP. This observation is based on the fact that not all patients with obstructed main pancreatic duct experience pain relief after ductal decompression. It

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has been documented that around 30% of the patients treated with decompressive surgery still exhibit recurrent attacks of pain.[7] Furthermore, Manes et al.[8] could not find a definite relationship between pain score and pancreatic pressure, although the intrapancreatic pressure correlated well with ductal changes in CP. It thus seems that increased pancreatic pressure is not always closely associated with pain in CP. These observations have made researchers look more closely at additional pathways and mechanisms that are active in CP. The causes, both morphological and molecular, are provided in Table 1.[9–16] Thus, these factors, independently or in combination, appear to be operational in the pain syndrome of small duct CP.

Management of Pain in Small Duct CP

The different treatment modalities for management of pain in small duct CP are surgery, therapeutic endoscopy, and medical measures. Patients should be evaluated with appropriate laboratory tests and by CT scans or magnetic resonance cholangiopancreatography or endoscopic retrograde cholangiopancreatography. Furthermore, a detailed pain history is crucial. These tests provide an excellent roadmap to the location and to the extent of the disease process, as well as the status of endocrine and exocrine functions, and enable the clinician to chart an appropriate treatment plan.

Surgical Resection

Surgery for pain in small duct CP is broadly divided into resectional and drainage procedures. Resectional procedures should be tailored according to location and extent of the disease process.

Small duct CP with dominant head mass. Pancreaticoduodenectomy (PD) has been the standard surgical treatment for pancreatic head-related complications of CP and for isolated small duct CP. In 2005, it is accepted as a safe procedure, especially when performed in high-volume centers of excellence where mortality rates are well below 5%.[17] Whereas the classical Whipple procedure achieves excellent pain relief apart from removing other complications and sequelae associated with a pancreatic head mass, the long-term results (i.e., quality of life, digestive function, diabetes mellitus) of this procedure for CP have been questioned.[18] The pylorus-preserving PD (ppPD), compared to the classical Whipple resection, has theoretical benefits by preserving the pylorus and the first part of the duodenum,[19] which have, however, never been proven in controlled trials. Irrespective, after ppPD, 85%–95% long-lasting pain relief during the first 5 years has also been documented in CP patients.[20] Nonetheless, remnant pancreatitis does occur in approximately one out of four patients.[21] In recent decades, there has been a shift from these resectional procedures toward more organ-preserving operations such as the duodenum-preserving pancreatic head resection (DPPHR),[22] the Freýs procedure,[23] and more recently the Bern modification of the DPPHR.[24] These modifications are a reflection of the understanding that PD primarily originated as an operation to treat malignancies of the periampullary region, rather than as a procedure to treat a predominantly benign disease like CP. Furthermore, despite the marked improvement in morbidity and mortality after PD, it remains a challenging and formidable operation; any procedure that offers comparable, if not better, outcome as a PD with a potential for less morbidity and mortality across centers with varying experience in pancreatic surgery, is expected to stand the test of time and find favor in the surgical community. Table 2[25–28] gives details of pain relief in randomized trials comparing the above-mentioned surgical techniques for CP. It must be pointed out, however, that evidence to support the belief that DPPHR preserves pancreatic function is not yet available. However, the overall results confirm that DPPHR and its modifications can be strong contenders as standard procedures for small duct CP with head predominance.

Small duct CP with dominant body and tail disease. This scenario presents a challenge to the clinician. In patients where pain remains uncontrolled with analgesic medications, and in those requiring increasing dosages of analgesics/narcotics, a distal pancreatectomy is the only treatment option available. However, it is critical to document that disease is focally located in the left pancreas before offering a distal pancreatectomy. Sawyer and Frey[29] recorded excellent pain relief when distal pancreatomy was

Table 1. Potential factors that might be involved in the pathogenesis of pain in small duct chronic pancreatitis

Alterations in pancreatic nerves (damaged perineurium, enlarged pancreatic nerves)
Neuroimmune interaction (perineural inflammatory cells, increased expression of neuropeptides)
Increased pressure in pancreatic ducts and pancreatic tissue
Pancreatic ischaemia
Pancreatic fibrosis
Pancreatic pseudocysts
Duodenal stenosis
Common bile duct stenosis
Maldigestion

Table 2. Randomized controlled trials comparing surgical techniques for chronic pancreatitis

Author	Trial	Pain outcomes	Other significant outcomes
Klempa et al.[25]	clWhipple vs. DPPHR	DPPHR: better pain relief	DPPHR: better weight gain
Büchler et al.[26]	ppPD vs. DPPHR	DPPHR: better pain relief	DPPHR: better weight gain
lzbicki et al.[27]	Frey vs. DPPHR	Equal pain relief	
lzbicki et al.[28]	ppPD vs. Frey	Equal pain relief	Frey: better QoL, less morbidity
Mackowiec et al. (unpublished)*	ppPD vs. DPPHR	Equal pain relief	DPPHR: better weight gain

clWhipple = classical pancreaticoduodenectomy; DPPHR = duodenum-preserving pancreatic head resection; Frey = Frey procedure (local resection of pancreatic head combined with lateral pancreaticojejunostomy); ppPD = pylorus preserving pancreaticoduodenectomy; QoL = quality of life.
*Makowiec F, Riediger H, Hopt UT, Adam U. Randomized controlled trial of pylorus-preserving Whipple versus duodenum-preserving pancreatic head resection in chronic pancreatitis. Presented at the 38th Annual Meeting of the Pancreas Club, New Orleans, Louisiana, May 16, 2004.

performed for disease limited to the body or tail of the pancreas. However, in the same series, a number of patients with diffuse disease did not enjoy similar benefits; of seven patients with severe pain preoperatively and diffuse disease or disease localized to the head of the pancreas, six required further hospitalization and resection or drainage procedures for severe, recurrent pain. Another study by Rattner et al.[30] observed that sustained pain relief was difficult to achieve after distal pancreatectomy for disease mainly located in the left pancreas (70% pain relief). However, they achieved better results with distal pancreatomy when associated pseudocysts were present. On the background of limited experience documented about small duct CP with dominant body and tail disease, it currently seems prudent to subject this uncommon group of patients to a distal pancreatectomy. An added advantage of this resectional procedure is that some patients with focal left sided small duct CP harbor unsuspected pancreatic cancer. Whereas the operative morbidity remains low, the attendant metabolic complications of distal pancreatectomy may warrant lifelong medical support and management.

The role of near-total pancreatectomy. This procedure[31] has also been attempted for small duct disease or for recurrent pain after a duct drainage procedure or limited pancreatic resection. However, the results of pain relief[32] after near-total pancreatectomy are far from satisfactory (75% success in pain relief) compared with other resection procedures. This observation is baffling, more so on the background that much better pain relief has been documented with the PD or DPPHR. Clearly, extrapancreatic and as yet unknown mechanisms are at work in the pain syndrome of CP. Furthermore, the metabolic consequences of this operation are significant (82% new onset diabetes mellitus). Hence, this procedure should be reserved only for those extreme situations when all other means of controlling pain in small duct CP have been exhausted.

Drainage Procedures

The role of drainage procedures in treatment of small duct CP remains unclear. Traditionally, these procedures have been used with limited success for a dilated and obstructed main pancreatic duct. However, some studies, keeping in mind the need for parenchyma conservation, have evaluated the feasibility of drainage procedures in small duct CP.

Lateral pancreaticojejunostomy. In a study of 17 patients where lateral pancreaticojejunostomy was performed for a main pancreatic duct size of less than 7 mm, lateral pancreaticojejunostomy provided unsatisfactory results, with 76% of the patients reporting that their pain was the same, if not worse than, before the operation. The study concluded that lateral pancreaticojejunostomy offered little benefit with respect to pain relief, subsequent hospitalization, continued narcotic use, or overall health status.[33] Another study reported better results, with as many as 86% of patients remaining pain free with a mean follow-up of 3.5 years.[34] However, a drawback of this study was the definition employed to document nondilated pancreatic ducts, once again reflecting the lack of a universal definition for small duct CP. Of the 28 patients evaluated in the study, 25 had minimal dilatation that was recorded as less than 8 mm. It thus seems that whereas this study has managed to convey the message that lateral pancreaticojejunostomy provides satisfactory results, even in minimally dilated ducts, these results cannot be extrapolated to small duct CP where duct size should be less than 4–5 mm.

Combination of Resection and Drainage Procedures

Longitudinal V-shaped excision of the ventral pancreas. Izbicki et al.[35] reported a new technique where patients with small duct CP underwent longitudinal V-shaped excision of the ventral pancreas,

and the resultant cavity with secondary and tertiary ducts was drained into a Roux-en-Y loop as a pancreaticojejunostomy. This technique is thus based on the Partington-Rochelle procedure[36] and its modification of longitudinal pancreaticojejunostomy combined with limited local excision of the pancreatic head, as described by Frey and Amikura.[37] The limited resection of the pancreatic head in the Frey procedure is more an outcome of an effort to drain the main pancreatic duct in the head region of the pancreas, an area where it tends to run deep compared to its course in the body and tail of the CP gland. Though this seems to be a rational concept, definite evidence to support this belief remains elusive. Complete relief from pain was observed in 92% of patients, and the median pain score decreased by 95%. Furthermore, 77% of patients gained more than 10% of their pretherapy body weight. On the background that increased parenchymal pressure and a glandular compartment syndrome are related to the pain syndrome of CP,[38,39] this technique assumes significance. It attempts to drain the secondary and tertiary pancreatic ducts, while preserving sufficient pancreatic tissue to maintain exocrine and endocrine function. Nonetheless, longer follow-up and larger (multicenter) trials, as yet unavailable, are needed to adequately judge the position of this method in the armamentarium of the pancreatic surgeon.

Therapeutic Endoscopy

Endoscopic treatment of CP has been put forth as an attractive alternative to surgery because it is inherently less invasive with shorter periods of hospitalization and can often be performed under intravenous sedation. An indication for pancreatic duct stenting can be seen in patients with a solitary prepapillary stenosis without stenosis of side branches, or as success control for a planned surgical intervention. In these clinical scenarios, however, there is usually pancreatic ductal obstruction with a degree of dilation of the main pancreatic duct (6 mm or more). There is a solitary randomized controlled trial comparing endoscopy with surgery in treatment of pain in CP,[40] which reported that surgery is superior to endotherapy for long-term pain reduction in patients with painful obstructive chronic pancreatitis. Due to its low degree of invasiveness, however, it was suggested that endotherapy could be offered as a first-line treatment, with surgery being performed in case of failure and/or recurrence. But in case of pain in small duct CP, the very nature of pancreatic morphological alterations (as also the poor outcomes associated with standard surgical drainage procedures) results in a very limited role for therapeutic endoscopy. In 2005, therapeutic endoscopy may have a role in the management of pseudocysts in small duct CP in certain selected patients. Recently patients of small duct CP were subjected to progressive transampullary duct dilation with subsequent placement of a wall stent, followed by lateral pancreaticojejunostomy 2 weeks later.[41] Seventy-one percent of patients reported an improvement in pain compared with their preprocedure levels, and 25% of patients discontinued their narcotic medications. With these uncertain results, only more experience can better define the role of this procedure in the management of pain in small duct CP. Furthermore, endoscopic ultrasonography (EUS) can be used for EUS-guided celiac plexus blocks in the management of pain in small duct CP.[42] Based on the hypothesis that small duct CP is just a developmental stage in the ultimate evolution of CP rather than a distinctive variant of CP, EUS may provide some answers in the coming years. It remains to be seen whether detection of an "early" small duct CP by EUS can somehow influence subsequent treatment options for pain in small duct CP.

Other Treatment Modalities

Denervation of pancreatic sympathetic pain afferents has been suggested as an alternative to more extensive resection procedures for management of pain in small duct CP. Sympathetic nervous system carries almost all the pancreatic pain afferents, and hence, surgical approaches have been mainly focused on these pathways. However, the results are mixed and the observed variability is attributed to a combination of poor patient selection, incomplete knowledge about pancreatic pain, and inadequate knowledge of pancreatic neuroanatomy.

Coeliac ganglion blockade. Temporary celiac plexus blocks have been used to predict whether a patient can benefit from sympathetic ablation by surgical means.[43] Permanent celiac plexus block can be performed with 50% alcohol. Whereas some authors have reported good success rates in a small number of patients with pain in small duct CP,[44] others have reported inconsistent outcomes with only short-term pain relief.[45] The reasons for the failure of celiac ganglion blockade to consistently control pain in small duct CP include difficulty in precise placement of the needles for injection, restricted diffusion of the neurolytic agent due to peripancreatic inflammation and fibrosis, and other unknown factors.

Thoracoscopic splanchnicectomy. In 1994, Cuschieri[46] reported the first thoracoscopic approach to the splanchnic nerves in patients with CP. The experience with thoracoscopic splanchnicectomy was reviewed by Bradley and Bem in 2003.[47] This

collected experience showed a 78.4% improvement in the perceived level of pancreatic pain. Furthermore, Howard et al.,[48] in a prospective study, concluded that bilateral thoracoscopic splanchnicectomy worked best in patients who had no prior endoscopic or operative interventions, compared with patients who had undergone some sort of intervention where quality of life and pain scores returned to baseline levels postoperatively and remained poor throughout the study. Because thoracoscopic splanchnicectomy can reduce the pain in some, but not all, patients with CP, clearly more experience with this approach needs to be accumulated to make a rational evaluation of the role of this technique in the management of pain in small duct CP.

Centrally acting analgesic drugs. Centrally acting analgesic drugs represent a pharmacological approach to the central control of pancreatic pain. It has been reported that at least half of the patients with CP will require narcotic analgesics at some time during the course of the disease. Addiction and other side effects of narcotic analgesics are well-documented, thus preventing them from being an ideal long-term solution for the pain of small duct CP.[47]

Pancreatic enzyme supplementation. Pancreatic enzyme supplementation as a means of pain control in small duct CP remains a controversial issue. The rationale for enzyme use lies in the observed negative feedback mechanism regulating pancreatic stimulation. Six randomized, controlled trials have evaluated the use of pancreatic enzyme replacement therapy in pain management of CP.[49–54] The results have been disappointing with two trials[49,50] utilizing enzymes in tablet form and reporting some benefit, whereas the remaining four failed to show any benefit.[51–54] The patients more likely to benefit were females with idiopathic pancreatitis and less advanced disease.[55] As an interesting observation, gastroparesis has been frequently observed (44%) in patients with small duct CP, and it has been suggested that patients with small duct CP whose abdominal pain does not respond to pancreatic enzyme therapy should be evaluated for gastroparesis.[56] Despite the uncertain benefit obtained, many centers attempt a trial of pancreatic enzyme supplementation at some stage of pain management in small duct CP. The various treatment options for management of pain in small duct CP are summarized in Table 3.

Autoimmune Pancreatitis

Autoimmune pancreatitis was first introduced as a distinctive entity in 1995 by Yoshida et al.[57] It is a nonalcoholic, chronic lymphoplasmacytic pancreatitis that is characterized by periductal infiltration of CD4-positive T cells, fibrosis, and acinar atrophy. Imaging studies often reveal a diffuse narrowing of the pancreatic main duct and swelling of the pancreatic head, wrongly suggesting the presence of a malignant tumor. Clinical signs include mild abdominal pain, jaundice, and recurrent episodes of acute pancreatitis. Steroids seem to be effective in improving clinical symptoms as well as in the resolution of pancreatic and bile duct narrowing, which separates autoimmune pancreatitis from other forms of pancreatitis.[58] Nonetheless, autoimmune pancreatitis patients are frequently treated with surgical procedures because of the presently incomplete understanding of this disease entity, the difficult diagnosis, and the low awareness of this disease. Clearly, greater awareness and more knowledge of this unique entity are necessary and awaited.

SUMMARY

Management of small duct CP should be tailored according to the symptoms of the patient, which are assessed by objective tests and by sound radiological imaging precisely documenting the dominant focus and extent of the disease. This information is crucial in the context that pain in small duct CP seems to be

Table 3. Current treatment options in management of pain in small duct CP

Clinical scenario	Treatment modality
Small duct CP + head mass of uncertain pathology	ppPD
Small duct CP with head dominant disease	DPPHR and its modifications
Small duct CP with pseudocyst	Drainage (endoscopic or surgical) or resection (depending on location and extent of CP)
Small duct CP with body and tail dominant disease	Distal pancreatectomy
Small duct CP involving the whole organ	Longitudinal V-shaped resection of the ventral pancreas
Recurrent pain after surgery for small duct CP	Identify residual cause of disease (resurgery, analgetics, nerve blocks)
Early stage small duct CP	Trail of pancreatic enzyme supplementation followed by surgery in case of failure

more difficult to manage than pain in CP with a dilated main pancreatic duct. The problem gets further accentuated by the absence of a universal definition of small duct CP; as a result, different studies addressing this issue are difficult to compare and evaluate, and there is a paucity of reliable published data.

Radical pancreatic resection (PD) provides good results for pain management in small duct CP. These procedures are especially useful when a malignant neoplasm must be excluded in small duct CP with a head dominant mass. With over 90% long-term pain relief and results of randomized trials in favor of DPPHR and its modification, these organ-preserving procedures should be the benchmark against which all alternative treatment modalities for pain management of small duct CP with head dominant disease should be evaluated. These demanding procedures are best undertaken in high-volume, experienced centers with excellent results. The causes of recurrent or persistent pain after drainage procedures are complex, multifactorial, and as yet are not well understood. They can be due to inadequate drainage of the head of the gland, failure to drain small ducts, and associated perineural inflammation. Procedures for pain management in small duct CP that have combined the advantages of resectional surgeries with those of drainage procedures have gained ground in recent times. Pending larger series and long-term results, resectional procedures may be tested by procedures such as the longitudinal V-shaped excision of the ventral pancreas, an extensive drainage procedure that offers the benefits of resection without its attendant morbidity. Procedures such as thoracoscopic splanchnicectomy and newer endoscopic procedures such as endosonography-guided celiac plexus blocks, though attractive concepts, require larger and longer controlled trials before their routine implementation. And finally, advances in molecular biology, with development of novel treatment options, are expected to throw more light on the pain mechanisms in small duct CP.

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Glutamine Does Not Protect Against Hepatic Warm Ischemia/Reperfusion Injury in Rats

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The administration of glutamine before experimental ischemia/reperfusion (I/R) has been shown to protect intestinal, pulmonary, and myocardial tissue by inducing heat shock proteins (HSP). However, it is not known whether glutamine is protective for all organs. We therefore tested whether pretreatment with glutamine reduces injury following hepatic I/R in rats. Male lean Zucker rats were pretreated with either glutamine (0.75 g/kg intraperitoneally, n = 6) or saline (n = 6), 24 and 6 hours before ischemia. Seventy percent of the liver was exposed to 75 minutes of warm ischemia followed by 24 hours reperfusion. Liver enzymes, histology, neutrophil accumulation, survival, and heat shock protein (HSP) 70 induction were examined. Glutamine administration did not reduce liver injury. In both groups, 5 of 6 animals survived 24 hours of reperfusion. There was no difference in serum transaminase levels with AST 15113 ± 4336 U/L (glutamine) vs. 17695 ± 8531 U/L (control, $P > 0.05$), and ALT 7763 ± 2524 (glutamine) U/L vs. 5884 ± 2063 U/L (control, $P > 0.05$). The degree of neutrophil accumulation and necrosis was not different between groups at 24 hours of reperfusion. Pretreatment did not result in HSP70 upregulation in any of the groups. Pretreatment with glutamine did not reduce hepatic ischemia/reperfusion injury. The lack of protection was associated with an absence of HSP70 upregulation prior to ischemia. (J GASTROINTEST SURG 2006;10:234–239)

KEY WORDS: Liver surgery, heat shock protein, inflammation, myeloperoxidase

Liver surgery is associated with hepatic ischemia/reperfusion (I/R) injury, which may result in postoperative organ dysfunction and even organ failure. Pre-existent liver disease such as cirrhosis or liver steatosis further increases the risk of surgery-induced I/R injury. Therefore, protective techniques are needed that are aimed at increasing hepatic tolerance to I/R injury.

Glutamine, a conditionally essential amino acid, has been shown repeatedly to protect from a variety of stressful stimuli. In different experimental protocols, glutamine administration has been proven to provide protection against infection and septic shock. This is demonstrated by enhanced immune function, decreased bacteremia, inhibited mucosal gut atrophy, and improved survival.[1–3] In addition, glutamine can ameliorate injury from intestinal[4] and myocardial I/R.[5,6] The protective effects of glutamine on cardiomyocytes are associated with an upregulation of heat shock protein (HSP) 70.[7] Glutamine is known to induce HSP70 expression in several other vital organs, such as the lung and the liver.[8]

Upregulation of HSP70, as part of the cellular stress response, renders cells more resistant to subsequent potentially lethal insult. Several agents and gene-transfer techniques have been shown to induce HSP70 expression in stressed and unstressed conditions, which results in protection from endotoxin shock,[9] acute lung injury,[10] organ protection in kidney and pancreatic islet cells transplantation,[11,12] and myocardial ischemia.[13] In the liver, heat shock

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protein induction by heat stress[14] or ischemic preconditioning[15] protects against subsequent warm hepatic ischemia. It is unknown whether the pharmacological induction of HSP with glutamine would be equally protective to the liver. Protective effects of glutamine that are possibly independent of HSP induction include decreased inducible nitric oxide synthase (iNOS) expression,[16] reduced oxidative stress, increased glutathione synthesis,[17] and reduced neutrophil recruitment.[18]

We have investigated whether pretreatment with glutamine results in HSP induction and subsequent protection of the liver from I/R injury. We have also assessed whether glutamine pretreatment reduces the I/R-evoked inflammatory response.

MATERIALS AND METHODS

Animal Model

All animal experiments were carried out with approval of the local committee on animal research. The animal care was in agreement with the National Institutes of Health guidelines for ethical research. Inbred male lean Zucker rats (Harlan Sprague Dawley, Indianapolis, IN) were used for this study. All animal weights on arrival at our facility were approximately 250–300g. Animals had access to standard laboratory diet and were maintained on a light-dark cycle.

Rats were divided into two groups. HSP induction following glutamine administration appears to be dose-dependent.[19] The glutamine group (n = 6) was pretreated with 2 doses of glutamine (0.75 g/kg intraperitoneally) 24 and 6 hours prior to warm ischemia. In the control group (n = 6), saline was injected 24 and 6 hours prior to warm ischemia.

Animals were anesthetized with isoflurane and the liver was exposed through a midline incision. Temperature was monitored continuously with a rectal probe to maintain animals at normothermia.

Applying a 70% liver ischemia model, vascular structures to the left and median lobes were identified and clamped for 75 minutes using a bulldog clamp. Appropriate clamping was confirmed by inspection of the ischemic lobes. The unoccluded right and caudate lobes allowed outflow from the splanchnic circulation to avoid venous congestion. Following reperfusion, animals were given 5 mL of normal saline intraperitoneally, and the incision was closed in two layers. Surviving animals were sacrificed following a 24 hour observation period at which time blood and tissue were harvested. The tissue was immediately frozen in liquid nitrogen and stored at -80° C until further processing.

Subsequently, 6 animals were pretreated with either glutamine (n = 3) or saline (n = 3), as described above, to determine the HSP70 expression evoked by pretreatment alone. After completion of the pretreatment, rats were sacrificed and the liver tissue was harvested for HSP assessment.

Animal Recovery After Surgery and Survival

Rat recovery was closely monitored over the next 24 hours. Signs of poor clinical condition were lethargy, ruffled fur, guarding upon abdominal palpation, lack of grooming, and decreased food intake. Animals that appeared to be doing poorly were sacrificed before the 24 hour reperfusion endpoint. Upon sacrifice, all animals underwent necropsy. Animals with intra-abdominal pathology such as hemorrhage were excluded from the study.

Assessment of Hepatocellular Injury

Serum levels of AST, ALT, and alkaline phosphatase (AP) were determined (IDDEX Veterinary Services, Sacramento, CA) in rats surviving 24 hours of reperfusion.

Determination of Necrosis

The ischemic lobes were excised immediately after the animals were sacrificed. Tissues were fixed in 10% formalin and then embedded in paraffin in preparation for light microscopy analysis. Sections were cut at 5μ and stained with hematoxylin and eosin to allow histological examination. The analyses were performed on randomly selected specimens from each group. The pathologist who performed the analyses was blinded to the experimental group of the animals. Light microscopic examination was performed under standard conditions at 10× and 20× magnification. Histological criteria for the assessment of hepatic necrosis after ischemia reperfusion were as follows: less than 5%, 5%–25%, 25%–50%, 50%–75%, and greater than 75% necrosis.

Assessment of HSP70 induction. Validation of HSP70 was performed using a quantitative ELISA as it is more sensitive and quantifiable than the Western blot technique.[20] Tissue was placed in homogenizing buffer (HSP70 extraction reagent, StressGen Biotechnologies, Victoria, BC, with complete protease inhibitor, Roche Diagnostics, Indianapolis, IN). For each 0.5 mL piece of tissue, 1 mL of homogenizing buffer was used. Tissues were dissociated using a tissue homogenizer, and cells were lysed by exposure to extraction reagent. The cell lysates were transferred to microfuge tubes and centrifuged

at 30,000 g for 10 minutes at 4° C. Supernatants were removed and stored at −20° C until time of assay.

HSP70 was measured from tissue lysates using a commercially available ELISA for rat HSP70 (StressGen Biotechnologies). Tissue total protein was measured using a commercially available bicinchoninic acid assay (Sigma-Aldrich Co., St. Louis, MO). The concentration of HSP70 protein per unit of total protein (pg/μg) is presented.

Intrahepatic neutrophil accumulation assessment. Activity of myeloperoxidase (MPO), an enzyme stored in the azurophilic granules of neutrophils, was used to measure tissue neutrophil sequestration. We used a spectrophotometric method to assay tissue MPO activity. In brief, frozen livers were thawed and extracted for MPO following homogenization and sonication. The assay is based on the oxidation of 3,3′, 5,5′-tetramethyl benzydine (TMB) by MPO in the presence of H_2O_2. Units of MPO activity were calculated using a standard curve derived from a myeloperoxidase standard sample (Calbiochem, EMD Biosciences Inc., La Jolla, CA). Myeloperoxidase activity is expressed as mU/min/mg protein.

Statistical Analysis

All data are presented as mean ± SD. Comparison between groups was performed applying two-tailed unpaired *t*-test. *P* values < 0.05 were considered to be statistically significant.

RESULTS

Animal Recovery and Survival

One animal in each group died during the observation period. All surviving animals in the control group were judged to be in poor clinical condition, which was in contrast to those in the glutamine group. Blood samples taken from the control-group animal that was sacrificed 23 hours after reperfusion were included in subsequent analysis.

Hepatocellular Injury

In the glutamine group (n = 5), AST levels after 24 hour reperfusion were 17694 ± 8530 IU/L, which is similar to those in control rats (n = 6) who had AST levels of 15113 ± 4336 IU/L ($P = 0.53$, Fig. 1). The pattern of ALT levels followed the distribution of AST levels. ALT levels were similar in both groups (7763 ± 2523 IU/L vs. 5884 ± 2062 IU/L, $P = 0.2$, Fig. 1).

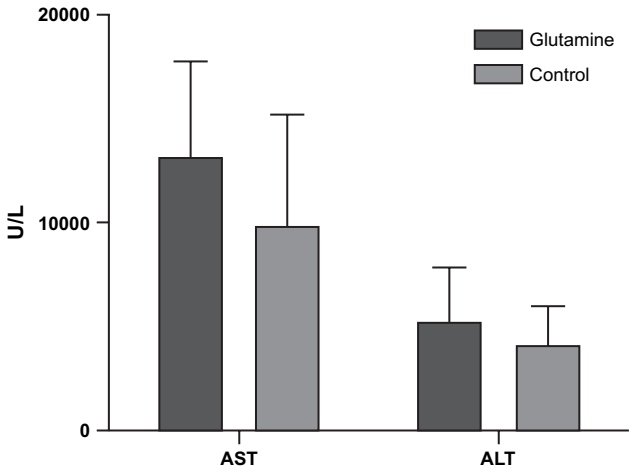

Fig. 1. Postischemic transaminase concentration of animals pretreated with glutamine and saline (control) after 24 hours of reperfusion.

Hepatic Necrosis

Liver necrosis after 24 hour of reperfusion was not different between groups. It was consistently greater than 75% in rats treated with glutamine or saline (Fig. 2). Necrosis was panacinar, but small foci of pericentral areas and some portal areas were spared. In addition, a zone of subcapsular liver remained viable.

HSP Expression

After 24 hours of reperfusion, HSP70 concentrations were dramatically increased in both groups. However, there was no difference between study groups (Table 1). Pretreatment with glutamine did not result in HSP70 induction before ischemia. The samples obtained from pretreated livers that did not undergo ischemia had HSP concentrations that were lower than the defined sensitivity of the test.

Myeloperoxidase Assay

Myeloperoxidase activity following 24 hours of reperfusion was elevated in both groups, but not different between groups (Fig. 3).

DISCUSSION

In the present study, pretreatment with glutamine did not result in increased hepatic HSP expression and hepatic protection during ischemia reperfusion injury when compared to a control group. Similarly, glutamine pretreatment did not ameliorate intrahepatic neutrophil accumulation, which has been suggested to be possibly HSP independent.

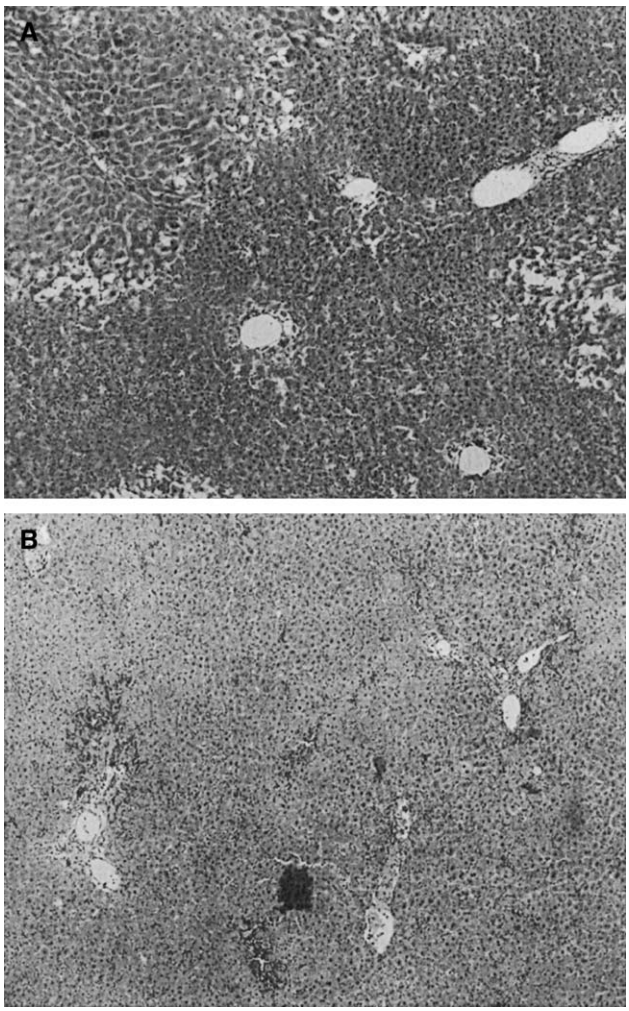

Fig. 2. (**A**) Representative histology from a control rat. One viable focus in the left upper part of the slide shows intact, normal hepatocytes. (**B**) Representative histology from a glutamine-treated rat. Signs of inflammatory changes and necrosis are noted throughout the slide.

Hayashi et al. demonstrated previously that glutamine pretreatment for 1 week resulted in profound HSP70 induction in lung, heart, and liver and subsequently attenuated cardiopulmonary bypass induced inflammatory response in rats.[8] However, their treatment regimen was laborious in that it required three injections (100 mg/kg) per day for 1 week. This regimen is unlikely to be acceptable in a clinical setting. Heat shock protein induction can be achieved within hours, and its maximum is usually achieved within 24 hours.[19] We were interested in determining whether or not a short course of glutamine over 24 hours would be able to induce HSP70.

Our total dose of glutamine (1.5 g/kg) is lower than the cumulative dose (2.1 g/kg) that was administered by Hayashi, and ours was administered intraperitoneally instead of intravenously. It is reasonable to assume that peak concentrations of glutamine were higher in our study (albeit not in steady state). Using this short dosing protocol, we were unable to detect any differences between HSP70 expression or hepatic protection in glutamine-treated and control animals 24 hours after ischemia/reperfusion. Subsequently, we also determined the HSP70 expression levels in glutamine-treated and control animals that were not exposed to ischemia/reperfusion injury. In both groups (n = 3, each group), HSP70 expression was below the recommended limit of detection for the assay (Table 1). The lack of glutamine-induced HSP70 induction in the liver appears to be dependant on the duration of glutamine treatment, rather than on the cumulative dose or peak glutamine levels.

Table 1. Heat shock protein 70 concentrations

	Control	Glutamine
HSP70 following pretreatment (pg/mg protein)	< 50	< 50
HSP70 following reperfusion (pg/mg protein)	1782 ± 1375	1060 ± 1036

Furthermore, the concept of pre-ischemic HSP induction as a prerequisite for protection by glutamine is challenged by reports that demonstrate protection by postischemic glutamine application.[5] In one study survival was improved by the pretreatment with glutamine-supplemented parenteral nutrition for 5 days prior to intestinal ischemia.[4] Another study demonstrated that glutamine-supplemented parenteral nutrition that was administered after intestine I/R injury was protective.[21] However, a different study demonstrated an increased degree of I/R injury when glutamine was infused after ischemia.[22] The protective effects of glutamine appear to depend

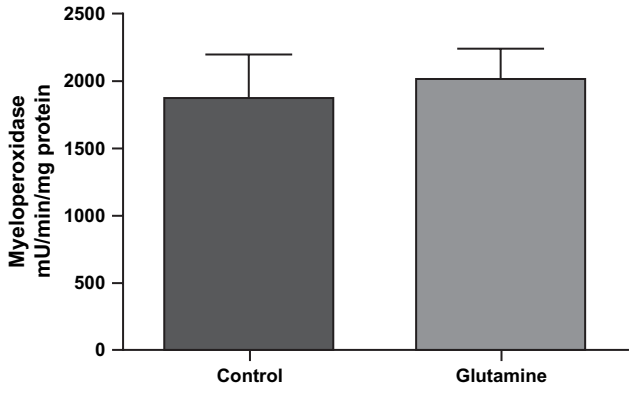

Fig. 3. Myeloperoxidase activity after 24 hours of reperfusion.

on the experimental design. Differences in timing, dose, and/or mode of the glutamine administration may account for the differing results.

Heat shock protein induction by ischemic preconditioning is very effective in the experimental setting.[15] However, it has not been widely used in the clinical setting despite observed beneficial effects with hepatic surgery in humans.[23] Pharmacological preconditioning with a relatively nontoxic substance would be a desirable alternative. Several clinically available substances have been shown to protect against experimental hepatic I/R injury by decreasing the associated inflammatory response: immunosuppressants such as cyclosporine, azathioprine,[24] and FK 506,[25] as well as pentoxifylline[26] and ATII receptor antagonists.[27] In the clinical setting, none of these substances are routinely used for hepatic protection.

Evidence for HSP-independent protection derives from the finding that glutamine is known to protect intestinal tissue from I/R injury,[4] although it is not able to induce HSP in the ileum in vivo.[19] Glutamine is known to induce an anti-inflammatory response that results in reduced neutrophil recruitment,[18] and HSP induction has been associated with a decreased production of pro-inflammatory cytokines.[3] However, in our study the pretreatment with glutamine did not ameliorate the inflammatory response as evaluated by measuring intrahepatic MPO activity.

If glutamine were protective against hepatic ischemia reperfusion injury, its safe implementation into clinical practice could be accomplished very quickly. However, the currently available information does not establish that glutamine has a consistently protective effect against hepatic ischemia/reperfusion injury.

CONCLUSION

In our study the pretreatment with glutamine did not protect against hepatic ischemia/reperfusion injury. This lack of protection was associated with a failure to increase HSP70 expression in the liver. The role of HSP70 as a mediator for pharmacological preconditioning in the liver remains unclear.

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Debunking Dogma: Surgery for Four or More Colorectal Liver Metastases Is Justified

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Treatment of four or more colorectal liver metastases (CRLMs) is controversial and remains a relative contraindication to surgery at some institutions. We sought to assess the outcome of patients with four or more CRLMs treated with surgery. Patients (159) with four or more CRLMs were treated surgically at a single institution. The median number of treated lesions was 5 (range, 4–14). The majority of patients received neoadjuvant chemotherapy (89.9%). Forty-six (29.0%) patients underwent resection only, 12 (7.5%) underwent radiofrequency ablation (RFA) only, and 101 (63.5%) underwent resection plus RFA. The 5-year actuarial disease-free and overall survival rates were 21.5% and 50.9%, respectively. Patients who underwent RFA as part of their surgical procedure (hazard ratio [HR] = 1.81, $P = 0.03$) and those with a positive surgical resection margin (HR = 1.52, $P = 0.01$) were more likely to have a shorter time to recurrence. Patients who did not have a reduction in tumor size following neoadjuvant chemotherapy had a higher likelihood of death following surgical treatment (HR = 2.53, $P = 0.01$). Patients with four or more CRLMs should be considered for aggressive surgical treatment, including liver resection with or without RFA, in order to improve the chance of long-term survival. Certain clinicopathologic factors, including lack of response to neoadjuvant chemotherapy, were associated with a worse prognosis. (J GASTROINTEST SURG 2006;10:240–248)

KEY WORDS: Colorectal metastasis, multiple tumors, radiofrequency ablation, resection, outcome

Hepatic resection remains the only potentially curative therapeutic option for patients with colorectal liver metastasis (CRLM), with reported 5-year survival rates of 25–58% in patients with liver-only disease.[1–6] Traditionally, primary tumor stage, preoperative carcinoembryonic antigen (CEA) level, hepatic tumor size, number of CRLMs, and presence of extrahepatic disease have been reported to be independent predictors of long-term survival after resection.[3,7–9] In particular, the number of intrahepatic metastases has been considered to be one of the major prognostic factors after resection of CRLM, with a decrease in survival as tumor number increases—especially for patients with at least four CRLMs.[8,10–12] As such, although the efficacy of surgical resection of one to three CRLMs has been established,[1,8,12] resection of four or more metastatic lesions is controversial.[13–19]

Because the suitability of patients with four or more CRLMs for surgery is not well defined, the objective of the current study was to assess the efficacy of surgery—including resection and/or radiofrequency ablation (RFA)—in a large cohort of patients with at least four CRLMs treated at a single institution. Additionally, we sought to determine which clinicopathologic factors were associated with disease-free and overall survival.

PATIENTS AND METHODS

Between April 1996 and September 2004, 159 patients with four or more CRLMs underwent surgical treatment (resection, RFA, or combination of both modalities) at The University of Texas M. D. Anderson Cancer Center, Houston, Texas. Only patients with histologically confirmed colorectal hepatic metastases treated with curative intent were included in the current study. Patients were evaluated with a baseline history and physical examination; serum laboratory tests, including serum CEA; computed

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tomography (CT) scanning or magnetic resonance imaging (MRI) of the abdomen and pelvis; and a chest radiograph. Patients were excluded from surgical consideration if their bilirubin was greater than 2 mg/dl, platelet count was less than 40,000/μl, or the prothrombin time was prolonged more than 1.5 times above normal. If platelet or fresh-frozen plasma transfusions corrected the abnormal laboratory values to meet these criteria, the patient received treatment. All patients had no clinical, radiographic, or intraoperative evidence of extrahepatic disease. Following surgery, all patients were regularly followed and prospectively monitored for recurrence by serum CEA levels, a CT or MRI scan of the abdomen, and a chest radiograph every 3–4 months up to 2 years and then every 6 months thereafter.

Surgical Resection

At the time of laparotomy, an initial exploration was performed to exclude the presence of extrahepatic disease. An intraoperative hepatic ultrasound was then performed to identify, count, and characterize the nature and vascular proximity of the malignant hepatic lesions. The location of the metastases and their relation to surrounding vascular and biliary structures dictated whether a formal anatomic resection was performed. In general, the extent of surgery was determined by a combination of the estimated hepatic functional reserve, which was assessed by a combination of preoperative liver biochemistry, the distribution of the metastatic disease, and the predicted remnant liver volume after resection. Resection was classified as less than a hemihepatectomy (e.g., segmentectomy or subsegmentectomy), hemihepatectomy, or extended hepatectomy (five or more liver segments).[20]

Radiofrequency Ablation

RFA of hepatic lesions was performed at the time of laparotomy according to a standardized treatment algorithm.[21–23] Intraoperative ultrasonography was used to place the RF needle into the lesions to be treated with RFA. RFA was administered using the RF 2000 or 3000 generator system (Boston Scientific Co., Natick, MA), a LeVeen monopolar needle electrode (4.0-cm maximum array diameter), and four dispersive grounding pads applied to the patient's skin. The LeVeen needle electrode is a 15-gauge, 12- to 15-cm-long insulated cannula that contains 10–12 individual hook-shaped electrode arms that are deployed in situ. For tumors less than 2.5 cm in diameter, the multiple array was deployed into the center of the tumor. For larger lesions, the array was first deployed at the most posterior interface ultrasonographically between the tumor and normal liver parenchyma and subsequently withdrawn and redeployed at 1.5-cm intervals in the tumor. Each tumor or area within a large tumor was treated with a one-phase application of RF power before retracting the multiple array and repositioning or removing the needle electrode. The electrode was optimally positioned to achieve complete destruction of the tumor and at least a 1-cm zone of normal liver parenchyma when possible.

Data Collection

The following data were collected for each patient: demographics; laboratory data; administration of neoadjuvant chemotherapy; type of chemotherapy; response to neoadjuvant chemotherapy; tumor number, size, and location; operative details; complication details; disease status; date of last follow-up; and death date. Data were recorded as follows: clinical features, present or absent; CEA level prior to surgery, less than 5 ng/ml, 5–100 ng/ml, or greater than 100 ng/ml; largest tumor size, less than or equal to 3 cm versus greater than 3 cm; tumor location, unilobar versus bilobar; number of lesions ablated, one, two to four, or at least five. Tumor size and number were defined by the resection specimen and/or by intraoperative ultrasonographic measurement. Neoadjuvant chemotherapy was classified based on the main agent used in the regimen: 5-fluorouracil (5-FU), oxaliplatin, irinotecan (CPT-11), a combination of oxaliplatin and CPT-11, and floxuridine (FUDR) via hepatic artery infusion pump. Response to neoadjuvant chemotherapy was defined as at least a 25% reduction in tumor bidimensional measurements demonstrated on preoperative CT or MRI. Data on recurrence were categorized as intrahepatic only, distant only, or both intrahepatic and distant. The specific site of distant recurrence was also noted.

Statistical Analyses

All data are presented as percentages of patients or the median value with ranges. Statistical analyses were performed using univariate tests (χ^2, log-rank) to test for differences in variables with regard to disease-free and overall survival. Factors that appeared to be significantly associated with survival were entered into a Cox proportional hazards model to test for significant effects while adjusting for multiple factors simultaneously. Actuarial survival was calculated using the Kaplan-Meier method. Differences in survival were examined using the log-rank test. A P value <0.05 was considered significant.

RESULTS

Clinicopathologic Characteristics

Table 1 shows the clinicopathologic features of the 159 patients in the study. The median patient age was 56 years (range, 35–74 years). The median number of treated lesions was 5 (range, 4–14) and the median size of the largest lesion was 3.5 cm (range, 0.3–15 cm). Most patients (n = 117, 73.6%) had bilobar disease.

The majority of patients received neoadjuvant chemotherapy (n = 143; 89.9%) prior to surgical treatment of the hepatic metastases. The types of chemotherapy administered included 5-FU (n = 40; 28.0%), oxaliplatin (n = 16; 11.2%), CPT-11 (n = 68; 47.5%), oxaliplatin and CPT-11 (n = 10; 7.0%), and FUDR via hepatic artery infusion pump (n = 9; 6.3%). Of the 143 patients who received neoadjuvant chemotherapy, the majority (n = 104; 72.7%) had a reduction in the size (⩾ 25% decrease in tumor bidimensional measurements) of the hepatic metastases. Patients who received 5-FU alone (56.1%) were less likely to have a preoperative reduction in tumor size than were patients who received other chemotherapy regimens (77.7%) (P = 0.008) (Table 2).

At the time of operation, surgical treatment was resection only in 46 (29.0%) patients, RFA only for tumors in unresectable locations in 12 (7.5%) patients, and resection of large or dominant lesions combined with RFA of smaller lesions (an approach used in patients otherwise considered unresectable) in 101 (63.5%) patients (Table 3). Of the 147 patients who had a surgical resection, the extent of hepatic resection was less than a hemihepatectomy in 33 patients (22.4%), a hemihepatectomy in 72 patients (49.0%), and an extended hepatectomy in 42 patients (28.6%). On final pathologic analysis, margin status was positive in 19 patients (12.9%), negative by 1–9 mm in 51 (34.7%), and at least 1.0 cm in 77 (52.4%). Patients with four tumors (2.1%) were

Table 1. Clinical and pathologic features of patients (n = 159)

Variable	
Age, median (yr)	56
Gender, n (%)	
Female	52 (32.7)
Male	107 (67.3)
Tumor number, n (%)	
Median	5
4	57 (35.8)
5–7	82 (51.6)
>7	20 (12.6)
Largest tumor size	
Median	3.5 cm
>3 cm, n (%)	85 (53.5)
Tumor location, n (%)	
Unilobar	42 (26.5)
Bilobar	117 (73.5)
Preoperative CEA level, n (%)	
Median	8.4 ng/mL
<5 ng/mL	53 (33.3)
5–100 ng/mL	75 (47.2)
>100 ng/mL	31 (19.5)

CEA = carcinoembyronic antigen.

Table 2. Details of the response to neoadjuvant chemotheraphy (n = 143)

Type of Neoadjuvant Chemotherapy	Reduction in Tumor Size		*P* Value
	No.	%	
5-Fluorouracil only	23	56.1	
Oxaliplatin	14	87.5	
CPT-11	50	73.5	
Combination oxaliplatin and CPT-11	8	80.0	
Hepatic artery pump (FUDR)	8	88.9	
5-Flurouracil only	23	56.1	.008
Other chemotherapy regimens	82	77.7	

CPT-11 = irinotecan; FUDR = floxuridine.

Table 3. Details of surgical treatment

	No. of Patients (%)
Type of surgical treatment (n = 159)	
Resection only	46 (29.0)
RFA only	12 (7.5)
RFA plus resection	101 (63.5)
Details of RFA (n = 113)	
Median number of tumors ablated	2
1	47 (41.6)
2–4	55 (48.7)
⩾5	11 (9.7)
Details of surgical resection (n = 147)	
Type of resection	
Wedge	12 (8.2)
Left lateral segmentectomy	21 (14.3)
Right hemihepatectomy	51 (34.7)
Left hemihepatectomy	22 (14.9)
Extended right hepatectomy	29 (19.7)
Extended left hepatectomy	12 (8.2)
Status of surgical margin	
1–9 mm	51 (34.7)
⩾10 mm	77 (52.4)
Positive	19 (12.9)

RFA = radiofrequency ablation.

less likely to have a positive surgical margin than were patients who had more than four tumors (19.1%) ($P = 0.007$). The extent of surgical resection did not predict margin status ($P = 0.09$). Patients who underwent less than a hemihepatectomy were more likely to be treated by RFA (n = 29; 87.9%) compared with patients who had a more extensive resection (n = 72; 63.2%) ($P = 0.005$).

The perioperative complication rate was 26.4% (42 complications in 37 patients) (Table 4). The median length of stay was 7.0 days (range, 2–49 days). Three patients died within 90 days of treatment for a perioperative mortality rate of 1.9%.

Patterns of Recurrence

With a median follow-up of 32.4 months, 111 patients (69.8%) had developed recurrent disease: 37 patients (33.3%) with intrahepatic only disease, 24 patients (21.7%) with distant only disease, and 50 patients (45.0%) with a combination of intrahepatic and distant metastases. The median time to development of recurrence was 10.5 months (range, 2.2–68.4 months). The most common site of distant metastatic failure was the lung (57 of 74 patients with distant disease; 77.0%).

Statistical analyses revealed two clinicopathologic factors that were associated with risk of overall recurrence. Patients who had not received neoadjuvant chemotherapy were more likely to recur than patients who had received preoperative chemotherapy (80.0% versus 69.2%, respectively; $P = 0.01$). Patients who were treated with RFA only were also more likely to recur (90.9%) compared with patients who underwent resection alone (54.3%) or resection plus RFA (70.2%) (each $P < 0.05$). Other factors including preoperative CEA, type of neoadjuvant chemotherapy, type of surgical resection, number of tumors treated, number of lesions ablated, pathologic margin status, tumor number, and tumor size did not affect the chance of overall recurrence (all $P > 0.05$).

In addition to overall recurrence, analyses were performed to determine which, if any, factors were associated with an increased risk of intrahepatic recurrence. Among all the factors examined, only the number of lesions ablated by RFA was associated with an increased risk of local recurrence. Specifically, patients who had at least five lesions ablated by RFA experienced an increased rate of intraheptic recurrence (100.0%) compared with patients who had fewer lesions ablated (62.8%) ($P = 0.02$).

Table 4. Perioperative complications (42 complications in 37 patients)

Complication	No. of Patients (%)
Ascites	1 (0.6)
Cardiac arrhythmia	3 (1.9)
Symptomatic fluid collection/biloma	6 (3.8)
Hepatic insufficiency	2 (1.3)
Perihepatic abscess	2 (1.3)
Symptomatic pleural effusion	2 (1.3)
Pneumonia	6 (3.8)
Pneumothorax	1 (0.6)
Portal vein thrombosis	2 (1.3)
Postoperative bleed	1 (0.6)
Prolonged postoperative ileus	4 (2.5)
Pulmonary embolus	3 (1.9)
Urinary tract infection	4 (2.5)
Wound infection	5 (3.0)
Total	42 (26.4)

Disease-Free Survival

The 1-, 3-, and 5-year actuarial disease-free survival rates were 51.9%, 27.8%, and 21.5%, respectively (Fig. 1). On univariate analysis, a number of factors were associated with a decrease in disease-free survival: preoperative CEA greater than 5 ng/ml, RFA as part of the surgical procedure, size of largest metastatic lesion greater than 3 cm, and a positive surgical margin (Table 5). Other factors, including number of tumors treated, were not associated with disease-free survival (all $P > 0.05$).

On multivariate analysis, the type of surgical procedure and the pathologic status of the surgical

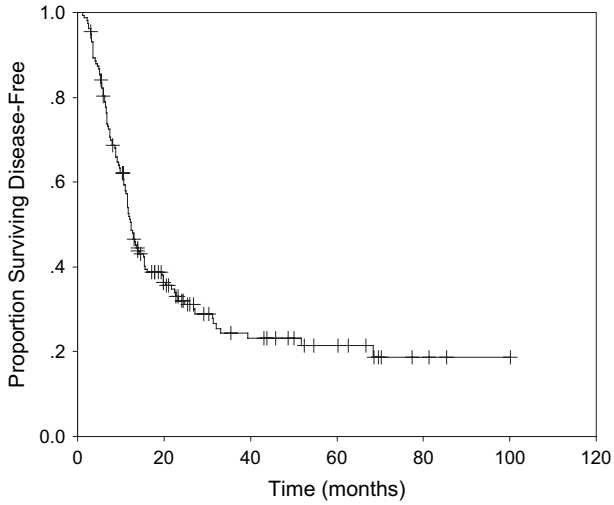

Fig. 1. With a median follow-up of 32.4 months, the median disease-free survival for patients with four or more CRLMs treated with surgery was 10. 4 months. The 1-, 3-, and 5-year actuarial disease-free survival rates were 51.9%, 27.8%, and 21.5%, respectively.

Table 5. Clinicopathologic factors associated with disease-free survival on univariate analysis

Variable	5-Year Disease-Free Survival Rate (%)	*P* Value
CEA level prior to surgery (mg/mL)		
<5	29.2	.04
⩾5	13.3	
Type of surgical procedure		
RFA only	10.2	.03
Resection only	41.4	
RFA plus resection	14.3	
Largest tumor size (cm)		
⩽3	26.9	.03
>3	16.7	
Margin status		
Negative	23.4	.02
Positive	0	

CEA = carcinoembryonic antigen; RFA = radiofrequency ablation.

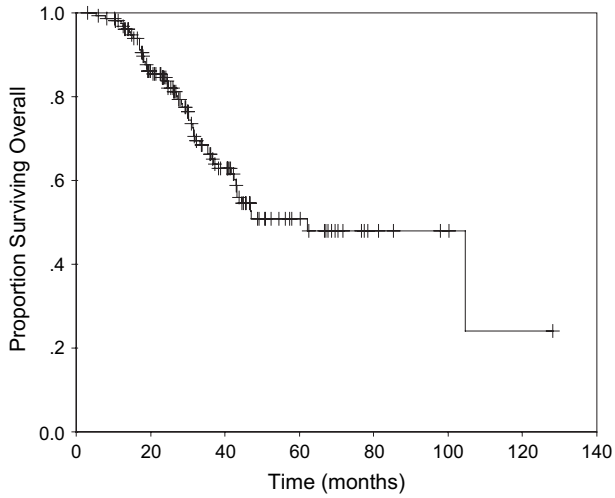

Fig. 2. The median overall survival was 62.1 months and the 1-, 3-, and 5-year actuarial overall survival rates were 97.4%, 66.2%, and 50.9%, respectively.

margin remained independent predictors of a shortened disease-free survival interval. Patients who underwent an RFA as part of their surgical procedure (hazard ratio [HR] = 1.81, 95% confidence interval [CI], 1.26–2.35, $P = 0.03$) and those with a positive surgical resection margin (HR = 1.52, 95% CI, 1.18–1.86, $P = 0.01$) were more likely to have a shorter time to recurrence.

Overall Survival

The median overall survival was 62.1 months and the 1-, 3-, and 5-year actuarial overall survival rates were 97.4%, 66.2%, and 50.9%, respectively (Fig. 2). Univariate analyses revealed five factors that were associated with overall survival. Two factors associated with long-term survival related to the patient's response to preoperative chemotherapy (Fig. 3). Patients with a preoperative CEA less than 5 ng/ml had a longer median overall survival (not reached) than patients with a CEA level at least 5 ng/ml (36.6 months) ($P = 0.007$). Similarly, patients who had a preoperative reduction in the size of their hepatic metastases (104.7 months) fared significantly better than patients who had no decrease in tumor size in response to preoperative chemotherapy (32.0 months) ($P = 0.002$). Other factors associated with overall survival related to tumor burden (Fig. 4). Patients who had one lesion ablated had a median overall survival of 101.4 months compared with a median overall survival of 41.4 months for patients who had multiple lesions treated with RFA ($P = 0.03$). Tumor size also affected survival. Patients whose size of the largest metastatic lesion was less than or equal to 3 cm had a longer median survival (not reached) compared with patients who had a metastatic lesion measuring greater than 3 cm (38.2 months) ($P = 0.001$). Patients with unilobar disease also had a longer median survival (not reached) than patients with bilobar metastases (46.8 months) ($P = 0.04$). Other factors, including number of tumors treated, did not affect overall survival (all $P > 0.05$).

On multivariate analysis, response to neoadjuvant chemotherapy was the only factor that remained independently associated with a longer overall survival. Patients who did not have a reduction in tumor size following neoadjuvant chemotherapy had a higher likelihood of death following surgical treatment (HR = 2.53, 95% CI, 1.85–3.27, $P = 0.01$).

DISCUSSION

To date, most studies reporting on the surgical treatment of CRLM have included patients with a wide array of clinicopathologic characteristics.[1–6,24–26] A number of these studies have found a decrease in patient survival with increasing number of tumors.[1,8,10–12,19] These studies, however, have been criticized for including only a small number of patients who actually had multiple metastases. Additionally, some reports on resection of multiple metastases have been difficult to interpret because clusters of small lesions in close proximity or satellite lesions around a large metastasis were classified as multiple metastases. More recently, a few studies have specifically investigated the outcome of patients with multiple (four or more) metastases following

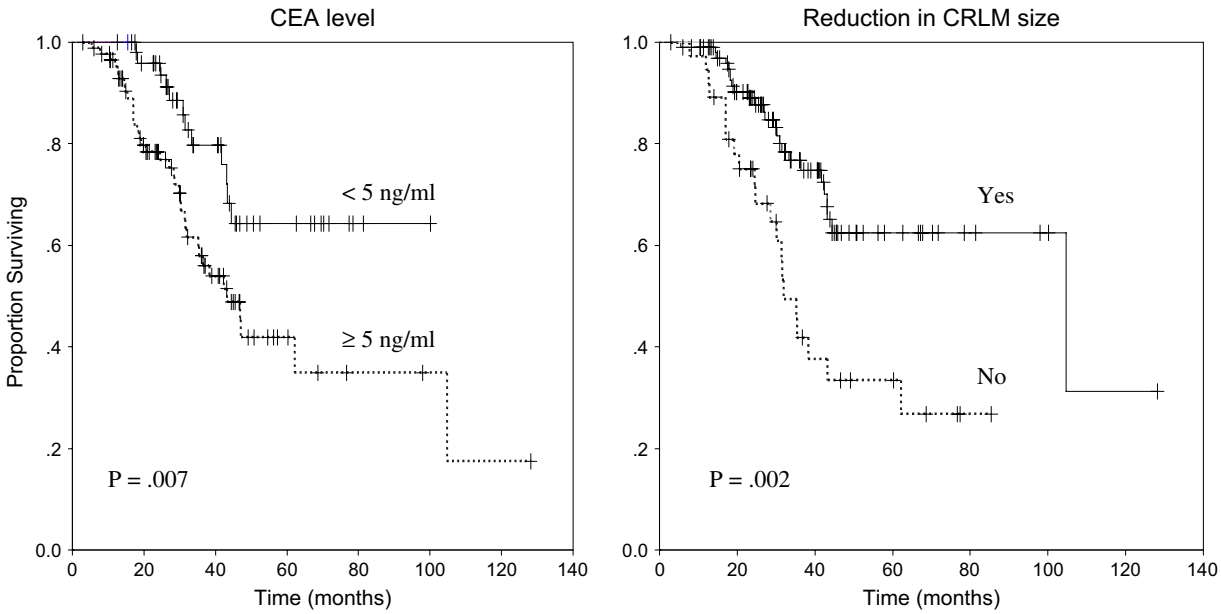

Fig. 3. Two factors associated with long-term survival related to the patient's response to preoperative chemotherapy. Patients who had a CEA level less than 5 mg/ml and those who had a preoperative reduction in tumor size following neoadjuvant chemotherapy had better overall survival rates.

hepatic resection.[13,18,19,27] To our knowledge, however, the current study is the first to include patients who were treated with RFA, hepatic resection, or a combination of both modalities. Unlike most previous studies, the current study also specifically examined the impact of neoadjuvant chemotherapy on the prognosis of patients with four or more CRLMs. As such, we were able to evaluate not only the feasibility and safety of each treatment strategy but also to assess more directly the disease-specific

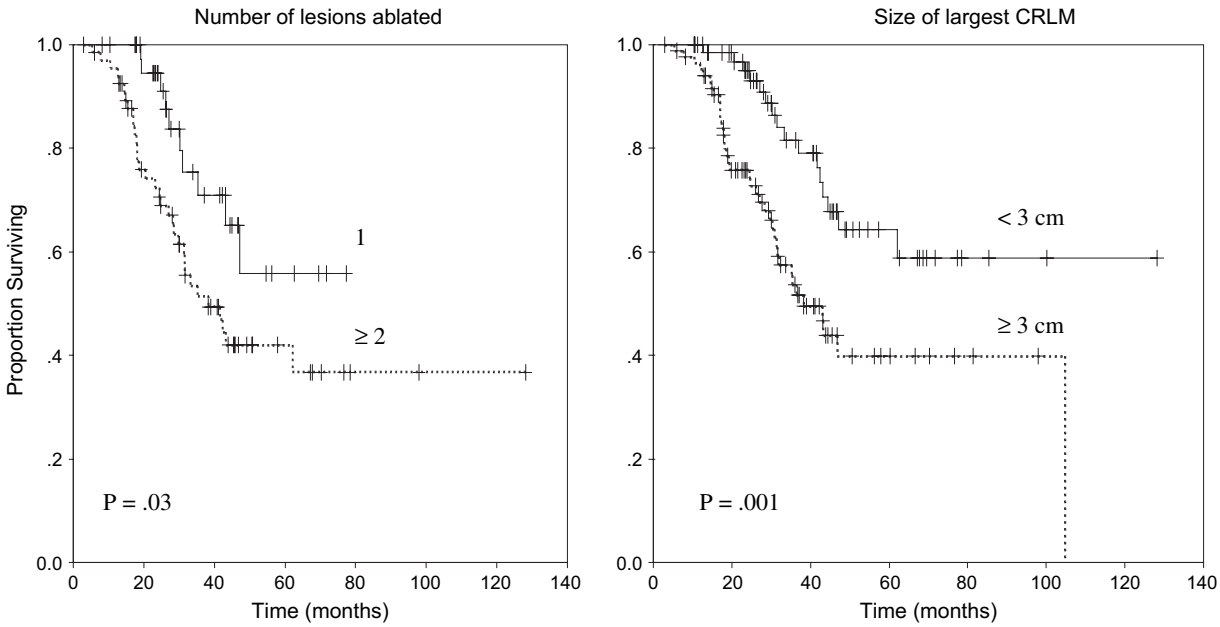

Fig. 4. Other factors associated with overall survival related to tumor burden. Patients who had only one lesion ablated or whose size of the largest metastatic lesion was equal to or less than 3 cm were noted to have an improved overall survival.

outcome of patients with four or more CRLMs treated with neoadjuvant chemotherapy and surgery.

The complication and mortality rates for the 159 patients who underwent surgical treatment were 26.4% and 1.9%, respectively. Consistent with our previously reported experience with combined RFA and resection for advanced hepatic malignancies,[23] patients in the current study who underwent combined modality treatment did not have an increased risk of perioperative morbidity or mortality. Other investigators have similarly reported low perioperative morbidity and mortality rates following hepatic resection of multiple hepatic metastases.[13,18] These data provide convincing evidence that surgical treatment of four or more CRLMs is not only feasible but also well tolerated and safe when performed at a major hepatobiliary center.

RFA has become a widely used ablative technique to provide treatment for patients who are not candidates for hepatic resection.[28–31] Although RFA has been mostly utilized as an isolated, alternative therapy for unresectable CRLM, it can be combined with hepatic resection.[23] By combining RFA with resection, more patients may become candidates for surgical treatment, as the surgeon can resect larger tumors while ablating residual smaller lesions. In the current study, 12 patients underwent RFA alone while 101 patients were treated with RFA plus resection. RFA was used alone in patients who had lesions that were in unfavorable locations or who were judged to be unable to tolerate a major parenchymal resection. Those patients who were treated with RFA alone did have a significantly higher rate of recurrence (90.9%) compared with patients who underwent resection alone (54.3%) or resection plus RFA (70.2%) (each $P < 0.05$). In addition, every patient who had five or more lesions ablated developed an intrahepatic recurrence. Although direct comparisons between patients who undergo resection, RFA, and combined RFA and resection are difficult, these results imply that patients who harbor disease the extent of which cannot be adequately treated with resection and/or limited ablation are at an increased risk of intrahepatic and systemic recurrence. The use of RFA in combination with surgery for four or more CRLMs therefore is justified, but the use of RFA as a sole modality to treat four or more CRLMs appears to have marginal therapeutic benefit.

The overall recurrence rate of 69.8% reported in the current study was similar to that published in most other studies investigating resection of four or more CRLMs.[13,18,19] Kokudo et al.[14] described a 5-year recurrence rate of 76.9%, while Weber et al.[18] noted a 78.7% recurrence rate at the time of last follow-up. While the overall recurrence rate is instructive and confirms the suspicion that patients with multiple lesions are at an increased risk of recurrence, the more significant finding of the current study may be that two thirds (66.7%) of the patients recurred with distant disease as a component of their recurrence pattern. Because of the high incidence of systemic failure after surgical treatment of multiple hepatic metastases, we do not advocate the use of liver directed regional chemotherapy. Rather, with the introduction of effective intravenous and oral chemotherapeutic agents, we believe that systemic chemotherapy is more appropriate. In general, patients in the current study received additional adjuvant chemotherapy following surgery using the same chemotherapy regimen as they had received preoperatively, as long as they had demonstrated a response to neoadjuvant therapy. In the event that the patient failed to respond to neoadjuvant therapy (i.e., stable or progressive disease), a different chemotherapeutic regimen was used postoperatively.

The surgical literature contains conflicting reports with regard to the impact that four or more CRLMs has on overall survival. In a study of 800 patients with CRLM, Hughes et al.[3] reported that the 5-year survival rate for patients with one or two metastases was 37% compared with 18% for patients with three or more metastases. In this series, there were only three long-term survivors with four or more metastatic lesions. Both Cady et al.[32] and Ekberg et al.[11] have similarly described poor outcomes for patients with multiple tumors. Cady et al.[32] reported that no patient with three or more metastatic lesions survived disease free for longer than 48 months. Ekberg et al.[11] noted no 3-year survivors with more than three lesions. Because of these findings, many surgeons have considered four or more lesions a relative contraindication to hepatic resection. More recently, other centers have found no difference in overall survival as the number of tumors increase, with 5-year survival rates of 40–50%.[8,19,33] Similar to other contemporary reports, in the current study, the overall actuarial 5-year survival rate for patients undergoing surgery for four or more CRLMs was 50.9%. This favorable overall survival rate most likely relates to the fact that patients included in the current analysis were highly selected. Every patient had no extrahepatic disease at the time of initial surgical treatment, most received neoadjuvant chemotherapy (89.9%), almost two thirds (72.7%) had a reduction in tumor size following preoperative chemotherapy, all patients underwent thorough intraoperative ultrasonography to avoid missing small hepatic lesions, and only 19 patients had a positive surgical resection margin. On

univariate analyses, factors associated with overall survival related to response to neoadjuvant chemotherapy (preoperative CEA level immediately prior to surgery, radiographic reduction in tumor size following neoadjuvant chemotherapy) and tumor burden (number of lesions ablated by RFA, size of largest metastasis, bilobar location of disease). Of note, the number of tumors treated did not affect survival. On multivariate analysis, the only factor that remained an independent predictor of overall survival was the response to neoadjuvant chemotherapy. These data suggest that tumor biology (response to cytotoxic therapy) rather than morphologic criteria (tumor number, size) determine long-term prognosis. Thus, we believe that tumor number should not be accepted dogmatically as an exclusion criterion for surgical treatment of CRLM in an era of active chemotherapy agents and advanced surgical techniques.

In recent years, response rates observed with 5-FU have been significantly increased by its combination with oxaliplatin and/or CPT-11.[34–38] These higher response rates have allowed 15–20% of patients with initially unresectable disease to be secondarily resected with 5-year survival rates of 30–40%.[4,39] It has been our approach, as well as the policy of others,[27] to manage patients with multiple tumors using neoadjuvant chemotherapy irrespective of whether the lesions are initially resectable. The rationale of this therapeutic strategy has been supported by reports of better prognosis obtained with neoadjuvant chemotherapy and surgery versus immediate surgery in patients with multinodular colorectal liver metastases.[40] More recently, the actual effect of tumor response to chemotherapy has been evaluated. Allen et al.[41] reported that patients whose disease did not progress while they were receiving neoadjuvant chemotherapy experienced improved survival after liver resection as compared with patients who did not receive chemotherapy. In a study by Adam et al.,[27] patients who had tumor progression while receiving neoadjuvant chemotherapy had only an 8% overall 5-year survival rate versus 37% for patients who had an objective tumor response to preoperative chemotherapy. The current study corroborates the prognostic importance of tumor control prior to surgery with neoadjuvant chemotherapy. Specifically, we noted that patients who failed to have a measurable reduction in tumor size following neoadjuvant chemotherapy had over a 2.5 times higher likelihood of cancer-related death following surgical treatment. Taken together, these data call into question the utility of hepatic resection in patients with multiple CRLMs who fail to respond to neoadjuvant therapy and may have important implications for the therapeutic strategy adopted by medical oncologists and surgeons.[27]

CONCLUSION

Tumor number—even tumors numbering four or more—should not be used as an arbitrary criterion to exclude patients from surgical treatment. Rather, patients with four or more CRLMs should be considered for liver resection, RFA, or both in order to improve the chance of long-term survival. Although recurrence is common, 30.2% of patients in the current study had not recurred at the time of last follow-up and the 5-year actuarial disease-free survival rate was 21.5%. Systemic chemotherapy should be used to consolidate therapy following surgery as the majority of patients who recur will have distant metastases as a component of their pattern of recurrence. Long-term survival can be achieved, however, response to neoadjuvant chemotherapy plays an important role in determining the potential therapeutic benefit of liver resection in patients with multiple metastases. Future studies should strive to identify molecular markers that define the underlying biology of colorectal metastases better than currently available clinical, morphologic, and pathologic factors.

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Transarterial Chemoembolization With Degradable Starch Microspheres, Irinotecan, and Mitomycin-C in Patients With Liver Metastases

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Degradable starch microspheres (DSMs) provide transient occlusion of small arteries and are thought to improve the therapeutic effect of anticancer drugs. Irinotecan (CPT-11) is one of the most effective anticancer agents. We herein report cases with liver metastases treated with transarterial chemoembolization with DSM, CPT-11, and mitomycin-C (DSM-CPT therapy). Five patients underwent DSM-CPT therapy for liver metastases that originated from colorectal cancer for four and gastric cancer for one. They all lack indication for surgery. They were all male with an age range of 42–78 years (mean, 55.2 years). Three of them had pretreatment histories with 5-fluorouracil or related agents, and four of them had combined systemic or local chemotherapy at the period. Required doses for stasis of whole blood flow of hepatic artery of DSMs were used with CPT-11 and mitomycin-C. After one to six injections, four patients had a partial response and the disease progressed in one patient with gastric cancer origin. Two of the partial response patients underwent surgery after 2 months of the partial response period. Carcinoembryonic antigen and CA19-9 levels in partial response patients decreased to 16.1% and 19.3% of the level before treatment, respectively. DSM-CPT therapy can be a potential therapy for liver metastases. (J GASTROINTEST SURG 2006;10:249–258)

KEY WORDS: Transarterial chemoembolization, degradable starch microsphere, irinotecan, mitomycin-C, liver metastases

Degradable starch microspheres (DSMs) are agent to be developed for transient occlusion of small arteries, which are readily degraded in the blood by alpha-amylase.[1,2] The microspheres are $45 \pm 7 \times 10^{-6}$ m in diameter, and descend to lodge the arteriole/capillary level.[3] An anticancer drug coadministered with DSM to the hepatic artery is selectively trapped and concentrated with DSMs in the area, and it is expected that DSM enhanced the anticancerous activity.[3,4] Several studies in metastatic liver tumors indicate that transarterial therapy with DSMs and anticancer drug improved the therapeutic effects.[5–8]

Irinotecan (CPT-11) is a potent inhibitor of topoisomerase I and an anticancer agent mainly for adenocarcinomas. Several randomized phase III studies have shown the potent effect of CPT-11 for advanced colorectal cancer (CRC).[9–12] Nowadays, it is one of the key drugs of therapeutic strategy for CRC with 5-fluorouracil (5-FU), leucovorin (LV), and oxaliplatin (L-OHP).[10,12,13] Mitomycin-C (MMC) has also been demonstrated to be effective against metastatic CRC.[14,15] Preclinical studies have shown that a combination of MMC and CPT-11 synergistically inhibits tumor growth in vitro.[16,17]

We herein report cases with liver metastases of adenocarcinoma (with the origins of CRC for four and gastric cancer for one) treated with transarterial chemoembolization with the combination of DSM, CPT-11, and MMC (DSM-CPT therapy) and discuss the possibilities of the therapy for neoadjuvant chemotherapy and chemotherapy for the patients with advanced disease mainly in the liver, on an outpatient basis.

PATIENTS AND METHODS

Five patients underwent DSM-CPT therapy for liver metastases that originated from CRC for four

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and gastric cancer for one. They all had advanced disease that initially lacked indication for surgery (all have multiple liver metastases spread out in the whole liver and three have extrahepatic distant metastases). They were all males with an age range of 42–78 years (mean age, 55.2 years). Three of them (cases 2–4) had pretreatment histories with 5-FU or related agents, and four of them had combined systemic (cases 3 and 4) or local (cases 1 and 2) chemotherapy during the period of DSM-CPT therapy.

Protocol

Doses of 200–1200 mg (required doses for stasis of the whole blood flow of the hepatic artery, which were determined for each patient) of DSM, 80 mg of CPT-11, and 8 mg of MMC were used for a single injection via the catheter or the reservoir port. The first two patients (cases 1 and 2) with the lesions only in the liver received a single injection of DSM-CPT therapy, followed by daily continuous 5-FU (1000 mg/24 hr) + LV (100 mg/3 hr) arterial infusion for 7 and 6 days (disrupted early in case 2 with side effects), started 7 days after DSM-CPT therapy. Cases 3 and 4 with extrahepatic lesions (lung, lymph node, etc.) received repeat injections of DSM-CPT therapy combined with systemic chemotherapies (CPT-11 and tegafur/uracil for case 3, S-1 and CDDP for case 4). Case 5, who had suspicious but not defined peritoneal lesions, underwent short-term repeat solo therapy of DSM-CPT for the liver and was observed to have change in the suspicious peritoneal lesions.

Case 1

A 55-year-old man was referred to our department with giant and multiple liver tumors. Sigmoid colon cancer was revealed, and the liver tumors were diagnosed as synchronous metastases. After single transarterial chemotherapy with carboplatin, doxorubicin, and MMC for the liver, he underwent sigmoidectomy February 27, 2004. DSM (1200 mg) -CPT therapy was performed on March 17, followed by daily continuous 5-FU (1000 mg/24 hr) + LV (100 mg/3 hr) arterial infusion for 7 days. After the string of therapies, the levels of tumor markers were markedly decreased and the follow-up computed tomography (CT) examination and positron-emission tomography (PET) scan revealed massive (8–90%) necrosis of the liver tumor (Fig. 1). After 2 months of a good PR (partial response) period, he underwent surgery for brain metastasis in December 2004 (7 months after the hepatectomy). He is now well without recurrence in the liver at 1 year 2 months after the hepatectomy.

Case 2

A 50-year-old man with a history of left hemicolectomy for descending colon cancer was referred to our department with metachronous multiple liver metastases, in which the inferior vena cava was widely buried. He had undergone 5-FU therapy in the other hospital without response. DSM (270 mg)-CPT therapy was performed August 20, 2004, followed by daily continuous 5-FU (1000 mg/24 hr) + LV (100 mg/3 hr) arterial infusion for 6 days (disrupted with side effects: nausea, diarrhea, and leukopenia). After the string of therapies, the levels of tumor markers were markedly decreased (Fig. 2) and the follow-up CT examination and the PET scan revealed decreased size of the liver tumor. After percutaneous transhepatic portal embolization for the hypertrophy of the residual liver volume and 2 months of good PR period, he underwent surgery. (However, he died of disseminated intravascular coagulation as a complication of the surgery, and his long-term outcome after the surgery could not be evaluated.)

Case 3

A 52-year-old man was referred to our department for rectal cancer, synchronous multiple liver metastases, and a lung metastasis. After the surgery for the rectum, systemic and transarterial chemotherapies with 5-FU and LV were given from September 29, 2003, which resulted in progressive disease and side effects (leukopenia and nausea). From December 2003, DSM (200 mg)-CPT therapy was performed combined with periodical systemic CPT-11 administration and oral daily administration of tegafur-uracil (UFT). Four DSM-CPT-11 therapies and two half-dose DSM-CPT-11 therapies resulted in PR of the disease (Fig. 3). After 4 months of a PR period, side effects (abdominal pain and nausea) prevented him from the transarterial therapy (last injection was at March 25). Thereafter, although he was received various chemotherapies, systemic injection of CPT-11, cisplatin (CDDP), and oral UFT and S-1, the disease gradually progressed and he died November 29, 2004.

Case 4

A 42-year-old man with a history of low anterior resection for rectal cancer was referred to our department with growing multiple liver metastases, which were treated with 5-FU/LV systemic and transarterial therapies for 10 months. The CT examination after the admission revealed multiple lung metastases and hepatic hilar lymph node metastases, in addition

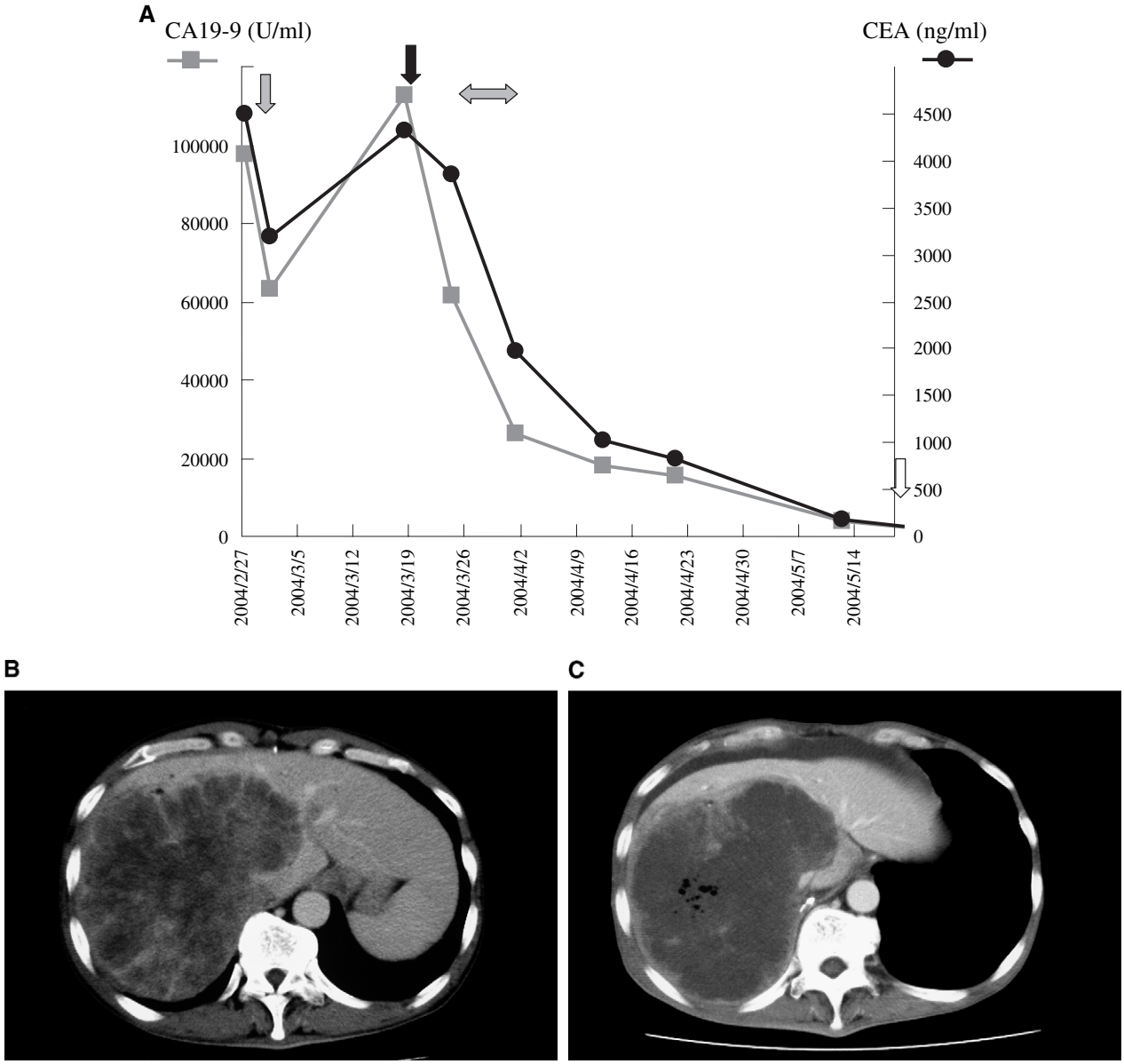

Fig. 1. CA19-9 and CEA levels (**A**) and the CT examination with contrast (**B**: pretreatment, **C**: posttreatment) in case 1. (**A**) *Black vertical arrow* indicates single injection of DSM-CPT therapy. *Gray vertical arrow* indicates the sigmoidectomy for the original site. *White vertical arrow* indicates hepatic resection for metastases. *Horizontal mark* shows the period of continuous infusion of 5-FU/LV. After the single string of therapies, the levels of tumor markers were markedly decreased. After 2 months of a good PR period, he underwent surgery. (**B, C**) The CT examination with contrast in case 1 showed the enhancement inside the tumor, which was observed before the therapy, disappeared in the whole area of the tumor, and air density areas appeared in the center of the tumor.

to the liver metastases. He underwent systemic chemotherapy with oral S-1 and intravenous CDDP from April 13, 2004. Since the effect was insufficient, DSM (600 mg)-CPT therapy was added to the combination from June 4. With repeated (three times) DSM-CPT therapies, he obtained a 3-month PR period (Fig. 4). However, repeated cholangitis due to the common bile duct obstruction by the lymph node disturbed the therapy. After September, although he received systemic chemotherapies with S-1, UFT, LV, CPT-11, and/or CDDP, the disease gradually progressed and he died on January 17, 2005.

Case 5

A 77-year-old man with a history of distal gastrectomy for gastric cancer was referred to our department with growing multiple liver metastases, which

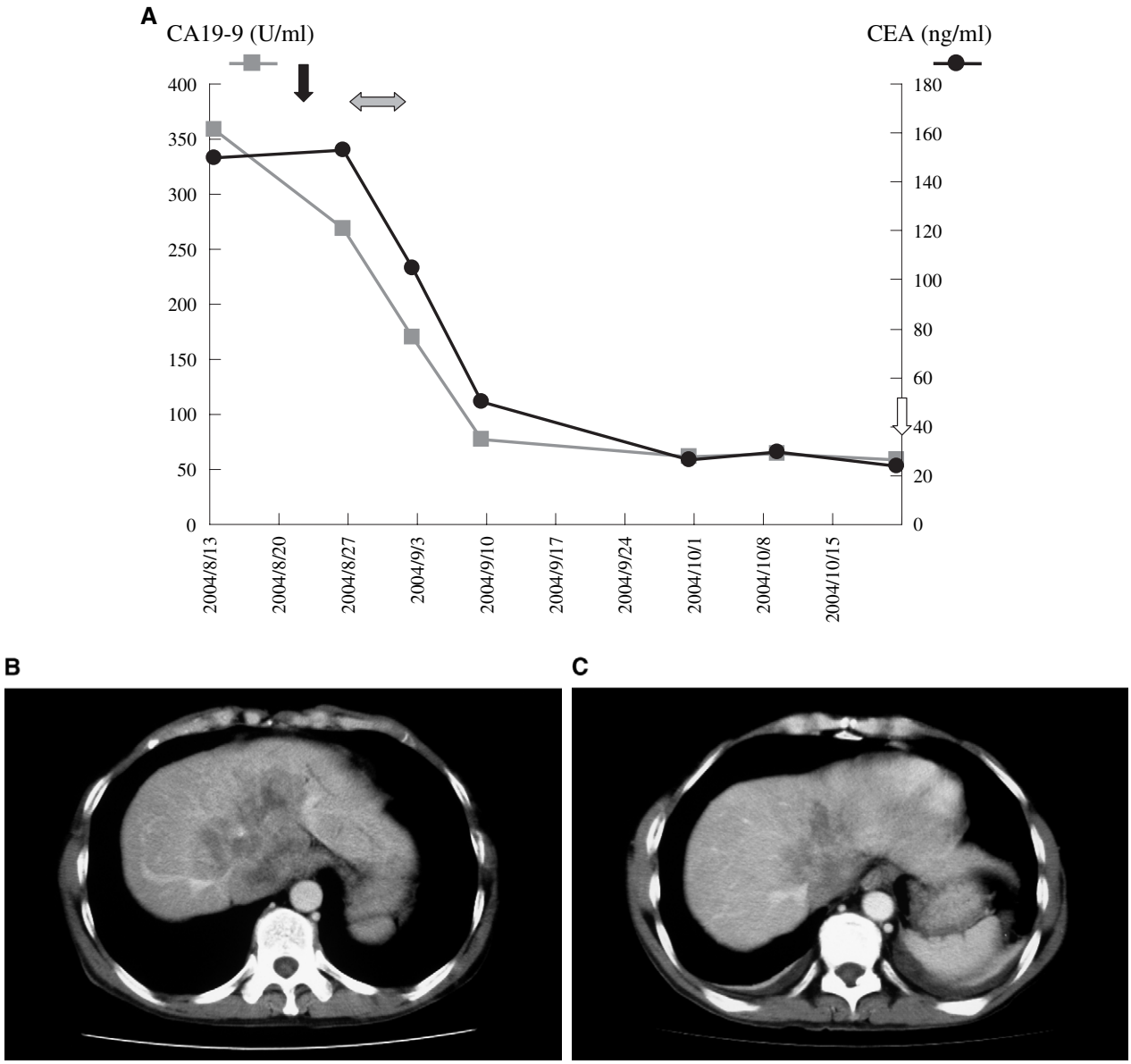

Fig. 2. CA19-9 and CEA levels (**A**) and the CT examination with contrast (**B**: pretreatment, **C**: posttreatment) in case 2. (**A**) *Black vertical arrow* indicates single injection of DSM-CPT therapy. *White vertical arrow* indicates hepatic resection for metastases. *Horizontal mark* shows the period of continuous infusion of 5-FU/LV. After the single string of therapies, the levels of tumor markers were markedly decreased. (**B, C**) The CT examination with contrast in case 2 showed the tumor diameter regression of 55.7% (PR). After percutaneous transhepatic portal embolization for the hypertrophy of the residual liver volume and 2 months of a good PR period, he underwent surgery.

had been treated with S-1 for 2 months. Since the effect of S-1 therapy was decreasing and he also developed side effects (decrease of leukocytes and platelet), DSM (200 mg)-CPT therapy was introduced. Although the level of tumor markers, which had been increasing rapidly, was sustained for the short duration with three DSM-CPT therapies (Fig. 5), peritoneal metastases (which had been suspicious, but not defined) became clear on the follow-up CT examination and the therapy was disrupted. He died of the disease on April 14, 2005.

Summary of cases is given in Table 1.

Injection Methods

For injection of the drugs, the femoral artery was catheterized while patients were under local

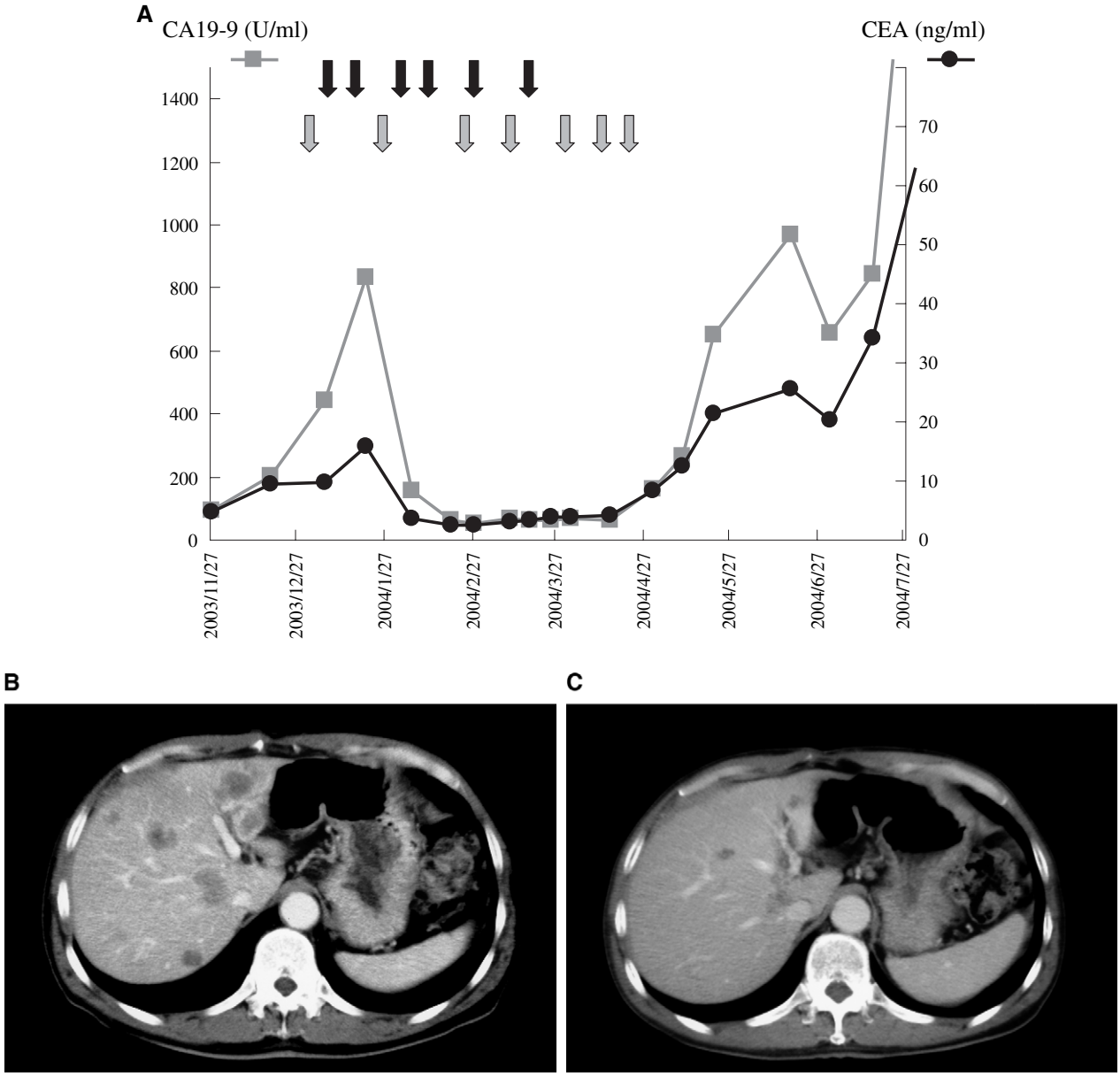

Fig. 3. CA19-9 and CEA levels (**A**) and the CT examination with contrast (**B**: pretreatment, **C**: posttreatment) in case 3. (**A**) From December 2004 (after the failure of systemic and transarterial chemotherapies with 5-FU and LV), DSM (200 mg)-CPT therapy (*black vertical arrows*) was performed combined with periodical systemic CPT-11 administration (*gray vertical arrow*) and oral daily administration of tegafur-uracil (UFT). (**B, C**) The CT examination with contrast in case 3 showed the tumor diameter regression of 65.6% (PR). After 4 DSM-CPT therapies and 2 half-dose DSM-CPT therapies with 4-month PR of the disease, side effects (abdominal pain and nausea) prevented him from the transarterial therapy. Thereafter, although he was received various chemotherapies, the disease gradually progressed and he died on November 29.

anesthesia. To obtain information about hepatic arteries, tumors, tumor thrombi, and portal hemodynamics before embolization, digital subtraction angiography was performed selectively in the celiac and proper, right, or left hepatic artery, and portography was performed from the superior mesenteric artery. All collateral flows to the liver were embolized. The tip of the catheter was placed in the proper hepatic artery or right and/or left hepatic artery to inject embolic agents at the first treatment and the reservoir port was placed. The tip of the port catheter was placed in the gastroduodenal artery with coils, and a side hole of the catheter for injection was placed at the bifurcation of the

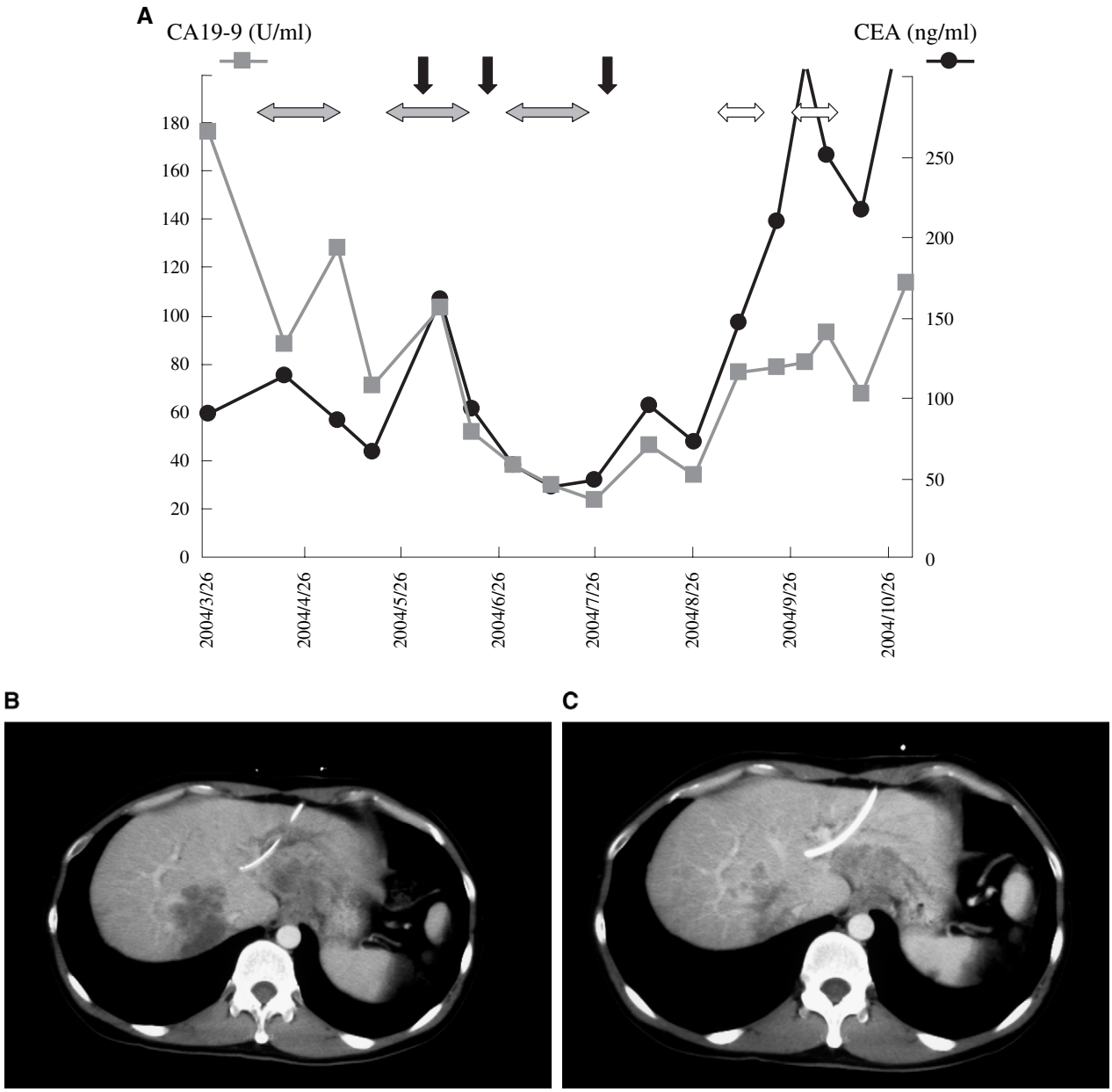

Fig. 4. CA19-9 and CEA levels (**A**) and the CT examination with contrast (**B**: pretreatment, **C**: posttreatment) in case 4. (**A**) He underwent systemic chemotherapy with oral S-1 and intravenous CDDP (*gray horizontal marks*) from April 13, 2004 (after the failure of systemic and transarterial chemotherapies with 5-FU and LV). Since the effect was insufficient, DSM (600 mg)-CPT therapy (*black vertical arrows*) was added. (**B, C**) The CT examination with contrast in case 4 showed the tumor diameter regression of 50% (PR). With repeated (3 times) DSM-CPT therapies, he obtained 3-month PR period. However, repeated cholangitis due to the common bile duct obstruction with lymph node gradually progressed and disturbed the therapy. After September, although he received systemic chemotherapies with S-1, UFT, LV, CPT-11, and/or CDDP (*white horizontal marks* indicate systemic therapies with S-1 and CPT-11 in **A**), the disease gradually progressed and he died on January 17, 2005.

proper hepatic artery and the gastroduodenal artery to obtain the injection flow only to the liver. At the first injection, DSMs were administered to ascertain the optimal DSM dosage per patient for the treatment.

Evaluation of the Effects and Statistics

The antitumor response was assessed with dynamic CT and also tumor marker levels according to the RECIST guideline in 2000.[18] Toxic effects were

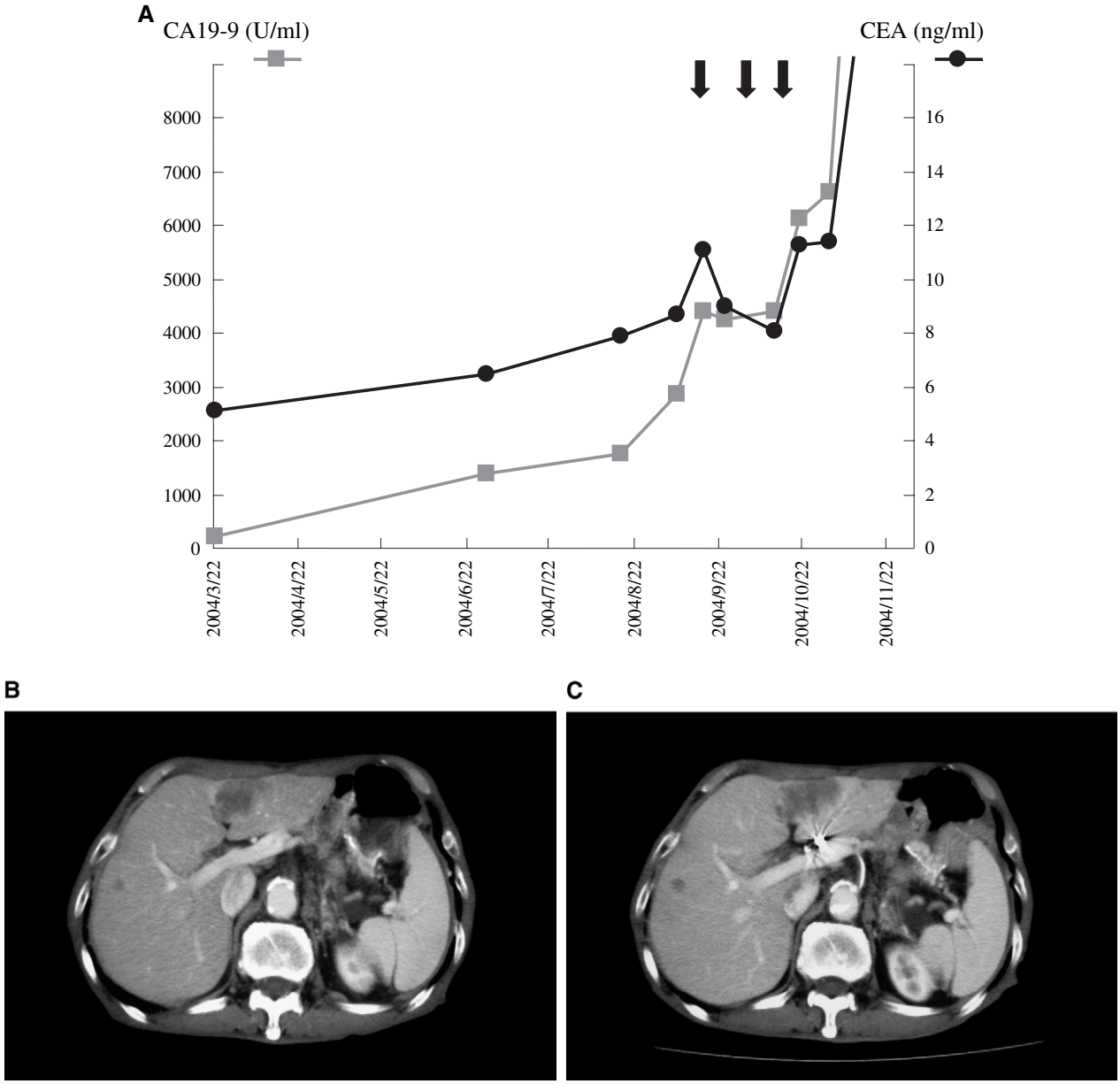

Fig. 5. CA19-9 and CEA levels (**A**) and the CT examination with contrast (**B**: pretreatment, **C**: posttreatment) in case 5. (**A**) DSM (200mg)-CPT therapy (*black vertical arrows*) was introduced after the failure of S-1 therapy. Although the level of tumor markers, which had been increasing rapidly, was sustained for the short duration with 3 injections of DSM-CPT therapies, evident peritoneal metastases became clear in the follow up CT examination and the therapy was disrupted. (**B, C**) The CT examination with contrast in case 5 showed the tumor diameter regression of 0%. He died of the disease on April 14, 2005.

evaluated using the National Cancer Institute (USA) Common Terminology Criteria for Adverse Events v3.0 in December 2003.

RESULTS

Response

After one to six injections, four of the patients with CRC cancer origin (cases 1–4) had PR, which persisted longer than 2 months, and the disease progressed in one patient with gastric cancer origin (case 5). Two of the PR patients underwent surgery after 2 months of PR (Table 1). Carcinoembryonic antigen (CEA) and CA19-9 levels in the patients with PR decreased to 16.1% and 19.3% of the level before the treatment, respectively (Figs. 1–5).

The evaluation using enhanced CT with contrast shows the tumor diameter regression of 13.1% (case 1, Fig. 1), 55.7% (case 2, Fig. 2), 65.6% (case 3, Fig. 3), 50% (case 4, Fig. 4), and 0% (case 5,

Table 1. Summary of the cases

Patient	Age (yr) Gender	Origin	Time of Diagnosis	Extrahepatic Diseases	Pretreatments	Combination Therapies	Dose of DSM (mg)	No. of DSM Injections	Response	Adverse Events (> Grade 2)
1	55/M	S colon	Synchronous	No	Carboplatin, ADR, MMC IA	5-FU/LV IA	1200	1	PR	No
2	50/M	D colon	Metachronous	No	5-FU IV	5-FU/LV IA	270	1	PR	Leukopenia
3	52/M	Rectum	Synchronous	Lung	5-FU/LV IV, IA	CPT-11 IV, UFT PO	200	6	PR	Abdominal pain
4	42/M	Rectum	Metachronous	Lung, LN	5-FU/LV IV, IA, CDDP IV + S-1 PO	CDDP IV + S-1 PO	600	3	PR	No
5	77/M	Stomach	Metachronous	? (Peritoneum)	S-1 PO	No	200	3	PD	No

DSM = degradable starch microspheres; ADR = doxorubicin; MMC = mitomycin - C; 5-FU = 5-fluorouracil; LV = leucovorin; CDDP = cisplatin; S-1 = tegafur/CDHP/potassium oxonate; CPT-11 = irinotecan; UFT = tegafur/uracil; PR = partial response; PD = progressive disease.

Fig. 5), respectively. Although the tumor diameter regression of case 1 was measured as 13.1%, the enhancement with the contrast of the tumor was disappeared in the whole area and the air density area appeared in the tumor of case 1 after the therapy. It was confirmed that a 90% area of the tumor showed the necrosis in the histologic examination with the surgical specimen. Therefore, the response of case 1 after the therapy was evaluated as PR. In case 5, the response was evaluated as PD, because of peritoneal lesions.

Toxicity

In the course of repeated injections, two patients (cases 3 and 4) developed time-limiting (less than 1 hour) abdominal pain (case 3, grade 3; case 4, grade 1), occurred immediately after the injection, and nausea (grades 2 and 1). Vomiting was also observed in case 3 (grade 1).

Leukopenia was observed in all patients (grade 3 in case 2, grade 2 in cases 3–5, grade 1 in case 1). Grade 1 alopecia developed in cases 2 and 3. Grade 2 diarrhea occurred in case 2. There were no significant elevations observed in the levels of serum aspartate aminotransferase, alanine aminotransferase, bilirubin, γ-glutamyl transpeptidase, and alkaline phosphatase.

DISCUSSION

The standard first-line therapy for advanced CRC, including liver metastases out of surgical indications, had been 5-FU/LV for two decades. The therapy has a response rate of 23% and a median survival time (MST) of 11.5 months.[19] Two recent randomized phase III trials have shown that a combination of CPT-11, 5-FU, and LV is associated with higher response rates, a longer TTP, and longer overall survival than 5-FU/LV therapy.[10,12] In another randomized phase III trial, the MST was 18.6 months with L-OHP/5-FU/LV, 14.1 months with CPT-11/5-FU/LV, and 16.5 months with L-OHP/CPT-11.[13] Nowadays, CPT-11 has become one of the key drugs of therapeutic strategy for advanced CRC, including liver metastases out of surgical indications, with 5-FU, LV, and L-OHP. MMC has also been demonstrated to be effective against metastatic CRC.[14,15] A combination of protracted venous infusion (PVI) 5-FU and MMC in chemotherapy-naive patients results in a higher response rate and longer TTP than PVI 5-FU alone (response rate 54% versus 38%, median TTP 7.9 months versus 5.4 months).[15] When used as a second-line treatment for patients with CRC refractory to bolus

5-FU/LV, PVI 5-FU and MMC show modest activity with acceptable toxicity.[14] There are also preclinical studies, which have shown that a combination of MMC and CPT-11 synergistically inhibits tumor growth in vitro.[16,17]

DSMs were developed to provide transient occlusion of small arteries.[1,2] The occlusive effect of the agent lasts up to 80 minutes before its degradation by alpha-amylase in the blood and presents time-limiting effect of embolization with less damage to the vessels.[5] An anticancer drug coadministered with DSMs to hepatic artery is selectively trapped and concentrated in the area, and thus the enhancement of agent's activity is expected.[3,4] Several studies in metastatic liver tumors indicate that transarterial therapy with DSMs and anticancer drug improved the therapeutic effects, compared with an anticancer drug alone.[5–8] It is reported that CPT-11 is converted to its active form SN38 mainly in the liver.[20] Thus, it is possible that the stasis of CPT-11 in the liver with DSMs leads to the enhancement of the activity, not only with the higher concentration in the area. The present combination of DSM, CPT-11, and MMC has a possibility of synergy effects of those drugs. Furthermore, DSMs with transient occlusion of the blood flow cause less damage to the arteries.[5] The fact may make the repeated (for example, weekly or biweekly) administrations possible with the reservoir port on an outpatient basis. DSM-CPT therapy may become a potential therapy for patients with metastases mainly in the liver.

Although the dose of DSM was determined as the dose required for the stasis of whole blood flow of the hepatic artery, the dose of CPT-11 for transarterial injection was reported varied. There are two different phase II studies, which use five consecutive days CPT-11 injection of 20 mg/m^2/day and every 3 weeks CPT-11 injection of 200 mg/m^2.[21,22] We set our dose (80 mg) of CPT-11 in counting on the enhancing effect of coadministered DSMs and also aiming outpatient basis treatment with weekly or biweekly injection.

In cases 1 and 2, single injection followed by 7-day transarterial 5-FU/LV infusion was extremely effective and the persisted effect for 2 months leads to the surgical removal of the tumors. These cases may indicate that the therapy can be potent neoadjuvant chemotherapy for some patients. Moreover, the short duration of the preoperative chemotherapy should be an advantage as a neoadjuvant therapy. Although patient 1 survived 1 year 2 months without recurrence in the liver, he developed a brain metastasis, and patient 2 was dead from complications of the surgery. The long-term outcome after the surgery cannot be evaluated from these cases, and further investigation will be needed. Patient 2 developed relatively severe adverse events, which were not observed in the other four patients. Since the events were similar to the side effects listed as those from CPT-11 and MMC, they seem to be caused by the effect mainly of anticancer drugs, not DSMs. It is thought that patients with low hepatic uridine diphosphate glucuronosyltransferase 1A1 activity may be at an increased risk for CPT-11 toxicity.[23] Further investigation is necessary to clarify whether these events in case 2 were special events for this individual or due to possible common events. And the necessity and type of the combination therapies to DSM-CPT therapy, such as 5-FU/LV, and their long-term results after the surgery should be also investigated.

Cases 3 and 4 showed the possibility of weekly or biweekly outpatient basis treatment of DSM-CPT. They were introduced to DSM-CPT therapy in poor and rapidly deteriorating general conditions and survived for 11 and 7 months with 4- and 3-month PR periods after the failure of their pretreatments. The fact was considerable as a salvage therapy. However, adverse events (abdominal pain, nausea, vomiting, etc.) developed in both cases 3 and 4 after multiple injections. They seemed to be caused by DSM-CPT therapy itself (especially by DSMs), because the onsets are immediate after the injection and time limiting. Those may be caused by the ischemic attack. Repeated injection might have caused decreased blood flow to the tumors and thus overflow of DSMs to the other organs. Periodical angiography from the reservoir port might be needed to avoid these events. However, evident liver or biliary damage was not observed after the multiple injections.

Although the fact that DSM-CPT was less effective in case 5 who had gastric cancer origin may mean that the therapy is more effective for CRC metastasis, more investigations are also needed.

CONCLUSION

DSM-CPT therapy can be a potential therapy for patients with advanced disease mainly in the liver, especially for patients with CRC origin. It could be one of the candidate therapies for the neoadjuvant and/or the outpatient basis second-third line after the failure of combination therapies with 5-FU/LV, CPT-11, and/or L-OHP for those patients.

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Effect of Gastric Bypass on Barrett's Esophagus and Intestinal Metaplasia of the Cardia in Patients With Morbid Obesity

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Gastric bypass in patients with morbid obesity should be an excellent antireflux procedure, because no acid is produced at the small gastric pouch and no duodenal reflux is present, due to the long Roux-en-Y limb. Five hundred fifty-seven patients with morbid obesity submitted to resectional gastric bypass, and routine preoperative upper endoscopy with biopsy samples demonstrated 12 patients with Barrett's esophagus (2.1%) and three patients with intestinal metaplasia of the cardia (CIM). An endoscopic procedure was repeated twice after surgery, producing seven patients with short-segment Barrett's esophagus (BE) and five patients with long-segment BE. Body mass index (BMI) decreased significantly, from 43.2 kg/m^2 to 29.4 kg/m^2 2 years after surgery. Symptoms of reflux esophagitis, which were present in 14 of the 15 patients, disappeared in all patients 1 year after surgery. Preoperative erosive esophagitis and peptic ulcer of the esophagus healed in all patients. There was regression from intestinal metaplasia to cardiac mucosa in four patients (57%) with short-segment BE, and in one patient (20%) with long-segment BE. Two (67%) of three cases with CIM had regression to cardiac mucosa. There was no progression to low- or high-grade dysplasia. Gastric bypass in patients with Barrett's esophagus and morbid obesity is an excellent antireflux operation, proved by the disappearance of symptoms and the healing of endoscopic esophagitis or peptic ulcer in all patients, which is followed by an important regression to cardiac mucosa that is length-dependent and time-dependent. (J GASTROINTEST SURG 2006;10:259–264)

KEY WORDS: Gastric bypass, Barrett's esophagus, cardia intestinal metaplasia

Barrett's esophagus is an acquired condition, secondary to chronic duodeno-gastroesophageal reflux, in which the distal esophageal mucosa is replaced by columnar mucosa with intestinal metaplasia.[1–4] The main importance of this metaplastic change at the distal esophagus is that there is a significant increase in the risk of developing adenocarcinoma.[1–3,5] On the other hand, patients with morbid obesity have an increased incidence and severity of gastroesophageal reflux.[6–9] Gastric bypass is the "gold standard" operation for patients with morbid obesity.[10–14] In this operation, no acid is produced when a small gastric pouch is created with a total capacity less than 20 ml, and the presence of a long Roux-en-Y limb presents duodenal and intestinal content from refluxing into the gastric pouch and into the esophagus. Therefore, it seems an "ideal operation" for the treatment of chronic reflux in patients with Barrett's esophagus.

The purpose of the present prospective study was to determine the behavior of intestinal metaplasia at the distal esophagus (Barrett's esophagus) or at the cardia (intestinal metaplasia of the cardia) after gastric bypass in morbidly obese patients.

MATERIAL AND METHODS

Patients Studied

This prospective study started in August 1999 and ended on October 23, 2004, and included 557

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patients with morbid obesity who were submitted to open resectional gastric bypass. The details of the whole group and the surgical technique have been published extensively elsewhere.[15,16] All patients were submitted to preoperative endoscopy and biopsy studies. From this group, 15 patients had intestinal metaplasia at the distal esophagus or cardia and were divided in two groups: (a) 12 (2.1%) patients with Barrett's esophagus with a mean of 47.4 years (range, 33–62), consisting of five men and seven women with a mean body mass index (BMI) of 43.9 kg/m^2 (range, 35–61; there was no exclusion in this group); and (b) three (0.5%) patients with intestinal metaplasia of the cardia (CIM) with a mean age of 48.6 years (range, 43–56), consisting of all women with a mean BMI of 37.8 kg/m^2 (range, 35–39).

All patients with a BMI below 40 kg/m^2 had an associated comorbidity such as diabetes or arterial hypertension.

Endoscopic Examination

One author (A.C.) performed all endoscopic procedures, employing an Olympus GIFXQ-20 endoscope (Olympus, Tokyo, Japan). After a 12-hour night fast and pharyngeal anesthesia with lydocaine, the scope was introduced through the mouth with the patient in supine left lateral position. Special care was taken to measure the exact location of the squamous-columnar junction at the beginning and at the end, avoiding the "push" and "pull" effect of the endoscope.[17] The length of the columnar-lined distal esophagus was measured as the distance between the squamous-columnar junction and the endoscopically located lower esophageal sphincter, which is the point where the proximal extent of the gastric rugal folds meet with the tubular esophagus.[17,18] The presence of erosions proximal to the squamous-columnar junction was carefully recorded. For the present study, endoscopic procedure was performed before surgery and at least two times after surgery in each patient. During this procedure, in every patient, 4-quadrant biopsy samples were taken 5 mm distal to the squamous-columnar junction. In patients with short-segment Barrett's esophagus (BE), two more samples were taken 2 cm distal. Among patients with long-segment BE, four samples were taken, each 2 cm distally according to the length of the columnar mucosa. Therefore, between 6 and 16 samples were taken in each patient during each endoscopic procedure, with a mean of 8.5 samples per patient. Also, in each patient, two samples were taken at the antrum.

Patients were divided into two groups[10]: (a) short-segment Barrett's esophagus, (length $\geqslant$ 30 mm) and (b) long-segment Barrett's esophagus, (length $\geqslant$31 mm).

Regression of BE

We defined the regression of BE as: (1) the loss of intestinal metaplasia with the presence of only cardiac and/or fundic mucosa on two consecutive endoscopic examinations with biopsy specimens and (2) the decrease in length of columnar-lined mucosa at the distal esophagus, at least 3 cm in length.

Histologic Analysis

All samples were submerged immediately in 10% formalin solution, sent for histologic examination, and stained with hematoxylin-eosin and Alcian blue stain at pH 2.5; fundic mucosa was identified by the presence of parietal and chief cells at the deep glandular layer, and cardiac mucosa was identified by the presence of mucous-secreting columnar cells. Intestinal metaplasia was defined by the presence of goblet cells. The presence of Helicobacter pylori was also evaluated at the columnar-lined mucosa with intestinal metaplasia.

Monitoring of Esophageal Exposure to Acid Reflux

All examinations were performed after 12 hours of fasting. The details of this procedure have been extensively detailed in previous publications.[19–21] The results of acid reflux test were expressed as the percentage of time during which the intraesophageal pH was less than 4 (normal values less than 4%).

Surgical Procedure

In all patients, a resectional open gastric bypass was performed as previously described.[15,16] A small gastric pouch with a total capacity less than 20 ml was constructed with the use of linear staplers (Tyco Healthcare, Norwalk, CT). The gastrojejunal anastomosis was performed with a circular Stapler 25 (Tyco Healthcare). The length of the Roux-en-Y loop varied between 125 and 150 cm.

Statistical Analysis

The chi-square test and the variance analysis test were employed, with $P < 0.05$ as significant.

Follow-up

All patients with intestinal metaplasia of the distal esophagus or cardia were carefully followed and there was no loss of patients. Endoscopic control was performed at 12 to 14 months after surgery.

RESULTS

After operation, all patients had an uneventful recovery, and no postoperative complications were observed in any patient. Eleven patients out of 12 with BE (91.7%) had symptoms of chronic gastroesophageal reflux of more than 2 years duration, whereas all three patients with CIM had gastroesophageal (GE) reflux symptoms. Upper endoscopy before operation was normal in all patients with CIM, whereas among patients with BE, seven (58.3%) had erosive esophagitis and two of them also had a peptic ulcer of the esophagus (16.7%). Hiatal hernia was present in three patients (25%). Patients with BE were divided in two groups: (a) short-segment BE with seven patients (58.3%) and (b) long-segment BE with 5 patients (41.7%).

The 24-hour pH study performed on four patients with BE had a mean percent time with pH < 4 of 40.2% (range, 19–75), whereas resting lower esophageal sphincter pressure, performed in five patients, showed a mean pressure of 10.2 mmHg (range, 3–17).

Table 1 shows the endoscopic and histologic findings before and several times after surgery in all patients included in the present study. The mean follow-up for patients with BE was 24 months; patients with CIM had a mean follow-up of 29 months. The BMI, before and at 24 months after surgery, is also included.

The mean BMI among patients with BE decreased significantly, from 43.2 kg/m^2 to 29.4 kg/m^2 ($P < 0.0001$). The same occurred among patients with CIM. The results of antral biopsies showed no intestinal metaplasia in any patients. H pylori was present in three patients with BE (25%) and in one CIM patient (33%) at the antrum.

After surgery, symptoms of GE reflux disappeared in all patients; the endoscopic study at 1 and 2 years after surgery showed esophageal mucosa was normal in all patients. At 12 months after surgery, erosive esophagitis or peptic ulcer of the esophagus had healed in all. The careful histological analysis of the columnar-lined mucosa revealed that among seven patients with short-segment BE, four (57%) patients showed regression of IM to cardiac mucosa at a mean time of 25 months after surgery. Among five patients with long-segment BE, one (20%) patient showed regression to oxyntic cardiac mucosa. There was no progression to low- or high-grade dysplasia. Of two patients with low-grade dysplasia and short-segment BE, one patient regressed to cardiac mucosa, whereas the other patient showed disappearance of low-grade dysplasia with persistence of intestinal metaplasia after surgery. The evaluation of the presence of H pylori at the columnar-lined mucosa revealed that before surgery, it was present in two patients, and after surgery, it was absent in both of them; however, it appeared in two patients, one with cardiac and one with oxyntic cardiac mucosa.

Among the three patients with CIM, two (67%) patients showed regression to cardiac mucosa at a mean of 14 months after surgery. H pylori was present in one patient before surgery and disappeared after surgery; however, it appeared in one other patient after surgery.

DISCUSSION

The results of the present study suggest that gastric bypass, in patients with morbid obesity and the presence of Barrett's esophagus or intestinal metaplasia of the cardia, is an excellent antireflux operation, resulting in regression of IM to cardiac mucosa in a significant number of patients.

We have reviewed a large number of publications concerning obesity and GE reflux, the effect of bariatric surgery on GE symptoms, and functional studies. However, very few mention the specific study of patients with BE before operation, and there is no publication concerning the effect of bariatric surgery on BE or CIM. There is only one study from Balsiger et al.[22] from the Mayo Clinic, which mentions that the endoscopic control in seven patients with BE and gastric bypass has shown no progression to severe dysplasia, but makes no mention of any regression. Several years ago, we published a study concerning the incidence of obesity in control subjects, patients with reflux esophagitis and patients with BE.[23] We noticed that 25% of patients with BE were obese, compared to 5% of control or reflux esophagitis patients. Later Ovrebo et al.[24] found no BE in any of 38 patients with morbid obesity who were submitted to surgery. Furthermore, Balsiger et al.[22] reported that of 25 patients submitted to conversion from vertical banded gastroplasty to Roux-en-Y gastric bypass, 29% showed histologic changes of BE at the distal esophagus. Finally, Surter et al.[25] reporting on the endoscopic findings among 344 patients with morbid obesity, found BE in four patients (1.2%).

Table 1. Endoscopic and histologic findings at the distal esophagus before and after gastric bypass for morbid obesity in patients with Barrett's esophagus or intestinal metaplasia of the cardia

Sex	Age	BMI before surgery	BMI after surgery (24 mo)	Length BE (mm)	Endoscopy		Histological findings				H pylori at columnar mucosa		Time for regression (mo)
					Before surgery	After surgery	Before surgery	I Control (mo)*	II Control (mo)*	III Control (mo)*	Before surgery	After surgery	
Barrett's esophagus													
Woman	46	37	29	20	Esophagitis	Normal	IM	Carditis (14)	Carditis (28)		(−)	(−)	14
Man	33	61	42	20	Esophagitis	Normal	IM	IM (26)			(+)	(−)	No
Woman	52	35	22	20	Normal	Normal	IM + LGD	IM + LGD (24)	IM (36)	Carditis (48)	(−)	(−)	48
Woman	60	38	26	30	Esophagitis	Normal	IM	Carditis (24)	Carditis (36)		(−)	(−)	24
Woman	54	39	27	20	Normal	Normal	IM	IM (12)	IM (24)		(−)	(−)	No
Man	38	48	31	20	Normal	Normal	IM	Carditis (14)	Carditis (28)		(+)	(+)	14
Man	46	51	28	20	Normal	Normal	IM + LGD	IM + LGD (12)	IM + LGD (24)	IM (48)	(−)	(−)	No
Man	39	45	27	40	Normal	Normal	IM	IM (28)	IM (40)		(−)	(−)	No
Woman	56	36	31	40	Peptic ulcer	Normal	IM	Funditis (24) + Carditis	Funditis (36) + Carditis		(−)	(+)	24
Woman	44	40	29	60	Esophagitis	Normal	IM	IM (18)	IM (28)		(−)	(−)	No
Man	39	45	32	80	Esophagitis	Normal	IM	IM (33)	IM (72)		(−)	(−)	No
Woman	62	44	29	120	2 Peptic ulcer	Normal	IM	IM (12)	IM (24)		(−)	(−)	No
		Mean 43.2	29.4										
Intestinal metaplasia of the cardia													
Woman	43	36	22	—	Normal	Normal	IM	Carditis (14)	Carditis (28)		(−)	(−)	14
Woman	56	39	25	—	Normal	Normal	IM	IM (12)	IM (24)		(−)	(+)	No
Woman	47	38	23	—	Normal	Normal	IM	Carditis (14)	Carditis (34)		(+)	(−)	14
		Mean 37.7	23.3										

BMI = body mass index (Kg/M2); BE = Barrett's esophagus; IM = intestinal metaplasia; LGD = low-grade dysplasia.
*Endoscopy performed after surgery (mo).

There is no mention in any publication concerning patients with CIM or the effect of surgery on BE among patients submitted to gastric bypass or any other bariatric procedure. We have performed routine endoscopic and histologic assessment in 557 patients with morbid obesity and have found 2.1% of patients with BE and 0.5% of patients with CIM. This seems a low figure, which probably is due, in part, to the young age of patients with morbid obesity, but the importance of treating BE supports the need for endoscopic and histologic surveillance.

The most important point of our study refers to the postoperative findings concerning the behavior of the metaplastic epithelium after gastric bypass in patients with morbid obesity. The pathophysiological effect of this type of surgery is very similar to what we have used for patients with Barrett's esophagus.[20,26] In this operation, acid secretion is abolished after bilateral vagotomy plus partial distal gastrectomy, and duodenal content is diverted by the addition of a Roux-en-Y loop of 60 to 70 cm long. We have published the results concerning the decrease in acid secretion,[20] the significant decrease in acid reflux into the esophagus,[19,20,26–28] and the complete and permanent abolition of reflux of duodenal content into the esophagus.[20,27] Gastric bypass completely reproduces the following pathophysiological consequences: (a) gastric acid secretion is reduced to a minimal output because there is a large reduction in parietal cell mass with the creation of the gastric pouch[29,30] and (b) the long Roux-en-Y limb (125 to 150 cm) produces a permanent and complete abolition of reflux of duodenal or intestinal content into the esophagus. Therefore, if both types of surgical procedures produce similar pathophysiological effects, the behavior of intestinal metaplasia at the distal esophagus should be similar. In another study,[31] we have examined the effect of acid suppression and duodenal diversion in 78 patients with BE, all surveyed more than 5 years after the primary operation. Thirty-one patients had short-segment BE and 47 patients had long-segment BE. Among them, the regression of intestinal metaplasia to cardiac or fundic mucosa occurred in 66% and 60%, respectively. The mean time for regression was about 44 months after surgery.

In the present study, the regression of IM to cardiac mucosa occurred in 57% of patients with short-segment BE, at a mean of 25 months after surgery. Among patients with long-segment BE, there was regression in 20%. The present report may have a possibility of sampling error, which is present in any study of this kind. Although we tried to obtain at least eight samples during every endoscopic procedure, the possibility of sampling error cannot be excluded. A much longer follow-up (more than 5 years) than the present study (24 months) is needed to reach definitive conclusions. However, the importance of the present study is that if the refluxate material is eliminated (which is harmful to the distal esophagus, i.e., acid and duodenal content, even without performing any antireflux procedure to improve the function of the lower esophageal sphincter), intestinal metaplasia, and therefore the eventual risk of developing adenocarcinoma, can regress to cardiac or oxyntic cardiac mucosa, which is length-dependent (inversely related to the length of BE) and time-dependent (it is directly related to the length of follow-up). We have not found any previous reference to the behavior of intestinal metaplasia of the cardia after gastric bypass, and therefore we cannot compare our results with other reports. We challenge other surgical groups performing gastric bypass to determine an objective surveillance and to conduct several endoscopic and histologic studies among patients with BE and morbid obesity.

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Diagnosis and Therapy of Primary Hypertrophic Pyloric Stenosis in Adults: Case Report and Review of Literature

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Adult idiopathic hypertrophic pyloric stenosis (AIHPS) is a misleading anatomic and radio-clinical entity of unknown etiology. Only about 200 cases have been reported in the literature. It is a benign disease resulting from hypertrophy of the circular fibers of the pyloric canal. Despite the recent progress in radiography and endoscopy, it is very hard to define hypertrophic stenosis in adults. Differentiation of primary from secondary pyloric stenosis is frequently a task of the pathologist rather than the surgeon. The main therapy is surgical, although endoscopic dilatation has been tried. There remains controversy over the best surgical approach. A case is reported of a 48-year-old male patient with AIHPS who was subjected to distal gastrectomy. This paper discusses the possible causes of the disorder, the recommended diagnostic steps, and the different surgical approaches. (J GASTROINTEST SURG 2006;10:265–269)

KEY WORDS: Primary hypertrophic pyloric stenosis, adults, idiopathic

CASE REPORT

A 48-year-old Caucasian male without significant prior medical or surgical history presented with a gradual onset of early satiety, nausea, vomiting, and the inability to digest solid food for the past 6 months. The patient gradually modified his diet to pureed foods and several cans of Ensure daily without experiencing any weight loss. He denied any history of peptic ulcer disease or gastroesophageal reflux. A barium upper gastrointestinal tract (UGI) study showed delayed emptying of the stomach. A narrow and slightly elongated pyloric channel was noted, as well as indentations at the base of the duodenal bulb causing a mushroomlike deformity (Fig. 1). No ulcerations or defects were found. A CT scan of the abdomen suggested narrowing and thickening of the distal stomach and proximal duodenum. The upper endoscopy showed narrowing of a short segment at the duodenal bulb, giving it a cervix-like appearance (Fig. 2). Mild superficial erosions at the area of narrowing were noted. Biopsies revealed acute and chronic inflammation with superficial ulceration and epithelial reparative changes but were negative for malignancy. Subsequently, the patient agreed to surgery. After thorough exploration of the abdomen, findings were limited to a thickened pylorus. A distal gastrectomy and Billroth II reconstruction in an isoperistaltic anticolic fashion using a stapled anastomosis was performed. Pathology demonstrated marked hypertrophy and hyperplasia of the pyloric musculature without any fibrosis. The thickness of the muscularis propria was 1.5 cm. Prominent hypertrophy of the muscularis mucosae with muscle strands within the lamina propria was also noted (Fig. 3). Ganglion cells appeared normal. Minimal chronic gastritis was present; however, there was no evidence of significant peptic ulcer disease or malignancy. These findings are consistent with idiopathic hypertrophic pyloric stenosis in the adult. The patient tolerated the procedure without any complications and remains asymptomatic 2 years after surgery.

DISCUSSION

To the present, approximately 200 cases of primary idiopathic pyloric stenosis in the adult have been reported in the literature.[1,2,3] The disease was

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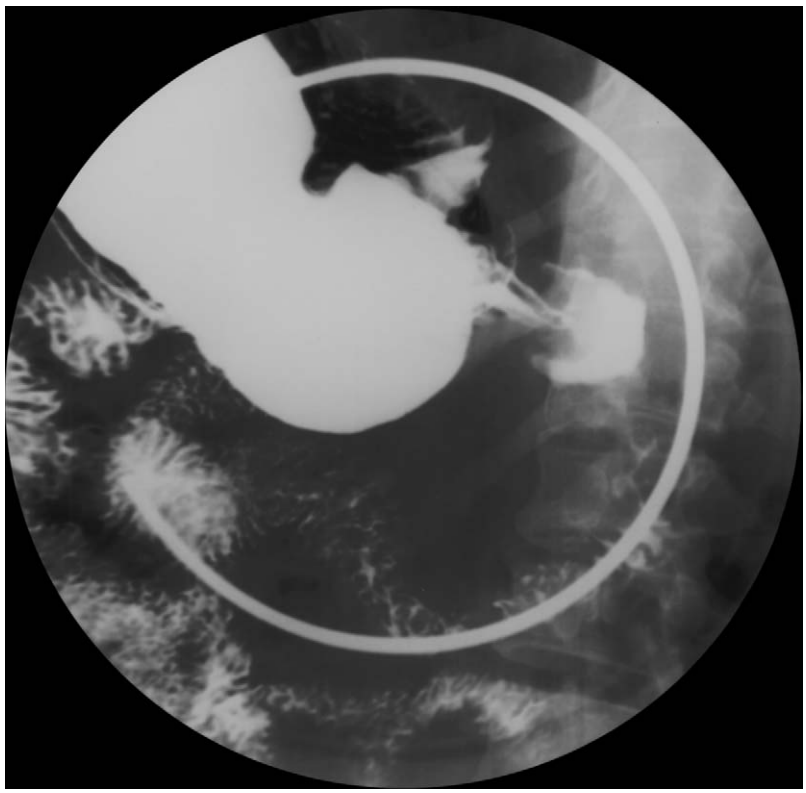

Fig. 1. UGI series with barium shows a narrow and slightly elongated pyloric channel. A convex indentation at the base of the duodenal bulb can be seen causing a mushroomlike deformity (Kirklin's sign).

first described by Cruveilhier in 1835. Kirklin and Harris[4] were the first to define the classic radiologic findings in 1933.

The etiology of adult hypertrophic pyloric stenosis remains in doubt.[1–3,5] Differentiation of primary from secondary pyloric stenosis is frequently the task of the pathologist rather than the surgeon.[6] Secondary pyloric stenosis is considered to be secondary to other diseases in the upper gastrointestinal tract such as hypertrophic gastritis, peptic ulcer disease, or malignancy. The secondary type is the most common and is recognized by a long-standing history of gastrointestinal complaints. In a series of 100 patients with pyloric outlet obstruction, the obstruction was secondary to peptic ulcer disease in 37% and to malignancy in 42% of the patients. Only a single patient with primary stenosis was identified.[6] Most authors believe that hypertrophic pyloric stenosis of the adult is due to persistence of the infantile form into adult life.[1,3–5,7] Hypertrophy may not become manifest until a complicating factor such as edema, spasm, or inflammation precipitates pyloric occlusion.[2,8] Nevertheless, controversy still persists whether the muscle hypertrophy is a cause or effect of other conditions such as ulcers or gastritis.[6,7,9] Ulcers may develop as a result of chronic obstruction.[2,5,6,8] It has been experimentally shown in dogs that occlusion of the pylorus produces gastric ulceration.[10] The fact that, in the majority of gastric ulcers, pyloric hypertrophy is not present supports this theory. Other possible discussed etiologies include protracted pylorospasm, neuromuscular incoordination due to changes in Auerbach's plexus, and vagal hyperactivity.[2,3,5,8,11,12]

Anatomically, the pylorus is composed of a thicker inner circular layer that is contiguous with the inner circular muscle layer of the stomach, but is completely separated from the circular muscle of the duodenum. These muscle fibers form two distinct loops, which are united in a muscle torus and constitute a palpable band in the pylorus. An outer thinner longitudinal muscle layer is contiguous with the longitudinal layer in the stomach and continues to travel into the duodenum.[6,13] The normal pyloric channel measures about 0.8–1 cm in length and has a wall thickness of 3–8 mm with an average of 4 mm.[1,5,6,9,13] In adult idiopathic hypertrophic pyloric stenosis (AIHPS), the pylorus is bulbous or fusiform, with its thickest portion at the pyloroduodenal junction.[6,13] It is generally accepted that muscle 1 cm or thicker and persistent canal elongation of more than 2 cm are abnormal.[1,4,5] A resected hypertrophied pylorus

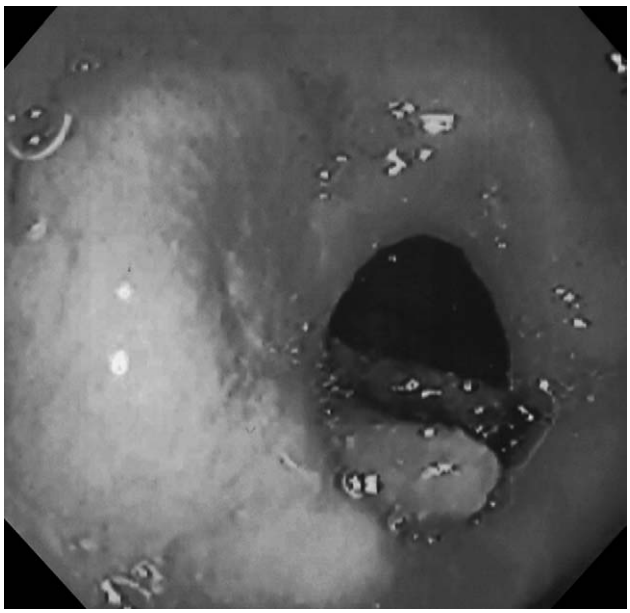

Fig. 2. Endoscopic picture showing narrowing at the duodenum resembling a cervix. Pieces of solid food material are seen in the duodenal bulb area.

looks much like the cervix.[1] Microscopically, there is marked hypertrophy and hyperplasia of the circular and occasionally of the longitudinal muscle in a focal or diffuse fashion.[1,6,13,14] Varying degrees of inflammatory changes or edema and degenerative changes in the ganglion cells of the myenteric plexus have been reported.[1,12,14] In this patient, the thickness of the muscularis propria in the pylorus was 1.5 cm. Prominent hypertrophy of the muscularis mucosae with muscle strands within the lamina propria was noted as well (Fig. 3).

Criteria for diagnosis depend on several features. Typical clinical findings include symptoms of delayed gastric emptying. The absence of pain at the onset of symptoms is a significant diagnostic point. Other diseases that can cause delayed gastric emptying such as scleroderma, diabetes mellitus, gastroparesis, or secondary pyloric stenosis need to be ruled out.[3,15] The radiologic and gastroscopic findings are often nonspecific. A barium UGI study is indicated in the evaluation of gastric outlet obstruction, but cannot unequivocally differentiate hypertrophic pyloric stenosis from neoplasm or inflammatory disorders.[1,2] The diagnosis should be suspected if the radiologic studies show elongation of the pyloric canal to 2–4 cm with varying degrees of obstruction. In AIHPS, the contour of the pyloric canal and the distal antrum is smooth with no ulcerations or defects.[1,6,9,13] The canal is frequently eccentric in relation to the antrum, located on the side of the lesser curvature.[1,6,16] The narrowed pyloric canal may be seen as an extremely thin line of barium (string

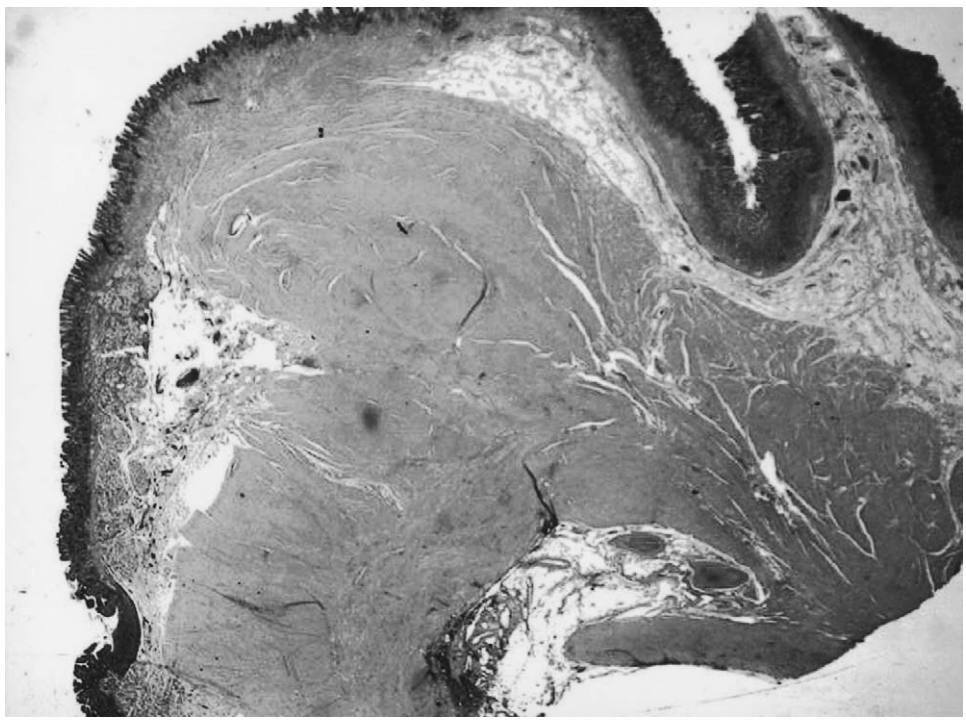

Fig. 3. Low-power view of the entire pylorus (hematoxylin and eosin stain). The thickness of the muscularis propria in the pylorus is 1.5 cm. Also noted is a prominent hypertrophy of the muscularis mucosae.

sign).[17] Often a convex indentation at the base of the duodenal bulb is produced by the marked thickening of the pyloric muscle, causing a mushroomlike deformity (Kirklin's sign[4]; Fig. 1). The presence of a barium-filled cleft between the hypertrophied muscle and the fibers of the pylorus itself can project into either one or both sides of the pylorus proximal to the base of the bulb (Twining's sign).[18] It must be stressed that the radiographic diagnosis should be based on the presence of several findings because none of the individual signs described above are pathognomic. Transabdominal sonography is successfully used for the diagnosis of infantile hypertrophic pyloric stenosis. Its usefulness for diagnosis of adult hypertrophy is also described.[6] The classic finding at gastroscopy is a fixed, markedly narrowed pylorus with a smooth border. This appearance is described as a "donut" or as the cervix sign (Fig. 3).[1,13,19] Such a picture may be simulated by temporary pylorospasm, but persists in hypertrophy after anticholinergic medication.[1] An additional feature is the failure of the pylorus to close completely.[13,20] Intubation of the duodenum with the endoscope may be impossible.

The aims of treatment are to rule out carcinoma histologically, to cure the obstruction, and to treat any accompanying complications. Simson et al.[21] suggest that a full-thickness biopsy is necessary for exclusion of malignancy. Most authors agree that a histologic confirmation of the disease is recommended to safely rule out malignancy. Therefore, the treatment of choice for this disorder remains surgical. Endoscopic pyloric dilatation has been attempted but is associated with a high rate of recurrence. It should only be considered for patients with high operative risk, as it may palliate symptoms.[1,20] Histologic diagnosis cannot be obtained by this procedure. The Ramstedt pyloromyotomy, commonly performed in children, is criticized in this situation because of the possibility of diverticulum formation, pyloric scarring with only partial relief, and mucosal laceration.[2,3,5,6,22] Gastroenterostomy bears the serious disadvantage of precluding visualization of the pylorus, high recurrence rate, and lack of histologic confirmation.[6,14,23] Finney or Heineke-Mikulicz pyloroplasty has been suggested because they are short and curative procedures.[1–3,5,6,20] Both procedures have technical disadvantages in patients with very thick muscle and show tendency for recurrent obstruction.[22] Brahos et al. introduced the double pyloroplasty technique, in which a posterior myotomy is combined with the usual Weinberg modification of the Heineke-Mikulicz procedure.[22] This technique enables a tension-free, transverse closure of the initial anterior longitudinal myotomy incision. The pylorus can be inspected for neoplasm, biopsies can be obtained, and a gastric resection can be avoided, especially in patients who would not tolerate such. Danikas et al.[20] describe in their paper a laparoscopic pyloroplasty. The authors suggest that the laparoscopic technique offers a safe and effective approach, with earlier postoperative recovery and shorter hospitalization. Most authors, however, favor a limited distal gastric resection with Billroth I or II anastomosis.[1,2,8,13,14,21,24] This technique is especially recommended if the pylorus wall is very thick, making a pyloroplasty technically difficult.

CONCLUSION

Primary hypertrophic pyloric stenosis in adults is an unusual entity. The diagnosis can only be established after the exclusion of more common causes of gastric outlet obstruction. There is no consensus as to the most efficacious therapy. However, a gastric resection seems to be favored by most authors.

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Effect of Roux-en-Y Gastric Bypass on Satiety and Food Likes: The Role of Genetics

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Among factors influencing the outcome of bariatric surgery may be genetics and familial risk. The purpose of this study was to assess the etiology of obesity and its impact on hunger, satiety, and food likes in obese patients undergoing Roux-en-Y gastric bypass (RYGB). This study was based on 76 patients undergoing RYGB procedures performed by a single surgeon. A previously described 100-point obesity risk index (ORI) was used to assess familial obesity risk. Hunger and satiety were assessed using a standardized Visual Analog Scale "Snickers" test, and food preferences for regular vs. low-fat potato chips were measured preoperatively and postoperatively. Patients were stratified preoperatively into high ORI (n = 34) and low ORI (n = 42) groups. Before operation, high-ORI patients preferred high-fat (regular) potato chips to low-fat (baked) potato chips, whereas the low-ORI patients liked both food types equivalently ($P < 0.05$). After operation (n = 43), both groups showed lower preferences for high-fat potato chips ($P < 0.05$ for high-ORI group). As anticipated, hunger was dramatically suppressed after RYGB. However, there was more satiety in the high-ORI group ($P < 0.05$, ANOVA). Most patients undergoing bariatric surgery had a strong familial or genetic component to their disease. RYGB in high-ORI patients was associated with a significant decline in preference of fatty food and a significantly prolonged drop in hunger ratings after a fast and after a standard 282 kcal meal. The success of bariatric surgery may be influenced by the etiology of obesity. (J GASTROINTEST SURG 2006;10:270–277)

KEY WORDS: Morbid obesity, Roux-en-Y gastric bypass

The Roux-en-Y gastric bypass procedure results in weight loss, primarily by a process that seems to be due to reductions in meal size. The mechanical effects produced by the small gastric reservoir, a small anastomosis, and the decreased clearance of the Roux-en-Y limb combine to produce early and prolonged satiety. However, the precise mechanisms whereby the gastric bypass procedure produce satiety and/or decrease hunger are poorly understood. Furthermore, the effects of this procedure on hunger or satiety have not been well documented. Objective measures of hunger or satiety in gastric bypass patients have not been measured.

Understanding of the pathophysiology of obesity has increased markedly over the last decade.[1–3] Scores of candidate genes have been described with obvious links to the obese phenotype.[1,4,5] Genes affecting obesity, energy balance, feeding behavior, leptin levels, satiety, hunger, and adipocyte differentiation have all been described.[1–3] The treatment of obesity is problematic, with most medical interventions being ineffective. Many factors likely affect the success of medical interventions or bariatric surgery in obese humans.[3,6,7] However, despite the major effect of genetics on human fatness, few studies have assessed the impact of genetics on the medical or surgical treatment of obesity. To our knowledge, the role of genetics on satiety and hunger has not been investigated. The first purpose of the present study, therefore, was to assess the association between a global assessment of genetics in obese individuals, and hunger or satiety in patients undergoing Roux-en-Y gastric bypass. The second purpose was to assess the effects the genetics on weight loss.

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METHODS

Between November 2000 and September 2001, 76 patients scheduled to undergo gastric bypass for morbid obesity were asked to enter this prospective study. Entry criteria included age greater than 18 years, body mass index (BMI) greater than 40kg/m^2, or BMI greater than 35kg/m^2 with a reversible life-threatening comorbidity. The previously described obesity risk index (ORI) was applied to all patients at initial surgical consultation (Table 1).[8] This ORI was devised to quantify the genetic contribution to an individual's weight (0 = no genetic component, 100 = maximum possible genetic risk for obesity). Six elements measured the genetic influence on a patient's weight: three personal weight milestones (BMI at age 10, 20, and 30 years) and three family history factors (parents' BMI, siblings' BMI, and secondary degree relatives' BMI).[9] Patients were subsequently placed into two genetic risk groups, low and high risk, if less than or greater than the mean ORI for the study.

Hunger/Satiety

Hunger was measured using a 9-point Likert scale. Subjects were asked to make a mark along a 9-point scale with "extremely full" = 1 and "extremely hungry" = 9 at the extremes. Subjects were studied in response to a standard gastric load, with a 2.16-ounce Snickers bar (282 calories, 126 fat calories, 16 protein calories, and 140 carbohydrate calories). Subjects consumed a Snickers bar at 10:00 A.M. after an overnight fast. The hunger/satiety Likert scale was recorded at time = 0, and then every 30 minutes for 3 hours. No eating or drinking was permitted during the 3-hour measurement. The hunger/satiety test was repeated at least 3 months postoperatively.

Table 1. Obesity risk index

Element	Result	Points
Personal history		
Age 10	BMI < 23	0
	23 < BMI < 26*	10
	BMI ⩾ 26†	20
Age 20	BMI < 30	0
	30 < BMI < 40	10
	BMI > 40	20
Age 30	BMI < 35	0
	35 < BMI < 50	5
	BMI > 50	10
Family history		
Mother	BMI < 30	0
	30 < BMI < 40	7
	BMI > 40	14
Father	BMI < 30	0
	30 < BMI < 40	7
	BMI > 40	14
Siblings	Mean BMI < 30	0
	30 < Mean BMI < 40	6
	Mean BMI > 40	12
Second degree relatives	Each with BMI > 35	2 to maximum 10 points (N = 5)

BMI = Body mass index in kg/m^2.
*If not known, substitute "very obese."
†If not known, substitute "fattest kid in class."

Food Likes/Dislikes

All patients consumed, in random order, a single serving bag of regular fried (high fat) and baked (low fat) potato chips on consecutive days, both preoperatively and postoperatively. A 9-point Likert scale was completed with 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

Surgical Procedures

All patients underwent Roux-en-Y gastric bypass using a standardized open technique by the same surgeon.[10–12] A 15 cc proximal gastric pouch was constructed using three superimposed firings of a heavy wire linear stapler (Ethicon Endosurgery, Cincinnati, OH). A 75 cm Roux-en-Y limb was constructed with a 10 mm hand-sewn running polypropylene suture technique for the pouch-jejunal anastomosis. Postoperatively, all patients were asked to comply with a nutritional supplement regimen of (1) multivitamins plus iron, 2 pills daily; (2) vitamin B12, 500 μg daily; and (3) calcium, 1200 mg daily.

Statistical Analysis

Postoperative weight or current weight was recorded, allowing computation of postoperative BMI and calculation of percent change in BMI (preop BMI – postop BMI/preop BMI). We also calculated the absolute change in BMI, as well as comparison to ideal BMI (postop BMI – 24/24). Data was analyzed by group or paired *t* tests, or ANOVA with Fishers post hoc test, as appropriate. Relationships of multiple variables to outcome were calculated using linear regression with *P* values less than 0.05 considered statistically significant. Data are presented as mean ± standard error (± SEM).

RESULTS

Seventy-six patients were entered into the trial and completed preoperative hunger/satiety and

food like/dislike studies. Postoperative testing (greater than 3 months) was completed in 43 patients. Long-term follow-up was achieved in 68 patients, eight patients were lost to follow-up. The average follow-up was 32 months. The preoperative BMIs and preoperative Snickers test results were compared in the patients who did or did not complete postoperative testing. There was no difference ($P = 0.4$) in BMI, hunger levels, or ORI ($P = 0.75$) in these groups. Thus, the group that completed postoperative testing is the same population as those that did not. Postoperative testing was also performed later than 3 months in several patients. There was no relationship between the change in BMI and how much later the test was recorded ($P = 0.55$). There were 60 females and 16 males. The average age was 43 years (range, 18–67 years); the average weight was 324 pounds (range, 210–448 pounds), with an average BMI = 52 kg/m^2 (range, 38–76 kg/m^2). The mean ORI was 38 ± 3 (range, 0–83). Therefore, the low- and high-ORI groups were less than and ⩾38, respectively. Similar to our previous work,[8] about 15% of patients had little or no evidence of genetic risk for obesity (ORI less than 10).

Food Likes/Dislikes

The relationship between ORI and preferences for foods of all 76 patients studied preoperatively is summarized in Table 2. Preoperative and postoperative studies in the 43 patients who completed both studies are illustrated in Fig. 1. As shown in Table 2, patients with high ORIs greatly preferred regular potato chips to baked potato chips (7.2 + 0.3 vs. 4.9 + 0.4). On the other hand, patients with low ORIs reported similar preference scores for regular and baked potato chips. The mean scores for baked potato chips in low-ORI and high-ORI patients were 5.8 ± 3.4 and 4.9 ± 0.4, respectively ($P < 0.05$). Preoperative and postoperative scores were compared in 43 patients. The key statistically significant finding is in the high-ORI group. Before surgery, these patients preferred high-fat (regular) potato chips. After surgery, the preference for fatty foods decreased ($P < 0.05$) in the high-ORI patients. As shown in Fig. 1, all patients seemed to like regular and baked potato chips the same. The loss of preference for the high-fat potato chips after surgery in the high-ORI patients was statistically significant.

Table 2. Relationship of obesity risk index to likes/dislikes of high-fat or low-fat foods in 76 patients studied preoperatively (1 = dislike extremely, 9 = like extremely)

	Total (n = 76)	Low ORI (n = 42)	High ORI (n = 34)
Regular Potato Chips	7.0 ± 0.2	6.8 ± 0.4	7.2 ± 0.3
Baked Potato Chips	5.4 ± 0.3	5.8 ± 0.4	4.9 ± 0.4*

ORI = obesity risk index.
*$P < 0.05$ vs. low ORI, group t test.

Fasting Hunger

The mean score for fasting hunger in all 76 patients was 6.7 ± 0.2 (i.e., T = 0 in preoperative Snickers bar test; Fig. 2). The values in low-ORI and high-ORI patients were 6.5 + 0.3 and 6.9 ± 0.2, respectively ($P = 0.25$). In the 43 patients studied postoperatively (Fig. 3), the mean fasting hunger score was 6.1 ± 0.2, significantly less than the preoperative fasting hunger score ($P = 0.04$, paired two-tailed t test). The differences in preoperative and postoperative fasting hunger scores in the low-ORI (n = 21) patients were not significant, whereas the decrease in hunger in the high-ORI (n = 22) patients was significant. The clinical significance of this statistically significant decrease in hunger is unknown.

Satiety

The results of the preoperative Snickers bar test in all 76 patients are illustrated in Fig. 2. Using linear regression, we found a statistically significant negative relationship between long-term satiety and excess BMI ($P = 0.026$). In other words, patients with higher BMIs were less full (or more hungry) 3 hours after a meal. Of interest, hunger or satiety scores at 3 hours were significantly greater than the fasting score in preoperative patients ($P < 0.0001$). That is, preoperative patients were hungrier 3 hours after consuming a Snickers bar than after a fasting interval of at least 10 hours. The scores in the 43 patients studied both preoperatively and postoperatively are shown in Fig. 3. As noted above, there was a small but statistically significant decrease in hunger at T = 0. As expected, satiety was greatly enhanced in patients after gastric bypass procedures. The average scores one-half hour after consumption of the Snickers bar, preoperatively and postoperatively, were 5.2 + 0.2 and 3.0 + 0.2, respectively. Satiety resolved in a linear fashion in all groups (Fig. 3). As discussed above, preoperative patients were more hungry 3 hours after the test meal than after a 10-hour fast. In contrast, postoperative patients were less hungry 3 hours after a Snickers bar than at T = 0. That is, satiety seems to be maintained for prolonged time after a small meal. As

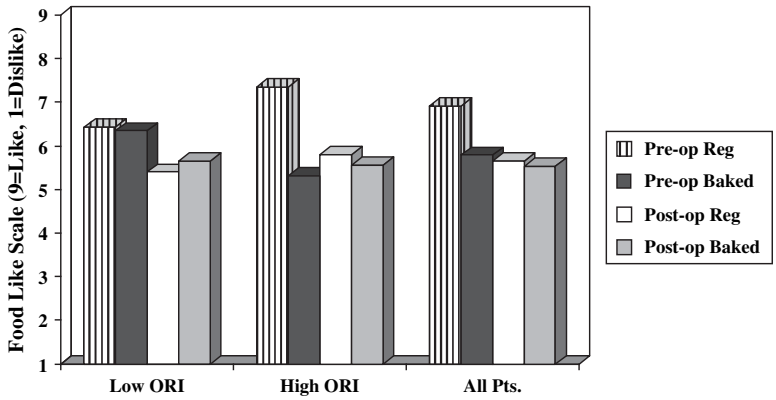

Fig. 1. Likes or dislikes of low-fat (baked potato chips) and high-fat (regular potato chips) food in 43 patients studied preoperatively and postoperatively (1 = dislike extremely, 9 = like extremely). High-ORI patients preferred high-fat food preoperatively but not postoperatively. ORI = obesity risk index; Pts = patients.

shown in Fig. 3, patients with high ORIs had significantly less hunger (or greater satiety) at each time interval after 1 hour.

Weight Loss

With an average follow-up of 32 months, the average weight was 224 pounds; the average BMI was 36.4 ± 1.0. The percent change in BMI was 28.7 ± 1.6%. The average percent loss of excess BMI was 53 ± 3.0%. We could not demonstrate a statistically significant relationship between the scores on the Snickers bar test and the postoperative change in BMI. Furthermore, using regression analysis, we did not find a relationship between the ORI and the change in BMI ($P = 0.236$). After adjusting for age, the relationship between ORI and change in BMI became stronger, approaching statistical significance ($P = 0.085$), and there was a statistically significant effect of ORI on the absolute change in BMI. However, ORI is correlated with preoperative BMI (i.e., high-ORI patients have higher BMIs), so that the relationship between absolute change in BMI and ORI becomes nonsignificant when we adjust for the preoperative BMI value.

After adjusting for ORI and age, we found no relationship between the preoperative hunger level (fasting score on the Snickers test) and the relative change in BMI. The satiety value at 30 minutes tended to correlate with the relative change in BMI, adjusting for ORI and age ($P = 0.068$). However, long-term satiety (satiety value at 180 minutes) had no apparent relationship to relative change in BMI. In summary, we did not find strong evidence that ORI predicted the amount of change in BMI. Similarly, the preoperative Snickers test did not predict the change in BMI.

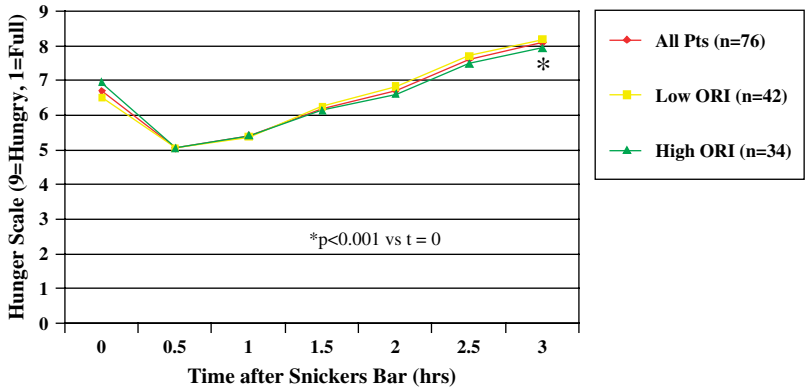

Fig. 2. Hunger after a fast (t = 0) and satiety for 3 hours after a 282 calorie (Snickers bar) preload in all 76 patients studied preoperatively (1 = extremely full, 9 = extremely hungry). Patients were more hungry 3 hours after the preload than after a 12-hour fast. ORI = obesity risk index.

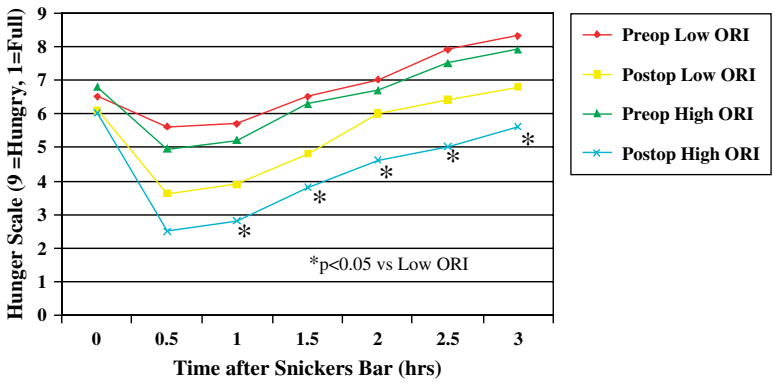

Fig. 3. Effect of a 282 calorie meal (Snickers bar) on satiety in 43 patients studied preoperatively and postoperatively. Patients with high ORI had significantly enhanced satiety (*$P < 0.05$, ANOVA). ORI = obesity risk index.

DISCUSSION

The primary purpose of the present study was to examine the relationship between a global measure of the genetic contribution to body weight and hunger, satiety, and food likes in morbidly obese patients. Second, we hoped to measure objectively the effects of gastric bypass procedures on hunger, satiety, and food likes. Finally, we wished to examine the hypothesis that genetics may play a role in the outcomes after gastric bypass procedures.

The unavoidable flaw of the present study is that there is not a single test that can identify our crucial output variable of genetically determined obesity. Obesity is a disease with multifactorial inheritance. Human obesity results from contributions from many genes, with loci on different chromosomes being centers for genetic abnormalities.[1,5,13] Because at least 20 different aberrations in brain-gut peptides have been linked to the risk for obesity, it is unlikely that any single obesity test will ever be available. Previous studies have designed models for risk of diseases with multifactorial inheritance.[9] Models of occurrence of diseases with multifactorial inheritance assume additive genetic effects and factor in the presence of the disease in parents, siblings, and second-degree relatives. In addition, age at onset of obesity and degree of obesity has been shown to correlate with established genetic defects known to affect human fatness.[9,14] For example, the effect of overfeeding on weight gain in children is significantly influenced by familial obesity factors.[14] We believe our obesity risk index is the best tool available to identify patients who have genetically determined obesity.

Studies of genetic epidemiology, such as adoption studies and twin studies, conclude that genetic factors account for at least 80% of the obesity risk.[15–17] However, many nongenetic factors influence hunger, satiety, and subsequent food intake.[18–19] Hedonistic qualities of food (e.g., taste, texture, and smell), environmental factors (e.g., food availability, cost), and emotional factors (e.g., eating when bored, depressed, stressed, happy) are important.[20,21] Macronutrient content (e.g., high-density foods, fat, or carbohydrates) of the diet is also a factor.[19,21,22] Previous studies have concluded that overweight or obese individuals show a tendency toward a greater liking and selection of energy-dense foods, which may contribute to the development and maintenance of obesity.[20,23–25] Data support the possibility that preference for fatty foods is a predisposing factor for obesity, rather than a result of it.[23] These investigators concluded, however, that difficulties in weight control might actually reflect problems with queues and motivations to eat (e.g., hunger), rather than heightened pleasure derived from eating. Reviews of the success of dietary programs focus on these hedonistic, environmental, emotional, and macronutrient factors.[26] For example, Freedman and co-workers[22] concluded that "dietary compliance is likely a function of psychological issues" and that "successful weight loss maintenance may be predicted by an individual's belief system."[22] Similarly, Ness-Abramof and Apovian,[27]in a review of the medical therapy of obesity, concluded that "failure to adhere to permanent lifestyle changes is responsible for the high rate of recidivism of obesity." Explanations for lack of efficacy in dietary programs focus on these psychological issues, seemingly ignoring the primary pathophysiology of obesity.[28] Complex interactions of brain-gut peptides control satiety and hunger, and genetics plays a crucial role in eating behavior.

It is likely that genetically determined abnormalities in satiety are clinically more important than

abnormalities in hunger or meal initiation in obese humans.[2] Until recently (see ghrelin discussion below), most studies have concluded that there is little physiologic evidence that appetite and meal initiation (i.e., hunger) are controlled by metabolic or hormonal signals.[2] It has been proposed that, under normal circumstances, meal initiation is largely based on learned associations such as habit and social environment.[2,22] Obesity research has focused, therefore, on the importance of how much is eaten or meal cessation (i.e., satiety), concluding that these signals are mediated by gut neuropeptides.[2] These studies suggest that aberrations in gut peptides and/or peptide receptors such as resistance to leptin or insensitivity to, or abnormal levels of, other satiety signals such as cholecystokinin are crucial in the regulation of body weight and have assumed that patients with obesity may demonstrate some or all of these aberrations.[2]

The identification of ghrelin as a "hunger" peptide may challenge the concepts stated above, and a recently published study suggested that fasting serum ghrelin levels (and by inference hunger) were suppressed in patients after gastric bypass procedures.[29–30] Our study found a small but statistically significant decrease in hunger after gastric bypass. Furthermore, high-ORI patients were hungrier after a fast than were low-ORI patients. To our knowledge, the present study is the first ever to measure objectively hunger after gastric bypass. Empirically, we have observed that most patients after gastric bypass procedures report decreased hunger. In contrast to the theories reviewed above regarding obesity in humans, our data indicates that this phenomenon of decreased hunger likely has a role in weight loss after gastric bypass. Because hunger in a fasted state is unlikely to reflect a mechanical effect of the gastric bypass, these findings support the proposal that hunger may be suppressed by hormonal mechanisms such as ghrelin. The present study also indicates that gastric bypass procedure affects food likes and dislikes. Patients with high genetic risk for obesity significantly preferred high-fat potato chips to baked potato chips at their initial visit. This is consistent with the literature reporting preferences for "energy dense" foods in obese humans.[23] After surgery, however, preference for fatty and nonfatty foods equalized. The etiology for this change is unclear. The Roux-en-Y gastric bypass as performed in this series produces minimal malabsorption. However, many patients report that they no longer enjoy fatty foods after the gastric bypass procedure. Whether this represents conditioned behavior to gastrointestinal intolerance of fatty foods, or a physiologic change in brain-gut peptides is unknown. Regardless, this statistically significant decrease in preference for fatty (energy dense) foods may contribute to weight loss after the gastric bypass procedure.

As anticipated, there was significant enhancement of satiety after the gastric bypass procedure. The presumed primary method of producing weight loss is a mechanical one, with significant restriction of food consumption and prolonged satiety due to delayed gastric emptying of the small pouch through the Roux-en-Y limb. The intriguing finding of the present study was that this enhancement of satiety is significantly greater in patients with high genetic risk for obesity. This finding may not be clinically significant and may not translate into better outcomes, as we could not demonstrate improved success in terms of weight loss in our genetically obese patients. It may, however, translate into improved satisfaction with the procedure. That is, patients with genetic obesity may more easily cope emotionally with the restricted ability to consume foods and enhanced satiety.

Previous investigators have attempted to identify factors that might predict success with medical or surgical treatment of obesity. Long-term success of nonoperative treatment of morbid obesity is unsatisfactory.[31–33] A review of 161 articles on the management of obesity concluded that, on average, 1%–2% of people taking part in weight loss programs maintain initial weight loss for 5 years.[32] Similarly, other studies claiming to demonstrate the importance of behavioral therapy and physical activity in weight loss programs actually showed increased weight 5 years after initiation of these programs.[34] Regardless, few authors have addressed the vital significance of genetics and presumed physiological aberrations in satiety and hunger in treating patients with morbid obesity.[26,33–36] Surgical series have attempted to identify factors that might predict their outcomes after surgery.[7,37] Younger patients with high activity levels demonstrate improved outcomes. Others have suggested that patients with preferences for sweets experience less weight loss after gastric bypass procedures compared to nonsweet eaters.[7] Our hypothesis, which we did not prove, was that patients with genetic obesity would demonstrate greater weight loss compared to those without apparent genetic predisposition to obesity (and presumed behavioral abnormalities). We believe that patients with genetic obesity consume excess calories, largely because of decreased satiety and/or increased hunger signals, which are corrected by surgery. On the other hand, nongenetic obese patients may consume calories due to nonphysiologic signals that may not be affected by surgery. Our data indicate that patients with a greater component of genetic obesity did, in

fact, report greater satiety after a standard meal. However, these findings did not clearly translate into greater weight loss. Future studies will investigate the impact of genetics on other outcomes such as patient satisfaction and health-related quality of life.

CONCLUSION

The present study objectively confirmed that the Roux-en-Y gastric bypass (RYGB) procedure markedly enhances satiety for at least 3 hours after a meal. We also demonstrated that RYGB patients are less hungry after a fast. Using a global measure of genetic contribution to obesity (ORI), we found that patients with high ORIs preferred fatty foods before but not after RYGB. We also demonstrated that patients with high ORIs had significantly greater satiety after a meal. However, we were unable to demonstrate a significant association between ORI and weight loss after RYGB.

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Surgical Management and Complex Treatment of Infected Pancreatic Necrosis: 18-Year Experience at a Single Center

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Infected pancreatic necrosis (IPN), the most severe form of acute pancreatitis, is responsible for most cases of pancreatitis-related morbidity and mortality. Since 1986, 220 patients with IPN have been treated. The surgical treatment was performed on average 18.5 days (range, 8–25 days) after the onset of acute pancreatitis and consisted of wide-ranging necrosectomy, combined with widespread drainage and continuous lavage. In 108 of the 220 cases, some other surgical intervention (distal pancreatic resection, splenectomy, total pancreatectomy, cholecystectomy, colon resection, etc.) was also performed. Following surgery, the supportive therapy consisted of immunonutrition (glutamine and arginine supplementation) and modification of cytokine production with pentoxifylline and dexamethasone. Continuous lavage was applied for an average of 44.5 days (range, 21–95 days), with an average of 9.5 L (range, 5–20 L) of saline per day. The bacteriologic findings revealed mainly enteral bacteria, but *Candida* infection was also frequently detected (21%). Forty-eight patients (22%) had to undergo reoperation. The overall hospital mortality was 7.7% (17 patients died). In our experience, IPN responds well to adequate surgical treatment, continuous, long-standing widespread drainage and lavage, together with supportive therapy consisting of immunonutrition and modification of cytokine production, combined with adequate antibiotic and antifungal medication.
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KEY WORDS: Infected pancreatic necrosis, surgical treatment, fungal infection, immunonutrition, pentoxifylline

The pancreatic necrosis combined with septic conditions is the leading cause of mortality in acute pancreatitis. Although aggressive organ-system support has resulted in an improved survival rate in the early stage of the disease, patients continue to die at a later stage from necrotic and septic complications, culminating in multiorgan failure.[1,2] The reported mortality rate of these complications ranges from 8% to 80%.[3–8] During the last 10 years, it has been recognized that pancreatic digestive enzymes may not play such a predominant role in the pathogenesis of complicated pancreatitis, which seems rather to result from the release of various inflammatory mediators from activated leukocytes. In fact, multiple organ failure and septic complications in acute pancreatitis are no different from the systemic complications of other diseases or injuries (sepsis, trauma, and burn) that do not involve the release of digestive enzymes from the pancreas. The pathophysiologic concept of severe acute pancreatitis is based on leukocyte activation as a key step leading from local to systemic inflammation culminating to the systemic inflammatory response syndrome.[9] This concept allows us to recognize that activated leukocytes have an important role in the multisystem involvement during severe acute pancreatitis and infected pancreatic necrosis (IPN).[10–13]

This report analyzes the results of our surgical strategy and complex treatment of IPN and complications, which are wide-ranging necrosectomy combined with other surgical interventions, continuous widespread lavage, and drainage together with

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supportive therapy consisting of immunonutrition and modification of cytokine production, combined with adequate antibiotic and antifungal medication.

MATERIAL AND METHODS

Patients

From 1986 to 2004, a total of 220 patients underwent surgery for IPN in our department. There were 168 males and 52 females with an average age of 44.3 years (range, 21–79 years). The etiology of the underlying acute pancreatitis was attributed to alcohol in 65%, biliary tract disease in 20%, and hyperlipidemia in 7%, and it was idiopathic in 8%. The underlying acute pancreatitis was particularly severe in these patients; the average number of APACHE II score was 15.5 (range, 11–32). The criteria for the diagnosis of IPN included the confirmation of previous or present pancreatitis and clinical and laboratory signs of sepsis as follows: fever (temperature $>38.4^\circ$ C) or hypothermia ($<35.9^\circ$ C), tachycardia (>110/min), tachypnea (>25/min), leukocyte count $>15 \times 10^9$/L or $<3 \times 10^9$/L, and at least one of the following: hypoxemia (PaO_2 <70 mm Hg), oliguria (urine output <30 ml/hr), metabolic acidosis, a change in mental status, and the recovery of microorganisms before and at the time of the operation, or pathologic examination of tissue following resection of the inflammatory mass. Pancreatic parenchymal necrosis combined with inflammatory complications was confirmed by contrast-enhanced computed tomography scanning. Sonography-guided fine-needle aspiration (FNA) and determination of procalcitonine (PCT) were also applied in the last 10 years.

Special Laboratory Investigations

To investigate which of the cytokine-related laboratory tests correlated with the severity or the lethal outcome of IPN, the following laboratory investigations were performed (1) titration of tumor necrosis factor (TNF) (TNF-α ELISA; BioSource Europe SA, Nivelles, Belgium); (2) interleukin (IL)-6 assay (performed using the IL-6–dependent mouse hybridoma cell line B-9 and the activities were calibrated against rhIL-6 [Amersham, London, England]); and (3) stimulation of leukocytes (white blood cells [5 $\times$ 106/ml] were incubated for 24 hours at 37° C with *Escherichia coli* LPS [0111 B:4; Sigma-Aldrich GmbH, Munich, Germany]). The supernatants were tested for the presence of TNF and IL-6.

Concentration of PCT was measured by using an immunoluminometric assay, with LUMI-test PCT kit (BRAMS Diagnostica, Berlin, Germany).

Clinical Management

After admission and during the early postoperative period, all patients were treated intensively. Medical treatment included total parenteral nutrition, adequate fluid resuscitation, cardiac and respiratory monitoring, and antibiotic therapy in accordance with the bacteriologic findings in the abdomen, blood, and bronchial system. In all of the patients, the total parenteral nutrition consisted of immunonutrition (supplementary administration of glutamine and arginine) and additional pentoxifylline (PTX) (400 mg/day) and dexamethasone (10 mg/day) therapy to modify and decrease the cytokine production.[14–16] Furthermore, patients with established organ failure, as indicated earlier, were treated as follows: renal failure by hemodialysis, respiratory failure by adequate mechanical ventilation, circulatory failure by fluid resuscitation and inotropic agents, and hepatic failure and coagulopathy by adequate replacement with fresh frozen plasma and platelets.

Surgical Treatment

The surgical treatment was performed on average 18.5 days (range, 8–25 days) after the onset of acute pancreatitis. In all patients (220 patients), the operative management consisted of wide-ranging necrosectomy through the whole affected area, using bilateral subcostal laparotomy. The abdomen was explored for classification of the extent of pancreatic and extrapancreatic necrosis. For accurate exploration of the retroperitoneum, Kocher's mobilization of the duodenum and mobilization of the right and left colon were performed. In our cases, the infected necrotizing process was situated in the right and left retrocolic area in 143 patients (65%), and either in the left subphrenic area in 47 patients (21%) or in the retroduodenal and subhepatic area in 28 patients (12%). Debridement or necrosectomy were done either by digital means or by the careful use of an instrument. Continuous normal saline lavage were done by syringe applied in this region to facilitate removal of all demarcated devitalized tissue, while preserving the vital pancreatic tissue and removing the infected, necrotic tissue from the whole affected retroperitoneal area.[17] After surgical debridement, meticulous hemostasis and extensive intraoperative lavage with 8 to 12 L of normal saline were applied, and for postoperative closed continuous local lavage, 4–11 large silicone rubber tubes were inserted into the entire affected area (Fig. 1). They are inserted only into the pancreatic region and the retroperitoneal spaces, without any connection with the intraabdominal region.[18,19]

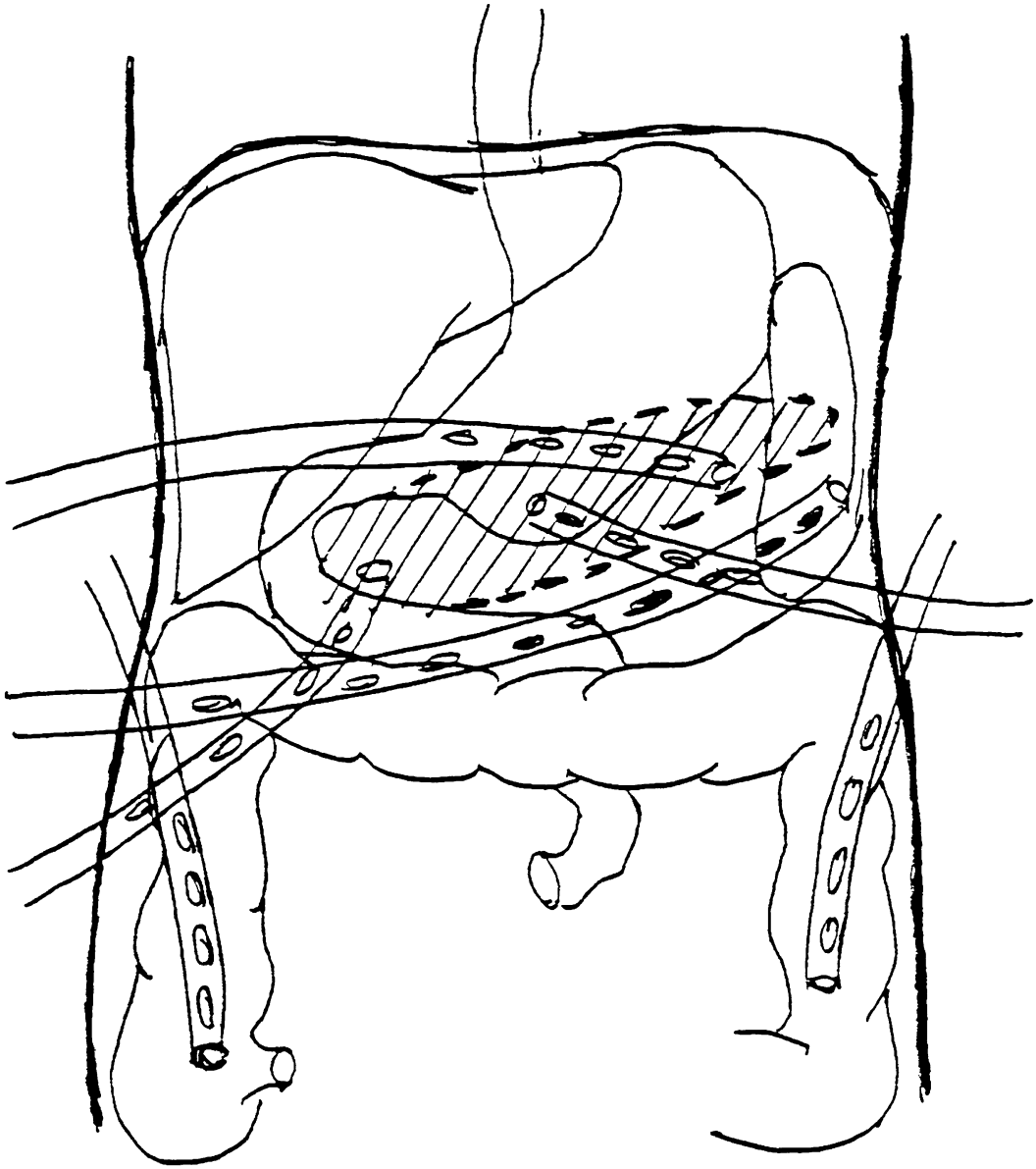

Fig. 1. Positions of the tubes in the retroperitoneal areas for continuous lavage. These inserted tubes have no connection with the intra-abdominal region.

In 108 of the 220 cases (49%), some other surgical intervention—distal pancreatic resection and splenectomy (n = 35), splenectomy (n = 5), subtotal pancreatectomy (n = 11), total pancreatectomy (n = 3), colon resection (n = 8), cholecystectomy (n = 45), cholecystectomy and bile duct drainage (n = 5), sphincteroplasty (n = 2), partial hepatic resection (n = 1), or appendectomy (n = 2)—was also performed. Continuous lavage was applied for an average of 44.5 days (range, 21–95 days), with a median of 9.5 L (range, 5–20 L) of normal saline per 24 hours. In the first few postoperative days, the amount of lavage fluid was generally 15–20 L, which was later reduced, depending on the clinical course. The criteria for discontinuing the lavage management were the absence of any signs of acute pancreatitis and abscess formations and complete cleansing and closing of the infected necrotic cavity. These were confirmed by measuring amylase in the lavage fluid and by the appearance and quality of the outflowing liquid without any devitalized tissue.

RESULTS

Treatment

Table 1 details the surgical procedures performed and the outcome. A total of 113 patients underwent

Table 1. Clinical results following different surgical procedures

Initial procedure	No. of patients	Death (n)
Necrosectomy and widespread lavage	113	10
Necrosectomy, widespread lavage and other surgical intervetion (distal pancreatic resection, splenectomy, colon resection, cholecystectomy, etc.)	107	7
Total	220	17 (7.7%)

necrosectomy with widespread drainage and lavage, with 10 deaths. In addition to 107 cases, necrosectomy and widespread lavage were combined with additional procedures (distal pancreatic resection, splenectomy, colon resection, cholecystectomy, etc.) because of the extent of the necrotizing process. Death occurred in 7 of the 107 patients who had undergone extensive surgical intervention.

Cultures of the necrotic tissue, aspirated and lavage fluid, blood, and bronchial system discharge were performed. Polymicrobial infections were found in 90.5% of all patients, and monomicrobial ones were found in only 9.5% (Table 2). The bacterial findings revealed mainly enteral bacteria, but in 46 of the 220 cases, *Candida* infection was also detected. The incidence of fungal infection was 21%. In all of the *Candida*-infected patients, *Candida* was isolated in a mixed culture (fungal and bacterial infection). The positive fungal result was obtained from different sites either intraoperatively or postoperatively. Of the 46 patients, 25 displayed fungal colonization, whereas the other 21 patients had disseminated fungal infection on the basis of Burchard's criteria.[20]

Table 2. Microbiological findings from preoperative, intraoperative, and postoperative specimens

Germs	n
Polymicrobial infection	199 (90.5%)
Single microbial infection	21 (9.5%)
Organisms isolated (%)	
Enterobacter	54
Pseudomonas	46
Streptococcus	43
Klebsilla	28
Staphylococcus	24
Escherichia coli	16
Anaerobes	16
Acinetobacter	14
Other	9
Candida albicans	21

No mortality was noted in the colonization group, but there were nine deaths in the disseminated group. In 21 disseminated *Candida*-infected patients, flucytosine, amphotericin B, or fluconazole was administered in 8, 4, and 9 patients, with deaths of 8, 1, and 0 patients, respectively.

Complications

Major complications are detailed in Table 3. Forty-eight patients (21,8%) had to undergo reoperation: 37 of them had developed a secondary abscess in the area of the original necrosis cavity and 5 had developed a colonic fistula, which was cured by large bowel resection, while massive diffuse local bleeding was responsible in 6 patients. Pancreatic fistulas were observed in 24 patients; in 16 cases, they closed spontaneously, but in the remaining 8 cases, the fistulas became longstanding, high-output ones with high amylase concentration (mean, 435,500 units/L). In all of these eight patients, octreotide therapy (3 × 0.1 mg/day) was combined with total parenteral nutrition, and 13 days (range, 7–19 days) of this treatment led to complete closure of the fistulas.[21] Systemic complications occurred mainly in connection with the septic condition, local complications, and reoperation. In 94 patients, the respiratory failure required mechanical ventilation for over 24 hours. Renal and circulatory insufficiency developed in 27 and 55 patients, respectively.

Seventeen patients died following operation; the overall mortality rate was 7.7% (17 of 220 patients). The cause of death was bacterial sepsis in five patients, bacterial and fungal sepsis in eight patients, fungal sepsis in one patient, and myocardial infarction in one patient. Two of the reoperated cases died following reoperation of local bleeding. The

Table 3. Major complications and outcome following surgical management

Complications	No. of patients	Reoperations (n)	Death (n)
Local			
Abscess	37	37	14
Pancreatic fistula	24	—	—
Colonic fistula	5	5	—
Bleeding	6	6	2
Systemic			
Respiratory failure	94	—	—
Renal failure	27	—	—
Cardiac insufficiency	55	—	1
Hospital mortality of 220 patients			17 (7.7%)

hospital stay of surviving patients amounted to a median of 45.5 days (range, 21–95 days).

Measurements

A total of 45 of the 220 patients with IPN were studied. All patients had positive cultures from the necrotic tissue and retroperitoneal discharge with Gram-negative rods. Of the 45 patients, 28 had positive blood cultures as well. Initial serum samples for TNF-α and IL-6 determination were obtained between 12 and 24 hours after the onset of sepsis, following by daily sampling. The serum samples of only 14 of the patients (31%) contained detectable TNF-α, but the IL-6 serum levels were almost always above the normal range in all of the patients. The mean level of TNF-α was 11,000 U/ml (range, 8,500–25,000 U/ml), and there was no significant difference between survivors and nonsurvivors. The mean levels of IL-6 seemed to be correlated with the severity of the illness (250 versus 400 U/ml). Stimulation of white blood cells with *E. coli* LPS led to a high TNF-α production (mean level, 150,000 U/ml; range, 145,000–165,000 U/ml) in all of the patients. The follow-up study of the septic patients revealed that the in vitro TNF-α production decreased under the normal range (500–1,500 U/ml) in the later phase of disease significantly, in correlation with the severity of the illness. This might be of prognostic value; the unresponsiveness to in vitro stimuli seemed to be transient after the recovery, but it was irreversible in the fatal cases (n = 4). The same tendency, that is, a biphasic response to in vitro stimuli, was observed in the case of IL-6 production. As a consequence of PTX therapy, the TNF-α production dropped to the normal level on day 2 and did not decrease beyond the normal level. The severity of the illness was evaluated in accordance with the APACHE II score system. PTX therapy resulted in a decreasing tendency in the scores, which tended to change inversely with the improvement in the clinical status, and the laboratory parameters. This immunomodulation is only one aspect of the successful treatment of IPN; adequate surgical treatment obviously determines the outcome of this serious disease.

DISCUSSION

It has become a generally accepted fact[22–25] that one major prognostic factor in patients with necrotizing pancreatitis is bacterial infection of the necrosis. In a prospective clinical trial, the overall contamination rate of pancreatic tissue necrosis was approximately 40%. Infected necrosis was detected in 25% of the patients who had had the disease for only 1 week and in 45% of the patients with a duration of 2 weeks. Currently, of the three infected complications of acute necrotizing pancreatitis (IPN, pancreatic abscess, and infected pseudocyst), IPN is the most common infectious complication, it is the most severe, and it carries the highest mortality rate.[2,5,25,26] As many as 5–10% of all patients with acute pancreatitis may develop this life-threatening complication. Accordingly, the identification and assessment of the severity of infected complications in patients with acute pancreatitis have become imperative for optimum management.

During the past 10 years, it has been recognized that inflammatory mediators and cytokines produced by activated leukocytes have an important role in the multisystem involvement during acute pancreatitis. Activated leukocytes are thus a pathogenetic factor in the severity of the disease, and factors released by activated leukocytes therefore reflect the severity of the disease.[9,12] TNF plays a pivotal role in the initiation of septic syndrome. TNF is produced mainly by monocytes and macrophages in response to various stimuli, of which endotoxins derived from Gram-negative bacteria are the most potent. We therefore measured the serum TNF and IL-6 levels and the TNF-producing capacity of the leukocytes in patients with IPN following necrotizing pancreatitis. ELISA revealed circulating TNF in 31% of patients with presumed sepsis following pancreatitis. There was no clear association between the TNF level and the development of shock or the fatal outcome of the disease, and TNF was detectable only at the onset of the clinical responses. The exact time that elapsed between the onset of symptoms and the collection of sera could not be assessed. The TNF-mediated cytotoxicity of the patients' leukocytes was significantly higher than that in the healthy control group. The in vitro TNF-producing capacity was also higher in the study group. The decrease in inducibility before the fatal outcome of the disease might be due to the exhaustion of the leukocytes or a refractory condition of the leukocytes, which had been in a stimulated condition for a prolonged time in vivo. Paradoxically, therefore, the prolonged upregulation of the TNF-producing cells was accompanied by a poor in vitro reactivity. This decrease in responsiveness might be of prognostic value. There was a close correlation between IL-6 levels and sepsis. However, the higher IL-6 levels reflect a consequence of cytokine cascade activation rather than a cause of the pathogenesis of sepsis. Our results suggest that determination of the TNF-producing capacity of the leukocytes might be more informative then measurement of the serum

TNF level in evaluation of the severity or prognosis of septic complications following necrotizing pancreatitis.[13]

Bacterial infection is a very important risk factor in necrotizing pancreatitis. Thorough screening for bacterial infection is essential. FNA is a reliable and harmless procedure to determine the bacterial contamination of the necrosis,[27–30] and FNA is accepted as a gold standard in the diagnosis of IPN. Procalcitonine is a new diagnostic marker of systemic bacterial infection.[31–33] This laboratory test can be also used for differential diagnosis of bacterial and nonbacterial inflammatory reaction; therefore, determination of PCT serum concentration is a suitable method for diagnosis of IPN and is a helpful marker facilitating a decision concerning surgical intervention.[34–36] Recovery of microorganisms at the time of operation and in the postoperative period is also important for choosing adequate antibiotics against the bacterial infection. The origin and the route of the bacteria to result in pancreatic infection are still unclear. However, reasonable ways are direct transluminal penetration from the colon and/or spreading along the lymphatics from the gallbladder or the colon. On the other hand, there is no doubt that the virulence of the microorganisms and the reduced defense capacity of the patient are essential.[37] In our cases, the preoperative, intraoperative, and postoperative microbiological findings showed a high presence of Gram-negative strains of intestinal origin, and polymicrobial infections (90.2%) were identified. It was also noteworthy that the incidence of *Candida* infection in the overall infected necrotic pancreatic cases was 21%. In all patients, *Candida* was isolated in a mixed culture (fungal and bacterial infection).[18,19] In agreement with various authors,[38–41] bacterial sepsis and disseminated fungal infection are not mutually exclusive processes; medications were applied against both of the agents. In our disseminated fungal infection, fluconazole therapy was a highly effective and well-tolerated drug.[42]

A variety of approaches have been advocated for the surgical management of IPN; they include different techniques ranging from tissue-sparing methods to aggressive, extensive resection. During the 1980s, three main patterns of management could be identified in the surgical management of necrotic and infective complications of acute pancreatitis: (1) "conventional" treatment, including resection of the involved pancreatic tissue or necrosectomy, followed by drainage; (2) "lavage" treatment, in which necrosectomy is followed by permanent local irrigation or lavage of the involved retroperitoneal area; and (3) an "open abdominal management" (laparostomy), involving resection or necrosectomy followed by various combinations of planned and staged reoperations. The summarized results of the three patterns were reviewed by Rau et al.[43] According to that report, when only patients with documented IPN were considered, the mortality rate was 42% (range, 24–84%), 19.8% (range, 14–27%), and 21% (range, 11–55%) when "conventional" treatment, "lavage" treatment, and the "open abdominal management," respectively, were applied. In the last 5 years, a few centers have published a limited number of patients applying necrosectomy by "minimal invasive procedures" with the same mortality as "open abdominal management." Therefore, "minimal invasive procedures" may be of value but probably only in a select subgroup.[44–47]

In our practice, "lavage" treatment was adopted. We applied a bilateral-subcostal incision because this laparotomy allows a wide and safe exploration when extensive infected necrosis is present. The surgical management of our patients with IPN was directed toward removal of the devitalized intrapancreatic and extrapancreatic tissue in all affected areas. It seems very important to explore every possibly infected site because ineffective debridement can endanger the recovery of the patients and increase the likelihood of reoperations. In accord with several authors, it is not necessary to remove every small part of the devitalized tissue, because any necrotic or necrotizing tissue is washed out by the lavage fluid later in the postoperative period.[2,3,17,18] The successfulness of postoperative closed continuous lavage depends on the number and the size of the drainage tubes.[48,49] Generally, we applied 4–11 large silicone tubes inserted to all affected places. As infected necrotic processes can extend into intrapancreatic and extrapancreatic areas, other surgical interventions can also be advised. This reason explains our surgical strategy; that is, in 108 of 220 patients (49%), the necrosectomy and continuous lavage were combined with several surgical interventions (distal pancreatic resection, splenectomy, cholecystectomy, colon resection, etc.). Reoperations were necessary in 48 patients (22%), mainly in consequence of residual abscesses. The overall mortality decreased significantly to 7.7% (17 patients died), and this mortality rate is significantly better than recently published data.[43] An improved rate of survival in infected necrosis is provided by adequate surgical debridement combined with continuous widespread lavage and drainage in all affected areas. A large volume of saline solution for continuous lavage through multiple drainage is a safe and atraumatic procedure that can eliminate the infected, necrotic tissue. If necessary, other surgical intervention is also advised.

Effective surgical treatment was supplemented with supportive therapy, including glutamine, arginine, PTX, and dexamethasone. Several observations support the concept that glutamine supplementation improves the organ function, and glutamine is a critical nutrient for the gut mucosa and immune cells.[50,51] It was recently clearly demonstrated that glutamine-enriched parenteral nutrition exerts protein anabolic effects, improves the gut structure and immune cell number and function, and reduces morbidity.[52,53] PTX is a well-known vasoactive drug, a phosphodiesterase inhibitor with proven clinical efficiency in various circulatory disorders. PTX raised new interest because it had been demonstrated to prevent or to attenuate the release of TNF-α–induced lipopolysaccharide.[54] PTX is a potent inhibitor of TNF-α messenger RNA. PTX also exerts direct inhibitory effects on various neutrophil functions, and it may influence other inflammatory cytokines.[54] It was also demonstrated that dexamethasone is a selective inhibitor of TNF-α production.[16] These drugs may therefore improve the therapeutic strategy in the treatment of sepsis syndrome following necrotizing pancreatitis. The combined application of dexamethasone and PTX may cause a greater suppression of TNF biosynthesis that can be achieved by either agent alone.[16]

In conclusion, this improved result can be achieved by adequate surgical treatment and continuous, long-standing drainage and lavage, together with supportive therapy, including immunonutrition, modifying the cytokine production, combined with adequate antibiotic and antifungal medication. This surgical strategy provides the possibility for recovery in cases of necrotizing pancreatitis combined with septic complications.

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Palliative Surgery for Unresectable Pancreatic and Periampullary Cancer: A Reappraisal

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This study aimed to reappraise short-term and long-term results of palliative biliary and gastric bypass surgery in patients with unresectable pancreatic head carcinoma found at explorative laparotomy. We retrospectively analyzed 83 consecutive patients whose pancreatic head carcinoma appeared unresectable at laparotomy (vascular involvement [57%], liver metastases [24%], distant metastatic lymph nodes [11%], peritoneal implants [8%]) and who underwent palliative surgical concomitant biliary and gastric bypass. Postoperative mortality and morbidity rates were 4.8% and 26.5%, respectively. Postoperative-delayed gastric emptying occurred in 9 patients (10%). Antecolic (46%) and retrocolic (54%) gastrojejunostomies did not differ for the duration of nasogastric suction, the delay of oral intake, and the incidence of delayed gastric emptying. Mean hospital stay was 16 ± 8 days. Median survival was 9 months (range 1–44). Late cholangitis occurred in 2 patients (2.4%) treated medically. One recurrent jaundice required transhepatic stenting 9 months from surgery. Four late gastric outlet obstructions occurred (4.8%) with a mean delay of 8 months from surgery. These data demonstrate that, in patients with unresectable pancreatic head carcinoma at laparotomy, palliative concomitant biliary and gastric bypass in a single procedure is safe and long-term efficient. This strategy remains to be compared to endoscopic palliation in this setting. (J GASTROINTEST SURG 2006;10:286–291)

KEY WORDS: Pancreatic carcinoma, palliative care, gastric bypass, biliary bypass

Only 5–20% of pancreatic head carcinoma are resectable at the time of presentation.[1–3] For patients with obvious nonresectable disease, endoscopic techniques have been developed as alternatives to traditional surgical management. Biliary stenting, and more recently duodenal self-expandable endoprostheses, have been promoted as the treatment of choice because of their low morbidity.[4–9] However, despite improvement in imaging procedures, assessing unresectability still remains difficult in some cases, and purely nonsurgical palliation may, in these cases, overlook resectable tumors. Furthermore, pancreatic biopsies performed under radiologic or endoscopic ultrasound guidance, which have to be obtained before starting a palliative treatment by chemotherapy or radiotherapy, fail to prove the diagnosis of adenocarcinoma in 10–20% of attempted procedures. In these questionable cases where unresectability of the tumor is not proven, our policy today remains in favor of a surgical approach where palliative surgical bypass is performed if the tumor appears unresectable at laparotomy.

Historical series reported high morbidity (up to 50%) and mortality (up to 30%) rates of bypass surgery,[10,11] but more recent series reported reduced morbidity and mortality rates of less than 30% and 10%, respectively.[3,12–14] Whereas endoscopic palliation is widely used today, this study aimed to reappraise the short-term and long-term results of a surgical palliation policy combining biliary-enteric bypass and gastrojejunostomy in patients with unresectable pancreatic head carcinoma at laparotomy.

MATERIAL AND METHODS

Patients

Between June 1996 and December 2003, 340 patients without obvious contraindication to resection detected by preoperative imaging assessment

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(distant metastases, distant lymph nodes involvement, unresectable vascular involvement) underwent a surgical exploration for pancreatic head adenocarcinoma. If such contraindication was found intraoperatively, a biopsy was done to prove malignancy, and surgical palliation was performed in a single surgical procedure. Among the 340 patients, 257 (76%) underwent a pancreaticoduodenectomy. The remaining 83 patients (24%), who underwent surgical palliation for malignant pancreatic disease found unresectable at laparotomy, were retrospectively reviewed in this study. There were 38 women and 45 men with a mean age of 64 ± 11 years. The following preoperative symptoms were recorded at first presentation: jaundice (77%), abdominal pain (54%), loss of weight more than 10% (25%), and vomiting or nausea (13%). Mean delay between the onset of symptoms and operation was 9 ± 9 weeks. Resectability was routinely assessed preoperatively by chest X-ray, abdominal ultrasonography, and abdominal-computed tomography (helicoidal CT scan was used from 1998 to 2003). Echoendoscopy was not routinely performed.

For 62% of the patients, the resectability of the tumor was preoperatively judged as doubtful. For the others (38%), the preoperative assessment judged the tumor as resectable. Histologic confirmation of the diagnosis of adenocarcinoma was obtained intraoperatively in 73 patients (88%). For the remaining patients (12%), either the intraoperative biopsies were not performed (n = 4) or were negative (n = 6), but proof of malignancy was obtained by follow-up and demonstrated disease progression in these 10 patients. In this specific setting, the sensitivity of biopsy was 92%.

Surgical Palliative Procedure

Reasons for unresectability indicating surgical palliation are shown in Table 1. The palliative procedure included, routinely, both a biliary-enteric bypass and a gastrojejunostomy. Biliary bypass was a hepaticoduodenostomy in 69 patients (83%). A Roux and Y hepaticojejunostomy was performed in 14 patients (17%) because the duodenum could not be used due to tumor volume or duodenum involvement. Gastrojejunostomy was antecolic in 46 patients (antecolic group) and retrocolic in 37 patients (retrocolic group), depending on the surgeons. Both were isoperistaltic. A chemical splanchnicectomy with alcohol was performed in 5 patients (6%) because preoperative pain was not controlled by oral analgesics. Mean duration of surgical procedures was 203 ± 67 minutes. Postoperatively, 35 patients (42%) had no other therapy, 25 patients (30%) received postoperative chemotherapy, and 23 patients (28%) postoperative chemoradiation.

Table 1. Indications for surgical palliation (n = 83 patients)

Reasons for unresectability	No. of patients (%)
Vascular invasion*	47 (57)
Liver metastases	20 (24)
Distant metastatic lymph nodes	9 (11)
Peritoneal implants	7 (8)

*Vascular invasion included arterial encasement or involvement of the superior mesenteric artery, the celiac axis, or the hepatic artery, and unresectable venous involvement of the mesenteric and portal veins.

Outcome

Postoperative mortality, postoperative morbidity, resumption of oral diet, delay of flatus passage, postoperative hospital stay, and long-term survival were assessed. Postoperative mortality was defined as death within 30 days after operation. Postoperative-delayed gastric emptying was diagnosed when a normal diet was not tolerated within 10 days of surgery.[12]

Follow-up

Follow-up information was obtained through direct patient or referring physician contacts, from hospital charts, and by contacting the French civil status registry office. Mean length of follow-up was 9 ± 9 months.

Statistical Analysis

Data were presented as mean ± standard deviation or median (range). Statistical analysis was carried out using statistical software (Statview 5.0, SAS Institute Inc., Cary, NC). Differences between groups were evaluated using chi-square analysis, Fisher's exact test, or Student's *t*-test when appropriate. Survival analysis was performed with the Kaplan-Meier method. The log-rank test was used to evaluate differences in survival between groups. A P value < 0.05 was considered as statistically significant.

RESULTS

Short-term Outcome

Mortality rate was 4.8%, and the overall postoperative morbidity rate was 26.5% (Table 2). Postoperative deaths were due to one hepatic failure

Table 2. Postoperative mortality and morbidity (n = 83 patients)

	No. of patients (%)
Mortality*	4 (4.8)
Morbidity	22 (27)
Delayed gastric emptying	9
Wound infection	3
Intra-abdominal abscess	1
Ascites	4
Pneumonia	2
Urinary infection	2
Cholangitis	1

*Defined as death within 30 days after operation.

in an alcoholic patient, one mesenteric arterial infarction, one gastrojejunal anastomotic leak, and one pneumonia complicated by multiorgan failure. Age of the patients did not correlate with the overall postoperative complication rate ($P = 0.47$). One patient (1.2%) was reoperated to drain a wound abscess. The most common complication was the postoperative-delayed gastric emptying occurring in 9 patients (10%). Seven patients (8%) received red cell units perioperatively. Overall mean hospital stay was 16 ± 8 days (median 13 days, range 8–60).

Comparison Between Antecolic and Retrocolic Gastrojejunostomies

No significant difference in patient demographics, preoperative symptoms, and surgical findings was observed between the two groups, as shown in Table 3. There was no statistical difference between the two groups for postoperative length of nasogastric suction, passage of flatus, resumption of oral intake, and postoperative-delayed gastric emptying (Table 4). Delayed gastric emptying was not correlated with preoperative symptoms of gastric outlet obstruction ($P = 0.92$).

Comparison Between Hepaticoduodenostomy and Hepaticojejunostomy

No significant difference in patient demographics, preoperative symptoms, and short-term outcomes was observed between the two groups (data not shown). The surgical time was significantly longer in patients with a hepaticojejunostomy than with a hepaticoduodenostomy (251 ± 88 minutes vs. 193 ± 58 minutes; $P = 0.002$).

Table 3. Clinical and surgical findings in the two groups of gastrojejunostomy

	Antecolic group (n = 46)	Retrocolic group (n = 37)	*P*
Male/Female	25/21	20/17	0.97
Age	64 ± 11	62 ± 12	0.43
Delay first symptoms operation (days)	10 ± 11	8 ± 6	0.37
Preoperative symptoms			
Jaundice	33 (72)	31 (84)	0.30
Abdominal pain	25 (54)	20 (54)	0.97
Vomiting/Nausea	7 (15)	6 (16)	0.90
Weight loss >10%	17 (37)	8 (22)	0.20
Reasons for unresectability			
Vascular invasion	29 (63)	18 (49)	0.25
Liver metastases	10 (22)	10 (27)	0.60
Metastatic lymph nodes	3 (7)	6 (16)	0.90
Peritoneal implants	4 (8)	3 (8)	0.30
Duration of surgical procedure (min)	207 ± 72	197 ± 62	0.53
Biliary bypass			
Hepaticoduodenostomy	40 (87)	29 (78)	0.45
Hepaticojejunostomy	6 (13)	8 (22)	

Values in parentheses are percentages.
Continuous data are presented as mean ± standard deviation.

Long-term Outcome

Median postoperative survival was 9.2 months (range 1–44; Fig. 1). There was no statistical difference in survival between patients without postoperative treatment (median 7.1 months, range 1–44), patients treated by postoperative chemotherapy (median 10.6 months, range 1–35), or postoperative chemoradiation (median 10.3 months, range 1–30). Two patients (2.4%) developed cholangitis 8 months and 17 months, respectively, after initial palliative surgery and were efficiently treated by antibiotics. These two patients had undergone a hepaticoduodenostomy. One patient (1.2%) developed recurrent jaundice 9 months after initial surgery with a hepaticojejunostomy. He was treated by biliary transhepatic stenting. There was no significant difference in terms of late biliary complications between patients with hepaticoduodenostomy and patients with hepaticojejunostomy (3% vs. 7%, NS). Four patients (4.8%) had late gastric outlet obstruction with a mean delay of 8 months from the initial palliative surgery. Three of them had an antecolic gastrojejunostomy and one had a retrocolic gastrojejunostomy (NS). One of them underwent a subsequent second gastroenterostomy, whereas the others had medical support only. Thus, jaundice and gastric outlet obstruction were efficiently prevented

Table 4. Postoperative course following antecolic and retrocolic gastrojejunostomies

	Antecolic group n = 46	Retrocolic group n = 37	*P*
Nasogastric suction (days)	7 ± 5	7 ± 4	0.96
Passage of flatus (days)	5 ± 2	5 ± 1	0.64
Oral intake (days)	7 ± 3	7 ± 3	0.52
Postoperative delayed gastric emptying	6 (13%)	3 (8%)	0.71
Morbidity	11 (24%)	11 (30%)	0.17
Mortality*	4 (8.6%)	0 (0%)	0.12
Hospital stay (days)	16 ± 8	16 ± 9	0.90
Late outlet obstruction	3 (7 ± 3 months)	1 (10 months)	0.76

Continuous data are presented as mean ± standard deviation.
*Was defined as death within 30 days after operation.

by this surgical palliative procedure, until death, in 78 patients (94%).

DISCUSSION

Despite improvements in preoperative diagnosis and staging, pancreatic head carcinoma remains frequently found to be unresectable at the time of the laparotomy, leading to an intraoperative shift from an initial curative intent toward a palliative procedure. This study confirms, first, that both biliary and gastric bypasses can be performed in this setting, with low morbidity (26.5%) and low mortality (4.8%) rates. Second, it shows that antecolic and retrocolic gastrojejunostomy yield similar morbidity, including postoperative delayed gastric emptying. Finally, the low rates of late cholangitis (1.2%), recurrent jaundice (2.4%), and late gastric outlet obstruction (4.8%) confirm the long-term efficacy of the palliative procedure.

Procedure-related morbidity and mortality rates in our study (26% and 4.8%, respectively) compare favorably with those in the three prospective randomized studies comparing biliary bypass surgery to endoscopic biliary stenting; surgical morbidity rates were reported ranging from 26% to 40% and high mortality rates from 15% to 31%.[15–17] It is noteworthy that significantly lower morbidity (11% vs. 29%; $P = 0.02$) and lower procedure-related mortality rates (3% vs. 14%; $P = 0.006$) in patients treated endoscopically were only demonstrated in Smith's study.[17] Mortality did not differ between patients with endoscopic or surgical bypass in the meta-analysis of these trials by Taylor et al.[18] Patients included in these studies had a clear contraindication to resection that was preoperatively established, whereas these patients were excluded from our study. Our improved results may be due to improvement of preoperative imaging assessment and the use of endoscopic palliative procedures for patients whose preoperative workup detects a clear contraindication to surgical resection. Thus, the subgroup of patients who finally undergo surgery with a curative intent may have here a less extended disease than in these older studies. The resulting better general conditions may contribute to the better results of palliative surgery observed in our series. More recently, other groups reported reduced

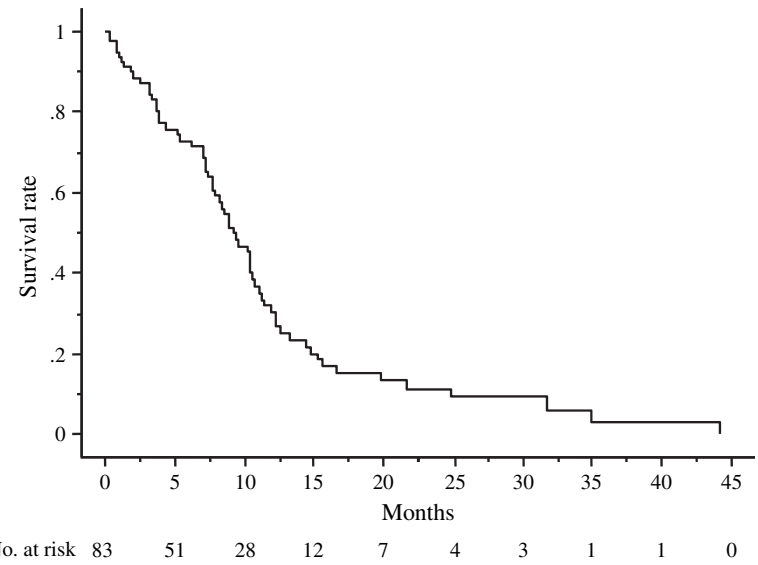

Fig. 1. Survival in unresectable patients. Kaplan-Meier survival curve (n = 83).

morbidity and mortality rates less than 30% and 5%, respectively, in patients with tumors appearing unresectable at laparotomy.[3,12–14]

In our series, the mean hospital stay was 16 days (median 13 days, range 8–60). This long hospital stay is partially explained by a frequent difficulty in our country, the lack of beds in rehabilitation centers that offer medical support to patients before they return home.

When the pancreatic tumor seems unresectable at laparotomy, the alternative would be to stop the surgical procedure and to consider endoscopic palliation. We do not use this strategy. Its first disadvantage is exposure of patients to several anesthesiology procedures, each with their own risk. Secondly, meta-analyses of randomized trials comparing biliary bypass surgery to endoscopic biliary stenting clearly demonstrated that more treatment sessions are required for recurrent jaundice after stent placement than after surgery, thus impairing the benefit of the endoscopic procedure.[18,19] The use of metallic expendable stents may reduce this difference with surgical bypass, but that has not been tested yet in a randomized trial.[20] Third, the systematic addition of a gastrojejunostomy to surgical biliary bypass efficiently prevents gastric outlet obstruction symptoms.[3,21–25] In our series only 4 patients (4.8%) developed late gastric obstruction. Two recent prospective randomized trials comparing concomitant biliary and gastric bypass with biliary bypass alone, in patients found at exploratory laparotomy to have unresectable periampullary carcinoma, did not show any added morbidity in the group with prophylactic gastric bypass combined with the biliary bypass (30%).[2,26] Follow-up demonstrated in the double bypass groups a dramatic drop of the incidence of late outlet obstruction (19–41% in patients without gastrojejunostomy vs. 0–5% in patients undergoing a gastrojejunostomy, $P < 0.001$ in both trials). Furthermore, morbidity rates are high, approaching 25%, in patients who require a second surgical procedure in this setting.[27]

The incidence of postoperative-delayed gastric emptying after prophylactic gastrojejunostomy combined with biliary bypass for palliative treatment has been reported to vary from 8–15%.[3,12,13,25] In the study by Lillemoe et al.,[12] there was no significant difference of incidence of delayed gastric emptying between retrocolic and antecolic gastrojejunostomy (6% vs. 17%, respectively; $P = 0.08$).

Recently, endoscopic or radiologic palliations of duodenal obstruction using large-caliber metallic stents have been considered as an alternative to surgical gastric bypass.[7–9,28–30] In the largest published series by Nassif et al.[9] including 63 patients, stent placement and resumption of oral diet were achieved in 95% and 92% of the patients, respectively. However, the median patency time of these expensive stents was only 5.5 weeks, and 20% of the patients experience recurrent gastric outlet obstruction despite a short median survival of 7 weeks.

The here-adopted surgical palliation strategy, simultaneously combining biliary and gastric bypasses in jaundiced patients with preserved general condition and nonobvious diffuse metastatic disease, yielded low procedure-related morbidity and mortality rates. It allows us to identify resectable tumors and reduces the need for expensive sophisticated preoperative assessment such as systematic echoendoscopy or laparoscopic exploration. In a single procedure, it obtains proof of malignancy by a conclusive surgical biopsy in 92% of the cases and provides a long-acting efficient palliation in patients whose survival may increase with new palliative chemotherapies. The cost-effectiveness of this strategy should be compared with more recent and frequently proposed complex procedures, which combine extensive imaging assessment and expensive repeated endoscopic procedures.

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Outcomes of Cholecystectomy After Endoscopic Sphincterotomy for Choledocholithiasis

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Laparoscopic cholecystectomy (LC) for treatment of symptomatic common bile duct stones (CBDS) after endoscopic sphincterotomy (ES) is associated with increased conversion and complications compared with other indications. We examined factors associated with conversion and complications of LC after ES. A retrospective study of 32 patients undergoing ES for CBDS followed by cholecystectomy was undertaken. Surgical outcomes for this group were compared with a control population of 499 LCs for all other indications. Factors associated with open cholecystectomy and complications in the ES group were analyzed. Patients undergoing LC preceded by ES had a significantly higher complication (odds ratio [OR] = 7.97; 95% CI, 2.84–22.5) and conversion rate (OR = 3.45; 95% CI, 1.56–7.66) compared with LC for all other indications. Pre-ES serum bilirubin greater than 5 mg/dL was predictive of conversion (positive predictive value = 63%, $P < 0.005$). Patients with symptomatic CBDS that undergo LC after ES have higher complication and conversion rates than patients undergoing LC without ES. Pre-ES serum bilirubin is useful in identifying patients who may not have a successful laparoscopic approach at cholecystectomy. (J GASTROINTEST SURG 2006;10:292–296)

KEY WORDS: Cholecystectomy, choledocholithiasis, endoscopic, sphincterotomy, outcomes

The classical management of symptomatic choledocholithiasis (CBDS) had been to perform a common bile duct exploration (CBDE) in conjunction with open cholecystectomy. Minimally invasive approaches, both laparoscopic and endoscopic, have changed this paradigm. Some centers advocate laparoscopic CBDE at laparoscopic cholecystectomy (LC) as a single-staged approach to this disease.[1–3] The disadvantages of this approach are that few surgeons are adept at laparoscopic CBDE, leading to conversion to an open procedure or the need for a post-LC endoscopic retrograde cholangiography (ERC) with endoscopic sphincterotomy (ES) and stone extraction.[1] With the widespread use of endoscopic approaches to the bile duct, ERC-ES with stone extraction has emerged in most centers as the initial approach for patients with CBDS.

For patients who present with symptomatic CBDS who are effectively treated with ES, there has been some debate as to whether cholecystectomy should be performed. The reported incidence of further gallstone complications ranges from 11%–47%.[4–8] A recent trial addressing this question randomized 120 subjects who underwent ES to immediate cholecystectomy versus a wait-and-see arm.[8] The study determined that 47% of subjects in the wait-and-see arm developed recurrent biliary symptoms leading to cholecystectomy within the 2-year follow-up period. The conversion and complication rates for cholecystectomy were significantly higher in the wait-and-see arm than in the immediate cholecystectomy arm, leading the authors to conclude that all patients who are medically fit should undergo LC after ES. However, an interesting observation is that the conversion and complication rates in both arms of the study were higher than rates traditionally reported for LC. The immediate LC arm had a conversion rate of 23% and complications rate of 14% versus the wait-and-see arm with a 55% conversion rate and 32% morbidity rate.

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Laparoscopic cholecystectomy is generally accepted as a safe procedure with low morbidity and mortality. In fact, many centers perform this operation on an outpatient basis. To better understand which patients are at higher risk for complications or conversion, we undertook a study of patients undergoing ES at our institution to assess whether timing of LC after ES, or other factors, influenced the rate of conversion and complications in this disease group.

MATERIALS AND METHODS

This study was approved by the institutional review board of the University of Alabama at Birmingham and granted exemption of informed consent and an HIPAA waiver. An analysis of a prospectively collected database of all patients undergoing ERC-ES was undertaken. Subjects who presented with symptomatic choledocholithiasis between September 1999 and October 2002 and underwent ES with stone extraction as their initial treatment were included in the study. Indications for ERC-ES were biliary dilation on CT or ultrasound, cholangitis, jaundice, or pancreatitis. Exclusion criteria were prior cholecystectomy, history, or ERC finding of sclerosing cholangitis, and diagnosis of concomitant pancreatic adenocarcinoma or cholangiocarcinoma. Only subjects with follow-up at our institution were included in the study.

The control population included all subjects undergoing cholecystectomy for any indication, including biliary colic, cholecystitis, and gallstone pancreatitis without ERC-ES during the same time period. All cholecystectomies were performed with attending surgeon supervision in the gastrointestinal surgical group. A chart abstraction was performed with the after variables recorded: age, race, gender, pre-ES serum bilirubin, pre-ES alkaline phosphatase, pre-ES white blood count (WBC), operative time, and length of stay. Operative notes were reviewed and the reason for conversion was recorded. The main outcome variables studied were conversion to or open cholecystectomy and major complications. Major complications were defined as major organ injury, retained stones, bile leak, infection/abscess, bleeding requiring transfusion, and prolonged intensive care stay.

Statistical Analysis

The control and study populations were compared using *t* tests when mean and standard deviation were available and chi-squared tests for categorical variables. Univariate analysis to identify factors associated with conversion and/or open procedure and complications was done using chi-squared and Wilcoxin rank sum tests. Because we were interested in analyzing how the procedure was completed, not how it was started, conversions to open cholecystectomy, and open cholecystectomy were combined in the analysis.

RESULTS

A total of 1210 ERCP procedures were performed during the study period. Only 118 (7.6%) ERCP procedures done in 92 subjects were eligible for the study. Follow-up information at our institution was available on 45 (49%) of the subjects. Of those 45 subjects, 32 (72%) underwent subsequent cholecystectomy at a median of 9 days (range, 1–70) after their last ES. The control population consisted of 499 cholecystectomies done for all other indications during the study period. The characteristics of the study and control populations are listed in Table 1. The two populations were similar in age, gender, American Society of Anesthesiologists class, and length of stay. The ES group had a significantly longer operative time compared with the control group.

The conversion and complication rates are shown in Table 2. The total open rate in the control group was 11%, compared with 31% in the study group ($P < 0.01$). The rate of conversion from laparoscopic to open cholecystectomy was 25% for the ES group compared with 4% for the control group. Reasons

Table 1. Characteristics of patients with and without ES before cholecystectomy

Variable	Without ES* (n = 499)	With ES† (n = 32)	*P* value
Gender (%)			
Male	134 (27)	8 (25)	—
Female	365 (73)	24 (75)	0.818
Mean age (±SD)	49 (17.3)	53 (20)	0.210
Mean ASA (±SD)	2.4 (0.7)	2.6 (0.6)	0.115
Mean LOS (±SD)	3.0 (4.8)	3.6 (3.6)	0.488
Mean operative time (±SD)	80 (30.2)	103.4 (52.8)	0.001
Race/ethnicity			
Caucasian	315 (63)	15 (47)	—
African American	176 (35)	14 (44)	—
Other	5 (1)	3 (9)	0.0004

ASA = American Society of Anesthesiologists; ERC = endoscopic retrograde cholangiography; ES = endoscopic sphincterotomy; LOS = length of stay.
*Controls included cholecystectomy cases between 1/1/2000 and 12/31/2002.
†Cases included patients who underwent ERC for choledocholitiasis and cholecystectomy from 9/1/1999 to 10/31/2002.

Table 2. Type of cholecystectomy, and complications for patients with and without ES before cholecystectomy

Variable	Without ES* (n = 499)	With ES† (n = 32)	OR‡	CI	*P* value
Cholecystectomy (%)					
Laparoscopic	441 (89)	22 (69)			
Converted to open	22 (4)	8 (25)			
Open	36 (7)	2 (6)			
Total open	58 (11)	10 (31)	3.45	1.56, 7.66	≤0.01
Complications (%)					
No	485 (97)	26 (81)			
Yes	14 (3)	6 (19)	7.97	2.84, 22.50	≤0.001

*Controls included cholecystectomy cases between 1/1/2000 and 12/31/2002.
†Cases included patients who underwent ERC for choledocholitiasis and cholecystectomy from 9/1/1999 to 10/31/2002.
‡Odds ratio.

for conversion to open in the ES group were inability to identify the anatomy (6), a recognized common bile duct injury (1), and a duodenal injury (1). The complication rate in the control group was 3% versus 19% in the study group ($P < 0.001$). Major complications were duodenal injury (1), common bile duct injury (1), pneumonia (1), and retained common bile duct stones (3). Subjects who underwent ES for symptomatic choledocholithiasis had a significantly higher conversion rate at cholecystectomy (odds ratio [OR] = 3.4; 95% CI, 1.56–7.66) and major complications (OR = 7.97; 95% CI, 2.84–22.5) than subjects who underwent cholecystectomy for all other indications.

Univariate analysis of factors associated with conversion or open procedure and complications in the ES group was performed. Based on risk factors for complications and conversion in other cholecystectomy outcome studies, we included number of days between ES and cholecystectomy, age, gender, pre-ES WBC, pre-ES serum alkaline phosphatase, and pre-ES serum total bilirubin in our data analysis. The results of the univariate analysis are shown in Table 3. The only significant variable under study for conversion to or open cholecystectomy was the pre-ES serum bilirubin (Table 3). The mean pre-ES serum bilirubin in cases that were successfully completed laparoscopically was 3.2 mg/dL versus 7.4 mg/dL in the combined-converted group and the open group ($P = 0.005$). A similar analysis was performed for major complications and none of the variables tested were associated with complications from cholecystectomy. We did a sensitivity analysis to find a cutoff for pre-ES serum bilirubin and the likelihood of an open or converted procedure greater than 50%. Pre-ES serum bilirubin greater than 5 mg/dL had a sensitivity of 63%, specificity of 86%, and positive predictive value of 63% for open/converted cholecystectomy.

Table 3. Univariate analysis of type of cholecystectomy following ES

Variable	Type of cholecystectomy: Laparoscopic (n = 22)	Type of cholecystectomy: Converted/ open (n = 10)	*t* test or χ^2 *P*
Median days from ES to cholecystectomy	3 (1–70)	14.5 (1–39)	0.753
Age (± SD)	52 (21)	55 (19)	0.707
Gender (% female)	16 (72.8)	8 (80)	0.659
Pre-ES WBC (± SD)	10.49 (4.76)	14.83 (11.11)	0.142
Pre-ES ALK PO4 (± SD)	308 (208)	263 (285.9)	0.626
Pre-ES serum BILI (± SD)	3.19 (1.90)	7.40 (5.67)	0.005

ALK PO4 = alkaline phosphatase; BILI = bilirubin; WBC = white blood count.

DISCUSSION

This study evaluates the outcome of cholecystectomy after ES for symptomatic choledocholithiasis. We found that this patient population had a significant increase in both major complications and conversion rates when compared with cholecystectomies done for all other indications. These findings are similar to outcomes of cholecystectomy after ES in a randomized trial of LC versus wait-and-see in patients with symptomatic CBDS.[8] Further analysis in our study demonstrated that pre-ES serum bilirubin was significantly associated with an open cholecystectomy.

Our study has several limitations. First, our sample size in the ES group is small. Because our institution is a tertiary care center, a large portion of the patients that underwent ERC plus ES for

CBDS returned to their local surgeons for additional treatment. The exclusion of subjects who received care outside our institution could bias our results. Those who received their definitive treatment at a tertiary care institution may represent a higher risk population and could limit the generalizability of our results. Because of the small numbers in our study group, multivariable analysis was not feasible. Second, this was a retrospective review of complications, and not all patients had documentation or measurement of the risk factors studied.

We attempted to define risk factors for conversion and complications for LC after ES. Our study found that elevated pre-ES serum bilirubin was associated with an increased likelihood of having an open or converted procedure. Pre-ES serum bilirubin greater than 5 mg/dL had a predictive value of 63% for open cholecystectomy. A large outcomes study of choledocholithiasis published in 1989, before the LC era, found that preoperative elevated serum bilirubin was independently associated with increase morbidity after cholecystectomy.[9] They also found that preoperative ES increased the risk for complications at cholecystectomy. Furthermore, a large study by Sarli et al.[10] looking at elective LC for asymptomatic CBDS reported a conversion rate of 8.3% in patients with a history of prior ES versus 3.4% in the control group. Our study further defines which patients with a history of ES are more likely to undergo conversion or open cholecystectomy in the LC era.

A recent study examined factors associated with conversion for LC done for acute cholecystitis found that male gender, age greater than 60 years, WBC greater than 18,000, duration of disease greater than 96 hours, palpable gallbladder, and severe inflammation at surgery were predictive of conversion.[11] In contrast, our study identified a single preoperative variable that was significantly associated with an open cholecystectomy in this patient population.

It is unclear why a higher pre-ES serum bilirubin is associated with an unsuccessful completion of a LC after ES. In several other studies on cholecystectomy outcomes for acute cholecystitis, elevated serum bilirubin was associated with increased infectious complications but not with conversion.[11–14] Elevated serum bilirubin is present in patients with cholangitis and has been shown to be a predictive factor for the severity of cholangitis.[15,16] It is possible that pre-ES serum bilirubin is a surrogate marker for the severity of cholangitis in our patient population. Cholangitis leads to increased inflammation around the portal structures and is likely to make the dissection of Calot's triangle more difficult. Because of the association of pre-ES serum bilirubin and converted/open cholecystectomy, we think the value is useful for planning the venue of the operative procedure. With many cholecystectomies being done in free-standing outpatient facilities, understanding that not all patients with choledocholithiasis can be successfully completed laparoscopically should help guide where the procedure is performed.

There were no significant predictors in our study of complications after cholecystectomy. However, we found that preoperative ERC-ES does not obviate the need for intraoperative cholangiography. Two-thirds of the complications in our series were related to the biliary tract, including bile duct injury (1) and retained CBDS (3). Furthermore, the main reason for converting to an open procedure in our series was the inability to adequately define the biliary anatomy at laparoscopy. Routine use of intraoperative cholangiography is associated with lower bile duct injury rates.[17] Our findings demonstrate that there is an important role for intraoperative cholangiography to verify anatomy and the absence of residual CBDS, despite preoperative ERC-ES.

Finally, we did not find any significant relationship between timing of cholecystectomy after ES and the likelihood of an open operation or complications. In the study by Boerma et al.,[8] subjects who underwent delayed cholecystectomy in the wait-and-see arm had a higher rate of complications and conversion than those in the cholecystectomy arm. These findings could be explained by the subjects' subsequent biliary pathology, such as acute cholecystitis, that led to cholecystectomy. This could also represent a selection bias, as not all subjects in the wait-and-see arm underwent cholecystectomy, and it is possible that if they had, the complication and conversion rates could be equivalent. Other studies on outcomes of cholecystectomy for acute cholecystitis have found that delayed operation increased the risk for conversion.[8,12,13] All but two of the cholecystectomies in our study population were performed within 6 weeks of ES, which was the cutoff for the LC group in the randomized trial. Future studies specifically looking at timing of cholecystectomy after ES for CBDS are warranted to answer this question.

These data provide important information for planning of the procedure and in obtaining informed consent for cholecystectomy after ES. Cholecystectomy for symptomatic choledocholithiasis represents a small fraction of patients undergoing cholecystectomy, only 6.5% in our study. Because this study found that patients undergoing LC after ES have higher conversion rates and higher complication rates compared with other indications, this information can be used when planning the venue of the operation (outpatient surgery center versus inpatient

hospital center). These are technically demanding cases, which often require an open procedure to confirm unclear anatomy. Pre-ES serum bilirubin may be useful in predicting which patients are at high risk for conversion. The outpatient surgery center setting should be reserved for carefully selected patients.

CONCLUSION

Cholecystectomy after endoscopic sphincterotomy for symptomatic CBDS proves to be more difficult, requires a longer operative time, and may require conversion to an open procedure.

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Multiple Gastrointestinal Stromal Tumors of the Ileum and Neurofibromatosis Type 1

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Type 1 neurofibromatosis, also known as von Recklinghausen disease, is one of the most common genetic disorders. Gastrointestinal associations have been well described in these patients, but the true incidence of gastrointestinal tumors and the proportion of these becoming clinically significant are not known. The most common gastrointestinal tumors are stromal tumors, most of which are located in the stomach and jejunum. We discuss the case of a female patient with neurofibromatosis whose initial diagnosis was an ovarian mass. During surgery the diagnosis of an intestinal stromal tumor was made. Operative findings were a multilobulated tumor arising from the ileal wall 50 cm from the ileocecal valve. The tumor did not originate from the nervous myenteric plexus or muscular layer of the small bowel wall; it originated from within the stromal cells of the intestinal wall. Mitotic count showed 3 mitoses per 10 high-power fields. Immunohistochemical stains of the tumor showed positive staining for CD117 and CD34 and negative staining for S100, α-smooth muscle actin, and desmin. The intestinal myenteric plexus showed positive staining for chromegranin A and S100. The histologic characteristics of this patient's tumor are compatible with an undifferentiated stromal tumor of nonneural or nonmuscular origin. (J GASTROINTEST SURG 2006;10:297–301)

KEY WORDS: Neurofibromatosis, gastrointestinal stromal tumors, ileum

Type 1 neurofibromatosis (NF1), also known as von Recklinghausen disease, was first fully described in 1882 by Frederick von Recklinghausen.[1] NF1 is one of the most common genetic disorders, with a frequency of 1:2500 to 1:5000 births.[2] Inherited in an autosomal dominant pattern with variable penetrance, the genetic abnormality has been localized to the long arm of chromosome 17 (17q11.2).[2,3] Approximately 50% of individuals with NF1 lack a family history and are presumed to represent new mutations; therefore, the frequency of NF1 does not vary among different races and ethnic groups.[2] Gastrointestinal associations of NF1 have been well described, but the true incidence of gastrointestinal stromal tumors (GISTs) and the proportion of these becoming clinically significant are not known.[3] Based on retrospective reviews and postmortem examination studies, some have estimated that 10−60% of patients with NF1 have gastrointestinal tumors, although less than 5% actually have associated symptomatology.[3–5] Gastrointestinal complications of NF1 arise during midlife, later than cutaneous lesions.[4] Gastrointestinal involvement in von Recklinghausen's disease commonly occurs in four principal forms: hyperplasia of the gut neural tissue, multiple GISTs, duodenal or periampullary endocrine tumors, and a miscellaneous group of other tumors[3,5]; GISTs are the most common of these.[3,5,7] GISTs associated with NF1 tend to be multiple, with some tumors being malignant and others benign.[3,4] GISTs associated with NF1 present with abdominal pain, palpable abdominal mass, bowel obstruction, intussusception, volvulus or perforation, and gastrointestinal hemorrhage.[3–11] Most NF1 GISTs develop in the wall of the stomach or in the first few centimeters of jejunum.[3,5,7] Some GISTs present as an ovarian mass,[12] although an NF1 GIST presenting as an ovarian mass is rare.

We discuss the case of a young female patient with NF1, whose initial diagnosis was an ovarian mass. During surgery, the diagnosis of a GIST of the ileum was made and was later confirmed by the histopathologic report. She has five of seven diagnostic criteria

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of the consensus criteria of the National Institutes of Health for neurofibromatosis.[13]

CASE REPORT

A 33-year-old female patient had a history of NF1 with multiple café-au-lait spots, axillary and inguinal freckling, multiple discrete cutaneous neurofibromas, a plexiform cutaneous neurofibroma located on the abdominal wall (Fig. 1), Lisch nodules, and a first-degree relative with NF1. She was admitted to the Department of Obstetrics and Gynecology of our institution because she had an ovarian mass diagnosed with ultrasonography. She had no other diagnostic imaging studies performed. Preoperative laboratory tests were normal. Operation was advised. Operative findings were an infiltrative tumor arising from the small intestinal wall at a site 50 cm proximal to the ileocecal valve (Fig. 2). The tumor had infiltrated the right ovary and the free border of the greater omentum and was adherent to but not infiltrating into the walls of the urinary bladder and sigmoid colon. Intestinal resection and end-to-end anastomosis were performed. The entire protruding tumor was resected en bloc together with the free border of the greater omentum. A right ovary resection was also carried out. Postoperative recovery was uneventful.

Pathologic and histologic examination of the surgical specimen demonstrated a large tumor, 12 cm in diameter, and five small satellite nodules ranging from 3 to 15 mm in diameter. The larger tumor comprised the entire intestinal wall thickness and infiltrated the serosal surface and mesentery (Fig. 3). The smaller satellite nodules were enclosed within the intestinal wall; they were subserosal and did not infiltrate the serosa (Fig. 2). The tumor did not originate from the myenteric plexus or the muscular layer of the small bowel wall; it originated from the stromal cells of the intestinal wall (Fig. 4). Mitotic count showed 3 mitoses per 10 high-power fields. Immunohistochemical stains were positive for CD117, CD34, and chromegranin A and negative for S100, α-smooth muscle actin (α-SMA), and desmin.

DISCUSSION

The GIST of our patient had a diameter of 12 cm and had 3 mitoses per 10 high-power fields. Tumors larger than 5 cm with mitotic counts greater than 2 mitoses per 10 high-power fields are considered high-risk tumors; GISTs presenting with mitotic counts greater than 10 mitoses per 10 high-power fields are classified as high-grade malignancy because they show aggressive behavior.[3,4,8,14,15] Even though we performed a complete radical resection (R0 resection),[8] we considered our patient's tumor to be a high-risk tumor.[8,14–16] We enrolled the patient into a follow-up program. Eventually she was referred for additional treatment with the competitive inhibitor of the tyrosine kinases associated with the KIT protein, imatinib mesylate.[14–19]

The Auerbach's myenteric plexus in NF1 patients may be the site of origin for GISTs.[5] The histology

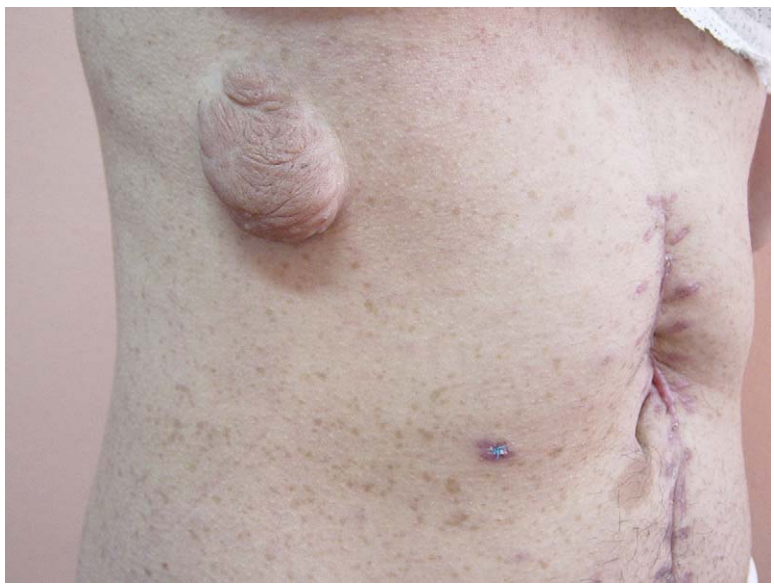

Fig. 1. Plexiform cutaneous neurofibroma (Upper left). Notice the hypertrophic abdominal midline scar from the recent surgery and the cutaneous freckling and *café-au-lait* spots.

Fig. 2. Small intestinal surgical specimen. The large gastrointestinal stromal tumor has a lobulated appearance, while four similar, but smaller, subserosal tumors protrude from the intestinal wall.

and immunochemistry of the tumor in our patient showed that its origin was not the intestinal myenteric plexus or muscular layer cells (Fig. 4). Immunohistochemical staining was negative for neural and muscular cells in the tumor. Chromegranin A and S100 stained the myenteric nervous plexus of the small bowel wall, but they did not stain the spindle cells of the tumor. All benign and malignant histologic GIST variants, except leiomyomas and schwannomas, exhibit CD117. Human CD117, also known as *c-kit*, is a transmembrane protein with receptor tyrosine kinase capacity for a growth factor called stem cell factor or mast cell growth factor.[2–5,7,8,14,15] Immunohistochemical staining for NF1 GISTs[2–7,20,21] stains positive for CD117, CD34, and S100. In this case, immunohistochemical stains of the tumor showed positive staining for CD117 and CD34. The tumor had negative staining for chromegranin A, S100,

Fig. 3. Longitudinal section of the ileal specimen. The intestinal lumen is narrowed by the large tumor, which invades the entire intestinal wall and adjacent mesentery.

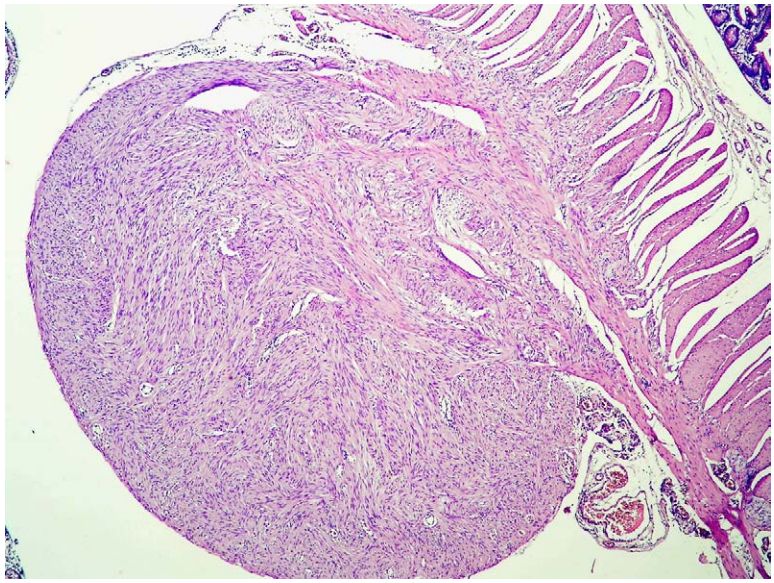

Fig. 4. Longitudinal histological section from one of the smaller, subserosal tumors (Hematoxylin/Eosin stain; magnification × 40), showing the tumor originating from the wall of the small bowel. Immunochemistry stains for S100 and chromegranine A were negative, indicating that the myenteric plexus is not the origin of the tumor.

α-SMA, and desmin. The intestinal myenteric plexus was stained by S100 and chromegranin A, demonstrating that this patient's GIST was not of neural or muscular origin; therefore, the GIST of our patient had no obvious differentiation. Most GISTs show smooth muscle differentiation in 30–40% of the cases, no obvious differentiation in 40%, neural differentiation in 10%, and combined smooth muscle and neural differentiation in 3%.[8]

Postoperatively, the patient underwent abdominal computed tomography (CT) scanning, brain magnetic resonance imaging (MRI), upper endoscopy, and colonoscopy; all examinations had normal results. Abdominal CT scan and brain MRI are the most useful imaging studies in the evaluation and follow-up of NF1 patients.[2,3,9] Other NF1-related tumors, such as optic pathway gliomas and astrocytomas, are diagnosed with brain MRI.[2,3] Abdominal CT scan identifies duodenal periampullary tumors.[3] Endoscopy and colonoscopy are useful for the diagnosis of gastric, duodenal, periampullary, and colonic tumors.[3,9]

CONCLUSION

This case illustrates various typical findings of patients with NF1. The patient had five of seven diagnostic criteria proposed by the National Institutes of Health for NF1.[13] Thus, whenever encountering a pelvic mass adjacent to ovaries in an NF1 patient, the suspicion of an NF1-related tumor should be raised. Because GISTs are the most common tumors related to NF1, this should be the first diagnostic consideration. The histologic and immunohistochemical characteristics of this patient's tumor are compatible with an undifferentiated GIST of nonneural, nonmuscular origin.

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Abdominal Drainage Was Unnecessary After Hepatectomy Using the Conventional Clamp Crushing Technique

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A prophylactic abdominal drainage catheter is routinely inserted by many surgeons in patients after hepatic resection. Between January 2002 and September 2004, 462 consecutive patients who had undergone hepatic resection using a clamp crushing method by the same surgical team were retrospectively divided into the drainage group (n = 357) and the nondrainage group (n = 105). There was no difference in hospital mortality between the two groups of patients (drainage group, 0.6% vs. nondrainage group, 0%; $P = 1.0$). However, there was a greater incidence of surgical complications in the drainage group (31.4% vs. 8.6%, $P < 0.001$), and greater incidence of wound complications and subphrenic complications in the drainage group compared to the nondrainage group (24.4% vs. 4.8%, $P < 0.001$). In addition, the mean (± SEM) postoperative hospital stay of the drainage group was 13 ± 6.5 days, which was significantly longer than that of the nondrainage group (9.7 ± 3.3 days, $P = 0.001$). On multivariate analysis, abdominal drainage and intraoperative bleeding were the independent risk factors that were significantly associated with the incidence of drainage-related complications. The results suggested that routine abdominal drainage is unnecessary after hepatic resection when the conventional clamp crushing method is used during parenchyma transection. (J GASTROINTEST SURG 2006;10:302–308)

KEY WORDS: Abdominal drainage, hepatic resection, postoperative complications

Prophylactic drainage of the peritoneal cavity after abdominal surgery has been widely used for centuries, although few data exist to scientifically support the practice.[1–3] Recent studies have suggested that many routine abdominal surgeries such as cholecystectomy, splenectomy, pancreatic resection, and standard gastrointestinal or colon resection, where drains were once widely used, can be performed safely without prophylactic drainage.[4–15] However, drainage is still conventionally inserted into the subphrenic or subhepatic space closed to the transection surface after hepatectomy, which is employed to release the intra-abdominal tension due to ascitic fluid accumulation and allows the monitoring of the occurrence of postoperative intra-abdominal bleeding as well as the detection and drainage of any bile leakage.[16] The use of drains after hepatic resection has been challenged recently.[17] There have been three prospective randomized trials performed demonstrating that abdominal drainage is unnecessary, or even contraindicated, after minor or major hepatectomy in patients with either a normal or a cirrhotic liver.[18–20] Drains have been reported to have such disadvantages as bowel injuries, increased rates of intra-abdominal and wound infection, increased abdominal pain, decreased pulmonary function, and prolonged hospital stay.[6,11,15,21,22] However, there was no study in mainland China to evaluate the significance of drainage after hepatectomy, where the transection technique was mainly based on the clamp crushing method and most patients were cirrhotic. Therefore, a retrospective study was performed to determine whether abdominal drainage is necessary after hepatic resection in our hospital.

PATIENTS AND METHODS

Five hundred eighty consecutive patients who underwent hepatic resection by the same surgeon group at Liver Cancer Institute and Zhongshan

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Hospital, Fudan University between January 2002 and September 2004 were recruited in this study. One hundred eighteen patients were excluded from this study because they were undergoing hepatic artery ligation and cannulation (n = 64), intraoperative radiofrequency ablation (n = 4), intraoperative ethanol injection (n = 3), cyst fenestration (n = 11), biliary-enteric anastomosis (n = 14), exploration alone (n = 5), or had major injuries to major bile duct or adjacent organs such as the stomach, colon, etc. (n = 17). Therefore, 462 patients were analyzed in this study. Before January 2004, drains were inserted in almost all patients after hepatectomy, except for a few cases (n = 17; 5.5%) who underwent local resection of small tumors. A closed-suction latex drain was inserted into the subhepatic or subphrenic space close to the transection surface before abdominal wound closure. A separate stab wound that was 5–7 cm away from the incision was made and dilated with a clamp to grasp the drain to bring it through the anterior abdominal wall near the right anterior axillary line. The drain was connected to a closed system without suction pressure. After January 2004, abdominal drains were only used in patients who had a concomitant biliary-enteric anastomosis or major injuries to the major biliary duct.

Preoperative investigation of the patients included blood biochemistry, alpha-fetoprotein assay, chest X-ray, percutaneous ultrasonography, computerized tomography or magnetic resonance imaging of the abdomen, and upper digestive tract barium examination to detect esophageal varices. In the drainage group, the abdominal drains were removed on postoperative day 3, unless there was obvious bile leakage greater than 20 ml/day. In patients who experienced leakage of ascites from the main wound or drain site, various methods including suturing of the drain site or leakage site from the main abdominal wound, pressure dressing, and oral diuretic were used to control the leakage. Routine transcutaneous ultrasonography was performed on all patients on postoperative day 5–7 by experienced radiologists to detect any subphrenic collection, ascitic fluid, and pleural effusion. All patients received the same perioperative care by the same team of surgeons and nurses. Clinical data of all patients were recorded in a computerized database, and the operative outcomes of the two groups of patients were compared.

DEFINITIONS

The nomenclature of types of hepatic resection was based on the Brisbane 2000 Terminology of Liver Anatomy and Resections.[23] Left and right hemihepatectomies, left and right trisectionectomies, and other resections involving three or more segments of the liver were considered for a "major resection" category. Operations classified as "minor resection" included wedge resections and segmentectomies for two or less segments. Bile leakage was defined as the presence of ongoing bilious drainage of any volume for more than 1 week postoperatively. Pleural effusion with the symptoms of fever and dyspnea and needing thoracentesis, as well as significant leakage of ascitic fluid from the abdominal drain site after drain removal of greater than 50 ml/day, were considered postoperative complications. According to the postoperative transcutaneous ultrasonography, if the thickness of fluid collection in hypogastric spaces was greater than 5 cm, ascites was defined.

STATISTICAL ANALYSIS

Statistical analysis was performed by the χ^2 test or the Fisher exact test to compare discrete variables. The *t* test was used to compare continuous variables. Multivariate analysis was performed using the logistic regression model to identify independent factors that were associated with postoperative morbidity. Statistical analyses were performed by SPSS 10.0 (SPSS Inc., Chicago, IL). A *P* value of less than 0.05 was considered to indicate statistical significance. Numeric values were expressed in mean ± standard error of mean unless otherwise stated.

RESULTS

There were 357 patients in the drainage group and 105 in the nondrainage group. The drainage group consisted of 276 (77.3%) males with an average age of 50.4 ± 0.6 years. The average age of the patients in the nondrainage group was 50.9 ± 1.2 years, which consisted of 80 (76.2%) males. There were 89 (24.9%) patients with comorbid diseases such as hypertension, diabetes mellitus, cardiovascular diseases, and esophageal varix in the drainage group, whereas 32 (30.5%) patients had comorbid diseases in the nondrainage group. Two hundred and fifty-two (70.6%) patients in the drainage group were found to have a positive serology for hepatitis B surface antigen and 269 (75.4%) patients to have a cirrhotic liver, and 73 (69.5%) and 76 (72.4%), respectively, in the nondrainage group (Table 1). The clinicopathologic parameters were comparable in both groups of patients (Table 2).

There were 103 (28.9%) patients in the drainage group and 21 (20.0%) in the nondrainage group

Table 1. Clinical and laboratory data of drainage and nondrainage groups of patients

Clinical parameter	Drainage group	Nondrainage group
No. of patients	357	105
Sex (male/female)	276/81	80/25
Age* (years)	50.4 ± 0.6	50.9 ± 1.2
Hepatitis B surface antigen serology positive	252 (70.6%)	73 (69.5%)
Hepatitis C surface antigen serology positive	4 (1.1%)	1 (1.0%)
Serum total bilirubin* (μmol/L)	17.9 ± 1.8	15.4 ± 0.6
Serum albumin* (g/L)	41.4 ± 0.3	42.7 ± 0.5
Serum glubulin* (g/L)	29.3 ± 0.3	28.9 ± 0.5
Aspartate aminotransferase* (U/L)	45.6 ± 2.5	44.3 ± 4.6
γ-Glutamyl transferase* (U/L)	118.4 ± 10.4	81.4 ± 7.5
Prothrombin time* (S)	11.3 ± 0.1	11.5 ± 0.1
Hemoglobin* ($\times 10^9$g/L)	132.2 ± 1.0	129.4 ± 1.7
Platelet* ($\times 10^9$g/L)	143.7 ± 3.6	127 ± 5.9
Leukocyte* ($\times 10^{12}$g/L)	5.4 ± 0.1	5.8 ± 0.6
Comorbid diseases	89 (24.9%)	32 (30.5%)
Cirrhosis	269 (75.4%)	76 (72.4%)
Ascitics	11 (3.1%)	5 (4.8%)

P value > 0.05 in all parameters.
*Values expressed as mean ± standard error of mean.

undergoing major hepatic resection with resection of ⩾3 Couinaud's segments (*P* = NS; Table 3). The intraoperative blood loss was 752.9 ± 54.8 ml, which was significantly more than that in the nondrainage group (396.8 ± 56.9 ml, *P* < 0.05; Table 4). Pringle's maneuver was used in 218 (61.1%) patients in the drainage group and 54 (51.4%) patients in the nondrainage group (*P* = NS). Hospital mortality occurred in two (0.6%) patients due to liver failure in the drainage group, and none in the nondrainage group (*P* = NS).

A total of 192 complications occurred in 135 patients, resulting in an overall operative morbidity rate of 29.2% (Table 5). The operative morbidity rate was 33.1% in the drainage group, which was significantly higher than that in the nondrainage group (16.2%, *P* = 0.001). There were 128 surgical complications (defined as any one of bile leak, subphrenic complications, pleural effusion, pneumothorax, intra-abdominal bleeding, wound infection, re-exploration, and leakage of ascites fluid from drain site) that occurred in 112 (31.4%) patients in the drainage group, which was significantly more than those in the nondrainage group (10 surgical complications occurred in nine (8.6%) patients, *P* < 0.001). Sixty-one (17.6%) patients in the drainage group had a total of 63 wound complications, which included wound infection (n = 12) and leakage of ascitic fluid from the drain site (n = 51). The incidence was significantly higher than that of the nondrainage group, in which no wound complications occurred (*P* < 0.001). In addition, the incidence of septic complications was similar between the drainage group and the nondrainage group (6.2% vs. 2.9%, *P* = NS). A total of 24 septic complications occurred in 22 (6.2%) patients in the drainage group. These included subphrenic infection (n = 7), wound infection (n = 12), and chest infection (n = 5). Two (2.9%) patients had a total of three septic complications in the nondrainage group.

Twenty-four (5.2%) patients required postoperative radiological interventions for subphrenic complications, including 20 patients (5.6%) in the drainage group and four patients (3.8%) in the nondrainage group (*P* = NS). Bile leakage was developed in six (1.7%) patients in the drainage group, and none in the nondrainage group. Among them, the bile leakage was detected by the abdominal drains in four patients, so the drains were kept until bile leakage stopped. However, another two patients developed symptoms and signs of intra-abdominal infection 5 days after hepatic resection and 2 days after removal of the abdominal drain. Percutaneous drainage of the collection yielded bile-stained fluid. Three (0.8%) patients had intra-abdominal hemorrhage in the drainage group and one (1.0%) patient in the nondrainage group. The drains detected the occurrence of postoperative intra-abdominal bleeding in the three patients in the drainage group. And the patient in the nondrainage group developed hypotension and a significant drop in hemoglobin on the first day after hepatic resection; subsequent abdominal paracentesis yielded uncoagulated blood. All patients were saved by hemostasis treatment

Table 2. The pathological data of drainage and nondrainage groups of patients

Pathological data	Drainage group	Non drainage group*
Hepatocellular carcinoma	273 (76.5%)	77 (73.3%)
Hemangioma	27 (7.6%)	6 (5.7%)
Cholangiocarcinoma	17 (4.7%)	3 (2.9%)
Focal nodular hyperplasia	5 (1.4%)	3 (2.9%)
Hepatocholangiocarcinoma	4 (1.1%)	1 (0.9%)
Inflammatory pseudotumor	2 (0.6%)	3 (2.9%)
Secondary liver cancer	14 (3.9%)	9 (8.5%)
Other	15 (4.2%)	3 (2.9%)

**P* value > 0.05 for all pathological data.

Table 3. Extent of hepatic resection of drainage and nondrainage groups of patients

Hepatic resection	Drainage group	Nondrainage group
⩾ 3 segments	103 (28.9%)	21 (20.0%)
Left lateral sectionectomy + segment 1 resection	1 (0.3%)	1 (1.0%)
Left hemihepatectomy + segment 1 resection	2 (0.6%)	0 (0.0%)
Left hemihepatectomy	44 (12.3%)	6 (5.7%)
Left trisectionectomy	5 (1.4%)	1 (1.0%)
Right hemihepatectomy	48 (13.5%)	11 (10.4%)
Right trisectionectomy	3 (0.8%)	2 (1.9%)
<3 segments	254 (71.1%)	84 (80.0%)
Left lateral sectionectomy	20 (5.6%)	11 (10.5%)
Right posterior sectionectomy	23 (6.4%)	2 (1.9%)
Wedge resection	188 (52.7%)	66 (62.8%)
Segmentectomy	23 (6.4%)	5 (4.8%)

and blood transfusion. One patient in the drainage group developed localized peritonitis 1 month after hepatic resection and underwent re-exploration to remove the necrotic tissues. The mean postoperative hospital stay of the drainage group was 13.3 ± 6.5 days, which was significantly longer than that of the nondrainage group (9.7 ± 3.3 days, $P < 0.05$).

There were 90 complications, including subphrenic collection and abscess, wound infection, and leakage of ascites fluid from the drain site, which were considered related to the presence of the abdominal drains. They occurred in 87 (24.4%) patients in the drainage group, whereas only five complications occurred in five (4.8%) patients in the nondrainage group ($P < 0.001$), showing that the drainage-related complications in the drainage group were significantly more than those in the nondrainage group.

Statistical analysis was performed on all 462 patients to identify independent factors that were associated with postoperative morbidity. The following thirteen factors were examined: age, sex, serum total bilirubin, serum albumin, aspartate aminotransferase, prothrombin time, hemoglobin, platelet, comorbid diseases, underlying liver cirrhosis, extent of hepatic resection, intraoperative blood loss, and abdominal drainage, in which underlying liver cirrhosis, intraoperative blood loss, and abdominal drainage were considered as the risk factors on postoperative morbidity. In multivariate analysis, the intraoperative blood loss greater than 500 ml was the only independent risk factor that was significantly associated with the incidence of postoperative complications ($P = 0.007$, relative risk = 1.908, 95% CI = 1.194–3.050), whereas the abdominal drainage was not the influencing factor. However, abdominal drainage and intraoperative blood loss ($P = 0.02$, relative risk = 0.534, 95% CI = 1.315–2.905) were independent risk factors in multivariate analysis of the incidence of drainage-related complications.

DISCUSSION

Currently, hepatic resection can be performed with mortality rates below 5% and with acceptable complication rates of 16% to 31%.[24–26] Subphrenic collections and biliary fistulas are still the most common intra-abdominal complications after liver resection,[25,27] and most surgeons around the world still use prophylactic drains obligatorily to prevent or detect these complications at an early stage.

The first study showing that abdominal drainage was unnecessary after hepatectomy was reported by Franco et al.[17] Three randomized control trials failed to show any advantage of prophylactic drainage.[18–20] Two of them were performed in Western countries, where most hepatectomies were performed in patients without cirrhosis.[18,19] As a supplement, Liu et al.[20] investigated the effect of abdominal drainage in 104 cirrhotic patients after liver resection. There was a significantly greater rate of infection complications, wound complications, and longer hospital stays in patients in the

Table 4. Intraoperative and postoperative data of drainage and nondrainage groups of patients

Intraoperative and postoperative data	Drainage group	Nondrainage group	*P*
Resection > 3 segments	103 (28.9%)	21 (20.0%)	0.080
Intraoperative blood loss* (ml)	752.9 ± 54.8	396.8 ± 66.9	<0.05
Pringle's maneuver	218 (61.1%)	54 (51.4%)	0.091
Postoperative hospital stay* (days)	13.3 ± 6.5	9.7 ± 3.3	0.001
Hospital mortality	2 (0.6%)	0 (0.0%)	1.000
Operative morbidity	118 (33.1%)	17 (16.2%)	0.001

*Value expressed as mean ± standard error of mean.

Table 5. Postoperative complications in drainage and nondrainage groups of patients

Complications	Drainage group	Nondrainage group	*P*
Surgical complications	128 (35.9%)	10 (9.5%)	<0.001
Bile leak	6 (1.7%)	0 (0.0%)	0.345
Subphrenic complications	27 (7.6%)	5 (4.8%)	0.320
Subphrenic collection	20 (5.6%)	4 (3.8%)	0.619
Subphrenic infection	7 (2.0%)	1 (1.0%)	0.689
Pleural effusion	26 (7.3%)	4 (3.8%)	0.263
Pneumothorax	2 (0.6%)	0 (0.0%)	1.000
Intra-abdominal bleeding	3 (0.8%)	1 (1.0%)	1.000
Wound infection	12 (3.4%)	0 (0.0%)	0.077
Re-exploration	1 (0.3%)	0 (0.0%)	1.000
Leakage of ascites fluid from drain site	51 (14.3%)	—	—
No. of patients with surgical complications	112 (31.4%)	9 (8.6%)	<0.001
Medical complications	38 (10.6%)	16 (15.2%)	0.198
Liver failure	5 (1.4%)	3 (2.9%)	0.315
Ascites	13 (3.6%)	6 (5.7%)	0.400
Cardiac arrhythmia	2 (0.6%)	2 (1.9%)	0.223
Chest infection	5 (1.4%)	2 (1.9%)	0.660
Cardiac dysfunction	8 (2.2%)	2 (1.9%)	1.000
Cerebrovascular disorders	2 (0.6%)	1 (1.0%)	0.539
Renal failure	1 (0.3%)	0 (0.0%)	1.000
Other	2 (0.6%)	0 (0.0%)	1.000
No. of patients with medical complications	33 (9.2%)	11 (10.5%)	0.705
Total complications	166 (46.5%)	26 (24.8%)	<0.001
No. of patients with complications	118 (33.1%)	17 (16.2%)	0.001
No. of patients	357	105	

A total of 166 complications occurred in 118 patients in the drainage group, and 26 complications occurred in 17 patients in the nondrainage group. Total complication rates in the drainage group and the nondrainage group were 33.1% and 16.2%, respectively ($P = 0.001$).

drainage group. Recently, another prospective study reported by Liu et al.[28] demonstrated that abdominal drainage was not mandatory after donor hepatectomy in live donor liver transplantation. Most of these investigations were conducted in highly specialized institutions where the Cavitron Ultrasonic Aspirator (CUSA; CUSA Technologies, Salt Lake City, UT) was routinely used during parenchyma transection and most patients had chronic liver diseases. Because CUSA and other transection apparatus were not routinely used in mainland China, our current study was to evaluate the necessity of abdominal drainage in patients after hepatic resection using the conventional clamping crush method.

Monitoring of the occurrence of postoperative intra-abdominal bleeding and the detection and drainage of any bile leakage are two of the theoretical advantages of abdominal drainage.[16] However, the placement of abdominal drains may provide a false sense of security to surgeons. Drains can be blocked by blood clot in the presence of postoperative intra-abdominal bleeding, and they may not be in the correct position to detect any biliary leakage, which further delays appropriate intervention. In our study, we failed to detect bile leakage in two patients until they developed symptoms of intra-abdominal infections. To monitor of the occurrence of postoperative intra-abdominal bleeding, we always kept a close watch on the blood pressure and hematocrit instead of observing the effluent of the drain, because the drain was often mixed with ascites or it was occasionally blocked. By observing the blood pressure and the hematocrit, all four patients who had intra-abdominal bleedings were treated opportunely and efficaciously in our study. Twenty (5.6%) patients in the drainage group needed another percutaneous drainage because of high fever; four (3.8%) patients in the nondrainage group needed this procedure. With advances in the radiological interventions, the consequence of a subphrenic collection or biliary fistula in an nondrained patient can be solved by ultrasound-guided percutaneous drainage.[29–34] Therefore, sometimes drainage was useless because it did not always drain abdominal fluid accumulations, nor did it always detect the postoperative intra-abdominal bleeding and bile leakage.

Although retrograde bacterial contamination along abdominal drains has been observed in some

studies,[22,35,36] our current study showed that the incidence of septic complications was similar between the drainage group and the nondrainage group.

The multivariate analysis in our study showed that only the intraoperative blood loss was the independent risk factor that was significantly associated with the incidence of postoperative complications, whereas the abdominal drainage was not the influencing factor, which suggested the postoperative morbidity will not increase without drainage after hepatic resection.

However, in our present study the incidence of wound complications in the drainage group (17.1%) was significantly higher than that in the nondrainage group (0.0%). Significant leakage of ascitic fluid from the drain site was observed in 14.3% of the patients. Before removing the drainage, the ascitic fluid leaked from the abdominal cavity around the drainage catheter when it failed to drain any ascitic fluid collections because of blocking. More leakages of the ascitic fluid, which led to a continuous soaking of the abdominal wound, also led to difficulties in the application of wound dressing and nursing care, and discomfort and mental distress of the patients were observed in patients after removing the drain. Continuous soaking of the abdominal wound with possible contaminated ascitic fluid might also contribute to an increase in the incidence of wound infection. In multivariate analysis, the abdominal drainage was one of the independent risk factors that were significantly associated with the incidence of drainage-related complications. Additionally, the postoperative hospital stay in the drainage group was longer than that in the nondrainage group, which predicates more hospital costs in the drainage group.

In this study, improvement in liver resection techniques in our group, such as anatomical resection, outflow control, methylene blue test for bile leaks, especially meticulous hemostasis, resulted in significant reduction of intraoperative blood loss after January 2004, and undoubtedly helped to prevent postoperative complications. The decreased post operative complications founded the basis of a "no-drain" policy, which further decreased the drainage-related complications.

CONCLUSION

In summary, this study showed that abdominal drainage was unnecessary because it may fail to detect hemorrhage or bile leakage in the surgical site, and may produce drainage-related complications.

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Neoadjuvant Radiochemotherapy for Patients With Locally Advanced Rectal Cancer Leads to Impairment of the Anal Sphincter

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Neoadjuvant radiochemotherapy (RCTx) has become an acceptable therapy for patients with locally advanced rectal cancer. However, little is known about the effect of the RCTx on the function of the anal sphincter. Forty-one consecutive patients with locally advanced rectal cancer (cT3, N+) underwent neoadjuvant RCTx with subsequent resection. All patients were examined clinically and by anal manometry for their anal sphincter function. A multichannel water-perfused catheter system was used, and resting pressure, maximum squeeze pressure, and length of the anal high-pressure zone were determined prior to the neoadjuvant therapy and before the operation. The length of the high-pressure zone did not change after the neoadjuvant therapy. However, resting and maximum squeeze pressure decreased significantly after preoperative RCTx. This effect was more pronounced for the resting pressure rather than the maximum squeeze pressure, indicating that the internal sphincter is primarily affected. These results correlated with the clinical data showing an impaired continence status in patients treated with neoadjuvant therapy. Neoadjuvant RCTx leads to impairment of the anal sphincter predominantly in the internal sphincter. This effect may enhance the surgical impairment of continence after curative resection.
(J GASTROINTEST SURG 2006;10:309–314)

KEY WORDS: Anal sphincter, neoadjuvant therapy, anal manometry

Neoadjuvant radiochemotherapy has become an acceptable therapeutic tool for the treatment of locally advanced rectal cancer.[1–3] It may increase the rate of curative resection by downstaging[2,3] and may provide sphincter preservation in tumors located in the distal rectum.[4–6] Additionally, by applying irradiation preoperatively, oxygen tension in the tumor is higher due to untouched blood supply, leading to a more effective treatment.[7]

Several national randomized studies demonstrated a beneficial effect of neoadjuvant radiochemotherapy compared with adjuvant treatment or surgery alone. The Swedish Rectal Cancer Trial demonstrated an improved survival with a preoperative 5 × 5-Gy regimen compared with surgery alone.[8] The Dutch Colorectal Cancer Group showed better local tumor control with preoperative irradiation leading to a reduction in local recurrences.[9] The German Trial CAO/ARO/AIO-94[10] presented data from an interim-analysis including 805 patients randomly assigned to neoadjuvant radiochemotherapy or adjuvant radiochemotherapy, showing that preoperative radiochemotherapy does not bear a higher risk for complications in regard to postoperative morbidity compared with the adjuvant regimen and has a favorable impact on survival.

However, little is known about the effect of preoperative radiochemotherapy on the anal sphincter function. Most of the published studies addressing anal sphincter function were done after the resection of the rectum, making it impossible to distinguish between the solely effect of radiochemotherapy prior to the resection or the detrimental effect of surgery in addition to the neoadjuvant therapy.[11,12] It is well known that surgery alone, meaning low anterior resection, leads to a poor functional outcome; even so, anal sphincter function is normal in these studies.[13,14]

Knowledge of the potential detrimental effect of pelvic irradiation comes largely from studies in patients treated for prostate or bladder cancer.[15] Some studies suggest that anal sphincter impairment occurs often after irradiation, whereas others claim that the

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anal sphincter is unaffected.[16–20] Gervaz and colleagues[21] nicely showed that irradiation leads to a radiation-induced fibrosis based on an overproduction of fibrogenic cytokines, such as transforming growth factor-β1. This leads to the overproduction of collagen, fibronectin, and integrins. Histologically, endothelial damage, collagen deposition, and sclerosis can be observed.[22–24]

The effect of neoadjuvant radiochemotherapy on anal sphincter function in patients with rectal cancer is largely unknown. This effect might add to the poor functional outcome after low anterior resection.

PATIENTS AND METHODS

The study population consisted of 41 consecutive patients with locally advanced rectal cancer (uT3) considered for neoadjuvant radiochemotherapy followed by curative anterior or low anterior resection. Primary tumor staging was done with magnetic resonance imaging, abdominal ultrasonography, and endoscopy with ultrasound. All patients underwent clinical examination and manometry of the anal sphincter prior to the neoadjuvant radiochemotherapy and before the resection. Continence status was assessed by a modified Wexner score. The continence score postoperatively was measured 2–4 weeks after closure of the protective ileostomy.

Neoadjuvant Radiochemotherapy

Chemotherapy consisted of six cycles of 5-fluorouracil as continuous infusion in doses of 250 mg/m^2. Radiotherapy was administered with a total dose of 45 Gy in fractions at 1.5 Gy per day treated daily, 5 days a week. The irradiation is designed to include the entire tumor bed up to the internal iliac nodes.

Surgery

The operation was performed 4–6 weeks after the end of the neoadjuvant radiochemotherapy. Resection was done according to the principles of total mesorectal excision, meaning sharp dissection under direct vision along the parietal pelvic fascia, preserving the pelvic hypogastric nerve supply. Reconstruction was done by stapled end-to-end anastomosis with the level of the anastomosis reaching from 2 cm up to 8 cm from the anal verge depending on the location of the carcinoma. No pouch reconstruction or other type of new reservoir was created.

Manometry

Anorectal manometry was performed by using an eight-channel water-perfused catheter system. The catheter was connected to a polygraph and monitored and analyzed with Polygram for Windows, Version 2.0 (Synectics Medical, Medtronic, Middlesex, England). The patients were placed in a left lateral position. After insertion of the catheter within the rectum, a 3-minute adaptation period was allowed. Hereafter, the catheter was pulled back through the sphincter region in 1-cm increments to measure the length of the sphincter. Resting pressure was assessed by placing the manometry catheter within the high-pressure zone and leaving it there for 2 minutes. For evaluation of the maximum squeeze pressure, the catheter was placed within the high-pressure zone and the patients were asked to maximal squeeze. Attention was paid to the fact that only the sphincter muscles were used without squeezing the gluteal muscles. All measurements were done at least five times, and the mean was calculated. In 14 of 41 patients, manometry data were available after the resection and replacement of a potential protective stoma.

Statistical Analysis

For comparison of the manometry data, a two-tailed *t* test was used; for comparison of the continence score, a Mann-Whitney *U* test for nonparametric data was used. $P < 0.05$ was considered statistically significant.

RESULTS

There were 10 women and 31 men (median age, 61 years; age range, 34–81 years). All patients were diagnosed with locally advanced rectal cancer (uT3) and completed the entire neoadjuvant radiochemotherapy protocol. The control group consisted of 30 people not having any symptoms regarding anal sphincter problems. Anal manometry was performed prior to the radiation and chemotherapy and before the operation, meaning 4–6 weeks after the radiochemotherapy. Manometry results of the control group were not significantly different compared with the group of patients before the neoadjuvant radiochemotherapy (Fig. 1). As seen in Figures 1 and 2, the length of the anal sphincter did not decrease significantly after the radiochemotherapy, whereas resting pressure and maximum squeeze pressure decreased significantly after neoadjuvant radiochemotherapy. The resting pressure decreased by almost 21% compared with a 14% decrease for the maximum squeeze pressure, demonstrating a more pronounced effect of the radiochemotherapy on the internal anal sphincter. This decrease in sphincter pressure was even more pronounced after the operation and closing of the diverting stoma. These manometric

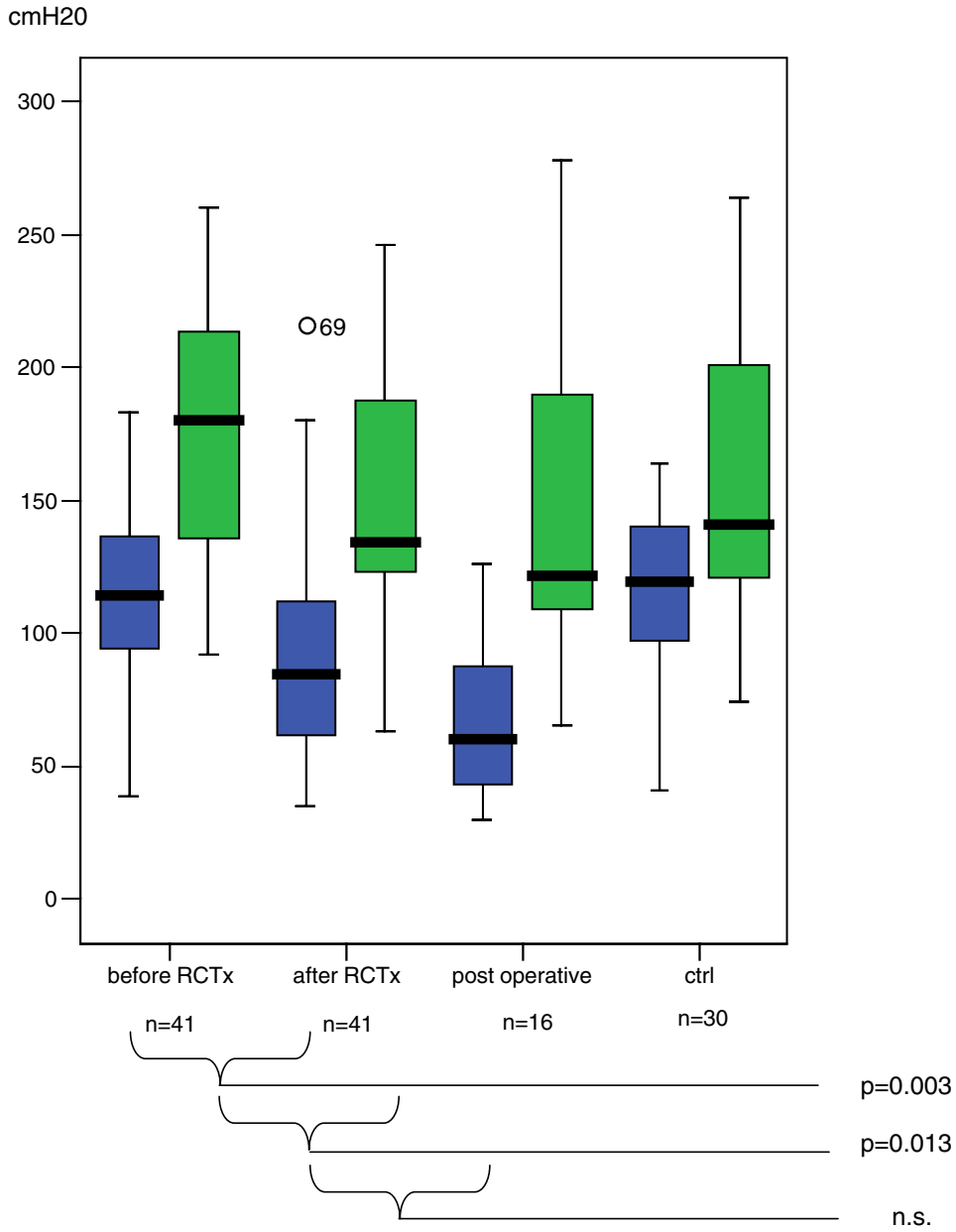

Fig. 1. Manometry results before and after radiochemotherapy (RCTx), resting pressure: box plots on the left in the respective groups; maximum squeeze pressure: box plots on the right in the respective groups.

results correlate to the clinical data showing a pronounced effect of neoadjuvant therapy on the continence status (Table 1). Sixteen patients underwent anal manometry after the resection and replacement of a potential protective stoma. The diverting stoma was closed approximately 6 weeks after the initial operation. In contrast to the other results, sphincter length decreased significantly postoperatively (Fig. 2).

DISCUSSION

This study shows objective evidence that neoadjuvant radiochemotherapy impairs anal sphincter function, leading to some degree of incontinence independent of the detrimental effect of an anterior resection, which consequently will enhance this effect. Although the oncologic benefit of neoadjuvant therapy in addition to radical curative resection is undoubted, functional aspects have not been addressed frequently but play an important role in the quality of life after a combined treatment.

The loss of rectal pooling capacity after radical resection leads to incontinence to liquid and solid feces in one of four patients[13,14]; even so, the sphincter function itself is not hampered. Adding the detrimental effect of radiochemotherapy to this situation

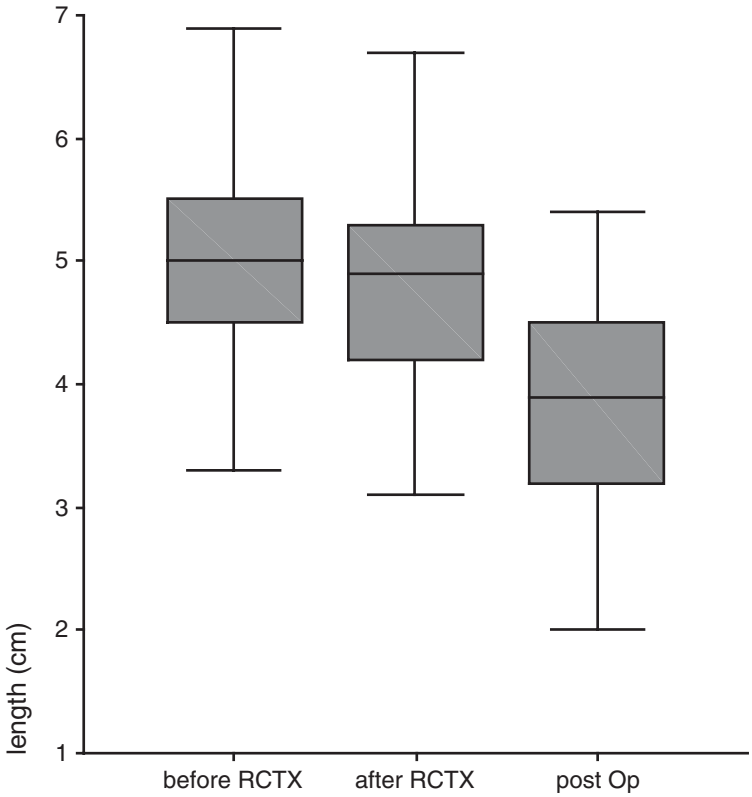

Fig. 2. Length of anal sphincter before and after radiochemotherapy (RCTx) and postoperatively (post Op) (n = 16 in the post Op group and 41 in the other groups). There was a statistically significant decrease in length postoperatively ($P < 0.05$).

as described in this study may enhance the functional outcome considerably. Whether the increase in continence score is due to the pelvic irradiation alone cannot be answered by our study. It may well be that impairment of reservoir function considerably contributes to this effect because all patients underwent straight stapled anastomosis. Studies have demonstrated that by creating a new reservoir such as a pouch reconstruction, functional outcome may improve.

The decrease in sphincter pressure after closure of the iliostomy may be due to the development of fibrosis. However, these results are based on only 16 of the total of 41 patients and there was only one time point of manometry data available postoperatively. Therefore, conclusions cannot be drawn from these data. Further measurements are needed to clarify this point. There might be long-term adaptation developing in these patients, and improvement is possible with changes in diet and bowl management and sphincter exercises.

Table 1. Continence Score in the Three Groups Based on a Modified Wexner Score.

	Before RCTX (n = 41)	After RCTX (n = 41)	Postoperative (n = 14)
Score	1 (0–3)	7 (4–10)	4 (3–11)

Values given as median (range). There was a statistically significant increase in the continence score before and after radiochemotherapy (RCTX). No significant difference was present between the group and the other two groups.
From Vaizey et al.[34]

Biofeedback has been a valuable tool in improving functional anal sphincter disorders. Because the effect of neoadjuvant radiochemotherapy occurs predominantly on the internal sphincter, biofeedback may not play a significant role in improving functional outcome.

Even though radiotherapists try to spare the sphincter region when administering radiation, current protocols of neoadjuvant radiochemotherapy still involve the anal canal. Recently, sphincter-preserving radiation therapy has been introduced, avoiding the sphincter region by using a special "sphincter block" to reduce the treatment volume distally.[21] However, this method has not been studied prospectively with objective parameters, such as anal manometry.

The fact that in our study the reduction in the resting pressure after neoadjuvant treatment was more pronounced in contrast to the maximum squeeze pressure indicates that the internal sphincter is affected primarily by radiation. This is in conjunction to other studies suggesting that the internal sphincter is the predominant sphincter at risk when applying radiation to the lower pelvis.[25] Studies aimed at the correlation of manometric data with quality of life index suggested that reduction in internal sphincter function is closely associated with a poor quality of life.[26]

The mechanisms responsible for these changes remain unclear. Physiologic data from the use of anal canal electromyography showed no change after radiation, suggesting a myogenic effect rather than a neurogenic effect.[27] Another theory comes from data on brachial plexopathy as a well-known long-term complication after irradiation for breast cancer.[28] This neuropathy is closely related to fibrosis around the nerve trunks. In patients treated for rectal cancer, this theory could not be proved with electromyography data.

Even the studies that report a beneficial effect of neoadjuvant radiochemotherapy in regard to local tumor control and survival describe a high rate of post-treatment bowel dysfunction, up to 30% in comparison to 10% after surgery alone.[8]

The effect on anal sphincter function is dose dependent. Kusunoki and colleagues[29] demonstrated that after applying high-dose radiation (80 Gy), resting pressure and maximum squeeze pressure decrease was more pronounced than after low-dose irradiation (30 Gy). Other studies did not show any correlation of pelvic irradiation and impaired anorectal function but lack homogeneous methodology and interpretation such as different target organs and different dosage to the anal canal.[18,30]

Whether the sphincter impairment is a permanent effect remains largely unknown. Controversial studies have been published, some demonstrating a permanent impairment of anal sphincter function[31,32]; others report a sphincter recovery after 1 year.[27] Histologic studies showed a time-dependent increase in collagen deposition after radiochemotherapy with a fibrosis in about 80% of the patients.[33] These changes may be responsible for the sphincter disturbances observed in these patients. Our data only provide a trend toward a persistent impairment of anal sphincter function due to the low number of patients[16] studied postoperatively. Perhaps the development of fibrosis explains the long-term effect of radiochemotherapy. Of note is that postoperatively, the length of the sphincter seems to decrease. This may be due to a scar developed within the sphincter area.

Despite the beneficial oncologic effect of preoperative radiochemotherapy, functional aspects play a considerable role for patients after this multimodal therapy and have been underestimated in the past. Posttreatment functional disturbances after multimodal therapy for rectal cancer remain a complex problem. Preserving sphincter function plays an important role in functional outcome together with reconstruction of a reservoir.

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Cyclooxygenase-2 Expression and Clinical Outcome in Gastrointestinal Stromal Tumors

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The significance of cyclooxygenase-2 (COX-2) expression in mesenchymal tumors has not been completely described. We analyzed clinicopathologic variables and COX-2 protein expression in all mesenchymal tumors of the GI tract that were treated at our institution between 1990 and 2002. Paraffin-embedded specimens were immunohistochemically stained for KIT and COX-2 protein. KIT-positive tumors were diagnosed as gastrointestinal stromal tumors (GIST). Among 42 available specimens, 38 tumors were diagnosed as GIST and four were non-GIST mesenchymal GI tumors (KIT negative). The median overall survival for the GIST patients was 34 months. Ninety-two percent of GIST expressed COX-2 protein. COX-2 protein was not expressed in any of the non-GIST tumors. GIST patients with negative or low COX-2 expression developed disease recurrence and/or died of their disease in 37% of the cases, compared with 18% for GIST patients with high COX-2 expression (difference not statistically significant). The vast majority of mesenchymal tumors of the GI tract are GIST that express COX-2 protein. As opposed to known predictors of GIST behavior such as tumor size and mitotic count, levels of COX-2 protein expression did not correlate with clinical outcome. (J GASTROINTEST SURG 2006;10:315–319)

KEY WORDS: Gastrointestinal stromal tumors, cyclooxygenase-2

Gastrointestinal stromal tumors (GIST) are tumors of mesenchymal origin that arise from the interstitial cell of Cajal and that occur throughout the GI tract. Most commonly, GIST occur in the stomach (60%–70%), but they may also develop in the small bowel (25%–35%), colon and rectum (5%), and esophagus (less than 2%).[1] GIST are characterized by the presence of constitutively activated KIT protein, a receptor tyrosine kinase. The mutated protein performs its tyrosine kinase activity in the absence of its ligand, stem cell factor.[2] This gain of function may be the result of mutations in exon 9, 11, 13, or 17 of the c-KIT gene, or a mutation in the PDGF receptor alpha gene.[1] Although KIT-positive tumors encompass a spectrum of histologic cell types and tumor behavior, GIST represent the majority of mesenchymal tumors occurring in the GI tract with the exception of true smooth muscle tumors such as esophageal leiomyosarcoma and colonic leiomyosarcoma, as well as glomus tumor, inflammatory fibroid polyp, and schwannoma.[3] Identification of GI mesenchymal tumors as GIST versus non-GIST has become more imperative with the advent of imatinib mesylate (Gleevec, Novartis, Geneva, Switzerland), a KIT tyrosine kinase inhibitor which has been shown to improve survival in cases of metastatic or unresectable tumors.[4]

Despite a common origin and a similar mechanism of carcinogenesis, GIST tumors demonstrate a variety of clinical behavior patterns that range from relatively benign and indolent to frankly malignant. Size greater than 5 cm, mitotic index, male sex, incomplete resection, and unresectability have been recognized to have clinical significance as predictors of malignant tumor behavior.[5–8] In addition to these clinical characteristics of GIST tumors, on the molecular level, the particular type of mutation leading to the activation of the KIT protein seems to correlate with clinical outcome and response to imatinib therapy.[1,5,9] However, predicting clinical behavior patterns on the basis of the mutation type would be costly and time consuming. There is a need to establish easily identifiable biologic markers of GIST behavior that may help establish clinical prognosis.

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Recent data implicates the overexpression of cyclooxygenase-2 (COX-2) protein in both carcinogenesis and progression of a wide range of human malignancies. The level of expression of COX-2 has been shown to be elevated in premalignant lesions of the gastric and colonic mucosa, indicating that there is involvement with carcinogenesis of those tumors.[10,11] In association with vascular endothelial growth factor (VEGF), COX-2 has also been shown to promote tumor angiogenesis and thereby influence tumor growth and metastases.[12] This effect has been documented in both gastric carcinoma[13] and bladder cancer.[14] Correlations with decreased survival have been made in oligodendroglial neoplasms[15] and small cell lung carcinoma,[16] although no relationship can be established for colonic neoplasms.[9]

Although there exists a growing body of evidence regarding the significance of COX-2 expression in carcinomas, very little is known about the role of COX-2 in tumors of mesenchymal origin.[17] There is evidence that COX-2 is expressed in varying degrees in soft tissue sarcoma, although no correlation can be drawn between the level of COX-2 expression and other clinicopathologic features.[5] Specifically, the role of COX-2 in GIST has only recently been addressed. Sheehan et al.[18] found that COX-2 is indeed overexpressed in varying degrees in GIST tumors, especially in the epithelioid histologic variant. Although that same group was able to identify higher expression of COX-2 in malignant tumors and tumors located in the stomach, the clinical implications of COX-2 overexpression in GIST tumors remain relatively unknown. In the present study, we have analyzed clinicopathologic variables of patients with gastrointestinal mesenchymal tumors who were treated at our institution. We have correlated those variables with the pattern of tumor-associated COX-2 protein expression in an attempt to establish the role of COX-2 as a potential prognostic factor in patients with GIST.

MATERIALS AND METHODS

All patients included in this study consented to the use of their tissues for medical research. The medical records of all patients who underwent surgical resection of nonepithelial tumors of the gastrointestinal tract between 1990 and 2002 were reviewed. Forty-two paraffin-embedded tumor specimens were identified out of a possible sixty patients treated at the University of Alabama at Birmingham Hospital during the specified time period. Five-micron slices were mounted on glass slides for staining. A panel of stains is needed to separate GIST from other tumors.[19] Accordingly, specimens were stained for KIT (CD117), CD34, smooth muscle actin, S-100, and COX-2 protein using standard immunohistochemical techniques, including the ABC universal kit (Vector Laboratories, Burlingame, CA). Rabbit monoclonal antihuman KIT was supplied by Dako (Carpenteria, CA), whereas mouse monoclonal antihuman COX-2 was obtained from Cayman Chemical (Ann Arbor, MI).

All specimens staining positive for KIT protein were considered GIST tumors, whereas those staining negative for KIT were considered non-GIST tumors. The immunohistochemical stain scoring was performed by two pathologists (N.J. and B.R.) who independently scored each specimen based on a combination of the percentage of positive tumor cells and the intensity of stain. The proportion of COX-2 expressing cells varied from 0%–100%, and the cytoplasmic staining intensity varied from none (0) to very strong (+4). In addition, the immunostaining scores of the two observers were combined to obtain an average percent positive score, as well as staining intensity for each case. These stringent assessment criteria were taken to eliminate the individual observer bias in reporting the staining intensity.

Having been assigned a score reflecting the level of COX-2 protein expression, clinical and pathologic parameters were obtained for each specimen. Parameters included tumor size, tumor location, mitotic count, and overall survival. Tumor size greater than 5 cm (for gastric GIST) and greater than 2 cm (for nongastric GIST) as well as mitotic count greater than 5/50 high power fields were considered as factors indicative of malignant potential.[19] Descriptive statistics as well as correlation of those outcome measures with the level of COX-2 protein expression were performed using the Fisher exact test, *t* test, log-rank, and life table methods.

RESULTS

Immunohistochemical staining was performed on 42 tumor specimens for this study. Based on the presence or absence of KIT staining, of those 42 tumor specimens, 38 were designated GIST tumors, whereas four were designated non-GIST mesenchymal tumors. The non-GIST tumors were identified as one leiomyosarcoma, one leiomyoma, one schwannoma, and one inflammatory polyp. Most tumors were located in the stomach (64%), with a significant portion in the small bowel (31%) and the remainder in the large bowel (5%). Twelve of the tumors (29%) were greater than 10 cm.

Median follow-up time was 24 months. Of the 38 patients with GIST, 11 patients died of disease and three are alive with recurrence. Median survival for patients with GIST was 34 months. Five-year actuarial survival rate for patients with GIST tumor was 37% (Fig. 1).

Most GIST tumors were found to express COX-2 protein in varying degrees. Among the 38 GIST specimens, 35 (92%) expressed COX-2 protein. Of those 35 specimens, 69% had a low COX-2 expression level (Fig. 2, *A*), whereas the remaining 31% had a high expression level (Fig. 2, *B*). None of the non-GIST mesenchymal tumors expressed COX-2 (Fig. 2, *C*). Level of COX-2 expression did not correlate to tumor location (gastric vs. nongastric) or tumor size (less than 5 cm vs. greater than 5 cm). However, GIST-associated mitotic count was found to vary inversely with COX-2 expression ($P = 0.03$). Patient demographics as well as tumor characteristics and their relationship to levels of COX-2 expression are depicted in Table 1. GIST patients with negative or low COX-2 expression died of disease and/or developed disease recurrence in 10/27 cases (37%), compared to 2/11 cases (18%) of those with high COX-2 expression. This difference however, did not reach statistical significance.

DISCUSSION

In recent years, the aberrant expression of COX-2 protein by epithelial tumors of the GI tract has been well documented. Furthermore, the expression of COX-2 seems to correlate with poor clinical outcome in these tumors. There is also a growing body of evidence that COX-2 inhibition has therapeutic implications in colon carcinoma.[20] Despite the recognition of the association between COX-2 expression and epithelial tumors, little is known about the role of COX-2 in carcinogenesis, progression, and prognosis in tumors of mesenchymal origin.

Precise identification of tumors of the GI tract as GIST or non-GIST has become particularly important with the advent of Gleevec. The most reliable molecular marker to date has been c-KIT, which is universal among GISTs, whereas cell type is widely variable and may include spindle cell, epithelioid, and pleomorphic.[3] COX-2 protein, which has been shown to be constitutively expressed in the interstitial cell of Cajal, may be a second marker for GIST.[18] After immunohistochemical staining, our findings indicate that GIST selectively display COX-2 protein when compared to non-GIST mesenchymal tumors of the GI tract. None of the other types of tumors, including leiomyosarcoma, leiomyoma, or schwannoma, had detectable levels of COX-2 protein. About 95% of GIST express KIT, and that is currently the main diagnostic criteria for this kind of tumor. GIST are also positive for CD34 (approximately 60% of cases) and typically negative for S100 protein and desmin. COX-2 may be useful as an additional molecular marker to aid in the identification of GIST, particularly in those cases with epithelioid histology or where KIT is

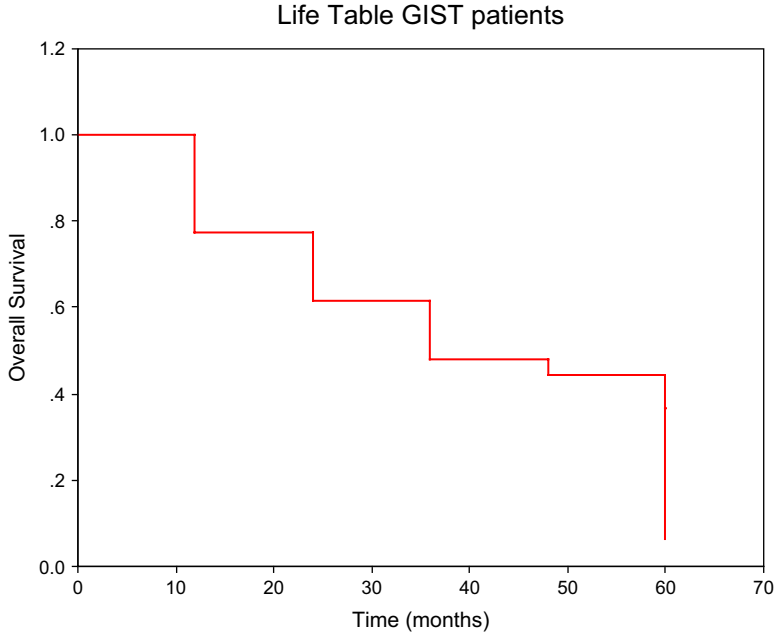

Fig. 1. Life table of GIST patients.

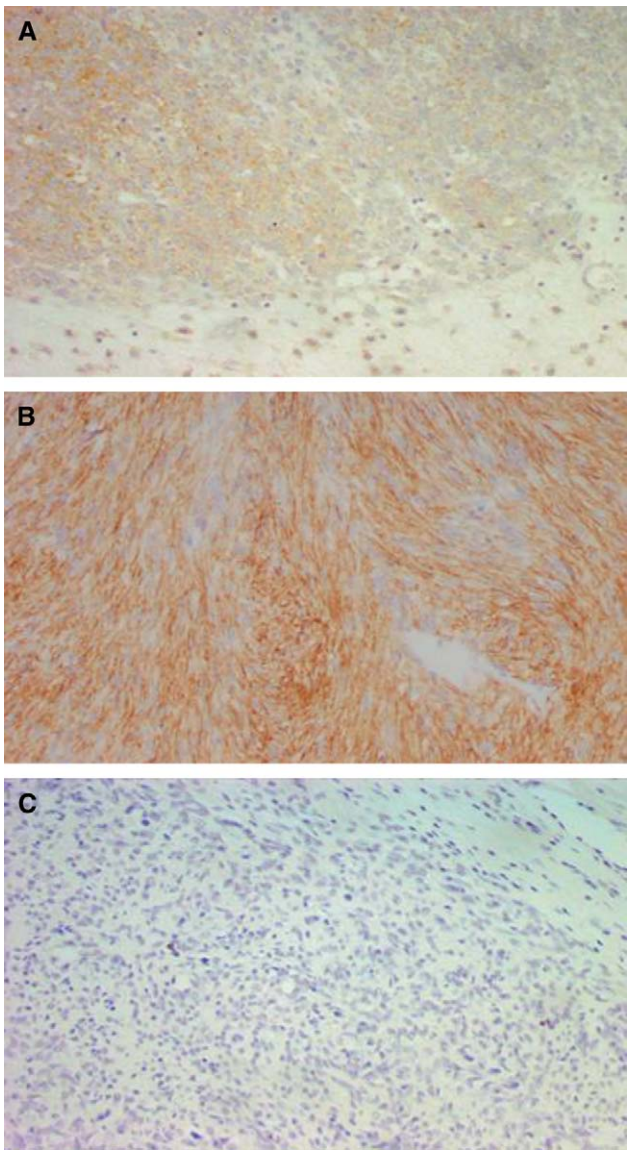

Fig. 2. Immunohistochemical staining for COX-2 protein (X20). (**A**) GIST with low level of COX-2 expression; (**B**) GIST with high level of COX-2 expression; (**C**) Leiomyosarcoma with undetectable COX-2 expression.

Table 1. Clinical characteristics of GIST with respect to expression of COX-2

	Negative/low (n = 27)	High (n = 11)
	n (%)	n (%)
Age		
>50	21 (78)	2 (18)
Sex		
Female	15 (56)	6 (55)
Male	12 (44)	5 (45)
Size		
<5 cm	8 (30)	3 (27)
5–10 cm	7 (26)	2 (18)
>10 cm	8 (30)	4 (37)
Unknown	4 (14)	2 (18)
Margins		
Neg	23 (85)	9 (82)
Pos	3 (11)	1 (9)
Neg/mets	1 (4)	1 (9)
Mitotic index*		
<5/10 hpf	12 (44)	9 (82)
>5/10 hpf	15 (56)	2 (18)
Disease recurrence		
Yes	10 (37)	2 (18)
No	17 (63)	9 (82)

*$P = 0.03$.

only weakly or focally positive. COX-2 staining may also help to identify GIST when KIT expression is falsely negative due to technical problems. The potential value of COX-2 staining in the identification of KIT negative (less than 5%) GIST must also be mentioned, though that specific subpopulation of tumors was not addressed in this study.

The significance of COX-2 expression in carcinogenesis and tumor progression of mesenchymal tumors remains unclear. Although correlations between COX-2 expression and tumor progression have been observed in epithelial tumors, no such correlation has been observed in mesenchymal tumors in general, and GIST in particular. Among carcinomas, COX-2 has been shown to localize to the tumor-associated stroma in the early stages of carcinogenesis (colon polyps), with COX-2 being expressed by tumor cells once the malignant phenotype has been established (invasive adenocarcinoma). A size and invasion-dependent increase in COX-2 levels has been described in human colorectal carcinomas, and a relationship between COX-2 expression and survival has been suggested.[21,22] The significance of COX-2 expression by mesenchymal tumors, on the other hand, has not been established.[17]

Higher levels of COX-2 have been associated with GIST epithelioid phenotypes and gastric location, both of which are favorable prognostic indicators.[18] Our data support this observation, indicating that high COX-2 protein expression may be associated with a more favorable clinical prognosis in GIST. Although no correlation can be drawn between level of COX-2 protein expression, tumor location, and tumor size, mitotic count is lower in those tumors designated high expressers of the enzyme, indicating that those tumors may be relatively less aggressive. Supporting this statement is the trend toward improved survival and fewer instances of recurrence in patients whose GIST express high levels of COX-2 protein. Thirty-seven percent of patients

with low COX-2 expression developed disease recurrence and/or died of disease, whereas only 18% of patients whose GIST tumors highly expressed COX-2 had those clinical outcomes. Although not statistically significant in this small group of patients, the trend supports the speculation that the higher the level of COX-2 protein expression, the less aggressive GI mesenchymal tumors may be. Elevated COX-2 protein expression level also seems to be more prevalent among young patients with smaller tumors. Our small patient numbers preclude us from establishing a statistically significant association between these parameters. Analysis of COX-2 expression in a larger population of GIST patients will be required to confirm if there is indeed a significant inverse relationship between patient age, tumor size, and level of tumor-associated COX-2 protein expression. It is clear that all these suggestions need to be verified by larger studies, as well as correlative in vitro investigations to better define the role of COX-2 in the pathogenesis of mesenchymal tumors in general, and GIST in particular. Whereas we await the results of those ongoing studies, we can only speculate on the potential use of COX-2 inhibitors in the treatment of GIST.

CONCLUSION

We conclude that the vast majority of mesenchymal tumors of the GI tract are GIST that express COX-2 protein. COX-2 expression may help differentiate GIST from other GI mesenchymal tumors. As opposed to known predictors of GIST biologic behavior, such as tumor size and mitotic count, levels of tumor-associated COX-2 protein expression did not correlate with clinical outcome.

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