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EDITORIAL

Keith A. Kelly, MD

Coeditor 1997–2007

John L. Cameron

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Keith A. Kelly

Almost 12 years ago, I was approached to consider becoming an editor of the Society of Surgery of the Alimentary Tract's newly proposed journal, the *Journal of Gastrointestinal Surgery*. On several occasions I declined, until I learned there would be a coeditor, Dr. Keith Kelly. I immediately accepted to have the opportunity to work with Dr. Kelly. It was a wise decision. There have been two of us, but Keith has been the principal architect, conscience, soul, and driving force of the journal and has been the major factor behind its meteoric rise to become the outstanding surgical journal in the world devoted to alimentary tract surgery. Now, after 11 successful and productive years, Dr. Kelly has decided to step down.

Dr. Kelly has had a distinguished career in surgery. He received his undergraduate degree from the University of Illinois and his medical degree from the University of Chicago. His surgical training was obtained at the University of Washington School of Medicine Affiliated Hospitals. His career as an outstanding operating surgeon and a productive and insightful surgical investigator was spent at The Mayo Clinic, both in Rochester and in Scottsdale. In both locations, he rose to chair the Department of Surgery. He developed into one of the most outstanding and respected alimentary tract surgeons in the country and became known throughout the world for his contributions to clinical and investigative surgery. Dr. Kelly has received many honors, including the Founders Medal of the SSAT, and he served as President of the SSAT in 1992. He has received numerous teaching awards and has a teaching award named after him! He has served as visiting professor at institutions throughout the world. He directed a productive research laboratory investigating motility disorders of the digestive tract that was funded by NIH for over 20 years.

This background provided a unique set of qualifications for what became his most outstanding accomplishment, the creation and nurturing of the *Journal of Gastrointestinal Surgery*. The SSAT and JOGS have benefited immensely from Keith's leadership, and the journal stands as a monument to his career. His careful and compulsive editing, his vision for the structure and mission of the journal, his constant ideas and suggestions for its improvement have all led to its success. He has also educated his coeditor to the point where Dr. Kelly feels it is safe to step down! So now there is one. In the wings, however, are two associate editors, Dr. Jeffrey B. Matthews and Dr. Charles J. Yeo, who, again, will make it two coeditors when the remaining editor steps down. They undoubtedly will elevate the journal to even greater heights. Thank you, congratulations, and God Speed to Dr. Keith Kelly.

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Vaccine Impedes the Development of Reflux-Induced Esophageal Cancer in a Surgical Rat Model: Efficacy of the Vaccine in a Pre-Barrett's Esophagus Setting

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Abstract
Background & Aims We developed a granulocyte-macrophage-colony-stimulating factor (GM-CSF) tumor vaccine for esophageal cancer. We evaluated the effectiveness of the vaccine as a prevention option in a surgical reflux rat model of esophageal cancer.
Methods A surgical model involving a jejuno-esophagostomy was used to create Barrett's esophagus and esophageal cancer in rats. No carcinogen exposure was utilized. Cell lines derived from these tumors were stably passaged *in vitro*. GM-CSF-secreting tumor cells were generated using stable transfection. All rats underwent a total gastrectomy, followed by a jejuno-esophagostomy. The surgery promoted the reflux of duodenal contents into the esophagus. All animals were administered either a GM-CSF secreting whole cell vaccine or a phosphate-buffered saline (PBS) placebo injection 4, 6, 14, and 16 weeks post-surgery.
Results While 15 of 16 animals in the non-vaccinated placebo group developed esophageal cancer, 94% (15 of 16), animals in the vaccine group had an incidence of cancer of 25% (4 of 16) ($p<0.05$). Barrett's esophagus was seen in 100% (16 of 16) of the controls and 83% (13 of 16) of the vaccinated animals.
Conclusions A GM-CSF-secreting whole cell tumor vaccine impeded esophageal tumor growth, but not the development of Barrett's esophagus, in a clinically relevant surgical reflux model.

Keywords Vaccine · Esophageal cancer · GM-CSF · Barrett's esophagus

Abbreviations

APC	Antigen-presenting cell
GM-CSF	Granulocyte-macrophage-colony-stimulating factor

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ELISA	enzyme linked immunosorbent assay
H&E	hematoxylin and eosin
PBS	Phosphate-buffered saline

Introduction

Animal models of carcinogenesis are valuable tools and have been of particular interest to our group. To investigate novel approaches to manage Barrett's esophagus and esophageal cancer, an animal model of surgically created esophageal reflux was utilized.[1–5] The model has the distinct advantage of promoting rat esophageal tumor growth without carcinogen that resembles human esophageal carcinogenesis. In a previous study, we examined tumors extracted from rats subjected to the surgical reflux model. The harvested tumors exhibited histologic subtypes of adenocarcinoma and squamous cell carcinoma and the presence of premalignant and malignant tumors. Through cytogenetic and karyotype analyses, tumor cells harvested from rats subjected to the surgical reflux model were found to share common morphologies, mucin features, pathways, and the expression of differentiation markers with human esophageal cancer.[4,5] With the availability of an *in vivo* clinically relevant surgical model, and thus, the ability to test new therapies for Barrett's esophagus and esophageal cancer, we proceeded to investigate a tumor vaccine approach to treat esophageal cancer.

Using our clinically relevant reflux model, we created three cancer cell lines that appear histologically as squamous cell carcinoma, but retain certain molecular features of adenocarcinoma.[4] Tumors were harvested and grown *in vitro*. In development of this vaccine, rat tumor cells growing *in vitro* were genetically modified to express granulocyte-macrophage-colony-stimulating factor (GM-CSF) to attract professional antigen-presenting cells (APCs), such as macrophages or dendritic cells, to the site of the vaccine. While multiple cytokines may be used to activate APCs, previous research has demonstrated that GM-CSF-producing vaccines give the most potent, specific, and longest lasting anti-tumor immunity.[6]

Our group pioneered the use of GM-CSF secreting whole-cell allogenic tumor vaccines for the treatment of pancreatic,[7] breast,[8] renal,[9] prostate cancers,[10] and melanoma.[11] Based on the promising work with these diseases, we aimed to produce a similar vaccine for Barrett's esophagus and esophageal cancer. In a concurrent study, we found our vaccine to be effective at impeding the development of esophageal cancer when it was administered after the development of Barrett's esophagus (Miyashita et al., in preparation). In the present paper, we describe the testing of the GM-CSF vaccine as a preventive treatment strategy. The vaccine was administered before the development of Barrett's esophagus in a clinically relevant rat surgical reflux model of esophageal carcinogenesis to evaluate its efficacy.

Methods

All procedures were approved by the Animal Care and Use Committee of Johns Hopkins University, and animals received humane care in compliance with the "Guide for Care and use of Laboratory Animals", published by the National Research Council (National Academy Press, 1996). Animals were acclimatized in a central animal facility for 2 weeks before the experiments.

Establishment of Cell Lines

Tumors were harvested 9 months after surgical creation of reflux in rats.[4,5] Tumors were mechanically minced and enzymatically digested using collagenase. The disaggregated cells were plated in appropriate growth media as primary cultures. The culture plates were checked for the presence of cancer cells everyday. After adequate growth, a single clone was picked using a cloning ring and transferred to 96-well plates. We froze and stored aliquots of the original lineage. Other cells continued to undergo passages (over 40) without changing phenotype.[4,5]

Vaccine Creation

To create the vaccine cells, we used electroporation to transfect a DNA expression vector, pCDNA3 [non-vaccinated phophate-buffered saline (PBS) placebo] or mGM-pCDNA3.1 (with GM-CSF), from Invitrogen Life Technologies (Carlsbad, CA) as previously described (Miyashita et al., in preparation). The efficiency of the transfection was checked by estimating the amount of GM-CSF secreted by the transfected cells using a Quantikine enzyme linked immunosorbent assay (ELISA) kit (R & D systems, Minneapolis, MN).

Animal Vaccination

Vaccine cells were irradiated with 5,000 Gy to prevent their propagation. Animals were injected with 1×10^7 cells/0.5 ml of irradiated tumor cells secreting GM-CSF or an equivalent amount of phosphate buffer solution (PBS) into both the upper limbs and the lower left limb. The rats were vaccinated according to the schedule described in Fig. 1.

Rat Surgical Reflux Model

The rat reflux model was created by performing a total gastrectomy on Sprague–Dawley rats, followed by a jejuno-

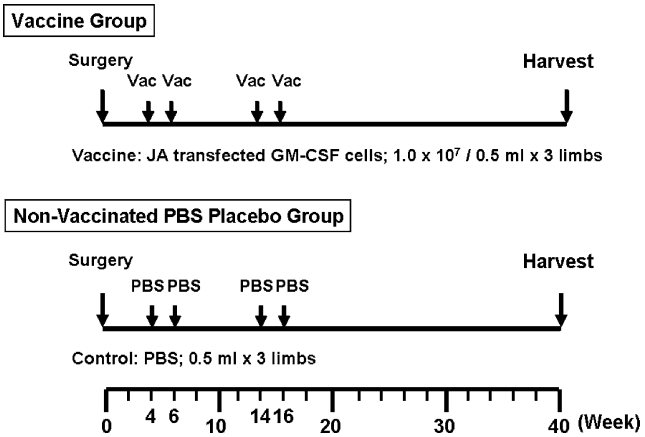

Figure 1 Experimental timeline. A vaccination or PBS injection was delivered 4, 6, 14, and 16 weeks after surgery. The experiment ended 40 weeks after post-surgery, when all animals were sacrificed and any remaining tumor masses were harvested from the animals.

esophagostomy, as established by Miwa et al. in 1996.[2] Fifty-four animals were randomly divided into two groups: a vaccine group (n=30) and a non-vaccinated PBS placebo group (n=24). Rats were vaccinated 4, 6, 14, and 16 weeks after surgery with 3×10^7 irradiated vaccine cells secreting GM-CSF or an equivalent amount of PBS as shown in Fig. 1. All rats were weighed every 4 weeks throughout the experiment. All surviving animals were sacrificed 40 weeks after surgery, and their esophagi were examined.

Tissue Preparation

The thyroid cartilage and the site of anastomosis were used as landmarks during the resection of the esophagus and jejunum. The specimen was cut longitudinally into three 1-mm wide slices of the esophageal mucosa. The slices were fixed in 10% formalin for 24 h and then embedded in paraffin for hematoxylin and eosin (H&E) staining.

Pathological Assessment

As previously described, pathological assessment was carried out on 5 μm H&E stained sections from each block (Miyashita et al., in preparation). The pathological progression due to duodeno-esophageal reflux was categorized as one of the following:

Proliferative squamous hyperplasia Proliferative hyperplasia is a condition characterized by a thickened epithelium to twice that of a normal epithelium with acanthosis, elongation of the papillae, and parakeratosis. Other features include the thickening of the basal layer of the squamous epithelium and the preservation of a stratified appearance (Fig. 2a).

Barrett's metaplasia Barrett's metaplasia is defined by the presence of columnar-lined epithelium with intestinal metaplasia replacing the esophageal squamous epithelium (Fig. 2b).

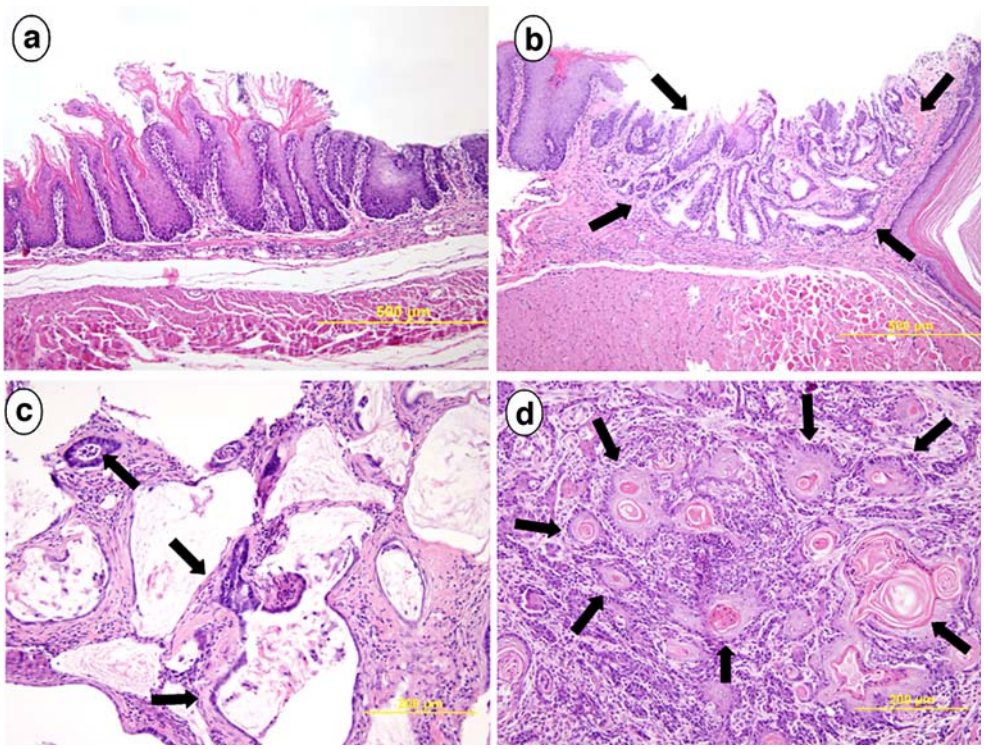

Figure 2 A representative photomicrograph of an esophageal carcinoma which resulted from surgically induced duodenal contents reflux **a** Proliferative hyperplasia, **b** Barrett's metaplasia (*arrow*), **c** Adenocarcinoma (*arrow*), **d** Squamous cell carcinoma with keratin pearls (*arrow*).

Carcinoma Epithelial growth with cellular and structural atypia, invading into the submucosal layer was defined as carcinoma. Adenocarcinoma consists of malignant-appearing glandular cell growth with both atypia and invasiveness that has two types of histology: tubular adenocarcinoma and mucinous adenocarcinoma (Fig. 2c). Squamous cell carcinoma is a well-differentiated carcinoma marked by cellular and structural atypia and squamous pearls (Fig. 2d).

Statistical Analysis

A Fischer's exact test was used for statistical analysis on the incidence of pathological findings. Data management and statistical analysis was performed using Sigma Stat software (Systat Software Co., San Jose, CA), and differences were considered significant when the p value was <0.05.

Results

Rat Surgical Reflux Model

Of the 54 operated animals, 32 rats survived 40 weeks post-surgery and were included in the study. Of these, 16 were part of the non-vaccinated PBS placebo group, while the remaining 16 were subjected to a vaccine. A total of 22 rats (8 in the non-vaccinated PBS placebo group and 14 in the vaccine group) died of surgical complications, such as malnutrition, pneumonia, and unknown causes. No differences in mortality were noted between the two groups. Body weight did not differ between the two groups (Table 1).

Table 1 Comparison of Outcomes and Histopathological Findings

Parameters	Non-vaccinated PBS placebo group	Vaccine group
Total surgeries	24	30
Survivors	16	16
Average weight±SE	272±81 g	273±73 g
Cancer	15 (94%)*	4 (25%) *
Adenocarcinoma	14	4
Squamous cell carcinoma	1	0
Barrett's metaplasia	16 (100%)	13 (82%)
Proliferative hyperplasia	16 (100%)	16 (100%)

The incidence of cancer was significantly lower in the vaccine group, as compared to the non-vaccinated PBS placebo group (*$p<0.05$).

Macroscopic

In the non-vaccinated PBS placebo group, there was pronounced thickening of the esophagi in all rats. The esophageal epithelia displayed areas of irregularity (Fig. 3a). On the other hand, vaccinated rats displayed smooth distal portions of the esophagus with normal width (Fig. 3b). These results are similar to our previously reported findings (Miyashita et al., in preparation).

Microscopic

While 94% (15 of 16) of animals in the non-vaccinated PBS placebo group developed esophageal cancer, animals in the vaccine group had an incidence of cancer of 25% (4 of 16). Animals in the vaccine group had a significantly lower chance of developing cancer than the placebo group ($p<0.05$) (Table 1). In the placebo group, 14 rats displayed adenocarcinoma, and one rat developed squamous cell carcinoma, while four rats in the vaccine group developed adenocarcinoma. Barrett's metaplasia was found on 100% (16 of 16) of the rats in the placebo group, but there was a protective tendency in the vaccinated group with 82% (13 of 16) of the rats displaying signs of Barrett's metaplasia.

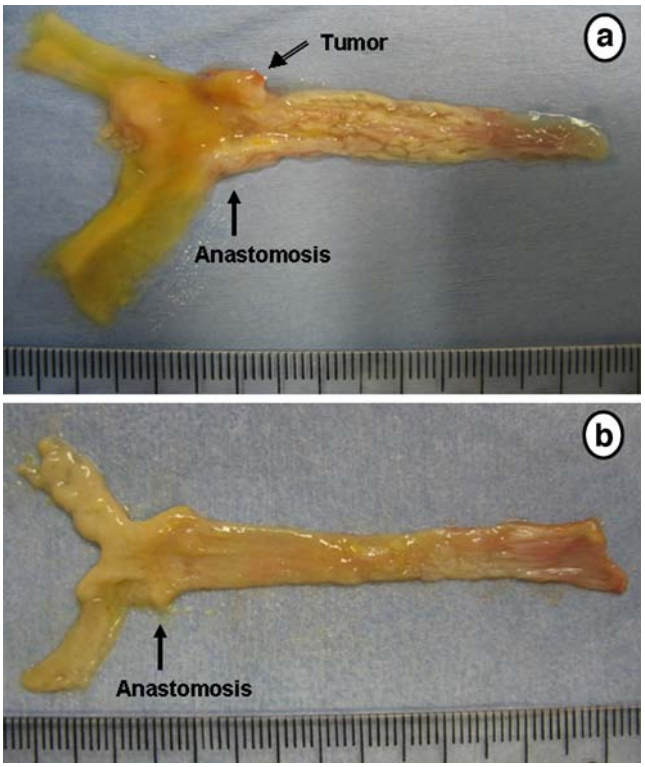

Figure 3 Macroscopic findings. **a** The distal portion of an esophagus in a non-vaccinated PBS placebo rat thickened. The epithelium was rough and a transparent tumor (*thick arrow*) is visible near the anastomosis (*thin arrow*). **b** The esophagus of a rat from the vaccine group exhibited a slightly uneven surface, but displayed no tumor.

All rats in the vaccine and placebo groups developed proliferative hyperplasia.

Discussion

We have created an animal model of esophageal cancer that enabled the establishment of stable tumor cell lines for use in *in vitro* and *in vivo* studies of this devastating disease.[4,5] From this model, we created a whole-cell, GM-CSF secreting tumor vaccine for the prevention of Barrett's esophagus and esophageal cancer in clinically relevant animal surgical models. To create the vaccine, tumor cells were transfected using electroporation to stably express GM-CSF. Those cells were irradiated and used to vaccinate animals.

We have confirmed the efficacy of the vaccine against esophageal carcinogenesis when it is administered after the development of Barrett's esophagus. In a previous study, we transplanted tumors subcutaneously in rats vaccinated with the GM-CSF secreting whole cell vaccine used in this experiment (Miyashita et al., in preparation). The vaccine produced inflammatory infiltrates, granulomas, and eosinophils at the vaccination site. Histological examinations of the implantation site showed infiltration with $CD4^+$ and $CD8^+$ T cells around the tumor and tumor cell death. The immune response effectively impeded tumor growth at the implantation site. We confirmed in a clinically relevant surgical reflux model the results we observed in our prior subcutaneous tumor transplant study. Rats vaccinated after the development of Barrett's esophagus developed cancer at a lower rate than rats administered an equivalent injection of saline (Miyashita et al., in preparation).

In the present study, we aimed to determine the efficacy of our vaccine when it is administered before the development of Barrett's esophagus. All rats underwent a total gasterectomy, followed by an esophago-jejunostomy. As reported previously, rats subjected to this surgical model develop Barrett's esophagus 20 weeks post-surgery.[12] Animals were vaccinated or injected with a saline equivalent before the development of Barrett's esophagus or 4, 6, 14, and 16 weeks post-surgery. Regardless of treatment, nearly all rats displayed signs of Barrett's esophagus. The vaccine was not effective at preventing the development of Barrett's esophagus. However, the vaccine impeded the progression of Barrett's esophagus to cancer. These results suggest the potential of use of our vaccine as a preventive option for Barrett's associated esophageal cancer.

In considering the application of this type of vaccine in humans, it is important to consider the similarities between our surgically induced rat reflux tumors and human esophageal cancers. Through karyotype analyses, we found highly aneuploid cell populations with the derangement of key chromosomes that encode for a variety of oncogenes involved in esophageal carcinogenesis. The neoplastic nature and gene expression profile of these rodent reflux-induced tumors was found to be comparable to human esophageal cancer.[4] The expression levels of insulin growth factor, vascular endothelial growth factor, polo-like factor, cyclin-dependent kinase 4, hypoxia-inducible factor 1, and the deletion of the Y chromosome were all similar to findings observed in human esophageal cancer.[4] A recent paper by Su et al. showed that the tumors in the rat model share critical features with human disease including mucin features in the Barrett's like lesions and keratin as well as p53 and cyclooxygenase 2 expression in the cancers.[13] Therefore, the rat model can be appropriately used to study the processes involved in esophageal carcinogenesis in humans. For further reference, the differentially expressed genes are deposited at the NCBI's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through the GEO series accession number GSE1707.

This project was the first to develop a successful whole-cell GM-CSF esophageal cancer vaccine for rodents. Other investigators have successfully developed whole cell vaccines and begun early clinical trials for pancreatic,[7] breast,[8] renal,[9] prostate cancers,[10] and melanoma.[11] It is clear from our experience with the whole cell vaccine approach that the cells used in a vaccine do not have to be identical to the tumor cells against which the vaccine is directed. For instance, with pancreatic cancer, the initial approach in humans was to create a vaccine using autologous cells from each patient's tumor, but then, it became apparent that a nonspecific allogenic cell line could be used with similar success to the autogenous cells and with much greater convenience.[7] There are at least two well-known human esophageal adenocarcinoma cell lines, namely, BIC and SEG. These cell lines would be candidates for use in a future human trial.

Potential targets for the esophageal cancer vaccine would be patients with an elevated risks of developing esophageal cancer. This could include patients with known risk factors such as gastro-esophageal reflux disease. The vaccination schedule and lack of toxicity of the vaccine provides physicians the opportunity to vaccinate individuals before any clinical signs of Barrett's esophagus. The use of this vaccine has the potential to reduce the reliance on therapies that have many unintended adverse effects.

Conclusion

In summary, this project was the first to develop a whole-cell GM-CSF vaccine to prevent esophageal cancer in animals. We were able to generate a stable esophageal cancer cell line.

Although a GM-CSF-secreting cancer vaccine derived from these cells was unsuccessful in preventing the development of Barrett's esophagus, the vaccine successfully inhibited carcinogenesis in a surgical rat reflux model. Our vaccine may represent a safe and potent option to prevent the development of esophageal cancer.

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References

1. Byrnes CK, Bahadursingh A, Akhter N, Parinandi NL, Natarajan V, Montgomery E, Tihan T, Duncan MD, Nass PH, Harmon JW. Duodenal reflux produces hyperproliferative epithelial esophagitis—a possible precursor to esophageal adenocarcinoma in the rat. J Gastrointest Surg 2003;7:172–180.
2. Miwa K, Sahara H, Segawa M, Kinami S, Sato T, Miyazaki I, Hattori T. Reflux of duodenal or gastro-duodenal contents induces esophageal carcinoma in rats. Int J Cancer 1996;67:269–274.
3. Buttar NS, Wang KK, Leontovich O, Westcott JY, Pacifico RJ, Anderson MA, Krishnadath KK, Lutzke LS, Burgart LJ. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. Gastroenterology 2002;122:1101–1112.
4. Bonde P, Sui G, Dhara S, Wang J, Broor A, Kim IF, Wiley JE, Marti G, Duncan M, Jaffee E, Montgomery E, Maitra A, Harmon JW. Cytogentic characterization and gene expression profiling in the rat reflux induced esophageal tumor model. J Thorac Cardiovasc Surg 2007;133:763–769.
5. Sui G, Bonde P, Dhara S, Broor A, Wang J, Marti G, Feldmann G, Duncan M, Montgomery E, Maitra A, Harmon JW. Epidermal growth factor receptor and hedgehog signaling pathways are active in esophageal cancer cells from rat reflux model. J Surg Res 2006;134:131–9.
6. Thomas MC, Greten TF, Pardoll DM, Jaffee EM. Enhanced tumor protection by granulocyte-macrophage colony-stimulating factor expression at the site of an allogeneic vaccine. Hum Gene Ther 1998;9:835–843.
7. Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, Lillemoe KD, O'Reilly S, Abrams RA, Pardoll DM, Cameron JL, Yeo CJ. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol 2001;19:145–156.
8. Emens LA, Armstrong D, Biedrzycki B, Davidson N, Davis-Sproul J, Fetting J, Jaffee E, Onners B, Piantadosi S, Reilly RT, Stearns V, Tartakovsky I, Visvanathan K, Wolff A. A phase I vaccine safety and chemotherapy dose-finding trial of an allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin. Hum Gene Ther 2004;15:313–337.
9. Simons JW, Jaffee EM, Weber CE, Levitsky HI, Nelson WG, Carducci MA, Lazenby AJ, Cohen LK, Finn CC, Clift SM, Hauda KM, Beck LA, Leiferman KM, Owens AH, Jr., Piantadosi S, Dranoff G, Mulligan RC, Pardoll DM, Marshall FF. Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. Cancer Res 1997;57:1537–1546.
10. Simons JW, Mikhak B, Chang JF, DeMarzo AM, Carducci MA, Lim M, Weber CE, Baccala AA, Goemann MA, Clift SM, Ando DG, Levitsky HI, Cohen LK, Sanda MG, Mulligan RC, Partin AW, Carter HB, Piantadosi S, Marshall FF, Nelson WG. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer. Cancer Res 1999;59:5160–5168.
11. Soiffer R, Lynch T, Mihm M, Jung K, Rhuda C, Schmollinger JC, Hodi FS, Liebster L, Lam P, Mentzer S, Singer S, Tanabe KK, Cosimi AB, Duda R, Sober A, Bhan A, Daley J, Neuberg D, Parry G, Rokovich J, Richards L, Drayer J, Berns A, Clift S, Cohen LK, Mulligan RC, Dranoff G. Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. Proc Natl Acad Sci U S A 1998;95:13141–13146.
12. Miyashita T, Ohta T, Fujimura T, Ninomiya I, Fushida S, Hattori T, Miwa K. Duodenal juice stimulates oesophageal stem cells to induce Barrett's oesophagus and oesophageal adenocarcinoma in rats. Oncol Rep 2006;15:1469–1475.
13. Su Y, Chen X, Klein M, Fang M, Wang S, Yang CS, Goyal RK. Phenotype of columnar-lined esophagus in rats with esophagogastroduodenal anastomosis: similarity to human Barrett's esophagus. Lab Invest 2004;84:753–765.

Discussion

John G. Hunter, M.D. (Portland, OR): This is unique and innovative work. I read this manuscript, and thank you for sending it ahead of time, with more excitement than about 99% of all manuscripts I read these days, and I read a few. We have had some experience with this model of esophageal cancer induction when S. P. Bowers was exploring the relationship of iron metabolism and DNA hypermethylation in Barrett's esophagus. We had some trouble with the model, tangentially discussed in the manuscript and in the presentation. So, several of my questions may deal with the model. Here we go:

When sewing the small intestine to the esophagus, how does one know whether the intestinalization that develops is truly metaplastic, i.e., Barrett's, and not overgrowth of the intestinal epithelium above the anastomosis? Can you demonstrate differences in gene prevalence or protein expression between the intestinal and Barrett's epithelium?

Rats subjected to esophagojejunostomy developed adenocarcinoma, adenosquamous carcinoma, and squamous type cancers. The cancer vaccine was actually developed from a tumor line with a distinctly squamous morphology. Does the frequent appearance of squamous carcinoma in this model suggest a mechanism of carcinogenesis that is different from human adenocarcinoma developing as a consequence of long-standing reflux?

In the first set of experiments, the tumor injected into the mouse was the tumor from which the vaccine was created. The effects were remarkable. This might have some human application if "auto" vaccines could be developed from primary esophageal tumors before neoadjuvant therapy and reintroduced after resection and recovery. When the vaccine was used to prevent de novo Barrett's and adenocarcinoma in a rat with an esophagojejunostomy, your second set of experiments, it was somewhat less effective. Would you comment on why you think the vaccine was less effective in this model?

Lastly, the mechanism for tumor kill is clearly and convincingly demonstrated through the action of helper T cells, but the mechanism for preventing esophageal metaplasia is not so clear, as, at least in humans, metaplasia is thought to be a healing response to esophageal injury in a low pH environment with bile acid exposure. So what is the proposed mechanism for intestinal metaplasia in this model where no acid is present and where metaplasia can be suppressed by an antitumor vaccine?

Like most great papers, this paper asks more questions, but I am out of time. I enjoyed it a lot. Thank you very much, and congratulations.

Tomoharu Miyashita, M.D. (Baltimore, MD): The vaccine cells were derived from the rat cell line that expressed mainly squamous cell carcinoma features. However, a positive feature of the rat cell line is that it shared important cytogenetic features with human adenocarcinoma cells, which are not characteristic of Barrett's epithelium. This is a tumor vaccine, so it affected the adenocarcinoma and not the Barrett's. The incidence of Barrett's was not greatly affected by the vaccine, but the incidence of cancer was significantly reduced.

John W. Harmon, M.D. (Baltimore, MD): I will continue to address the series of thoughtful questions raised by Dr Hunter.

How do you tell the Barrett's? Our pathologist, Elizabeth Montgomery is the authority on this. At the anastomotic junction between the jejunum and the esophagus, it is a difficult call. The intestinal cells in the jejunum look like Barrett's epithelium. But when we see nests of columnar epithelium appearing in areas of squamous epithelium, we can show convincingly that there is Barrett's metaplasia. The ones that are convincing are the ones that are clearly separated from the anastomosis, where you see a nest of columnar cells surrounded by squamous epithelium.

Regarding the issue of squamous carcinoma being produced in this model, it is true. You do see a few adenosquamous cancers in humans also, but many more here. It is just a fact of life with this model. But we found that using our cell lines, which were mainly squamous, we were nonetheless able to protect the animals from developing adenocarcinoma. Our cytogenetic analysis, published previously, shows a lot of similarities between our rat squamous cancer cells and human adenocarcinoma cells.

Regarding using the patient's own tumor as an autovaccine, you raise an important point. In the first series, we used the animal's own tumor, grown from the cell line that was used for the vaccine, and we got a really good effect. When we were not using cells from the tumor itself, we got less effect. Interestingly, in the adjuvant setting with humans, it would be possible to use the patient's own tumor, as you suggested. You should know that this approach is being used at Hopkins for both breast and pancreas. I know that in the pancreas trial, they having been doing that. They have been taking the resected specimen, and making the vaccine from those cells. This is now a phase 2 clinical trial.

David I. Soybel, M.D. (Boston, MA): I also had a question about the model. Presumably, what you want to do with the vaccine is to see if it will benefit a patient who has something like a T3 lesion and positive nodes. You would want to give the vaccine after resection to prevent recurrence. So I am wondering, is there an animal model, which gives you that kind of opportunity to look at recurrence to really see the clinical applicability? I imagine that with an implanted tumor, even in this preventive model, the applicability to the general population of people with esophageal cancer might be limited. So, is there an animal model where you can address that exact issue?

Dr. Harmon: I don't think we have a good model that mimics the adjuvant setting at this point. We are certainly thinking of it. We think that is one place where the vaccine could be useful. The other place for the vaccine would be somebody with a high grade Barrett's dysplasia.

We like the idea of using the vaccine in places where there is no big tumor burden. I think that it is asking an awful lot of a vaccine to wipe out a big tumor burden.

Reginald V. N. Lord, M.D. (Sydney, Australia): It is likely that the reflux model rats that were given the vaccines at later time points would already have developed one of these types of tumors. My question is, was there a difference in the proportion of tumors in the rats according to the different time points at which they were given the vaccine? The answer, of course, is important for considering the possibility that the vaccine

could be effective against established tumors rather than just prevention.

Dr. Harmon: We have two series, this series and the Post-Barrett's vaccination series. In our model, rats get Barrett's at 20 weeks. So, these animals were vaccinated presumably before the time that they actually got a cancer, and before Barrett's, and had the results that you saw. In a subsequent experiment, we got slightly less efficacy when we used the vaccine after week 20. After week 20, the rats would have already developed Barrett's metaplasia. When the vaccine was used after week 20, post-Barrett's, we still got a statistically significant reduction in the incidence of cancer.

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Endoscopic Ultrasound and Computed Tomography Predictors of Pancreatic Cancer Resectability

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Abstract
Introduction This study investigates the ability of endoscopic ultrasound (EUS) and computed tomography (CT) to predict a margin negative (R0) resection and the need for venous resection in patients undergoing pancreaticoduodenectomy (PD).
Methods Patients with pancreatic head adenocarcinoma undergoing surgery with intent to resect during the last 5 years were identified. EUS and CT data on vascular involvement were collected. Preoperative imaging was compared to intraoperative findings and final pathology. Contingency table analysis using Fisher's exact test identified imaging features of EUS and CT associated with unresectability and positive margins.
Results Seventy-six patients met study criteria. Forty-seven (62%) underwent potentially curative PD. The R0 resection rate was 70%. There were 16 unresectable patients because of locally advanced disease. Venous involvement >180° and arterial involvement >90° by CT had 100% positive predictive value for failure to achieve R0 resection ($p<.01$). If patients with prestudy biliary stents were excluded, EUS venous abutment or invasion also predicted R0 failure ($p=.02$). Combined but not individual EUS and CT findings were predictive of need for vein resection.
Conclusions Pancreas protocol CT imaging appears to be a better predictor of resectability compared to EUS. EUS accuracy is affected by the presence of biliary stents.

Keywords Ultrasound · Computed tomography · Resectability · Pancreatic cancer

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States. In 2007, there will be an estimated 37,000 new cases and 33,000 deaths. It remains one of the few cancers in which the death rate almost parallels its incidence, and for all stages, 1- and 5-year survival is a dismal 26% and 5%, respectively.[1]

The best chance for long-term survival occurs in patients with localized disease undergoing surgical resection. Numerous prognostic variables associated with survival have been identified in surgical series such as tumor size, tumor differentiation, lymph node positivity, and margin status.[2] Although there is evidence that an adequate lymphadenectomy is important for pathologic staging purposes,[3] the issue of radical or extended lymphadenectomy is controversial and in metaanalysis has not been found to benefit survival.[4,5] Thus, within the surgeon's purview, the primary goal of surgical therapy is to achieve a margin-negative R0 resection with minimal postoperative complications.[6] A secondary goal is to avoid unnecessary laparotomy discovering unresectable disease.

In this context, a preoperative assessment of R0 resectability becomes critically important. Radiologic stag-

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Table 1 Scoring Rubric for Pancreas Protocol Evaluation of Pancreatic Head Masses

Vascular Involvement				
	None	<90	90–180	181–360
SMA				
SMV/Portal vein				
Celiac artery				
Hepatic artery				

Vascular involvement is characterized as the circumferential degrees of involvement by tumor.
SMA Superior Mesenteric Artery; *SMV* Superior Mesenteric Vein

ing with endoscopic ultrasound (EUS) and computed tomography (CT) is currently used to identify patients who may be resectable. In addition to fine-needle aspiration (FNA) for tissue diagnosis, EUS offers high-resolution, local imaging of the pancreas and surrounding structures. Current state-of-the-art CT utilizes a multiphase, multidetector technique to acquire thin image slices through the abdomen. Imaging criteria for resectability that can be seen by both EUS and CT include no evidence of distant metastasis; no arterial involvement at the celiac axis, hepatic artery, and SMA distributions; and a patent portal and superior mesenteric vein.

Most older studies found EUS superior to CT in the detection and American Joint Committee on Cancer (AJCC) T-staging of pancreatic cancers.[7,8] However, with the recent 2002 AJCC 6th Edition[9] update, which establishes more explicitly the relationship between T-stage and resectability, recent comparative studies between EUS and CT examining this issue present more varied and conflicting data.[10–12] The purpose of this study was to investigate the ability of EUS and CT to predict a margin-negative R0 resection in patients undergoing pancreaticoduodenectomy (PD). Tumor positivity at the retroperitoneal and vascular margins as well as finding a patient unresectable at laparotomy because of locally advanced disease were considered failures of local primary tumor staging; thus, we sought to identify EUS and CT features in these patients that may have altered their surgical management. Image findings associated with the need for portal-venous resection were also determined.

Materials and Methods

A retrospective review of patients from 2001 to 2006 with adenocarcinoma of the pancreatic head undergoing surgery with the intent to resect was performed. Patients were identified using a pancreatic cancer database compiled from the institutional cancer registry and surgical clinic records under institutional review board approval. They were required to have had both pancreas protocol CT and EUS studies at our institution available for review. General patient data recorded included age, sex, age at diagnosis, time to surgery from diagnosis, and survival. Additional specific information was noted regarding the presence of preimaging biliary stenting, the use of neoadjuvant therapy, and the presence of gastric outlet obstruction as documented in clinic notes. All procedure notes and surgical pathology reports were reviewed for operative findings and surgical results.

EUS was performed by one of two experienced gastroenterologists with a linear-scanning echoendoscope (Olympus America, Inc., Melville, NY). Blinded to prior imaging results and clinical outcome, they reviewed the procedure video or note and classified EUS findings by involvement of the superior mesenteric artery, superior mesenteric vein, celiac axis, hepatic artery, portal vein, lymph nodes, and liver. Vessel "abutment" was defined as a loss of the hyperechoic interface between the tumor and vessel, whereas vessel "invasion" was defined as visualization of tumor within the lumen, vessel encasement, or vessel occlusion. Lymph nodes were considered malignant if they were hypoechoic, well-defined, round, and larger than 5 mm in diameter. Likewise, well-defined hyper- or hypoechoic lesions within the left hepatic lobe were considered suspicious for metastasis. When possible, FNA was used to sample these extrapancreatic lesions for diagnosis.

Pancreas protocol CT was defined as a dedicated multidetector contrast CT of the abdomen, with 3 mm cuts through the pancreas. Image acquisition was obtained in the noncontrast, arterial, and portal-venous phases of contrast administration. Two experienced gastrointestinal CT radiologists blinded to prior imaging results, clinical history, and clinical outcome then reviewed and scored the CT scans according to a rubric designed to quantify the degree of vascular involvement by tumor from 0° to 360° (Table 1). Ascites, liver lesions, local invasion, and suspicious adenopathy at specific locations were also noted (Table 2).

Table 2 Scoring Rubric for Pancreas Protocol Evaluation of Pancreatic Head Masses

	Yes	No
Liver metastasis		
Peritoneal metastasis		
Ascites		
Invades stomach		
Invades duodenum		
Periportal nodes		
Peripancreatic nodes		
Celiac nodes		
Aortocaval nodes		
Mesenteric nodes		
Stent present		

The two groups for statistical analysis were then the patients who received an R0 resection compared to those who had R1 resections at the retroperitoneal and vascular margins or were found to be locally advanced and unresectable at operation. EUS and CT findings separately and combined were compared to intraoperative findings and surgical pathology. Contingency tables were constructed to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of imaging features. Fisher's exact test was used to analyze associations between these categorical variables, with $p<.05$ considered statistically significant. All calculations were performed using STATA (Stata Corporation, College Station, TX).

Results

Seventy-six patients met study criteria (Table 3). The mean age at diagnosis was 64 years and there were 45 males (59%) and 31 females. Five patients had undergone neoadjuvant chemoradiation protocols before surgery. Two patients had a final histologic diagnosis of mucinous adenocarcinoma and the remainder had ductal adenocarcinoma.

Mean time to surgery from date of diagnosis, excluding the patients who underwent neoadjuvant therapy, was 25 days. The final stage distribution of patients is summarized in Table 3 and the surgical results shown in Table 4. Forty-seven PDs including 17 with additional vein resection (PD-vein) were performed for an overall resectability rate of 62%. Of these, an R0 resection was obtained in 33 (70%). Of the 14 patients with a positive resection margin, 13 (93%) were at the retroperitoneal margin and one was at the vascular margin associated with a portal vein resection. Median survival for R0 resection, R1 resection, and unresectable patients was 16.5, 8.1, and 9 months, respectively.

For the 29 patients found unresectable at operation, eight (28%) were caused by liver metastases; three (10%) from carcinomatosis; and two (7%) from distant nodal metastasis outside the normal bounds of resection (one celiac node, one para-aortic node). Sixteen (55%) patients were unresectable because of locally advanced disease and an inability to clear the tumor from the mesenteric or portal vasculature. Of note, nine (31%) patients in the unresectable group had signs and symptoms of gastric outlet obstruction or could not be palliated with an endobiliary stent, but all were thought to have insufficient evidence by imaging alone to preclude exploration with intent to resect. Only four (25%) of the 16 patients with locally advanced unresectable disease exhibited gastric outlet obstruction. Diagnostic laparoscopy was utilized in 22 cases (35% of patients without other indications to go to the operating room such as gastric outlet obstruction). For the unresectable patients, laparoscopy identified two of three (67%) liver metastases and one of four (25%) locally advanced tumors.

Table 3 Demographics of Study Patients Including Stage Distribution

Characteristic	Number (%)
Total number of patients	76
Male	45 (59)
Female	31 (41)
Mean age	64 yrs
Neoadjuvant chemoradiation	5 (7)
Ductal adenocarcinoma	74 (97)
Mucinous adenocarcinoma	2 (3)
Stage	
1A	5 (9)
1B	4 (5)
2A	8 (10)
2B	29 (37)
3	16 (21)
4	14 (18)
Mean time to surgery[a]	25 days
Median survival, R0 resection	16.5 months
Median survival, R1 resection	8.1 months
Median survival, unresectable	9 months

[a] From the time of original diagnosis by imaging and not receiving neoadjuvant therapy

Table 4 Results of Surgery

Procedure (Total = 76)	Number (%)	Margin/Node Status	Number (%)
Pancreaticoduodenectomy	30 (39)	R0N0	9
		R0N1	14
		R1N0	1
		R1N1	6
PD + vein	17 (22)	R0N0	4
		R0N1	6
		R1N0	1
		R1N1	6
All resections	47 (62)	R0	33 (70)
		R1	14 (30)
		N0	15 (32)
		N1	32 (68)
Unresectable	29 (38)	Cause	
		Liver metastasis	8 (28)
		Carcinomatosis	3 (10)
		Distant nodal metastasis	2 (7)
		Locally advanced	16 (55)

R0: Margin Negative Resection; R1: Margin Positive Resection; PD: Pancreaticoduodenectomy (Whipple procedure)

Table 5 EUS Predictors of Resectability

Vascular Involvement	*N* (%)	Margin Status	*n*	*p*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
	Total = 63								
Venous									
Free	35 (56)	R0	22	.08	57	67	61	63	62
		$R1^+$	13						
Abuts/Invades	28 (44)	R0	11						
		$R1^+$	17						
Arterial									
Free	57 (90)	R0	30	1	10	91	50	53	52
		$R1^+$	27						
Abuts/Invades	6 (10)	R0	3						
		$R1^+$	3						
Pre-stent EUS	Total = 27								
Venous									
Free	12 (44)	R0	9	.02	79	69	73	75	74
		$R1^+$	3						
Abuts/Invades	15 (56)	R0	4						
		$R1^+$	11						
Arterial									
Free	24 (89)	R0	12	1	14	92	67	50	52
		$R1^+$	12						
Abut/Invades	3 (11)	R0	1						
		$R1^+$	2						

$R1^+$: Retroperitoneal margin positivity or locally advanced and unresectable at operation

EUS and CT Predictors of Resectability

Sixty-three patients either resected or found unresectable due to locally advanced disease were analyzed. EUS findings associated with R1 margins or locally advanced unresectability are shown in (Table 5). Neither portal vein or superior mesenteric vein abutment or invasion, separately or in combination, was significantly associated with failure to obtain an R0 resection. EUS findings of arterial involvement were even less accurate, with three of six

Table 6 CT Predictors of Resectability

Feature	*N* (%) Total = 63	Margin Status	*n*	*p*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Venous									
Free	28 (44)	R0	14						
		$R1^+$	7						
>90°	31 (49)	R0	12	.09	73	54	61	67	63
		$R1^+$	19						
>180°	7 (11)	R0	0	<.01	50	100	100	67	75
		$R1^+$	7						
Arterial									
Free	50 (79)	R0	29						
		$R1^+$	21						
>90°	3 (5)	R0	0	<.01	28	100	100	58	64
		$R1^+$	8						
Duodenal invasion	38 (60)			.20					
Stomach invasion	3 (5)			1					
Indeterminate liver lesions	12 (19)			.20					
Ascites	8 (13)			.71					

$R1^+$: Retroperitoneal positivity or locally advanced and unresectable at operation

patients originally determined to have arterial abutment or invasion still achieving an R0 margin. For those three patients, CT had not identified any SMA involvement in two and <90° involvement in the third. EUS did identify one patient with a concerning liver lesion that was later confirmed as a metastasis.

To determine if the presence of a biliary stent could contribute to the inaccuracy of EUS, patients who did not have a pre-EUS stent were examined as a subgroup (Table 5). There were 27 such evaluable patients, and in this situation EUS venous abutment or invasion, but not arterial involvement, was significantly associated with incomplete resection, giving a sensitivity, specificity, PPV, NPV, and accuracy of 79%, 69%, 73%, 75%, and 74%, respectively.

Suspicious adenopathy was noted in eight patients for the entire group, only two of which had a stent pre-EUS. Three went on to R1, node positive resections, and one received an R0 resection. Four were completely unresectable, with one caused by celiac node metastasis found at laparotomy. The association between suspicious EUS adenopathy and R0 resectability was suggestive, but not significant, with $p=.09$, sensitivity 97%, specificity 17%, PPV 58%, NPV 83%, and accuracy 60%.

In contrast, CT findings for vascular involvement were associated with surgical outcome (Table 6). No patient with mesenteric venous involvement greater than 180° had successful R0 resection, with four being locally advanced and three with R1 margin. This resulted in 50% sensitivity, 100% specificity, 100% PPV, 67% NPV, and 75% accuracy. CT venous involvement greater than 90° approached statistical significance as well with $p=.09$. Similarly, arterial involvement in any distribution greater than 90° was significantly associated with no patient achieving R0 resection giving 28% sensitivity, 100% specificity, 100% PPV, 58% NPV, and 64% accuracy for surgical failure.

To examine if the presence of a biliary stent affected CT results, the subgroup of 34 patients was also evaluated, but with no significant change in outcome (data not shown). Interestingly, biliary stents were associated with CT adenopathy in general. In 42 patients with stents before CT exam, all were found to have adenopathy by CT (PPV 100%, $p<.01$). However, adenopathy alone did not correlate with node positivity ($p=1$) or R0 resection ($p=.70$). The rate of node involvement was exactly the same (5 of 7, 71%) after resection in unstented patients whether CT identified adenopathy or not. Although not the primary focus of the study, other CT findings such as stomach invasion ($p=1$), duodenal invasion ($p=.20$), indeterminate liver lesions ($p=.20$), and ascites ($p=.71$) were observed infrequently and not associated with local resection failure. Five of the eight (62%) patients unresectable because of liver metastasis did have CT evidence for suspicious or indeterminate liver lesions.

EUS and CT separately were not sensitive or specific enough to predict the need for vein resection, even when the presence of a biliary stent was considered (Table 7). However, when EUS and CT findings were combined such

Table 7 EUS and CT Predictors of Vein Resection During Pancreaticoduodenectomy (PD)

Modality	Feature	*N* (%) total=47	Resection	*n*	*p*	PPV (%)	NPV (%)	Accuracy (%)
EUS	Vein-free	29 (62)	PD	21				
			PD + vein	8				
	Abutment	9 (19)	PD	5	.42	44	72	66
			PD + vein	4				
	Invasion	9 (19)	PD	4	.23	56	72	68
			PD + vein	5				
	Abutment/Invasion	18 (38)	PD	9	.21	50	72	64
			PD + vein	9				
CT	Vein-free	17 (36)	PD	14				
			PD + vein	3				
	Any involvement	30 (64)	PD	16	.06	47	82	60
			PD + vein	14				
	>90°	20 (43)	PD	11	.09	45	82	62
			PD + vein	9				
	>180°	3 (6)	PD	1	.14	67	82	80
			PD + vein	2				
EUS-CT	Any involvement by CT and EUS	17 (36)	PD	8	.07	53	81	67
			PD + vein	9				
	CT >90° and EUS abutment/invasion	10 (21)	PD	4	.05	60	81	73
			PD + vein	6				

that they had to agree on venous involvement, then the finding of CT involvement more than 90° in addition to EUS abutment or invasion had 67% sensitivity, 76% specificity, 60% PPV, 81% NPV, and 73% accuracy for needing vein resection. Stricter combinations such as CT involvement >180° with EUS invasion to increase the specificity of the tests resulted in too few observations for meaningful analysis.

Discussion

This study examined our failures with pancreaticoduodenectomy in pancreatic cancer, specifically R1 resections at the retroperitoneal/vascular margin and locally advanced unresectable disease, by reanalyzing the EUS and CT studies that had already been used prospectively to proceed with attempted resection. On critical review, especially with systematic CT evaluation, it appears that there were indeed imaging findings that would have predicted an inability to achieve an R0 margin. These are the CT presence of tumor venous involvement greater than 180° and arterial involvement greater than 90°. Review of the original radiology reports reveals that often little remark was made on the degree of vascular involvement in such quantitative terms. Our findings, however, are in agreement with recent radiology literature addressing the issue of clinically significant vascular involvement on CT in pancreatic cancer.[13,14]

EUS is a dynamic study much more dependent than CT on operator experience as well as patient factors that may make a study difficult. Recent literature has suggested that EUS is not as accurate as once thought when determining vascular invasion with positive predictive values as low as 28%.[15,16] It is also not clear how often the presence of a biliary stent is considered in these studies. It is not unexpected that biliary stenting contributes to local inflammation and adenopathy and thus potentially obscures EUS or CT visualization. When patients with stents before EUS were excluded, we found that EUS venous abutment or invasion was accurate to 74% and significantly associated with local unresectability. From our results, adenopathy cannot otherwise be used as a surrogate for unresectability.

Both EUS and CT demonstrated limited specificity predicting the need for vein resection, which was unexpected, particularly as CT had performed so well with resectability. They clearly have higher negative predictive values, and thus are much better classifying vessels free of tumor encasement rather than vessels involved with tumor. When combined, the modalities can be complimentary and we did obtain a significant result 73% accurate for vein resection. However, this study did not observe consistent correspondence between EUS and CT across most features.

Conclusion

Pancreas protocol CT currently appears more accurate than EUS in determining local resectability of pancreatic head adenocarcinomas. We have identified specific CT findings associated with failure to clear the retroperitoneal or vascular margin. When prestudy biliary stenting is excluded EUS vascular involvement is also predictive of resectability. Although the R1 margin in 14 patients may have resulted in part from surgeon-related technical factors, the 12 patients with locally advanced unresectable disease and no gastric outlet obstruction could potentially have been spared the morbidity of operation without the fear of not giving them the benefit of the doubt that resection could be achieved. Laparoscopy has been proposed as one method to identify unresectable patients before laparotomy. As we saw with this series, laparoscopy can help identify liver metastasis, but it has more difficulty ruling out locally advanced disease. Indeed, laparoscopy precluded further operation in only one of four patients with locally advanced unresectable disease, and here laparoscopic ultrasound was also utilized.

To our knowledge, this is the first study to examine the issue of local resectability as it relates to current standards of preoperative staging because this is the primary concern of the surgeon attempting curative resection. The study population was relatively small, but statistically significant results were obtained, and with more study power we assume that additional predictive factors will be identified, in particular those combinations of imaging findings most specific for resectability.

Yovino et al.[17] recently described a scoring system to predict unresectability of pancreatic cancer. This study examined essentially the same EUS and CT features as we do now, although the authors considered both local and metastatic unresectability. The scoring system will also need to be subject to external validation. Particularly because our EUS results are less consistent, we will likewise need to validate our findings in a prospective manner. As patients judged unresectable generally do not undergo surgical exploration and confirmation of their staging, the best validation for these types of investigations is then to look for an improvement in the overall resectability and R0 resection rates as well as the associated survival of patients undergoing operation with intent to resect.

References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer Statistics. CA Cancer J Clin 2007;57:43–66.
2. Winter JM, Cameron JL, Campbell KA, Arnold MA, Chang DC, Coleman J, Hodgin MB, Sauter PK, Hruban RH, Riall TS, Schulick RD, Choti MA, Lillemoe KD, Yeo CJ. 1423 pancreatic-

oduodenectomies for pancreatic cancer: a single-institution experience. J Gastrointest Surg 2006;10:1199–1211.
3. Schwarz RE, Smith DD. Extent of lymph node retrieval and pancreatic cancer survival: Information from a large US population database. Ann Surg Oncol 2006;13:1189–1200.
4. Michalski CW, Kleeff J, Wente MN, Diener MK, Buchler MW, Friess H. Systematic review and meta-analysis of standard and extended lymphadenectomy in pancreaticoduodenectomy for pancreatic cancer. Br J Surg 2007;94:265–273.
5. Peiper M, Bolke E, Orth K, Hosch SB, Rehders A, Matthaei H, Knoefel WT. Current status of radical systematic lymphadenectomy in pancreatic cancer—A review of the literature. Eur J Med Res 2007;12:47–53.
6. Howard T, Krug JE, Yu J, Zyromski NJ, Schmidt CM, Jacobson LE, Madura J, Wiebke EA, Lillemoe KD. A margin-negative R0 resection accomplished with minimal postoperative complications is the surgeon's contribution to long-term survival in pancreatic cancer. J Gastrointest Surg 2006;10:1338–1346.
7. Hunt GC, Faigel DO. Assessment of EUS for diagnosing, staging, and determining resectability of pancreatic cancer: A review. Gastrointest Endosc 2002;55:232–237.
8. Gress FG, Hawes RH, Savides TJ, Ikenberry SO, Cummings O, Kopecky K, Sherman S, Wiersema M, Lehman GA. Role of EUS in the preoperative staging of pancreatic cancer: A large single-center experience. Gastrointest Endosc 1999;50:786–791.
9. Greene FL. Exocrine pancreas. AJCC cancer staging manual. 6th Ed. New York: Springer-Verlag, 2002: 179–187.
10. Yusoff IF, Mendelson RM, Edmunds SE, Ramsay D, Cullingford GL, Fletcher DR, Zimmerman AM. Preoperative assessment of pancreatic malignancy using endoscopic ultrasound. Abdom Imaging 2003;28:556–562.
11. DeWitt J, Devereaux B, Chriswell M, McGreevy K, Howard T, Imperiale TF, Ciaccia D, Lane KA, Maglinte D, Kopecky K, LeBlanc J, McHenry L, Madura J, Aisen A, Cramer H, Cummings O, Sherman S. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. Ann Intern Med 2004;141:753–763.
12. Soriano A, Castells A, Ayuso C, Ayuso JR, de Caralt MT, Gines MA, Real MI, Gilabert R, Quinto L, Trilla A, Feu F, Montanya X, Fernandez-Cruz L, Navarro S. Preoperative staging and tumor resectability assessment of pancreatic cancer: prospective study comparing endoscopic ultrasonography, helical computed tomography, magnetic resonance imaging, and angiography. Am J Gastroenterol 2004;99:492–501.
13. Phoa SS, Reeders JW, Stoker J, Rauws EA, Gouma DJ, Lameris JS. CT criteria for venous invasion in patients with pancreatic head carcinoma. Br J Radiol 2000;73:1159–1164.
14. Li H, Zeng MS, Zhou KR, Jin DY, Lou WH. Pancreatic adenocarcinoma: Signs of vascular invasion determined by multi-detector row CT. Br J Radiol 2006;79:880–887.
15. Aslanian H, Salem R, Lee J, Andersen D, Robert M, Topazian M. EUS diagnosis of vascular invasion in pancreatic cancer: Surgical and histologic correlates. Am J Gastroenterol 2005;100:1381–1385.
16. Rosch T, Dittler HJ, Strobel K, Meining A, Schusdziarra V, Lorenz R, Allescher HD, Kassem AM, Gerhardt P, Siewert JR, Hofler H, Classen M. Endoscopic ultrasound criteria for vascular invasion in the staging of cancer of the head of the pancreas: A blind reevaluation of videotapes. Gastrointest Endosc 2000;52:469–477.
17. Yovino S, Darwin P, Daly B, Garofalo M, Moesinger R. Predicting unresectability in pancreatic cancer patients: The additive effects of CT and endoscopic ultrasound. J Gastrointest Surg 2007;11:36–42.

DISCUSSION

Attila Nakeeb, M.D. (Indianapolis, IN): In the surgical management of pancreatic cancer, our objective is to achieve a margin negative R0 resection. Therefore, it is incumbent on surgeons to be able to accurately stage these patients preoperatively. Endoscopic ultrasonography has become the favorite tool of the gastroenterologist for staging pancreatic cancer, whereas most surgeons still feel that a CT scan is really all we need to determine resectability. I believe your data confirm this opinion. I have got a couple of questions that I would like to ask you.

Almost a third of your resected patients actually underwent venous resection and reconstruction. Can you tell us the margin status of those patients and were you able to achieve an R0 resection in those patients? Your data show that if you see venous abutment or invasion on your endoscopic ultrasound, it is a coin flip as to whether or not that patient can be resected or not and does not give us any added information.

What it really comes down to is EUS may overestimate tumor stage. The real question is are we overusing this technology? If you have a patient that shows less than 90% invasion on your CT scan of the vein, is there any reason to get an EUS on that patient? Does it really change how you are managing your patients? Should we go to our endoscopist and tell them if you want to do an EUS, don't put the stent in first so we can get some accurate information out of it.

I really enjoyed the presentation.

Philip Bao, M.D. (Nashville, TN): Thank you for your comments and questions. For our 17 patients who had vein resection, we had one with a positive vascular margin. So I think we were doing well at least for this patient cohort in clearing the vascular margin.

As to the question of the utility of EUS, I don't think by any means we are arguing that EUS should be removed, mostly because EUS-FNA is so important in obtaining a tissue diagnosis. It is also sensitive identifying small pancreatic lesions that may not be detected by CT. For better or for worse, because of our referral patterns, most of these patients already have had EUS and a biliary stent by the time they see us in clinic, so it is hard to take the study back. But I agree, it appears that it would be preferable for endoscopists who first see these patients to perform the EUS before stenting.

I think as a diagnostic modality EUS will remain important, but certainly as a staging modality, at least with these results, we would trust a CT more.

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Telomere Shortening and Telomerase Expression during Multistage Carcinogenesis of Intraductal Papillary Mucinous Neoplasms of the Pancreas

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Abstract Intraductal papillary mucinous neoplasm (IPMN) of the pancreas has been increasingly identified as a precursor to infiltrating ductal adenocarcinoma. Telomerase activation in response to telomere crisis followed by telomere shortening is thought to be a crucial event in the development of most human cancers. The aim of this study was to determine when this event occurs in the context of histologically defined IPMN progression. We analyzed telomerase expression in 68 IPMN samples and assessed telomere length by quantitative fluorescence in situ hybridization in samples taken from 17 sequential IPMN patients that included 37 individual loci. Samples from pancreatic ductal adenocarcinomas (PDACs, $n=15$) and chronic pancreatitis patients ($n=10$) were also examined. Telomeres were significantly shortened in 36 (97.3%) of 37 IPMN loci, with average telomere length decreasing with IPMN progression. Notably, even adenoma IPMNs demonstrated a 50% reduction of telomere length in 7 of 14 foci examined. Marked telomere shortening was observed from the in situ IPMN carcinoma stage ($P<0.001$; vs borderline IPMNs) through the invasive stage, although telomerase had been activated, indicating that telomeres had shortened to a critical length by this histological grade. Up-regulated human telomerase reverse transcriptase expression was detectable and increased gradually with cancer development and was primarily observed at the borderline IPMN stage and then in more advanced histopathologies. Progressive telomere shortening predominantly occurs during early IPMNs carcinogenesis before telomerase activation and progression from borderline to carcinoma in situ IPMNs is the critical stage of IPMNs carcinogenesis at which telomere dysfunction occurs.

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Abbreviations

IPMN	intraductal papillary mucinous neoplasm
hTERT	human telomerase reverse transcriptase
IHC	immunohistochemistry
Q-FISH	quantitative fluorescence in situ hybridization
TRAP	telomeric repeat amplification protocol
PDAC	pancreatic ductal adenocarcinoma
TS	*telomerase substrate*
TPG	total product generated
TFI	telomere fluorescence intensity
ALT	alternative lengthening for telomeres

Introduction

Intraductal papillary mucinous neoplasm (IPMN) of the pancreas has been increasingly recognized as a cystic neoplasm of the pancreas[1,2] and is characterized by intraductal papillary growth and mucin hypersecretion, resulting in cystic dilation of the pancreatic ducts.[3–5] The pathogenesis of IPMNs is thought to involve multiple stages, with development from initial adenoma to borderline lesions, followed by carcinoma in situ and ultimately invasive carcinoma.[3,6,7] However, little is known of the initiating genetic events within the pancreatic ductal epithelium that facilitate the progression of IPMN cancers.

Reported genetic alterations in IPMNs include mutations in the *TP53*, *KRAS*, *MUC*, and *DPC4* genes, loss of heterozygosity at several chromosomal loci,[8–12] and aberrant DNA methylation patterns that may contribute to the inactivation of a subset of tumor suppressor genes.[13] Recently, telomere dysfunction has emerged as a crucial trigger for the complex chromosomal abnormalities that occur in carcinogenesis.[14–17] Thus, investigation into the dynamic changes in telomere length and a temporal relationship with telomerase activation during IPMN development is a critical question.

Human telomeres are nucleoprotein complexes consisting of 8–15 kb of TTAGGG repeats along with specific binding proteins and are located at chromosomes ends.[18,19] These structures prevent chromosome termini from being recognized as double-stranded DNA breaks and are essential to genomic stability.[17] In somatic cells, telomeres progressively shorten during each round of cell division through replication-dependent loss of DNA termini until one or more telomeres become dysfunctional.[20,21] Consecutive telomere shortening ultimately leads to excessive telomere erosion, loss of telomere capping function, and eventually genetic instability and cellular senescence when telomeres become critically short.[14] Consequently, epithelial cells with excessive telomere shortening are mostly eliminated by protective mechanisms.[17] However, rarely some cells escape from this surveillance by activation of telomerase and develop additional cancer hallmarks through accumulation of multiple genomic and epigenetic aberrations, which restore the minimal length of telomeres required to maintain their function.[22] Thus, telomere crisis, defined as events that occur when cells lose telomere function as a result of extended proliferation in the absence of telomerase, is a critical rate-limiting and promoting event for cancer progression.[23,24] Telomeres can be maintained through recombination or, as seen in the most human cancers, by telomerase activation.[18] Telomerase is an RNA-dependent DNA polymerase that is generally inactivated in normal human somatic cells and is under control of the *human telomerase reverse transcriptase* (*hTERT*) gene that encodes the catalytic component of telomerase.[25,26] Similarly, telomerase activation and up-regulated hTERT expression have been implicated in malignant cell transformation in human tumor tissues.[20,24,27] Accordingly, ectopic hTERT expression precedes transformation in human cancer development, which leads to unlimited yet not tumorigenetic growth, and therefore, hTERT detection has been proposed as a promising marker for carcinogenesis even if at precancerous stage.[28]

In the present study, we examined hTERT expression by immunohistochemistry (IHC) and telomere length by quantitative fluorescence in situ hybridization (Q-FISH) in tissue microarrays containing a variety of IPMN precursor lesions. We also analyzed telomerase activity semi-quantitatively using the telomeric repeat amplification protocol (TRAP) assay to investigate the relationship between telomere dysfunction and histologic type or malignancy grade, thereby identifying the role of telomere biology in the carcinogenesis of IPMN of the pancreas.

Materials and Methods

Patients and Tissue Samples

Human tissue sections were collected from 68 patients who underwent pancreatic resection for IPMN of the pancreas at the Department of Surgery, Hiroshima University Hospital, from January 1990 to December 2005. A retrospective, consecutive, and single-center review of the clinicopathological factors of these cases was performed. Subsequently, samples from different patients with pancreatic ductal adenocarcinomas (PDACs; $n=15$) and chronic pancreatitis

(n=10) were obtained from the resected pancreas tissues, along with adjacent non-cancerous pancreatic tissues from each patient (n=83). The study group included 15 patients with IPMNs already published.[8] Written informed consent was obtained from all patients, and the study was conducted according to the guidelines of the Helsinki Declaration. When histologically defined IPMNs included various grades of lesions, sometimes even within the same tumor, we defined the highest dysplastic lesions as the final diagnosis. All pathologic specimens were reviewed by two independent pancreatic pathologists (F. S. and Y. H.). Tumors were classified as adenoma (adenoma IPMN), borderline lesion (borderline IPMN), carcinoma in situ (carcinoma in situ IPMN), or invasive carcinoma derived from IPMN (invasive IPMN) according to established criteria of the World Health Organization on tumors of the digestive system.[29]

Tissue samples were obtained immediately after surgical resection. In each case, a portion of the tissue samples was stored at −80°C until use for detecting telomerase activity, while the remaining portion was fixed in 10% buffered formalin and embedded in paraffin. Tissue microarrays were constructed using a manual Tissue Puncher/Arrayer (Beecher Instruments, Silver Spring, MD) and representative serial sections cut into 4-μm-thick sections for IHC and Q-FISH, with adjacent sections used for H&E staining for reference.

Cell Culture

Four tumor cell lines derived from pancreatic ductal cancers, PK-1, PK-8, PK-9, and PK-59, were kindly donated by the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University, Miyagi, Japan. The cell lines were maintained in RPMI 1640 medium (Gibco BRL, Rockville, MD), supplemented with 10% (v/v) fetal bovine serum (Gibco BRL) and 10 μg/ml antibiotic–antimycotic (Gibco BRL) in the presence of 5% CO_2 at 37°C. The cell lines were used as positive controls for IHC, and protein extracts from PK-1 cells were used in each polymerase chain reaction (PCR) run as a positive control. Cytospin slides to confirm the staining properties of IHC were produced using 1×10^6 cells. Slides were air dried for 30 min and fixed in 95% ethanol until use.

Quantification of Telomerase Activity

Extraction of telomerase protein and evaluation of its activity were performed using the TRAP assay as previously described.[30] Telomerase activity levels were measured using the TRAPeze® XL telomerase detection kit (Chemicon International, Temecula, CA) according to the manufacturer's protocol. Briefly, extracts from 40 to 100 mg samples of frozen pancreatic tissue were homogenized in 100–200 μl CHAPS lysis buffer and incubated on ice. Lysates were centrifuged at 12,000×g for 20 min at 4°C, and the supernatants rapidly frozen and stored at −80°C. Aliquots of extract containing 1 μg protein were then used for TRAP assay. Detection of telomerase activity involves initial telomerase extension of the telomerase substrate (TS) primer, which serves as the substrate for the telomerase enzyme, followed by PCR amplification of the telomerase products using reverse primers labeled with fluorescein (telomerase-specific products) and sulforhodamine: TS forward (5′-AATCCGTC GAGCAGAGTT-3′) and fluorescein-labeled Amplifluor RP reverse primers (5′-CCCTTACCCTTACCCTTACCC TAA-3′). Extension reactions by telomerase were performed at 30°C for 30 min with PCR cycling parameters of 94°C for 5 min to activate the Taq polymerase followed by 36 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 60 s, and a final extension at 55°C for 25 min followed by incubation at 4°C. Fluorescent signals produced by the amplification were directly proportional to the amount of TRAP products generated. A fluorescence plate reader (Wallac 1420 ARVOsx, Perkin-Elmer, Wellesley, MA) was used to detect the levels of fluorescein and sulforhodamine. Telomerase activity levels were quantified as the ratio of fluorescein intensity of the entire TRAP ladder to the sulforhodamine intensity of the internal control after correction for the negative control and background fluorescence, and expressed as total product generated (TPG) units. All samples were run in duplicate. Reaction mixtures with 2 μl CHAPS lysis buffer instead of sample extract were used as negative controls.

Immunohistochemical Detection of Human Telomerase Reverse Transcriptase

IHC was performed using the streptavidin-peroxidase technique and Dako EnVision+system (Dako Cytomation GmbH, Hamburg, Germany) as described previously.[31] An affinity-purified polyclonal rabbit antibody against hTERT (EST21A) was raised against a 16-amino-acid peptide sequence that maps to the middle of hTERT (Alpha Diagnostic International, San Antonio, TX) and used at a concentration of 5 to 10 μg/ml. The validity of the antibodies used in this has been discussed in previous reports on Western blotting and immunostaining.[31] Tissues were cut as 4-μm serial sections from tissue microarray paraffin blocks, deparaffinized in xylene, and rehydrated through an alcohol dilution series. After antigen retrieval by autoclaving (121°C, 15 min in 0.01 M citrate buffer), sections were immersed in methanol containing 3% hydrogen peroxide for 10 min and incubated in protein

blocking solution (Dako, Carpinteria, CA) for 60 min. Sections were incubated with primary hTERT antibody at a dilution of 5 to 10 μg/ml for overnight at 4°C, and then incubated in labeled streptavidin-biotin polymer (Envision Plus, Dako), followed by 0.05% 3,3′-diaminobenzidine (DAB) in PBS with hydrogen peroxide as a substrate for 10 min. Sections were lightly counterstained with Mayer's hematoxylin and then mounted. Tissues with pancreatic cancer known to be positive for hTERT and a pancreatic-derived cell line were used as external positive controls. Negative controls consisted of sections incubated without the primary antibody.

Immunohistochemical Evaluation

IHC intensity was evaluated by light microscopy and Image-Pro Plus version 4.0 software (Media Cybernetics, Silver Spring, MD). Immunohistochemical evaluation of hTERT expression was performed independently by two authors (Y. H. and E. F.). As hTERT nuclear and/or nucleolar staining formed specific patterns of staining intensity, sections were classified into one of three categories as previously described:[31] + + (strong, IHC signals strongly detected throughout the nucleus but not the nucleolus), + (weak, IHC signals weakly detected or appearing as a speckled/dotted pattern in the nucleus), and – (no staining).

Quantitative Fluorescence In Situ Hybridization

Telomere lengths were evaluated by Q-FISH, which measures the proportional length of telomeric TTAGGG DNA repeats[32] using a flourescein isothiocyanate (FITC)-labeled telomere peptide nucleic acid FISH kit and the GenPoint Fluorescein system (Dako, Glostrup, Denmark) according to the manufacturer's protocol. Quantification of telomere fluorescence intensity (TFI) was performed on consecutive sections as used for H&E staining, allowing quantification of TFI in different areas of histological distribution. Briefly, deparaffinized 4-μm sections of tissue microarray blocks underwent heat-induced antigen retrieval in Dako Target Retrieval solution (Dako), proteinase K pretreatment solution (diluted 1:1,000) added, and then hybridized with a FITC-labeled, telomere-specific peptide nucleic acid probe. Slides were then denatured using an OmniSlide thermal cycler (Hybaid, Middlesex, UK) at 90°C for 5 min and hybridized for overnight at 45°C.

Slides were washed at 52°C for 20 min with wash solution (diluted 1:50) and incubated with 200 μl anti-FITC-HRP solution (diluted 1:100) at room temperature for 20 min. After incubation with 200 μl fluorescyl tyramide solution at room temperature in the dark, slides were counterstained with 4′-6-diamidino-2-phenylindole (DAPI; Vysis, Downers Grove, IL).

Quantitative Analysis of Digital Images

For telomere length assessment, serial H&E-stained slides were used as guides for examination of Q-FISH slides. Signal intensities were visualized with a Nikon Eclipse E600 fluorescence microscope (Nikon, Tokyo, Japan) equipped with the appropriate fluorescence filter sets. Fluorescent images were captured with a charge-coupled device camera using Leica ver. 4 software (Leica Microsystem Imaging Solutions, Cambridge, UK) and digitized fluorescent telomere signals quantified using TFL-TELO V1.0 image analysis software, developed by P. Landsdorp. In this method, the number of telomere spots and intensities of each telomere spot in a given cell nucleus in the FITC channel were automatically assessed and normalized to the total DAPI signal. Unmodified black and white images were used to assess mean TFI.

Statistical Analysis

For analysis of telomerase activity and telomere signals, quantitative data were expressed as the mean±SD. Differences between categorical variables were evaluated by Fisher's exact test, and the Mann–Whitney U test used for comparisons of telomerase activity levels and mean ratios of telomere signal to DAPI between the various cell types using Statview-J 4.11 software (Abacus, Concepts, Berkley, CA). Statistical significance was defined as $P<0.05$.

Results

Patients and Tissue Samples

Sixty-eight representative tumor samples from IPMN patients were examined for telomerase activity and hTERT expression (Table 1). The samples included adenoma ($n=19$), borderline lesions ($n=14$), carcinoma in situ ($n=20$), and invasive IPMNs ($n=15$). Patients consisted of 51 male (75.0%) and 17 female (25.0%), and median age was 66.5 (range, 35–84).

In the analysis of telomere length by Q-FISH, lesion telomeres were directly compared to internal standard within the same tissue section. Cases in which detectable telomere signals were not found in controls or in which excessive autofluorescence precluded assessment of telomere signals were excluded from the study. Consequently, we examined telomere signals in sequential samples from 17 patients with IPMNs of different histological grades (Table 2): All of these samples were subjected to appropriate fixation conditions that warrant the quality of the Q-FISH analysis. We evaluated 37 individual loci by Q-

Table 1 hTERT Expression in Intraductal Papillary Mucinous Neoplasms (IPMNs) of the Pancreas, Pancreatic Ductal Adenocarcinoma (PDAC), and Chronic Pancreatitis

Histopathologic diagnosis[a]	hTERT Expression[b]			Positive Rate of hTERT Expression[c]
	−	+	++	
IPMNs				
Adenoma (n=19)	16	3	0	0.158
Borderline (n=14)	9	3	2	0.357[d]
Carcinoma in situ (n=20)	3	11	6	0.850[e]
Invasive (n=15)	2	7	6	0.867
PDACs (n=15)	1	6	8	0.933
Chronic pancreatitis (n=10)	10	0	0	0.000

hTERT Human telomerase reverse transcriptase; I*PMN* intraductal papillary mucinous neoplasm; *PDAC* pancreatic ductal adenocarcinoma
[a] This table includes 68 representative tumor samples with IPMNs from each patient, which defined the highest dysplastic lesions as the diagnosis of each case.
[b] Immunohistochemical staining. The intensity of individual signals was assessed as follows: − no staining; + weak; and ++ strong expression.
[c] ++ , + positive; − negative.
[d] P=0.2379 for borderline vs adenoma IPMNs
[e] P=0.0048 for carcinoma in situ vs borderline IPMNs, Fisher's exact test

FISH, including adenoma (n=14), borderline (n=8), carcinoma in situ (n=11), and invasive (n=4) IPMN loci.

Observed telomerase activity and hTERT expression increased gradually with cancer development, and were primarily observed from the borderline IPMN stage through to more advanced histopathologies (Fig. 1; Table 1). Telomeres were significantly shortened in 36 (97.3%) of 37 IPMN loci and showed a gradual decrease with cancer development. Notably, even adenoma IPMNs, the earliest precursor lesion, demonstrated a 50% reduction in telomere length in 7 of 14 foci examined (Table 2). Above the level of carcinoma in situ, no further telomere shortening was observed, such that carcinoma in situ IPMNs exhibited significant telomere shortening compared to borderline IPMNs (P<0.001) but not compared to PDACs. This suggested that telomeres had shortened to a critical length at the carcinoma in situ histological grade.

Telomerase Expression in Chronic Pancreatitis and Non-cancerous Pancreatic Tissues

Levels of telomerase activity were assessed in samples of chronic pancreatitis tissues and non-cancerous pancreatic tissue (summarized in Fig. 1). Telomerase activity levels were 2.42±2.98 TPG units in chronic pancreatitis tissues compared to 0.57±0.48 TPG units in adjacent non-cancerous pancreatic tissues, which represented a significant difference (P=0.0018). In contrast, no hTERT expression was detectable in adjacent non-cancerous pancreatic epithelia or tissues with chronic pancreatitis, although activated lymphocytes in tissues from severe chronic pancreatitis expressed intense hTERT signals, with one sample reaching 10.26 TPG units.

Telomerase Expression in Invasive Ductal Adenocarcinoma of the Pancreas

Samples of PDAC showed significantly higher levels of telomerase activity (64.14±37.09 TPG units) than both adjacent non-cancerous pancreatic tissues (P<0.0001) and chronic pancreatitis tissues (P=0.0002). Intense hTERT expression was detectable in 14 (93.3%) of 15 PDACs (Table 1).

Telomerase Expression in Intraductal Papillary Mucinous Neoplasms of the Pancreas

Telomerase activity levels were 1.20±1.30 TPG units in adenoma, 4.42±3.54 in borderline lesions, 14.66±12.01 in carcinoma in situ, and 35.92±21.81 in invasive IPMNs, such that levels increased with IPMN progression. Levels of telomerase activity in invasive IPMNs were significantly higher than in carcinoma in situ or borderline IPMNs and approached that of PDAC (Fig. 1; P=0.0184 for carcinoma in situ vs borderline IPMNs, P=0.0022 for invasive vs carcinoma in situ IPMNs).

hTERT expression was detectable in 3 (15.8%) of 19 adenoma, 5 (35.7%) of 14 borderline, 17 (85.0%) of 20 carcinoma in situ, and 13 (86.7%) of 15 invasive IPMNs (Table 1; Fig. 2). Cases positive for malignant IPMNs showed significantly higher expression than borderline or adenoma IPMNs (Table 1; P=0.0048 for carcinoma in situ vs borderline IPMNs). Intensities of individual hTERT signals in malignant IPMNs and those detected in PDAC cells were stronger than those detected in adenoma IPMNs, and appeared to reflect the biological invasiveness of each tumor cell (Fig. 2).

Table 2 Summary of Telomere Length and Telomerase Expression from 17 Patients with Intraductal Papillary Mucinous Neoplasms (IPMNs) of the Pancreas

Patient No.	Age/ Sex	Diagnosis[a]	hTERT Expression[b]	Telomere Fluorescence Intensity (Q-FISH)[c]				
				IPMN mean	IPMN SD	Normal mean	Normal SD	IPMN: Normal ratio[d]
1	37/M	Invasive carcinoma in situ adenoma	++	154.24	21.94	1,135.81	152.76	0.14
			++	148.43	10.07			0.13
			+	1021.59	181.06			0.90
2	76/M	Invasive carcinoma in situ borderline lesion	++	82.32	5.24	1,076.12	45.89	0.08
			+	281.82	12.81			0.26
			+	566.84	142.81			0.53
3	75/M	Invasive adenoma	+	105.37	13.71	1,136.53	75.35	0.09
			−	604.90	139.48			0.53
4	72/M	Invasive borderline lesion borderline lesion adenoma	++	82.03	5.98	961.76	50.52	0.09
			+	798.06	126.89			0.83
			+	198.34	81.12			0.21
			−	906.18	90.74			0.94
5	54/F	Carcinoma in situ borderline lesion adenoma	−	1306.32	54.60	1,243.38	92.16	1.05
			−	1167.04	149.27			0.94
			−	375.38	76.54			0.30
6	53/M	Carcinoma in situ adenoma	++	99.76	17.92	992.45	53.30	0.10
			−	470.70	102.48			0.47
7	66/M	Carcinoma in situ borderline lesion adenoma	++	116.44	10.45	1,364.88	92.16	0.09
			ND	278.96	94.88			0.20
			+	519.37	107.05			0.38
8	69/M	Carcinoma in situ adenoma	−	105.36	12.88	910.86	32.46	0.12
			−	243.47	69.79			0.27
9	74/F	Carcinoma in situ borderline lesion	+	172.37	12.88	1,127.00	60.88	0.15
			−	199.13	63.55			0.18
10	80/M	Carcinoma in situ adenoma	++	169.90	14.27	1,076.95	57.33	0.16
			−	339.05	26.47			0.31
11	73/M	Carcinoma in situ adenoma	+	85.37	36.26	1,046.41	72.79	0.08
			ND	331.43	61.06			0.32
12	72/F	Carcinoma in situ borderline lesion	+	112.25	12.83	1,098.62	32.45	0.10
			−	234.64	77.81			0.21
13	68/M	Carcinoma in situ adenoma	++	429.37	33.21	1,212.99	100.52	0.35
			−	614.49	128.58			0.51
14	76/M	Borderline lesion adenoma	−	412.35	81.24	1,223.08	33.17	0.34
			−	578.97	139.81			0.47
15	65/M	Adenoma	−	717.17	72.99	980.33	40.45	0.73
16	57/M	Adenoma	−	802.51	73.31	1,210.79	37.63	0.66
17	65/M	Adenoma	+	720.09	99.38	995.44	66.32	0.72

IPMN Intraductal papillary mucinous neoplasms; *ND* not done

[a] The diagnoses of IPMNs of the pancreas were established criteria by the World Health Organization on tumors of the digestive system, classified as adenoma, borderline lesion, carcinoma in situ, and invasive carcinoma.[29]

[b] Immunohistochemical staining. The intensity of individual signals was assessed as follows: − no staining; +, weak; and ++ strong expression.

[c] Telomere hybridization was performed, and the maximum intensity projections of the acquired images were analyzed using the image analysis software TFL-TELO V1.0, a telomere image analysis program developed by P. Landsdorp. Telomere signals in 10 to 25 nuclei from normal or different pathological regions were measured separately, and then normalized by DAPI fluorescence intensity in corresponding areas.

[d] Telomere length in IPMN cells was expressed as ratios of the specific telomere fluorescent signals between them and adjacent normal control cells.

In the same patients, hTERT expression was negative in adenoma but positive in carcinoma in situ and invasive IPMNs, apparently reflecting disease progression (Table 2). About 3 of 16 adenoma IPMN samples showed hTERT expression, and in most samples, telomerase activity was below 1.0 TPG units (data not shown). Interestingly, abrupt transitions of hTERT expression were observed between adenoma IPMN and hTERT-negative normal ductal cells (Fig. 2e). Furthermore, histologically "uniform" pancreatic epithelial cells of adenoma IPMN exhibited heterogenous

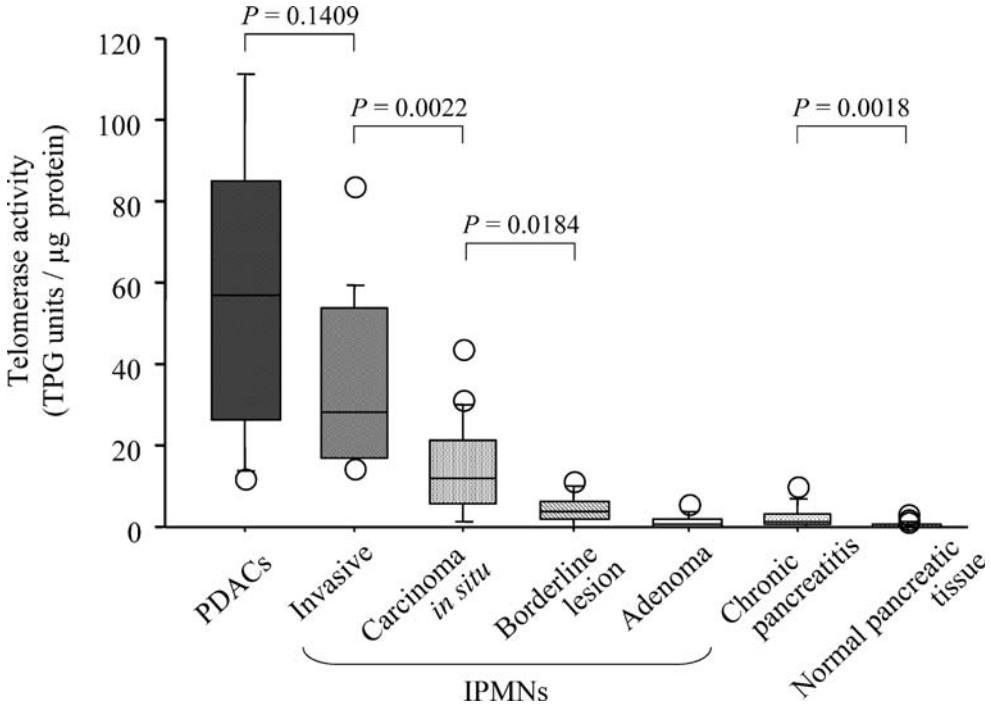

Figure 1 Quantitative analysis of telomerase activity in intraductal papillary mucinous neoplasms (*IPMNs*) of the pancreas compared with pancreatic ductal adenocarcinoma (*PDACs*), and chronic pancreatitis. Results are presented as a *box-and-whisker* plot. Telomerase activity levels gradually increase with progression from adenoma to invasive IPMNs. Invasive IPMNs show significantly higher levels of telomerase activity than other types of IPMN with lower-grade atypia (P=0.0022 for invasive IPMNs vs carcinoma in situ IPMNs), and approach that of pancreatic ductal adenocarcinomas (*PDACs*; P= 0.1409). Samples with chronic pancreatitis show significantly higher telomerase activity levels than samples of adjacent non-cancerous pancreatic tissues (P=0.0018), with 10.26 TPG units observed in a patient with severe chronic pancreatitis.

hTERT expression with no visible differences in cellular appearance (Fig. 2f).

Quantitative Telomere Assessment by Telomere Fluorescence In Situ Hybridization

To determine whether telomere dysfunction occurred during the development of IPMNs, 17 representative samples of IPMNs that included 37 different loci with various histological grades were examined (Table 2). Individual telomere lengths were analyzed for 10 to 25 nuclei of each locus. Cases in which detectable telomere signals were not found in normal epithelial cells or in which excessive background precluded assessment of telomere signals were excluded from the analysis. The pancreatic cancer cell line PK-1 was used as control for telomere length and analyzed along with each sample and gave a constant 378.2±17.9 TFI. Telomeric signals produced a widely distributed speckled pattern of nuclear signals. Telomeres in IPMN lesions were directly compared to those within normal pancreatic ductal epithelia and pancreatic acini (12 of 17 loci, 70.6%), or fibroblasts and endocrine cells (5 of 17 loci, 29.4%) within the same tissue sections.

Figures 3 and 4 show representative Q-FISH images. We observed intense cellular TFI that were comparable between the different IPMN histological grades. No significant differences in TFI were observed in regions of normal pancreatic epithelia from patients with IPMNs (1,105.49± 66.57 TFI), chronic pancreatitis (1,132.60±60.22 TFI), or PDACs (1,084.53±55.66 TFI). Lymphocytes and exocrine cells displayed stronger TFI than pancreatic epithelial cells, with lymphocytes exhibiting the most intense signals (data not shown). TFI was significantly reduced in PDAC samples and was scattered, weak, or absent in pancreatic epithelial nuclei of all 15 PDAC loci examined (108.93± 31.53 TFI).

Figure 5 summarizes the ratios of average epithelial to control telomere length obtained for each loci examined. In IPMN samples, 36 (97.3%) of 37 loci from the 17 IPMNs cases showed markedly reduced average telomere length ratios. With disease progression to more advanced stages, average telomere length ratios for the different IPMN histological grades became significantly reduced for each case investigated (r=0.55; P<0.0001).

Adenoma IPMN, the earliest precursor lesion, demonstrated a 50% reduction in average telomere length ratio in

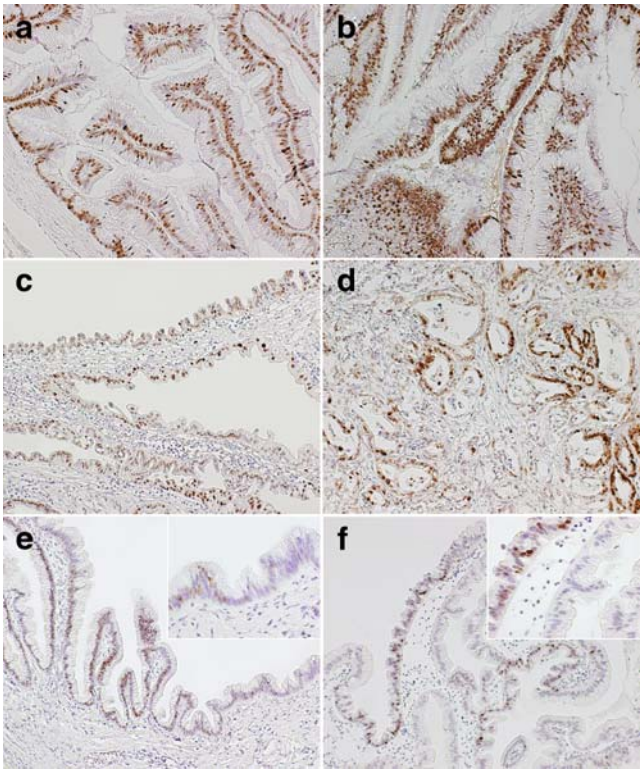

Figure 2 Immunohistochemical analysis of hTERT expression in intraductal papillary mucinous neoplasms (*IPMNs*) of the pancreas. hTERT expression in adenoma (**a**), borderline (**b**), carcinoma in situ (**c**), and invasive (**d**) IPMNs. Strong nuclear immunoreactivity for hTERT protein was observed in most of tumor cells. Note that the signal intensities for each cell are weaker in adenoma IPMN compared more advanced stages. **e** Abrupt transition of hTERT expression from normal pancreatic ducts to adenoma IPMNs (*inset*, magnified view of transition area). **f** Despite the "uniform" appearance of pancreatic epithelial cells from a case with adenoma IPMN, heterogenous hTERT expression is clearly visible. Magnified view demonstrating abrupt loss of hTERT protein expression with no visible alteration in cellular appearance (*inset*). Original magnifications, ×100 (*a–f*), ×400 (*inset*, **f**, **e**).

7 (50.0%) of 14 foci examined, but overall was highly variable (0.54±0.22) such that two patients (no. 1 and 17) showed high telomere length ratios even with demonstrated hTERT expression (Table 2). Notably, loci from borderline IPMNs had an average telomere length ratio of 0.43±0.30, whereas most epithelial cells within carcinoma in situ IPMN loci exhibited significantly weaker telomere signals (0.24±0.28) although telomerase had been activated. This indicated that telomeres had reached a critically short length at this histological grade (Fig. 5; $P<0.001$ for carcinoma in situ vs borderline IPMNs).

In patient 4, borderline IPMN loci demonstrated intratumoral heterogeneity in telomere lengths over approximately half of the lesional nuclei. This indicated that the premalignant cells had undergone further molecular or genetic alterations despite unchanged histological manifestations (Figs. 4a,b). Interestingly, a sample of carcinoma in situ IPMN from patient 5 showed much stronger telomere signals than loci with adenoma IPMN in the same tissue sections without detectable hTERT expression, which suggested the possibility of an alternate mechanism for telomere lengthening (Figs. 4c,d).

Discussion

Telomerase activation, responsible for the elongation of telomeric DNA sequences and transition through telomere crisis, is thought to be a crucial event in the development of the vast majority of human cancers, including pancreatic

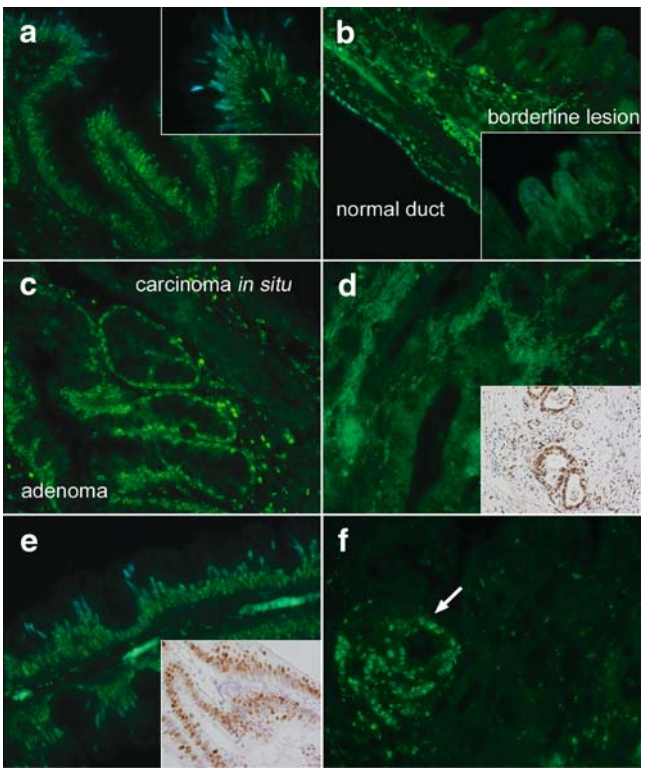

Figure 3 Quantitative fluorescence in situ hybridization (*Q-FISH*) for assessment of telomere lengths performed on IPMNs of the pancreas. Telomeres are visualized by hybridization of FITC-labeled anti-telomeric probes giving bright green colored spots. **a** Adenoma IPMN without hTERT expression show intense telomeric signals in the nuclei (*inset*, higher magnification). **b** Borderline IPMN shows weak telomeric signals in the nuclei (*inset*), whereas intense telomeric signals are retained in the adjacent normal epithelium. **c** Adjoining areas of adenoma and carcinoma in situ IPMN. Weak telomeric signals in carcinoma in situ (*upper right*) and adenoma with intense signals (*left below*). **d** In invasive IPMN samples, telomeric signals are significantly reduced and are scattered, weak, or absent in pancreatic epithelial nuclei although intense hTERT expression is maintained (*inset*). **e** Adenoma IPMN retaining intense telomeric signals shows hTERT expression (Table 2, patient 1; *inset*). **f** Invasive IPMN exhibits significantly decreased signals, compared with entrapped non-cancerous endocrine cells (*arrow*). DAPI counterstain; original magnifications, ×400 (**a**–**f**), ×1,000 (*inset*, **a**, **b**), ×200 (*inset* **d**, **e**).

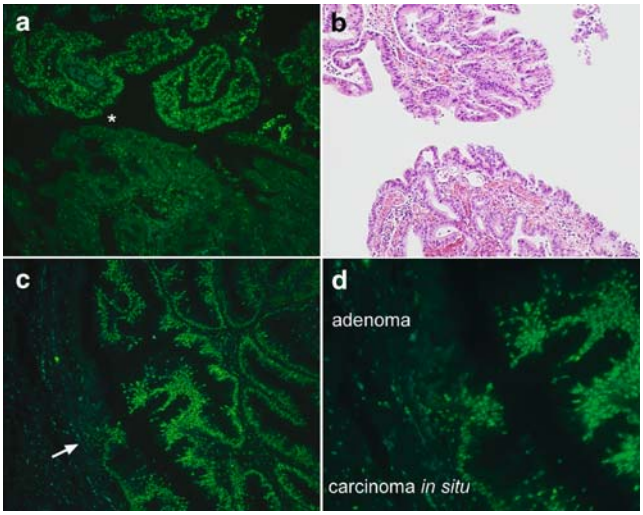

Figure 4 Telomere length heterogeneity in IPMNs of the pancreas. **a** Telomere length heterogeneity is clearly visible in a case with borderline IPMN (Table 2, patient 4). **b** Same region as shown in **a**, stained for H&E. Histologically "uniform" pancreatic epithelia with no visible alterations in cellular appearance (*asterisk*, location marker for comparison with **b**). **c** Transition area between adenoma and carcinoma in situ IPMN (*arrow*). **d** High power image of the transition between adenoma and carcinoma in situ IPMN (Table 2, patient 5). DAPI counterstain; original magnifications, ×100 (**a**, **c**), ×200 (**b**), ×400 (**d**).

cancer.[33,34] Consistent with these findings, we detected up-regulated hTERT expression more often in cases of malignant IPMNs than in borderline IPMNs, but only rarely in adenoma IPMNs, such that hTERT expression increased with progression. Additionally, epithelial cells in 36 of 37 IPMN loci (97.3%) showed marked telomere shortening even at the adenoma stage, the earliest stage of precursor lesion, similar to that observed for PDACs. This result, together with the finding of hTERT expression associated with advanced IPMN stages indicated a time lag between telomere erosion and onset of telomere activation, which could accelerate telomere loss during the early stages of IPMN carcinogenesis. Although activation of telomerase was universally observed in malignant IPMNs, malignant cells showed abnormally short telomeres compared with precursors cells, which suggested that the telomerase activity acquired by malignant cells maintains telomere equilibrium at shortened levels rather than mediating the lengthening of TTAGGG repeats.

The timing of telomeric dysfunction during the IPMN development has not been clearly determined. Meeker et al.[35] reported that telomere abnormalities occurred even at the early stages of the oncogenic process in various types of human cancers, such as prostate, breast, and pancreatic cancers, and revealed dramatic telomere shortening in 97.1% of cases with preinvasive stages of human epithelial carcinogenesis.[34] In the present study, disease progression from borderline to carcinoma in situ IPMNs led to significant telomerase activation, which indicated that telomerase activity may reach a critical point at the carcinoma in situ stage, and could be prominent during latter stages that display significant decreases in telomere length (Fig. 6). Although the level of telomerase activity in borderline IPMNs was confirmed to be significantly lower than that observed in malignant IPMNs by TRAP assay, immunohistochemical analysis revealed up-regulated hTERT expression primarily at the stage of borderline IPMNs and increasing gradually during progression, with the difference between borderline and adenoma IPMNs being not statistically significant. Taken together, our data suggested that borderline IPMNs with short telomeres represent the earliest truly neoplastic lesion in the progression of IPMN carcinogenesis that could serve as the representative form from which the carcinogenesis originated. Once telomerase activation was acquired at the borderline stage IPMN development, it appeared to be sustained through subsequent stages.

Interestingly, abrupt transitions in hTERT expression were observed at junctions between adenoma IPMNs and

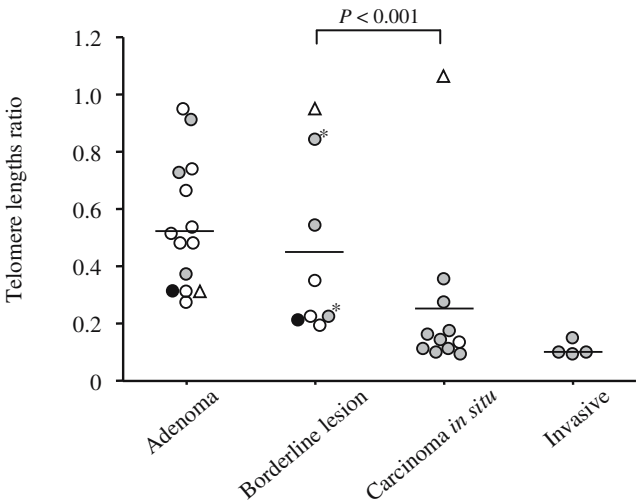

Figure 5 Mean telomere length of IPMN nuclei was measured for each individual sample and expressed as an epithelial/control ratio. For each sample, 10 to 25 nuclei were analyzed. Each mark represents the mean value of an individual sample. Average telomere length ratios decrease with increasing disease progression for each case investigated (r=0.55; P<0.0001). At the carcinoma in situ IPMN stage, no telomere shortening is found, similar to invasive IPMNs (P< 0.001 for carcinoma in situ vs borderline IPMNs). Note that significantly higher telomere lengths ratios are observed in loci with carcinoma in situ IPMNs compared to adenoma IPMNs in the same sample from patient 5 (*triangles*), suggesting the activation of an alternate mechanism for telomere lengthening. Intratumoral heterogeneity is observed in borderline lesion from patient 4 (*asterisk*, marker indicating individual borderline loci in the same patient). *Gray circles* Loci with hTERT expression; *white circles* hTERT-negative loci; *black circles* not examined hTERT expression. The total mean value for each group is shown (*horizontal line*).

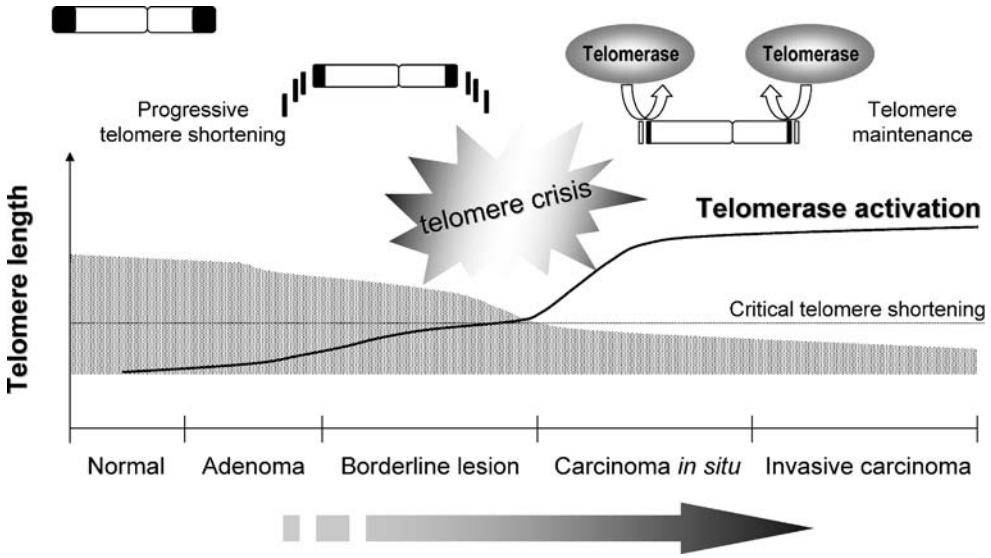

Figure 6 Schematic diagram representing telomere dynamics during the carcinogenesis of intraductal papillary mucinous neoplasms (*IPMNs*) of the pancreas. During development of IPMN lesions, progressive telomere shortening occurs at the adenoma IPMN stage before telomerase activation and further cell division that requires activation of telomerase to maintain telomere length at sufficient levels, supporting the "telomere hypothesis" of human carcinogenesis.[21] With disease progression from borderline to carcinoma in situ IPMNs, telomerase is substantially up-regulated after critical telomere shortening, indicating that transition from borderline to carcinoma in situ IPMNs may be the critical stage at which telomere dysfunction occurs during IPMN carcinognesis.

normal pancreatic ductal cells, with hTERT expression associated with disease progression. The low proportion of adenoma IPMN cells that expressed hTERT but without detectable telomerase activity was probably due to the telomerase levels dropping below the sensitivity of the TRAP assay. In addition, heterogeneous hTERT expression was observed in the histologically "uniform" pancreatic epithelia with no visible alterations in cellular appearance, indicating the presence of histologically undetectable immortal precancerous cells within these lesions. Therefore, we propose that ectopic expression of hTERT in premalignant pancreatic epithelia is indicative of extensive tumor cell proliferation that may precede transformation as a "regional tumorigenesis" event, predisposing that lesion to cancer development.[28] Clinically, up-regulated hTERT expression in premalignant cells would be a useful marker for detecting the subset of patients at risk of carcinoma development, such that lesions positive for hTERT expression would be evaluated aggressively to exclude malignancy.

The role of telomerase in cancer progression seems to be involved in telomere stabilization, preventing telomere exhaustion.[17] However, more recent evidence points to a novel function of *hTERT* in contributing to carcinogenesis independent of telomere-stabilization. Transgenic mice that expressed *TERT* in a broad variety of tissues promoted spontaneous development of mammary intraepithelial neoplasia and invasive mammary carcinomas independent of telomere length.[33] In addition, hairpin siRNA that targeted human telomere RNA rapidly inhibited growth of human cancer cells independently of p53 or telomere length.[36] In the present study, two cases of adenoma showed hTERT expression although telomere length was maintained, which suggested that telomerase may affect proliferation of cells not only by stabilizing telomeres, but also by affecting the expression of growth-controlling genes.[37]

However, we also identified a patient with abnormally long telomeres that expressed undetectable hTERT protein. Nonetheless, significant telomere shortening was still found in adenoma loci obtained from the same patients. This result might suggest the presence of alternative lengthening for telomeres (ALT), an alternate mechanism for telomere extension activated during relatively late phases of IPMN carcinogenesis.[38]

In summary, we studied the temporal sequence of telomere shortening and hTERT expression during development of IPMNs of the pancreas. Our findings suggested that progressive telomere shortening represented an early and prevalent genetic abnormality acquired during IPMNs carcinogenesis and that hTERT expression was up-regulated after substantial losses of telomeric DNA. Notably, transition from borderline to carcinoma in situ IPMNs appeared to be the critical stage at which telomere dysfunction occurred during IPMNs carcinogenesis. Progressive telomere shortening, together with telomerase activation may eventually drive the full transformation of IPMNs.

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References

1. Sohn TA, Yeo CJ, Cameron JL, Hruban RH, Fukushima N, Campbell KA, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. Ann Surg. 2004;239:788–797. (discussion 797–799).
2. Couvelard A, Sauvanet A, Kianmanesh R, Hammel P, Colnot N, Levy P, Ruszniewski P, Bedossa P, Belghiti J. Frozen sectioning of the pancreatic cut surface during resection of intraductal papillary mucinous neoplasms of the pancreas is useful and reliable: a prospective evaluation. Ann Surg. 2005;242:774–778. (discussion 778–780).
3. Klöppel G, Solcia E, Longnecker DS, Capella C, Sobin LH. World Health Organization International Histological Typing of Tumours of the Exocrine Pancreas. Berlin: Springer, 1996.
4. Tanaka M, Chari S, Adsay V, Fernandez-del Castillo C, Falconi M, Shimizu M, Yamaguchi K, Yamao K, Matsuno S. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology. 2006;6:17–32.
5. Murakami Y, Uemura K, Ohge H, Hayashidani Y, Sudo T, Sueda T. Intraductal papillary-mucinous neoplasms and mucinous cystic neoplasms of the pancreas differentiated by ovarian-type stroma. Surgery. 2006;140:448–453.
6. Adsay NV, Longnecker DS, Klimstra DS. Pancreatic tumors with cystic dilatation of the ducts: intraductal papillary mucinous neoplasms and intraductal oncocytic papillary neoplasms. Semin Diagn Pathol. 2000;17:16–30.
7. Tanaka M. Intraductal papillary mucinous neoplasm of the pancreas: diagnosis and treatment. Pancreas. 2004;28:282–288.
8. Uemura K, Hiyama E, Murakami Y, Kanehiro T, Ohge H, Sueda T, Yokoyama T. Comparative analysis of K-ras point mutation, telomerase activity, and p53 overexpression in pancreatic tumours. Oncol Rep. 2003;10:277–283.
9. Sessa F, Solcia E, Capella C, Bonato M, Scarpa A, Zamboni G, Pellegata NS, Ranzani GN, Rickaert F, Klöppel G. Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms: an investigation of tumour cell differentiation and K-ras, p53 and c-erbB-2 abnormalities in 26 patients. Virchows Arch. 1994;425:357–367.
10. Biankin AV, Biankin SA, Kench JG, Morey AL, Lee CS, Head DR, Eckstein RP, Hugh TB, Henshall SM, Sutherland RL. Aberrant p16(INK4A) and DPC4/Smad4 expression in intraductal papillary mucinous tumours of the pancreas is associated with invasive ductal adenocarcinoma. Gut. 2002;50:861–868.
11. Z'Graggen K, Rivera JA, Compton CC, Pins M, Werner J, Fernandez-del Castillo C, Rattner DW, Lewandrowski KB, Rustgi AK, Warshaw AL. Prevalence of activating K-ras mutations in the evolutionary stages of neoplasia in intraductal papillary mucinous tumors of the pancreas. Ann Surg. 1997;226:491–498. (discussion 498–500).
12. Sohn TA, Yeo CJ, Cameron JL, Iacobuzio-Donahue CA, Hruban RH, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an increasingly recognized clinicopathologic entity. Ann Surg. 2001;234:313–321. (discussion 321–322).
13. Sato N, Ueki T, Fukushima N, Iacobuzio-Donahue CA, Yeo CJ, Cameron JL, Hruban RH, Goggins M. Aberrant methylation of CpG islands in intraductal papillary mucinous neoplasms of the pancreas. Gastroenterology. 2002;123:365–372.
14. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. Embo J. 1992;11:1921–1929.
15. Hiyama K, Ishioka S, Shirotani Y, Inai K, Hiyama E, Murakami I, Isobe T, Inamizu T, Yamakido M. Alterations in telomeric repeat length in lung cancer are associated with loss of heterozygosity in p53 and Rb. Oncogene. 1995;10:937–944.
16. Hackett JA, Greider CW. Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. Oncogene. 2002;21:619–626.
17. Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature. 2000;406:641–655.
18. Blackburn EH. Structure and function of telomeres. Nature. 1991;350:569–573.
19. van Steensel B, de Lange T. Control of telomere length by the human telomeric protein TRF1. Nature. 1997;385:740–743.
20. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266:2011–2015.
21. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990;345:458–460.
22. O'Hagan RC, Chang S, Maser RS, Mohan R, Artandi SE, Chin L, DePinho RA. Telomere dysfunction provokes regional amplification and deletion in cancer genomes. Cancer Cell. 2002;2:149–155.
23. Maser RS, DePinho RA. Connecting chromosomes, crisis, and cancer. Science. 2002;297:565–569.
24. Hiyama E, Hiyama K. Clinical utility of telomerase in cancer. Oncogene. 2002;21:643–649.
25. Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR. Telomerase catalytic subunit homologs from fission yeast and human. Science. 1997;277:955–959.
26. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998;279:349–352.
27. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33:787–791.
28. Miyazu YM, Miyazawa T, Hiyama K, Kurimoto N, Iwamoto Y, Matsuura H, Kanoh K, Kohno N, Nishiyama M, Hiyama E. Telomerase expression in noncancerous bronchial epithelia is a possible marker of early development of lung cancer. Cancer Res. 2005;65:9623–9627.
29. Longnecker DS, Adler G, Hruban RH, Klöppel G. Intraductal papillary-mucinous neoplasms of the pancreas. In: Hamilton SR Aaltonen LA, editor. World health organization classification of tumors. Pathology and genetics of tumors of the digestive system. Lyon: IARC Press; 2000. p. 237–241.
30. Hiyama E, Yamaoka H, Matsunaga T, Hayashi Y, Ando H, Suita S, Horie H, Kaneko M, Sasaki F, Hashizume K, Nakagawara A, Ohnuma N, Yokoyama T. High expression of telomerase is an independent prognostic indicator of poor outcome in hepatoblastoma. Br J Cancer. 2004;91:972–979.
31. Hiyama E, Hiyama K, Yokoyama T, Shay JW. Immunohistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. Neoplasia. 2001;3:17–26.
32. Meeker AK, Gage WR, Hicks JL, Simon I, Coffman JR, Platz EA, March GE, De Marzo AM. Telomere length assessment in

human archival tissues: combined telomere fluorescence in situ hybridization and immunostaining. Am J Pathol. 2002;160: 1259–1268.
33. Artandi SE, Alson S, Tietze MK, Sharpless NE, Ye S, Greenberg RA, Castrillon DH, Horner JW, Weiler SR, Carrasco RD, DePinho RA. Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc Natl Acad Sci U S A. 2002;99: 8191–8196.
34. Meeker AK, Hicks JL, Iacobuzio-Donahue CA, Montgomery EA, Westra WH, Chan TY, Ronnett BM, De Marzo AM. Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. Clin Cancer Res. 2004;10:3317–3326.
35. Meeker AK, Hicks JL, Platz EA, March GE, Bennett CJ, Delannoy MJ, De Marzo AM. Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. Cancer Res. 2002;62:6405–6409.
36. Li S, Rosenberg JE, Donjacour AA, Botchkina IL, Hom YK, Cunha GR, Blackburn EH. Rapid inhibition of cancer cell growth induced by lentiviral delivery and expression of mutant-template telomerase RNA and anti-telomerase short-interfering RNA. Cancer Res. 2004;64:4833–4840.
37. Smith LL, Coller HA, Roberts JM. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. Nat Cell Biol. 2003;5:474–479.
38. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nat Med. 1997;3:1271–1274.

Discussion

C. Max Schmidt, M.D. (Indianapolis, IN): Dr. Hashimoto, congratulations on a very elegant study and important contribution to the literature. IPMN is a poorly understood clinical entity and its treatment remains controversial. Recently, IPMN treatment guidelines were published based upon the recommendations of an international consensus conference. The treatment guidelines, as pointed out in presentations at this meeting, are suboptimal. This underscores why studies like yours correlating biomarkers with grade of IPMN dysplasia are so important. I have two questions for you.

Your study was conducted in surgical pathology specimens. Optimally, surgeons would have information about stage of dysplasia before operating on patients to help guide clinical decision-making. How do you see your study findings being applied in the clinical setting? Will telomerase activity be able to be determined reliably by tissue biopsy? How about in pancreatic ductal or cyst fluid?

The management of side branch IPMNs as opposed to main duct IPMNs is more controversial predominantly because the incidence of cancer is considerably less. Have you looked specifically at the subgroup of side branch IPMNs to see if telomerase activity or telomere shortening is effective in that subgroup of patients with IPMN?

Yasushi Hashimoto, M.D. (Hiroshima, Japan): Thank you for your kind comments. The primary goal of our study is to find better diagnostic markers for cancer diagnosis. As you know, telomerase activity or *hTERT* mRNA detections are highly sensitive and reliable biomarkers for cancer diagnosis, but the assays are so complicated that we couldn't apply these assays to clinical usage. Moreover, the assays are limited in its usage for whole tissue analysis, so we cannot detect a small amount of cells or heterogeneous activity due to the sensitivity of these assays. Besides, immunohistochemistry can examine at in situ levels. Immunohistochemistry offers some advantage in terms of labor and cost-time saved. As you know, accuracy of a conventional cytological examination is quite low, so we would like to use this assay to improve diagnostic accuracy. This assay would also be applicable for detecting high risk patients as an adjunct to EUS-FNA or pancreatic juice cytology, perhaps even more reliable than telomerase activity. I'm afraid I do not have enough data to your question whether the assay is feasible, and is not, for pancreatic cystic fluid analysis. The current studies are ongoing prospectively, and I will be able to show you data soon.

For the second question, as I understand your question correctly, you are referring to the difference of hTERT expression between main duct type and branch duct type? With reference to the difference of hTERT expression between main duct type and branch duct type, as you know, branch duct type do have a lower incidence of invasive carcinoma than main duct type, but not by much. We have an incidence of 40% malignant disease in overall IPMNs in the last 70 IPMN patients, but the risk of invasive malignancy of branch duct type is 7%, only one case. From our study, we could not see any difference of the telomerase activity levels between branch duct type and main duct type. The answer to this question is going to need further study.

Michael G. Sarr, M.D. (Rochester, MN): Dr. Hashimoto, what a beautiful presentation, and I would challenge anyone in the audience to give as beautiful a presentation in Japanese as you did in English, okay?

My question is this. Is this a marker of malignancy or are you suggesting that telomerase activity actually causes malignant transformation?

Dr. Hashimoto: Thank you for your kind comments. Your referring to the hTERT expression to be a biomarker?

Dr. Sarr: Yes.

Dr. Hashimoto: In our previous study, telomerase activity in the pancreatic juice is a highly sensitive biomarker. In IPMN, one of the impressive findings of this study relates to the distribution of hTERT expression in the precursor regions. Concerning hTERT-positive adenoma, such lesions appear to be biologically closely related to

borderline or more aggressive lesions rather than to adenomas. Therefore, I suppose hTERT expression precedes transformation in cancer development, so we propose that ectopic hTERT expression in pre-malignant cells is indicative of extensive proliferation, and such lesions are likely to develop invasive disease, which is to say, a promising diagnostic marker including high-risk patients.

Dr. Sarr: And along the therapeutic lines that Max asked, have you looked at intracystic fluid and analyzed telomerase activity or even expression of that protein in the cells in non-resected specimens?

Dr. Hashimoto: The study is ongoing to examine the pancreatic juice and cystic fluid for hTERT analysis preoperatively. I do not have enough data to show you.

L. William Traverso, M.D. (Seattle, WA): Dr. Hashimoto is being sent by the Japanese Society of HBP Surgery to study in this country for 18 months, and I look forward to continued expert activity like you have shown here today. Nice job.As was mentioned, main pancreatic duct IPMN has a high incidence of cancer, and therefore, all those patients should be resected if a surgical candidate. In addition, side branch disease has less chance of cancer, therefore, observation in many of these patients is recommended if they are asymptomatic. Preoperatively, if one takes the pancreatic juice, Dr. Hashimoto, and looks for a telomere crisis, how do we know that in the pancreatic juice that this would be coming from a side branch or a main duct origin?

Dr. Hashimoto: That is a very important question. It is a matter of concern as to where I could pick up these cells, by ERCP or FNA. I do not have enough of that to examine which part of the cells we picked up, and we are now thinking of these methods.

Dr. Traverso: One last question. In reverse logic, it appears that your telomere studies have justified the WHO classification. The borderline WHO type would have no telomere crisis. More than borderline then there is a telomere crisis. With your work we should become more comfortable using the WHO classification. Would you agree?

Dr. Hashimoto: I suppose so. I did not have enough time to present today. As you mentioned, we found the transitions of hTERT expression between borderline lesions and carcinoma in situ, indicating the disease progression. As we presented in this study, some lesions showed heterogeneous expressions in telomere length and hTERT expression in histologically “uniform” appearing ducts, possibly, I think, indicating that histologically undetectable immortal pre-cancerous cells, in turn, biological invasiveness. Once telomerase is activated, it appears to be sustained through subsequent stages.

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Targeting MEK is Effective Chemoprevention of Hepatocellular Carcinoma in TGF-α-Transgenic Mice

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Abstract Hepatocellular carcinoma (HCC) causes >600,000 mortalities per year worldwide. Previous studies from our lab provide evidence for altered mitogen-activated protein kinase and extracellular signal-regulated kinase kinase (MEK) signaling in HCC pathogenesis. We hypothesized that pharmacologic targeting of MEK may prevent HCC. Transforming growth factor-alpha-transgenic mice (CD1-MT42) exposed to diethylnitrosamine were randomized to 20 (trial I) or 35 (trial II) weeks of MEK inhibitor PD0325901 (1, 10 mg/kg) or control via orogastric gavage. Ten HCC (44%) formed in *trial I* controls versus 0 in treatment arms ($p<0.05$). Fourteen HCC (50%) formed in *trial II* controls versus 1 (9%) in treatment arms ($p<0.05$). Mean HCC volume was 578 mm^3 in control versus 46 mm^3 in the single tumor formed in *trial II*. In *trial I*, foci of altered hepatocytes (FAH) formed in 78% of control versus 40% and 0% (1 and 10 mg/kg PD0325901) in treatment arms ($p<0.05$). In *trial II*, incidence of FAH was 80% in control versus 20% and 50% (1 and 10 mg/kg PD0325901) in treatment arms ($p<0.05$). Hepatocyte expression of phosphorylated extracellular signal-regulated kinase dose-dependently decreased in *trial I* but remained the same in *trial II*. Control and treated HCC demonstrated similar proliferation rates, but apoptosis appeared increased with treatment. MEK targeting is effective HCC chemoprevention, perhaps by lowering the apoptotic threshold.

Keywords Hepatocellular carcinoma · Liver cancer · MEK/ERK · PD0325901 · Chemoprevention

The data herein were presented at the Society for Surgery of the Alimentary Tract/Digestive Disease Week, May 19–24, 2007, Washington, DC.

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Introduction

Worldwide, hepatocellular carcinoma (HCC) is the fifth most common solid organ malignancy and the third leading cause of cancer-related death.[1,2] HCC had the highest increase in incidence of any solid organ malignancy in the USA from 1980–2000, and its incidence is predicted to rise with the prevalence of hepatitis C virus infections.[3] Risk factors for HCC include infections with hepatitis B or C viruses (HBV, HCV); exposure to alcohol, tobacco, aflatoxin, oral contraceptives, or fluorinated hydrocarbons; genetic metabolic enzyme deficiencies; bile stasis; obesity; and type II diabetes.[4,5] HCC mortality rates remain unchanged despite advances in chemotherapeutics, surgical techniques, and other minimally invasive procedures because of the tumor's insidious onset and late presentation at diagnosis. The most promising therapeutic option for HCC is orthotopic liver transplantation, but few patients are candidates, and the donor population size remains inadequate.

Transforming growth factor-alpha (TGF-α) is an inflammation-induced cytokine upregulated in liver regeneration after partial hepatectomy and hepatic insult and regulates cellular proliferation, migration, and differentiation.[6,7] TGF-α overexpression alone has been demonstrated to cause HCC tumorigenesis in aged mice,[6] classifying TGF-α as a proto-oncogene.[8] TGF-α is a member of the epidermal growth factor (EGF) mitogen superfamily and binds to the EGF receptor, the upstream intramembrane receptor of the mitogen-activated protein kinase kinase/extracellular regulated kinase (MEK/ERK) pathway, which is upregulated in human HCC.[2,6] Recent reports have implicated the two most common risk factors for HCC tumorigenesis, HBV and HCV, in hepatocyte TGF-α overexpression,[7,9] resulting in increased levels of phosphorylated ERK (P-ERK, activated ERK).

The MEK/ERK pathway is a promising target in HCC chemoprevention, and several small molecule inhibitors of this pathway have been developed. MEK inhibitors, including PD98059, PD184161, PD184352 (CI-1040), and PD0325901 inhibit MEK1/2 by binding to an ATP-independent binding site. PD0325901 is a derivative of the first MEK inhibitor to demonstrate in vivo tumor growth inhibition (CI-1040) and has demonstrated in vitro effectiveness in melanoma cell lines harboring mutations of upstream ERK effectors.[10,11]

In the present study, we hypothesized that short- and long-term inhibition with the novel MEK inhibitor PD0325901 would demonstrate chemopreventative effects on HCC and/or precancerous foci of altered hepatocytes (FAH) in an HCC developmental animal model employing TGF-α-transgenic mice. We demonstrate that PD0325901 has chemopreventative effects on gross HCC and microscopic FAH formation, possibly by increasing intra-tumor apoptosis rates.

Materials and Methods

Animal Model

A TGF-α-transgenic mouse model of HCC has been previously characterized in the literature.[12] Briefly, a vector composed of the human TGF-α gene and a metallothionein promoter was transfected into CD1 mice, creating the TGF-α-transgenic (MT42) mouse. Without co-carcinogens, HCC develops in 65% of mice age 10–15 months. With single-dose DEN injection (5 mg/kg body weight) at age 15 days, carcinogenesis can be accelerated to as early as age 23 weeks.[13]

Developmental Protocol

Forty-two male TGF-α-transgenic mice were bred and housed at a maximum of three per cage in American Association for Accreditation for Laboratory Animal Care (AAALAC)-approved facilities, and animal research and handling were in strict conformance with federal Institutional Animal Care and Use Committee (IACUC) guidelines. At age 14 days, mice were weaned and fed a basal diet of mouse chow. At age 15 days, animals received an intraperitoneal injection (5 mg/kg body weight) of the carcinogen diethylnitrosamine (DEN). At age 10 weeks, animals were randomized to trial I (20 weeks, 20 mice) or trial II (35 weeks, 22 mice). Within each trial, animals were randomized to vehicle (hydroxymethylethylcellulose) or MEK inhibitor PD0325901 (1 or 10 mg/kg body weight suspended in vehicle with sonication, kindly provided by Pfizer Global Research and Development) administered via orogastric lavage once daily. After 20 or 35 weeks on protocol (ages 30 or 45 weeks, respectively), animals were sacrificed via lethal intraperitoneal injection of pentobarbital, and livers were harvested. At sacrifice, livers were inspected for tumor formation, and tumor size was assessed using calipers measuring to the nearest 0.1 mm. For each animal, three representative liver sections from different lobes were fixed in 10% formalin (Sigma, St. Louis, MO, USA). After 48 h, fixed tissues were transferred to 70% ethanol, paraffin-embedded, and serially cut (5 μm).

Tissue Staining

Liver tissue slides were stained with hematoxylin and eosin (H & E) according to standard protocol. Liver tissue slides were immunostained for Ki-67 (rabbit anti-human Ki-67 IgG, LabVision, Fremont, CA, USA) and P-ERK (mouse anti-human IgG, Santa Cruz Biotechnology, Santa Cruz, CA, USA) as follows. Slides were deparaffinized and hydrated under running water. Slides were placed in antigen retrieval citrate, pH 6.0 (Dako North America, Carpinteria, CA, USA), in a pressure cooker for 15 min, 3% hydrogen

peroxide for 10 min, and Protein Block (Dako North America) for 15 min. Slides were then incubated in either P-ERK antibody (1:500, 60 min) or Ki-67 antibody (1:200, 30 min). Slides were incubated in mouse (P-ERK) or rabbit (Ki-67) envision polymer for 30 min (Dako North America), after which, slides were counterstained and dehydrated. Liver tissue slides were stained for DNA fragmentation using ApopTag® Peroxidase In Situ Apoptosis Detection Kit (Millipore, Billerica, MA, USA) per manufacturer's protocol.

Lesion Analysis

Fixed liver tissues were microscopically inspected (Micromaster Microscope, Fisher Scientific, Waltham, MA, USA) for foci of altered hepatocytes (FAH), adenoma, and HCC using the established descriptions of Frith and Ward.[14] FAH were subtyped as eosinophilic (increased hepatocyte size with granular cytoplasm), basophilic (decreased hepatocyte size without granular cytoplasm), vacuolated (hepatocytes with vacuoles of varying sizes and nuclei located peripherally), or clear cell ("lacy" or "ground glass" cytoplasm suggestive of glycogen and nuclei located centrally).

FAH, adenoma, and HCC previously identified on H & E-stained slides were located on Ki-67-stained slides. Lesion-specific proliferative rates were assessed by counting the positively staining nuclei per high-power field [×200 magnification, 1 high-power field (HPF)/lesion], and a percentage was calculated (positive nuclei/total nuclei, ×100). P-ERK and ApopTag®-stained slides were analyzed in similar fashion. Non-lesion (normal) tissues were analyzed with counts from three randomly selected fields of hepatocytes per animal.

Biostatistical Analysis

Biostatistical data analyses were performed using Prism 3.02 software (Graphpad, San Diego, CA, USA). Data were analyzed using Fisher's exact test or Student's *t* test at the 95% confidence interval.

Results

Drug Toxicity and Animal Deaths

We observed no drug-induced toxicity or treatment-related mortality during this study. Furthermore, there were no differences in animal weights among treatment groups. We observed one death in the short-term trial (placebo) and five deaths in the long-term trial (four in 1 mg/kg PD0325901, one in 10 mg/kg PD0325901). We attribute these deaths to traumatic orogastric lavage and/or the known frail constitution of these homozygous transgenic mice. The systemic effects of PD0325901 have been extensively characterized, with toxicity at doses over 100 mg/kg body weight and death at doses over 300 mg/kg body weight.[15]

P-ERK and Ki-67 in Progressive Hepatocyte Dysplasia

Figure 1 depicts P-ERK and Ki-67 expression in progressively dysplastic hepatocellular lesions. In controls, mice at age 30 weeks (*trial I*) demonstrated an eightfold increase in P-ERK expression within HCC hepatocytes compared to "normal" hepatocytes. At age 45 weeks (*trial II*), P-ERK expression within HCC hepatocytes was elevated 4.5-fold compared to normal hepatocytes. Adenoma had P-ERK expression increased 3.5-fold compared with normal hepatocytes in mice age 30 weeks, and by age 45 weeks, adenoma P-ERK expression approached normal levels. FAH P-ERK expression levels were similar to those of

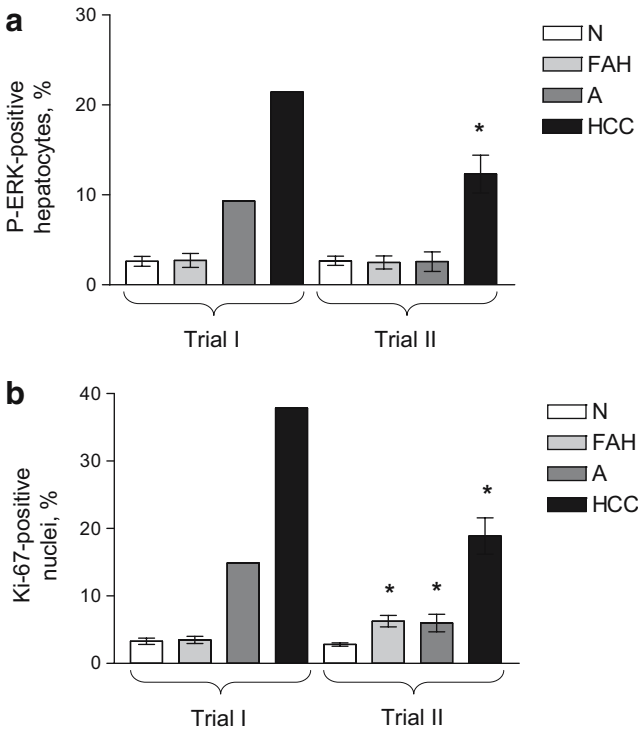

Figure 1 P-ERK and Ki-67 in progressive hepatocyte dysplasia. *Columns* represent the average counts of all identifiable untreated lesions (*FAH* foci of altered hepatocytes, *A* adenoma, *HCC* hepatocellular carcinoma) or three randomly selected fields of normal hepatocytes (*N*) per animal for trials I and II. *Error bars* represent the standard error of the mean (SEM). **a** *P-ERK immunohistochemistry* demonstrated increasing hepatocyte expression in progressively dysplastic lesions, particularly in HCC in trials I and II. n=24, 8, 1, and 1 for N, FAH, A, and HCC in trial I, and n=24, 10, 6, and 6 for N, FAH, A, and HCC in trial II. **b** Ki-67 immunohistochemistry demonstrated increased proliferation rates in progressively dysplastic lesions similar to P-ERK expression. *p<0.05 compared to normal hepatocytes. n=24, 12, 1, and 1 for N, FAH, A, and HCC in trial I, and n=24, 9, 9, and 6 for N, FAH, A, and HCC in trial II.

normal hepatocytes at ages 30 and 45 weeks. Ki-67 expression within progressively dysplastic hepatocellular lesions mirrored the trends observed with P-ERK staining.

PD0325901 Biologic Activity

P-ERK immunohistochemistry of normal hepatocytes on short-term (*trial I*) MEK inhibition with PD0325901 demonstrated a dose-dependent P-ERK decrease in treatment arms compared to control animals ($p<0.05$). P-ERK suppression, however, was not observed in treatment arms receiving long-term (*trial II*) MEK inhibition (Fig. 2).

Gross Findings

In *trial I*, gross tumor incidence was 44% (4/9) in the control arm compared to 0 and 0% in the treatment arms (0 of 5 and 0 of 5; $p<0.05$, combined treatment arms versus control). Overall, there were ten HCC in the controls compared to 0 in the combined treatment arms (Fig. 3a). Control tumor multiplicity was 1.1±0.5 tumors/animal compared to zero tumors per animal in the treatment arms ($p<0.05$, combined treatment arms versus control; Fig. 3b). Mean volume of control tumors was 163±145 mm^3 (Fig. 3c). In *trial II,* gross tumor incidence was 50% (5 of 10) in the control arm compared to 9% in treatment arms ($p<0.05$, combined treatment groups compared to control). Overall, there were 14 HCC in control animals, compared to 0 in the 1 mg/kg arm and 1 in the 10 mg/kg arm (Fig. 3d). Control tumor multiplicity was 1.4±0.5 tumors per animal compared to 0 and 0.17±0.17 tumors per animal in 1 and 10 mg/kg arms ($p<0.05$, combined treatment groups compared to control; Fig. 3e). The mean volume of *trial II* control tumors was 578±309 mm^3 compared to a single tumor volume of 46 mm^3 on 10 mg/kg PD0325901 (Fig. 3f). Histopathologic analysis confirmed gross tumor findings.

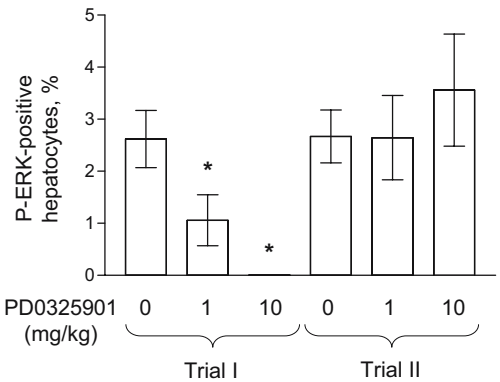

Figure 2 PD0325901 biologic activity. P-ERK-positive normal hepatocyte counts were used to confirm biologic activity of PD0325901. *Columns* represent the average counts of three randomly selected fields of normal hepatocytes per animal for *trials I* and *II*. *Error bars* represent the standard error of the mean (*SEM*). In *trial I*, P-ERK-positive normal hepatocytes demonstrated a dose-dependent decrease in expression ($p<0.05$) with PD0325901; however, P-ERK suppression was not observed in normal hepatocytes in *trial II*. *$p<0.05$ compared to normal hepatocytes. $n=27$, 15, and 15 for 0, 1, and 10 mg/kg PD0325901 in *trial I*, and $n=24$, 6, and 9 for 0, 1, and 10 mg/kg PD0325901 in *trial II*.

Proliferation and Apoptosis within HCC

Analysis of proliferation and apoptosis in control and treatment arm tumors was limited by the profound absence of tumors in treated animals. In the single HCC which formed on long-term 10 mg/kg PD0325901, the proliferation rate (Ki-67 staining) was 17.9%, similar to the average proliferation rate of 18.9±2.7% in the control HCCs. The apoptosis rate (ApopTag® staining) of control HCCs was 0.4±0.04%, whereas in the 10 mg/kg arm, the HCC apoptosis rate was 95%.

Foci of Altered Hepatocytes Analysis

In rodents, foci of altered hepatocytes represent an early stage of neoplastic development, and FAHs are used frequently as endpoints in carcinogenicity testing and as indicators of chemoprevention.[16] In *trial I*, FAH incidence dose-dependently decreased from 78% (7 of 9) in the control arm to 40% (2 of 5) and 0% (0 of 5) in 1 and 10 mg/kg arms, respectively ($p<0.05$, combined treatment groups compared to control; Fig. 4a). Control FAH multiplicity was 3.6±1.6 lesions per animal compared with 2.6±2.1 and 0 lesions per animal in 1 and 10 mg/kg arms, respectively (Fig. 4b). In *trial II*, FAH incidence was 80% (8 of 10) in the control arm compared to 20% (1 of 5) and 50% (3 of 6) in 1 and 10 mg/kg PD dose groups ($p<0.05$, combined treatment groups compared to control; Fig. 4a). Control FAH multiplicity was 1.7±0.4 lesions per animal compared to 0.2±0.2 and 1.2±0.6 in 1 and 10 mg/kg arms ($p<0.05$, combined treatment groups compared to control; Fig. 4b).

FAHs have been classified into four histologic subtypes (eosinophilic, basophilic, vacuolated, clear cell), with basophilic foci having the greatest proliferation rates and closest association with HCC.[17,18] We analyzed the effect of PD0325901 on FAH histologic subtype prevalence. Only two animals in the treated arms of *trial I* had FAH; thus, we only analyzed FAH in *trial II*. In *trial II*, the control arm FAH histologic subtypes were 77.5% basophilic (11 of 15) and 22.3% eosinophilic (4 of 15) compared to 37.5% basophilic (3 of 8) and 62.5% eosinophilic (5 of 8) in the treatment arms.

Discussion

HCC is one of the deadliest and most prevalent cancers worldwide because of its difficulty to detect and treat. In

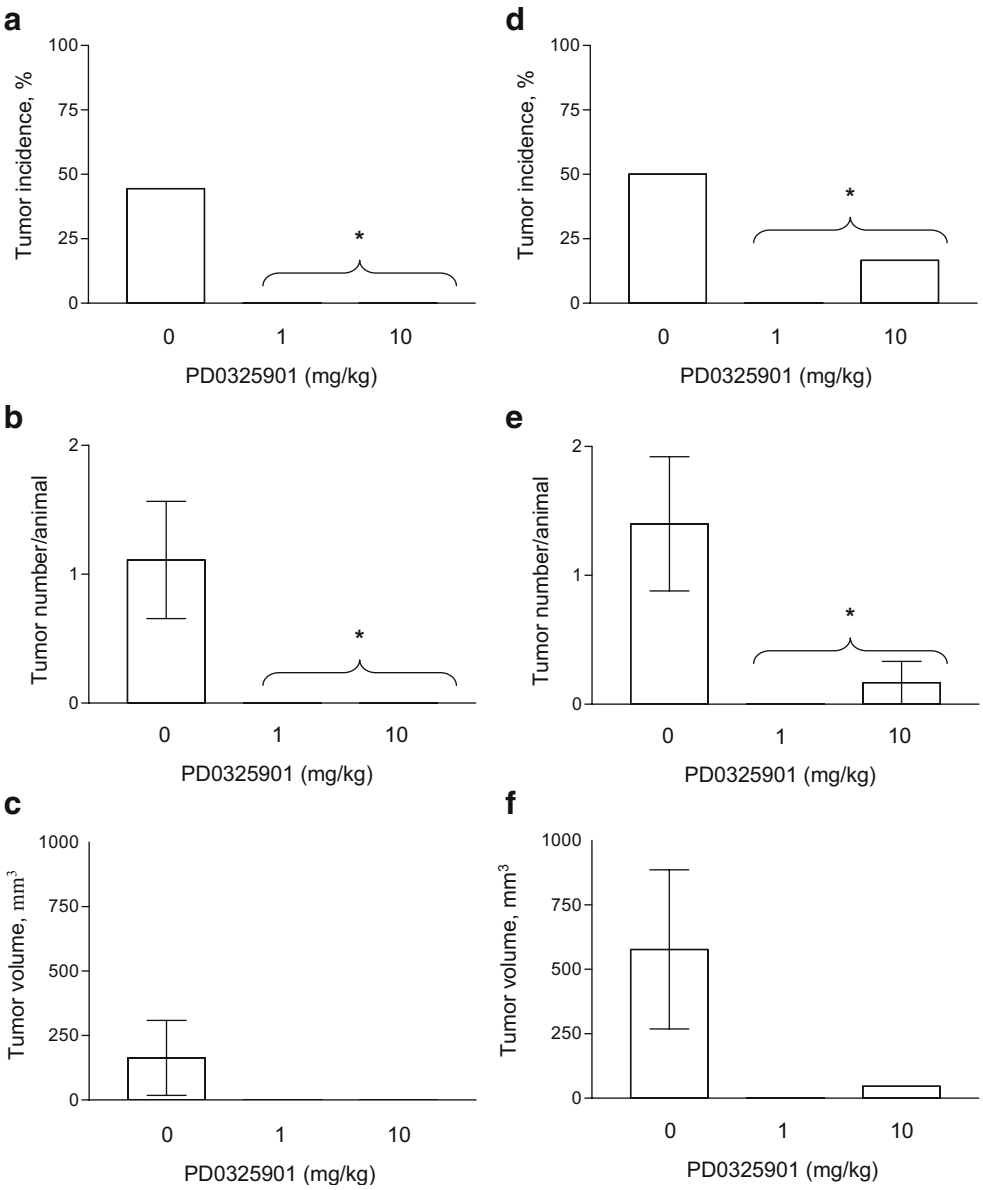

Figure 3 Gross tumor data. *Columns* represent the incidence (**a** and **d**), multiplicity (**b** and **e**), and volume (**c** and **f**) of tumors, and *error bars* represent the SEM (**b**, **c**, **e**, **f**). *$p<0.05$ combined treatment arms compared to control. **a** Trial I gross tumor incidence. $n=9$, 5, and 5 animals per group for 0, 1, and 10 mg/kg PD0325901. **b** Trial I tumor multiplicity. $n=10$, 0, and 0 tumors per group for 0, 1, and 10 mg/kg PD0325901. **c** Trial I average tumor volume. $n=10$, 0, and 0 tumors per group for 0, 1, and 10 mg/kg PD0325901. **d** Trial II gross tumor incidence. $n=10$, 5, and 6 animals per group for 0, 1, and 10 mg/kg PD0325901. **e** Trial II tumor multiplicity. $n=14$, 0, and 1 tumors per group for 0, 1, and 10 mg/kg PD0325901. **f** Trial II average tumor volume. $n=13$ and 1 tumors per group for 0 and 10 mg/kg PD0325901.

the developing world, aflatoxin-contaminated foods and hepatitis can be unavoidable risk factors, whereas in the USA, HCC prevalence is expected to escalate with the HCV epidemic. Thus, it is prudent to examine potential chemopreventative measures for high-risk populations through targeted drug therapy. The MEK/ERK pathway is upregulated in the majority of human HCC tumors and represents a potential chemoprevention target.

Clinically, primary prevention of human HCC has been through public health efforts to minimize risk factors, namely HBV infection, with universal childhood HBV vaccination and heightened blood donor screening. Since its inception, HBV vaccination has markedly reduced the childhood infection rates in Taiwan, Gambia, Malaysia, South Africa, and the USA, and childhood mortality from HCC in Taiwan has decreased 60–70%.[19] Secondary HCC prevention or prevention of recurrence after orthotopic liver transplantation has focused on HBV and HCV antiviral therapies (interferons, lamivudine), with success in select patient populations.[20–22]

Relatively few studies of primary HCC chemoprevention in experimental animal models exist. Chemopreventative agents have included retinoids, tamoxifen, and COX-2 inhibitors. The use of retinoids as HCC chemoprevention has yielded contradictory results, with one study demonstrating marked decreases in HCC formation, whereas another study demonstrated increased numbers of HCC and the development of more clinically aggressive angiosarcomas in some cases.[23,24] Tamoxifen has been investigated in clinical trials as HCC chemotherapy with minimal

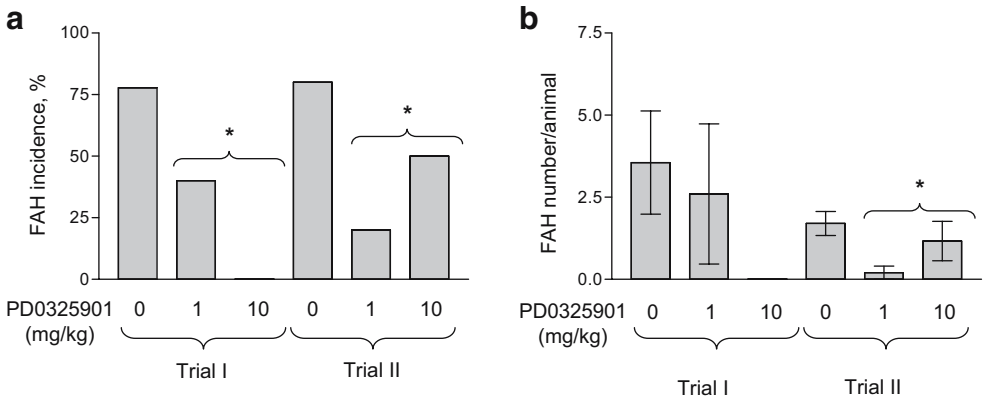

Figure 4 FAH analysis. **a** FAH incidence in trials I and II. *$p<0.05$ combined treatment arms compared to the control. $n=9$, 5, and 5 animals per group for 0, 1, and 10 mg/kg PD0325901 in trial I, and $n=10$, 5, and 6 animals per group for 0, 1, and 10 mg/kg PD0325901 in trial II. **b** FAH multiplicity in trials I and II. $n=32$, 13, and 0 FAH/group for 0, 1, and 10 mg/kg PD0325901 in trial I, and $n=17$, 1, and 7 FAH/group for 0, 1, and 10 mg/kg PD0325901 in trial II. *$p<0.05$ treatment arms compared to control.

success,[25,26] but a single study investigating its chemopreventative potential has demonstrated promising results.[24] COX-2 inhibitors are potential chemopreventative agents, given the inflammatory basis of most cases of HCC, and one *in vivo* study of racemic etodolac's chemopreventative effects in a mouse model of fatty liver demonstrated decreased HCC formation in low- and high-dose treatment groups.[27]

In this study, we examined the chemopreventative effects of the novel MEK inhibitor PD0325901 in an HCC developmental animal model overexpressing TGF-α. P-ERK expression and proliferation rates increased in progressively dysplastic lesions in controls, supporting MEK as a plausible chemoprevention target. Dose-dependent decreases in P-ERK with short-term treatment demonstrate that PD0325901 is biologically active in mouse liver. Relatively low-dose (1, 10 mg/kg) PD0325901 was effective HCC chemoprevention in TGF-α-transgenic mice in short- and long-term trials. Additionally, precancerous FAH were decreased in the treatment arms of both trials compared to the control, and with long-term PD0325901, there was a shift away from highly transformed basophilic FAH to less-transformed subtypes. FAH have been observed in human livers and in animal models of HCC, with strong inter-species similarities regardless of the inciting agent,[28] and are closely linked to cirrhosis and HCC.[29] Thus, this information may provide further support of the chemopreventative role of MEK inhibition in HCC.

Our lab has further studied the effects of PD0325901 in a nude mouse xenograft model employing Hep3B flank tumors. This model differs from the TGF-α-transgenic model in that TGF-α is not constitutively overexpressed, and the Hep3B cell line is human-derived and is infected with the hepatitis B virus. We observed significantly decreased tumor growth rates with 5 weeks of orogastric lavage with PD0325901 in low- and high-dose treatment groups. These data will be published in a separate report and further support the efficacy of PD0325901.

ERK has a number of well-characterized effector proteins, including c-myc and beta-catenin. We did not study the effects of MEK inhibition on these downstream proteins; however, other groups have elegantly analyzed c-myc and beta-catenin in hepatocellular carcinogenesis. C-myc is a proto-oncogene upregulated in many human tumors, including HCC.[30] C-myc is phosphorylated, activated, and stabilized by P-ERK. Co-expression of c-myc and TGF-α has been frequently observed in human HCC, and in double-transgenic mice, co-expression synergistically accelerated HCC formation and decreased survival.[31]

Aberrant expression and mutations of beta-catenin can be observed in a subset of human and animal HCCs.[32] This protein is critical for embryonic development and is involved in cell adhesion, anti-apoptotic, and proliferative processes. In studies of tumors of c-myc/TGF-α double-transgenic mice treated with phenobarbital, nuclear beta-catenin was localized to cells forming the invasion front. Beta-catenin-positive tumors showed increased proliferation and tumor sizes compared to beta-catenin-negative tumors, whereas apoptosis rates were unchanged.[32] Additionally, others have demonstrated that the HBX protein from the hepatitis B virus causes ERK phosphorylation and subsequent upregulation of beta-catenin through inactivation of its degradation pathways.[33] In vitro studies employing anti-fibroblast growth factor antibodies demonstrated inhibition of ERK and beta-catenin.[34] In summary, the intracellular signaling in HCC shows complexity and redundancy, which suggests that multi-targeted chemotherapy may be an optimal therapeutic strategy for patients.

Conclusion

In this study, we show that P-ERK dose-dependently decreased in short-term PD0325901 treatment groups compared to the control. Suppression of P-ERK in long-term treatment groups was not observed. Short-term MEK inhibition with PD0325901 resulted in a complete absence of HCC tumor formation in low- and high-dose treatment groups. Similarly, short-term MEK inhibition resulted in decreased FAH formation compared to the control. Long-term MEK inhibition with PD0325901 resulted in near-complete absence of tumors, with a single small tumor forming in the high-dose treatment group. Furthermore, FAH formation was decreased in treated animals compared to the control. With high-dose, long-term treatment, FAH subtypes shifted away from highly transformed basophilic foci to less-transformed types. Tumor proliferation rates were similar between the control and the long-term high-dose PD0325901, whereas tumor apoptosis was markedly increased with long-term high-dose drug compared to the control. Thus, in this study, both short- and long-term MEK inhibition with PD0325901 significantly abrogates tumor and FAH formation, possibly by lowering the apoptosis threshold, and in longer-term treatment, there appears to be a relative loss of sensitivity to drug.

To the best of our knowledge, this is the first HCC chemoprevention study targeting the MEK/ERK pathway in a developmental animal model. The chemopreventative benefits of PD0325901 demonstrated in this study may prompt investigation of this drug in human HCC chemoprevention.

Acknowledgment The authors are grateful to Lee Ann Baldridge for her immunostaining assistance.

Discussion Staveley-O'Carroll, M.D. (Hershey, PA): I want to congratulate you on an excellent study and a very nice presentation. In particular, I am impressed with the model that you have chosen to test the efficacy of this drug. I mean, arguably, hepatocellular cancer is the most deadly cancer worldwide, but it occurs in a population that is easily identified, the cirrhotic, and they might be amenable to this kind of chemopreventive strategy. I have a couple of questions for you.

I was surprised to see that phospho-ERK did not go down in your long-term trials, although you showed efficacy of your drug long-term. Do you have any explanations for that?

Sabrina C. Wentz, M.D. (Indianapolis, IN): We think there may be a loss of sensitivity with long-term administration, as evidenced by one tumor forming with long-term treatment. We have used this drug in some human HCC cell lines, and we have seen a similar loss of sensitivity with long-term administration.

Dr. Staveley-O'Carroll: So you have looked in other models, human cell lines being transferred into nude mice?

Dr. Wentz: We have looked at cell lines *in vitro*, and we have also looked at the Hep3B cell line in a nude mouse, and we have also seen a regression of tumor size with administration of PD0325901.

Dr. Staveley-O'Carroll: Is this drug being tested in humans in any phase I or II clinical trials in hepatocellular cancer or any other cancers?

Dr. Wentz: It has been studied in phase I trials, and there is not much toxicity. As far as phase II trials, it is being studied for melanoma, which has upregulation of the MEK–ERK pathway in these cases. As far as HCC, not yet. Our institution is working hard to get a phase II trial started, but it has not happened quite yet.

Dr. Staveley-O'Carroll: Thank you very much.

Roderich E. Schwarz, M.D. (Dallas, TX): I much enjoyed your presentation and your model as well, but your model, I think, is precisely focused on TGF-α. So, the question is in the human correlate, as Dr. Stavely-O'Carrol has mentioned, that is hepatitis B and hepatitis C driven, which role does TGF-α play in the early carcinogenesis mechanism versus the later progression of cancer mechanism? Because most of the viral implications rely on dysfunction of p53 and other oncogenic signaling events, whereas other growth promoting factors may only start to be overexpressed in an established invasive hepatocellular cancer and then lead to quicker growth of the established cancer. So, in other words, can you use an approach like this to prevent cancer, or would you speculate that TGF-α is possibly a later factor that gets overexpressed and you can in fact use it for treatment?

Dr. Wentz: I cannot comment on TGF-α and its role in early versus late disease progression, but as I mentioned earlier, we looked at this in the Hep3B cell line, which does have the hepatitis B virus, and we found that the drug was effective there. We know that in hepatitis B and hepatitis C, the MEK–ERK pathway is upregulated. So, the drug would possibly work in those patients.

Dr. Schwarz: And the frequency of the TGF-α overexpression or the MEK and ERK pathway activation is a common one, or is it seen in a subset of the human hepatocellular cancer?

Dr. Wentz: In human hepatocellular carcinoma, the MEK–ERK pathway is upregulated in approximately 80% of the cases. For TGF-α, it is a similar percentage.

References

1. Marrero JA. Hepatocellular carcinoma. Curr Opin Gastroenterol 2005;21:308–312.
2. Schmidt CM, McKillop IH, Cahill PA, Sitzmann JV. Increased MAPK expression and activity in primary human hepatocellular carcinoma. Biochem Biophys Res Commun 1997;236:54–58.
3. Lopez LJ, Marrero JA. Hepatocellular carcinoma. Curr Opin Gastroenterol 2004;20:248–253.
4. Marrero JA. Hepatocellular carcinoma. Curr Opin Gastroenterol 2006;22:248–253.
5. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004;127:S5–S16.
6. Sandgren EP, Luetteke NC, Qiu TH, Palmiter RD, Brinster RL, Lee DC. Transforming growth factor alpha dramatically enhances oncogene-induced carcinogenesis in transgenic mouse pancreas and liver. Mol Cell Biol 1993;13:320–330.
7. Jakubczak JL, Chisari FV, Merlino G. Synergy between transforming growth factor alpha and hepatitis B virus surface antigen in hepatocellular proliferation and carcinogenesis. Cancer Res 1997;57:3606–3611.
8. Pahlavan PS, Feldmann RE Jr, Zavos C, Kountouras J. Prometheus' challenge: molecular, cellular and systemic aspects of liver regeneration. J Surg Res 2006;134:238–251.
9. Sato Y, Kato J, Takimoto R, Takada K, Kawano Y, Miyanishi K, Kobune M, Sato Y, Takayama T, Matunaga T, Niitsu Y. Hepatitis C virus core protein promotes proliferation of human hepatoma cells through enhancement of transforming growth factor alpha expression via activation of nuclear factor-kappaB. Gut 2006;55:1801–1808.

10. Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. Nat Rev Cancer 2004;4:937–947.
11. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N. BRAF mutation predicts sensitivity to MEK inhibition. Nature 2006;439:358–362.
12. Lee GH, Merlino G, Fausto N. Development of liver tumors in transforming growth factor alpha transgenic mice. Cancer Res 1992;52:5162–5170.
13. Tamano S, Merlino GT, Ward JM. Rapid development of hepatic tumors in transforming growth factor alpha transgenic mice associated with increased cell proliferation in precancerous hepatocellular lesions initiated by *N*-nitrosodiethylamine and promoted by phenobarbital. Carcinogenesis 1994;15:1791–1798.
14. Frith CH, Ward JM. A morphologic classification of proliferative and neoplastic hepatic lesions in mice. J Environ Pathol Toxicol 1979;3:329–251.
15. Brown AP, Carlson TC, Loi CM, Graziano MJ. Pharmacodynamic and toxicokinetic evaluation of the novel MEK inhibitor, PD0325901, in the rat following oral and intravenous administration. Cancer Chemother Pharmacol 2007;59:671–679.
16. Bannasch P, Nehrbass D, Kopp-Schneider A. Significance of hepatic preneoplasia for cancer chemoprevention. IARC Sci Publ 2001;154:223–240.
17. Bannasch P, Enzmann H, Klimek F, Weber E, Zerban H. Significance of sequential cellular changes inside and outside foci of altered hepatocytes during hepatocarcinogenesis. Toxicol Pathol 1989;17:617–628 (discussion 629).
18. Tiniakos DG, Brunt EM. Proliferating cell nuclear antigen and Ki-67 labeling in hepatocellular nodules: a comparative study. Liver 1999;19:58–68.
19. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiol Rev 2006;28:112–125.
20. Mazzaferro V, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, Capussotti L, Calise F, Pellicci R, Belli G, Tagger A, Colombo M, Bonino F, Majno P, Llovet JM. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. Hepatology 2006;44:1543–1554.
21. Colombo M, Donato MF. Prevention of hepatocellular carcinoma. Semin Liver Dis 2005;25:155–161.
22. Hino K, Okita K. Interferon therapy as chemoprevention of hepatocarcinogenesis in patients with chronic hepatitis C. J Antimicrob Chemother 2004;53:19–22.
23. Sano T, Kagawa M, Okuno M, Ishibashi N, Hashimoto M, Yamamoto M, Suzuki R, Kohno H, Matsushima-Nishiwaki R, Takano Y, Tsurumi H, Kojima S, Friedman SL, Moriwaki H, Tanaka T. Prevention of rat hepatocarcinogenesis by acyclic retinoid is accompanied by reduction in emergence of both TGF-alpha-expressing oval-like cells and activated hepatic stellate cells. Nutr Cancer 2005;51:197–206.
24. Di Bisceglie AM, Osmack P, Brunt EM. Chemoprevention of hepatocellular carcinoma: use of tamoxifen in an animal model of hepatocarcinogenesis. J Lab Clin Med 2005;145:134–138.
25. Gallo C, De Maio E, Di Maio M, Signoriello G, Daniele B, Pignata S, Annunziata A, Perrone F. Tamoxifen is not effective in good prognosis patients with hepatocellular carcinoma. BMC Cancer 2006;6:196.
26. Nowak AK, Stockler MR, Chow PK, Findlay M. Use of tamoxifen in advanced-stage hepatocellular carcinoma. A systematic review. Cancer 2005;103:1408–1414.
27. Liu W, Nakamura H, Tsujimura T, Cheng J, Yamamoto T, Iwamoto Y, Imanishi H, Shimomura S, Yamamoto T, Hirasawa T, Inagaki S, Nishiguchi S, Hada T. Chemoprevention of spontaneous development of hepatocellular carcinomas in fatty liver Shionogi mice by a cyclooxygenase-2 inhibitor. Cancer Sci 2006;97:768–773.
28. Bannasch P, Haertel T, Su Q. Significance of hepatic preneoplasia in risk identification and early detection of neoplasia. Toxicol Pathol 2003;31:134–139.
29. Su Q, Benner A, Hofmann WJ, Otto G, Pichlmayr R, Bannasch P. Human hepatic preneoplasia: phenotypes and proliferation kinetics of foci and nodules of altered hepatocytes and their relationship to liver cell dysplasia. Virchows Arch 1997;431:391–406.
30. Marampon F, Ciccarelli C, Zani BM. Down-regulation of c-Myc following MEK/ERK inhibition halts the expression of malignant phenotype in rhabdomyosarcoma and in non muscle-derived human tumors. Mol Cancer 2006;5:31.
31. Calvisi DF, Thorgeirsson SS. Molecular mechanisms of hepatocarcinogenesis in transgenic mouse models of liver cancer. Toxicol Pathol 2005;33:181–184.
32. Calvisi DF, Factor VM, Ladu S, Conner EA, Thorgeirsson SS. Disruption of beta-catenin pathway or genomic instability define two distinct categories of liver cancer in transgenic mice. Gastroenterology 2004;126:1374–1386.
33. Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, Bartholomeusz G, Li Y, Pan Y, Li Z, Bargou RC, Qin J, Lai CC, Tsai FJ, Tsai CH, Hung MC. Erk associates with and primes GSK-3beta for its inactivation resulting in upregulation of beta-catenin. Mol Cell 2005;19:159–170.
34. Desnoyers LR, Pai R, Ferrando RE, Hotzel K, Le T, Ross J, Carano R, D'Souza A, Qing J, Mohtashemi I, Ashkenazi A, French DM. Targeting FGF19 inhibits tumor growth in colon cancer xenograft and FGF19 transgenic hepatocellular carcinoma models. Oncogene 2007 (in press).

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Complete Endoscopic Closure of Gastric Defects Using a Full-Thickness Tissue Plicating Device

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Abstract Obtaining endolumenal closure of hollow visceral defects may complement conventional, incision-based, surgical alternatives and benefit the experimental field of natural orifice translumenal endoscopic surgery (NOTES). Endoscopic tissue plicating devices (TPD) represent a promising closure technology; however, the long-term integrity of resultant closures is uncertain. Swine (n=10) underwent survival transgastric NOTES peritoneoscopy procedures with TPD gastrotomy closure while device performance and closure integrity were evaluated. Following uncomplicated procedures, no animals revealed leakage on upper gastrointestinal contrast fluoroscopy immediately following closure and on postoperative days 2 and 7. Necropsy performed on the 14th postoperative day revealed a subclinical colonic injury for one animal; the remaining nine animals had no complications. Gastric burst testing revealed the strength of closure was comparable to that of nonsurgical control stomachs (85.1 vs. 85.3 mm Hg, p=0.98). For six of nine (66%) TPD animals, bursting occurred remote to the closure site in nonsurgical tissue, indicating that closure strength equaled that of native tissue. Endoscopic TPD closure of standardized NOTES gastric defects results in strong, leak-proof closure; however, injuries can occur. These findings support evaluation of TPD closure in human trials involving noncontrolled gastric defects.

Keywords Endoscopy · Minimally invasive surgery · Stomach · NOTES

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Introduction

Obtaining endolumenal closure of hollow visceral defects may be an attractive alternative to conventional, incision-based surgery. Perhaps the largest beneficiary of an endolumenal defect closure technique may be natural orifice translumenal endoscopic surgery (NOTES). NOTES is a unique surgical platform promising abdominal surgical access without external skin incisions by allowing transmural passage of flexible endoscopes through hollow viscera into the peritoneal cavity.[1] NOTES has largely been relegated to experimental practice for lack of reliable endoscopic devices to provide closure at the transvisceral point of peritoneal access.[2]

The current standard of care dictates that anastomotic leaks, gastrogastric fistulas, and gastroduodenal perforations requiring surgery are repaired via laparoscopy or laparotomy, which can subject patients to wound complications such as infection, pain, hernia, and the physiologic burdens of surgery and anesthesia. Moreover, these gastric defects may

be postoperative complications typically set in a formidable surgical field with active infection and adhesions. An endolumenal approach to managing such defects may spare patients the added morbidity of reoperation in these often complicated and contaminated reoperative fields.

The tissue plicating device (TPD) (NDO Plicator, NDO Surgical, Mansfield, MA, USA) (Fig. 1) is an FDA-approved endoscopic device that provides endolumenal treatment of gastroesophageal reflux disease.[3–5] The reusable flexible device is introduced per oral access into the stomach and used to cinch the esophagogastric junction (GEJ) under endoscopic visualization by plicating adjacent gastric tissue. Preloaded, polytetrafluoroethylene (PTFE)-pledgeted, polypropylene suture implants are placed with the assistance of an integrated tissue grasper, ensuring full-thickness serosal approximation of the plicated tissue. Plicated augmentation of the GEJ requires 180° retroflexion of the TPD for adequate visualization. Using the device in an anteflexed configuration, however, has resulted in unique endoscopic applications. The TPD has demonstrated feasibility and conceptual proof of gastric defect closure in the acute swine model, but the long-term efficacy of TPD closure in chronic surviving models is uncertain.[6] The goal of this study was to evaluate the long-term efficacy of closing standardized gastric defects in a NOTES animal model. Acutely created, transmural, gastric defects were used to simulate perforations, fistulae, or points of NOTES access, and they underwent attempted TPD closure. A secondary goal was to characterize intraoperative performance of the TPD by quantitatively assessing the time and number of implants necessary to obtain closure of each defect.

Materials and Methods

Experiments were conducted following approval from Case Western Reserve University's Institutional Animal Care and Use Committee (Protocol #2006-0109). Ten, 40-kg female domestic swine were obtained from a USDA-approved local vendor and underwent a 7-day quarantine and acclimation period at the authors' institution. During this period of time, veterinary personnel evaluated each animal to ensure baseline health. All animals were subjected to the same husbandry procedures. Animals were individually caged with woodchip bedding, fed the same diet, and had unlimited access to water.

Animals were removed from woodchip bedding 72 h prior to the scheduled date of surgery. Animals were fasted from solid food 24 h prior to surgery but allowed to drink water ad libitum. Following restraint, animals were sedated with 8 mg/kg intramuscular tiletamine HCl and zolazepam (Telazol, Fort Dodge, Animal Health, Fort Dodge, IA, USA). Endotracheal intubation was then performed with a #6 French endotracheal tube, and animals were mechanically ventilated at 12 respirations/minute, with a tidal volume of 15 mL/kg, and FiO_2 of 100%. Inhaled isoflurane (AErrane, Baxter Healthcare, Deerfield, IL, USA) was administered at 1.5% for the duration of the surgery. Following intubation, all animals underwent preprocedural 5-L gastric lavage with 0.9% sterile saline via a 40 French stomach evacuator/lavage tube (Lavacuator II, Mallinckrodt, St. Louis, MO, USA) to assist with removing gastric bezoars and debris.

Creation of a Standardized Gastric Defect

Following gastric lavage, NOTES peritoneoscopy was performed using the previously described CASE-T technique.[7] A standard forward-viewing single-channel video endoscope (Olympus, Center Valley, PA, USA, EVIS Type 100 Q140) was passed via the mouth and gastroscopy was performed. Utilizing transillumination verified by external pressure dimpling performed by an assistant, a 12-gauge needle and catheter were passed through the left upper quadrant abdominal wall into the stomach under direct endoscopic visualization. Once the needle tip was intralumenal, a 0.89-mm×400-cm access Jag wire (Boston Scientific, Natick, MA, USA) was passed through the needle and secured intraluminally by an endoscopic snare. The needle and catheter

Figure 1 The TPD (NDO Plicator, NDO Surgical).

were subsequently removed, and the Jag wire, snare, and endoscope were removed as one unit through the mouth. With the transabdominal access wire coursing from the external abdomen through the abdominal wall into the stomach and out through the mouth, the endoscope was reintroduced alongside the wire into the stomach and the gastric site containing the access wire entrance was visually inspected. A novel modified endoscopic needle knife electrocautery/access wire incised the adjacent gastric tissue surrounding the wire, resulting in a 3-mm transmural slit enveloping the transabdominal access wire. The needle knife/access wire was then advanced 10 cm through the enlarged gastric defect into the peritoneal cavity. An 18- to 20-mm esophageal/colonic dilating balloon (CRE ESO 18 to 20 mm×240 cm balloon #5850, Boston Scientific, Cork, Ireland) was passed over the electrocautery/access wire and dilated the 3-mm gastrotomy slit to a 12-mm-diameter circular gastrotomy. The balloon and needle knife were then withdrawn from the endoscope and the endoscope tip was directed through the gastric defect into the abdominal cavity. Previous studies have demonstrated that the resultant gastric defect is consistently circular and 12 mm in diameter.[8] Systematic NOTES peritoneoscopy was performed to exclude the presence of intra-abdominal injury caused from NOTES access.

Upon the completion of NOTES peritoneoscopy, the endoscope was withdrawn from the peritoneal cavity into the stomach. The percutaneous transabdominal access wire was intentionally left coursing from the anterior abdominal wall through the defect and out through the mouth to assist with both endoscopic and subsequent radiographic identification of the closure site. A Savary spring-tipped metal guidewire (Cook Medical, Bloomington, IN, USA) was introduced into the stomach through the endoscopic accessory channel, and the endoscope was removed leaving the wire in place. Upon withdrawal of the endoscope, the distance from the GEJ to the incisors was noted. Care was taken to ensure the Savary wire did not protrude through the NOTES defect and remained in an intragastric position.

TPD Closure of the Gastric Defect

The TPD was advanced beyond the measured length of the GEJ over the Savary wire, and the wire was subsequently exchanged for a 5.9-mm-diameter pediatric flexible gastroscope (GIF-XP 160, Olympus) to provide endoscopic visualization. Each defect was closed with a variable number of sequential firings of the device until closure appeared adequate under endoscopic visualization, with the transabdominal access wire left in place. Ideally, the first implant location was selected to bisect the defect, and subsequent implants were placed to close residual gaps. The u-stitch design of the suture implant enabled approximation of the defect edges without entrapment of the transabdominal access wire.

Postoperative Evaluation of Closure

Once closure appeared complete, multiplanar, real-time, upper GI contrast fluoroscopy was performed under anesthesia in the operative suite. In the prone animal, a large bore orogastric tube was blindly passed into the stomach and 240 mL of water-soluble radiopaque contrast [MD-Gastroview (diatrizoate meglumine and diatrizoate sodium solution), Mallinckrodt] was infused. The percutaneous transabdominal access wire was left in place to serve as a radiopaque marker of the closure site to assist with fluoroscopic visualization. Anterior–posterior, lateral, and 45° oblique views of the stomach were obtained evaluating for intraperitoneal contrast leakage from the gastrotomy site. Results were immediately interpreted by the operating endoscopist. If leakage was noted, the closure process was repeated and additional implants were placed with the TPD. For animals with leak-proof closure on contrast fluoroscopy, anesthesia was terminated and the animal was recovered. The transabdominal access wire was then removed from each animal by simply pulling either extracorporeal end. All animals received one dose of Enrofloxacin 2.5 mg/kg (Baytril, Bayer Healthcare LLC, Shawnee Mission, KA, USA), a once-daily antibiotic administered intramuscularly.

Postoperative Recovery and Screening Upper GI Contrast Fluoroscopy

Animals were extubated when clinically appropriate and followed a standardized care path. Animals were permitted to eat and drink without restriction immediately following the surgery. All animals were evaluated daily by Case veterinary and surgical staff for food intake, pain, bowel and urinary function, and overall wellbeing. Pain medicine was not routinely administered and was dosed on a case-by-case basis at the shared discretion of surgical and veterinary personnel.

On postoperative days 2 and 7, animals received repeat upper GI contrast fluoroscopic exams (UGI). Conscious sedation was obtained following intramuscular injection of 4 mg/kg of intramuscular tiletamine HCl and zolazepam (Telazol, Fort Dodge, Animal Health). Animals were sedated to facilitate handling and examination; however, they remained conscious enough to swallow, cough, and maintain a protected airway. Each animal underwent repeated upper GI contrast fluoroscopy following the aforementioned techniques. The presence or absence of intra-abdominal leakage was recorded for each animal at each time point. Animals returned to their housing facility following each fluoroscopic

exam and were recovered. Following each exam, animals were permitted to eat and drink ad libitum.

Necropsy and Closure Bust Testing

After 14 days, animals were euthanized with intravenous sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI, USA) and a laparotomy was performed. The gastric closure was evaluated for injury to adjacent organs and adhesions. The peritoneal cavity was explored for abscess formation or evidence of infection. Following thorough intra-abdominal exploration, the foregut was excised en bloc and underwent pressurized burst testing of the stomach to evaluate long-term strength of the TPD closure. Pressurized oxygen gradually inflated the stomach through tubing fastened in the esophagus, while intragastric pressures were simultaneously recorded with a digital central venous pressure/arterial blood pressure transducer (Hewlett Packard, Palo Alto, CA, USA, model 68 #M1176a and #M1006b) secured in the duodenum. The stomach was submerged in a basin of water and failure was defined at the first sign of bubbling from the serosal surface of the stomach. Pressures were recorded in millimeters of mercury for each stomach at the point of failure, as well as the location of each failure. Gastric burst testing was also performed on a control group of 40-kg pigs (n=10) originally used for another series of nonsurgical experiments.

Endpoints and Data Analysis

In the postoperative period, clinical data were reported by veterinarians in conjunction with surgical staff. Evidence of peritonitis, pain, food intolerance, bowel movements, urination, and activity level were documented for each animal during the postoperative period, and treated as binary variables. All fluoroscopic imaging was reduced to binary data depending upon the presence or absence of leak. Variations from the experimental protocol were documented. At the end of the study period, all animals were weighed and postoperative weight gain was recorded as a binary variable.

Binary data collected at time of necropsy were presence of abscesses and injury to adjacent organs from TPD closure. Gastric bursting pressure was recorded as continuous numerical data expressed as mean +/− standard deviation and were compared between study and control groups. For study animals, the location of bursting failure was classified as occurring at the closure or remotely. Gastric burst pressure data was compared between study and control groups with a Student's t test. For the remainder of the categorical and binary data, statistical comparison was performed with Fisher's exact test. For all statistical comparisons, significance was defined to be $p<0.05$.

Intraoperatively, recorded endpoints for each animal were the categorical number of suture implants required and the binary presence of intra-abdominal injury from NOTES peritoneoscopy. The number of implants required for each closure was represented as median +/− standard deviation, whereas the presence of abscess was represented as percentage of total animals. Time required for each implant placement was calculated after each experiment upon time-coded videotape review and treated as a continuous variable listed in minutes:seconds format. The necessary time for implant placement was compared between first, second, and subsequent placements for each animal. Additionally, total closure time was compared between the first and the second half of animals to determine if a learning curve existed. Time per implant and total time of closure were converted to decimal format and were compared between groups using a Student's t test.

Results

Gastric NOTES access was easily achieved in all study animals, and peritoneoscopy revealed no injury related to obtaining peritoneal access. All animals appeared to thrive in the postoperative period. No animals required supplemental analgesics, and no animals deviated from the experimental protocol. Seventy percent of animals gained weight in the postoperative period; there were no deaths. Upper GI contrast fluoroscopy (Fig. 2—representative UGI) revealed no leakage from any animals on postoperative days 0, 2, and 7.

Necropsy revealed one iatrogenic injury introduced during gastric closure. A small piece of colonic serosa was found incorporated into one gastric closure, with a small degree of gastrocolic fistulization. The narrow (<2 mm) fistula tract appeared to involve one corner of a PTFE pledget. Three subcentimeter, well-circumscribed, walled-off abscesses were found in the omentum adjacent to the site of the fistula. There was no evidence of peritonitis, peritoneal bile staining, remote abscess, or obstruction. The remainder of the animals demonstrated only loose, flimsy adhesions to the serosal aspect of each closure (Fig. 3).

Following one exclusion for gastrocolic fistula, gastric burst testing was conducted on 9 of the study animals and 10 control animal stomachs. The mean failure pressure for study and control animals was 85.1+/−16 and 85.3+/−26.9 mm Hg, respectively (p=0.98). Six of nine (66.7%) study animals failed at a nonsurgical site remote to the healed closure defect. For study animals failing at the closure defect, the mean burst pressure was 83.3 mm Hg. There was no difference in mean burst pressure between study animals failing at remote sites vs. those failing at the closure (86.0+/−19.6 vs. 83.3+/−8.1 mm Hg, p=0.78).

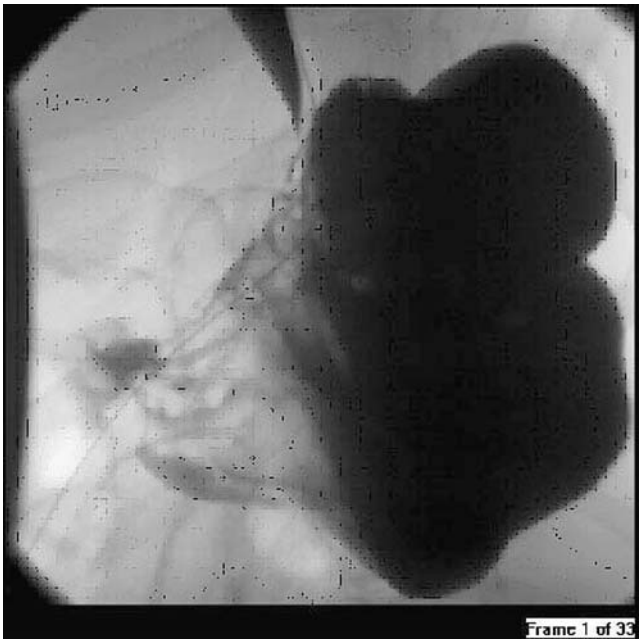

Figure 2 Upper GI fluoroscopy evaluating for leakage at the plicated gastric defect. The transabdominal access wire was left in place to guide fluoroscopic evaluation for contrast extravasation.

NOTES gastrotomies were closed with a median of 3+/−0.74 suture implants (range, 2 to 4), requiring a total mean time of 45:00+/−16:42 per animal (range=25:49–01:07:16); intraoperative data are listed in Table 1. Overall, mean time per implant placement was 14:28+/−09:00 (range=3:04 to 36:50). Time analysis was performed on only nine animals; videotape failure forced the exclusion of the first animal.

The mean time for first, second, and subsequent implant placements was analyzed across all animals and is listed in Table 2. For all animals, the mean time for the first implant was 23:19, whereas the mean time for second implant placement was 12:30 ($p<0.002$). As the number of implants required to close a particular gastrotomy increased, the time for each fire decreased. For closures requiring three implants ($n=8$) and four implants ($n=3$), mean time for each implant was 7:30 and 10:00, respectively ($p=0.59$). The time to fire

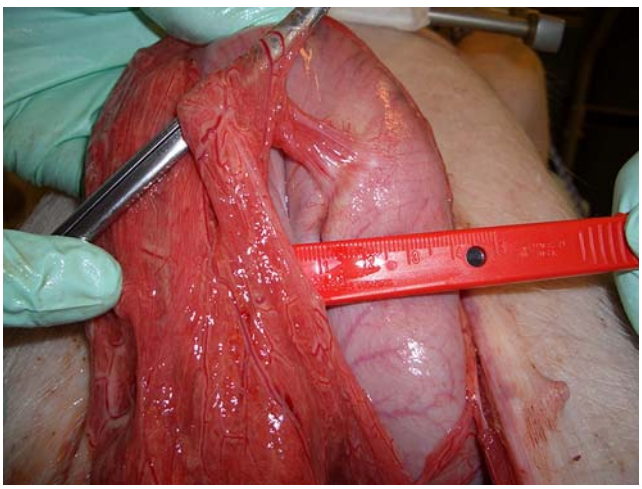

Figure 3 Representative sample of loose omental adhesion to external surface of gastrotomy closure.

Table 1 Intraoperative Data

Animal Number	Total Time for Closure	Implant Number	Time for Implant
1[a]	–	–	–
2	0:30:44	1	0:20:47
		2	0:03:12
		3	0:06:45
3	0:52:42	1	0:36:50
		2	0:15:52
4	1:07:16	1	0:21:49
		2	0:25:40
		3	0:12:45
		4	0:07:02
5	0:58:11	1	0:31:32
		2	0:15:05
		3	0:03:04
		4	0:08:30
6	0:20:25	1	0:13:21
		2	0:03:54
		3	0:03:10
7	1:01:49	1	0:17:48
		2	0:20:03
		3	0:09:30
		4	0:14:28
8	0:39:33	1	0:20:22
		2	0:07:00
		3	0:12:11
9	0:48:28	1	0:28:15
		2	0:15:09
		3	0:05:04
10	0:25:49	1	0:19:11
		2	0:06:38
Mean	0:45:00	3	0:14:28
Standard deviation	0:16:42	0.74	0:09:00

[a] Video recording malfunction, timed data not available. Three total fires used

Table 2 Implant Order vs. Time for Implant

Implant Firing Order	Number of Animals	Average Time[a]
1rst Implant placed	10	23:19[b]
2nd Implant placed	10	12:30
3rd Implant placed	8	7:30
4th Implant placed	3	10:00

[a] Timed closure data not available for one animal
[b] Time for 1st implant was longer than for 2nd, 3rd, and 4th implants ($p<0.005$)

Table 3 Postoperative Data

Animal Number	Gained Weight	Death	Abscess Present	Injury During Closure	Fluoroscopic Contrast UGI Study		
					Leak Day 0	Leak Day 2	Leak Day 7
1	No	No	No	No	No leak	No leak	No leak
2	Yes	No	No	No	No leak	No leak	No leak
3	Yes	No	No	No	No leak	No leak	No leak
4	Yes	No	No	No	No leak	No leak	No leak
5	Yes	No	No	No	No leak	No leak	No leak
6	Yes	No	No	No	No leak	No leak	No leak
7	Yes	No	Yes	Yes	No leak	No leak	No leak
8	Yes	No	No	No	No leak	No leak	No leak
9	No	No	No	No	No leak	No leak	No leak
10	No	No	No	No	No leak	No leak	No leak
Percent	70%	0%	10%	10%	0%	0%	0%

the first implant took significantly longer than that of the second, third, or fourth implants ($p<0.005$). Total closure time did not differ significantly between the first and the second half of study animals (52:13 vs. 39:13, $p=0.27$).

Discussion

Endolumenal therapies have long afforded alternatives to incision-based surgical approaches, sparing patients the added morbidity and mortality associated with conventional surgery. Endoscopic retrograde cholangiopancreatography, endoscopic colonic polypectomy, and percutaneous endoscopic gastrostomy tube are a few examples of surgical endoscopy that have revolutionized standards of surgical care, and each has became a powerful tool in the armamentarium of general surgeons. Tissue approximation and defect closure is an emerging application of contemporary endoscopic technology with a wide range of potential applications.

A variety of endoscopic tools are available to assist with closure or coverage of endolumenal defects. Endoscopic clips, plugs, stents, tissue fasteners, staplers, and suturing devices have been used to close tissue defects but no one technology has demonstrated superiority, as evidenced by a lack of routine clinical use.[9–18] This current report is an intermediate step in the continuation of our experiments to determine the long-term efficacy of the TPD in survival animal models. Potential applications include closure of transgastric NOTES access points, gastric anastomotic leaks, and gastric perforations. In previous reports, our group has developed a technique and demonstrated feasibility of using the TPD in an anteflexed configuration to provide leak-proof closure of gastrotomies in nonsurvival models.[6] The long-term efficacy of this technique, however, was unknown.

In this preclinical, survival swine NOTES model, standardized gastric defects were closed using the TPD. Intraoperative and postoperative UGI fluoroscopy demonstrated leak-proof closure for all animals intraoperatively and on postoperative days 2 and 7. All animals thrived in the postoperative period without clinical evidence of complications (Table 3). Upon ex vivo gastric burst testing, mean failure pressure of plicated closures was commensurate with controls consisting of normal, nonsurgical gastric tissue (Table 4). In the 10 stomachs closed with the TPD, six stomachs failed burst testing at a nonsurgical site remote to

Table 4 Ex Vivo Bust Testing

	Control Stomachs	TPD Closed Stomachs	
	Burst Pressure (mm Hg)	Burst Pressure (mm Hg)	Location of Failure
	56	76	Closure site
	105	106	Remote
	51	108	Remote
	91	82	Closure site
	115	92	Closure site
	53	87	Remote
	85	–	–
	131	86	Remote
	80	72	Remote
	86	57	Remote
Mean	85.3	85.1	
Standard deviation	26.9	16.1	
% Failure at closure site	–	–	33% (3 of 9)

Animal #7 from the plicated closure group had gastrocolic fistula, which precluded burst testing

the gastrotomy, indicating that the strength of the plicated closure equaled or exceeded that of native, nonsurgical gastric tissue. There was one subclinical injury noted at necropsy, as one pledget was found incorporated into adjacent colon with evidence of a possible gastrocolic fistula. The current study leads the authors to draw several conclusions regarding the new application of defect closure with the TPD.

Gastric defect closure with the TPD results in strong, leak-proof closure of gastric defects in the immediate postoperative period. Unlike other endoscopic closure technologies available, tissue plicating technology results in full-thickness serosal apposition and binding. Compared with other mucosa-based endoscopic closure techniques, such as endoscopic clips, the full-thickness purchase of the TPD is hypothesized to be the primary source of closure strength. The resultant robust closure is composed of the former defect edges invaginated into the gastric lumen bound with nonabsorbable, pledgeted, full-thickness sutures. The configuration of this closure results in a closure that becomes stronger as gastric distention pulls the edges apart. One risk of full-thickness closure is blindly incorporating adjacent organs into the closure. It is likely that the one injury encountered in this series of animals was due to colonic tissue inadvertently incorporated within the gastric closure. It is hypothesized that improvements with the device's tissue grasper would eliminate the risk of collateral damage by allowing for improved tissue manipulation and selectivity prior to implant delivery.

Using time as a surrogate for technical difficulty, endoscopic closure of these defects appears to be an advanced endoscopic skill. In the hands of two experienced endoscopists working simultaneously, plicated closure of gastric defects took, on average, 45 min. Several technical factors and properties inherent to the TPD add to the time necessary to perform this procedure. As illustrated in the previous feasibility studies performed by the authors, each single-fire suture implant requires an extracorporeal reloading of the device, which adds greatly to the time required to close each defect. Thus, for each implant placed, a guidewire needs to be replaced and the device is reintroduced. The mean time for each suture implant in this study was 14:28, which was is nearly 2.5 times longer than the time per implant in our previous acute study.

Also adding to the length of the procedure were difficulties initially visualizing the defect and apposing tissue. This may not necessarily reflect poor performance of the TPD device but, rather, difficulty inherent to the endoscopic nature of the procedure. The time to place the first implant for each closure took nearly twice as long as that of the second implants. This discrepancy indicates that obtaining initial visualization, apposition, and delivery of the first implant is the most challenging step of obtaining closure. The authors suspect that inadequate gastric insufflation, which is caused by intra-abdominal leaks through the open gastrotomy, is probably the single largest factor adding to the time necessary for closure. Moreover, improvements in the TPD's integrated tissue grasper may improve closure times by temporarily apposing defect edges prior to implant delivery and improving intragastric insufflation. Once one implant was placed, partial closure permitted adequate insufflation and visualization, as evidence by a marked reduction in time necessary to place subsequent implants. Additionally, the resultant invaginated tissue tent formed by placement of the first implant makes subsequent fires of the device easier because the tissue already protrudes into the jaws of the device, unlike the first firing.

Several limitations of the study model exist. First, the model utilized acutely created 12-mm circular NOTES defects with fresh tissue edges to simulate gastric perforations. Although adequate for studying device performance in NOTES, most gastric perforations and anastomotic defects and fistulas will not consist of fresh, clean-cut tissue edges. In the absence of a realistic, inflamed gastric perforation or fistula model, the current study demonstrates that the TPD is capable of closing defects with clean-cut, healthy tissue; however, device performance in the setting of potentially inflamed and friable tissue has not been studied. Predicated upon the encouraging findings of the current study, subsequent work will be necessary to validate actual TPD performance in non-NOTES applications involving potentially inflamed human tissue. Additionally, identifying the exact site of perforation or anastomotic leak solely with endoscopy may be difficult with the crude dissecting capabilities in the absence of haptics. Another limitation of the study is lack of long-term follow-up, as animals survived only to 14 days. The chronic performance of the nonabsorbable suture-pledget assembly was not evaluated. Migration, erosion, and fistulization may occur beyond the 2-week study period utilized in the current study. It should be noted, however, that human trials have demonstrated no implant migration at 36 months of follow-up.[5] Use of absorbable pledgets and suture materials may reduce the risk of such untoward events.

The notoriously low sensitivity, specificity, and diagnostic yield of contrast fluoroscopy to evaluate enteric leaks are other limiting factors for the present study. In the absence of standardized evaluation techniques to evaluate NOTES closures for leak, contrast fluoroscopy was chosen because of its familiarity, ease, and low cost. Both the surgical and radiological literature, however, is replete with reports describing the limitations of contrast fluoroscopy. The false negative rates of upper GI contrast following

esophagectomy approach 60%, whereas lower GI exams report false positive rates of 12%.[19,20] Multislice CT scanning may improve diagnostic yield; however, the costs of such studies limit experimental use.[21]

Conclusion

TPD closure of standardized gastric defects results in strong, leak-proof closure in the survival swine model. As with any closure technology, injury to adjacent organs can occur, and they may be minimized with subsequent improvements to the device platform. These results support the development of human trials testing the TPD's ability to close uncontrolled gastric defects such as fistulas, anastomotic leaks, and human NOTES access sites.

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References

1. McGee MF, Rosen MJ, Marks J, Onders RP, Chak A, Faulx A, et al. A primer on natural orifice transluminal endoscopic surgery: building a new paradigm. Surg Innov 2006;13:86–93.
2. Rattner D, Kalloo A. ASGE/SAGES working group on natural orifice translumenal endoscopic surgery. October 2005. Surg Endosc 2006;20:329–333.
3. Chuttani R, Kozarek R, Critchlow J, Lo S, Pleskow D, Brandwein S, et al. A novel endoscopic full-thickness plicator for treatment of GERD: an animal model study. Gastrointest Endosc 2002;56:116–122.
4. Chuttani R, Sud R, Sachdev G, Puri R, Kozarek R, Haber G, et al. A novel endoscopic full-thickness plicator for the treatment of GERD: a pilot study. Gastrointest Endosc 2003;58:770–776.
5. Pleskow D, Rothstein R, Kozarek R, Haber G, Gostout C, Lembo A. Endoscopic full-thickness plication for the treatment of GERD: long-term multicenter results. Surg Endosc 2007;21:439–444.
6. McGee M, Marks J, Onders R, Chak A, Jin J, Williams C, et al. Complete endoscopic closure of gastrotomy following natural orifice translumenal endoscopic surgery (NOTES) using the NDO plicator. Surg Endosc 2007; in press.
7. McGee MF, Rosen MJ, Marks J, Chak A, Onders R, Faulx A, et al. A reliable method for monitoring intraabdominal pressure during natural orifice translumenal endoscopic surgery. Surg Endosc 2007;21:672–676.
8. Rosen M, McGee M, Marks J, Chak A, Onders R, Faulx A, et al. Optimizing peritoneal access for natural orifice transvisceral endoscopic surgery (NOTES). Surg Endosc 2006;20:S365.
9. Minami S, Gotoda T, Ono H, Oda I, Hamanaka H. Complete endoscopic closure of gastric perforation induced by endoscopic resection of early gastric cancer using endoclips can prevent surgery (with video). Gastrointest Endosc 2006;63:596–601.
10. Disibeyaz S, Parlak E, Koksal AS, Cicek B, Koc U, Sahin B. Endoscopic treatment of a large upper gastrointestinal anastomotic leak using a prolene plug and cyanoacrylate. Endoscopy 2005;37:1032–1033.
11. Fong DG, Pai RD, Thompson CC. Transcolonic endoscopic abdominal exploration: a NOTES survival study in a porcine model. Gastrointest Endosc 2007;65:312–318.
12. Hausmann U, Feussner H, Ahrens P, Heinzl J. Endoluminal endosurgery: rivet application in flexible endoscopy. Gastrointest Endosc 2006;64:101–103.
13. Jagannath SB, Niiyama H, Chung SS, Cotton PB, Gostout CJ, Hawes RH, et al. Endoscopic gastrojejunostomy with survival in a porcine model. Gastrointest Endosc 2005;62:287–292.
14. Pham BV, Raju GS, Ahmed I, Brining D, Chung S, Cotton P, et al. Immediate endoscopic closure of colon perforation by using a prototype endoscopic suturing device: feasibility and outcome in a porcine model (with video). Gastrointest Endosc 2006;64:113–119.
15. Raju GS, Ahmed I, Xiao SY, Brining D, Poussard A, Tarcin O, et al. Controlled trial of immediate endoluminal closure of colon perforations in a porcine model by use of a novel clip device (with videos). Gastrointest Endosc 2006;64:989–997.
16. Sclabas GM, Swain P, Swanstrom LL. Endoluminal methods for gastrotomy closure in natural orifice transenteric surgery (NOTES). Surg Innov 2006;13:23–30.
17. Seaman DL, Gostout CJ, de la Mora Levy JG, Knipschield MA. Tissue anchors for transmural gut-wall apposition. Gastrointest Endosc 2006;64:577–581.
18. Sumiyama K, Gostout CJ, Rajan E, Bakken TA, Deters JL, Knipschield MA. Endoscopic full-thickness closure of large gastric perforations by use of tissue anchors. Gastrointest Endosc 2007;65:134–139.
19. Tirnaksiz MB, Deschamps C, Allen MS, Johnson DC, Pairolero PC. Effectiveness of screening aqueous contrast swallow in detecting clinically significant anastomotic leaks after esophagectomy. European surgical research. Eur Surg Res 2005;37:123–128.
20. Akyol AM, McGregor JR, Galloway DJ, George WD. Early postoperative contrast radiology in the assessment of colorectal anastomotic integrity. Int J Colorectal Dis 1992;7:141–143.
21. Power N, Atri M, Ryan S, Haddad R, Smith A. CT assessment of anastomotic bowel leak. Clin Radiol 2007;62:37–42.

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Influence of Imaging on the Negative Appendectomy Rate in Pregnancy

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Abstract Appendectomy is the most common non-gynecologic surgery performed during pregnancy. Little data exist on the accuracy of imaging studies in the diagnosis of appendicitis in pregnancy. The objective of this study was to evaluate the probability of ultrasound and computed tomography (CT) scan in diagnosing appendicitis in pregnancy, as reflected in the negative appendectomy rate. We retrospectively reviewed the charts of 86 pregnant women who underwent an appendectomy between January 1, 1997 and January 1, 2006. Patients were divided into three groups: clinical evaluation, ultrasound, and ultrasound followed by a CT scan. The clinical evaluation group had 13 patients, with a negative appendectomy rate of 54% (7/13). Fifty-five patients underwent an ultrasound alone, with a negative appendectomy rate 36% (20/55). In the ultrasound/CT group (n=13), the negative appendectomy rate was 8% (1/13). There was a significant reduction in the negative appendectomy rate in the ultrasound/CT scan group compared to clinical evaluation group (54 vs 8%, $p<0.05$). This reduction was not achieved in the ultrasound group when compared to the clinical evaluation group or the ultrasound/CT group ($p=0.05$). A significant reduction was achieved when the ultrasound/CT group was compared to the patients in the ultrasound only group who had a normal or inconclusive ultrasound ($p<0.05$). Our data documents a very high negative appendectomy rate in the pregnant patient. We recommend an ultrasound followed by a CT scan in patients with a normal or inconclusive ultrasound.

Keywords Appendicitis · Pregnancy · Ultrasound · CT

Introduction

The incidence of acute appendicitis during pregnancy ranges from 0.06 to 0.1%, making appendectomy the most common non-gynecologic surgery performed during pregnancy.[1,2] The anatomical and physiological changes that occur during pregnancy make the diagnosis of acute appendicitis in this patient population more challenging than in their non-pregnant counterparts.[3,4] Signs and symptoms such as anorexia, nausea and vomiting, and mild leukocytosis are seen in normal pregnancy, but they can also be symptoms of acute appendicitis.[1] It is imperative that acute appendicitis is rapidly diagnosed and managed in the pregnant patient.

Diagnostic imaging studies have been shown to be accurate techniques for detecting acute appendicitis in the general population.[6] Ultrasound is reported to have a sensitivity and specificity of 86 and 81%, respectively.[6] Although this test is non-invasive, rapid, inexpensive, and does not use ionizing radiation, it is operator-dependent and often difficult to interpret due to patient limitations, such as obesity or a retrocecal appendix. CT has been shown to be even more accurate in diagnosing acute appendicitis, with a reported sensitivity and specificity of 94 and 95%, respectively.[6] Although these imaging studies have been extensively studied and compared in the general population, their accuracy in diagnosing acute appendicitis in the pregnant population remains unknown.[3,7–9] Potential enthusiasm for routine use of CT scans in diagnosing

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appendicitis in pregnancy is of set by concerns for ionizing radiation exposure to the mother and fetus.[3,10]

In the general population, the reported overall negative appendectomy rate (NAR) after an appendectomy for clinical diagnosis of acute appendicitis without imaging is reported to be as high as 25%.[11] The increased use of ultrasound (US) and/or computed tomography (CT) scan has reportedly improved the overall NAR in the general population to <15%.[11] However, little data exist evaluating the impact of these imaging techniques on reducing the NAR in pregnancy. Our objective is to evaluate the influence of US and CT imaging in diagnosing acute appendicitis in pregnancy, as reflected in the NAR. We chose this measurement over other evaluations, such as predictive values, as it provides a readily meaningful clinical outcome measure.

Materials and Methods

Patients

After receiving institutional approval, we retrospectively reviewed and analyzed the charts of every pregnant woman who underwent an appendectomy at the Brigham and Women's Hospital between January 1, 1997 and January 1, 2006, for a total of 86 patients. Information collected included the age of patient, gestational age, white blood cell (WBC) count, any imaging modality that was used to help with the diagnosis, type of operation, length of stay, and the final pathology report. Patients were divided into three groups: those who underwent an appendectomy based on clinical evaluation alone (CLIN, $n=13$); based on clinical evaluation plus US ($n=55$), or based clinical evaluation and an US followed by a CT scan (US-CT, $n=13$). A small number of patients underwent a CT scan alone (CT, $n=5$) before appendectomy, but were excluded from further analysis.

Imaging

The US and CT imaging studies were interpreted as either diagnostic of appendicitis or as normal/inconclusive. All imaging studies were reviewed and interpreted by radiologists. The graded compression technique was used for the US studies. For each CT scan, the spiral helical technique was used scanning from the second lumbar vertebra to the pubic symphysis. With the exception of one, all patients received oral, intravenous, and/or rectal contrast for preparation. No patients had a magnetic resonance imaging (MRI).

Pathology

Definitive diagnosis of acute appendicitis was made by histo-pathological examination of the resected appendices. The pathological findings were classified as either a normal appendix (normal) or as acute appendicitis/perforation/gangrenous (appendicitis).

Statistical Analysis

The NAR was calculated for each group of patients (CLIN, US, US-CT, and CT) by dividing the number of patients with a pathologically confirmed normal appendix by the total number of patients in the group. To control the overall Type I error rate at 5%, Fisher's exact test for a 4×2 contingency table was used to simultaneously compare the four groups of patients (CLIN, US, US-CT, and CT) with respect to NAR, taking $p<0.05$ as significant. Comparisons between pairs of groups were performed used Fishers Exact test for (2×2) tables. In a second analysis, the US-CT was compared to each of the two subgroups of US using two separate Fisher's exact test; to control the overall Type I error rate at 5% for this second analysis, we used a Bonferonni adjustment so that each p value is significant if $p<0.025$. The data analysis was reviewed by our departmental statistician (see Acknowledgements).

Results

There was no difference between the groups of patients with respect to WBC on presentation, gestational age, or length of stay. Of the 86 patients, 13 had no imaging performed and underwent an appendectomy based on clinical evaluation alone (CLIN), 55 underwent US only (US), 13 had an US followed by CT scan (US-CT), and 5 underwent a CT scan alone (CT).

Pathology Correlation

Appendicitis was pathologically confirmed in 6 of the 13 in the CLIN, 35 of the 55 in the US group, 12 of the 13 in the US-CT group and 2 of 5 in the CT group.

In the US group, 18 of the 34 patients that had a normal or inconclusive US actually had acute appendicitis on pathology. Also in the US group, four patients who had an US reporting acute appendicitis were found to have normal appendices on pathology (Table 1). All patients in the US-CT group had a normal/inconclusive US. Nine of these patients had a CT report of acute appendicitis, all of which were confirmed to have acute appendicitis on pathological examination. The remaining four patients had a CT report of normal/inconclusive, one of which had a normal pathology report (Table 1). Three patients in the CT group had reports of acute appendicitis, one of which had a normal appendix on pathology. Interestingly, the two cases where the CT was interpreted as normal went to the

Table 1 Correlation of the Imaging and Pathology Findings in the US, US-CT, and CT Groups

Pathology	Imaging					
	US		US-CT		CT	
	Appendicitis	Norm/Incon	Appendicitis	Norm/Incon	Appendicitis	Norm/Incon
Appendicitis	17	18	9	3	2	0
Normal	4	16	0	1	1	2
Total	21	34	9	4	3	2
Group total	55		13		5	

operating room based on the surgeon's clinical evaluation and were found to have normal appendices on pathological examination.

Overall NAR

Independent of imaging, our overall NAR was 37% (32/86) compared to 25% in the general population. The overall NAR per year varied over the observed 10 years and had no correlation with the use of CT scans.

NAR of each Patient Group

Table 2 lists the NAR for each group (CLIN, US, US-CT, and CT). Of the 13 in the CLIN group, 7 had a pathologically normal appendix, with a NAR of 54%. The NAR in the US group was 36% (20/55). In the US-CT group, the NAR was 8% (1/13).

The overall p value for the 4×2 Fisher's exact test was significant at $p=0.038$. When performing the paired comparisons, there was a statistically significant reduction in the NAR in the US-CT group compared to the CLIN group (54 vs 8%, $p<0.05$). This reduction was not achieved in the US group when compared to the CLIN or when compared to the US-CT group ($p>0.05$). The small group of patients in the CT only group ($n=5$) was found to have an NAR of 60% (Table 2).

NAR based on Specific Imaging Findings

There was no statistically significant reduction in the NAR of the US group when compared to the US-CT group. A subgroup analysis was performed based on the specific findings in the US group (Table 3). In the US group, 21 patients had a report of acute appendicitis, 4 of which had a normal pathology. This calculated to a NAR of 19% (4/21). When this rate was compared to the NAR in the US-CT group (regardless of the CT results), no significant difference was appreciated (19 vs 8%, $p>0.025$; Table 3). However, there was a significant reduction when the NAR of US reporting normal/inconclusive findings [NAR=53% (18/34)] was compared to the NAR of the US-CT group (regardless of CT results; 53 vs 8%, $p<0.025$; Table 3).

Discussion

The use of diagnostic imaging, specifically US and CT, has helped to reduce the rate of negative appendectomies in the general population. We set out to determine the influence of US and CT on the NAR in suspected cases of acute appendicitis in our pregnant patient population. Our data document a very high NAR in the pregnant patient, which is substantially higher than that seen in the general population. This highlights the inaccuracy of clinical examination alone, especially in the pregnant patient, in diagnosing appendicitis. Imaging studies are essential in reducing the NAR in the general patient population. Our data also support the use of such imaging studies in pregnancy, showing a statistically significant reduction in the NAR of the US-CT group when compared to the CLIN group. The fact that we did not observe a statistically significant reduction in the NAR of the US group when compared to the US-CT suggests that an approach of an US

Table 2 Comparison of the NAR Between the Groups

Group	NAR
CLIN	54% (7/13)
US	36% (20/55)
US-CT	8% (1/13)*
CT	60% (3/5)

*$p<0.05$ CLIN vs US-CT

Table 3 Comparison of the NAR Between the US Group and the US-CT Group Based on the Specific Ultrasound Findings

	NAR		p value
$US_{Appendicitis}$ vs US-CT	19% (4/21)	8% (1/13)	0.6272
$US_{Norm/Incon}$ vs US-CT	53% (18/34)	8% (1/13)	*0.0166

*$p<0.05$ $US_{Norm/Incon}$ vs US-CT

followed by a CT is superior to obtaining an US alone. Some may suggest that going directly to a CT scan in all patients may be the best approach. This would, however, expose the patient and the developing fetus to potentially unnecessary radiation. Furthermore, data from our small group of patients who had a CT alone would suggest that this approach could still result in a high NAR. We clearly have to raise concerns about putting too much emphasis on the 60% NAR, as the numbers in this group were very small (n=5), and in the majority of the cases, the physicians overruled a negative CT. To help develop a diagnostic algorithm that avoids exposure of all patients to radiation and a costly test, we decided to perform a subgroup analysis in those patients that had an US only and compare it to the US-CT group. Our data showed that if the US suggested acute appendicitis, obtaining a follow-up CT did not significantly reduce the NAR (19 vs 8%, $p>0.05$). However, if the US was interpreted as normal or inconclusive, obtaining a follow-up CT did decrease the NAR significantly (53 vs 8%, $p<0.02$), suggesting an increased accuracy in the diagnosis of acute appendicitis in the pregnant patient when both imaging tools are used together. Therefore, we propose an algorithm that subjects pregnant patients suspected of acute appendicitis to an US first. If this cheap and radiation-free procedure diagnoses acute appendicitis, no further imaging is indicated, and the patient can go directly to surgery. However, if the US is normal or inconclusive, the specificity for appendicitis is low, and therefore, a CT should be obtained to definitively rule out appendicitis (Fig. 1). Of note, the negative predictive and positive predictive values of these imaging studies were neither calculated nor discussed. We aimed to measure a clinically meaningful outcome measure (NAR) and propose a practical algorithm whereby surgeons can rapidly decide on the most appropriate treatment for pregnant patients with acute appendicitis.

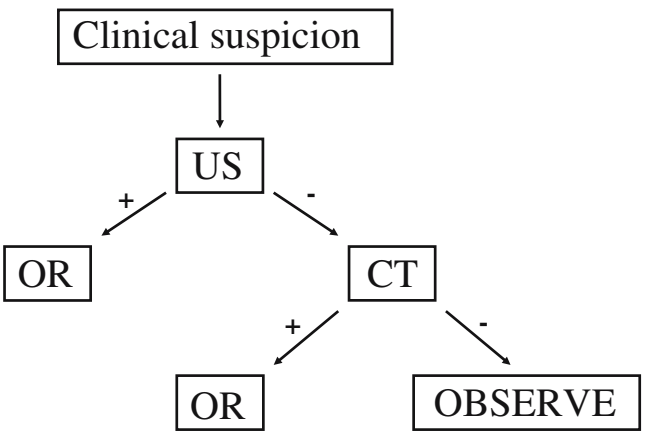

Figure 1 Algorithm for the management of acute appendicitis in the pregnant patient.

Because ionizing radiation is a potential hazard to the developing fetus, avoidance of unnecessary radiation exposure in a pregnant patient is standard practice. The radiation exposure during a CT scan is well below the level required to cause fetal malformations.[7] However, there is a slight increase in the risk of childhood cancer, specifically leukemia.[7] Therefore, radiation exposure via diagnostic medical imaging should be avoided in a pregnant patient and used only when absolutely necessary. Our results suggest that a CT should be obtained only when the US report is normal or inconclusive. In these cases, the potential risks of radiation to the fetus are outweighed by the serious, immediate complications that could result from the missed diagnosis of acute appendicitis in the pregnant patient. We also recognize that there are risks of general anesthesia to the developing fetus, such as teratogenic effects of anesthetic drugs, alterations in uteroplacental blood flow, and maternal hypoxia.[13] These risks are especially higher when surgery is performed during the first trimester, which denotes the period of organogenesis. Appendicitis in pregnancy most commonly presents during the second trimester, however.[2] The risks of anesthesia to the fetus can be minimized if meticulous care is taken in choosing the most appropriate drugs to administer, maintaining adequate uteroplacental blood flow and preventing maternal hypoxia.[13]

MRI is being used more often now as an alternative to CT. This is largely because it avoids ionizing radiation. In a recent study, MRI was reported to have a sensitivity and specificity of 100 and 93.6%, respectively, in the diagnosis of acute appendicitis in pregnancy.[12] The impact of such imaging modality on the NAR is not known. Furthermore, MRI is not readily available in every hospital. At our institution, MRI is not routinely used in the diagnosis of acute appendicitis, and none of the patients in our study had an MRI performed.

Because our study is a retrospective review, we were unable to determine if there were also patients who were examined and found not to exhibit signs and symptom of acute appendicitis. Obtaining this information would have allowed us to determine the negative predictive value of the clinical evaluation group. The retrospective analysis also prohibited us from knowing who examined the patients (junior/senior residents vs surgical attendings) and what determined whether a patient received an imaging study.

Conclusions

Based on our results, we suggest an algorithm, where every pregnant patient with a clinical suspicion of acute appendicitis undergoes an US first. If the US reports acute appendicitis, the patient should undergo an appendectomy

without further imaging. However, if the US report is normal or inconclusive, a CT scan should be obtained for confirmation, and the patient should be taken to the operating room based on the results of the CT scan. This could potentially reduce the number of unnecessary operations in the pregnant patient.

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References

1. Mourad J, Elliott JP, Erickson L, Lisboa L. Appendicitis in pregnancy: New information that contradicts long-held clinical beliefs. Am J Obstet Gynecol 2000;82:1027–1029.
2. Andersson REB, Lambe M. Incidence of appendicitis during pregnancy. Int J Epidemiol 2001;30:1281–1285.
3. Pastore P, Loomis D, Sauret J. Appendicitis in Pregnancy. J Am Board Fam Med 2006;19:621–626.
4. Andersen B, Nielsen TF. Appendicitis in pregnancy: diagnosis, management and complications. Acta Obstet Gynecol Scand 1999;78:758–762.
5. Bickell NA, Aufses AH Jr, Rojas M Bodian C. How time affects the risk of rupture in appendicitis. J Am Coll Surg 2006;202: 401–406.
6. Terasawa T, Blackmore CC, Bent S, Kohlwes RJ. Systematic review: computed tomography and ultrasound to detect acute appendicitis I adults and adolescents. Ann Intern Med 2004;141: 537–546.
7. Miller JC. Risks from ionizing radiation in pregnancy. Radiology Rounds 2004;2.
8. Styrud J, Josephson T, Eriksson S. Reducing negative appendectomy: evaluation of ultrasonography and computer tomography in acute appendicitis. Int J Qual Health Care 2000;12:65–68.
9. Poortman P, Lohle P, Shoemaker C, Oostvogel H, Teepen H, Zwinderman K, Hamming J. Comparison of CT and sonography in the diagnosis of acute appendicitis: A blinded prospective study. AJR Am J Roentgenol 2003;181:1355–1359.
10. Barloon TJ, Brown BP, Abu-Yousef MM, et al. Sonography of acute appendicitis in pregnancy. Abdom Imaging 1995;20:149–151.
11. Lim HK, Bae SH, Seo GS. Diagnosis of acute appendicitis in pregnant women: value of sonography. AJR Am J Roentgenol 1992;159:539–542.
12. Pedrosa I, Levine D, Eyvazzadeh AD, Siewert B, et al. MR Imaging evaluation of acute appendicitis in pregnancy. Radiology 2006;38:891–899.
13. Kuczkowski KM. Nonobstetric surgery during pregnancy: what are the risks of anesthesia? Obstet Gynecol Surv 2004;59:52–56.

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A Soft Pancreatic Remnant is Associated with Increased Drain Fluid Pancreatic Amylase and Serum CRP Levels Following Pancreatoduodenectomy

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Abstract The aim of this prospective study was to clarify differences in postoperative changes of serum or drainage fluid pancreatic amylase levels and serum C-reactive protein (CRP) levels between patients with a soft pancreatic texture and those with a hard pancreatic texture undergoing pancreatoduodenectomy (PD) with pancreaticogastrostomy. A total of 61 consecutive patients with resectable periampullary tumors undergoing PD were recruited. This population was divided into 27 patients with a hard pancreatic texture and 34 patients with a soft pancreatic texture. Drainage fluid total amylase or pancreatic amylase levels, serum total amylase or pancreatic amylase levels, and serum CRP levels were measured postoperatively. Clinicopathological data were also compared between two groups. Postoperative complications more frequently occurred in patients with a soft pancreatic texture compared with those with a hard pancreatic texture (P=0.029). Serum or drainage fluid pancreatic amylase levels and serum CRP levels of patients with a soft pancreatic texture were significantly higher than those of patients with a hard pancreatic texture after PD on postoperative days 1 and 2 (P<0.05). A soft pancreatic texture was identified as an only independent predictive factor of increased drainage fluid pancreatic amylase levels (P=0.006) and serum CRP levels (P=0.047). A soft pancreatic texture is closely associated with increased drainage fluid pancreatic amylase and serum CRP levels after PD. More careful post-PD management is needed for patients with a soft pancreatic texture.

Keywords Pancreatoduodenectomy · Soft pancreatic texture · Hard pancreatic texture · Drainage fluid pancreatic amylase · C-reactive protein

Introduction

The operative mortality rate after pancreatoduodenectomy (PD) is reported to range from 0 to 5% at major surgical institutes.[1–6] The major cause of operative death is sepsis and hemorrhage resulting from pancreaticoenteric anastomotic failure, which occurs in 0 to 18% of patients undergoing PD.[2–18] To prevent this unfortunate complication after PD, various modifications of pancreaticoenteric reconstruction have been proposed: pancreticojejunostomy or pancreaticogastrostomy, invagination or duct-to-mucosa, stented or nonstented, end-to-end or end-to-side, and use of fibrin glue or not. However, no universal consensus has been reached regarding the particular variation of pancreaticoenteric reconstruction that is safer and less prone to anastomotic failure.

Several investigators have attempted to find useful predictors of pancreatic fistula after PD, and a soft pancreatic texture has been reported to be one of the most important predictors, according to the previous literature.[6,13,17] However, to our knowledge, there have been few detailed comparative studies between patients with a soft pancreatic texture and those with a hard pancreatic

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texture undergoing PD. Shyr et al.[19] reported that a soft pancreatic parenchyma was associated significantly with higher drainage fluid amylase levels than a fibrotic pancreas in patients after PD. The aims of this prospective study were to clarify differences in postoperative changes of serum or drainage fluid pancreatic amylase levels and C-reactive protein (CRP) levels between patients with a soft pancreatic texture and those with a hard pancreatic texture undergoing PD.

Patients and Methods

Sixty-one consecutive patients with resectable periampullary tumors undergoing pylorus-preserving PD with pancreaticogastrostomy at the Hiroshima University Hospital were recruited into this study from December 2003 to December 2006. Appropriate informed consent was obtained from all patients. According to intraoperative findings of the texture of the remnant pancreas (pancreatic body or tail) and postoperative pathological findings of the pancreatic cut margins, this patient population was divided into two groups: patients with a hard pancreatic texture and patients with a soft pancreatic texture. All patients with a hard pancreatic texture developed obstruction of the main pancreatic duct at the head of the pancreas because of tumor invasion by preoperative imaging examinations and had a fibrotic pancreas because of obstructive pancreatitis pathologically, whereas all patients with a soft pancreatic texture did not. Data concerning patient characteristics (age, gender, pathological diagnosis, and preoperative HbA_{1c} level), operative procedures (partial portal vein resection), perioperative parameters (operating time, blood loss, and blood transfusion), and postoperative complications were recorded. A pancreatic fistula was defined as reported by the International Study Group on Pancreatic Fistula Definition [output via an operatively placed drain of any measurable volume of drain fluid on or after postoperative day (POD) 3, with an amylase content greater than three times the upper normal serum value].[20] A biliary fistula was defined as persistence of biliary drainage for more than 5 days, confirmed by fistulography.[16] Gastric emptying was considered delayed when postoperative gastric suction was required for more than 10 days or when the patient was not able to tolerate a solid diet on or before POD 14.[16,17] Operative mortality was any death occurring within 30 days of the procedure.

In all patients, total amylase levels or pancreatic amylase levels in the fluid from the drainage tube near the pancreaticogastric anastomosis were measured on POD 1, 2, and 5. The quality of the drainage fluid was also recorded. Serum total amylase levels, serum pancreatic amylase levels, and serum CRP levels were examined on POD 1, 2, 5, and 7. The cutoff value for normal serum total amylase levels and serum pancreatic amylase levels in our hospital were <110 and <46 U/l, respectively.

Pancreaticogastrostomy was performed using the duct-to-mucosa method. After pancreatoduodenal resection with or without preservation of the pylorus, the pancreatic stump was dissected from the superior mesenteric and splenic vein for a distance of 2 cm. An incision of 3 cm was made in the seromuscular layer of the posterior gastric wall, and a row of interrupted 4–0 nonabsorbable monofilament sutures was placed between the seromuscular layer of the posterior gastric wall and the anterior wall of the pancreas (approximately 0.5 cm from the cut edge of the pancreas). A 0.5-cm incision of the exposed gastric submucosa/mucosa was made at the opposite side of the main pancreatic duct. Then, a row of interrupted 5–0 absorbable monofilament sutures was placed between the gastric submucosa/mucosa and the main pancreatic duct, which was completed by a total of eight 5–0 absorbable monofilament sutures, including the posterior and anterior sutures. The duct-to-mucosa method was completed by applying a row of interrupted 4–0 nonabsorbable monofilament sutures between the seromuscular layer of the posterior gastric wall and the posterior wall of the pancreas. A pancreaticogastric stent was placed for an internal drain of the pancreatic juice to the stomach. This stent was, in most cases, removed spontaneously within 1 or 2 months after surgery. After pancreaticogastrostomy, end-to-side choledochojejunostomy and end-to-side duodenojejunostomy were performed in antecolic fashion. All patients had two drains placed at the time of surgery, one *near* the choledochojejunostomy and one *near* the pancreaticogastrostomy. These drains were removed on POD 6 if a pancreatic fistula and a biliary fistula were not found. All patients received histamine H2-receptor antagonists during their postoperative hospitalization as prophylaxis for marginal ulcerlation. However, no patients received somatostatin analogues during their perioperative periods.

Data were presented as mean ± standard deviation or mean ± standard error. Statistical differences between the groups were verified using the chi-square test and the Mann–Whitney *U* test for univariate analysis. Multivariate analysis was performed using a multiple logistic regression model. Significance was defined at the $P<0.05$ level. Statistical analysis was carried out using the Macintosh version of StatView (version 5.0; SAS Institute, Cary, NC).

Results

Pancreaticogastrostomy after PD was performed for a total of 61 patients (27 with a hard pancreatic texture and 34 with a soft pancreatic texture). There was no mortality in both groups. There was no significant difference between

the two groups in age, gender, and preoperative HbA_{1c} levels. Pancreatic carcinoma occurred more frequently ($P<0.001$) in patients with a hard pancreatic texture than in patents with a soft pancreatic texture. Partial resection of the portal vein was performed more frequently ($P<0.001$), and the operative time ($P=0.003$) and blood loss ($P=0.010$) was significantly higher in patients with a hard pancreatic texture than in patients with a soft pancreatic texture. More patients with a hard pancreatic texture than patients with a soft pancreatic texture required perioperative blood transfusion ($P<0.001$). Postoperative complications occurred more frequently ($P=0.029$) in patients with a soft pancreatic texture (surgical site infection in three, biliary fistula in four, enterocolitis in one, delayed gastric emptying in three, and pancreatic fistula in three) than in patents with a hard pancreatic texture (pancreatic fistula in two and biliary fistula in one). However, the incidence of pancreatic fistula did not differ between two groups (Table 1). The pancreatic fistulae of five patients (three with a soft pancreatic texture and two with a hard pancreatic texture) were judged as grade A according to the clinical grading system of the International Study Group on Pancreatic Fistula Definition.[20] The total drainage fluid amylase levels of the four patients with pancreatic fistula were 1,100, 767, 484, and 416 U/l after POD 3. However, they were managed by slow removal of the operatively placed drains, and no further treatment was required. The other patient, who had a hard pancreatic texture, developed pus-like drainage fluid on POD 5. The total amylase level and pancreatic amylase level of this patient were 13,400 and 5,670 U/l on POD 7 in the drainage fluid, respectively. The patient was treated conservatively by leaving the drain in place and was cured within 2 weeks.

Table 1 Perioperative Characteristics of Patients with a Hard or Soft Pancreatic Texture Undergoing Pancreatoduodenectomy

	Pancreatic texture		*P* value
	Hard ($n=27$)	Soft ($n=34$)	
Age (years)	65±12	67±12	0.293
Gender			
Male	15	22	0.467
Female	12	12	
Pathological diagnosis			
Pancreatic carcinoma	21	3	<0.001
Non-pancreatic carcinoma	6	31	
Preoperative HbA1c levels (%)	6.4±2.0	5.4±1.0	0.058
Partial portal vein resection			
Yes	11	2	<0.001
No	16	32	
Operative time (minutes)	423±88	360±68	0.003
Blood loss (ml)	1,481±1,798	934±683	0.010
Blood transfusion			
Yes	11	2	<0.001
No	16	32	
Pancreatic fistula			
Yes	2	3	0.841
No	25	31	
Postoperative complications			
Yes	3	12	0.029
No	24	22	

Data are presented as mean ± standard deviation.

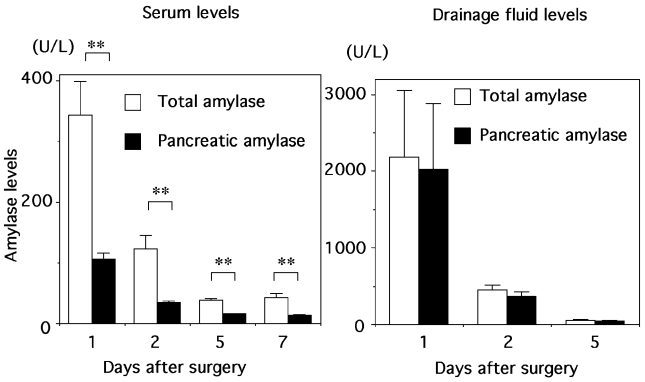

Figure 1 Comparison between total amylase levels and pancreatic amylase levels in patients undergoing pancreatoduodenectomy. Data are presented as mean ± standard error. $^{**}P<0.01$.

In all 61 patients, serum total amylase levels were significantly higher than serum pancreatic amylase levels on POD 1, 2, 5, and 7 ($P<0.001$). In 22 of 61 patients (36%), serum total amylase levels were more than three times the serum pancreatic amylase levels on POD 1. However, there was no significant difference between the drainage fluid total amylase levels and the drainage fluid pancreatic amylase levels during the study period (Fig 1).

In comparison between patients with a soft pancreatic texture and those with a hard pancreatic texture, drainage fluid pancreatic amylase levels of patients with a soft pancreatic texture were significantly higher than those of patients with a hard pancreatic texture on POD 1, 2, and 5 ($P<0.001$). However, the drainage fluid pancreatic amylase levels of both groups decreased to lower levels on POD 5. In addition, serum pancreatic amylase levels of patients with a soft pancreatic texture were significantly higher than those of patients with a hard pancreatic texture on POD 1, 2, 5, and 7 ($P<0.001$). The serum pancreatic amylase levels of patients with a soft pancreatic texture decreased to the normal range on POD 5 (Fig 2). Serum CRP levels of patients with a soft pancreatic texture were significantly higher than those of patients with a hard pancreatic texture

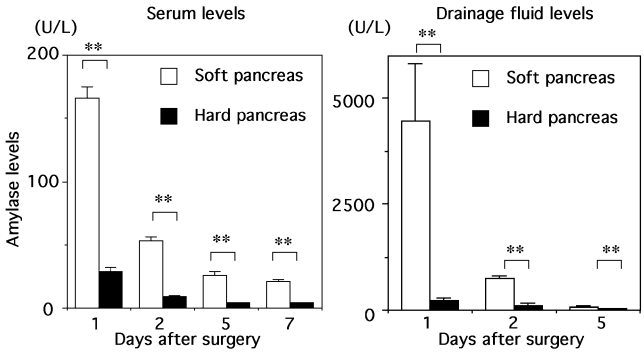

Figure 2 Comparison of serum and drainage fluid pancreatic amylase levels between patients with a soft pancreatic texture and those with a hard pancreatic texture undergoing pancreatoduodenectomy. Data are presented as mean ± standard error. $^{**}P<0.01$.

on POD 1 ($P=0.044$) and 2 ($P<0.018$). No significant difference existed between the two groups on POD 5 and 7 (Fig 3).

Table 2 lists univariate analysis of perioperative factors influencing postoperative maximum drainage fluid pancreatic amylase levels undergoing PD. Preoperative serum HbA_{1c} levels (<5.5%; $P=0.014$), absence of partial resection of the portal vein ($P=0.002$), a soft pancreatic texture ($P<0.001$), absence of blood transfusion ($P=0.039$), presence of postoperative complications ($P=0.003$), and a pathological diagnosis of non-pancreatic carcinoma ($P<0.001$) were significantly associated with higher drainage fluid pancreatic amylase levels (Table 2). These six factors were entered into multivariate analysis with a multiple logistic regression model. A soft pancreatic texture was identified as an only independent factor influencing postoperative maximum drainage fluid pancreatic amylase levels ($P=0.007$, relative risk 28.4, and 95% confidence interval of 2.6–313.4), additionally, preoperative serum HbA_{1c} levels (<5.5%; $P=0.029$) and a soft pancreatic texture (P were significantly associated with higher maximum serum CRP levels by univariate analysis (Table 3). A soft pancreatic texture was identified as an only independent factor influencing postoperative maximum serum CRP levels ($P=0.047$, relative risk 3.1, and 95% confidence interval of 1.0–9.7).

Discussion

Postoperative hyperamylasemia has been noted in a variety of surgical procedures, including cardiac surgery[21] and abdominal surgery.[22,23] However, elevation of the serum total amylase concentration does not reflect pancreatic injury because the elevated serum total amylase concentration consists of both pancreatic and salivary amylase. Several authors reported that hyperamylasemia during the early days after surgery was frequently of salivary origin.[21–23]

In this study, serum total amylase levels were significantly higher than serum pancreatic amylase levels on POD 1, 2, 5, and 7, although no significant difference existed between drainage fluid total amylase levels and drainage fluid pancreatic amylase levels. Additionally, in 36% of the patients, the serum total amylase levels were more than three times the serum pancreatic amylase levels on POD 1. Based on these results, measurement of the pancreatic amylase in serum or drainage fluid is mandatory for evaluating pancreatic injury in patients undergoing PD. For these reasons, we analyzed not the total amylase concentration but the pancreatic amylase concentration in the current study.

There has been only one report regarding the daily change in drainage fluid total amylase levels after PD in the published literature.[19] According to this report, drainage fluid total amylase was significantly higher in the group with a soft pancreatic parenchyma than in the group with a hard pancreatic parenchyma on POD 1–7. The results of the current study were similar to that report. Not only drainage fluid pancreatic amylase levels but also serum pancreatic amylase levels of patients with a soft pancreatic texture were significantly higher than those of patients with hard pancreatic texture throughout the study periods. In addition, a soft pancreatic texture was an only independent factor influencing increased drainage fluid pancreatic amylase levels after PD. Based on these results, we believe that soft pancreatic parenchyma is closely associated with higher drainage fluid pancreatic amylase levels after PD.

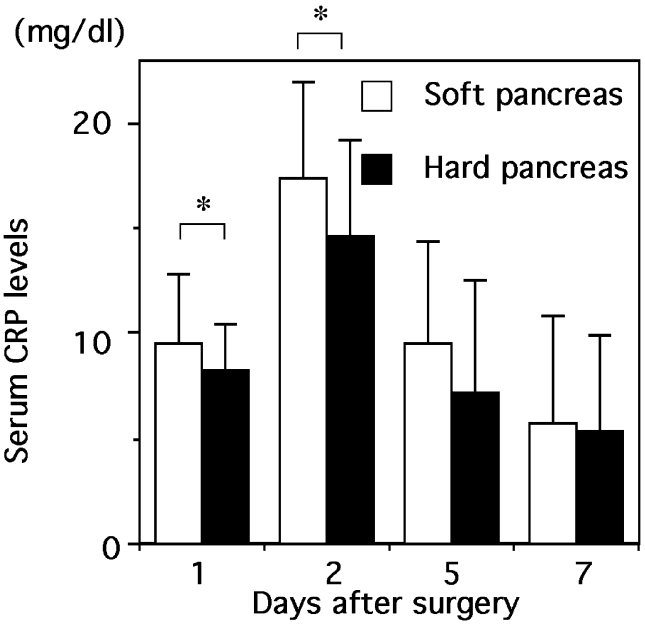

Figure 3 Comparison of serum CRP levels between patients with a soft pancreatic texture and those with a hard pancreatic texture undergoing pancreatoduodenectomy. Data are presented as mean ± standard deviation. $^{*}P<0.05$.

Table 2 Univariate Analysis of Perioperative Factors Influencing Postoperative Maximum Drainage Fluid Pancreatic Amylase Levels in Patients Undergoing Pancreatoduodenectomy

	Maximum drainage fluid pancreatic amylase levels (U/l)	*P* value
Preoperative serum HbA1c level (%)		
<5.5 (*n*=29)	3,374±9,335	0.014
≥5.5 (*n*=32)	826±1,562	
Partial portal vein resection		
Yes (*n*=13)	295±687	<0.001
No (*n*=48)	2,513±7,314	
Pancreatic texture		
Soft (*n*=27)	3,552±8,448	<0.001
Hard (*n*=34)	184±322	
Operative time (minutes)		
<380 (*n*=33)	1,353±1,899	0.422
≥380 (*n*=28)	2,810±9,374	
Blood loss (ml)		
<1,000 (*n*=30)	1,560±2,034	0.113
≥1,000 (n=31)	2,475±8,922	
Blood transfusion		
Yes (*n*=13)	559±840	0.039
No (*n*=48)	2,440±7,330	
Postoperative complications		
Yes (*n*=15)	5,153±12,992	0.003
No (*n*=46)	1,083±1,790	
Pathological diagnosis		
PCA (*n*=24)	370±608	<0.001
Non-PCA (*n*=37)	3,141±8,276	

Data are presented as mean ± standard deviation.
PCA Pancreatic carcinoma

To our knowledge, there have been no previous English reports concerning the relationship between pancreatic texture and serum CRP levels after PD. Many investigators have used postoperative serum CRP levels as a systemic inflammation parameter to compare the invasiveness of various surgical procedures.[24–26] In this study, serum CRP levels of patients with a soft pancreatic texture were significantly higher than those of patients with a hard pancreatic texture on POD 1 and 2 after PD. In addition, a soft pancreatic texture was identified as an only independent factor influencing postoperative higher serum CRP levels, whereas operative time, blood loss, blood transfusion, and postoperative complications did not correlate with elevation of postoperative serum CRP levels. A soft pancreatic texture appears to be one of the most important factors that accelerates elevation of serum CRP levels after PD.

In this study, patients with a soft pancreatic texture developed postoperative complications more frequently than patients with a hard pancreatic texture, although the incidence of pancreatic fistula did not differ between both groups. As mentioned above, patients with a soft pancreatic texture exhibited higher levels of drainage fluid pancreatic amylase and serum CRP. Much of intraperitoneal pancreatic amylase induces acceleration of locoreginal inflammation, and higher serum CRP levels indicate acceleration of systemic inflammation. Higher incidence of postoperative complications in patients with a soft pancreatic texture after PD might be caused by acceleration of rocoreginal and systemic inflammation.

Conclusions

In conclusions, a soft pancreatic texture accelerates not only elevation of serum or drainage fluid pancreatic amylase levels but also serum CRP levels after PD. Postoperative complications occurred more frequently in patients with a pancreatic texture than in patients with a hard pancreatic texture. More careful post-PD management is needed for patients with a soft pancreatic texture.

Table 3 Univariate Analysis of Perioperative Factors Influencing Postoperative Maximum Serum CRP Levels in Patients Undergoing Pancreatoduodenectomy

	Maximum serum CRP levels (mg/dl)	*P* value
Preoperative serum HbA1c level (%)		
<5.5 (*n*=29)	17.4±4.3	0.029
≥5.5 (*n*=32)	15.0±4.5	
Partial portal vein resection		
Yes (*n*=13)	14.9±4.7	0.283
No (*n*=48)	16.5±4.5	
Pancreatic texture		
Soft (*n*=34)	17.4±4.5	0.018
Hard (*n*=27)	14.6±4.6	
Operative time (minutes)		
<380 (n=33)	16.0±4.9	0.772
≥380 (*n*=28)	16.2±4.3	
Blood loss (ml)		
<1,000 (*n*=30)	15.7±5.0	0.415
≥1,000 (*n*=31)	16.6±4.1	
Blood transfusion		
Yes (*n*=13)	16.4±4.1	0.805
No (*n*=48)	16.1±4.7	
Postoperative complications		
Yes (*n*=15)	16.8±4.7	0.525
No (*n*=46)	15.9 ±4.6	
Pathological diagnosis		
PCA (*n*=24)	15.0±4.7	0.104
Non-PCA (*n*=37)	16.9±4.4	

Data are presented as mean ± standard deviation.
CRP C-reactive protein, *PCA* pancreatic carcinoma

References

1. Trede M, Schwall G, Saeger HD. Survival after pancreatoduodenectomy. 118 consecutive resections without an operative mortality. Ann Surg 1990;211:447–458.
2. Cullen JJ, Sarr MG, Ilstrup DM. Pancreatic anastomotic leak after pancreatoduodenectomy: incidence, significance, and management. Am J Surg 1994;168:295–298.
3. Marcus SG, Cohen H, Ranson JH. Optimal management of the pancreatic remnant after pancreatoduodenectomy. Ann Surg 1995; 221:635–645.
4. Yeo CJ, Cameron JL, Sohn TA, Lillemoe KD, Pitt HA, Talamini MA, Hruban RH, Ord SE, Sauter PK, Coleman J, Zahurak ML, Grochow LB, Abrams RA. Six hundred fifty consecutive pancreatoduodenectomies in the 1990s: pathology, complications, and outcomes. Ann Surg 1997;226:248–257.
5. Tsuji M, Kimura H, Konishi K, Yabushita K, Maeda K, Kuroda Y. Management of continuous anastomosis of pancreatic duct and jejunal mucosa after pancreaticoduodenectomy: historical study of 300 patients. Surgery 1998;123:617–621.
6. Sato N, Yamaguchi K, Yokohata K, Shimizu S, Morisaki T, Mizumoto K, Chijiiwa K, Tanaka M. Preoperative exocrine pancreatic function predicts risk of leakage of pancreaticojejunostomy. Surgery 1998;124:871–876.
7. Roder JD, Stein HJ, Bottcher KA, Busch R, Heidecke CD, Siewert JR. Stented versus nonstented pancreaticojejunostomy after pancreatoduodenectomy: a prospective study. Ann Surg 1999;229:41–48.
8. Takao S, Shimazu H, Maenohara S, Shinchi H, Aikou T. Modified pancreaticogastrostomy following pancreaticoduodenectomy. Am J Surg 1993;165:317–321.
9. Kim SW, Youk EG, Park YH. Comparison of pancreatogastrostomy and pancreatojejunostomy after pancreatoduodenectomy performed by one surgeon. World J Surg 1997;21:640–643.
10. Fabre JM, Arnaud JP, Navarro F, Bergamaschi R, Cervi C, Marrel E, Domergue J. Results of Pancreatogastrostomy after pancreatoduodenectomy in 160 consecutive patients. Br J Surg 1998;85:751–754.
11. Kapur BM, Misra MC, Seenu V, Goel AK. Pancreaticogastrostomy for reconstruction of pancreatic stump after pancreaticoduodenectomy for ampullary carcinoma. Am J Surg 1998;176:274–278.
12. Hyodo M, Nagai H. Pancreatogastrostomy (PG) after pancreatoduodenectomy with or without duct-to-mucosa anastomosis for the small pancreatic duct: short- and long-term results. Hepatogastroenterology 2000;47:1138–1141.
13. Aranha GV, Hodul PJ, Creech S, Jacobs W. Zero mortality after 152 consecutive pancreaticoduodenectomies with pancreaticogastrostomy. J Am Coll Surg 2003;197:223–231.
14. Payne RF, Pain JA. Duct-to-mucosa pancreaticogastrostomy is a safe anastomosis following pancreaticoduodenectomy. Br J Surg 2006;93:73–77.
15. Takano S, Ito Y, Watanabe Y, Yokoyama T, Kubota N, Iwai S. Pancreaticojejunostomy versus pancreaticogastrostomy in reconstruction following pancreatoduodenectomy. Br J Surg 2000;87:423–427.
16. Bassi C, Falconi M, Molinari E, Salvia R, Butturini G, Sartori N, Mantovani W, Pederzoli P. Reconstruction by pancreaticojejunostomy versus pancreaticogastrostomy following pancreatectomy: results of a comparative study. Ann Surg 2005;242:767–771
17. Yeo CJ, Cameron JL, Maher MM, Sauter PK, Zahurak ML, Talamini MA, Lillemoe KD, Pitt HA. A prospective randomized trial of pancreaticogastrostomy versus pancreaticojejunostomy after pancreaticoduodenectomy. Ann Surg 1995;222:580–592.
18. Duffas JP, Suc B, Msika S, Fourtanier G, Muscari F, Hay JM, Fingerhut A, Millat B, Radovanowic A, Fagniez PL, French Associations for Research in Surgery. A controlled randomized multicenter trial of pancreatogastrostomy or pancreatojejunostomy after pancreatoduodenectomy. Am J Surg 2005;189:720–729.
19. Shyr YM, Su CH, Wu CW, Lui WY. Does drainage fluid amylase reflect pancreatic leakage after pancreaticoduodenectomy? World J Surg 2003;27:606–610.
20. Bassi C, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J, Neoptolemos J, Sarr M, Traverso W, Buchler M, International Study Group on Pancreatic Fistula Definition. Postoperative pancreatic fistula: an international study group (ISGPF) definition. Surgery 2005;138:8–13.
21. Ihaya A, Muraoka R, Chiba Y, Kimura T, Uesaka T, Morioka K, Matsuyama K, Tsuda T, Nara M, Niwa H. Hyperamylasemia and subclinical pancreatitis after cardiac surgery. World J Surg 2001;25:862–864.
22. Adam DJ, Milne AA, Evans SM, Roulston JE, Lee AJ, Ruckley CV, Bradbury AW. Serum amylase isoenzymes in patients undergoing operation for ruptured and non-ruptured abdominal aortic aneurysm. J Vasc Surg 1999;30:229–235.
23. Tsuzuki T, Shimizu S, Takahashi S, Iio H. Hyperamylasemia after hepatic resection. Am J Gastroenterol 1993;88:734–736.
24. Nishiguchi K, Okuda J, Toyoda M, Tanaka K, Tanigawa N. Comparative evaluation of surgical stress of laparoscopic and open surgeries for colorectal carcinoma. Dis Colon Rectum 2001;44:223–230.
25. Nguyen NT, Goldman CD, Ho HS, Gosselin RC, Singh A, Wolfe BM. Systemic stress response after laparoscopic and open gastric bypass. J Am Coll Surg 2002;194:557–567.
26. Yamaguchi K, Yokohata K, Nakano K, Ohtani K, Ogawa Y, Chijiiwa K, Tanaka M. Which is a less invasive pancreatic head resection: PD, PPPD, or DPPHR? Dig Dis Sci 2001;46:282–288.

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Angiotensin II Induces Vascular Endothelial Growth Factor in Pancreatic Cancer Cells Through an Angiotensin II Type 1 Receptor and ERK1/2 Signaling

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Abstract Vascular endothelial growth factor (VEGF) is a crucial pro-angiogenic component in pancreatic ductal adenocarcinoma (PDA), and its high expression levels have been correlated with poor prognosis and early postoperative recurrence. We have recently shown that high levels of angiotensin II (AngII) type 1 receptor (AT1R) correlate and colocalize with VEGF in invasive PDA and that AngII induces VEGF expression in PDA cell lines. In this study, we explored the signaling mechanisms involved in the AngII-mediated VEGF induction and correlated AT1R and VEGF expression in noninvasive precursor lesions. An AT1R antagonist significantly ($p<0.05$) inhibited the AngII-mediated induction of VEGF messenger RNA and protein in all PDA cell lines. AngII-VEGF induction was inhibited by the tyrosine kinase inhibitor genistein, suggesting a mitogen-activated protein kinase signaling mechanism. AngII activated the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), but not p38 or c-*Jun* NH2-terminal MAP kinases. Inhibition of ERK1/2 activation reduced the AngII-induced VEGF synthesis. Immunohistochemical analysis of precursor lesions showed increased expression of AT1R in most ductal cells undergoing metaplasia. Pancreatic intraepithelial neoplasms showed more intense AT1R staining when compared to intraductal papillary mucinous neoplasms, which showed heterogeneous immunoreactivity. VEGF followed the same distribution pattern of AT1R in both lesions. AT1R expression in the premalignant pancreatic lesions suggests its involvement in tumor progression and angiogenesis. Our mechanistic findings provide the first insight into an AngII-initiated signaling pathway that regulates PDA angiogenesis. An AT1R-mediated VEGF induction suggests the possibility of AT1R blockade as a novel therapeutic strategy to control angiogenesis in PDA.

Keywords Angiotensin II · Vascular endothelial growth factor · Pancreatic cancer · Angiogenesis

Introduction

Pancreatic ductal adenocarcinoma (PDA) is a devastating disease with long-term survival limited only to patients with early stage tumors who are candidates for surgical resection. The poor prognosis typically associated with PDA is related to several factors including the delayed symptomatic presentation, the tumor's aggressive propensity to metastasize early, and its resistance to conventional therapies.[1] Thus, effective strategies for early diagnosis and treatment are urgently needed to improve survival.

Vascular endothelial growth factor (VEGF) has been associated with tumor progression and resistance in several types of cancers including PDA.[2,3] VEGF acts as a survival factor for certain tumor cells, causing them to be more radioresistant.[4] Recent studies have shown that targeting

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angiogenesis in PDA animal models could have a tumor-suppressing effect.[5,6] Thus, targeting VEGF and the various pathways involved in tumor angiogenesis in PDA may offer effective therapeutic strategy.

The circulating renin–angiotensin system (RAS) is well known to play important roles in the nervous, cardiovascular, and renal systems. The circulatory RAS cascade contains several key components, namely, the precursor angiotensinogen, the critical Zn^{2+}-dependent metallopeptidase, angiotensin I-converting enzyme, angiotensin I (AngI), and the bioactive octapeptide AngII, as well as multiple G protein-coupled receptor subtypes including AngII receptors 1 and 2 (AT1R, AT2R).[7] AT1R and AT2R belong to the heterotrimeric G protein-coupled receptor superfamily. Activation of AT1R leads to coupling with heterotrimeric G proteins and activation of phospholipase C-β, receptor tyrosine kinases, and non-receptor tyrosine kinases.[8] AngII signaling through AT1R activates mitogen-activated protein (MAP) kinase,[9] activates nuclear factor-kappa B (NF-κB), and increases the expression of NF-κB-dependent genes.[10] NF-κB triggers the expression of angiogenic genes such as VEGF.[11] AT2R activation is associated with increased tyrosine phosphatase activity.[12,13]

In addition to the circulating RAS, numerous tissues, including the pancreas,[14,15] possess their own AngII-generating systems that may finely tune specific functions via paracrine/autocrine actions.[16,17] Recent data from our lab have demonstrated that AngII plays a role in PDA angiogenesis through induction of VEGF.[18] The aim of the present work was to investigate the potential role of AT1R in PDA progression and angiogenesis. We explored the signaling mechanisms involved in AngII-mediated induction of VEGF. We also investigated the expression and localization of AT1R with VEGF in two noninvasive precursor lesions of PDA: the pancreatic intraepithelial neoplasms (PanINs) and intraductal papillary mucinous neoplasms (IPMNs).

Materials and Methods

Materials AngII was purchased from AnaSpec Inc. (San Jose, CA, USA). Losartan was from Merck (Whitehouse Station, NJ, USA), and PD123319 was from Sigma (St. Louis, MO, USA). Genistein and sodium orthovanadate were obtained from Sigma, and the mitogen-activated protein kinase (MAPK) inhibitor PD098059 and MAPK/extracellular signal-regulated protein kinase (ERK) kinase 1/2 (MEK1/2) inhibitor U0126 were from Cell Signaling Technology, Inc. (Beverly, MA, USA). Goat polyclonal immunoglobulin G (IgG) antibodies for AT1R were from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and human-specific VEGF enzyme-linked immunosorbent assay (ELISA) kit was from Assay Design (Ann Arbor, MI, USA). Horseradish peroxidase-conjugated donkey anti-goat and anti-rabbit IgG were from Vector Laboratories, Inc. (Burlingame, CA, USA). Rabbit polyclonal antibodies for total and phospho-ERK1/2 (Thr^{185}/Tyr^{189}), total and phospho-c-*Jun* NH2-terminal protein kinase (phospho-JNK; Ser^{473}), and total and phospho-p38 (Thr^{180}/Tyr^{182}) were purchased from Cell Signaling Technology.

Cell lines and culture We used the PK9 and Panc 10.05 cells, originally established from a primary PDA,[19] and HS766T and AsPC-1 cells, originally established from PDA metastasis. The PK9 and HS766T cells were generously donated by Dr. Scott Kern, Johns Hopkins University School of Medicine, Baltimore, MD, and the Panc10.05 and AsPC-1 cells were purchased from the American Type Culture Collection. Cells were cultured at 1×10^4 to near confluence in 96-well plate and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in a humid atmosphere of 5% CO_2/95% air. Cells were treated with AngII (10^{-7} mol/l) for 3 h then collected and examined for VEGF messenger RNA (mRNA) expression. To examine VEGF protein secretion in the media, cells were treated with AngII for 48 h, after which the media were harvested and analyzed. To examine AT1R protein expression, cells were treated with AngII for 24 h, after which the cells were collected and examined. To evaluate the effect of AngII blockade on VEGF production, cells were pre-incubated for 1 h with or without an AT1R blocker, losartan (10 μM) before stimulation with AngII. Cells were also pre-incubated with the AT2R blocker, PD123319 (10 μM). The concentrations used were based upon our preliminary concentration studies, with references to the values of VEGF mRNA and protein expression and based upon our previous studies.[18]

Immunostaining of PDA cells PDA cells grown on the coverglasses were washed with Hanks balanced salt solution, pH 7.4, at 37°C after aspiration of the culture medium. Cells were fixed in 4% formaldehyde in phosphate-buffered saline (PBS) at room temperature for 10 min, washed three times with PBS for 2 min each, and incubated in blocking solution (1% bovine serum albumin in PBS) for 30 min. Cells were then incubated for 1 h at room temperature with AT1R rabbit polyclonal antibody diluted 1:100 in blocking solution. Negative controls were incubated with blocking solution without primary antibody. Cells were then washed three times for 5 min each with PBS and incubated for 30 min at room temperature with biotinylated goat anti-rabbit IgG, diluted 1:200 in PBS (Vector Laboratories), as the secondary antibody. 3,3′-Diaminobenzidine tetrahydrochloride chromogenic substrate (Vector Laboratories) was used according to the

manufacturer's protocol to visualize the chromogenic reaction. Cells were rinsed three times for 5 min each with PBS, counterstained with hematoxylin, mounted on glass slides, and viewed by light microscopy.

RNA extraction and real time reverse transcription polymerase chain reaction Total RNA was isolated from cells using Trizol reagent (Life Technologies, Gaitherburg, MD, USA), according to the manufacturer's protocol. RNAs were quantified, and input amounts were optimized for each amplicon. Primers and probes were designed with the help of Primer Express Software (Applied Biosystems, Foster City, CA, USA). The specificities of AT1R primers were validated using semi-quantitative polymerase chain reaction (PCR). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the house keeping gene. VEGF and GAPDH were purchased as 'assays on demand' (Applied Biosystems) and were utilized according to the manufacturer's instructions. Complementary DNA was prepared, diluted, and subjected to real-time PCR using the TaqMan technology (7500 Sequence Detector; Applied Biosystems). Probes were labeled with a reporter and a quencher. Each sample was analyzed in at least two independent assays with duplicate samples, and the corresponding no-reverse transcriptase mRNA sample was included as a negative control. The GAPDH primers were included in every plate to avoid sample variations. The mRNA level of each sample for each gene was normalized to that of the GAPDH mRNA. The relative mRNA levels were presented as unit values of 2 $[C_{T\ (GAPDH)} - C_{T\ (gene\ of\ interest)}]$, where C_T is the threshold cycle value defined as the fractional cycle number at which the target fluorescent signal passes a fixed threshold above baseline.

Enzyme-linked immunosorbent assay VEGF concentration in the media was measured using a human-specific ELISA kit (Assay Design). Spectrophotometric evaluation of VEGF levels was made by Synergy HT multi-detection Microplate reader (BioTeck, Winooski, VT, USA).

Protein isolation and Western blot analysis Cells were lysed in modified radioimmunoprecipitation assay lysis buffer,[20] and the protein concentrations in the supernatant were determined using the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL, USA). Equal protein concentrations (40 μg) were denatured in a gel loading buffer at 85°C for 5 min, loaded onto 10% sodium dodecyl sulfate-polyacrylamide slab gels, transferred to polyvinylodene difluoride membranes, and incubated at 4°C overnight with rabbit polyclonal antibodies diluted in PBS-Tween 20 against AT1R (1:2,000) and VEGF (1:2,000). Proteins from PDA cells treated with or without AngII were blotted with anti-total and anti-phospho-ERK1/2 MAPK, anti-total and anti-phospho-p38 MAPK, and anti-total and anti-phospho-stress-activated protein kinase (SAPK)/JNK (Cell Signaling Technology). Lysates from the same experiment were separated on two gels and probed with either phospho-specific or total antibody as control for sample variation in protein content. To avoid sample loading errors, β-actin expression was determined in the blots to adjust and normalize the amount of sample loaded. The protein bands were visualized with enhanced chemiluminescence reagents (ECL Plus Western Blotting Detection System; Amersham Pharmacia Biotech), analyzed and intensity quantified using Kodak Electrophoresis Documentation and Analysis System 290 (EDAS 290).

Tissue acquisition Histologically confirmed human PanINs (n=5), IPMNs (n=5), and nonmalignant tissues were obtained from patients who underwent surgical resection at Thomas Jefferson University Hospital between 2005 and 2007. Tissue samples were fixed in neutral formaline for histological processing. All patients signed an appropriate consent for tissue acquisition and study. The study was approved by the Institutional Review Board at Thomas Jefferson University.

Immunohistochemistry To localize AT1R and study its relationship to VEGF in normal tissues and in the PanIN and IPMN lesions, formalin fixed, paraffin-embedded tissue blocks were prepared. Serial sections at 5 μm were incubated overnight at 4°C with rabbit polyclonal antibodies against human AT1R (1:100) and human VEGF (1:500; Santa Cruz Biotechnology). A VectaStain universal elite ABC kit and 3,3′-diaminobenzidine tetrahydrochloride chromogenic substrate (Vector Laboratories) was used according to the manufacturer's protocol to visualize the tissue reaction. Antibody specificities were validated with nonimmune isotype serum. Negative control sections where the primary or secondary antibodies were omitted were also prepared. For quantitative assessment of staining of AT1R and VEGF, images were captured using a color SPOT camera (Diagnostic Instruments, Inc, Sterling Heights, MI, USA) and analyzed using ImagePro plus software. Digital images were captured, and color segmentation was performed to highlight the stained area. The software then calculated this as a percentage of the total defined area. All histological assessment and image analysis were performed on coded, randomized sections by a blinded observer.

Statistical analysis All experiments were performed four to six times. Data were analyzed for statistical significance by analysis of variance (ANOVA) with post hoc Student's t test analysis. These analyses were performed with the assistance of a computer program (JMP 5 Software SAS

Campus Drive, Cary, NC, USA). Differences were considered significant at $p \leq 0.05$.

Results

AngII-mediated stimulation of VEGF mRNA accumulation and protein secretion in PDA cell lines is AT1R dependent We have shown previously that AngII at the physiological concentration of 10^{-7} mol/l significantly induces VEGF mRNA accumulation and protein secretion in PDA cell lines.[18] In this study, in addition to reproducing our data, we determined the receptor that mediates the AngII-induced VEGF gene expression. The AT1R blocker, losartan, and the AT2R blocker PD123319 were added for 1 h before addition of AngII to the cells. Losartan at 10 μM significantly prevented the increase in VEGF by AngII. Pretreatment of the cells with PD123319 had a similar effect on two cell lines (HS776T and Panc 10.05) and no effect on the other cell lines (PK9 and AsPC-1; Fig. 1a). Addition of losartan or PD 123319 alone did not affect VEGF mRNA levels.

Extracellular VEGF protein concentration showed between five- to sevenfold increase in PDA cells after 48 h of AngII stimulation. Losartan at 10 μM prevented the increase in VEGF by AngII in all PDA cell lines (Fig. 1b). The results for AngII-treated cells were expressed as a fold-induction of VEGF protein, taking control (no AngII added) value as 1. These data suggest that the induction of VEGF gene and protein expression in PDA cells by AngII is mediated through AT1R.

AT1R protein expression in PDA cells To evaluate the expression and localization of AT1R in PDA cells, we analyzed the basal AT1R protein expression by Western immunoblotting and stained the cells by immunohistochemistry. As see in Fig. 2a, there was a considerable amount of AT1R protein in PDA cells. AT1R immunore-

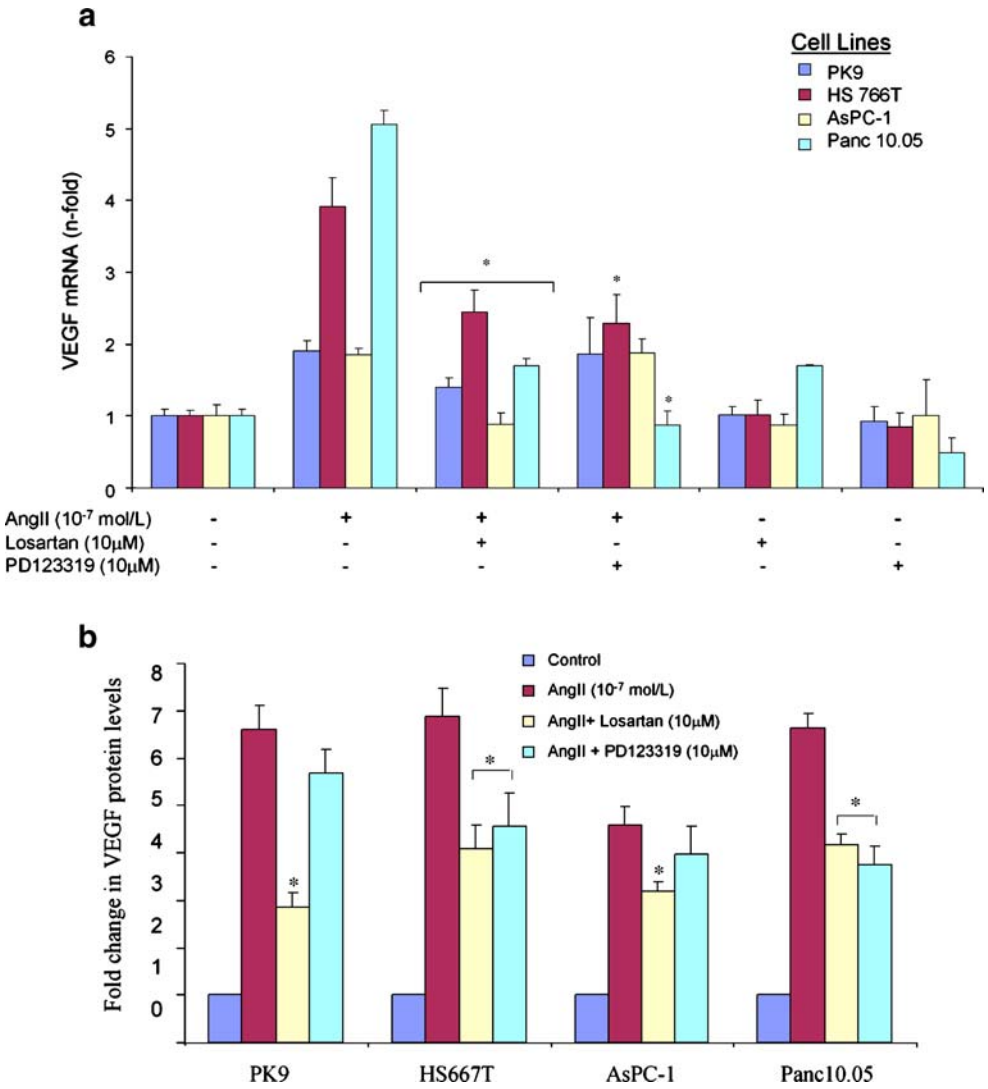

Figure 1 a AngII-induced VEGF mRNA expression in PDA cells is blocked by an AT1R blocker. Cells were pre-incubated for 1 h with AT1R blocker losartan or the AT2R blocker PD123319 before addition of AngII (10^{-7} mol/l) for 3 h. VEGF mRNA content was analyzed by real time reverse transcriptase PCR. Values are expressed as mean±SEM of three experiments. *$p<0.05$ vs. AngII alone treated cells using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t*-test. **b** VEGF protein in culture media was measured using human-specific ELISA kit after adding losartan (10 μM) or PD123319 (10 μM). Significant reduction of VEGF protein secretion by losartan is seen in all PDA cells at 48 h. Relative protein secretion was calculated as a fold-induction of taking control (no AngII added) value as 1. Each experiment was performed three times and repeated three times for reproducibility. *$p<0.05$ vs. AngII treatment, using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t*-test.

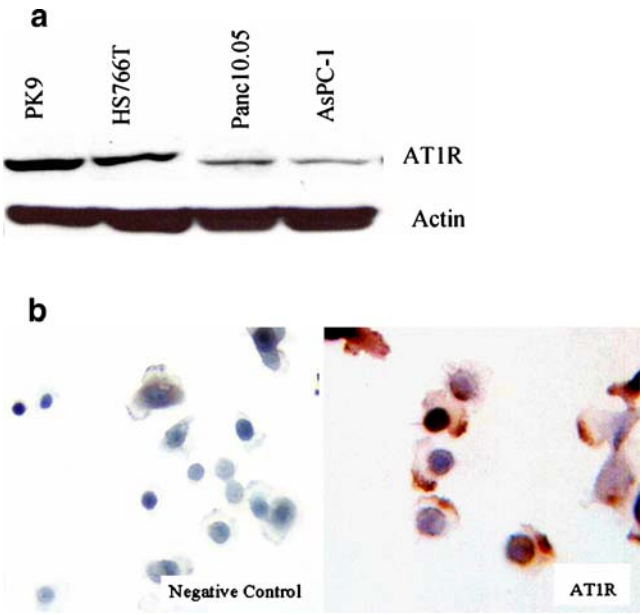

Figure 2 Expression of AT1R protein in PDA cells. **a** Representative Western immunoblot showing the expression of variable levels of AT1R protein in PDA cells. **b** Immunohistochemical staining of PDA cells shows that AT1R is localized mainly to the cell membrane with some intracytoplasmic expression (×200 original magnification).

activity was seen localized mainly on the cell membrane and, to a lesser extent, in the cytoplasm (Fig. 2b).

AngII induces AT1R mRNA and protein expression in PDA cell lines It is not known whether AngII itself could have a regulatory effect on AT1R in PDA cells, an effect that could consequently affect the AngII-VEGF induction. Thus, we evaluated the mRNA expression levels of AT1R in PDA cells in the presence or absence of AngII. Adding AngII induced a significant increase in AT1R mRNA levels (Fig. 3a). These data suggest that increasing AT1R expression by AngII could be one of the mechanisms through which it influences VEGF expression. Analysis of the AT1R protein levels indicated that AngII induced ~1.5-fold upregulation of AT1R protein levels after 24 h (Fig. 3b).

AngII-induced VEGF gene expression requires tyrosine kinase activity AngII signals through AT1R and induces protein tyrosine phosphorylation in many cell types.[21] We investigated whether a similar signaling pathway mediates AngII-induced VEGF gene expression in PDA cells. To determine the involvement of protein kinase in AngII-induced VEGF expression, PDA cells were pretreated with or without the specific tyrosine kinase inhibitor genistein (60 μM) for 30 min. AngII-induced VEGF mRNA accumulation was significantly inhibited by genistein in all PDA cell lines (Fig. 4). Genistein alone also reduced the basal VEGF mRNA levels (Fig. 4). To determine whether an increase in tyrosine phosphorylation induces VEGF mRNA accumulation, PDA cells were exposed to the protein tyrosine phosphatase inhibitor, sodium orthovanadate (100 μM) for 3 h. Sodium orthovanadate induced a

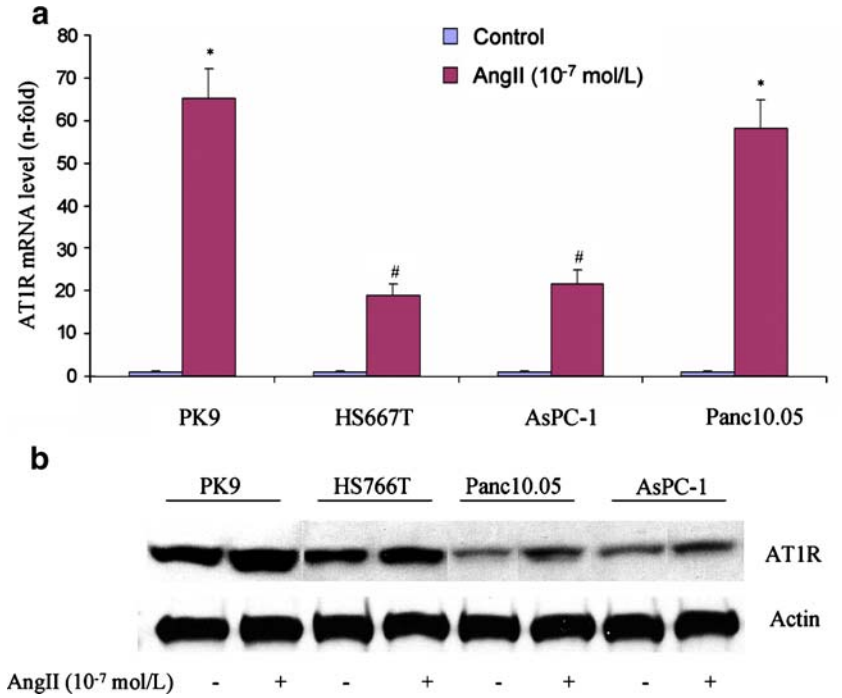

Figure 3 AngII induces AT1R mRNA in PDA cells. Cells were treated with AngII (10^{-7} mol/l) for 3 h. Twenty- to 60-fold upregulation of AT1R mRNA in PDA cells. Values are expressed as mean±SEM of three experiments. *$p<0.002$, #$p<0.05$ vs. control levels. **b** Representative Western immunoblot showing ~1.5-fold induction of AT1R protein in PDA cells. Cells were treated with AngII (10−7mol/L) for 24 h.

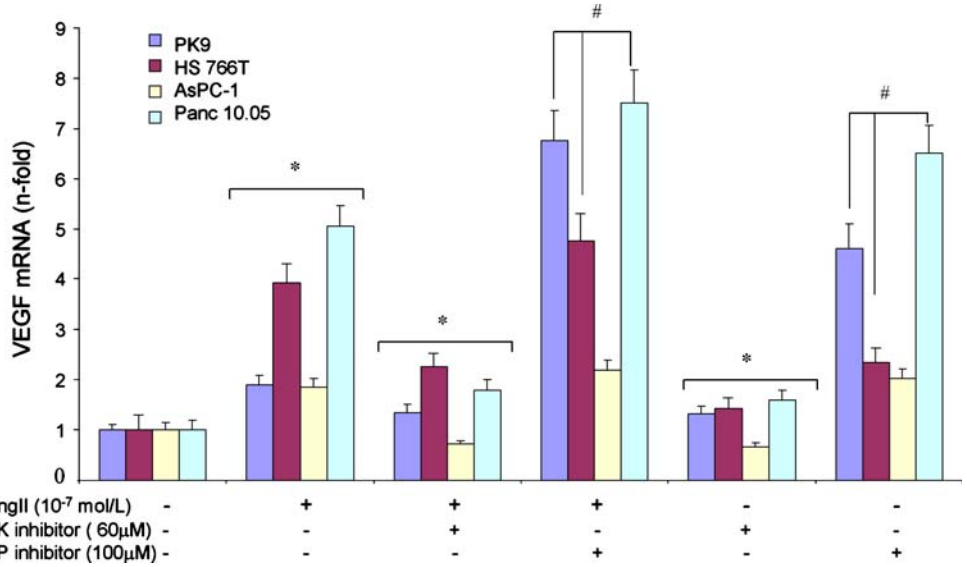

Figure 4 AngII-induced VEGF gene expression requires tyrosine kinase activity. PDA cells were pretreated with protein tyrosine kinase inhibitor genistein (60 μM) for 1 h or protein tyrosine phosphatase inhibitor sodium orthovanadate (100 μM) for 2 h then exposed to AngII (10^{-7} mol/l) for 3 h. VEGF mRNA levels were determined by real time PCR. Sodium orthovanadate induced VEGF mRNA accumulation in PDA cells. *$p<0.05$, #$p<0.02$ vs. AngII treated cells using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t*-test.

significant increase in VEGF mRNA accumulation in all PDA cell lines (Fig. 4). Sodium orthovanadate alone also significantly increased VEGF mRNA expression. These data suggest that protein tyrosine phosphorylation is essential for the induction of VEGF by AngII.

AngII-induced VEGF gene expression requires ERK1/2 MAP kinase activity AngII signaling through AT1R activates members of the MAPK family, ERK1/2, p38 MAPK, and JNK/SAPK in different tissues.[22,23] We tested whether AngII-induced VEGF mRNA expression involves activation of MAPK in PDA cells. After washing, the cells were lysed and 40-μg protein aliquots were subjected to Western blot analysis. We probed the blots with antibodies specific for phosphorylated ERK1/2 (Thr183/Tyr185), phosphorylated p38 (Thr180/Tyr182), and phosphorylated JNK/SAPK (Thr183/Tyr185).

AngII increased ERK1/2 phosphorylation within 5–30 min of treatment in the different cell lines (Fig. 5a), but not p38 or JNK (data not shown). Preincubation of the cells with MAPK inhibitor PD098059 or the MEK1/2 selective inhibitor U0126 abolished the AngII-induced VEGF gene expression (Fig. 5b). This suggests that specific activation of ERK1/2 kinase may play an important role in AngII-induced VEGF expression in PDA cells.

Expression of AT1R and VEGF in PDA precursor lesions As shown in Fig. 6a, in the normal pancreas, immunoreactivity of AT1R was focally detected in some ductal cells and in some stromal cells, whereas VEGF expression could be detected in the connective tissue surrounding the ducts and in most of the ductal cells. AT1R protein showed increased expression in the ductal cells undergoing metaplasia. In PanIN-3 lesions, intense expression of AT1R could be seen in the transforming cells. VEGF expression followed the same pattern. In IPMNs, a more heterogeneous expression of AT1R and VEGF could be seen; however, the two proteins maintained their colocalization in all the ductal cells. The close proximity between an AngII receptor and VEGF supports their potential paracrine/autocrine interaction in the premalignant lesions in vivo. Figure 6b shows quantitation of ductal AT1R (I) in PanIN and IPMN lesions. Both lesions showed higher immunoreactivity than normal ducts with more significant difference in the PanINs. Similar data are shown for ductal VEGF (II).

Discussion

The molecular and cellular mechanisms that contribute to the insidious nature of PDA are poorly understood. VEGF is an angiogenic factor that plays an important role in supporting the aberrant growth of PDA through maintaining its blood supply.[2,24,25] Previous studies have shown that high levels of VEGF correlate with lymph node metastasis and poor prognosis in PDA.[25,26] We recently demonstrated that AngII is a potent stimulator of VEGF expression in PDA cells.[18] In the current study, we investigated the signaling cascades involved in regulation of VEGF by AngII. We provide the first insight into an AngII-initiated signaling pathway that regulates PDA angiogenesis and introduce AT1R as a novel participant in PDA progression and angiogenesis.

We show here that AngII-induced VEGF gene expression occurs through an AT1R-mediated mechanism (Fig. 1a and b). Addition of losartan, an AT1R antagonist prevented the AngII-VEGF production in all PDA cell lines, an effect that was observed in only two of the cell lines when PD123319, an AT2R antagonist, was added. The fact that

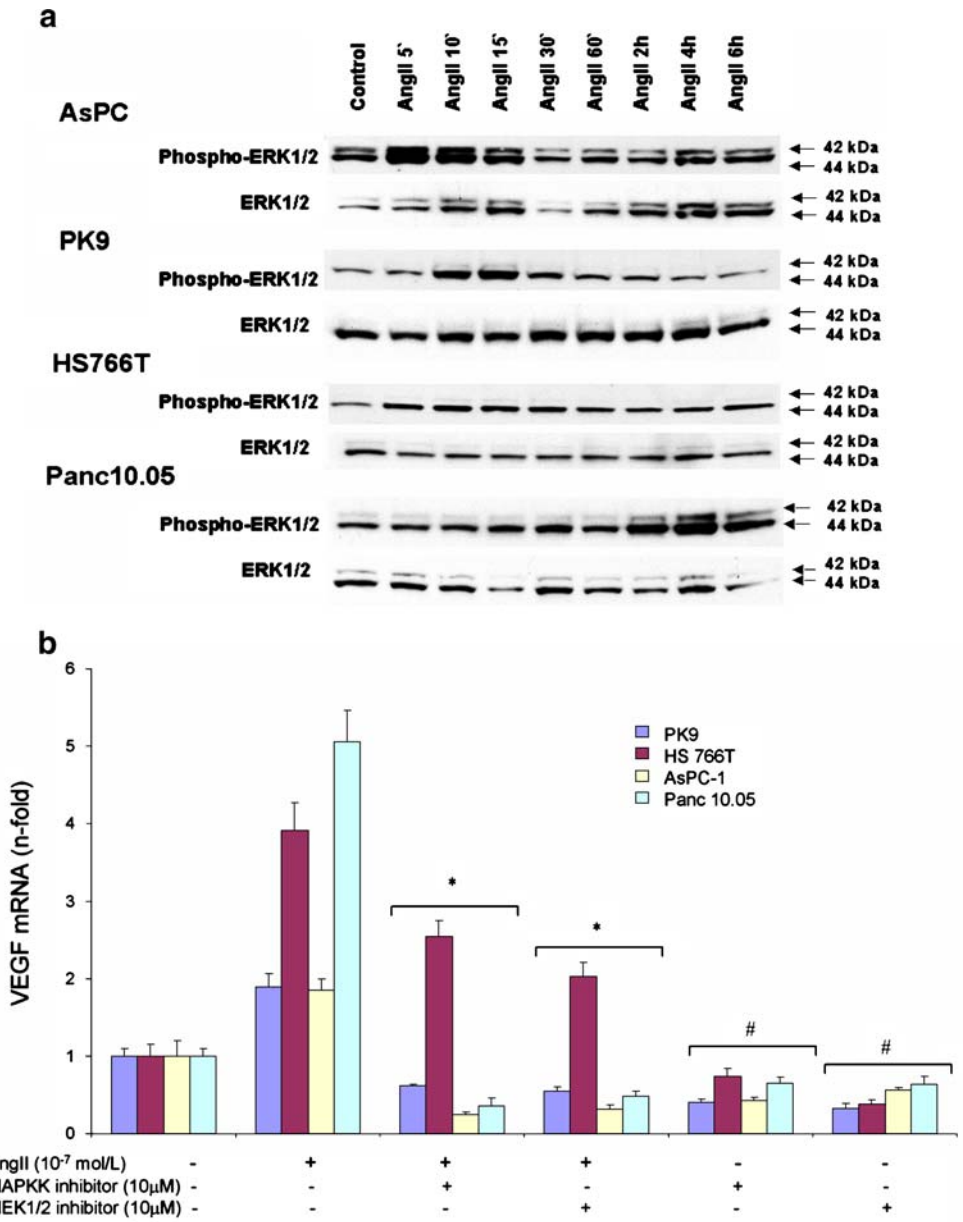

Figure 5 AngII-induced VEGF gene expression requires ERK1/2 MAPK activity. **a** Time-dependent activation of ERK1/2 MAPK signaling pathway by AngII in PDA cells. Representative Western blot probed with phospho-antibody against the activated from of ERK1/2 showing increased phosphorylation of ERK1/2 after incubation of AngII (10^{-7} mol/l) between 5 and 30 min. Blots were stripped and developed with anti-total ERK1/2 as control for equal protein loading. **b** Effect of MAPK kinase inhibitor PD098059 and MEK1/2 inhibitor, U0126, on AngII induced increase in VEGF mRNA. Cells were pretreated with the inhibitors (10 μM) for 10 min before incubation with AngII (10^{-7} mol/l) for 3 h. Data represent three independent experiments. *$p<0.02$, #$p<0.05$ vs. AngII treated cells using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t*-test.

each cell line expresses different levels of AT1R receptor might explain their different responses to the receptor antagonists. AT1R and AT2R belong to the heterotrimeric G protein-coupled receptor superfamily. Several studies have shown that elevated levels of AT1R mRNA increase the cell functional response to AngII.[27,28] We hypothesized that AngII could have a regulatory effect on AT1R in PDA cells that could influence stimulation of VEGF by AngII. Our data show that all PDA cell lines express AT1R mRNA and protein. AT1R protein was localized mainly on the cell membrane and, to a lesser extent, in the cytoplasm (Fig. 2b). We demonstrate for the first time that the addition of AngII to PDA cells significantly increased AT1R mRNA and protein expression, suggesting that upregulation of AT1R could be one of the mechanisms by which AngII induces VEGF in PDA cells. It would be of interest to investigate the effect of AngII on the expression levels of AT2R and to determine whether AngII-mediated regulation of AT2R is another mechanism of influencing VEGF production and contributing to the final cell response. Studies in this regard are currently ongoing in our laboratory.

MAPKs encoded by the ERK genes are a family of serine/threonine protein kinases activated as early responses to a variety of stimuli involved in cell growth, transformation, and differentiation.[29] They are also involved in the activation of AP-1 and NF-κB.[30] Two isoforms of ERK referred to as p44 (ERK1) and p42 (ERK2) are activated by phosphorylation of threonine and tyrosine residues by MAPK, also called MEK.[31,32] AngII rapidly activates MAPKs, particularly ERK1 and ERK2, in vascular smooth

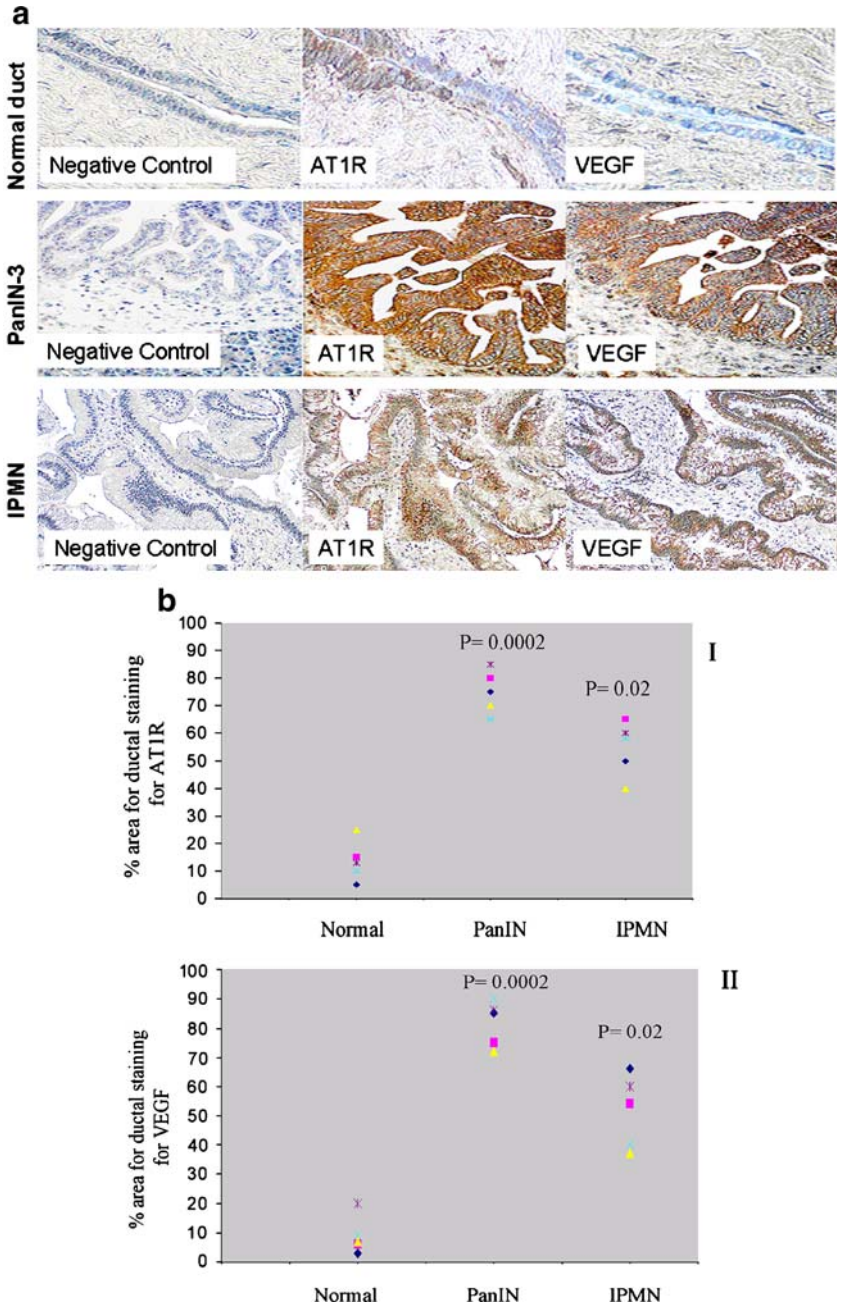

Figure 6 **a** Localization of AT1R and VEGF in normal human pancreas and PDA precursor lesions. Paraffin-embedded pancreatic serial sections were stained with AT1R and VEGF antibodies. In the normal tissues, AT1R expression is seen focally in some ductal cell. Normal pancreatic ductal epithelial cells express VEGF. In PanIN lesions, AT1R staining was more intense when compared to IPMNs, which showed heterogeneous immunoreactivity. VEGF colocalized with AT1R in both lesions. Negative control samples where the primary antibody was not added did not show nonspecific reaction (×200 original magnification). **b** Pancreatic ductal staining of AT1R (I) and VEGF (II). Staining was analyzed by image analysis and expressed as percentage area of ductal cells stained as described in "Materials and Methods." There was minimal staining in normal ducts. PanIN lesions showed markedly significant increase in ductal staining.

muscle cells.[29,33] Using selective inhibitors for MAPK activation, PD098059 and U0126, we demonstrated that AngII-induced VEGF mRNA occurs through a MEK-sensitive mechanism (Fig. 4b). AngII had no effect within this time period on the phosphorylation of either p38 or SAPK/JNK. Further studies are now required to delineate the specific signaling pathway by which AngII ultimately modulates VEGF synthesis in the PDA cells.

Morphologic studies in conjunction with molecular analysis have helped establish distinct noninvasive precursor lesions of PDA: the microscopic PanINs, which usually involve ducts smaller than 5 mm, and the macroscopic IPMNs, which usually involve the main duct and are associated with mucin accumulation and pancreatic ductal dilatation. Diagnosis of pancreatic lesions at the precursor level and their resection is often curative.[34] No data have been reported previously concerning the regulation of AT1R in PDA precursor lesions and its correlation with VEGF. In the present study, we demonstrate for the first time that high levels of AT1R are constitutively expressed in PanIN and IPMN lesions, colocalizing with VEGF, and suggesting an endogenous autocrine/paracrine interaction.

Several studies have shown that the use of angiotensin blockade therapies could reduce tumor growth and metas-

tasis in xenograft models of cancer.[7,35] The mechanism(s) mediating these effects are not clearly understood. AngII itself influences cell survival and proliferation,[18,36] but the impact of the long-term effects of AngII on the precursor lesions in the pancreas is yet to be determined.

The novel data from our study add new information about the role of the AT1R as an effective participant in PDA angiogenesis and suggest that targeting AT1R could be used as a novel therapeutic strategy to reduce VEGF levels in the ductal cells. Such studies are currently ongoing in our lab.

Our study demonstrates that AngII elicits a proangiogenic response in PDA cells by stimulation of VEGF production through an AT1R-ERK1/2-dependent mechanism. We also show that high expression levels of AT1R colocalize with VEGF in PDA precursor lesions. The existence of AT1R as a potential mediator of AngII induction of VEGF in PDA cells is unique and could provide a novel target for suppressing angiogenesis in PDA postoperatively or in individuals with a strong family history of pancreatic cancer.

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References

1. Sohn TA, Yeo CJ, Cameron JL, Koniaris L, Kaushal S, Abrams RA, Sauter PK, Coleman J, Hruban RH, Lillemoe KD. Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. J Gastrointest Surg 2000;4:567–579.
2. Korc M. Pathways for aberrant angiogenesis in pancreatic cancer. Mol Cancer 2003;7:2–8.
3. Seo Y, Baba H, Fukuda T, Takashima M, Sugimachi K. High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. Cancer 2000;88:2239–2245.
4. Gupta VK, Jaskowiak NT, Beckett MA, Mauceri HJ, Grunstein J, Johnson RS, Calvin DA, Nodzenski E, Pejovic M, Kufe DW, Posner MC, Weichselbaum RR. Vascular endothelial growth factor enhances endothelial cell survival and tumor radioresistance. Cancer J 2002;8:47–54.
5. Harmey JH, Bouchier-Hayes D. Vascular endothelial growth factor (VEGF), a survival factor for tumour cells: implications for anti-angiogenic therapy. Bioessays 2002;24:280–283.
6. Hotz HG, Reber HA, Hotz B, Sanghavi PC, Yu T, Foitzik T, Buhr HJ, Hines OJ. Angiogenesis inhibitor TNP-470 reduces human pancreatic cancer growth. J Gastrointest Surg 2001;5:131–138.
7. De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger TH. The angiotensin II receptors. Pharmacol Rev 2000;52:415–472.
8. Kim S, Iwao H. Stress and vascular responses: mitogen-activated protein kinases and activator protein-1 as promising therapeutic targets of vascular remodeling. J Pharmacol Sci 2003;9:177–181.
9. Zhang A, Ding G, Huang S, Wu Y, Pan X, Guan X, Chen R, Yang T. c-*Jun* NH2-terminal kinase mediation of angiotensin II-induced proliferation of human mesangial cells. Am J Physiol Renal Physiol 2005;88:F1118–F1124.
10. Amaya K, Ohta T, Kitagawa H, Kayahara M, Takamura H, Fujimura T, Nishimura G, Shimizu K, Miwa K. Angiotensin II activates MAP kinase and NF-kappaB through angiotensin II type I receptor in human pancreatic cancer cells. Int J Oncol 2004;4:849–856.
11. Schmidt D, Textor B, Pein OT, Licht AH, Andrecht S, Sator-Schmitt M, Fusenig NE, Angel P, Schorpp-Kistner M. Critical role for NF-kappaB-induced JunB in VEGF regulation and tumor angiogenesis. EMBO J 2007;26:710–719.
12. Takahasi K, Bardhan S, Kambayashi Y, Shirai H, Inagami T. Protein tyrosine phosphatase inhibition by angiotensin II in rat pheochromocytoma cells through type 2 receptor. Biochem Biophys Res Commun 1994;198:60–66.
13. Bottari SP, King IN, Reichlin S, Dahlstroem I, Lydon N, de Gasparo M. The AT_2 receptor stimulates protein tyrosine phosphatase activity and mediates inhibition of particulate guanylate cyclase. Biochem Biophys Res Commun 1992; 183:206–211.
14. Tahmasebi M, Puddefoot JR, Inwang ER, Vinson GP. The tissue renin-angiotensin system in human pancreas. J Endocrinol 1999;161:317–322.
15. Leung PS, Chappell MC. A local pancreatic renin–angiotensin system: endocrine and exocrine roles. Int J Biochem Cell Biol 2003;35:838–846.
16. Campbell DJ, Habener J. Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. J Clin Invest 1986;78:31–39.
17. Campbell DJ. Circulating and tissue angiotensin systems. J Clin Invest 1987;79:1–6.
18. Arafat HA, Gong Q, Chipitsyna G, Rizvi A, Saa CT, Yeo CJ. Antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor blockers in pancreatic ductal adenocarcinoma. J Am Coll Surg 2007;20:996–1005.
19. Kobari M, Hisano H, Matsuno S, Sato T, Kan M, Tachibana T. Establishment of six human pancreatic cancer cell lines and their sensitivities to anti-tumor drugs. Tohoku J Exp Med 1986;150:231–248.
20. Gould KL, Hunter T. Platelet-derived growth factor induces multisite phosphorylation of pp60c-src and increases its protein-tyrosine kinase activity. Molec Cell Biol 1988;8:3345–3356.
21. Menard J, Clauser E, Bouhnik J, Corvol P. Angiotensinogen: biochemical aspects. In Robertson JIS, Nichollas MS, eds. The renin angiotensin system. London: Gower Medical Publishing, 1993, pp 8.1–8.10.
22. Kim S, Iwao H. Stress and vascular responses: mitogen-activated protein kinases and activator protein-1 as promising therapeutic targets of vascular remodeling. J Pharmacol Sci 2003;9:177–181.
23. Zhang A, Ding G, Huang S, Wu Y, Pan X, Guan X, Chen R, Yang T. c-*Jun* NH2-terminal kinase mediation of angiotensin II-induced proliferation of human mesangial cells. Am J Physiol Renal Physiol 2005;88:F1118–F1124.
24. Itakura J, Ishiwata T, Shen B, Kornmann M, Korc M. Concomitant over-expression of vascular endothelial growth factor and its receptors in pancreatic cancer. Int J Cancer 2000;85:27–34.
25. Itakura J, Ishiwata T, Friess H, Fujii H, Matsumoto Y, Buchler MW, Korc M. Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. Clin Cancer Res 1997;3:1309–1316.
26. Buchler P, Reber HA, Buchler MW, Friess H, Hines OJ. EGF-RII influences the prognosis of pancreatic cancer. Ann Surg 2002;23:738–749.
27. Nickenig G, Sachinidis A, Michaelsen F, Bohm M, Seewald S, Vetter H. Upregulation of vascular angiotensin II receptor gene expression by low-density lipoprotein in vascular smooth muscle cells. Circulation 1997;95:473–478.

28. Chipitsyna G, Gong Q, Gray CF, Haroon Y, Kamer E, Arafat HA. Induction of monocyte chemoattractant protein-1 expression by angiotensin II in the pancreatic islets and beta-cells. Endocrinology 2007;148:2198–2208.
29. Duff JL, Marrero MB, Paxton WG, Schieffer B, Bernstein KE, Berk BC. Angiotensin II signal transduction and the mitogen-activated protein kinase pathway. Cardiovasc Res 1995;30:511–517.
30. Hirano M, Osada S, Aoki T, Hirai S, Hosaka M, Inoue J, Ohno S. MEK kinase is involved in tumor necrosis factor alpha-induced NF-kappaB activation and degradation of IkappaB-alpha. J Biol Chem 1996;271:13234–13238.
31. Crews CM, Alessandrini A, Erikson RL. The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. Science 1992;258:478–780.
32. Cobb MH, Goldsmith EJ. How MAP kinases are regulated. J Biol Chem 1995;270:14843–14846.
33. Duff JL, Berk BC, Corson MA. Angiotensin II stimulates the pp44 and pp42 mitogen-activated protein kinases in cultured rat aortic smooth muscle cells. Biochem Biophys Res Commun 1992;188:257–264.
34. Hruban RH, Wilentz RE, Maitra A. Identification and analysis of precursors to invasive pancreatic cancer. Methods Mol Med 2005;103:1–13.
35. Tokunaga T, Abe Y, Tsuchida T, Hatanaka H, Oshika Y, Tomisawa M, Yoshimura M, Ohnishi Y, Kijima H, Yamazaki H, Ueyama Y, Nakamura M. Ribozyme mediated cleavage of cell-associated isoform of vascular endothelial growth factor inhibits liver metastasis of a pancreatic cancer cell line. Int J Oncol 2002;21:1027–1032.
36. Won SM, Park YH, Kim HJ, Park KM, Lee WJ. Catechins inhibit angiotensin II–induced vascular smooth muscle cell proliferation via mitogen–activated protein kinase pathway. Exp Mol Med 2006;38:525–534.

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Indocyanine Green Plasma Disappearance Rate During the Anhepatic Phase of Orthotopic Liver Transplantation

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Abstract Non-invasive pulse spectrophotometry to measure indocyanine green (ICG) elimination correlates well with the conventional invasive ICG clearance test. Nevertheless, the precision of this method remains unclear for any application, including small-for-size liver remnants. We therefore measured ICG plasma disappearance rate (PDR) during the anhepatic phase of orthotopic liver transplantation using pulse spectrophotometry. Measurements were done in 24 patients. The median PDR after exclusion of two outliers and two patients with inconstant signal was 1.55%/min (95% confidence interval [CI]=0.8–2.2). No correlation with patient age, gender, body mass, blood loss, administration of fresh frozen plasma, norepinephrine dose, postoperative albumin (serum), or difference in pre and post transplant body weight was detected. In conclusion, we found an ICG-PDR different from zero in the anhepatic phase, an overestimation that may arise in particular from a redistribution into the interstitial space. If ICG pulse spectrophotometry is used to measure functional hepatic reserve, the verified average difference from zero (1.55%/min) determined in our study needs to be taken into account.

Keywords Indocyanine green · Pulse spectrophotometry · Orthotopic liver transplantation · Small-for-size liver remnant

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Introduction

In addition to the expected volume of the liver remnant,[1] quantitative (or "dynamic") liver function tests have been used to predict the risk of hepatic failure after liver resection.[2,3] Indocyanine green (ICG) is a nontoxic dye extracted exclusively by hepatic parenchymal cells without enterohepatic circulation and excreted entirely into the bile.[2,4] The ICG elimination test was originally invented to determine hepatic blood flow, but it is in fact an integrative measure of both hepatic perfusion and excretory function.[5–7] Therefore, ICG elimination testing has been used for the past 20 years in the context of hepatic resection[8] and to assess liver functional reserve in hepatic injury and septic states; in addition, it was found to be as accurate as more complex scores (e.g., APACHE II and SAPS II) with respect to outcome prediction.[9–11] Moreover, ICG elimination tests are intended to determine the suitability of a potential donor liver and to recognize early graft dysfunction resulting from, for example, primary delayed or nonfunction of the graft, technical complications, and acute or chronic graft rejection in the transplant setting.[12–14]

Table 1 The Demographic Data of all Patients

N	Diagnosis	Age (years)	Gender	BMI (kg/m^2)
1	CHC	64	F	25.8
2	CHC/HCC	49	M	28.0
3	CHC	47	M	27.0
4	CHC/ETC	39	F	25.1
5	CHB/HCC	48	M	27.9
6	NASH	53	M	46.0
7	CHC/HCC	66	M	20.8
8	CHC/CHB	50	F	29.3
9	IP	40	F	20.9
10	CHC	62	M	29.4
11	PBC	63	F	22.1
12	CC	56	F	24.0
13	ETC	55	M	24.1
14	MCLC	62	F	25.5
15	CHC	57	M	34.8
16	ETC	53	F	24.7
17	IP	57	F	25.0
18	CHB/HCC	49	F	30.4
19	CHC/HCC	68	M	27.0
20	PBC	59	F	19.0
21	AIH	69	F	22.0
22	UC	61	M	24.0
23	CHB	41	M	28.0
24	NASH	59	F	29.0
Median[a]	–	56 (49–62)	–	26.4 (24.7–28.0)

CHC: chronic hepatitis C, *CHB*: chronic hepatitis B, *ETC*: ethyltoxic cirrhosis, PBC: primary biliary cirrhosis, *NASH*: nonalcoholic steatohepatitis cirrhosis, *CC*: cryptogenic cirrhosis, *MCLC*: multiple congenital liver cysts, *IP*: intoxication with paracetamol, *AIH*: autoimmune hepatitis, *UC*: diagnosis unclear, *F*: female, *M*: male, *BMI*: body mass index

[a] Median is without patients 11 and 15 (the outliers), and 9 and 12 (unstable signal). Values in parentheses are the 95%CI.

Noninvasive pulse spectrophotometry[15] allows the monitoring of appearance and disappearance of ICG by a photometric device performing transcutaneous measurements in real time. It is supposed to correlate well with the conventional invasive ICG clearance test.[16–18] In the postoperative course, a plasma disappearance rate (PDR) >5.0%/min seems to indicate hepatic recovery after extended liver resections.[19] In particular, in small-for-size liver remnants (<0.8% of body weight)[1,20] and in fulminant hepatic failure, real-time prognostic tests are urgently needed as a decision aid for indication of artificial liver support systems like the molecular adsorbent recirculating system (MARS).[21,22] To date, clear indications for the postoperative initiation of MARS treatment and criteria for the discontinuation of therapy have not been established.[19]

Unfortunately, data are thus far lacking about the precision of ICG elimination tests in situations with limited residual hepatic functional reserves.[5,7] We sought to test the accuracy of ICG-PDR measurements by pulse spectrophotometry in a situation without hepatic perfusion and/or excretion and thus tested the hypothesis that ICG-PDR is zero or close to zero in patients during the anhepatic phase of orthotopic liver transplantation (OLT).

Materials and Methods

A total of 24 consecutive patients who underwent OLT between March 2005 and April 2007 in our institution were included in the current study after giving informed consent (institutional approval: 1.05.01.30.-17).

During the anhepatic phase of OLT, noninvasive measurement of ICG-PDR was performed in all patients. We rapidly injected 25 mg of ICG (Monopeak Indocyanine Green, SERB SA, Paris) in a constant volume of 10 ml saline via the jugular catheter. Urine concentrations of ICG were measured in four patients after ICG injection.

ICG Pulse Spectrophotometry

Pulse-dye densitometry (LiMON, Pulsion Medical Systems, Munich, Germany) was used for transcutaneous noninvasive measurement of the blood ICG concentration. During the first 5–10 min after intravenous injection, ICG concentrations were monitored at every pulse interval via pulse spectrophotometry. ICG-PDR as a relative measure describes fading of the initial ICG concentration (normalized to 100%) and is expressed as percentage change over time. Because ICG-PDR$=100\times k=100\times \ln 2 / t^{1/2}$, k (elimination rate constant) and $t^{1/2}$ (half-life), which have been

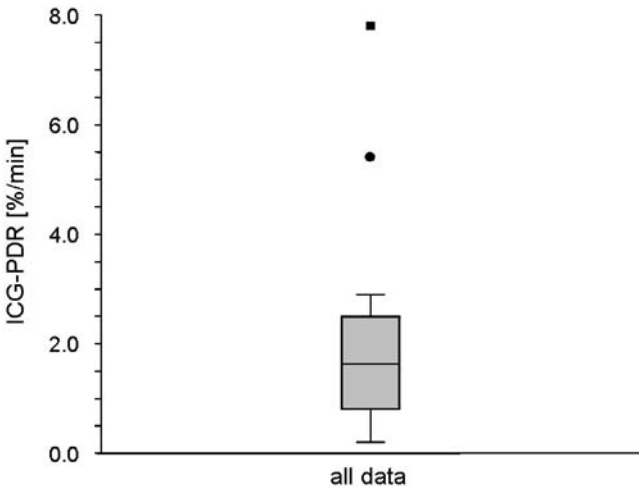

Figure 1 The indocyanine green plasma disappearance rate (ICG-PDR) values during the anhepatic phase of orthotopic liver transplantation in 22 patients are shown. The *extent of the box* indicates the 25th and 75th percentiles (inner-quartile range). The *line inside the box* shows the value of the 50th percentile (median). *Capped bars* indicate the last observations that are within the inner fence (25th or 75th, respectively, ±1.5 times the inner-quartile range), and *symbols* mark outliers (beyond inner fence).

Table 2 Results of Spectrophotometry and Possible Influencing Parameters

N	ICG PDR (%/min)	PCS[a]	NE[b] (μg/h)	Blood loss[c] (ml)	FFP[d] (unit)	Albumin[e] (g/l)	Δ weight[f] (kg)
1	2.2	Yes	1,200	1,000	0	26	4.3
2	2.6	No	0	300	0	25	3.8
3	0.2	Yes	1,000	1,000	4	30	0.2
4	2.5	Yes	800	2,000	6	24	0.2
5	0.8	No	400	300	0	25	4.1
6	1.0	No	1,500	2,500	22	24	−1.0
7	0.9	No	0	500	0	43	4.2
8	1.9	Yes	1,200	1,500	2	19	8.0
9	i.s.	No	1,000	200	0	n.a.	n.a.
10	2.5	Yes	800	1,700	4	18	−0.9
11	5.4	Yes	600	150	0	10	3.7
12	i.s.	Yes	400	1,200	3	n.a.	n.a.
13	0.8	Yes	240	1,700	0	25	0.5
14	2.9	Yes	140	1,200	0	20	5.6
15	7.8	Yes	80	5,000	9	28	−0.2
16	0.2	Yes	0	2,250	0	25	9.1
17	2.0	No	100	1,300	0	21	0.7
18	2.5	Yes	0	1,000	0	21	0.8
19	1.5	No	600	2,300	0	22	−3.0
20	0.6	Yes	100	800	5	21	n.a.
21	1.0	Yes	200	1,000	0	19	5.6
22	1.7	Yes	360	500	8	27	0.7
23	1.6	Yes	50 PE	2,300	5	24	3.1
24	0.7	Yes	180	3,000	5	21	1.1
Median[g]	1.55 (0.8–2.2)	n.a.	240 (100–800)	1,250 (1,000–2,000)	0 (0–5)	24 (21–25)	1.1 (0.2–4.2)
Correlation with ICG-PDR (R^2)[h]	n.a.	$p>0.05$[i]	0.003 ($p>0.05$)[j]	0.023 ($p>0.05$)[j]	0.021 ($p>0.05$)[j]	0.112 ($p>0.05$)[j]	0.008 ($p>0.05$)[j]

ICG-PDR: indocyanine green plasma disappearance rate, *i.s.*: instable signal, *PCS*: portacaval shunt, *NE*: norepinephrine, *PE*: phenylephrine, *FFP*: fresh frozen plasma, *n.a.*: not available

[a] Application of PCS during anhepatic phase.

[b] Application of NE dose during anhepatic phase.

[c] Blood loss from the beginning of the operation until the anhepatic phase.

[d] Administered units of FFP from the beginning of the operation until the anhepatic phase.

[e] Albumin (serum) on third postoperative day.

[f] Δ weight = difference of body weight before and 3 days after the operation.

[g] Median is without patients 11 and 15 (the outliers), and 9 and 12 (unstable signal). Values in parentheses are the 95%CI.

[h] Coefficient of determination.

[i] Wilcoxon rank-sum test.

[j] *t*-test.

used in earlier publications, can easily be derived. Normal values for ICG blood clearance are considered to be >18%/min.[6]

Spectrophotometric Measurement of ICG in Urine

Urine was collected for 115 to 210 min after injection of the dye. Samples were afterwards centrifuged. The ICG concentration in the supernatant was read against the blank at 800 nm on a Perkin Elmer Lambda 15 (Waltham, MA, USA) photometer.

Renal ICG-PDR (ICG-PDR$_r$) was calculated from the absolute ICG amount in urine (u) collected over a time period (t) after the injection of a dose (d) according to the equation:

$$\text{ICG-PDR}_r = 100 \times \left(1 - ((d-u)/d)^{1/t}\right)$$

Statistical Analysis

Data are presented as the median with the 95%CI in parentheses. Values beyond the inner fence (25th or 75th

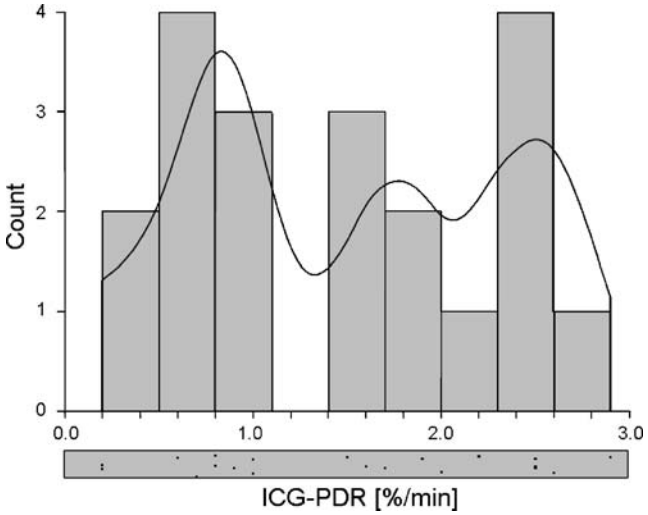

Figure 2 Histogram of ICG-PDR values with density trace (*black line*) and dot plot along the horizontal axis (raw data).

percentiles, respectively, ±1.5 times the inner-quartile range) were defined as outliers. Normality of data was proved with the Kolmogorov–Smirnov and D'Agostino Kurtosis tests. Descriptive statistics, group comparisons (Wilcoxon rank-sum test), and linear regressions were made using statistical software (NCSS 2004, Kaysville, Utah, USA). To test the linear regression, the coefficient of determination (R^2=square of the correlation coefficient) was determined and its significance tested with a *t*-test.

Results

The indications for OLT and the demographic data of all 24 patients are shown in Table 1. Two patients had to be excluded from further analysis because of an unstable percutaneous ICG signal.

After exclusion of two outliers as shown in Fig. 1 (ICG-PDR=5.4%/min and 7.8%/min), the median ICG-PDR was 1.55%/min (95%CI=0.8–2.2).

We performed univariate linear regression analysis with the following independent variables: norepinephrine during the anhepatic phase of OLT, blood loss, units of fresh frozen plasma given until the anhepatic phase, serum albumin on the third postoperative day, body weight before and 3 days after the operation, the time course of liquid balance (not depicted), and the tidal volume during the anhepatic phase (not depicted). As Table 2 shows, we identified no correlation of any of these variables with ICG-PDR (%/min); all coefficient of determination (R^2) values were close to zero and $p>0.05$. Furthermore, we found no difference in ICG-PDR between the groups with or without application of a temporary portacaval shunt ($p>0.05$).

The renal ICG-PDR$_r$ was found to be minute in comparison to the ICG-PDR values derived from the pulse spectrometer (Fig. 2, Table 3).

Discussion

Using a commercially available bedside monitor system, we unexpectedly found that ICG-PDR values showed considerable variability and differed from zero in the anhepatic phase of OLT in 22 patients. Of all the examined clinical cofactors, we found no correlation with ICG-PDR. Because neither blood loss nor administration of fresh frozen plasma before the anhepatic phase seemed to influence ICG-PDR, dilution during the measurement as a consequence of volume challenge after hemorrhage does not explain the ICG-PDR values different from zero. Alterations of the otherwise high protein binding of ICG as a consequence of volume or fresh frozen plasma administration are thus also very unlikely as an explanation for our findings.[23]

In addition, the tidal volume and therefore hypocapnia or hypercapnia do not affect ICG elimination by mechanisms other than changes in hepatic flow and hepatic function, as has already been established in an animal model.[24] Catecholamines and a temporary portacaval shunt seem to influence ICG-PDR only via changes in hepatic perfusion.[25,26] The extremely low amounts of ICG found in urine in our experiments also exclude renal excretion as a reason for the observed plasma disappearance of ICG. Technical failure because of detecting probe malpositioning and patient movement are excludable because the pulse spectrophotometer system used in this study does not allow measurements with weak or inconsistent signals. Spontaneous decay of ICG is also a rather implausible explanation

Table 3 ICG Measurement in Urine in Four Patients

ICG-PDR[a] (%/min)	Time[b] (min)	ICG in urine[c] (mg)	ICG-PDR$_r$[d] (%/min)
1	115	0.050	0.0017
1.7	120	0.011	0.0004
1.6	120	0.003	0.0001
0.7	210	0.047	0.0009

[a] ICG-PDR was measured by pulse spectrophotometry (LiMON).
[b] Urine was collected during roughly (time) 2 h after ICG injection.
[c] The total amount of ICG in urine was determined by photospectrometry.
[d] Based on this amount of ICG, the renal ICG-PDR$_r$ was then calculated according to the equation described in the "Materials and Methods" section and was found to be minute in comparison to the ICG-PDR values computed by LiMON.

for the disappearance of ICG because it remains stable for at least 4 h.[27]

However, our results are consistent with the findings of Stehr et al.[23] who described in a hyperdynamic porcine endotoxemia model an ICG-PDR increase although hepatic function assessed by measurement of biliary ICG excretion clearly decreased. Diffusion of the free or even the albumin-bound fraction of ICG to the interstitial space through an endothelial leak in the context of a perioperative systemic inflammatory response syndrome may explain the overestimation of ICG-PDR.[28] Furthermore, as reported by other authors, considerable temporary redistribution of ICG in animal models into extrahepatic–extravascular tissue may also assume importance in this context.[29,30] To explain the outcome in this study, therefore, we postulated a capillary leak with extravasation of protein-bound ICG into the interstitial space.[31,32] Thus, we examined the correlation of ICG-PDR to (1) sequential postoperative serum albumin values, (2) differences in body weight before and after OLT, and (3) the post-OLT course of liquid balances. Neither albumin nor the clinical parameters correlated statistically with the ICG-PDR values. However, the difficulty of quantitatively assessing the capillary leak is well recognized.[33]

According to our findings, the precision of the correlation of ICG-PDR and functional hepatic reserve must be questioned. Our results are even more remarkable because hepatic perfusion was eliminated as a parameter influencing PDR. Overestimation of residual liver function may occur in situations with a possible capillary leak (e.g., sepsis, anaphylaxis, or perioperative systemic inflammatory response syndrome). Because misinterpretation of hepatic function is a crucial issue, especially in the small-for-size liver remnant scenarios with marginal residual hepatic reserves, a possible inaccuracy of up to 1.55%/min on average has to be taken into account if ICG pulse spectrophotometry is used to determine functional hepatic reserve. It is important to recognize that this value corresponds to 31% of an ICG-PDR of 5.0%/min, which is considered the crucial cutoff point for a favorable outcome after major liver resection.[19]

Conclusion

During the anhepatic phase of OLT, ICG-PDR values were unexpectedly found to be considerably different from zero. Apart from hepatic perfusion and functional hepatic reserve, other factors seem to influence real-time ICG-PDR determined by pulse spectrophotometry. These cofactors increase in importance with low ICG-PDR values and must be taken into account when determining the functional hepatic reserve in liver insufficiency or when assessing the minimally required hepatic mass during extended liver resection.

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References

1. Shoup M, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH, Tuorto S, Blumgart LH, Fong Y. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. J Gastrointest Surg 2003;7:325–330.
2. Leevy CM, Mendenhall CL, Lesko W, Howard MM. Estimation of hepatic blood flow with indocyanine green. J Clin Invest 1962;41:1169–1179.
3. Tygstrup N. Determination of the hepatic galactose elimination capacity after a single intravenous injection in man: the reproducibility and the influence of uneven distribution. Acta Physiol Scand 1963;58:162–172.
4. Paumgartner G. The handling of indocyanine green by the liver. Schweiz Med Wochenschr 1975;105:1–30.
5. Skak C, Keiding S. Methodological problems in the use of indocyanine green to estimate hepatic blood flow and ICG clearance in man. Liver 1987;7:155–162.
6. Kuntz H, Schregel W. Indocyanine green: evaluation of liver function—application in intensive care medicine. In Lewis F, Pfeiffer U, eds. Fiberoptics in Critical Care Monitoring. Berlin: Springer, 1990, pp 57–62.
7. Uusaro A, Ruokonen E, Takala J. Estimation of splanchnic blood flow by the Fick principle in man and problems in the use of indocyanine green. Cardiovasc Res 1995;30:106–112.
8. Ohwada S, Kawate S, Hamada K, Yamada T, Sunose Y, Tsutsumi H, Tago K, Okabe T. Perioperative real-time monitoring of indocyanine green clearance by pulse spectrophotometry predicts remnant liver functional reserve in resection of hepatocellular carcinoma. Br J Surg 2006;93:339–346.
9. Gottlieb ME, Stratton HH, Newell JC, Shah DM. Indocyanine green. Its use as an early indicator of hepatic dysfunction following injury in man. Arch Surg 1984;119:264–268.
10. Kimura S, Yoshioka T, Shibuya M, Sakano T, Tanaka R, Matsuyama S. Indocyanine green elimination rate detects hepatocellular dysfunction early in septic shock and correlates with survival. Crit Care Med 2001;29:1159–1163.
11. Sakka SG, Reinhart K, Meier-Hellmann A. Prognostic value of the indocyanine green plasma disappearance rate in critically ill patients. Chest 2002;122:1715–1720.
12. Diaz S, Perez-Pena J, Sanz J, Olmedilla L, Garutti I, Barrio JM. Haemodynamic monitoring and liver function evaluation by pulsion cold system Z-201 (PCS) during orthotopic liver transplantation. Clin Transplant 2003;17:47–55.
13. Jochum C, Beste M, Penndorf V, Farahani MS, Testa G, Nadalin S, Malago M, Broelsch CE, Gerken G. Quantitative liver function tests in donors and recipients of living donor liver transplantation. Liver Transpl 2006;12:544–549.
14. Hori T, Iida T, Yagi S, Taniguchi K, Yamamoto C, Mizuno S, Yamagiwa K, Isaji S, Uemoto S. K(ICG) value, a reliable real-time estimator of graft function, accurately predicts outcomes in adult living-donor liver transplantation. Liver Transpl 2006; 12:605–613.
15. Shinohara H, Tanaka A, Kitai T, Yanabu N, Inomoto T, Satoh S, Hatano E, Yamaoka Y, Hirao K. Direct measurement of hepatic

indocyanine green clearance with near-infrared spectroscopy: separate evaluation of uptake and removal. Hepatology 1996; 23:137–144.
16. Faybik P, Krenn CG, Baker A, Lahner D, Berlakovich G, Steltzer H, Hetz H. Comparison of invasive and noninvasive measurement of plasma disappearance rate of indocyanine green in patients undergoing liver transplantation: a prospective investigator-blinded study. Liver Transpl 2004;10:1060–1064.
17. Sakka SG, Reinhart K, Meier-Hellmann A. Comparison of invasive and noninvasive measurements of indocyanine green plasma disappearance rate in critically ill patients with mechanical ventilation and stable hemodynamics. Intensive Care Med 2000;26:1553–1556.
18. Tsubono T, Todo S, Jabbour N, Mizoe A, Warty V, Demetris AJ, Starzl TE. Indocyanine green elimination test in orthotopic liver recipients. Hepatology 1996;24:1165–1171.
19. Inderbitzin D, Muggli B, Ringger A, Beldi G, Gass M, Gloor B, Uehlinger D, Regli B, Reichen J, Candinas D. Molecular absorbent recirculating system for the treatment of acute liver failure in surgical patients. J Gastrointest Surg 2005;9:1155–1161.
20. Schindl MJ, Redhead DN, Fearon KC, Garden OJ, Wigmore SJ. The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. Gut 2005; 54:289–296.
21. Heemann U, Treichel U, Loock J, Philipp T, Gerken G, Malago M, Klammt S, Loehr M, Liebe S, Mitzner S, Schmidt R, Stange J. Albumin dialysis in cirrhosis with superimposed acute liver injury: a prospective, controlled study. Hepatology 2002;36:949–958.
22. Sen S, Williams R, Jalan R. Emerging indications for albumin dialysis. Am J Gastroenterol 2005;100:468–475.
23. Stehr A, Ploner F, Traeger K, Theisen M, Zuelke C, Radermacher P, Matejovic M. Plasma disappearance of indocyanine green: a marker for excretory liver function? Intensive Care Med 2005;31: 1719–1722.
24. Fujita Y, Sakai T, Ohsumi A, Takaori M. Effects of hypocapnia and hypercapnia on splanchnic circulation and hepatic function in the beagle. Anesth Analg 1989;69:152–157.
25. Birnbaum J, Lehmann C, Taymoorian K, Krausch D, Wauer H, Grundling M, Spies C, Kox WJ. [The effect of dopexamine and iloprost on plasma disappearance rate of indocyanine green in patients in septic shock]. Anaesthesist 2003;52:1014–1019.
26. Pomier-Layrargues G, Huet PM, Villeneuve JP, Marleau D. Effect of portacaval shunt on drug disposition in patients with cirrhosis. Gastroenterology 1986;91:163–167.
27. Landsman ML, Kwant G, Mook GA, Zijlstra WG. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. J Appl Physiol 1976;40:575–583.
28. Ishihara H, Matsui A, Muraoka M, Tanabe T, Tsubo T, Matsuki A. Detection of capillary protein leakage by indocyanine green and glucose dilutions in septic patients. Crit Care Med 2000;28:620–626.
29. Ott P, Bass L, Keiding S. The kinetics of continuously infused indocyanine green in the pig. J Pharmacokinet Biopharm 1996;24:19–44.
30. Burczynski FJ, Pushka KL, Sitar DS, Greenway CV. Hepatic plasma flow: accuracy of estimation from bolus injections of indocyanine green. Am J Physiol 1987;252:H953–H962.
31. Ernest D, Belzberg AS, Dodek PM. Distribution of normal saline and 5% albumin infusions in septic patients. Crit Care Med 1999;27:46–50.
32. Margarson MP, Soni NC. Changes in serum albumin concentration and volume expanding effects following a bolus of albumin 20% in septic patients. Br J Anaesth 2004;92:821–826.
33. Ibla JC, Khoury J. Methods to assess tissue permeability. Methods Mol Biol 2006;341:111–117.

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Short Bowel Syndrome After Continence-Preserving Procedures

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Abstract The short bowel syndrome (SBS) can result from a variety of conditions, including postoperative complications and malignancy. Continence-preserving operations are generally performed for either ulcerative colitis (UC) or familial polyposis (FAP). These procedures can be associated with high morbidity and the potential for future malignancy. Our aim was to determine the causes and consequences of SBS in patients undergoing these procedures. Twenty-four patients (12 men and 12 women) 18 to 64 years of age were identified with SBS after continence-preserving procedures. Eighteen had pelvic procedures, and six had continent ileostomies. All SBS patients had a proximal ostomy. Remnant length measured <60 cm in five patients, 60–120 cm in ten patients, and >120 cm in nine patients. Overall 13 patients required long-term PN. Four FAP patients with desmoid tumors died. One patient with UC underwent intestinal transplant and expired. Follow-up ranges from 6 to 192 months. Overall 14 patients had UC, nine had FAP, and one had functional disease. Eight patients with an initial diagnosis of UC had subsequent Crohn's disease necessitating further resection and pouch excision. Eight patients (five with UC, two FAP, and one with functional disease) had postoperative complications, including obstruction or mesenteric ischemia requiring resections. One UC patient developed adenocarcinoma in a continent ileostomy. Seven of the nine FAP patients required resection for desmoid tumors. Six of these underwent resection alone. Three died at 10, 11, and 13 months after SBS from liver failure and sepsis while awaiting transplant. One patient has recurrent desmoid at 30 months, another is alive and well at 48 months, and the other patient, who was not a transplant candidate, died from an unrelated cardiac operation at 23 months. A single patient underwent resection with simultaneous multivisceral transplantation. SBS can develop after continence-preserving procedures. This occurs with inflammatory bowel disease when unsuspected Crohn's disease is present or complications occur. SBS related to desmoid tumors has a poor prognosis in patients undergoing resection alone. A more aggressive approach to intestinal transplantation in these patients may be warranted.

Keywords Short bowel syndrome · Familial polyposis

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Introduction

The short bowel syndrome (SBS) can result from a variety of conditions, including postoperative complications and malignancy.[1] While most instances of postoperative SBS occur after fairly common surgical procedures, certain operations might be expected to be at higher risk. Continence-preserving operations are generally performed for either ulcerative colitis (UC) or familial polyposis (FAP). These procedures can be associated with high morbidity and the potential for future malignancy.[2] Our aim was to determine the causes and consequences of SBS in patients undergoing these continence-preserving procedures.

Methods

We reviewed the records of 300 adult patients with SBS evaluated at the University of Nebraska Medical Center between January 1982 and March 2007. The SBS was defined as an intestinal remnant <180 cm with associated malabsorption. The causes of the SBS in this group of patients are given in Table 1. Twenty-four patients developed SBS after continence-preserving procedures (pelvic pouch and continent ileostomy). Only three of the pouch procedures were performed at our institution. There were 12 women and 12 men ranging from 18 to 64 years of age. Records were reviewed to determine the initial operation, postoperative course and long-term outcome of SBS. Follow-up from the diagnosis of SBS ranged from 6 to 192 months with a mean of 43 months. Statistical comparisons were made using chi-square analysis with $P<0.05$ signifying statistical significance.

Results

Twenty-four patients were identified with SBS after continence-preserving procedures. Eighteen had pelvic procedures and six had continent ileostomies. Overall, 14 patients had an initial diagnosis of UC, 9 had FAP, and 1 had functional disease (Table 2). The interval from the continence-preserving procedure to the development of SBS ranged from 1 to 212 months with a mean of 69 months.

All SBS patients had a proximal ostomy with no residual colon. One patient still has the pouch in place. Remnant length is shown in Table 2. Patients with FAP were significantly more likely to have shorter remnants. Overall, 13 (54%) patients required long-term PN. Patients with FAP were more likely to be on long-term parenteral nutrition. Overall, five patients died. Four patients with FAP and desmoid tumors died. One patient with UC underwent intestinal transplantation and subsequently expired.

Eight (57%) of the 14 UC patients had the subsequent diagnosis of Crohn's disease necessitating pouch excision and further resection (Table 3). Five other UC patients had postoperative complications, including obstruction ($n=3$) or mesenteric ischemia ($n=2$) requiring resections. Two of the obstructions was due to adhesions and repeated operations, and the other was a volvulus involving the pouch. Two patients developed mesenteric vein thrombosis during the early postoperative period. The final UC patient developed adenocarcinoma in a continent ileostomy 14 years after construction. Excision of the pouch and subsequent enterectomy for obstruction led to SBS. Overall, four (29%) UC patients with remnant lengths of 30, 70, 90, and 100 cm required long-term PN, and one underwent intestinal transplantation.

Seven of the nine patients undergoing pelvic procedures for FAP required resection for desmoid tumors. The tumors were all based in the mesentery and occurred from 1 to

Table 1 Causes of Short Bowel Syndrome

Causes	Number
Postoperative	84 (28%)
Irradiation/malignancy	63 (21%)
Mesenteric vascular disease	63 (21%)
Crohn's disease	49 (16%)
Trauma	22 (8%)
Other	19 (6%)
Total	300

Table 2 Etiology and Outcome

Parameters	Ulcerative Colitis	FAP	Functional	Total
Number	14	9	1	24
Female	7 (50%)	4 (44%)	1 (100%)	12 (50%)
Age (mean, range)	40 (18–64)	34 (18–57)	40 (40)	38 (18–64)
Interval to SBS (months)	80 (1–180)	56 (1–212)	132 (132)	74 (1–212)
Remnant length (cm) small intestine				
<60	1 (7%)	4 (44%)*	0 (0%)	5 (21%)
60–120	5 (36%)	4 (44%)*	0 (0%)	9 (38%)
>120	8 (57%)	1 (12%)*	1 (100%)	10 (42%)
Parenteral Nutrition	4 (29%)	9 (100%)*	0 (0%)	13 (54%)
Death	1 (7%)	4 (44%)*	0 (0%)	5 (21%)

*$P<0.05$ vs others

Table 3 Etiology and Mechanism of SBS

Parameters	Ulcerative Colitis	FAP	Functional	Total
Procedure				
IPAA	10 (71%)	8 (89%)	0 (0%)	18 (75%)
Continent ileostomy	4 (29%)	1 (11%)	1 (100%)	6 (25%)
Cause of resection				
Crohn's disease	8 (57%)*	0 (0%)	0 (0%)	8 (33%)
Tumor	1 (7%)	7 (78%) *	0 (0%)	8 (33%)
Obstruction	3 (21%)	2 (22%)	1 (100%)	6 (25%)
Mesenteric Ischemia	2 (14%)	0 (0%)	0 (0%)	2 (8%)

*$P<0.05$ vs others

72 months postoperatively (1, 1, 12, 13, 24, 48, and 72 months). Six underwent resection alone. Five of these patients underwent near total enterectomy (<30 cm remnant) for obstruction or fistula with later intestinal transplantation planned. Three died from liver failure and/or sepsis 10, 11, and 13 months after resection while awaiting intestinal transplantation. One refused transplantation and has recurrent desmoid at 30 months. The other patient is alive and doing well on PN at 48 months. The other patient was not a transplant candidate and died from an unrelated cardiac operation at 23 months with persistent desmoid. A single patient with SBS before excision of the desmoid underwent multivisceral transplantation at the time of resection. The two FAP patients without desmoids had postoperative complications (volvulus and perforated) leading to further resection and the SBS. Both are alive and well on PN at 11 and 12 months.

The patient with functional disease had an initial colectomy for constipation. She eventually had an ileostomy converted to a continent ileostomy. She subsequently underwent resection for recurrent obstructions. She has a 150-cm remnant and was weaned off parenteral nutrition.

Discussion

SBS after continence-preserving procedures accounted for 8% of our total patients with SBS. However, SBS remains a fairly infrequent complication of these procedures. Fazio et al.[2] found only one patient with SBS out of 1,005 having ileal pouch–anal anastomosis (IPAA). This was due to recurrent obstruction, a recognized complication of this procedure. One-fourth of our patients had this etiology. Patients with inflammatory bowel disease are known to be hypercoagulable and are at risk for mesenteric vein thrombosis as well. Remzi et al.[3] reported that 45% of patients undergoing computed tomography (CT) scan after IPAA had evidence of portal vein thrombi. However, only one of their patients developed pouch ischemia, and no intestinal resections were required. Two of our patients had mesenteric ischemia as the cause of SBS.

Recurrent Crohn's disease in patients undergoing pouch procedures for presumed ulcerative colitis was the most frequent reason for subsequent resection and SBS in our patients. As reported previously, this misdiagnosis was the mechanism for SBS in 17% of patients who developed SBS with Crohn's disease.[4] Regimbeau et al.[5] found that 10% of patients undergoing IPAA for Crohn's disease required excision of the pouch. Prudhomme et al.[6] reported that 15% of their patients requiring pouch excision had Crohn's disease. Patients, 5–10%, undergoing pouch procedures will eventually be found to have Crohn's disease.[7] Thus, this is not in an unusual clinical situation.

One-third of our patients had pouch procedures for FAP. Desmoid tumors were the most common cause for SBS in this group, accounting for three-fourths of patients. Prudhomme et al.[6] found that desmoid tumors caused 60% of pouch excisions in FAP patients. In our series, FAP patients were more likely to have tumors, had shorter remnant length, and were more likely to require PN than the other groups. One-half of the patients with desmoid tumors expired during the follow-up period.

The poor outcome of resecting desmoid tumors after IPAA has been reported by others. Soravia et al.[8] found that desmoids occur in 12% of FAP patients having IPAA. They resected one-half of the tumors with significant morbidity (23%) and mortality (8%). Sogar et al.[9] found that desmoids developed in 6% of their IPAA patients. Mesenteric desmoids resulted in pouch failure in more than half of their patients.

Our surgical strategy in desmoid patients has been to resect the tumor, and then, plan intestinal transplantation as a staged procedure. We felt this was an appropriate approach when intestinal remnants would be <30 cm, and complications, e.g., fistula was present. However, three patients expired before transplantation could be achieved, and one refused transplantation. Two patients developed recurrent desmoids. More recently, we have performed excision of the tumor and multivisceral transplantation simultaneously. Chatzipetrou et al.[10] reported survival in seven of nine patients undergoing a similar approach with transplantation at the time of resection. Thus, a more aggressive approach to intestinal transplantation in these patients may be warranted.

One of our patients with UC developed adenocarcinoma in a continent ileostomy 14 years after construction. This resulted in extensive resection. This is an unusual complication which has also been reported in conventional ileostomies and pelvic pouches.[11–12]

Patients undergoing continence-preserving procedures are at risk for developing SBS. This develops after continence-preserving procedures for inflammatory bowel disease when Crohn's disease is present or complications occur. SBS related to desmoid tumors in FAP patients is another common cause. Resection alone in this group has a poor prognosis and simultaneous intestinal transplantation should be considered.

References

1. Thompson JS, DiBaise JK, Iyer KR, Yeats M, Sudan DL. Postoperative short bowel syndrome. JACS 2005;201:85–89.
2. Fazio VW, Ziv Y, Church JM, Oakley JR, Lavery IC, Milson JW, Schroeder TK. Ileal pouch anal anastomoses complications and function in 1005 patients. Ann Surg 1999;222:120–127.
3. Remzi FH, Fazio VW, Oncel M. Portal vein thrombi after restorative proctocolectomy. Surgery 2002;132:655–662.

4. Thompson JS, Iyer KR, DiBaise JK. Short Bowel Syndrome and Crohn's Disease. J Gastrointest Surg 2003;7:1069–1072.
5. Regimbeau JM, Panis Y, Pocard M. Long term results of ileal pouch–anal anastomosis for colorectal Crohn's disease. Dis Colon Rectum 2001;44:769–778.
6. Prudhomme M, Dehni N, Dozois R, Tiret E, Parc R. Causes and outcomes of pouch excision after restorative proctocolectomy. Br J Surg 2006;93:82–86.
7. Bach SP, Mortensen NJ. Revolution and evolution: 30 years of ileoanal pouch surgery. Inflamm Bowel Dis 2006;12:131–145.
8. Soravia C, Berk T, McLeod RS, Cohen Z. Desmoid disease in patients with familial adenomatous polyposis. Dis Colon Rectum 2000;43:363–369.
9. Sogar PM, Moslein G, Dozois RR. Management of desmoid tumors in patients with ileal pouch–anal anastomosis for familial adenomatous polyposis. Dis colon Rectum 1998;41: 1350–1375.
10. Chatzipetrou MA, Tzakis AG, Pinna AD. Intestinal transplantation for the treatment of desmoid tumors associated with familial adenomatous polyposis. Surgery 2001;129:277–281.
11. Achneck HE, Wong IY, Kim PJ. Ileostomy adenocarcinomas in the setting of ulcerative colitis. J Am Gastroenterol 2005;39:396–400.
12. Cox CL, Butts DR, Roberts MP. Development of invasive adenocarcinoma in a long standing Kock continent ileostomy. Dis Colon Rectum 1997;40:500–503.

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How Many Lymph Nodes Properly Stage a Periampullary Malignancy?

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Abstract The impact of lymphadenectomy in prognosis and staging in periampullary malignancies remains largely undefined. We examined all pancreaticoduodenectomies for periampullary carcinomas in the SEER cancer registry from 1993 through 2003. Overall, 5465 pancreaticoduodenectomies for nonmetastatic periampullary carcinomas were identified. The cohort was comprised of 62.5% pancreatic, 18.9% ampullary, 11.6% distal bile duct, and 7.0% duodenal cancers. A linear association between the number of lymph nodes (LNs) examined and overall survival was observed overall and for pancreas and ampullary cancers for node-negative (N0) disease. Median survival for all patients with localized, N0 disease improved from 24 to 31 months, with sampling of a minimum of 10 LNs, whereas 2 and 5-year survival improved from 52 and 29%, with <10 nodes examined to 58 and 37% with 10+ nodes examined ($P<0.001$). A 1-month median survival advantage was seen in patients with node-positive disease when more than 10 lymph nodes examined (15 versus 16 months, $P<0.001$). Significantly better median survival and cure rates are observed after pancreaticoduodenectomy for localized periampullary adenocarcinoma when a minimum of 10 lymph nodes are examined. This benefit likely represents more accurate staging. To optimize the prognostic accuracy and prevent stage migration errors in multicenter trials a minimum of 10 lymph nodes should be obtained and examined before the determination of node-negative disease.

Keywords Lymph node · Whipple · Periampullary · Outcomes · SEER

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Introduction

Periampullary cancers include those tumors arising in the head of the pancreas, periampullary duodenum, extrahepatic bile duct, and the ampulla of Vater.[1] Pancreatic adenocarcinoma accounts for 60% of tumors arising in the periampullary region.[2] The other three periampullary malignancies are often unable to be differentiated from pancreatic adenocarcinoma before surgery. Surgical resection by means of pancreaticoduodenectomy provides the only chance of cure for all patients with periampullary malignancies.[3–6] The impact of the degree of lymphadenectomy on patient outcomes remains undefined for these malignancies.[7,8] Studies examining this question in pancreatic cancer have suggested that examination and removal of additional lymph nodes may be associated with improved survival. Whereas both prospective and retrospective studies regarding the extent of extended lymph node dissection have disagreed, the best data suggest no survival advantage for pancreatic cancer when more than 10 to 15 lymph nodes are removed and examined.[9–14]

Of note, the reported number of lymph nodes examined in individual institutional series for pancreatic cancer and other periampullary malignancies along with patient outcomes vary greatly.[15–17] We hypothesized that differences in reported prognoses between institutional series are largely caused by a stage migration phenomenon secondary to differences in the extent of lymph node sampling. Our objective was to determine the effect of the number of lymph nodes examined on stage-specific prognoses for patients undergoing pancreaticoduodenectomy for periampullary adenocarcinomas. We further hypothesized that should a minimal number of lymph nodes be identified to more accurately stage patients, an improved framework to better examine patient therapies and outcomes, particularly between series, would result.

Material and Methods

The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (NCI) is an authoritative source of information on cancer incidence and survival in the United States.[18] SEER currently collects and publishes cancer incidence and survival data from 17 population-based cancer registries encompassing approximately 26% of the U.S. population. With just more than 6,100,000 incident cancer cases, SEER represents the largest cancer database in the country. The SEER program registries routinely collect data on patient demographics, primary tumor site, tumor morphology and stage at diagnosis, first course of treatment, and follow-up for survival status. The SEER program is the only comprehensive source of population-based information in the United States that includes stage of cancer at the time of diagnosis and patient survival data. NCI staff work with the North American Association of Central Cancer Registries (NAACCR) to guide all state registries to achieve data content and compatibility acceptable for pooling data and improving national estimates. The SEER program is considered the prevailing standard for quality among cancer registries around the world.

Statistical analysis was performed with SPSS Statistical Package version 14.0 (SPSS Inc., Chicago, IL). Correlations between demographic or clinical data and number of lymph nodes examined were made using the Chi-square test. Median, 2-year and 5-year overall survival rates were calculated by the Kaplan–Meier method. Survival was calculated from the time of the initial diagnosis to the date of last contact (or the date of death, if the patient was deceased). The effect of the number of lymph nodes examined on survival was tested by using the Log-rank test. A multivariate analysis by means of the Cox proportional hazards model was used to further test the significance of prognostic factors on survival. Specifically gender, age, race, tumor size, anatomic site, lymph node status, SEER summary stage, the inclusion of radiotherapy in treatment, and the number of lymph nodes examined were included in the multivariate analysis.

Table 1 Sample Selection Criteria

Selection Criteria	Number (*N*)
SEER 17 Regs Public Use, Nov 2005 Sub	6,113,961
↓	
Periampullary adenocarcinomas	60,396
↓	
Year of diagnosis 1993–2003	34,265
↓	
Pancreaticoduodenectomy	6,827
↓	
Complete staging data	6,755
↓	
M0 disease only	6,330
↓	
More than one LN examined	5,465

SEER = Surveillance, Epidemiology, and End Results; LN = lymph node

Results

The April 2006 release of the SEER data base was used to identify all incident cases of periampullary adenocarcinoma diagnosed from 1993 to 2003 based on the representative ICD-O-3 morphology codes.[19] Specifically, those cases arising in the periampullary duodenum, extrahepatic bile duct, ampulla of Vater or the head of the pancreas were included. A detailed algorithm outlining the data extraction process is shown in Table 1. Incident cases were limited to those undergoing pancreaticoduodenectomy for adenocarcinoma of the pancreatic head, ampulla of Vater, periampullary duodenum, or extrahepatic bile duct with at least

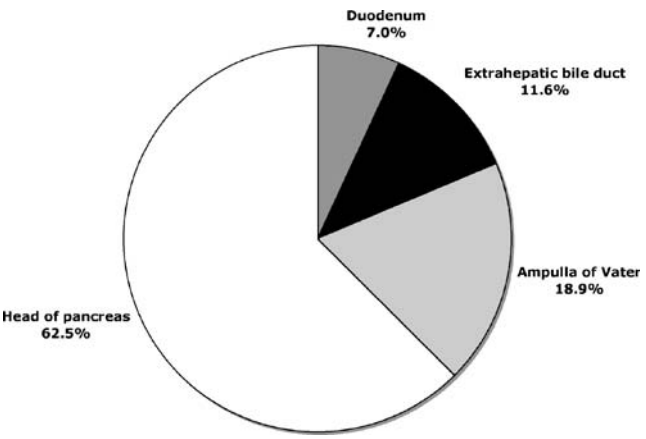

Figure 1 Anatomic distribution of tumors.

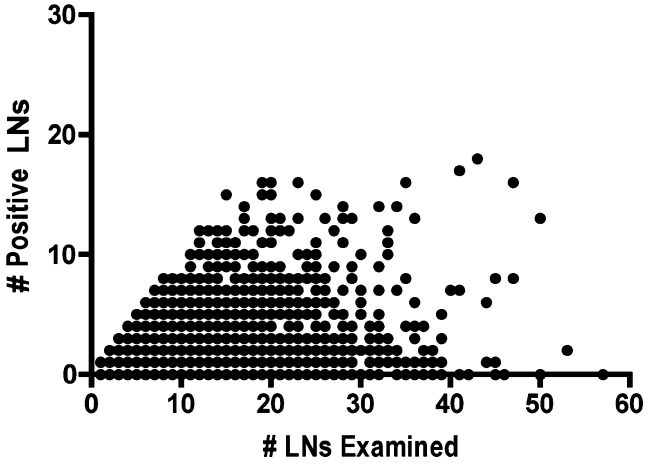

Figure 2 Comparison between the total number of lymph nodes examined and the total number of positive lymph nodes for each patient (LNs = lymph nodes).

one lymph node removed for pathologic examination. Patients with metastatic disease (M1) or incomplete staging data were excluded from the investigation. A total of 5,465 periampullary carcinoma cases were extracted for analysis. There were no duplicate cases in the study sample.

The fractional breakdown of cases by tumor anatomic location is shown in Fig. 1. Patients with disease arising from the head of the pancreas made up the majority of the cohort with 3,418 tumors (62.5%), whereas lesser numbers of tumors were found to arise from the ampulla of Vater (N= 1,031, 18.9%), extrahepatic bile ducts (N=633, 11.6%), and duodenum (N=383, 7.0%). The median follow-up time was 14 months for all patients and 19 months for survivors only.

Patient demographics and clinical data were extracted from the database. Figure 2 depicts a graphical comparison between the total number of lymph nodes examined and the total number of positive lymph nodes reported for each individual patient. A linear association between the number of lymph nodes examined and the number of positive lymph nodes was observed until an average of 10–15 lymph nodes were examined. To further define the impact of the number of nodes examined on prognosis, median survival was calculated for different patient groups using 5, 10, 15, 20, and 25 lymph nodes as the cutoff for the two groups (Table 2). Based on these observations, a minimum of 10 lymph nodes examined appeared to be associated with improved diagnostic accuracy in the staging of both node-negative and node-positive periampullary malignancies.

Demographic and clinical data were analyzed for all patients according to the number of lymph nodes examined—patients with one to nine and 10 or more lymph nodes were compared (Table 3). A slightly larger percentage of males were present in the one-to-nine LN group (54.1%) than in the ≥10-LN group (50.9%, P=0.020). The median age at the time of diagnosis was 67 years in the one-to-nine LN group compared to 65 years in the ≥10-LN group (P=0.004). There were no differences in the racial characteristics of the two groups. Patients in the one-to-nine LN group had twice the number of extrahepatic bile duct tumors than did those in the ≥10-LN group (14.7 vs. 7.2%, P<0.001), as well as significantly less pancreatic head tumors (58.3 vs. 68.4%, respectively, P<0.001).

The average tumor size was smaller in the one-to-nine LN group than in the ≥10-LN group (2.93 vs. 3.13 cm, P<0.001). There was a greater percentage of node positive disease in the ≥10-LN group than in the one-to-nine LN group (64.8 vs. 46.5%, P<0.001). Similarly, there was a greater percentage of regionally advanced disease in the ≥10-LN group than in the one-to-nine LN group (88.7 vs. 82.5%, P<0.001). Radiation therapy was used more often in the ≥10-LN group than in the one-to-nine LN group (45.3 vs. 35.9%, P<0.001). The percentage of those patients receiving preoperative radiation therapy was similar between the two groups (2.0% in the ≥10-LN group and 1.6% in the one-to-nine LN group).

Median, 2-year, and 5-year overall survival rates are shown in Table 4. Patients with 10 or more lymph nodes examined had an improved median survival compared to those patients with less than 10 lymph nodes examined in both N0 (31 vs. 24 months, P<0.001) and N1 subgroups (16 vs. 15 months, P=0.035). These results translated into similar improvements in both 2-year and 5-year survival rates in these same groups. In the group of individuals with adenocarcinoma of the head of the pancreas, those patients with 10 or more lymph nodes examined also had an

Table 2 Overall Median Survival by Number of Lymph Nodes Examined (All Tumor Locations)

Tumor Stage	Number of Lymph Nodes Examined				
	< 5 vs. ≥ 5	< 10 vs. ≥ 10	< 15 vs. ≥ 15	< 20 vs. ≥ 20	< 25 vs. ≥ 25
N0	23 vs. 28	24 vs. 31	25 vs. 37	26 vs. 36	26 vs. 42
P value	< 0.001	< 0.001	< 0.001	0.005	0.208
N1	14 vs. 15	15 vs. 16	15 vs. 16	15 vs. 16	15 vs. 16
P value	0.050	0.035	0.073	0.109	0.312

Values represent median survival in months for node-negative (N0) and node-positive (N1) disease. *P* value shown for Log rank test examining the effect of number of lymph nodes examined on median survival.

Table 3 Comparison of Demographic and Clinical Data by Number of Lymph Nodes Examined

	Number of LNs Examined		*P* value *
	1–9 *N*=3,178	≥10 *N*=2,287	
Gender			
Male	54.1	50.9	0.020
Female	45.9	49.1	
Age (years)			
Median	67	65	
< 50	11.4	12.9	0.004
51–60	19.1	21.3	
61–70	31.2	32.4	
71–80	32.0	28.3	
> 80	6.2	5.1	
Race			
White	81.7	82.8	0.119
Black	8.5	9.0	
Other	9.8	8.2	
Tumor size (cm)			
Mean	2.93	3.13	
<2.0 cm	22.7	15.8	<0.001
≥2.0 cm	77.3	84.2	
Primary tumor site			
Duodenum	7.1	6.9	<0.001
Extrahepatic bile duct	14.7	7.2	
Ampulla of Vater	19.8	17.5	
Head of pancreas	58.3	68.4	
LN Status			
N0	53.5	35.2	<0.001
N1	46.5	64.8	
SEER summary stage			
Localized	17.5	11.3	<0.001
Regional	82.5	88.7	
Radiotherapy			
Preoperative therapy	1.6	2.0	<0.001
Intraoperative therapy	0.6	0.6	
Pre and postoperative therapy	0.2	0.1	
Postoperative therapy	33.4	22.1	
Sequence unknown	0.3	0.3	
None given	64.1	54.7	

Values shown represent percentage of each group.
**P* value for Chi-square test examining the association between variables (LNs = lymph nodes, SEER = Surveillance, Epidemiology and End Results)

improved median survival compared to those patients with less than 10 lymph nodes examined in both N0 (22 vs. 18 months, $P<0.001$) and N1 subgroups (14 vs. 13 months, $P=0.002$). A similar, yet larger improvement was demonstrated in patients with an ampullary malignancy, in which median survival in N0 patients improved from 47 months in the one-to-nine LN group to 88 months in the ≥10-LN group ($P=0.044$). A trend toward improved overall survival with an increase in the number of lymph nodes examined was also observed for patients with N0 disease of the extrahepatic biliary ducts and the duodenum. Kaplan–Meier survival curves for these data are shown in Fig. 3.

Step-wise multivariate analysis of all demographic, clinical, and treatment variables was undertaken using the Cox regression model (Table 5). Increasing age at diagnosis, tumor size over 2.0 cm, and node-positive or regionally advanced disease were all independent predictors of lower overall survival. Duodenal, extrahepatic bile duct, and ampullary tumors were each independently associated with improved survival when compared to pancreatic head tumors. Radiation therapy was also independently associated with an improved outcome. Finally, examination of 10 or more lymph nodes was an independent predictor of improved overall survival.

Discussion

Accurate staging is of paramount importance in developing a therapeutic plan for cancer patients and in the evaluation of novel therapies. The number of lymph nodes examined during a curative surgical procedure has been shown to be very important in identifying the extent of disease for virtually all carcinomas. Such reports include those of improved survival rates with an increased lymph node evaluation for gastric,[20–23] colon,[24–27] and breast cancers.[28,29] Whether these results are simply reflective of a more accurate staging or an indication of improved clearance of regionally advanced disease remains debatable.

In spite of the unclear therapeutic benefit for increased lymphadenectomy, the impact of the number of lymph nodes examined on the accurate staging for adenocarcinomas of the periampullary region remains undefined. A 2006 study by Schwarz et al.[14] examining the extent of lymph node retrieval and pancreatic cancer in the SEER database found stage-based survival improvements with increasing numbers of lymph nodes examined. This study was limited to pancreatic cancers ignoring the other common malignancies of the periampullary region. We sought to extend the observations of Schwarz et al. and define the impact of the extent of lymph node examination to outcomes for periampullary cancers.

Additionally, the number of lymph nodes examined in individual series and survival data after resection reported to date can often vary greatly from series to series. Such inconsistencies may complicate and potentially invalidate comparisons made between these reports and make the interpretation of newer therapies difficult. For example, the benefit for 5-FU chemotherapy many years after its first report remains unclear.[16] We hypothesized that differences

Table 4 Overall 2-year, 5-year and Median Survival by Stage of Disease and Number of Lymph Nodes Examined

	1–9 Lymph Nodes						≥10 Lymph Nodes						
	N	2 years		5 years		Med	*N*	2 years		5 years		Med	*P**
		%	SE	%	SE			%	SE	%	SE		
Overall													
N0	1,701	52	.01	29	.01	24	804	58	.02	37	.02	31	<0.001
N1	1,477	30	.01	11	.01	15	1,483	34	.01	13	.01	16	0.035
HOP													
N0	894	39	.02	17	.02	18	487	47	.02	27	.03	22	<0.001
N1	960	24	.02	7	.01	13	1,077	31	.02	10	.01	14	0.002
EBD													
N0	293	60	.03	35	.04	31	70	67	.06	44	.07	46	0.077
N1	175	31	.04	10	.03	15	95	30	.05	10	.04	16	0.721
Amp													
N0	389	67	.03	43	.03	47	182	76	.03	52	.05	88	0.044
N1	241	45	.04	20	.03	19	219	43	.04	19	.04	20	0.961
Duo													
N0	125	71	.04	53	.05	60	65	80	.06	69	.07	NYR	0.087
N1	101	53	.05	27	.06	26	92	56	.06	31	.06	30	0.544

**P* value shown for Log rank test examining the effect of number of lymph nodes examined on median survival
HOP = head of pancreas, EBD = extrahepatic bile duct, Amp = ampulla of Vater, Duo = duodenum, SE = standard error, Med = median, NYR = not yet reached

in reported outcomes between institutional series might in part be largely caused by a stage migration phenomenon secondary to differences in the extent of lymph node sampling. Using a large multi-state population-based cancer registry, we sought to determine the effect of the number of lymph nodes examined on stage-specific prognosis for patients undergoing pancreaticoduodenectomy for periampullary adenocarcinoma.

A total of 5,465 patients in our study had between one and 57 lymph nodes examined at the time of pancreaticoduodenectomy for a periampullary adenocarcinoma. As expected, a linear association was observed between the number of lymph nodes examined and the number of positive lymph nodes reported (Fig. 2). However, this relationship appeared to deteriorate beyond 10–15 total lymph nodes examined, where additional lymphadenectomy failed to produce an increased number of positive nodes. A significant improvement in survival was noted with greater than 10 lymph nodes examined for all patients with periampullary adenocarcinoma (Tables 2 and 4). Although this benefit was most pronounced in the setting of node-negative disease (Fig. 3a), a statistically significant but clinically questionable survival improvement was also seen in patients with N1 cancers (Fig. 3b). Examination of at least 10 or more lymph nodes was also shown to be an independent predictor of decreased mortality by multivariate analysis (hazard ratio 0.847, $P<0.001$, Table 5).

We subsequently performed a subgroup analysis based on the type of periampullary cancer. Patients with lesions in the head of the pancreas demonstrated results similar to the entire cohort regarding the number of lymph nodes examined and survival, irrespective of their N status (Figs. 3c,d). Patients with N0 disease of the ampulla of Vater also benefited significantly from the examination of 10 or more lymph nodes versus the comparison group (Fig. 3g). Although no statistically significant survival benefits were seen for duodenal or extrahepatic bile duct tumors, a trend toward improved outcomes was observed for N0 disease in both locations (Fig. 3e,i).

The disproportionately increased survival benefit observed in the setting of N0 disease compared to N1 disease may be partly explained by a stage migration phenomenon. Patients with N0 status had fewer lymph nodes examined than did their N1 counterparts (data not shown), raising the possibility of a significant number of these individuals being understaged. Lesser numbers of lymph nodes analyzed increases the likelihood of missing a greater number of involved lymph nodes. Whereas we cannot dismiss the possibility of a therapeutic benefit to an extended lymphadenectomy for these patients, it seems more reasonable to propose that harvesting 10 or more lymph nodes provides improved staging information to more accurately guide subsequent treatment strategies.

It is important to address some important differences between our treatment groups. Those patients with greater than 10 lymph nodes examined had larger tumors, an increased incidence of N1 or regionally advanced disease,

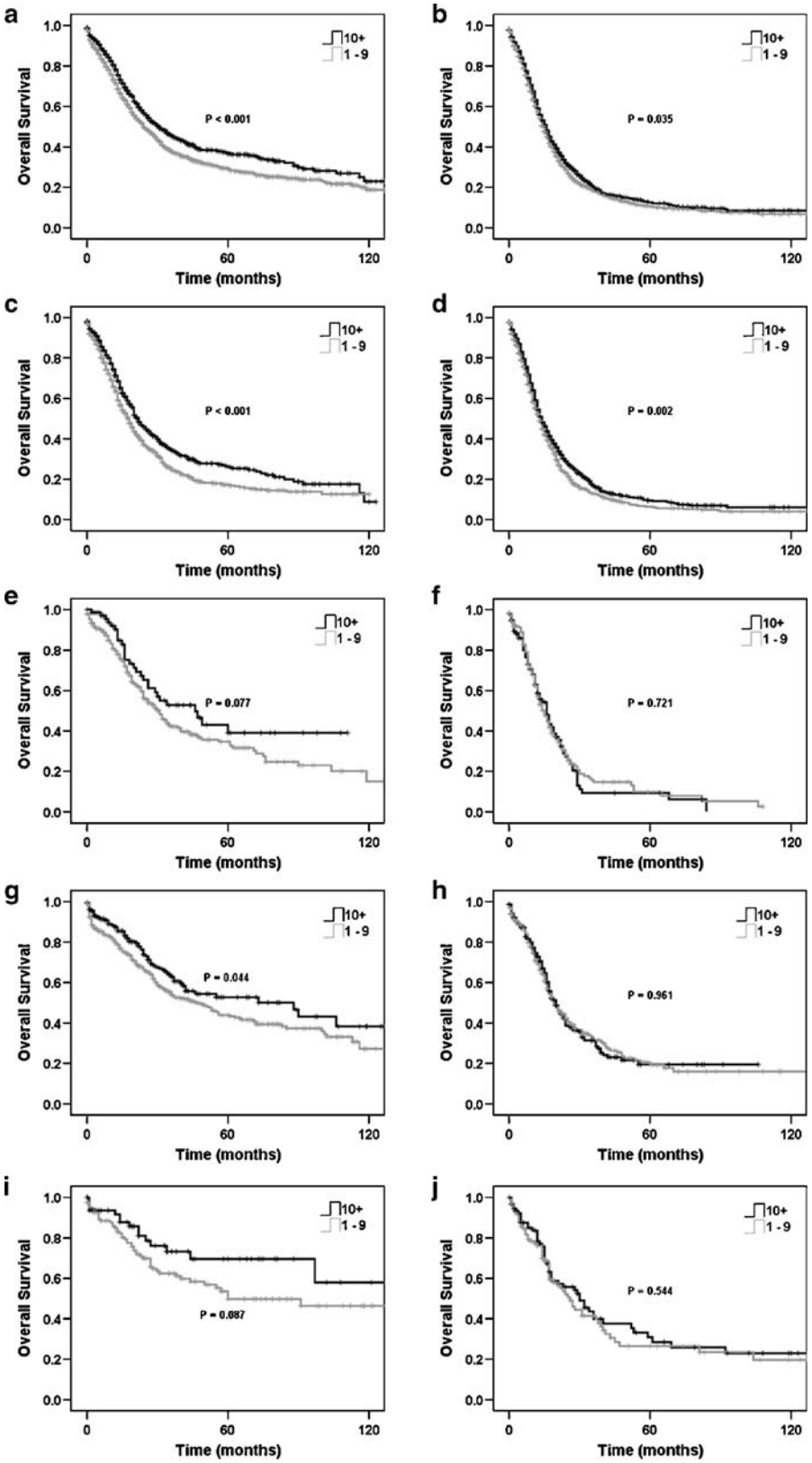

Figure 3 Kaplan–Meier curves comparing overall survival between patients with one to nine lymph nodes examined and ≥10 lymph nodes examined for (**a**) all periampullary tumors, N0 disease; (**b**) all periampullary tumors, N1 disease; (**c**) pancreatic tumors, N0 disease; (**d**) pancreatic tumors, N1 disease; (**e**) extrahepatic bile duct tumors, N0 disease; (**f**) extrahepatic bile duct tumors, N1 disease; (**g**) ampullary tumors, N0 disease; (**h**) ampullary tumors, N1 disease; (**i**) duodenal tumors, N0 disease; (**j**) duodenal tumors, N1 disease (*P* values shown for Log-rank test for association between median survival and treatment group).

Table 5 Cox Proportional Hazards Model for Risk of Death from All Periampullary Adenocarcinomas

	N	Hazard Ratio	95% CI	*P* value
Gender				
Male	2,435	Reference group	Reference group	Reference group
Female	2,247	0.943	0.876–1.014	0.115
Age				
< 50	549	Reference group	Reference group	Reference group
51–60	952	1.076	0.933–1.241	0.311
61–70	1,486	1.221	1.069–1.394	0.003
71–80	1,436	1.404	1.228–1.605	<0.001
80 +	259	1.515	1.243–1.846	<0.001
Race				
White	3,852	Reference group	Reference group	Reference group
Black	386	1.089	0.956–1.241	0.199
Other	444	0.997	0.876–1.136	0.969
Tumor size (cm)				
≤2.0	934	Reference group	Reference group	Reference group
>2.0	3,748	1.434	1.291–1.594	<0.001
Tumor site				
HOP	3,053	Reference group	Reference group	Reference group
Duo	332	0.350	0.295–0.416	<0.001
EBD	415	0.750	0.654–0.860	<0.001
Amp	882	0.502	0.449–0.560	<0.001
LN status				
N0	2,134	Reference group	Reference group	Reference group
N1	2,548	1.704	1.564–1.858	<0.001
SEER stage				
Localized	685	Reference group	Reference group	Reference group
Regional	3,997	1.288	1.135–1.463	<0.001
Radiotherapy				
Yes	1,927	Reference group	Reference group	Reference group
No	2,755	1.659	1.532–1.797	<0.001
# LNs examined				
1–9	2,652	Reference group	Reference group	Reference group
≥10	2,030	0.847	0.785–0.913	<0.001

Multivariate analysis is limited to patients with all variables available from the database (*N*=4,682)
CI = confidence interval, HOP = head of pancreas, EBD = extrahepatic bile duct, Amp = ampulla of Vater, Duo = duodenum, LN = lymph node, SEER = Surveillance, Epidemiology and End Results

and a larger proportion of pancreatic head tumors than those patients with nine or fewer lymph nodes examined. When one considers the number of lymph nodes examined as a surrogate for adequacy of surgical resection, it is reasonable to speculate that these patients in the 10+ lymph nodes group may have received higher quality or at least more aggressive surgical care than the comparison group. Whether such factors as hospital or surgeon procedure volume may be responsible for these differences in care are impossible to address using these data.

Differences in the utilization rate of adjuvant therapies were also observed between the two groups. Radiation therapy was more frequently administered in patients with greater than 10 lymph nodes examined than in the comparison group, with the majority of treatments given in the postoperative period (Table 3). These differences are likely because of the increased percentage of regionally advanced disease in this group. However, the rate of preoperative radiation therapy was virtually identical between the two groups, suggesting that neoadjuvant therapy was not a factor in down-staging patients from one treatment group to another.

The SEER registry, although considered a gold standard by many and an excellent database for comparative outcomes analysis, is nonetheless without its share of limitations. Many of the shortcomings in this study are similar in type and scope to those using other similar large cancer registries. Omissions in data reporting by individual registries to SEER have resulted in incomplete information on tumor size, stage, and grade. This results in a significant selection bias toward those patients who make it through the selection process. Unfortunately, no information regard-

ing the status of surgical resection margins or patient comorbidities was available from the registry. Finally, SEER does not include information on chemotherapy in its database.

Based on an analysis of the most recent SEER data, we nonetheless have demonstrated improved overall survival and cure rates after surgery for localized periampullary adenocarcinoma when a minimum of 10 lymph nodes are examined. This phenomenon is most likely representative of more accurate staging with increasing numbers of lymph nodes analyzed. A minimal benefit was also observed after additional lymphadenectomy in the setting of node-positive disease. Thus, we suggest that all patients after pancreaticoduodenectomy for periampullary malignancies should receive resections and pathological evaluation that includes 10 lymph nodes. This is particularly important in making the diagnosis of node-negative disease as without the evaluation of 10–15 lymph nodes diagnostic accuracy remains uncertain.

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References

1. Cameron JL. Current surgical therapy, 8th ed. Philadelphia: Elsevier Mosby, 2004.
2. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer statistics, 2006. CA Cancer J Clin 2006;56(2):106–130.
3. Buchler MW, Wagner M, Schmied BM, Uhl W, Friess H, Z'Graggen K. Changes in morbidity after pancreatic resection: Toward the end of completion pancreatectomy. Arch Surg 2003;138(12):1310–1314; discussion 1315.
4. Lillemoe KD, Yeo CJ, Cameron JL. Pancreatic cancer: State-of-the-art care. CA Cancer J Clin 2000;50(4):241–268.
5. Yeo CJ, Cameron JL, Lillemoe KD, Sitzmann JV, Hruban RH, Goodman SN, Dooley WC, Coleman J, Pitt HA. Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. Ann Surg 1995;221(6):721–731; discussion 731–733.
6. Diener MK, Knaebel HP, Heukaufer C, Antes G, Buchler MW, Seiler CM. A systematic review and meta-analysis of pylorus-preserving versus classical pancreaticoduodenectomy for surgical treatment of periampullary and pancreatic carcinoma. Ann Surg 2007;245(2):187–200.
7. Fortner JG. Regional resection of cancer of the pancreas: a new surgical approach. Surgery 1973;73(2):307–320.
8. Yeo CJ, Cameron JL, Lillemoe KD, Sohn TA, Campbell KA, Sauter PK, Coleman J, Abrams RA, Hruban RH. Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma: Part 2. Randomized controlled trial evaluating survival, morbidity, and mortality. Ann Surg 2002;236(3):355–366; discussion 366–368.
9. Satake K, Nishiwaki H, Yokomatsu H, Kawazoe Y, Kim K, Haku A, Umeyama K, Miyazaki I. Surgical curability and prognosis for standard versus extended resection for T1 carcinoma of the pancreas. Surg Gynecol Obstet 1992;175(3):259–265.
10. Kayahara M, Nagakawa T, Ueno K, Ohta T, Tsukioka Y, Miyazaki I. Surgical strategy for carcinoma of the pancreas head area based on clinicopathologic analysis of nodal involvement and plexus invasion. Surgery 1995;117(6):616–623.
11. Manabe T, Ohshio G, Baba N, Miyashita T, Asano N, Tamura K, Yamaki K, Nonaka A, Tobe T. Radical pancreatectomy for ductal cell carcinoma of the head of the pancreas. Cancer 1989;64 (5):1132–1137.
12. Pedrazzoli S, DiCarlo V, Dionigi R, Mosca F, Pederzoli P, Pasquali C, Kloppel G, Dhaene K, Michelassi F. Standard versus extended lymphadenectomy associated with pancreatoduodenectomy in the surgical treatment of adenocarcinoma of the head of the pancreas: a multicenter, prospective, randomized study. Lymphadenectomy Study Group. Ann Surg 1998;228(4):508–517.
13. Henne-Bruns D, Vogel I, Luttges J, Kloppel G, Kremer B. Ductal adenocarcinoma of the pancreas head: Survival after regional versus extended lymphadenectomy. Hepatogastroenterology 1998;45(21):855–866.
14. Schwarz RE, Smith DD. Extent of lymph node retrieval and pancreatic cancer survival: Information from a large US population database. Ann Surg Oncol 2006;13(9):1189–1200.
15. Sohn TA, Yeo CJ, Cameron JL, Koniaris L, Kaushal S, Abrams RA, Sauter PK, Coleman J, Hruban RH, Lillemoe KD. Resected adenocarcinoma of the pancreas—616 patients: Results, outcomes, and prognostic indicators. J Gastrointest Surg 2000;4(6):567–579.
16. Khanna A, Walker GR, Livingstone AS, Arheart KL, Rocha-Lima C, Koniaris LG. Is adjuvant 5-FU-based chemoradiotherapy for resectable pancreatic adenocarcinoma beneficial? A meta-analysis of an unanswered question. J Gastrointest Surg 2006;10(5):689–697.
17. White RR, Kattan MW, Haney JC, Clary BM, Pappas TN, Tyler DS, Brennan MF. Evaluation of preoperative therapy for pancreatic cancer using a prognostic nomogram. Ann Surg Oncol 2006;13 (11):1485–1492.
18. Surveillance E, and End Results (SEER) Program http://www.seer.cancer.gov) SEER*Stat Database: Incidence—SEER 17 Regs Limited-Use, Nov 2005 Sub (1973–2003), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006, based on the November 2005 submission.
19. Fritz AG. International classification of diseases for oncology: ICD-O, 3rd ed. Geneva: World Health Organization, 2000.
20. Smith DD, Schwarz RR, Schwarz RE. Impact of total lymph node count on staging and survival after gastrectomy for gastric cancer: Data from a large US-population database. J Clin Oncol 2005;23 (28):7114–7124.
21. Siewert JR, Bottcher K, Stein HJ, Roder JD. Relevant prognostic factors in gastric cancer: Ten-year results of the German Gastric Cancer Study. Ann Surg 1998;228(4):449–461.
22. Kodera Y, Yamamura Y, Shimizu Y, Torii A, Hirai T, Yasui K, Morimoto T, Kato T, Kito T. The number of metastatic lymph nodes: A promising prognostic determinant for gastric carcinoma in the latest edition of the TNM classification. J Am Coll Surg 1998;187(6):597–603.
23. Roukos DH, Lorenz M, Karakostas K, Paraschou P, Batsis C, Kappas AM. Pathological serosa and node-based classification accurately predicts gastric cancer recurrence risk and outcome, and determines potential and limitation of a Japanese-style extensive surgery for Western patients: A prospective with quality control 10-year follow-up study. Br J Cancer 2001;84(12):1602–1609.
24. Lee HY, Choi HJ, Park KJ, Shin JS, Kwon HC, Roh MS, Kim C. Prognostic significance of metastatic lymph node ratio in node-positive colon carcinoma. Ann Surg Oncol 2007;14(5):1712–1717.
25. Le Voyer TE, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, Haller DG. Colon cancer survival is associated

with increasing number of lymph nodes analyzed: A secondary survey of intergroup trial INT-0089. J Clin Oncol 2003;21 (15):2912–2919.
26. Swanson RS, Compton CC, Stewart AK, Bland KI. The prognosis of T3N0 colon cancer is dependent on the number of lymph nodes examined. Ann Surg Oncol 2003;10(1):65–71.
27. Goldstein NS, Sanford W, Coffey M, Layfield LJ. Lymph node recovery from colorectal resection specimens removed for adenocarcinoma. Trends over time and a recommendation for a minimum number of lymph nodes to be recovered. Am J Clin Pathol 1996;106(2):209–216.
28. Sosa JA, Diener-West M, Gusev Y, Choti MA, Lange JR, Dooley WC, Zeiger MA. Association between extent of axillary lymph node dissection and survival in patients with stage I breast cancer. Ann Surg Oncol 1998;5(2):140–149.
29. Polednak AP. Survival of lymph node-negative breast cancer patients in relation to number of lymph nodes examined. Ann Surg 2003;237(2):163–167.

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Morbidity and Mortality Following Multivisceral Resections in Complex Hepatic and Pancreatic Surgery

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Abstract
Complex multivisceral resections in major hepatic and pancreatic surgery are relatively infrequent, and information regarding the morbidity and mortality associated with such resections is scant. The purpose of this paper is to describe the outcomes following such aggressive surgical treatment. A retrospective review of the outcomes following multiorgan resection in the setting of major liver or pancreatic resection was conducted from 2002 until July 2006. Patients who had a major hepatic or pancreatic resection plus resection of at least one other organ were included. The primary outcome measures analyzed were the postoperative morbidity and mortality. Secondary outcomes included recurrence rates and survival. Twenty-seven patients met the inclusion criteria. There were two postoperative deaths (7%). Complications occurred in 59% of patients. Complications were minor in 26% and severe in 33%. Complications were more frequent in older patients and in patients with pancreatic resections. Mortality was significantly increased in the setting of a pancreaticoduodenectomy. These more aggressive procedures should be considered to carry a higher risk of complications, particularly in patients undergoing pancreaticoduodenectomies. Patients should be selected carefully when undertaking complex multivisceral resections in major hepatic and pancreatic surgery.

Keywords Multivisceral · Simultaneous · Liver · Pancreas · Surgery

Introduction

Multivisceral resections in hepatic and pancreatic surgery are relatively infrequent, and information regarding the morbidity and mortality associated with such resections is scant. These resections can occur in the setting of an en-bloc resection for tumors that have directly invaded other organs or in the setting of simultaneous resections of primary tumors along with separate sites of metastatic spread. En-bloc resection of the distal pancreas and spleen as part of a gastrectomy has been shown to significantly increase morbidity and mortality.[1,2]. With regards to the morbidity and mortality of pancreatic resections requiring en-bloc resection of adjacent organs, there are few available reports. For hepatic resections, simultaneous resections of primary tumors and distant sites of metastasis is the more common scenario. However, despite the fact that approximately 20% of patients with colorectal cancers are diagnosed with synchronous metastases at the time of presentation,[3,4] the performance of simultaneous multiorgan resections is relatively uncommon. The safety of such an aggressive approach remains a controversial subject, and patients should be very carefully selected when considering simultaneous multiorgan resection.[5]

In order to select patients appropriately when considering such aggressive treatment, it is imperative to consider the surgical risks. The purpose of this study was to describe the morbidity and mortality associated with complex

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multivisceral resections in major hepatic and pancreatic surgery to further aid patient selection.

Materials and Methods

Design

This was a retrospective analysis of the outcomes following multivisceral resection in complex hepatic and pancreatic surgery at a single tertiary-care hospital (Foothills Medical Centre, Calgary, Alberta, Canada). The study was approved by the University of Calgary Conjoint Health Research Ethics Board.

Subjects

All patients who underwent multivisceral resection in the setting of major hepatic resection or pancreatectomy from January 2002 until December 2006 were included. Eligibility criteria included patients with and without cancer diagnoses, although it was anticipated that the patient population would consist almost exclusively of patients with cancer. A multivisceral resection was defined as a major hepatectomy or pancreatic resection plus the resection of at least one other organ that is not routinely removed during the procedure. For example, patients who had removal of the distal stomach or gallbladder during a pancreaticoduodenectomy were not considered to have had a multivisceral resection and were not included in the study. Similarly, patients undergoing a distal pancreatectomy and splenectomy without removal of additional organs were not considered to have had a multivisceral resection. Major hepatectomy was defined as removal of at least two Couinaud segments, and patients undergoing pancreatectomy included patients having either a distal pancreatectomy or pancreaticoduodenectomy. The time frame of the review was chosen because the three hepatobiliary surgeons comprising the Hepatobiliary and Pancreatic Surgery Unit began working as a group in 2002.

Procedure

Eligible patients were identified through a database maintained by a surgical oncology center (Tom Baker Cancer Centre, Calgary, Alberta, Canada). The surgeons' own private charts as well as the hospital charts were reviewed. Demographic information, the diagnoses and indications for surgery, operative details, information about the hospital stay, postoperative complications, recurrence, and survival were extracted from the charts.

The primary outcome measure of the study was the morbidity and mortality after such resections. Recurrence rates and survival were secondary outcomes. Postoperative morbidity was graded according to a previously validated classification system.[6] Mortality was defined as either 30-day or in-hospital mortality, as mortality as a result of postoperative liver failure in major hepatic surgery may occur well beyond 30 days,[7] which is a commonly used end-point.

Results

A total of 27 patients were identified. There were 13 men and 14 women with a mean age of 61 years (median 64;

Table 1 Pathologies of Patients Undergoing Multivisceral Resection According to Primary Operations Performed

Pathology	*n*	Primary Operation Performed (*n*)		
		Liver Resection	Pancreaticoduodenectomy	Distal Pancreatectomy
Neuroendocrine Tumor	6	3	1	2
Pancreatic Adenocarcinoma	2			2
Pancreatic CystadenoCA	1		1	
Gastric Adenocarcinoma	3			3
Sarcoma[a]	4	1	2	1
Colorectal Adenocarcinoma	2	2		
Renal Cell Carcinoma	2	2		
Inflammatory Mass Mimicking Cholangiocarcinoma	2	2		
Cholangicarcinoma	1		1	
Adrenal Cortical Carcinoma	1	1		
Gall Bladder Adenocarcinoma	1	1		
Desmoid	1			1
Melanoma	1		1	
Total	24	12	6	9

[a] Includes one gastrointestinal stromal tumor, one angiosarcoma of the liver, one sarcoma of bile duct, and one retroperitoneal sarcoma

Table 2 Additional Organs Resected According to Primary Procedure performed

Organs Resected											
Primary Procedure Performed	Liver	Head of Pancreas	Duodenum	Distal Pancreas	Spleen	Stomach	Colon	Small Bowel	Kidney	Diaphragm	Peritoneum[a]
Liver Resection (n=12)	12			2	2	1	5	3	1		1
Pancreaticoduodenectomy (n=6)	4	6	6	1	1	2	4	1			
Distal Pancreatectomy (n=9)	1			9	9	5	6	6	3	1	

[a] This patient had peritoneal cytoreductive surgery at the same time as a liver and colon resection.

range 21 to 86). Twelve multivisceral resections were done in the setting of major liver resections (median of four segments resected), whereas six were performed in the setting of a pancreaticoduodenectomy, and nine were performed in the setting of a distal pancreatectomy. Three of the distal pancreatectomies were performed as part of a total gastrectomy. For one patient who had a right trisegmentectomy plus a pancreaticoduodenectomy for a cholangiocarcinoma, the pancreaticoduodenectomy was considered to be the primary operation, and the patient was included this group of six patients. Another patient who underwent both a right hepatectomy and distal pancreatectomy was included in the group of 12 patients who had liver resections as the primary procedure. Table 1 shows the primary procedure performed according to the various pathologies. Table 2 shows the organs resected as part of the primary procedure performed. A median of three organs was resected (range 2 to 7). In all but five cases, the multivisceral resection was mandated by the presence of direct local invasion into adjacent organs. In the remaining five cases, simultaneous resections occurred at two sites (the primary tumor and sites of metastatic disease).

Median operating time was 420 min (mean 448, range 191 to 683), and the median blood loss was 1,000 ml (mean 1,352 ml, range 300 to 5,000). Forty-eight percent of patients received blood transfusions in the operating room, with a median of 2 units transfused (mean 3, range 1 to 8). Three patients (11%) were admitted to the intensive-care unit (ICU) postoperatively for a median of 2 days (range 1 to 3). The median duration of hospital stay was 14 days (mean 22, range 7 to 60). There were no significant differences in these variables according to the primary type of operation performed.

There were two postoperative deaths (7% operative mortality). Postoperative complications occurred in 59% of patients and are listed in Table 3. These were minor in 26% and severe in 33%. One of the deaths occurred in a 75-year-old patient who underwent a pancreaticoduodenectomy in conjunction with a right trisegmentectomy for what proved to be a sarcoma of the bile duct. This patient was sent to a high-observation unit postoperatively, but when her condition deteriorated, she refused admission to the ICU and died on the seventh postoperative day due to postoperative liver failure, sepsis, and multisystem organ failure. The second postoperative death occurred in a 45-year-old patient with metastatic melanoma who underwent a pancreaticoduodenectomy in conjunction with a segmental liver resection (segments 4b and 5) and a right hemicolectomy. The patient died of recurrent disease 2 months after surgery.

Overall complications, including mortality, were more frequent in patients who underwent pancreatic resections compared to patients who underwent liver resections, occurring in 12 of 15 patients (80%) compared to 6 of 12 patients (50%). Similarly, patients over age 70 had more frequent complications, occurring in 6 of 7 patients (86%) compared to 11 of 19 patients (58%). Complications were not more frequent with increasing operating time or

Table 3 Most Frequent Complications Experienced[a]

Complication	n (%)
Mortality	2 (7)
Minor Morbidity	7 (26)
Major Morbidity	9 (33)
Intra-abdominal Abscess	5 (19)
Anastomotic Stricture	3 (11)
Pancreatic Fistula	3 (18)[b]
Line Infection	2 (7)
Pneumonia	2 (7)
Deep venous thrombosis	2 (7)

[a] Less frequent complications that occurred in one patient each (3%) include a pleural effusion, acute renal insufficiency, sepsis, an anastomotic leak, a *Clostridium difficile* infection, cholangitis, an empyema, a gastrointestinal bleed, an ileus, a urinary tract infection, ascites, and a wound infection. Some patients experienced more than one complication.

[b] The percentage refers to the percentage of the subgroup of 17 patients who underwent pancreatic resections.

increasing blood loss. Postoperative mortality was more common when a pancreaticoduodenectomy was performed.

After a mean follow-up of 10 months (median 6, range 0.3 to 46), six patients have suffered recurrent disease (local and/or distant recurrences) with a range of 2 to 46 months after surgery. Four patients died of disease between 2 and 46 months postoperatively. Disease-free and overall survival rates at 3 years were 68% (95% CI=37 to 86%) and 79% (95% CI=52 to 91%), respectively.

Discussion

Multivisceral resections in hepatic and pancreatic surgery are relatively infrequent, and evidence regarding the morbidity and mortality associated with such resections is scant. In our series, the postoperative mortality rate was 7%, and the overall rate of morbidity was 59%, with a minor complication rate of 26% and a major complication rate of 33%. This is in contrast to a mortality rate of 3% and an overall morbidity rate of 37% for patients who underwent liver resections in the same institution between 2001 and 2004.

Patients over the age of 70 and patients who underwent pancreatic resections tended to have more frequent complications. A pancreaticoduodenectomy in conjunction with resection of other organs was associated with a higher mortality rate than other types of resection in our series.

In a series of 17 patients who underwent either pancreaticoduodenectomy or distal pancreatectomy in conjunction with major hepatectomy for a variety of tumor types, D'Angelica et al.[8] noted a higher morbidity (47%) and mortality (18%) with such an aggressive approach. Similar to our series, all of the deaths occurred in patients who underwent a pancreaticoduodenectomy in conjunction with a liver resection, giving a mortality rate of 50% in this subset of patients. On the other hand, Sasson et al.[9] report no difference in morbidity and mortality between 79 patients who underwent a standard pancreatectomy and 37 patients who underwent simultaneous resection of other organs. The other resected organs were most commonly vascular structures but also included colon, adrenal, liver, and stomach. Morbidity and mortality in the group that had an extended resection were 35 and 2.7%, respectively.

In the setting of gastric cancer, distal pancreatectomy has been performed both as part of a D2 lymphadenectomy[1,2] and in cases of locally advanced disease.[10–12] Whereas some have found an increased morbidity with such multivisceral resections including pancreatectomy for gastric cancer,[1,10,12] others have not.[11] A large randomized trial comparing D1 to D2 lymphadenectomy found that the addition of splenectomy and distal pancreatectomy led to increased morbidity and mortality and possibly poorer overall survival.[1,13] These procedures have now largely been abandoned as part of routine D2 lymphadenectomy of gastric cancer. When multivisceral resection is needed for gastric cancer because of local invasion of other structures by advanced tumors, complications are also increased. When two or more organs, most commonly the pancreas and/or spleen, must be resected for gastric cancer, complications are increased significantly.[10]

For hepatic surgery, the most common scenario is that of colorectal cancer with synchronous liver metastases. Some authors recommend multiorgan resection in order to avoid repeat laparotomy. They have reported similar rates of morbidity and mortality regardless of whether the primary lesion and the liver metastases are resected together or as staged procedures, even when a major hepatectomy is performed in conjunction with removal of a left-sided primary.[14–18] However, patient selection appears to play a role in some of these reports as these resections were more commonly performed for right colonic primaries, for smaller and fewer liver metastases, and for less extensive liver resections.[17,18]

Other publications have documented significantly increased morbidity and mortality when hepatectomy is done in conjunction with other major organ resection. In a series of 67 patients who underwent major liver resection under total vascular isolation, complications were increased by up to 14 times when an associated gastrointestinal procedure was performed.[19]. Tanaka et al.[20] reported that complications are increased when more than one segment of liver is removed. The safety of such an aggressive approach remains a controversial subject, and patients should be very carefully selected when considering simultaneous multiorgan resection.[5]

There is a critical difference between en-bloc multivisceral resections that are performed for direct invasion of a tumor into contiguous organs and multivisceral resections performed for disease at different sites, as in the example of a colorectal cancer with synchronous liver metastases. In the former scenario, there is no option to perform a delayed, or staged, procedure. In such instances, the increased morbidity and mortality of a multivisceral hepatic or pancreatic resection is a necessary risk to the procedure.

In the latter scenario, there is an option to offer a staged procedure and avoid a simultaneous multivisceral resection. This decision can be quite difficult. Whereas in many cases, such an approach may be safe, properly defined selection criteria are lacking. Besides this question of which patients can be selected to safely undergo such resections, some have questioned whether certain patients would benefit from a staged procedure to allow adjuvant systemic treatments prior to liver resection. Fujita et al.[21] reported that the presence of six or more metastatic lymph nodes associated with a primary colorectal lesion was a poor

prognostic factor and suggested that perhaps such patients would benefit from systemic chemotherapy prior to liver resection for synchronous metastases. Unfortunately, this prognostic factor cannot be determined preoperatively. Capussotti et al.[22] have found that negative prognostic factors for overall survival in patients with synchronous colorectal liver metastases include T4 lesions, greater than three metastases, and invasion of contiguous structures by the metastases themselves. They suggest that perhaps such patients would benefit from neoadjuvant chemotherapy with the timing of resection of the primary lesion dictated by symptoms. At this point in time, however, validated selection criteria remain undefined.

This study has many limitations, and the results must be interpreted with these in mind. This is a relatively small, retrospective review with a heterogeneous patient population. Analyzing predictive factors of morbidity and mortality is difficult due to the small sample size. In our series, all but five patients had multivisceral resections due to local invasion of contiguous organs. For the remaining five patients, this study cannot answer the question of whether a staged procedure would have been safer. In addition, it is not possible to make comparisons to a control group of patients who underwent hepatic or pancreatic surgery without a multivisceral resection.

Nonetheless, the study does demonstrate that the risk of such aggressive surgical treatment is significant, and multivisceral resections should be considered to have increased morbidity and mortality. Patients undergoing a pancreaticoduodenectomy along with resection of other organs appear to be at a particularly high risk.

References

1. Cuschieri A, Weeden S, Fielding J, Bancewicz J, Craven J, Joypaul V, Sydes M, Fayers P. Patient survival after D1 and D2 resections for gastric cancer: long-term results of the MRC randomized surgical trial. Surgical Co-operative Group. Br J Cancer 1999;79:1522–1530.
2. Bonenkamp JJ, Songun I, Hermans J, Sasako M, Welvaart K, Plukker JT, van EP, Obertop H, Gouma DJ, Taat CW. Randomised comparison of morbidity after D1 and D2 dissection for gastric cancer in 996 Dutch patients. Lancet 1995;345:745–748.
3. Ries LA, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L et al. SEER Cancer Statistics Review, 1975–2002. Bethesda, MD: National Cancer Institute, 2004.
4. Mella J, Biffin A, Radcliffe AG, Stamatakis JD, Steele RJ. Population-based audit of colorectal cancer management in two UK health regions. Colorectal Cancer Working Group, Royal College of Surgeons of England Clinical Epidemiology and Audit Unit. Br J Surg 1997;84:1731–1736.
5. Schwarz RE. Visceral organ resections combined with synchronous major hepatectomy: examples of safety and feasibility. HPB 2003;5:27–32.
6. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004;240: 205–213.
7. Nagino M, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y. Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. Ann Surg 2006;243:364–372.
8. D'Angelica M, Martin RC, Jarnagin WR, Fong Y, DeMatteo RP, Blumgart LH. Major hepatectomy with simultaneous pancreatectomy for advanced hepatobiliary cancer. J Am Coll Surg 2004;198:570–576.
9. Sasson AR, Hoffman JP, Ross EA, Kagan SA, Pingpank JF, Eisenberg BL. En bloc resection for locally advanced cancer of the pancreas: is it worthwhile? J Gastrointest Surg 2002;6:147–157.
10. Martin RC, Jaques DP, Brennan MF, Karpeh M. Achieving RO resection for locally advanced gastric cancer: is it worth the risk of multiorgan resection? J Am Coll Surg 2002;194:568–577.
11. Carboni F, Lepiane P, Santoro R, Lorusso R, Mancini P, Sperduti I, Carlini M, Santoro E. Extended multiorgan resection for T4 gastric carcinoma: 25-year experience. J Surg Oncol 2005;90:95–100.
12. Piso P, Bellin T, Aselmann H, Bektas H, Schlitt HJ, Klempnauer J. Results of combined gastrectomy and pancreatic resection in patients with advanced primary gastric carcinoma. Dig Surg 2002;19:281–285.
13. Cuschieri A, Fayers P, Fielding J, Craven J, Bancewicz J, Joypaul V, Cook P. Postoperative morbidity and mortality after D1 and D2 resections for gastric cancer: preliminary results of the MRC randomised controlled surgical trial. The Surgical Cooperative Group. Lancet 1996;347:995–999.
14. Weber JC, Bachellier P, Oussoultzoglou E, Jaeck D. Simultaneous resection of colorectal primary tumour and synchronous liver metastases. Br J Surg 2003;90:956–962.
15. de SE, Lassalle FB, McCormack L, Pekolj J, Quintana GO, Vaccaro C, Benati M. Simultaneous colorectal and hepatic resections for colorectal cancer: postoperative and longterm outcomes. J Am Coll Surg 2002;195:196–202.
16. Chua HK, Sondenaa K, Tsiotos GG, Larson DR, Wolff BG, Nagorney DM. Concurrent vs. staged colectomy and hepatectomy for primary colorectal cancer with synchronous hepatic metastases. Dis Colon Rectum 2004;47:1310–1316.
17. Martin R, Paty P, Fong Y, Grace A, Cohen A, DeMatteo R, Jarnagin W, Blumgart L. Simultaneous liver and colorectal resections are safe for synchronous colorectal liver metastasis. J Am Coll Surg 2003;197:233–241.
18. Capussotti L, Ferrero A, Vigano L, Ribero D, Lo TR, Polastri R. Major liver resections synchronous with colorectal surgery. Ann Surg Oncol 2007;14:195–201.
19. Karoui M, Penna C, min-Hashem M, Mitry E, Benoist S, Franc B, Rougier P, Nordlinger B. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. Ann Surg 2006;243:1–7.
20. Tanaka K, Shimada H, Matsuo K, Nagano Y, Endo I, Sekido H, Togo S. Outcome after simultaneous colorectal and hepatic resection for colorectal cancer with synchronous metastases. Surgery 2004;136:650–659.
21. Fujita S, Akasu T, Moriya Y. Resection of synchronous liver metastases from colorectal cancer. Jpn J Clin Oncol 2000;30: 7–11.
22. Capussotti L, Vigano' L, Ferrero A, Lo TR, Ribero D, Polastri R. Timing of resection of liver metastases synchronous to colorectal tumor: proposal of prognosis-based decisional model. Ann Surg Oncol 2007;14:1143–1150.

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Neoadjuvant Chemotherapy and Radiation for Patients with Locally Unresectable Pancreatic Adenocarcinoma: Feasibility, Efficacy, and Survival

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Abstract
Background We evaluated the feasibility and efficacy of neoadjuvant chemotherapy and radiation for patients with locally unresectable pancreatic cancer.
Materials and Methods From October 2000 to August 2006, 245 patients with pancreatic adenocarcinoma underwent surgical exploration at our institution. Of these, 78 patients (32%) had undergone neoadjuvant therapy for initially unresectable disease, whereas the remaining patients (serving as the control group) were explored at presentation ($n=167$). All neoadjuvant patients received gemcitabine-based chemotherapy, often in conjunction with docetaxal and capecitabine in a regimen called GTX (81%). Seventy-five percent of neoadjuvant patients also received preoperative abdominal radiation (5,040 rad).
Results Neoadjuvant patients were younger than control-group patients (60.8 vs 66.2 years, respectively, $p<0.002$). Seventy-six percent of neoadjuvant patients were resected as compared to 83% of control patients (NS). Concomitant vascular resection was required in 76% of neoadjuvant patients but only 20% of NS ($p<0.01$). Complications were more frequent in the neoadjuvant group (44.1 vs 30.9%, $p<0.05$), and mortality was higher (10.2 vs 2.9%, $p<0.03$). Among the neoadjuvant patients, all but one of the deaths were in patients that underwent arterial reconstruction. Mortality for patients undergoing a standard pancreatectomy without vascular resection was 0.8% in this series. Of patients resected, negative margins were achieved in 84.7% of neoadjuvant patients and 72.7% of NS. Within the cohort of neoadjuvant patients, radiation significantly increased the complication rate (13.3 vs 54.6%, $p<0.006$), but did not affect median survival (512 vs 729 days, NS). Median survival for patients who received neoadjuvant therapy (503 days) was longer than NS that were found to be unresectable at surgery (192 days, $p<0.001$) and equivalent to NS that were resected (498 days).

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Conclusions Resection rate, margin status, and median survivals were equivalent when neoadjuvant patients were compared to patients considered resectable by traditional criteria, demonstrating equal efficacy. Surgical resection with venous reconstruction following neoadjuvant therapy for patients with locally advanced pancreatic cancer can be performed with acceptable morbidity and mortality. This approach extended the boundaries of surgical resection and greatly increased median survival for the "inoperable" patient with advanced pancreatic cancer.

Keywords Pancreas · Cancer · Neoadjuvant · Vascular · Gemcitabine

Introduction

Pancreatic adenocarcinoma is a lethal disease with an annual death rate that approaches the incidence.[1] Over half of the patients have metastases on presentation, whereas only 15% of patients will have resectable disease.[2] For every patient that presents with a resectable tumor, there are two patients that will have locally advanced but non-metastatic disease.[3] Strategies to recruit this patient population have the potential to triple the surgical impact on pancreatic cancer. Two such strategies include preoperative cytoreduction with neoadjuvant therapy and more aggressive surgery with vascular resection and reconstruction.

Gemcitabine-based chemotherapy for pancreatic cancer has shown a minor but significant improvement over previous therapies.[4] At our institution, we developed a three-drug regimen for patients with pancreatic cancer including gemcitabine, docetaxal, and capecitabine (RLF) called GTX.[5,6] Using this regimen in patients with metastatic disease to the liver and/or lung, we observed a 40% partial response rate and an 11% complete response rate at metastatic sites after nine cycles in previously untreated patients.[7] We also observed significant reduction in the primary tumor size in many of the patients while on therapy. With this preliminary data, we developed a neoadjuvant protocol for patients with locally advanced, nonmetastatic pancreatic cancer consisting of GTX for three cycles followed by standard conformal radiation to 5,040 rad with low-dose weekly gemcitabine as a radiosensitizer.

In this paper, we describe our total experience and outcomes with patients explored for pancreatic adenocarcinoma over the past 6 years. Patients explored after neoadjuvant therapy are specifically compared to those that went to surgery with lesions that appeared resectable at presentation.

Materials and Methods

Data Review

Data were gathered prospectively within our clinical database of patients with pancreatic neoplasms. After approval from the Columbia University Internal Review Board in accordance with Health Insurance Portability and Accountability Act regulations, the de-identified dataset was retrospectively reviewed. From October 2000 to August 2006, 245 patients with pancreatic adenocarcinoma underwent surgical exploration at our institution. Of these, 78 patients (32%) had undergone neoadjuvant therapy for initially unresectable disease, whereas the remaining patients (serving as the control group) were explored at presentation (n=167). The records were reviewed for demographic information, therapy delivered, operative findings and interventions, postoperative outcomes, pathologic findings, and overall survival.

Definition of Unresectable

The definition of unresectable varies among authors. For the purpose of this investigation, unresectable was defined as invasion of the superior mesenteric or portal vein or arterial abutment (superior mesenteric artery, hepatic artery, or celiac artery) of $\geq$180° of the vessel circumference. Venous invasion included those patients found to have invasion of the portal or superior mesenteric vein at the time of initial exploration, those with endoscopic ultrasonographic evidence of tumor within the wall or lumen of the vein, and patients with encasement of the vein on crossectional imaging. The unresectable group also included patients with thrombosis of the portal venous system. The patients are stratified based on spectrum of disease in Table 1.

Patients were found to be unresectable either by imaging studies including computed tomography with contrast, magnetic resonance imaging with gadolinium and endoscopic ultrasound (61%), or by previous surgical exploration (39%). All imaging studies were systematically reviewed by Columbia University surgeons, gastroenterologists, and radiologists during weekly conference. The quality of imaging studies was varied depending on the institution of origin, and studies were repeated at Columbia if necessary. Of the patients previously explored, 43% had their initial surgery at Columbia by a qualified pancreatic surgeon (JC, JA, BS), whereas the remaining patients were initially explored at outside institutions. Approximately half of these patients were explored at high-volume centers, whereas the remainder underwent their initial surgery in community hospitals.

Table 1 Patients Undergoing Surgical Exploration After Neoadjuvant Therapy Stratified by Extent of Disease

Extent of Disease	Number of Patients (%)
Invasion of SMV/portal vein only[a]	32 (43)
Short segment venous occlusion or arterial abutment <180°	12 (16)
Venous occlusion without technical options for reconstruction or >180° of arterial abutment	30 (41)

[a] Venous invasion included those patients found to have invasion of the portal or superior mesenteric vein at the time of initial exploration, those with endoscopic ultrasonographic evidence of tumor within the wall or lumen of the vein, and patients with encasement of the vein on cross-sectional imaging.

Neoadjuvant Protocol

Patients enrolled in the neoadjuvant protocol all had biopsy-proven adenocarcinoma of the pancreas. Neuroendocrine carcinoma was ruled out by immunohistochemical analysis. Patients determined to be unresectable by imaging studies alone were not laparoscoped prior to entry into the study. Chemotherapy consisted of gemcitabine, capecitabine, and doctaxal delivered as outlined in Table 2.

One cycle of chemotherapy consisted of the drug schedule outlined in Table 2 followed by 1 week of rest. Neoadjuvant protocol patients underwent three cycles of chemotherapy followed by chemoradiation using low-dose gemcitabine as a radiosensitizer. Abdominal radiation consisted of 4,500 cGy in 180 cGy fractions followed by 540 cGy in 180 cGy fractions focused on the mass using standard conformal techniques, resulting in a total dose of 5,040 cGy. Patients were restaged radiographically and then brought to the operating room for exploration if there was no evidence of disease progression after a 5-week rest period. The treatment algorithm is outlined in Fig. 1.

Nonneoadjuvant Patient Group

The nonneoadjuvant (control) group consisted of all patients explored with pancreatic adenocarcinoma during the index time interval who were thought to be resectable based on preoperative imaging studies.

Complications

For the purpose of this review, pancreatic fistula or leak was defined as the presence of amylase rich fluid in the abdominal drain greater than three times the serum amylase. Drain amylase was measured selectively based on the judgment of the operating surgeon and not routinely.

Delayed gastric emptying was defined as failure of the patient to tolerate solid food by the tenth postoperative day. Postoperative mortality was defined as any death within 30 days of the operation or death prior to discharge from the hospital.

Postoperative portal vein imaging by ultrasound was performed selectively based on the clinical course of the patient and the drain output in patients that underwent venous reconstructions.

Table 2 Drug Regimen Dosing and Timing for Patients Formally Enrolled in the Phase II Neoadjuvant GTX Protocol

Drug	Days Delivered	Dose
Capecitabine	1–14	750 mg/m^2/bid/po
Gemcitabine	4 and 11	750 mg/m^2 over 75 min
Docetaxal	4 and 11	30 mg/m^2 over 30 min

Days 15–21, patients are off all therapy.

Pathology Review

Over the 6-year time interval during which these patients underwent surgery, pancreatic resection specimens were evaluated by using a standard grossing protocol and reporting form. The following parameters were assessed in all cases: tumor size, degree of tumor differentiation, presence or absence of vascular invasion, presence or absence of lymph node metastases, and surgical margin status. Surgical margins evaluated included pancreatic neck, common bile duct, and posterior/radial margins.

Pancreatic resection specimens from patients that underwent vascular resection were also reviewed retrospectively. Hematoxylin and eosin (H&E) slides of the resection specimens were microscopically reviewed by two pathologists (HR, EV) to evaluate the viability index of the tumors. The viability index was determined by calculating the percent of viable tumor cells identified microscopically on a representative H&E slide sampling the largest focus of tumor. Percent pathologic response was defined by the percent of the tumor composed of fibrotic or necrotic tissue.

Survival

Overall survival was calculated from the time of diagnosis. For nonneoadjuvant patients, the date of diagnosis was defined as the day of operation. For the neoadjuvant patients, survival was calculated from the time neoadjuvant therapy was initiated.

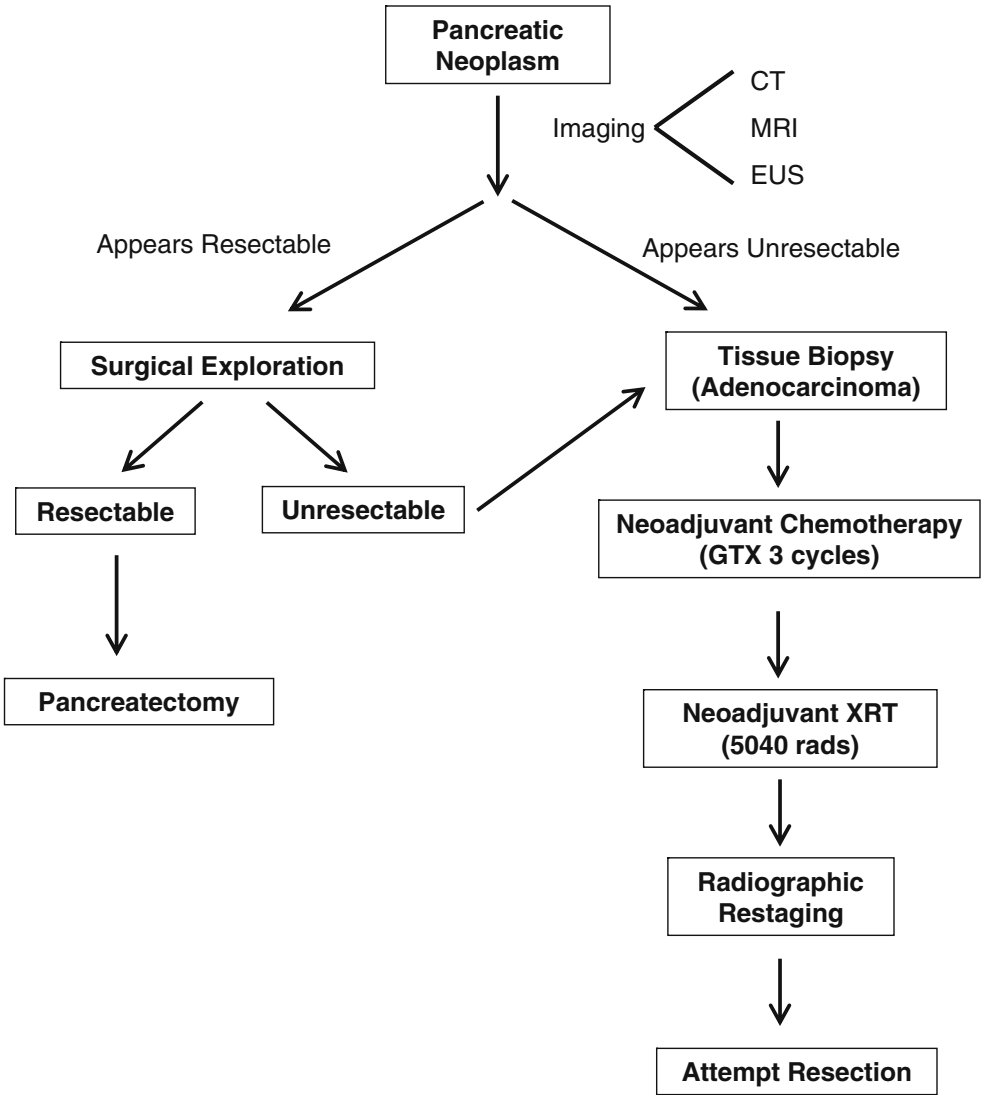

Figure 1 Treatment algorithm for patients presenting with pancreatic neoplasms.

Statistics

Student's *t* test was used to assess significance of differences in age and complication rate between the neoadjuvant therapy and nonneoadjuvant therapy groups. The Wilcoxon Mann–Whitney test was used to assess the differences in estimated blood loss, and chi-square analysis was used to determine differences in sex, resection rate, resection type, concomitant vascular resection, tumor viability, and mortality between the treatment groups. To evaluate the effect of neoadjuvant therapy on patient survival, life tables were constructed from survival data and Kaplan–Meier curves were plotted. Overall survival was measured from date of initial diagnosis to date of death or latest follow-up. For Kaplan–Meier curves, comparisons between groups were done using the log-rank test. All statistical analyses were done using Statistical Analysis Software (SAS) version 9.1 (SAS Institute, Cary, NC). Significance was set at $p<0.05$.

Table 3 Patient Demographics Stratified by Neoadjuvant Status

	Neoadjuvant	Nonneoadjuvant	$p\leq$
Gender			
Male	42 (55%)	79 (47%)	NS
Female	36 (45%)	88 (53%)	
Median Age (years)	60.8	66.6	0.002
Resection rate	59 (76%)	139 (83%)	NS

Results

Demographics

All neoadjuvant patients received gemcitabine-based chemotherapy, often in conjunction with docetaxal and capecitabine (81%). Fourteen patients (18%) were formally enrolled in a phase II prospective GTX-radiation neoadjuvant

Table 4 Operative Characteristics Stratified by Neoadjuvant Status

Surgical Variables	Overall	NA+Resected	NA+Not Resected	No NA+Resected	No NA+Not Resected[a]
Total Patients	245	59	19	139	28
Surgical Resection					
Whipple	141	47 (80%)		94 (68%)	
PPPD	19	1 (2%)		18 (13%)	
Distal Pancreatectomy	22	3 (5%)		19 (14%)	
Subtotal Pancreatectomy	1	1 (2%)			
Total Pancreatectomy	15	7 (12%)		8 (6%)	
Abandoned (overall)	47		19		28
Abandoned (local growth)	21		8 (42%)		13 (46%)
Abandoned (mets)	26		11 (58%)		15 (54%)
Vascular Resection					
Yes	73	45 (76%)*		28 (20%)	
No	172	14 (24%)		111 (80%)	
Previous Resection Attempt					
Yes		23 (39%)		5 (4%)	
No		36 (41%)		134 (96%)	
EBL ml (Median)	1,450	2,000		1,050	
Operative time (min)		472±171*		348±123	
Median Postoperative Length of Stay (days)		10	3	10	4.5

NA Neoadjuvant, *PPPD* pylorus preserving pancreaticoduodenectomy, *EBL* estimated blood loss.
*$p<0.01$ vs the nonneoadjuvant group.
[a] Excludes patients that subsequently received neoadjuvant therapy.

protocol (RLF), the remaining patients (82%) were treated off protocol. About half of the patients (45%) received their neoadjuvant therapy at outside institutions in consultation with the medical oncology faculty at Columbia University. Three quarters of the neoadjuvant patients also received preoperative abdominal radiation.

Patients were deemed unresectable either by radiologic imaging studies alone (61%) or by previous surgical exploration (39%). Of the patients that were found to have unresectable disease at the time of initial exploration, about half were explored at our institution (43%), and the remaining patients were explored at outside hospitals (57%).

Patient demographics are outlined in Table 3. Patients undergoing neoadjuvant therapy ($n=78$) were significantly younger than those brought to the operating room with resectable lesions (60.8 vs 66.6 years, $p<0.002$). Resection rate was similar in the neoadjuvant group (76%) and the nonneoadjuvant group (83%, NS). The gender makeup of both groups was similar.

Table 5 Pathologic Characteristics of Patients with Pancreatic Adenocarcinoma Who Underwent Surgical Resection of the Tumor ($n=198$)

Pathologic Variable	NA (%; $n=59$)	No NA (%; $n=139$)
Tumor Size (Mean/Range)	3.27 cm (0–6.5)	3.6 cm (0.5–11)
Differentiation		
Well	7 (11.9%)	8 (5.8%)
Moderate	23 (39.0%)	50 (36.0%)
Poor	21 (35.6%)	73 (52.5%)
Margin Status		
Positive Margin	9 (15.3%)	38 (27.3%)
Negative Margin	50 (84.7%)	101 (72.7%)
Node Status		
Positive	25 (42.4%)	103 (74.1%)
Negative	34 (57.6%)	36 (25.9%)
Neurovascular Invasion		
Yes	46 (78.0%)	112 (80.6%)
No	12 (20.3%)	26 (18.7%)

Surgical Variables

Operative findings and interventions are summarized in Table 4. All patients received octreotide subcutaneously preoperatively and for 24 h postoperatively. A similar percentage of patients underwent Whipple procedures in each group; however, it was more common for the pylorus to be preserved in the nonneoadjuvant setting (2 vs 16%, $p<0.01$). Operative blood loss was greater in the neoadjuvant group ($p<0.001$).

Table 6 Tumor Viability Index Stratified by Neoadjuvant Status

Tumor Viability (%)	Neoadjuvant (%)	Nonneoadjuvant (%)
0–10	12 (31.6)	0 (0)
11–50	13 (34.2)	2 (7.7)
51–89	5 (13.2)	1 (3.8)
90–100	8 (21.1)	23 (88)

$p<0.01$ by chi-square.

Table 7 Postoperative Complications and Length of Stay for Resected Patients (n=198) Stratified by Neoadjuvant Status

Postoperative Complication	Overall	NA (n=59)	Non-NA (n=139)	$p\leq$
Overall Complication Rate	69 (34.8%)	26 (44.1%)	43 (30.9%)	0.05
Intra-abdominal Hemorrhage	7 (3.5%)	3 (5.1%)	4 (2.9%)	
Portal Vein Thrombosis	7 (3.5%)	4 (6.8%)	3 (2.2%)	
Neurologic Dysfunction	4 (2.0%)	1 (1.7%)	3 (2.2%)	
Intra-abdominal Collection	7 (3.5%)	4 (6.8%)	3 (2.2%)	
Sepsis	10 (5.1%)	6 (10.2%)	4 (2.9%)	
Gastric Outlet Obstruction	4 (2.0%)	3 (5.1%)	1 (.7%)	
Anastamotic Leak	9 (4.5%)	5 (8.5%)	4 (2.9%)	
ARF	13 (6.6%)	6 (10.2%)	7 (5.0%)	
MI/Cardiac Event	7 (3.5%)	2 (3.4%)	5 (3.4%)	
Wound Infection	15 (7.6%)	4 (6.8%)	11 (7.9%)	
Pulmonary Compromise	20 (10.1%)	8 (13.6%)	12 (8.6%)	
Enteric Fistula	4 (2.0%)	4 (6.8%)	0	
GI Bleed	5 (2.5%)	1 (1.7%)	4 (2.9%)	
Reoperation	17 (8.6%)	10 (16.9)	7 (5.0%)	
Death	10 (5.1%)	6 (10.2%)	4 (2.9%)	0.03
Median Postoperative Length of Stay (Days)	10	10	10	NS

Complications—26 Patients with NA therapy had 57 complications overall. 43 patients without NA therapy had 65 complications overall.
NA Neoadjuvant, *Non-NA* nonneoadjuvant.

Vascular resection was required more often for the neoadjuvant patients (76%) than for nonneoadjuvant patients (20%, $p<0.01$). Forty-two percent of the neoadjuvant patients that underwent pancreatic resection but did not require vascular resection had been deemed unresectable by previous exploration. Of the 45 patients in the neoadjuvant group that underwent vascular resections, 34 patients (76%) had isolated venous reconstructions, 1 patient (2%) had an isolated arterial reconstruction, and 10 patients (22%) had combined venous and arterial reconstructions.

Pathology

Pathologic findings are summarized in Table 5. Tumor size, degree of differentiation, margin status, and nodal status were not different between groups.

For the cohort of patients who underwent vascular resection, the positive margin rate was significantly lower in the neoadjuvant group (25%) than in the nonneoadjuvant group (58% respectively, $p<0.008$). Tumor viability index was also significantly lower in the neoadjuvant group (see Table 6). A complete pathologic response (no viable tumor) was observed in the specimens from two patients (3%) in the neoadjuvant therapy group.

Complications

Complications are outlined in Table 7. The overall surgical complication rate was significantly higher in the neoadjuvant group (44.1%) than in the nonneoadjuvant group (30.9%, $p<0.05$). Operative mortality was also higher in the neoadjuvant group (10.2%) as compared to the nonneoadjuvant group (2.9%, $p<0.03$). All but one of the deaths in

Table 8 Overall Survival in Patients Stratified by Neoadjuvant and Resection Status

	Actual 1-Year Survival	Actual 2-Year Survival	Median Survival (days; 95% CI)
Overall (all patients; n=245)	57.2%	18.9%	445 (399, 524)
NA Therapy (78)	69.4%	23.2%	503 (403, 670)
No NA Therapy (167)	50.0%	16.1%	430 (356, 533)
Abandoned Resection (47)	34.3%	8.8%	296 (237, 410)
NA Therapy (19)	50.0%	13.3%	410 (296, 445)
No NA Therapy (28)	21.1%	5.3%	192 (133, 361)
Surgical Resection (n=198)	63.4%	22.0%	517 (422, 642)
NA Therapy (59)	76.1%	26.8%	533 (480, 714)*
No NA Therapy (139)	56.5%	19.1%	498 (376, 642)

*$p<0.001$ vs abandoned resection without neoadjuvant therapy.

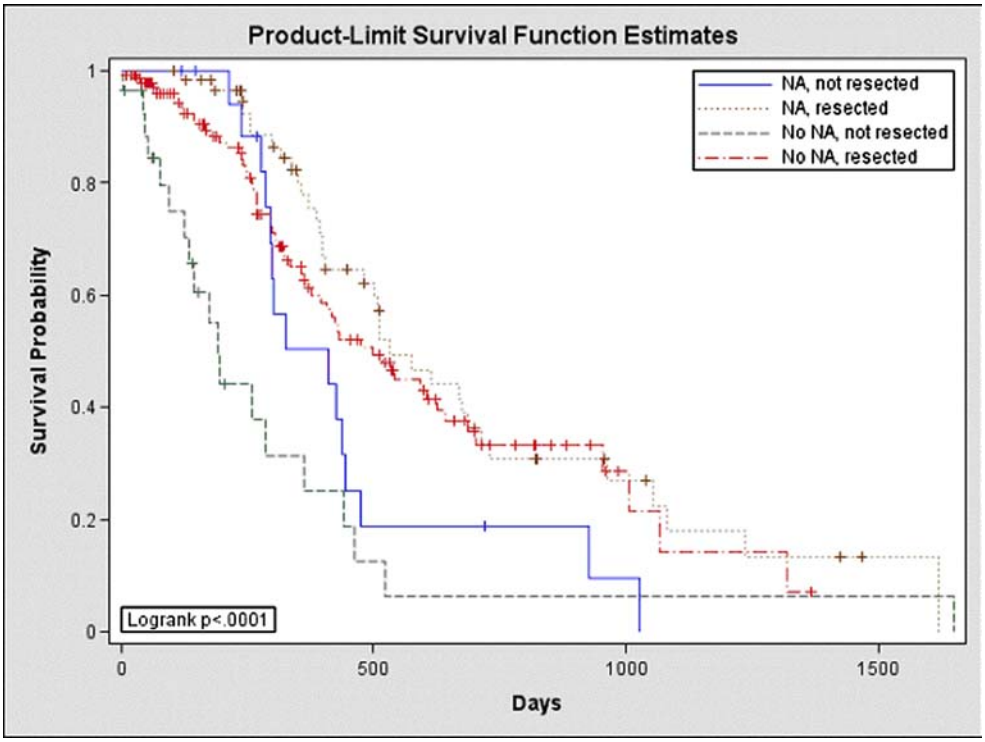

Figure 2 Kaplan–Meier curves for patients separated by resection status and neoadjuvant therapy status (NA = Neoadjuvant).

the neoadjuvant group were in patients that underwent arterial reconstruction, making it an independent risk factor for mortality ($p<0.01$). The mortality for patients undergoing an isolated venous reconstruction following neoadjuvant therapy was 3%. Among the neoadjuvant patients, the complication rate was dramatically increased in patients who received preoperative radiation therapy (54.5%) as compared to those who did not (13.3%, $p<0.006$).

Overall Survival

Overall survival is summarized in Table 8 and Fig. 2. The median survival for neoadjuvant patients that were successfully resected was 533 days, which was significantly better than the median survival of the nonneoadjuvant patients that were not resected (192 days, $p<0.001$) and similar to patients that were resected without neoadjuvant therapy who were initially deemed to be resectable (498 days, NS).

Discussion

Pancreatic adenocarcinoma is a challenging disease to manage and treat. Multiple series, including this one, have demonstrated that the best outcomes are realized when the tumor can be resected.[8–10] Unfortunately, only 15% of patients will present with a lesion that is resectable by traditional criteria. For every patient that presents with a resectable lesion, there will be two patients with locally advanced but nonmetastatic cancer. Strategies designed to recruit this patient population have the potential to triple the surgical impact on this disease. Two such strategies are preoperative cytoreduction with chemotherapy and/or radiation therapy, and more aggressive surgical resections with vascular reconstruction. Using both of these strategies, we have been able to achieve outcomes for selected patients with locally advanced pancreatic cancer that are similar to patients who present with initially resectable disease.

The definition of unresectable for pancreatic cancers differs among authors and practitioners.[11–14] At many institutions, the presence of any vascular invasion is inoperable disease. As the morbidity and mortality of pancreatic surgery has decreased, surgeons at several high-volume centers have been resecting and reconstructing portal veins more commonly. This has led to the group at M.D. Anderson to define lesions with limited portal or superior mesenteric vein involvement as ‘resectable’.[13] Borderline lesions are those that occlude the portal vein or abut arterial structures over less than 180° of the vessel circumference. Tumors are considered inoperable at M.D. Anderson when they occlude the portal vein without technical options for reconstruction, or when arterial structures have more than 180° of encasement.[13] Whereas this definition is helpful when comparing reported outcomes in patients enrolled in neoadjuvant protocols at high-volume centers, at most institutions portal vein invasion continues to be a contraindication to resection and for all practical purposes represents inoperable disease. It is for this reason that we

include those patients in our neoadjuvant group. For the purposes of comparison to other large series, the distribution of our patients based on the M.D. Anderson criteria is outlined in Table 1.

In this review, patients were deemed unresectable either by imaging studies alone (61%) or by previous surgical exploration (39%). All of the imaging studies were systematically reviewed by Columbia University surgeons, gastroenterologists, and radiologists during weekly conference. The quality of the imaging studies was varied, and studies were repeated at Columbia if necessary. All patients explored for pancreatic cancer at Columbia underwent a systematic intraoperative evaluation of their tumor for resectability as previously described.[15] About a fifth of the patients were deemed unresectable at the time of an initial surgical exploration which was done at an outside institution. Although these explorations were not standardized, many of them were performed at high-volume centers.

The results from this series demonstrate the feasibility of resecting locally advanced pancreatic cancers. The resection rate in the patients undergoing neoadjuvant therapy was 76% which was not different from the nonneoadjuvant patients (83%), demonstrating that it is possible to resect selected patients after neoadjuvant therapy. The complication rate (44.1 vs 30.9%, $p<0.05$) and mortality rate (10.2 vs 2.9%, $p<0.03$) were higher in the neoadjuvant group vs the nonneoadjuvant group but not prohibitively so. Furthermore, all but one of the deaths in the neoadjuvant group were in patients that underwent arterial resection, underscoring the need to be highly selective when choosing candidates for arterial reconstruction.

The efficacy of this approach is supported by the pathologic findings. The margin rate and resection rates were equivalent between groups. Furthermore, detailed histologic analysis of the patients that underwent vascular resection revealed considerable reduction in viable tumor cell mass in patients that underwent neoadjuvant therapy, with two patients having a complete pathologic response. These observations attest to the cytoreductive potential of gemcitabine-based regimens in patients with pancreatic adenocarcinoma.

The efficacy of this approach is further supported by the overall survival outcomes. Patients resected after neoadjuvant therapy lived significantly longer than bypassed patients (533 vs 192 days, $p<0.001$). In fact, survival for the neoadjuvant patients was equivalent to patients who were resected at initial presentation (533 vs 498 days, NS). Review of the Kaplan–Meier curves (Fig. 2) reveals that this approach pushes the survival curve of the ‘inoperable’ patient to the right such that it superimposes the curve of patients considered initially resectable by traditional criteria.

One might question the necessity of the neoadjuvant therapy, arguing that the patients in our neoadjuvant group simply underwent more aggressive resections with a high rate of vascular reconstruction (76%). However, when vascular resection was undertaken without neoadjuvant therapy, the positive margin rate was 58%. In sharp contrast, the positive margin rate was significantly reduced (25%, $p<0.008$) when patients underwent neoadjuvant therapy. The patients in the neoadjuvant group that did not require vascular resection (24%) either represent patients that were effectively downstaged by neoadjuvant therapy or patients that were overstaged at presentation. However, nearly half of the neoadjuvant patients that did not require vascular resection had been deemed unresectable by previous surgical exploration, suggesting that they were effectively downstaged by preoperative therapy.

The rate of disease progression for patients while receiving neoadjuvant therapy was low (4%) for patients formally enrolled in the protocol. However, many authors report a 5 to 10% incidence of metastatic disease found at the time of surgical exploration that was not seen on preoperative imaging studies.[16,17] Patients enrolled by radiologic criteria alone were not laparoscoped to rule out occult metastatic disease prior to entry into the study. Therefore, metastatic disease found at the time of exploration after neoadjuvant therapy represents a combination of occult metastatic disease that was present at the time of enrollment and true disease progression while on therapy.

Table 9 Selected Series of Neoadjuvant Therapy for Patients with Initially Unresectable Pancreatic Cancer

Author (year)	Number of Patients	XRT (cGy)	Chemotherapy	Number Surgically Resected	Negative Margins	Resected with Negative Margins
Jessup et al. (1993)[18]	16	4500	5-FU	2 (13%)	NR	NR
Kamthan et al. (1997)[19]	35	5400	5-FU, STZ, cisplatin	5 (14%)	100%	14%
Blackstock et al. (1999)[20]	17	5040	Gemcitabine	0	N/A	N/A
Epelbaum et al. (2002)[21]	20	5040	Gemcitabine	2 (10%)	66%	6.6%
Snady et al. (2000)[22]	68	5400	5-FU, STZ, cisplatin	20 (29%)	95%	28%
Ammori et al. (2003)[23]	67	5040	Gemcitabine (+/− cisplatin)	9 (13%)	66%	9%

XRT External beam abdominal radiation, *5-FU* 5-fluoruracil, *NR* not reported, *STZ* streptazocin.

This bias would be expected to result in an underestimation of the true patient survival in the neoadjuvant therapy group.

Our outcomes compare favorably to other series (Table 9) of neoadjuvant therapy for initially unresectable pancreatic cancer reported in the literature.[18–23] Using 5-FU combined with radiotherapy, Jessup et al.[18] reported a resection rate of 13%. Kamthan et al.[19] and Snady et al.[22] achieved resection rates of 14 and 29%, respectively, using similar protocols of 5-FU, streptozocin, and cisplatin combined with radiotherapy with a high rate of negative pathologic margins. In the present series of 78 patients, 59 (76%) of the patients explored underwent resection with a negative margin rate of 84.7%. Thus, the successful resection rate (resection with negative margins) in patients presenting with inoperable disease who underwent neoadjuvant therapy was 64.4% in our study.

This study is limited by its retrospective approach, nonstandardization of neoadjuvant therapy, and nonrandomization. Despite these shortcomings, the data support the feasibility and efficacy of the neoadjuvant approach with gemcitabine-based protocols for patients with locally advanced pancreatic cancer. Further work in prospective phase II and III trials is necessary to rigorously test individual regimens.

Conclusion

In conclusion, resection rate, margin status, and median survival were equivalent when neoadjuvant treated patients were compared to patients considered initially resectable by traditional criteria, demonstrating significant efficacy. Surgical resection following neoadjuvant therapy for patients with locally advanced pancreatic cancer can be performed with acceptable morbidity and mortality. This approach extended the boundaries of surgical resection and greatly increased median survival for the "inoperable" patient.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106–130.
2. Mancuso A, Calabro F, Sternberg CN. Current therapies and advances in the treatment of pancreatic cancer. Crit Rev Oncol/Hematol 2006;58:231–241.
3. Molinari M, Helton WS, Espat NJ. Palliative strategies for locally advanced unresectable and metastatic pancreatic cancer. Surg Clin North Am 2001;81:651–658.
4. Xiong HQ, Carr K, Abbruzzese JL. Cytotoxic chemotherapy for pancreatic cancer: Advances to date and future directions. Drugs 2006;66(8):1059–1072.
5. Fine RL, Fogelman DR, Sherman W. The GTX regimen: A biochemically synergistic combination for advanced pancreatic cancer (PC). Proc Am Soc Clin Oncol 2003;Abst # 1129.
6. Fine RL, Fogelman DR, Schreibman S, Guba S, Sharma J, Shapiro G. GTX chemotherapy for metastatic pancreatic cancer: Response, survival, and toxicity data. J Clin Oncol 2004;22:381s.
7. Fine RL, Fogelman DR, Schreibman SM, Desai M, Sherman W, Strauss J, Guba S, Andrade R, Chabot JC. The gemcitabine, docetaxel, and capecitabine (GTX) regimen for metastatic pancreatic cancer: A retrospective analysis. Cancer Chemother Pharmacol 2007 (in press).
8. Fogelman DR, Chen J, Chabot JA, Allendorf JD, Schrope BA, Ennis RD, Schreibman SM, Fine RL. The evolution of adjuvant and neoadjuvant chemotherapy and radiation for advanced pancreatic cancer: From 5-fluorouracil to GTX. Surg Oncol Clin N Am 2004;13:711–736.
9. Cress RD, Yin D, Clarke L, Bold R, Holly EA. Survival among patients with adenocarcinoma of the pancreas: A population-based study (United States). Cancer Causes Control 2006;17:403–409.
10. Yeo CJ, Cameron JL, Sohn TA, Lillemoe KD, Pitt HA, Talamini MA, Hruban RH, Ord SE, Sauter PK, Coleman J, Zahurak ML, Grochow LB, Abrams RA. Six hundred and fifty consecutive pancreaticoduodenectomies in the 1990's: Pathology, complications, and outcomes. Ann Surg 1997;226:248–260.
11. Cameron JL, Crist DW, Sitzmann JV, Hruban RH, Boitnott JK, Seidler AJ, Coleman J. Factors influencing survival after pancreaticoduodenectomy for pancreatic cancer. Am J Surg 1991;161:120–124.
12. Tashiro S, Uchino R, Hiraoka T, Tsuji T, Kawamoto S, Saitoh N, Yamasaki K, Miyauchi Y. Surgical indication and significance of portal vein resection in biliary and pancreatic cancer. Surgery 1991;109:481–487.
13. Varadhachary GR, Tamm EP, Abbruzzese JL, Xiong HQ, Crane CH, Wang H, Lee JE, Pisters PW, Evans DB, Wolff RA. Borderline resectable pancreatic cancer: Definitions, management, and role of preoperative therapy. Ann Surg Oncol 2006;13(8):1035–1046.
14. Poon RT, Fan ST. Opinions and commentary on treating pancreatic cancer. Surg Clin North Am 2001;81:625–636.
15. Evans DB, Lee JE, Pisters PWT. Pancreaticoduodenectomy (Whipple operation) and total pancreatectomy for cancer. In Nyhus LM, Baker RJ, Fischer JF, eds. Mastery of Surgery, 3rd ed. Boston: Little Brown, 1997, pp 1233–1249.
16. Holzman MD, Reintgen KL, Tyler DS, Pappas TN. The role of laparoscopy in the management of suspected pancreatic and periampullary malignancies. J Gastrointest Surg 1997;1:236–243.
17. Rumstadt B, Schwab M, Schuster K, Hagmuller E, Trede M. The role of laparoscopy in the preoperative staging of pancreatic carcinoma. J Gastrointest Surg 1997;1:245–250.
18. Jessup JM, Steele G Jr, Mayer RJ, Posner M, Busse P, Cady B, Stone M, Jenkins R, Osteen R. Neoadjuvant therapy for unresectable pancreatic adenocarcinoma. Arch Surg 1993;128:559–564.
19. Kamthan AG, Morris JC, Dalton J, Mandeli JP, Chesser MR, Leben D, Cooperman A, Bruckner HW. Combined modality therapy for stage II and stage III pancreatic carcinoma. J Clin Oncol 1997;15:2920–2927.
20. Blackstock AW, Bernard SA, Richards F, Eagle KS, Case LD, Poole ME, Savage PD, Tepper JE. Phase I trial of twice-weekly gemcitabine and concurrent radiation in patients with advanced pancreatic cancer. J Clin Oncol 1999;17:2208–2212.

21. Epelbaum R, Rosenblatt E, Nasrallah S, Faraggi D, Gaitini D, Mizrahi S, Kuten A. Phase II study of gemcitabine combined with radiation therapy in patients with localized, unresectable pancreatic cancer. J Surg Oncol 2002;81:138–143.
22. Snady H, Bruckner H, Cooperman A, Paradiso J, Kiefer L. Survival advantage of combined chemoradiotherapy compared with resection as the initial treatment of patients with regional pancreatic carcinoma. An outcomes trial. Cancer 2000;89:314–327.
23. Ammori JB, Colletti LM, Zalupski MM, Eckhauser FE, Greenson JK, Dimick J, Lawrence TS, McGinn CJ. Surgical resection following radiation therapy with concurrent ge mcitabine in patients with previously unresectable adenocarcinoma of the pancreas. J Gastrointest Surg 2003;7:766–772.

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AHPBA ANNUAL MEETING

CT vs MRCP: Optimal Classification of IPMN Type and Extent

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Abstract
Introduction Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are being diagnosed with increased frequency. CT scanning commonly serves as the primary imaging modality before surgery. We hypothesized MRCP provides better characterization of IPMN type/extent, which more closely matches actual pathology.
Methods Of 214 patients treated with IPMN (1991–2006), 30 had both preoperative CT and MRCP. Of these, 18 met imaging study criteria. Independent readers performed retrospective, blinded analyses using standardized criteria for IPMN type and extent.
Results A ductal connection was detected on 73% of MRCP scans and only 18% of CT. IPMN type was classified differently in seven (39%); four (22%) of which were read on CT as having main duct involvement where this was not appreciated on MRCP or found on surgical pathology. MRCP showed multifocal disease in 13(72%) versus only 9(50%) on CT. A different disease distribution was seen in 9(50%). Finally, 101 branch lesions were identified on MRCP compared to 46 on CT.
Conclusions CT falls short of MRCP in detecting a ductal connection, estimating main duct involvement, and identification of small branch duct cysts. These factors influence diagnostic accuracy, cancer risk stratification and operative strategy. MRCP should be employed for optimal management of patients with IPMN.

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Introduction

The management of pancreatic intraductal papillary mucinous neoplasms (IPMN) has been constantly evolving since the time of its initial description in 1982 by Ohhashi et al.[1] Intraductal papillary mucinous neoplasms of the pancreas are precancerous papillary, mucin-producing lesions which form within the pancreatic ducts. These lesions are being diagnosed with increased frequency.[2–6] As these lesions are highly variable in type and extent, sensitive and accurate imaging is important for their proper characterization. Specifically, imaging may assist in establishing a diagnosis of IPMN and discriminating it from other lesions, which may mimic IPMN such as mucinous cystic neoplasms (MCN).[7,8] Finally, imaging may also determine IPMN type and extent. These two factors have significant implications for the risk of malignancy[6,9] and provide risk stratification, which is essential for treatment decision making.

The optimal imaging for IPMN has not been well characterized in the literature.[10] With regard to CT and MRCP, limited existing literature has demonstrated comparable accuracy in the diagnosis of IPMN as well as the assessment of lesion size and main pancreatic duct (MPD) size.[11] Computed tomography (CT) is the study used most frequently to diagnose and characterize patients with IPMN.[12] Moreover, utilizing CT scanning as the sole source of imaging before definitive surgical management is not uncommon. In our experience, however, CT alone may not be sufficient to establish a diagnosis or accurately determine type and extent. Therefore other studies may be used in the management of these lesions. Endoscopic retrograde pancreatography (ERP), endoscopic ultrasound (EUS), intraductal ultrasound, and peroral pancreatoscopy are invasive options.[13,14] The ductogram provided by ERP is dependent upon pressure to fill all of the side branches. Small side branches or side branch mucus plugging may prevent ERP from fully characterizing IPMN type and extent.[15] Endoscopic ultrasound accurately determines type and extent, but has blind spots and does not provide the surgeon with a visual "roadmap" to reference in preoperative planning. Intraductal ultrasound and peroral pancreatoscopy are not fully characterized or widely available. Nevertheless, such invasive tests may be important for establishing a cytologic diagnosis of IPMN and characterizing the relative degree of cellular atypia. Magnetic resonance cholangiopancreatography (MRCP) is noninvasive and, unlike computed tomography (CT), provides a ductogram. The ductogram provided by MRCP, unlike ERP, is not dependent upon pressure to fill all of the fluid (mucous)-filled side branches. We therefore hypothesized that MRCP may provide better characterization of IPMN type and extent than CT and more closely match actual pathology.

Materials and Methods

Assurances

These studies were conducted in strict compliance with the Indiana University School of Medicine Institutional Review Board.

Patient Population and Study Criteria

This is a retrospective study of patients who underwent surgical resection for IPMN at Indiana University Hospital. Data were collected from a prospectively collected database as well as supplemented by chart review. Patients were selected for the study based upon having both pancreas protocol CT and MRCP performed within 12 months of primary operation for IPMN. No reoperative patients were included. Both pancreas protocol CT and MRCP had to be consistent with current quality standards for optimal imaging as defined in the method sections to follow. The CT and MRCP imaging for each patient was reviewed in a retrospective fashion, by independent readers. The readers were informed of the diagnosis of IPMN in each of the patients before review but were blinded to the results of the corresponding imaging test (i.e., CT or MRCP), surgical pathology, operative reports, or patient history. All images were accessed, viewed, and analyzed using the PACS/Centricity online imaging system at Indiana University Hospital. All measurements were taken using the same electronic measuring tools equipped in the system.

Parameters Assessed

The image analysis was carried out utilizing a standardized checklist that included IPMN type (main, mixed, or branch), anatomical location of the index lesion (head/uncinate, neck, body, or tail), overall distribution (i.e., localized in each of the aforementioned areas or diffuse). Presence of a ductal connection in these lesions was assessed based on imaging reports compiled before the confirmed diagnosis of IPMN, thereby limiting intrinsic bias. *Main-type* disease was radiologically defined as dilation of the main pancreatic duct (MPD) with a minimum allowable diameter of greater than 5 mm.[10–12] Segmental change in size, cystic character of dilation, and apparent filling defects were additional factors considered

in making the diagnosis of MPD involvement. *Branch type* was defined as a lesion characteristic of IPMN with a radiographically identifiable branch duct connection to the main or accessory pancreatic duct. *Mixed type* was defined as having radiographic characteristics of both main and branch duct IPMN. Any involvement of the main duct with associated branch cysts was considered mixed. Index lesion was defined as the most prominent (i.e. largest cross-sectional diameter) branch lesion in patients with branch or mixed disease unless there was isolated main pancreatic duct disease in which it refers to the location of main duct involvement. IPMN focality was classified as either unifocal or multifocal. Unifocal refers to one identifiable branch lesion (i.e., the index lesion). Multifocal refers to >1 identifiable and distinct lesion. Isolated MPD involvement was classified as unifocal. Distinct lesions were defined as those that did not share any common walls with the index lesion. Thus, a single branch lesion need not be mono-cystic, but could be septated or polycystic in appearance without being considered multifocal (i.e., multiple distinct lesions). Distribution was defined as localized or diffuse. Localized was defined as limited to one surgical site (e.g., head/uncinate of pancreas) whereas diffuse included >1 surgical site (e.g., head/uncinate, neck, body, tail). Surgical sites included head/uncinate (right of portal vein), neck (overlying portal vein), body (left of portal vein) and tail (left of splenic artery takeoff from celiac axis) of pancreas. Main pancreatic duct dilation was determined by maximal cross-sectional diameter (perpendicular to the long axis of the main duct). All measurements of the main pancreatic duct were obtained on images taken before the administration of secretin.[16,17]

Magnetic Resonance Cholangiopancreatography

MRCP is usually combined with routine contrast-enhanced MRI. For MRCP, the pancreatic duct is imaged using a single shot fast spin echo pulse sequence, with a single 40 mm thick coronal "slab" positioned over the pancreas. The matrix size is 256×256; the field of view varies from patient to patient, but is typically 22×22 cm. TE is ~750 ms. Fat saturation technique is used in all studies. Acquisition time is approximately 1–2 s per scan and obtained during breath-holding. A thin slice coronal T2 weighted sequence is also employed; a respiratory synchronized 3D turbo spin echo sequence (3D PACE, Siemens Medical Solutions, Malvern, PA) provides excellent details. This sequence is useful in detecting tiny side branches or connecting side branches between the cystic lesions such as side branch IPMN and main pancreatic duct, which can be obscured on the thicker slab images. Fasting for 4–6 h before the examination, and/or the oral administration of 300 ml of ferumoxsil oral suspension as a negative contrast agent (Gastromark, Mallinckrodt Medical, Raleigh, NC) was used to remove high signal from overlying stomach and duodenum.

Secretin is a 27-amino acid polypeptide hormone secreted by the duodenal mucosa in response to luminal acid, typically following a meal.[16] It has numerous physiological effects, including actions on the pancreas, the sphincter of Oddi, and the biliary tree. Secretin is given intravenously at a dose of 16 mcg in adults. At the commencement of injection, a baseline scan is obtained using a coronal single shot turbo spin echo image (2 s scan time); this is repeated every 30 s for 15 min. Secretin-induced distention of the pancreatic duct occurs typically within 4–7 min of administration in normal subjects.[17]

Pancreas Protocol Computed Tomography

CT studies were carried out with multidetector 16 (Brilliance 16) and 64 slice CT scanners (Brilliance 64; Philips Medical Systems). Water in lieu of oral contrast agent was used to avoid scattered artifact over and around the pancreas. The initial scan was a non-enhanced CT of the upper abdomen performed with 5-mm section thickness and 3-mm gap. A total of 120–150 ml of nonionic iodinated contrast material (Isovue 300 or 370), was injected into the antecubital vein at a rate of 4 ml/s with an 20-gauge angio-catheter and followed by a dual-phase CT of the pancreas. The initial post contrast study was a late arterial phase, which was obtained 35 s after administration of IV contrast and imaging parameters; 0.9-mm slice thickness and spacing between slices, 0.5 mm. These images were reformed to axial and coronal images, 3-mm slice thickness and 3-mm spacing between slices. The venous phase was performed at 70–80 s after initiation of IV contrast injection. Imaging parameters were 2-mm slice thickness and spacing between slices 0.6 mm. These images were reformed to axial and coronal 5-mm slice thickness and 3-mm spacing between slices. Tube voltage was 120 kvp and tube load was 200–250 mAs.

Pathologic Analysis

Pathologic analysis of the surgical specimens was carried out at the time of operative management of the IPMN. Specimens were also reanalyzed by a faculty pathologist (O.W.C.) experienced in the nomenclature and classification of IPMN. The final analysis of each sample was blinded to the results of any preoperative imaging. The specimens were characterized by presence/absence of main duct involvement and World Health Organization IPMN grade (adenoma, borderline, high grade/CIS, or invasive). In one case, there was no cystic dilation of the MPD present on pathologic (or radiographic) examination, but there was

some architectural change in the epithelium of the main duct. This case was classified as a main duct pancreatic intraepithelial neoplasia (PanIN) as opposed to a main duct IPMN.[18] The results of pathologic analysis were then compared preoperative imaging.

Results

From 1991–2006, 214 patients with IPMN were treated at a single, high-volume tertiary referral center (Indiana University Hospital). Of these patients 150 underwent a total of 157 operations for surgical management of IPMN. In the surgical group, 30 patients had undergone both preoperative abdominal CT and MRCP at Indiana University and were available for review. A review was undertaken of these 30 patients, and 18 met selection criteria for the study.

Of the 18 patients on study, 7 were male and 11 female (Table 1). The mean and median age at time of operation was the same at 66 years (range 49–79 years). The surgical management of these 18 patients occurred between 1999–2006. The mean (median) time from MRCP and CT to operation was 76 (67) and 78 (69) days, respectively. Operative management of patients included nine pancreaticoduodenectomies, six distal pancreatectomies, two total pancreatectomies, and one diagnostic laparoscopy. The latter patient underwent liver biopsy and confirmation of metastatic pancreatic cancer. This patient had MRI/MRCP 7 days before operative intervention. Fourteen patients had non-invasive IPMN. Five were graded as adenoma, and the remaining nine were graded as borderline. Four patients had invasive IPMN. No patients in this series had carcinoma *in situ* (Table 1).

Table 1 Demographics, Operation, IPMN Pathology

Parameters	Number
Age, years (range)	66 (49–79)
Gender	
Male	7
Female	11
Operation	
Pancreaticoduodenectomy	9
Distal Pancreatectomy	6
Total Pancreatectomy	2
Other	1
Pathologic Grade	
Adenoma	5
Borderline	9
Carcinoma in situ	0
Invasive	4

Table 2 IPMN Type

	CT	MRCP	Pathology
Type			
Branch	9	13	13[a]
Mixed	6	4	4
Main	3	1	1
Main duct component			
MPD component	9	5	5
No MPD component	9	13	13

[a] In one of the branch type IPMN there was neoplasia of the main duct epithelium without cystic dilation (PanIN)

A pancreatic lesion with an appearance characteristic of IPMN was identified on CT and MRCP in all patients. MRCP showed 13 (72%) branch, 4 (22%) mixed, and 1 (6%) main-duct-type IPMN. Conversely, CT showed 9 (50%) branch, 6 (33%) mixed, and 3 (17%) main-duct-type IPMNs. Overall, IPMN type was classified differently by MRCP compared to CT in 7(39%). Of these patients, four (22%) were read on CT as having main duct involvement where main duct disease was not appreciated on MRCP. Surgical pathology likewise did not identify main duct disease in any of these patients (Table 2).

A clear ductal connection, however, was identified on 73% of MRCP but only 18% of CT in those lesions of either mixed or branch type (Table 3). MRCP showed multifocal disease in 13(72%) versus only 9(50%) on CT. A different distribution was seen in 9(50%). MRCP showed non-diffuse disease in 6(33%) where corresponding CT imaging indicated diffuse disease. Conversely, MRCP showed diffuse disease in 3(17%) where corresponding CT imaging indicated non-diffuse disease. Finally, 101 branch lesions were identified on MRCP compared to 46 on CT. The mean main pancreatic duct (MPD) size observed in all patients was 5.7 mm for MRCP and 6.4 mm for CT. In lesions with an observed branch-type component, the average diameter of the index lesions was measured at 31.7 mm for MRCP and 27.8 mm for CT. On CT compared to MRCP, MPD and index lesion size measurements were not significantly different (Table 3).

Selected Imaging

Patient 1 In this case, CT characterized this IPMN as mixed. MRCP demonstrated isolated main duct IPMN. On CT, the branch-type cyst measured 13 mm which was roughly equivalent to the MPD diameter. Pathology confirmed isolated main duct IPMN. Thus, a dilated, tortuous main duct was misclassified as having a branch

Table 3 IPMN Diagnosis and Extent

	CT	MRCP
Ductal Connection(%)[a]	18.2	72.7
Focality		
Unifocal	9	5
Multifocal	9	13
Distribution		
Localized		
Head	5	6
Neck	0	0
Body	1	0
Tail	3	5
Diffuse	9	7
Main duct size (mm)*	6.4	5.7
Branch cyst size (mm)*	27.8	31.7
Branch Cyst Number	46	101

[a] Ductal connection was only assessed in branch or mixed type lesions
**NS*, $p>0.05$

component by CT. Main-type IPMN may have increased risk of invasion. Thus, the patient's preoperative risk (i.e., risk of cancer) stratification would not have been accurate based upon CT alone (Fig. 1a,b).

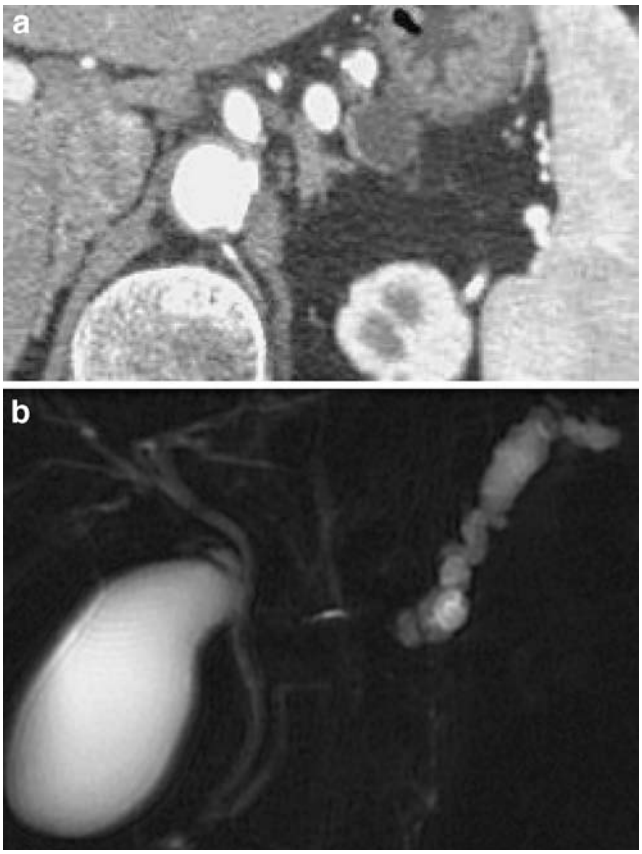

Figure 1 Imaging of patient with main type IPMN, **a** representative image from CT scan of the pancreas showing dilated main duct, **b** MRCP illustrating cystic dilation of main pancreatic duct.

Patient 2 This patient was classified by MRCP as multifocal branch-type IPMN in the tail of the pancreas without MPD involvement. The CT scan was read as having both branch and main duct involvement. A cluster of dilated branch cysts in the tail of the pancreas were identified on operative pathology. The MPD showed no evidence of IPMN involvement. Thus, the patient's preoperative risk

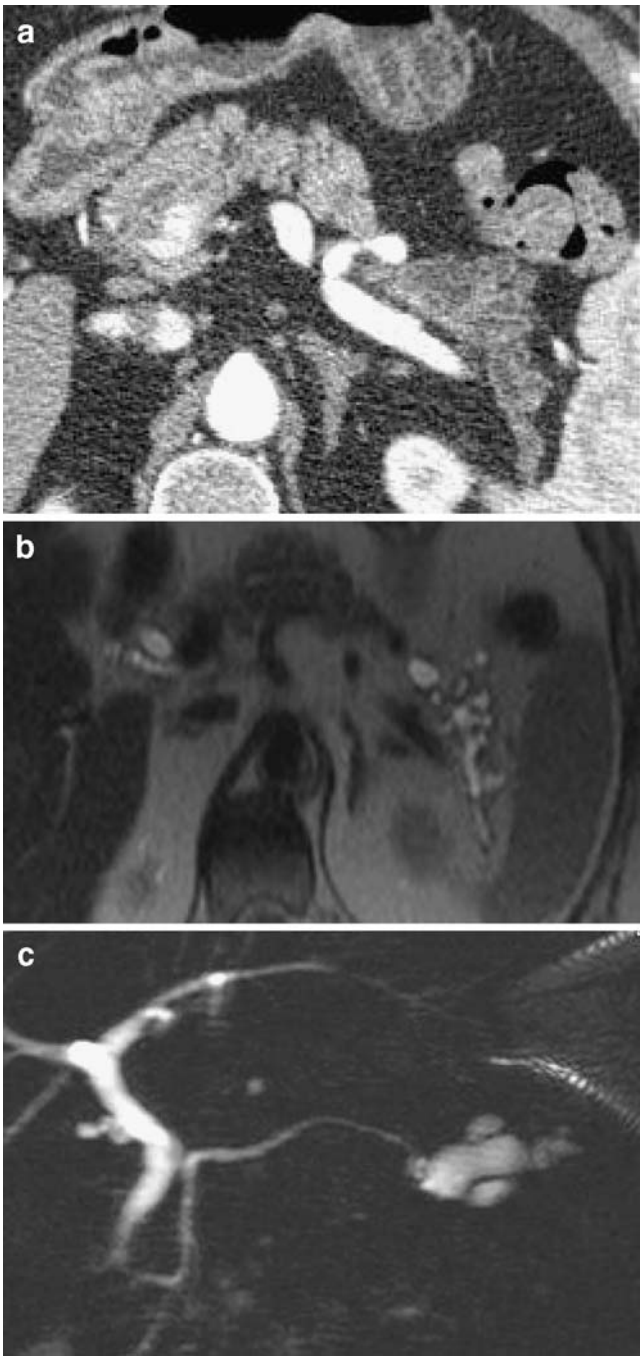

Figure 2 Imaging of patient with multiple branch type IPMN, **a** representative image from CT scan of the pancreas demonstrating tail involvement, **b** axial image from MRI demonstrating multicystic IPMN, **c** MRCP demonstrating multiple branch cysts.

(i.e., risk of cancer) stratification would not have been accurate based upon CT alone (Fig. 2a–c).

Patient 3 This patient was classified as branch-type IPMN on MRCP. CT scan, however, suggested a main duct component. Pathology confirmed isolated branch-duct-type IPMN. MRCP also demonstrated 6 distinct branch-type lesions compared to CT which demonstrated 4. Importantly, those lesions not identified on CT were more proximal which required resection of more pancreas to incorporate these lesions. Also pictured is the surgical specimen following distal pancreatectomy in this patient. Only the largest lesion was visible upon resection. The final pathology revealed multiple adenoma grade lesions including the presence of smaller branch-type cysts near the margin of the extended resection, which may have remained in the patient had MRCP not been performed (Fig. 3a–c).

Patient 4 This patient was identified as having branch-type IPMN by both CT and MRCP. The CT scan, however, failed to identify the cluster of small dilated cysts in the neck of the pancreas, which were clearly visible by MRI/MRCP. This finding highlights the importance of adequate preoperative imaging. Utilizing CT alone, the tail lesions may have been resected without knowledge of the proximal lesion. The MRCP visualization of these lesions allows for the decision to either follow or resect this small cluster of lesions based on patient comorbidities and other factors (Fig. 4a,b).

Patient 5 This patient was characterized as diffuse branch-type IPMN by both CT and MRCP. On CT, 9 distinct branch-type lesions were identified, whereas upon MRCP examination, 23 branch-type lesions where identified. This patient underwent total pancreatectomy. This image illustrates the advantage in identification of small branch-type lesions as well as differentiation of multiple closely related lesions by MRCP (Fig. 5a,b).

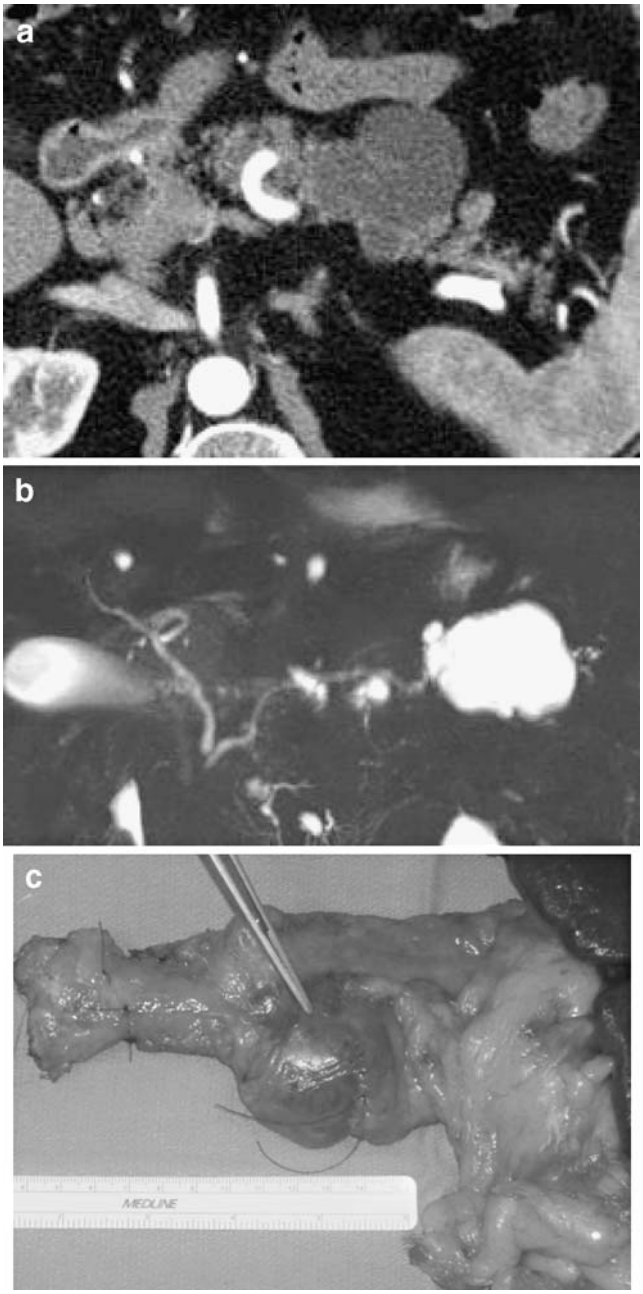

Figure 3 Imaging of patient with large cystic IPMN as well as multiple smaller foci of disease. 3**a**: Representative image from CT scan demonstrating single large cyst, 3**b**: MRCP demonstrating multifocal cysts, 3**c**: Surgical specimen with obvious large cyst.

Discussion

The management of IPMN of the pancreas continues to be a topic of considerable controversy. As the quality and availability of abdominal imaging, and the awareness in the medical community has improved, IPMN has become an increasingly recognized entity.[2–6] As IPMN becomes more commonly encountered, it will become important for clinicians to apply optimal imaging in these patients to accurately determine a diagnosis and characterize the type and extent of disease. All three of these factors have significant implications (particularly in elderly patients with associated co-morbidities) for proper pancreatic cancer risk stratification and treatment decision making.[10,19]

This study focused on patients who received both CT and MRCP before surgical management for pathologically confirmed IPMN of the pancreas. Each imaging study was retrospectively reexamined by reviewers blinded to the results of the corresponding imaging study and final pathology. IPMN type and extent were examined with standardized criteria. With time, IPMNs may progress or change their radiographic type and extent; thus, we

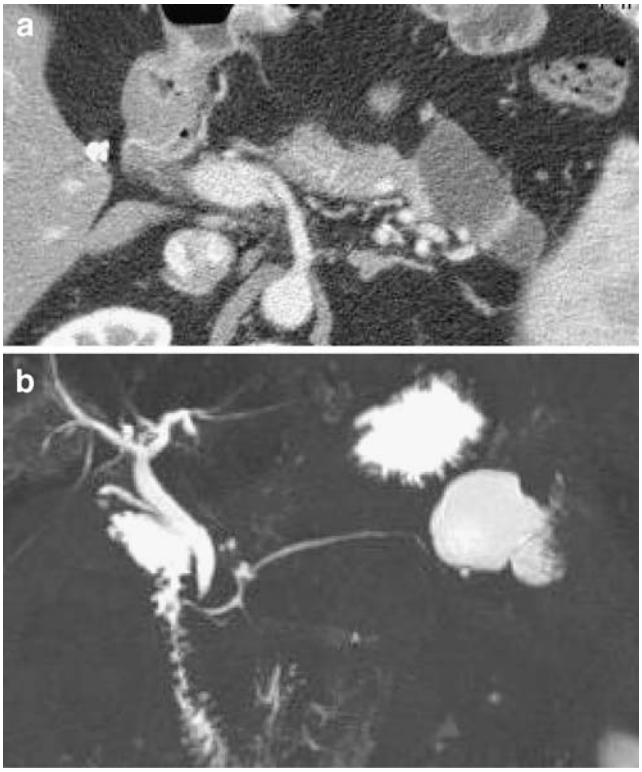

Figure 4 Imaging of patient with large branch type IPMN, 4**a**: Representative CT scan image demonstrating bilobed cystic mass, 4**b**: MRCP demonstrating large cyst and smaller cyst in neck.

excluded patients in this study whose imaging was remote from the operation. In addition, to avoid any lead time bias of one imaging study over another, we excluded patients with a significant time interval between CT and MRCP. The findings of our study suggest that while CT remains a more common imaging modality for IPMN of the pancreas, MRCP is superior with regard to the diagnosis and classification of IPMN type and extent. The advantage of MRCP over CT falls into three principle categories: (1) Diagnosis of IPMN (i.e., differentiation from other lesions which mimic IPMN); (2) IPMN type, specifically, evaluation of main duct involvement; (3) IPMN extent, specifically, identification of small branch-duct-type lesions.

The diagnosis of IPMN can be made pathologically or clinically. The clinical diagnosis of IPMN is based upon history, endoscopy/cytopathology, and radiography.[20] In the proper clinical setting (characteristic history±cytopathology), one of the most important distinguishing findings of IPMN on imaging is a ductal connection. In this study, MRCP was able to clearly demonstrate a ductal connection in the majority of patients whereas CT was unable to do so.

IPMN type has major implications for proper pancreatic cancer risk stratification and treatment decision making in patients with IPMN.[10] Our study demonstrated several examples where CT diagnosed a main duct component to IPMN where pathology did not confirm this finding. Conversely, MRCP correlated 100% with main duct component disease as determined by pathology.

The malignant potential of IPMN by type in order of decreasing potential for malignancy is main, mixed, and branch.[5] Thus, the presence of a branch component improves patient's pancreatic cancer risk. Main duct involvement may arguably be the most important radiographic criteria in determining a patient's risk of invasive cancer or malignancy. Thus, CT may overestimate the malignant or invasive potential of IPMN, which could misguide patients and care providers in overly aggressive treatment decision making. This fact is especially true in patients where existing comorbidities could warrant non-operative management of low-risk lesions.

IPMN extent of disease also has significant implications for proper pancreatic cancer risk stratification and treatment decision making in patients with IPMN. In addition, new evidence suggests that the multifocality or location may associated with malignancy in patients with branch-type lesions.[21] Small cystic lesions of the branch duct type are less often identified on the CT scan thereby increasing the likelihood that they may go undetected. MRCP is more sensitive at detecting smaller branch-duct-type lesions. Treatment decision making may be affected not only due to changes in pancreatic cancer risk stratification, but also

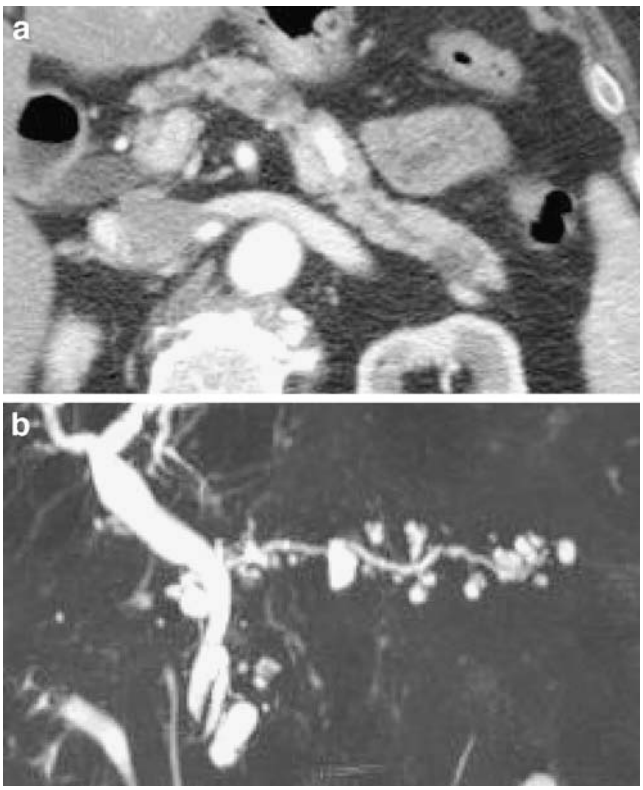

Figure 5 Imaging of patient with diffuse branch type IPMN throughout the pancreas. 5**a**: Representative CT scan image demonstrating diffuse cystic disease, 5**b**: MRCP showing multifocality and clear ductal connections.

in terms of planning the extent of resection in surgical candidates. This issue also has implications for surveillance testing as well as counseling of patients with evidence of multifocal disease, especially in those patients which the decision is made to leave small IPMN behind in the remanant pancreas. Clinically significant recurrence after resection is not an uncommon phenomenon in IPMN.[22] We speculate that the rate of "recurrence" of IPMN after resection may be influenced by the sensitivity of detection of IPMN preoperatively. In our experience, we have observed patients who had only CT preoperatively show evidence of previously undetected small branch-type lesions on initial MRCP postoperatively. This may yield a falsely elevated rate of recurrence, when in reality, the lesions were simply too small to be recognized by CT scan alone.

The door is open for further prospective study of this clinical question. A head to head prospective comparison of randomized patients given MRCP and CT before operative intervention would allow more substantial statistical analysis to be carried out. Based upon this carefully selected retrospective data, however, it is clear to us that high-resolution CT is not adequate as the sole method of surgical planning and management of IPMN. In addition, the appropriate imaging study for postoperative, or nonoperative surveillance[23] of these lesions has not been established in the literature and warrants further investigation.

Previous studies have not adequately addressed the optimal imaging modality for management of IPMN. A number of studies have examined each of the possible imaging modalities in isolation.[12,15,23] Significantly less data address these modalities in a head to head comparison. Sahani et al. recently addressed this topic in 2006. Their study demonstrated accuracy in assessment of MPD involvement by both CT and MRCP.[11] In contrast to our results, in the aforementioned study, the authors took a somewhat different approach. They classified any MPD greater than 4 mm as a main- or mixed-type IPMN. It is our contention that such a comparison determines the ability of CT and MRCP to accurately measure the MPD caliber rather than accurately assess the characteristics of this dilation, (i.e. cystic quality, filling defect, segmental size changes). The International Consensus Guidelines on IPMN state that MPD diameter greater than 1 cm is strongly suggestive of IPMN.[10] It is in the 4-mm to 1-cm range where qualitative assessment of the dilation is needed. Chronic pancreatitis, which coexists in many IPMN patients, may result in ectasia of the main pancreatic duct. In addition, downstream mucous plugging could result in some mechanical dilation of the upstream MPD. Either of these may lead to false classification and overestimation of main duct involvement. No dispute exists concerning the accuracy with which either of these modalities can be used to simply measure the MPD, but utilization of the ductogram provided by the MRCP allows for vastly improved characterization of the MPD, as evidenced by its consistency with the ultimate pathologic examination.

Another critical aspect in establishing a radiologic diagnosis of IPMN is the accuracy of detecting a ductal connection in branch- or mixed-type lesions. Sahani et al. demonstrated a sensitivity for detection of ductal connection of 83 and 87% for CT and MRCP, respectively,[11] compared with 18 and 73%, respectively, in our retrospective study. Data regarding ductal connection were obtained from radiologic analysis done before the confirmed diagnosis of IPMN in our series. In the prior study, analysis of ductal connection, a requisite characteristic for radiologic diagnosis of IPMN,[24] was carried out in the setting of known IPMN diagnosis. This could account for significant bias in assessing the presence of duct connection.

Currently, no clear consensus outlines an appropriate imaging approach to these complex patients. The International Consensus Guidelines for Management of IPMN and MCN of the Pancreas states that MRCP is (1) best to outline the gross appearance of these lesions and (2) helpful in determining a connection with the ductal system. This is an important distinction since the International Consensus Guidelines suggest that all MCN should be resected whereas asymptomatic branch-type IPMN <3 cm may be safely observed. The consensus guidelines equate MRCP and thin slice CT in regard to following index branch-type lesions with regard to changes in size.[10] The guidelines, however, do not directly address the preferred imaging modality in terms of accurately determining diagnosis, type and extent of these lesions preoperatively.

Conclusion

MRCP is superior in establishing a diagnosis of IPMN and determining type and extent of disease. MRCP is better able to distinguish branch-type IPMN from MCN. MRCP characterization of IPMN type will more accurately characterize the presence of a main duct IPMN component. This issue is clinically important for preoperative risk stratification due to cancer. MRCP characterization of extent will more accurately identify small branch-type lesions and multifocal branch disease. By giving a more complete picture of the extent of disease before surgical management, the surgeon will be better equipped to perform an effective and informed operative strategy. Our recommendation is that MRCP should be used as the predominate imaging modality, in conjunction with other modalities, to adequately characterize IPMN and guide further therapeutic decision making.

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References

1. Ohhashi K, Murakimi Y, Maruyama M, Takekoshi T, Ohta H, Ohhashi I. Four cases of mucous secreting pancreatic cancer [in Japanese]. Prog Dig Endosc 1982;20:348–351.
2. Spinelli KS, Fromwiller TE, Daniel RA, Kiely JM, Nakeeb A, Komorowski RA, Wilson SD, Pitt HA. Cystic pancreatic neoplasms: observe or operate. Ann Surg 2004;239(5):651–659.
3. Schmidt CM, Wiesenauer CA, Cummings OW, Yiannoutsos CT, Howard TJ, Wiebke EA, Goulet RJ Jr, McHenry LH, Sherman S, Lehman GA, Cramer H, Madura JA. Preoperative Predictors of Malignancy in Pancreatic Intraductal Papillary Mucinous Neoplasms (IPMN). Arch Surg 2003;138:610–618.
4. Sohn TA, Yeo CJ, Cameron JL, Iacobuzio-Donahue CA, Hruban RH, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an increasingly recognized clinicopathologic entity. Ann Surg 2001;234:313–322.
5. Sohn TA, Yeo CJ, Cameron JL, Hruban RH, Fukushima N, Campbell KA, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. Ann Surg 2004;239(6):788–799.
6. Salvia R, Fernandez-del Castillo C, Bassi C, Thayer SP, Falconi M, Mantovani W, Pederzoli P, Warshaw AL. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. Ann Surg 2004;239(5):678–687.
7. Itai Y, Ohhashi K, Nagai H, et al. "Ductectatic" mucinous cystadenoma and cystadenocarcinoma of the pancreas. Radiology 1986;161:697–700.
8. Madura JA., Schmidt CM., Yum MN. Mucin secreting cystic lesions of the pancreas: treatment by enucleation. Am Surg 2004;70(2):106–112.
9. Terris B, Ponsot P, Paye F, et al. Intraductal papillary mucinous tumors of the pancreas confined to secondary ducts show less aggressive pathologic features as compared with those involving the main pancreatic duct. Am J Surg Pathol 2000;24:1372–1377.
10. Tanaka M, Chari S, Adsay V, Fernandez-Del Castillo C, Falconi M, Shimizu M, Yamaguchi K, Yamao K, Matsuno S. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology 2006;6(1–2):17–32.
11. Sahani DV, Kadavigere R, Blake M, Fernandez-del Castillo C, Lauwers GY, Hahn PF. Intraductal papillary mucinous neoplasm of pancreas: Multi-detector row CT with 2D curved reformations-correlation with MRCP. Radiology 2006;238:560–569.
12. Kawamoto S, Lawler LP, Horton KM, Eng J, Hruban RH, Fishman EK. MDCT of intraductal papillary mucinous neoplasm of the pancreas: Evaluation of features predictive of invasive carcinoma. Am J Roentgenol 2006;186:687–695.
13. Cellier C, Cuillerier E, Palazzo L, et al Intraductal papillary and mucinous tumors of the pancreas: accuracy of preoperative computed tomography, endoscopic retrograde pancreatography and endoscopic ultrasonography, and long-term outcome in a large surgical series. Gastrointest Endosc 1998;47:42–49.
14. Wakabayashi T, Kawaura Y, Morimoto H, Watanabe K, Toya D, Asada Y, Satomura Y, Watanabe H, Okai T, Sawabu N. Clinical management of intraductal papillary mucinous tumors of the pancreas based on imaging findings. Pancreas 2001;22(4):370–377.
15. Fukukura Y, Fujiyoshi F, Sasaki M, Ichinari N, Inoue H, Kajiya Y, Nakajo M. HASTE MR cholangiopancreatography in the evaluation of intraductal papillary-mucinous tumors of the pancreas. J Comput Assist Tomogr 1999;23(2):301–305.
16. Chey WY, Chang TM. Secretin, 100 years later. J Gastroenterology 2003;38:1025–1035.
17. Fukukura Y, Fujiyoshi F, Sasaki M, Nakajo M. Pancreatic duct: morphologic evaluation with MR cholangiopancreatography after secretin stimulation. Radiology 2002;222:674–680.
18. Klein WM, Hruban RH, Klein-Szanto AJ, Wilentz RE. Direct correlation between proliferative activity and dysplasia in pancreatic intraepithelial neoplasia (PanIN): additional evidence for a recently proposed model of progression. Mod Pathol 2002;15 (4):441–447.
19. Yamao K, Ohashi K, Nakamura T, et al. The prognosis of intraductal papillary mucinous tumors of the pancreas. Hepatogastroenterology 2000;47:1129–1134.
20. Sakorafas GH, Sarr MG, van de Velde CJ, Peros G. Intraductal papillary mucinous neoplasms of the pancreas: a surgical perspective. Surg Oncol 2005;14(4):155–178.
21. White PB, Waters JA, Baker M, Cummings OW, Howard TJ, Pitt HA, Zyromski NJ, Nakeeb A, Lillemoe KD, Schmidt CM. Intraductal Papillary Mucinous Neoplasms (IPMN): Factors Predicting Malignancy. Ann Surg 2007 (in press).
22. Chari ST, Yadav D, Smyrk TC, DiMagno EP, Miller LJ, Raimondo M, Clain JE, Norton IA, Pearson RK, Petersen BT, Wiersema MJ, Farnell MB, Sarr MG. Study of recurrence after surgical resection of intraductal papillary mucinous neoplasm of the pancreas. Gastroenterology 2002;123(5):1500–1507.
23. Irie H, Yoshimitsu K, Aibe H, Tajima T, Nishie, Akihiro, Nakayama T, Kakihara D, Honda H. Natural history of pancreatic intraductal papillary mucinous tumor of branch duct type: Follow-up study by magnetic resonance cholangiopancreatography. J Comput Assist Tomogr 2004;28(1):117–122.
24. Schmidt CM, Lillemoe KD. IPMN—Controversies in an "Epidemic". J Surgical Oncology 2006;94(2):91–93.

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Long-Term Outcomes Following Liver Transplantation for Hepatic Hemangioendothelioma: The UNOS Experience from 1987 to 2005

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Abstract
Introduction Hepatic hemangioendothelioma (HEH) is a vascular neoplasm with intermediate malignant potential. Outcomes after liver transplantation have only been reported as small, single-institution experiences. The purpose of this study was to evaluate patient and allograft survivals after liver transplantation in a large, multi-institutional cohort of patients with HEH.
Methods Using the United Network for Organ Sharing (UNOS) database, we identified 110 patients with a diagnosis of HEH who underwent 126 transplants between 1987 and 2005. Patient and allograft survivals were calculated using Kaplan–Meier survival curves. Log rank tests were used to determine the influence of study variables on outcomes.
Results Of the 110 transplanted patients, 75 patients (68%) were female, 80 patients (73%) were Caucasian, and the median age was 36 years old (23% < 4 y.o., 71% > 18 y.o.). The 30-day posttransplant mortality rate was 2.4%. At a median patient follow-up interval of 24 months, 1- and 5-year patient and allograft survivals were 80% and 64%, and 70% and 55%, respectively. Pretransplant medical status, but not age, was found to statistically correlate with patient survival.
Conclusion These data indicate that survivals after transplantation for HEH are favorable. Given the propensity for recurrence after resection, these data support consideration of liver transplantation for all patients with significant intrahepatic tumor burden.

Keywords Orthotopic liver transplantation · Hemangioendothelioma · Survival analysis

Introduction

Epithelioid hemangioendothelioma is a rare tumor of vascular origin that can involve soft tissues as well as visceral organs, including the liver, lung, spleen, stomach, and heart. Hepatic epithelioid hemangioendothelioma (HEH) is a common clinical form of the disease. The presentation of HEH, and the corresponding clinical course can vary widely. Some HEH tumors behave similar to benign hepatic hemangiomas, whereas others have a clinical course resembling highly aggressive angiosarcoma.

Some patients with hepatic HEH will present with limited intrahepatic disease; however, the majority have multifocal disease at diagnosis with extensive intrahepatic tumor burden and/or extrahepatic metastases. In a recent review of 434 HEH cases reported in the world literature, 87% of patients presented with bilobar and multifocal disease, and 37% of patients presented with extrahepatic involvement, including secondary disease of the lungs, regional lymph nodes, peritoneum, bone, spleen, and diaphragm.[1] Patients with extensive intrahepatic and extrahepatic tumor burden tend to have a more fulminate course, with rapid disease progression and secondary end-organ dysfunction.

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Patients with the less common presentation of limited intrahepatic disease are candidates for hepatectomy or other local treatments. For patients with limited intrahepatic disease, the overall recurrence rates observed after limited local treatments appear to be acceptable.[1] However, aggressive recurrence has been reported after minor hepatic resection of presumed localized disease,[2] as well as after major hepatic resection of multifocal disease.[3]

The frequent treatment failures observed after local therapy has prompted the use of orthotopic liver transplantation (OLT) in selected cases.[4–9] For patients with unresectable intrahepatic disease with or without extrahepatic disease, OLT combined with systemic therapy is the only treatment option. Single center studies reporting on outcomes after OLT in patients with extensive tumor burden, have documented favorable outcomes after transplantation with 5-year survivals ranging from 48% to 76%.[7–9] Even in patients with known extrahepatic disease, OLT can prolong survival by preventing death from liver failure.[1,10]

The best treatment strategy for patients with borderline resectable HEH has not been determined. To assess the utility of OLT in patients with extensive intrahepatic disease, this study evaluated patient and graft survivals after transplantation in a large, multi-institutional cohort of patients with HEH.

Material and Methods

Analysis of the United Network for Organ Sharing (UNOS)/Organ Procurement and Transplantation Network (OPTN) database identified 110 patients with a diagnosis of HEH who underwent a total of 126 transplants between 1987 and 2005. The majority of patients received their initial transplant during the more recent 10-year period from 1996 to 2005 (77 patients, 70%).

Examined study variables included recipient age, race, gender, ABO blood group, era of transplant, pretransplant medical status, patient and allograft survivals, and cause of allograft failure or death (cancer-related vs. other causes). Kaplan–Meier survival curves were used to calculate survivals and log-rank tests were used to determine the influence of study variables on survivals. A p value<0.05 was considered statistically significant.

Results

Demographic Data

Of the 110 patients transplanted for HEH, 75 patients (68%) were female. The most frequent race was Caucasian (73%), followed by Hispanic (13%), African American (8%), and Asian (6%) (Table 1). The median age at the time of first transplant was 36 years (range 0–70 years). There were 25 patients less than 4 years old and seven patients between the ages of 4 and 18 at the time of first transplant. During the study period, the number of OLTs performed per year in the US for HEH has increased, peaking at 16 in 2002. This progression is documented in Fig. 1.

Pretransplant Medical Condition

The median wait list time before the first transplant was 36 days (range 0–2,655 days). The blood group distribution of recipients was O: 47 patients, A: 40 patients, B: 17 patients, and AB: 6 patients. At the time of the first transplant 43 of the 110 patients (39%) were hospitalized and 21 of these 43 patients were in an intensive care unit. Twenty-five patients were listed as Status 1 at the time of transplant. Of the 110 study patients, 92 (84%) were transplanted before 2002 (i.e., before utilization of the Model for End Stage Liver Disease (MELD)/Pediatric End-stage Liver Disease (PELD) scoring system). For the 18

Table 1 Demographic and Clinical Summary of UNOS Database Patients Transplanted for Hepatic Epitheliod Hemangioendothelioma from 1987 to 2005

Study Variable	No. of Patients	Percent
Total	110	100
Sex		
Male	35	32
Female	75	68
Race		
Caucasian	80	73
Hispanic	14	13
Black	9	8
Asian	7	6
Medical requirements at first OLT		
ICU Care	22	20
Ventilator	11	10
Non-ICU hospitalization	21	19
Status 1 at First OLT	25	23
ABO blood group		
A1	39	36
A2	1	1
AB	6	5
B	17	15
O	47	43
Labs at first OLT		
SGPT (U/L)	34	7–1,859
Creatinine (mg/dL)	0.7	0.1–4.0
Bilirubin (mg/dL)	0.7	0.2–43.2
Albumin (G/dL)	3.7	1.5–4.8

Abbreviations: No, number; OLT, orthotopic liver transplant; ICU, intensive care unit, SGPT, Serum glutamic pyruvic transaminase

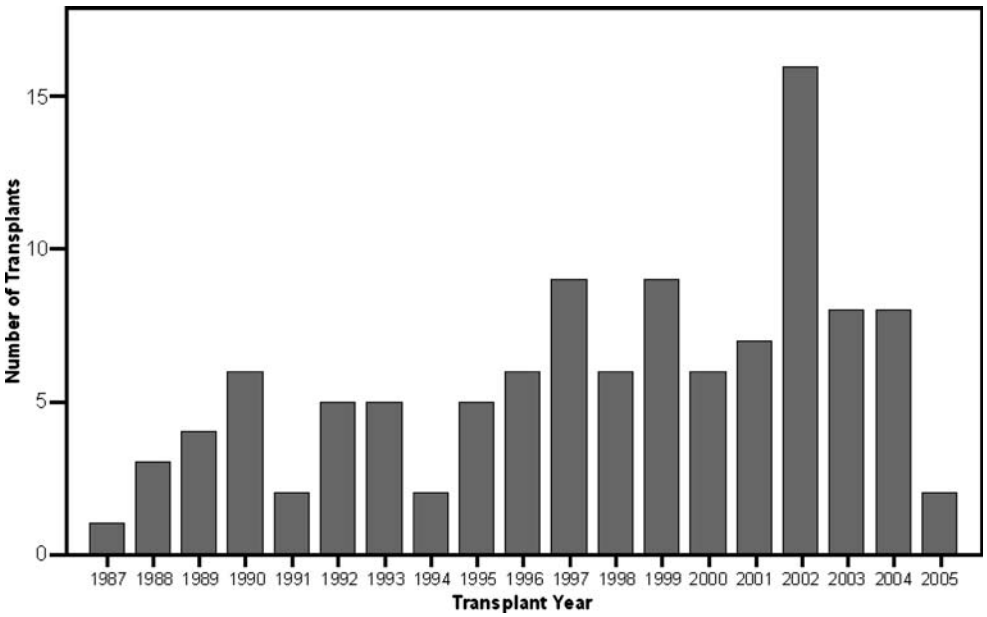

Figure 1 Progression of orthotopic liver transplantation for hepatic epithelioid hemangioendothelioma over time.

patients transplanted in the MELD/PELD era the median score was 27 (range 6–40). Biochemical parameters at the time of OLT for all study patients are recorded in Table 1.

Patient Survivals

The median length of posttransplant hospitalization was 16 days (range 0–156 days). There was one operative death, and two patients died within 30 days of transplant, yielding a 30-day mortality rate of 2.4%. At a median patient follow-up interval of 24 months (range 0–181 months), the 1-year, 3-year, and 5-year overall patient survivals were 80%, 68%, and 64%, respectively (Fig. 2). There were 31 actual 5-year survivors. Of the 38 patients who died during follow-up, 12 patients (32%) died of recurrent HEH, mainly involving distant sites.

Allograft Survivals

After first OLT, 12 patients (11%) required retransplantation, including four patients who received a third graft. Of the 17 operations for retransplantation, 10 (59%) were performed within 30 days of the previous operation. The indications for retransplant were available in 14 cases and included primary nonfunction (four transplants), vascular

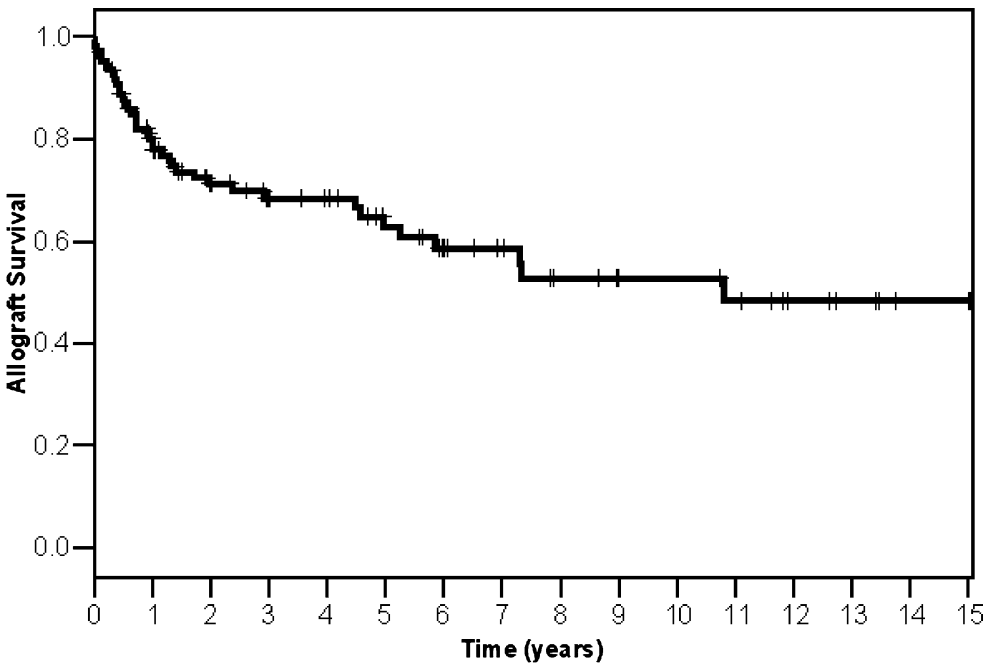

Figure 2 Kaplan–Meier plot of overall survivals for 110 patients treated with orthotopic liver transplantation for hepatic epitheliod hemangioma from 1987 to 2005.

thrombosis (three transplants), and biliary complications (two transplants). At a median allograft follow-up interval of 23 months (range 0–181 months), the 1-year, 3-year, and 5-year allograft survivals were 70%, 60%, and 55%, respectively (Fig. 3).

Patient Survivals Stratified by Age at First OLT

The patients presented in the UNOS/OPTN dataset had a bimodal age distribution. Of the 110 study patients, 25 patients (23%) were under age 4 (infantile HEH). Only seven patients (6%) were transplanted between the ages of 4 and 18 (pediatric HEH), and 78 patients (71%) were over age 18 (adult HEH). After OLT, patients with infantile HEH experienced 1-year, 3-year, and 5-year survivals of 68%, 61%, and 61%, respectively. Patients with adult HEH experienced similar outcomes with 1-year, 3-year, and 5-year survivals of 81%, 72%, and 67%, respectively (p=0.75) (Fig. 4, Table 2).

Analysis of Prognostic Factors

Univariate analysis was used to determine the impact of study variables on patient survivals. These comparisons determined that Status 1 designation, gender, age, and era of transplantation did not influence patient survivals. In contrast, other measures of pretransplant global medical condition, including the need for inpatient hospitalization and the need for intensive care unit management significantly impacted posttransplant survivals. Patients requiring hospitalization experience a 5-year posttransplant survival rate of only 44%, whereas outpatients experienced a 72% 5-year survival rate (p=0.01). Likewise, pretransplant intensive care unit patients had 5-year survivals of only 39%, compared to 65% for non-intensive care unit patients (p=0.02).

Discussion

Hepatic HEH is a rare tumor of vascular origin with variable malignant potential. No definitive etiologic factors have been confirmed, but possible causes include vinyl chloride, oral contraceptive use, asbestos, Thorotrast contrast, and alcoholic and viral hepatitis. Studies have consistently found that HEH occurs more frequently in women.[1,10] In one clinicopathologic study of 137 HEH cases, 61% of the patients were female and the mean age was 47 years.[10] The predominance of HEH in females, particularly during the reproductive years, suggests a hormonal association.[10]

Most patients present after the tumor burden becomes large enough to cause symptoms. In a recent series of 137 HEH patients, the diagnosis was made incidentally in only 22% of cases.[10] When symptomatic, the clinical presentation of HEH is nonspecific and includes right upper quadrant pain, hepatomegaly, and weight loss.[10] Tumor markers (AFP, CEA, CA 19-9) are usually in the normal range.[1] Computed tomography (CT) findings indicative of HEH include multiple hypo-attenuating tumors in both hepatic lobes that coalesce in a peripheral or subcapsular distribution with focal hepatic capsular retraction and, in larger lesions, a halo or target pattern of enhancement.[11,12]

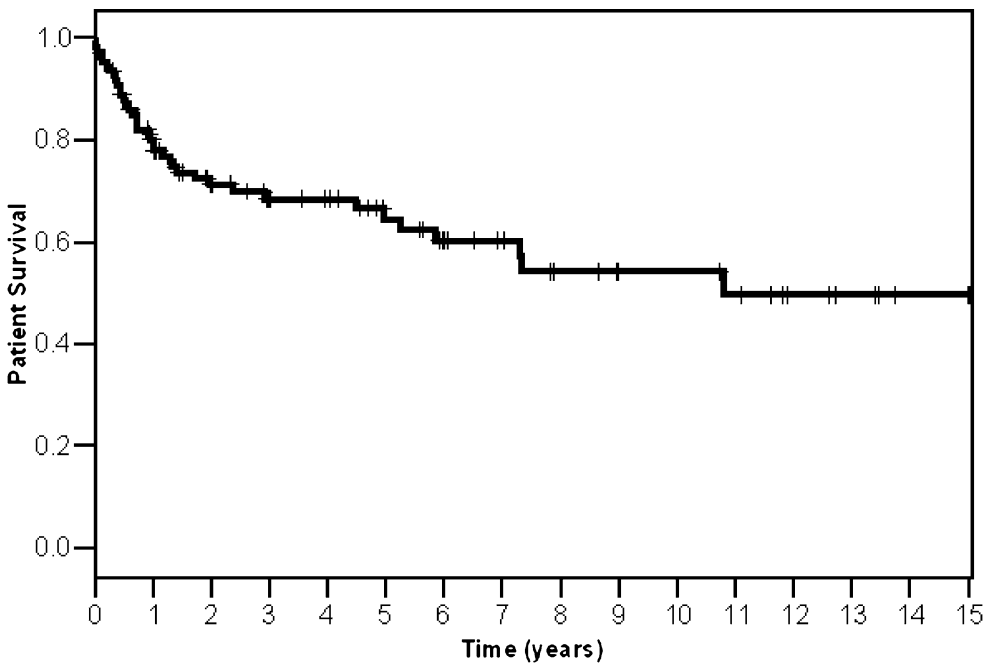

Figure 3 Kaplan–Meier plot of allograft survivals for 110 patients (126 transplants) treated with orthotopic liver transplantation for hepatic epitheliod hemangioma from 1987 to 2005.

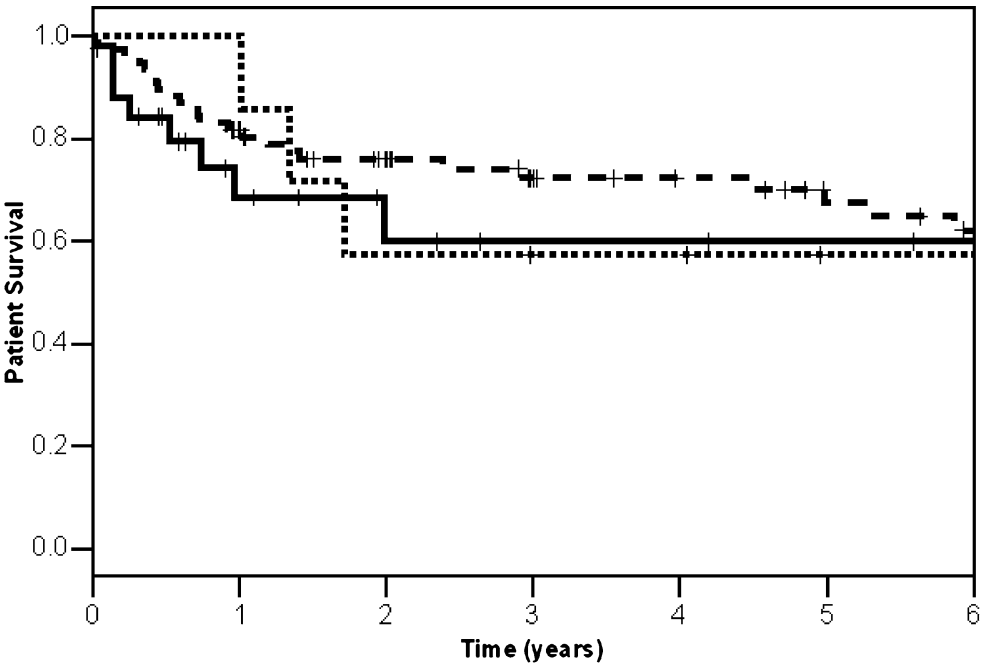

Figure 4 Patient survivals after orthotopic liver transplantation for hepatic epitheliod hemangioma stratified by age. Infantile (0–3 years old, *solid line*) vs. pediatric (4–18 years old, *dotted line*) vs. adult (>18 years old, *dashed line*).

A definitive diagnosis of HEH requires histopathologic assessment. Histologic characteristics include a well-developed basal lamina, the presence of Weibel–Palade bodies, and vascular invasion.[10,13] As histologic features of HEH may be similar to those of sclerosing hemangioma, angiosarcoma, cholangiocarcinoma, and metastatic carcinoma, the specificity of immunohistochemical examination is needed to supplement histologic examination.[10,13] Endothelial origin is confirmed by positive immuno-staining for factor VIII-related antigen and other endothelial cell markers.[13] Although helpful for diagnosis, no histopathologic features have been correlated with the disease's clinical behavior.

Currently, no prognostic clinical or histopathologic features have been identified that predict tumor aggressiveness. The only natural history data available for this disease comes from reports of untreated patients. In their review of the HEH world literature, Merhabi, et al.[1] determined that 25% of reported cases of HEH went untreated. More than half of these patients died of progressive disease and the 5-year survival for this cohort was only 5%. The unpredictable clinical course of HEH is further highlighted by specific reports of clinical outcomes after no treatment, including reports of extended survival with spontaneous tumor regression,[10,14] versus reports of rapid tumor progression leading to fulminant hepatic failure.[1,15]

For the rare patient who presents with limited intrahepatic disease, local treatment including liver resection is a logical treatment choice. Support for this approach comes from a recent review of all published series on HEH, which determined that the 5-year survival rate after resection for HEH was 75%.[1] However, because of the frequent multifocal nature of HEH at presentation, liver resection is rarely performed. In the above-mentioned review, only 19% of patients presented with unilobar disease, only 13% of patients presented with unifocal disease, and therefore, only 9.4% of these patients were candidates for liver resection.

Table 2 Posttransplant Survival Analysis for 110 Patients with Hepatic Hemangioendothelioma Stratified by Study Variables

Study Factor	*N*	Percent	Median Survival (months)	5-Year Survival (%)	*p* value
Sex					
Male	35	32	85.3	51	0.11
Female	75	68	>120	65	
Age					
Infantile (<4 years)	25	23	>120	61	0.75
Pediatric (4– 18 years)	7	6	>120	57	
Adult (19+ years)	78	71	>120	67	
Era					
1987–1995	33	30.0	>120	57	0.39
1996–2005	77	70.0	>120	57	
Preoperative medical condition					
Inpatient	43	39	63.7	44%	0.01
Outpatient	66	61	>120	72%	
ICU Care	22	20	22.8	39%	0.02
Non-ICU Care	87	80	>120	65%	
Status 1	25	23	>120	51%	0.36
Non-status 1	85	77	>120	63%	

Abbreviations: *N*, number of patients; ICU, intensive care unit

In the setting of more diffuse intrahepatic disease, major hepatic resection and OLT are the currently available treatment modalities.[2] Reports of tumor recurrence after liver resection are inconsistent. Whereas one case report has described success with major hepatic resection,[16] aggressive recurrence after resection of apparently localized lesions has also been reported.[2] It has been suggested that hepatotrophic, regenerative cellular signaling after hepatic resection may lead to rapid hyperproliferation of residual malignant disease in some patients.[2]

Instances of agressive disease recurrence and even fulminant hepatic failure have been reported following radical resection of multifocal HEH. In one recent study, radical resection of multifocal HEH was followed by multiple local recurrences, consumptive coagulopathy with thrombocytopenia, and death after resection.[3] Reports in the literature of "salvage" OLT, after noncurative resection, have also been disappointing.[2]

Based on the inability to predict the aggressiveness of HEH, the limited applicability of liver resection, and the reports of poor outcomes after resection of multifocal HEH, wider implementation of OLT should be considered. In the previously mentioned review of the world literature, 44.8% of patients were treated with OLT.[1] The 1-year and 5-year survivals in this cohort were 96% and 54%, respectively. Outcomes from the largest single-center cohort of patients transplanted for HEH were reported by Madariaga, et al. in 1995.[7] Five-year patient survival in this cohort of 16 patients was 71.3%. Five-year survival rates from other series in the literature include: Yokoyama et al.,[8] 48% (n=8) and Penn et al.,[17] 43% (n=21). In our analysis of the UNOS experience assessing 110 patients undergoing transplantation for HEH between 1987 and 2005, 1-year and 5-year allograft survivals were 70% and 55%, respectively, and 1-year and 5-year overall patient survivals were 80% and 64%, respectively.

Reported all-stage 5-year patient survival rates for HEH, regardless of therapy, are 41%,[1] 43%,[10] and 56%.[15] Given that the majority of patients who undergo OLT have advanced-stage disease with large tumor burden, achievement of 5-year patient survivals over 60% is remarkable. In addition, these outcomes compare favorably to those after OLT for other hepatic malignancies. Penn[17] reported that the HEH recurrence rate after OLT was only 33%, compared to 39% for non-incidental hepatoma, 44% for cholangiocarcinoma, 59% for metastatic tumors, and 64% for hemangiosarcoma. In our review of the UNOS database, 38 patients died during follow-up, with only 12 patients (11% of transplanted patients) having recurrent HEH as the primary cause of death.

The favorable outcomes after OLT in patients with HEH appear to be independent of patient age. Approximately 20% of the patients analyzed in this study were less than 4 years old. After OLT, this group with infantile HEH experienced survivals (5-year OS: 61%) similar to those of pediatric (5-year OS: 57%) and adult HEH patients (5-year OS: 67%). Although patient age did not have prognostic value, the analysis determined that the patient's medical condition before OLT was predictive of survival. Patients who were hospitalized, either in an ICU or non-ICU setting experienced significantly lower 5-year survival rates. These findings warrant consideration regarding patient selection for OLT listing and for donor-recipient matching.

Conclusions

This analysis indicates that liver transplantation for HEH is associated with favorable outcomes. Although liver resection and other local therapies may be reasonable choices in cases of small, focal lesions, the frequent presentation with multifocal disease often precludes the use of these treatments. Given the favorable outcomes after transplantation and the propensity for recurrence after liver resection, liver transplantation should be considered as a first-line treatment in patients who would otherwise require major hepatic resection, including selected patients with extrahepatic disease.

References

1. Mehrabi A, Kashfi A, Fonouni H, Schemmer P, Schmied BM, Hallscheidt P, Schirmacher P, Weitz J, Friess H, Buchler MW, Schmidt J. Primary malignant hepatic epithelioid hemangioendothelioma: A comprehensive review of the literature with emphasis on the surgical therapy. Cancer 2006;107(9):2108–2121.
2. Ben-Haim M, Roayaie S, Ye MQ, Thung SN, Emre S, Fishbein TA, Sheiner PM, Miller CM, Schwartz ME. Hepatic epithelioid hemangioendothelioma: Resection or transplantation, which and when? Liver Transpl Surg 1999;5(6):526–531.
3. Imanishi H, Kawata M, Yanagihara M, Nakayama N, Sato T, Furukawa Y, Fukunaga N, Kozuma T. Epithelioid hemangioendothelioma of the liver associated with thrombocytopenia and coagulopathy. Hepatogastroenterology 2002;49(48):1673–1675.
4. Lerut JP, Orlando G, Sempoux C, Ciccarelli O, Van Beers BE, Danse E, Horsmans Y, Rahier J, Roggen F. Hepatic haemangioendothelioma in adults: Excellent outcome following liver transplantation. Transpl Int 2004;17(4):202–207.
5. St Peter SD, Moss AA, Huettl EA, Leslie KO, Mulligan DC. Chemoembolization followed by orthotopic liver transplant for epithelioid hemangioendothelioma. Clin Transplant 2003;17(6):549–553.
6. Achilleos OA, Buist LJ, Kelly DA, Raafat F, McMaster P, Mayer AD, Buckels JA. Unresectable hepatic tumors in childhood and the role of liver transplantation. J Pediatr Surg 1996;31(11):1563–1567.
7. Madariaga JR, Marino IR, Karavias DD, Nalesnik MA, Doyle HR, Iwatsuki S, Fung JJ, Starzl TE. Long-term results after liver transplantation for primary hepatic epithelioid hemangioendothelioma. Ann Surg Oncol 1995;2(6):483–487.

8. Yokoyama I, Todo S, Iwatsuki S, Starzl TE. Liver transplantation in the treatment of primary liver cancer. Hepatogastroenterology 1990;37(2):188–193.
9. Marino IR, Todo S, Tzakis AG. Treatment of hepatic epitheliod hemangioendothelioma with liver transplantation. Cancer 1988; 62:2079–2084.
10. Makhlouf HR, Ishak KG, Goodman ZD. Epithelioid hemangioendothelioma of the liver: A clinicopathologic study of 137 cases. Cancer 1999;85(3):562–582.
11. Furui S, Itai Y, Ohtomo K, Yamauchi T, Takenaka E, Iio M, Ibukuro K, Shichijo Y, Inoue Y. Hepatic epithelioid hemangioendothelioma: Report of five cases. Radiology 1989;171(1):63–68.
12. Radin DR, Craig JR, Colletti PM, Ralls PW, Halls JM. Hepatic epithelioid hemangioendothelioma. Radiology 1988;169(1):145–148.
13. d'Annibale M, Piovanello P, Carlini P. Epitheliod hemangioendothelioma of the liver: Case report and review of the literature. Transplant Proc 2002;34:1248–1251.
14. Otrock ZK, Al-Kutoubi A, Kattar MM, Zaatari G, Soweid A. Spontaneous complete regression of hepatic epithelioid haemangioendothelioma. Lancet Oncol 2006;7:439–441.
15. Lauffer JM, Zimmermann A, Krahenbuhl L, Triller J, Baer HU. Epithelioid hemangioendothelioma of the liver. A rare hepatic tumor. Cancer 1996;78(11):2318–2327.
16. Mehrabi A, Kashfi A, Schemmer P, Sauer P, Encke J, Fonouni H, Friess H, Weitz J, Schmidt J, Buchler MW, Kraus TW. Surgical treatment of primary hepatic epithelioid hemangioendothelioma. Transplantation 2005;80(1 Suppl):S109–S112.
17. Penn I. Hepatic transplantation for primary and metastatic cancers of the liver. Surgery 1991;110(4):726–734; discussion 734–725.

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Outcomes Analysis for 280 Patients with Cholangiocarcinoma Treated with Liver Transplantation Over an 18-year Period

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Abstract Cholangiocarcinoma is an aggressive malignancy with 5-year survival rates <15%. Selected patients present with localized but unresectable disease and are candidates for orthotopic liver transplantation (OLT). The purpose of this study was to evaluate a multi-institutional experience with liver transplantation for this malignancy. Two hundred eighty patients with cholangiocarcinoma treated with OLT from 1987 to 2005 were identified in The United Network for Organ Sharing database. Patient and allograft survivals were calculated and the potential prognostic value of multiple clinicopathologic variables was assessed. At a median follow-up interval of 452 days (range: 0–6,166 days), 1- and 5-year patient survivals were 74 and 38%, respectively, with 49 actual 5-year survivors and 21 actual 10-year survivors. Posttransplant 1- and 5-year allograft survivals were 69 and 36%, respectively. Study variables associated with improved survivals included diagnosis of cholangiocarcinoma pre-OLT [5-year overall survival (OS): 68 vs. 20% for patients with incidental diagnoses at the time of OLT, $p<0.001$] and OLT after 1993 (5-year OS: 45 vs. 30% pre-1994, $p<0.01$). In contrast, the diagnosis of concomitant primary sclerosing cholangitis did not impact survivals (5-year OS: 41 vs. 50% without primary sclerosing cholangitis, $p=0.402$). Selected cholangiocarcinoma patients treated with OLT experience a survival benefit. Diagnosis of cancer prior to OLT allows for better staging and pre-OLT therapy that may translate into improved outcomes. These data support the continued development of multimodality cholangiocarcinoma treatment protocols that include OLT.

Keywords Bile duct cancer · Orthotopic liver transplantation · Survival analysis

Introduction

Cholangiocarcinoma is an uncommon, but aggressive, malignancy of the biliary tract. Unlike many other cancers, its incidence in the USA and worldwide is increasing. Between 1973 and 1997, the incidence increased by almost 10%, as did the mortality rate from the disease.[1,2] Although no specific etiologic factor can be found in most patients, there is an association between long-standing biliary inflammation [as in the case of primary sclerosing cholangitis (PSC)] and development of cholangiocarcinoma.[3] In the population of patients with PSC, the prevalence of cholangiocarcinoma ranges from 5 to 15%.[4]

Results of nonsurgical therapies for cholangiocarcinoma have been disappointing, with the majority of patients surviving less than 1 year after diagnosis.[5] In cases where complete resection is possible, 5-year patient survivals between 27 and 48% have been reported.[2] However, due to patient age, tumor location, distant disease, and/or underlying liver disease, candidacy for complete resection is more often the exception than the rule.[2] In selected cases of cholangiocarcinoma that are early-stage, but anatomically not resectable, orthotopic liver transplantation (OLT) has been investigated as a treatment modality.[6–8]

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Early experience with OLT in cholangiocarcinoma was disappointing, with reported 5-year survivals ranging from 18 to 25%.[9–11] However, more recent single-center reports indicate that 5-year patient survivals of over 80% can be achieved when liver transplantation is combined with neoadjuvant radiation and chemotherapy in patients with early-stage disease (stage I/II).[12,13] Given these recent favorable results, the aim of this study was to examine overall trends in outcomes following OLT for cholangiocarcinoma using the multi-institutional United Network for Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) patient database.

Materials and Methods

The UNOS/OPTN database encompassing 71,224 liver transplants from 1987 to 2005 was used for data collection. Analysis of this data set identified 280 patients who had a diagnosis of cholangiocarcinoma at listing (n=102) or at discharge (n=245) (67 patients were included in both groups). To determine allograft survival rates, data were collected on first OLTs (n=280) and subsequent retransplants (n=22) received by these patients, yielding a total of 302 analyzed transplants in 280 study patients.

Of the 280 patients analyzed, 101 patients (36%) were transplanted prior to 1994, before a standard system for listing pretransplant and posttransplant oncologic diagnoses was routinely utilized in the database. For these 101 patients, there was an overall treatment diagnosis listed as "cholangiocarcinoma," but knowledge of the presence of cholangiocarcinoma prior to transplant vs. incidentally found cholangiocarcinoma could not be confirmed. The remaining 179 patients (64%), transplanted after 1993, had clearly defined listing diagnoses in the UNOS database and, therefore, the analyses concerning indication for transplant were limited to these patients.

Examined study variables included recipient age, race, gender, indication for transplant, pretransplant clinical status, ABO blood group, allograft type, date of transplant, patient and allograft survivals, and cause of allograft failure or death (cancer-related vs. other cause). Kaplan–Meier curves were used to calculate survivals and log-rank tests were used to determine the influence of study variables on outcomes. A p value <0.05 was considered statistically significant.

Results

Demographics

Of the 280 patients who underwent liver transplantation for cholangiocarcinoma between April 1987 and December 2005, 64.3% were male. The most common race was Caucasian (86.8%), followed by African-American (4.6%) and Asian (2.2%). The median age of recipients was 48 years (range: 18–73 years). The recipient ABO blood group distribution was as follows: O, 128 patients; A, 107 patients; B, 37 patients; and AB, 8 patients.

Preoperative Status/Allograft Type

The median waitlist time for first-transplant recipients was 58 days (range: 0–3,147 days). One hundred and seventy-seven patients were transplanted before the model for end-stage liver disease (MELD) score was utilized for waitlist ranking. For the remaining 103 patients, the median lab MELD score was 14 (range: 6–47). At transplant, 35 patients (12.5%) were listed as status 1. One-hundred and four patients (37%) were hospitalized immediately prior to transplant. Of these patients, 37 were in the intensive care unit and 14 patients were requiring ventilator support. At time of transplant, the median serum creatinine was 0.9 mg/dL (range: 0.1–13 mg/dL), the median serum total bilirubin was 2.6 mg/dL (range: 0.2–53.7 mg/dL), and the median serum alanine aminotransferase was 70.5 U/L (range: 2–7,891 U/L). Two-hundred fifty-four recipients (91%) received whole cadaveric allografts, 21 patients (7%) received allografts from living donors, and 5 patients (2%) received reduced or split cadaveric allografts.

Indication for OLT

One hundred and two of the 179 patients (57%) with complete pretransplant oncologic diagnosis data had a pre-OLT listing diagnosis of cholangiocarcinoma. For 38 of these 102 patients with known cholangiocarcinoma, PSC was listed as a concomitant pre-OLT diagnosis. The remaining 64 patients with known pre-OLT cholangiocarcinoma were not reported to have PSC. Seventy-seven patients (43%) transplanted after 1993 had an incidental diagnosis of cholangiocarcinoma at the time of transplantation. The indications for OLT in these patients included PSC with liver failure in 31 (40%), other malignancy in 8 (11%), and nonmalignant, non-PSC-related liver failure in 38 (49%).

Patient Survivals

Twelve patients died within 30 days of primary transplant, yielding a 30-day mortality rate of 4.0%. At a median patient follow-up interval of 452 days (range: 0–6,166 days), 1- and 5-year patient survivals for all 280 study patients were 74% and 38%, respectively. There were 49 actual 5-year survivors and 21 actual 10-year survivors.

Patient Survivals: Impact of Demographic and Clinical Variables

Age, race, gender, and blood group had no impact on patient survivals. Allograft type and status 1 listing also had no impact on survivals. Clinical variables that were significant predictors of worse survival included inpatient hospitalization prior to transplant ($p=0.006$), ICU admission prior to transplant ($p<0.001$), serum creatinine ≥1.5 mg/dL ($p<0.001$), and serum bilirubin ≥2.0 mg/dL ($p=0.015$) (Table 1).

Patient Survivals Stratified by Indication

When stratified by indication for transplant, there were differences in patient survival. Those patients transplanted prior to 1994, without specific oncologic listing diagnoses in the database, had 1- and 5-year survivals of 67 and 30%, respectively. Patients transplanted from 1994 to 2005 with a listing diagnosis of cholangiocarcinoma in the setting of PSC had 1- and 5-year survivals of 90 and 79%, respectively. Patients with known cholangiocarcinoma in the absence of PSC had 1- and 5-year survivals of 90 and 64%, respectively. Those with known PSC but an incidental finding of cholangiocarcinoma had 1- and 5-year survivals of 79 and 18%, respectively, and those with no known PSC or cholangiocarcinoma at listing had 1- and 5-year survivals of 82 and 30%, respectively.

Patients could therefore be stratified into a historical group, transplanted prior to 1994 with cholangiocarcinoma listed as the overall diagnosis ($n=101$), those transplanted from 1994 to 2005 with known cholangiocarcinoma ($n=102$), and those transplanted from 1994 to 2005 with an incidental diagnosis of cholangiocarcinoma ($n=77$). When comparing survivals between these groups, the historic group and the incidental cholangiocarcinoma group had similar 5-year survivals (30 vs. 20%, respectively, $p=0.646$). However, patients with known cholangiocarcinoma prior to OLT experienced an improved 5-year survival rate of 68% (compared with historic survivals, $p<0.001$; compared with incidental diagnosis, $p<0.001$) (Fig. 1).

Patient Survivals Stratified by PSC Status

Of the 179 patients with a defined listing diagnosis, 69 (39%) patients were listed with a diagnosis of PSC. The 5-

Table 1 Posttransplant Survival Analysis Stratified by Study Variables

Variable	Strata	N	5-year OS	*p* Value
Age	<50 years	145 (52%)	42%	0.280
	≥50 years	135 (48%)	34%	
Sex	Male	178 (64%)	36%	0.213
	Female	102 (36%)	44%	
Race	Caucasian	243 (87%)	37%	0.365
	Other	37 (13%)	56%	
	Black	13 (5%)	69%	0.339
	Other	267 (95%)	37%	
Blood group	O	128 (46%)	46%	0.189
	A	107 (38%)	31%	
	B	37 (13%)	40%	
	AB	8 (3%)	30%	
Status 1	Yes	35 (13%)	37%	0.929
	No	245 (87%)	39%	
Pre-OLT location	Home	176 (63%)	45%	**0.006**
	Hospital	104 (37%)	29%	
	ICU	37 (13%)	13%	**<0.001**
	Other	243 (87%)	43%	
Allograft type	Cadaveric whole	254 (91%)	49% (3 year)	0.866
	Living donor	21 (8%)	58% (3 year)	
	Cadaveric split	5 (2%)	36% (3 year)	
Serum ALT	<200 U/L	166 (59%)	37%	0.281
	≥200 U/L	54 (19%)	36%	
Serum creatinine	<1.5 mg/dL	239 (85%)	42%	**<0.001**
	≥1.5 mg/dL	38 (14%)	20%	
Serum bilirubin	<2.0 mg/dL	119 (43%)	44%	**0.015**
	≥2.0 mg/dL	149 (53%)	35%	
MELD score	<15	55 (20%)	85% (2 year)	0.081
	≥15	48 (17%)	65% (2 year)	

Bold *p* values indicate statistical significance.
N = number of patients, OS = overall survival, ICU = intensive care unit, ALT = alanine aminotransferase

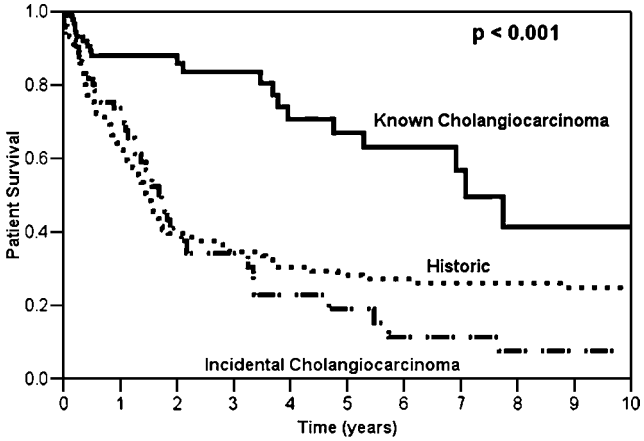

Figure 1 Patient survivals stratified by indication for liver transplantation. One hundred one patients transplanted prior to 1994 with no oncologic listing data in the UNOS database ("historic") are represented by the *dotted line*. The 102 patients with a known diagnosis of cholangiocarcinoma are represented by the *solid line*, and the 77 patients with incidental cholangiocarcinoma transplanted from 1994 to 2005 are represented by the *dotted-dashed line*.

year survival rate in patients with PSC was 41%, compared to 50% in patients without PSC (p=0.402).

Patient Survivals Stratified by Cause of Death

Of the 128 patients who died more than 30 days post-OLT, the cause of death was known in 114 of these 128 cases (89%). Of these, 55 patients died from locally recurrent (19 patients) or metastatic disease (36 patients), 24 patients died from infection, 13 patients died from allograft failure, and 22 patients died from other causes. Patients who died from recurrent disease had 1- and 5-year survival rates of 76 and 17%, respectively, with a median survival of 601 days compared with 1-year, 5-year, and median survivals of 44%, 3%, and 322 days for those who died from non-cancer causes (p<0.005) (Fig. 2).

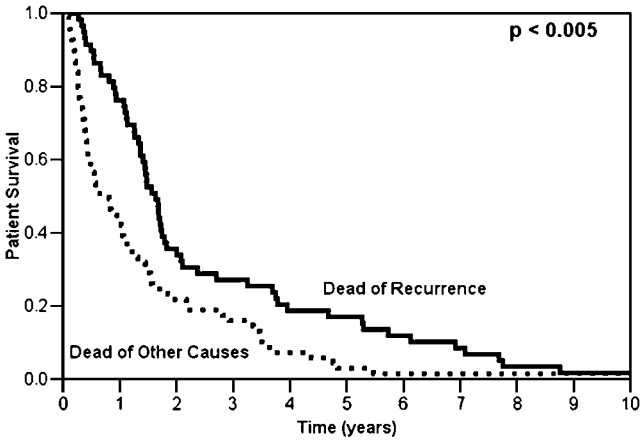

Figure 2 Patient survivals stratified by cause of death. Patients who died from cholangiocarcinoma local and/or distant recurrence are represented by the *solid line*, those who died from other causes are represented by the *dotted line*.

Patient Survival after Retransplant

Twenty patients underwent one retransplant after their initial transplant for cholangiocarcinoma, and two patients were transplanted a third time. Two patients died within 30 days of retransplant. Median survival after retransplant was 479 days. One- and 3-year patient survivals after retransplant were 50 and 37%, respectively. Comparing patient outcomes for those requiring retransplant (median survival: 595 days) to those not requiring retransplant (median survival: 1,069), overall survivals were statistically similar (p=0.407).

Era of Transplantation

For the 101 UNOS-listed patients transplanted prior to 1994, the 5-year survival rate was 30% and the median survival time was 587 days. For the 179 patients with complete diagnostic data for cholangiocarcinoma transplanted between 1995 and 2005, the 5-year survival rate was 45% and the median survival time was 1,413 days (p< 0.01) (Fig. 3). Dividing this group by era (1994–2000 vs. 2001–2005), no survival improvement was observed. The 66 patients transplanted from 1994–2000 experienced a 3-year survival rate of 58% and the median survival time was 1,367 days. The 3-year and median survivals for the 113 patients transplanted from 2001–2005 were 60% and 1,242 days, respectively (p=0.569).

Allograft Survivals

At a median graft follow-up interval of 390 days (range: 0–6,166 days), 1- and 5-year allograft survivals were 69 and 36%, respectively. Twenty patients required retransplant, including two patients who received a third allograft. Time to first retransplant ranged from 4 to 2,673 days, with a

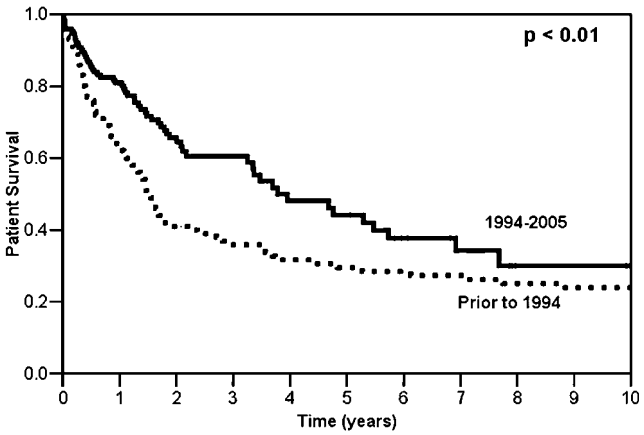

Figure 3 Patient survivals stratified by those transplanted from 1994 to 2005 (n=179, *solid line*), compared to those transplanted prior to 1994 (n=101, *dotted line*).

median of 44 days. The indication for retransplant was available in 9 of these 22 cases. Vascular thrombosis was the cause of allograft failure in six cases, whereas primary nonfunction was the cause in three cases, one of which was a second retransplant.

Discussion

Cholangiocarcinoma presents significant challenges to the hepatobiliary surgeon, the medical oncologist, and the radiation oncologist. The disease is often diagnosed at a stage or in a location that precludes complete resection. In addition, concomitant PSC is often considered a contraindication to resection due to the high likelihood of multifocal disease, clinically significant underlying liver dysfunction, and the high risk of recurrence following resection.[2] Based on these factors, liver transplantation has been used to treat patients with local disease.

Recent single-center studies have reported favorable outcomes in selected patients with hilar cholangiocarcinoma. The largest such series is from the Mayo Clinic.[8] Eligibility for their protocol is limited to patients with unresectable hilar cholangiocarcinoma or hilar cholangiocarcinoma in the setting of PSC. Operative exploration was used to rigorously confirm that patients were stage I or II, without nodal or other metastases, prior to OLT. The treatment included neoadjuvant chemoradiotherapy followed by a staging laparotomy in all patients. Of the 106 patients initially enrolled to this protocol, 11 patients died or had evidence of disease spread before neoadjuvant treatment was completed, and one was transplanted elsewhere. An additional 18 patients experienced disease progression diagnosed at laparotomy and were, therefore, excluded from the study. Of the remaining 87 patients, 65 transplants have been reported to date. In these 65 patients, 1- and 5-year survivals were 91 and 76%, respectively, with a median follow up interval of 18 months.[8]

In addition to the Mayo Clinic experience, there are two registry-based reports that have examined outcomes in patients transplanted for cholangiocarcinoma. In 2000, Pascher et al. reported on patients treated with OLT for cholangiocarcinoma recorded in the European Liver Transplant Registry and found a 5-year patient survival of 29%.[14] Likewise, a report from the Cincinnati Transplant Registry of 207 patients with cholangiocarcinoma who received liver transplants between 1968 and 1997 found a 5-year patient survival of 23%.[15] Since this report, there have been no US-based multicenter studies that have assessed more recent outcomes data.

Our analysis of 280 patients identified an overall 5-year patient survival of 38%, and a 60% 3-year survival rate in patients transplanted after 2000. In addition, 21 patients had actual survivals of greater than 10 years. The apparent improvement in overall outcomes over time may be related to several factors. These factors include improved staging, improved patient selection, and possibly more effective adjuvant therapies. With regard to staging, several groups have reported that nodal or other extrahepatic involvement and/or advanced disease stage (stage III or IV) are independent predictors of adverse outcomes following OLT.[8,16,17] Likewise, the favorable outcomes reported by the Mayo Clinic reflect the strict inclusion of patients with early-stage disease.[12,13,18] Combined, these findings support the use of a multimodality oncologic approach to patients with early-stage (stage I or II), but unresectable, cholangiocarcinoma that includes liver transplantation as part of a protocol-based treatment plan.

For the more recent cohort, who had complete data regarding the setting and timing of the cholangiocarcinoma diagnosis, several important clinical observations were made. Patients who were incidentally found to have cholangiocarcinoma had a significantly worse prognosis following transplant. The 5-year survival rate for patients with known cholangiocarcinoma was 68%, and the median survival time was not reached. In contrast, patients with incidental cholangiocarcinoma found in the explanted liver experienced 5-year survivals of only 20%, with a median survival time of only 640 days.

The prognostic value of the timing of cholangiocarcinoma diagnosis (pre-OLT vs. incidental at the time of OLT) was stronger than the presence or absence of PSC. Patients with PSC and known cholangiocarcinoma experienced higher survival rates compared to PSC patients with incidentally found cholangiocarcinoma. Likewise, patients without PSC were more likely to survive if their cholangiocarcinoma was identified prior to OLT.

The prognostic impact of cholangiocarcinoma developing in the setting of PSC is controversial. In this clinical situation, cholangiocarcinoma may be difficult to diagnose and is often an incidental finding during transplantation.[19] A review of the Canadian experience with incidental cholangiocarcinoma reported good short-term survivals, but this benefit was lost after the second year post-transplant.[20] Other single-center studies have found no difference in prognosis.[8,15,18]

These observations can be explained in several ways. First, independent of PSC status, patients with known cholangiocarcinoma may undergo additional staging studies to confirm the absence of metastases prior to OLT listing (i.e., selection bias). Patients with incidental cholangiocarcinoma may harbor undetected distant disease, have a higher stage at the time of transplant, and have a concomitant poorer prognosis. Second, only the patients with known cholangiocarcinoma can benefit from pre-OLT cancer therapies. The UNOS/OPTN database does not record the

presence or absence of pre-OLT chemoradiotherapy for cholangiocarcinoma, so direct evidence of its efficacy cannot be determined from this analysis.

Conclusions

This multi-institutional analysis of the US experience with liver transplantation for cholangiocarcinoma determined that outcomes following OLT for cholangiocarcinoma have improved over time with a 5-year survival rate of 45% during the most recent era of transplantation. Compared to outcomes in similar patients treated with medical therapy alone, patients with known cholangiocarcinoma that presents at an early, but unresectable, stage appear to benefit from OLT. However, patients incidentally found to have cholangiocarcinoma at the time of transplant, independent of the presence or absence of PSC, have a poorer prognosis.

References

1. Patel T. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. Hepatology 2001; 33:1353–1537.
2. Malhi H, Gores GJ. The modern diagnosis and therapy of cholangiocarcinoma. Aliment Pharmacol Ther 2006;23:1287–1296.
3. Lazaridis KN, Gores GJ. Primary sclerosing cholangitis and cholangiocarcinoma. Semin Liver Dis 2006;26:42–51.
4. Malhi H, Gores GJ. Cholangiocarcinoma: modern advances in understanding a deadly old disease. J Hepatol 2006;45:856–867.
5. Jarnagin WR, Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BJ, Youssef BM, Klimstra D, Blumgart LH. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. Ann Surg 2001;234:507–517.
6. Sudan DL. Transplantation for cholangiocarcinoma. Liver Transpl 2006;12:S83–S84.
7. Singh P, Patel T. Advances in the diagnosis, evaluation and management of cholangiocarcinoma. Curr Opin Gastroenterol 2006;22:294–299.
8. Heimbach JK, Gores GJ, Haddock MG, Alberts SR, Pedersen R, Kremers W, Nyberg SL, Ishitani MB, Rosen CB. Predictors of disease recurrence following neoadjuvant chemoradiotherapy and liver transplantation for unresectable perihilar cholangiocarcinoma. Transplantation 2006;82:1703–1707.
9. Goldstein RM, Stone M, Tillery GW, Senzer N, Levy M, Husberg BS, Gonwa T, Klintmalm G. Is liver transplantation indicated for cholangiocarcinoma? Am J Surg 1993;166:768–771.
10. Jeyarajah DR, Klintmalm GB. Is liver transplantation indicated for cholangiocarcinoma? J Hepatobiliary Pancreat Surg 1998;5:48–51.
11. Pichlmayr R, Lamesch P, Weimann A, Tusch G, Ringe B. Surgical treatment of cholangiocellular carcinoma. World J Surg 1995;19:83–88.
12. Heimbach JK, Gores GJ, Haddock MG, Alberts SR, Nyberg SL, Ishitani MB, Rosen CB. Liver transplantation for unresectable perihilar cholangiocarcinoma. Semin Liver Dis 2004;24:201–207.
13. Heimbach JK, Gores GJ, Nagorney DM, Rosen CB. Liver transplantation for perihilar cholangiocarcinoma after aggressive neoadjuvant therapy: a new paradigm for liver and biliary malignancies? Surgery 2006;140:331–334.
14. Pascher A, Jonas S, Neuhaus P. Intrahepatic cholangiocarcinoma: indication for transplantation. J Hepatobiliary Pancreat Surg 2003;10:282–287.
15. Meyer CG, Penn I, James L. Liver transplantation for cholangiocarcinoma: results in 207 patients. Transplantation 2000;69:1633–1637.
16. Shimoda M, Farmer DG, Colquhoun SD, Rosove M, Ghobrial RM, Yersiz H, Chen P, Busuttil RW. Liver transplantation for cholangiocellular carcinoma: analysis of a single-center experience and review of the literature. Liver Transpl 2001;7:1023–1033.
17. Robles R, Figueras J, Turrion VS, Margarit C, Moya A, Varo E, Calleja J, Valdivieso A, Valdecasas JC, Lopez P, Gomez M, de Vicente E, Loinaz C, Santoyo J, Fleitas M, Bernardos A, Llado L, Ramirez P, Bueno FS, Jaurrieta E, Parrilla P. Spanish experience in liver transplantation for hilar and peripheral cholangiocarcinoma. Ann Surg 2004;239:265–271.
18. Rea DJ, Heimbach JK, Rosen CB, Haddock MG, Alberts SR, Kremers WK, Gores GJ, Nagorney DM. Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. Ann Surg 2005;242:451–458.
19. Moreno Luna LE, Gores GJ. Advances in the diagnosis of cholangiocarcinoma in patients with primary sclerosing cholangitis. Liver Transpl 2006;12:S15–S19.
20. Ghali P, Marotta PJ, Yoshida EM, Bain VG, Marleau D, Peltekian K, Metrakos P, Deschenes M. Liver transplantation for incidental cholangiocarcinoma: analysis of the Canadian experience. Liver Transpl 2005;11:1412–1416.

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Comparison of Two Methods of Future Liver Remnant Volume Measurement

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Abstract
Background In liver transplantation, a minimum graft to patient body weight (BW) ratio is required for graft survival; in liver resection, total liver volume (TLV) calculated from body surface area (BSA) is used to determine the future liver remnant (FLR) volume needed for safe hepatic resection. These two methods of estimating liver volume have not previously been compared. The purpose of this study was to compare FLR volumes standardized to BW versus BSA and to assess their utility in predicting postoperative hepatic dysfunction after hepatic resection.
Methods Records were reviewed of 68 consecutive noncirrhotic patients who underwent major hepatectomy after portal vein embolization between 1998 and 2006. FLR (cubic centimeter) was measured preoperatively with three-dimensional helical computed tomography; TLV (cubic centimeter) was calculated from the patients' BSA. The relationship between FLR/TLV and FLR/BW (cubic centimeter per kilogram) was examined using linear regression analysis. Receiver operating characteristic (ROC) curve analysis was used to determine FLR/TLV and FLR/BW cutoff values for predicting postoperative hepatic dysfunction (defined as peak bilirubin level>3 mg/dl or prothrombin time>18 s).
Results Regression analysis revealed that the FLR/TLV and FLR/BW ratios were highly correlated (Pearson correlation coefficient, 0.98). The area under the ROC curve was 0.85 for FLR/TLV and 0.84 for FLR/BW (95% confidence interval, 0.71–0.97). Sixteen of the 68 patients developed postoperative hepatic dysfunction. The ROC curve analysis yielded a cutoff FLR/BW value of ≤0.4, which had a positive predictive value (PPV) of 78% and a negative predictive value (NPV) of 85%. The corresponding FLR/TLV cutoff value of ≤20% had a PPV of 80% and a NPV of 86%.
Conclusions Based on the strong correlation between the FLR measurements standardized to BW and BSA and their similar ability to predict postoperative hepatic dysfunction, both methods are appropriate for assessing liver volume. In noncirrhotic patients, a FLR/BW ratio of ≤0.4 and FLR/TLV of ≤20% provide equivalent thresholds for performing safe hepatic resection.

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Keywords Future liver remnant · Total liver volume · Hepatic insufficiency

Introduction

Advances in surgical technique and perioperative care have allowed more patients to be eligible for living donor liver transplantation (LDLT) and major hepatectomy with low perioperative mortality.[1,2] The graft volume in LDLT and liver remnant volume in major hepatic resection are critical for predicting postoperative outcome. A small-for-size graft and inadequate future liver remnant (FLR) may not meet

the hepatic metabolic demands after LDLT or major hepatectomy.[3,4] Preoperative volumetric analysis is essential to ensure sufficient functional liver parenchyma remains. In hepatic resection, preoperative portal vein embolization (PVE) is performed to increase the FLR volume and enhance the safety of major hepatectomy.[5] A highly accurate means of measuring graft volume and FLR preoperatively is three-dimensional computed tomography (CT).[6,7] In LDLT, the CT-measured graft volume is equated with graft weight, as liver density is 1 g/dl.[6,8] The graft weight is standardized to recipient body weight and defined as the graft-to-recipient weight ratio (GRWR) to determine the minimum graft requirement.[3,9] Because the liver normally composes 2 to 2.5% of the body weight (BW), a GRWR of 1 corresponds to 50% of liver volume, and a GRWR of 0.8 corresponds to 40% of liver volume.[10] In hepatic resection, the FLR volume is standardized to the total liver volume (TLV), which is based on patient body surface area (BSA).[11] The FLR/TLV ratio based on BSA used for liver resection and the GRWR based on BW used for liver transplantation have not been previously compared. The purpose of this study was to compare FLR volumes standardized to BSA or BW and to assess their ability to predict postoperative hepatic dysfunction after hepatic resection. We examined volumetric measurements and postoperative outcome in patients without cirrhosis who underwent preoperative PVE followed by major hepatectomy for hepatobiliary malignancies. Because the TLV bears a constant relationship to BSA and BW, we hypothesized that both methods could provide standard means to estimate liver function before hepatic resection.[12]

Materials and Methods

Between December 1998 and April 2006, 68 consecutive patients with hepatobiliary malignancies underwent PVE and measurement of the FLR volume using three-dimensional CT volumetry in preparation for major hepatectomy, as previously described.[13] The TLV was calculated from the patient's BSA using a mathematical formula $(\mathrm{TLV}[\mathrm{cm}^3] = -794.41 + 1,267.28 \times \mathrm{BSA}\ [\mathrm{m}^2])$, which was developed in a multicenter study of three-dimensional CT volumetric reconstructions in 292 adult patients who underwent CT for conditions unrelated to the hepatobiliary system.[11] This formula was validated in a meta-analysis comparing 12 formulas to estimate TLV and found to be the most precise and least biased compared to other formulas.[14] BSA was calculated according to Mosteller's formula: $[\mathrm{height\ (cm)} \times \mathrm{weight\ (kg)} \div 3,600]^{0.5}$.[15] Clinicopathological factors were reviewed, including age, gender, race, risk factors for liver injury, tumor histology, pre- and post-PVE measurements of FLR, extent of surgery, and postoperative course.

Hepatic parenchyma remote from the resected tumor was examined for pathologic findings of hepatic injury, defined as more than 30% steatosis, steatohepatitis Kleiner score≥4, grades 2 to 3 sinusoidal dilation, and Ishak grades 4 to 5 fibrosis.[16] Obesity was defined as body mass index≥30 kg/m^2.

Postoperative hepatic dysfunction was defined as peak bilirubin level>3 mg/dl or prothrombin time>18 s, based on prior studies showing a correlation between these parameters and postoperative complications.[17–19] Receiver operating characteristic (ROC) curve analysis was used to determine FLR/TLV and FLR/BW cutoff values for predicting postoperative hepatic dysfunction.[20,21] Cutoff values were determined by calculating the largest sum of the sensitivity and specificity values while maintaining the lowest probability of a negative test and the highest probability of a positive test.

Continuous data were expressed as means±standard deviations. Dichotomous variables were compared using the Chi-square test or the Fisher exact test where appropriate. Statistical significance was determined at $P<0.05$.

Results

The clinical and pathological characteristics of the 68 patients in this study are presented in Table 1. Median age was 60 years for the entire cohort, and 79% of the patients were male. Preoperative PVE with subsequent right or extended right hepatectomy was performed in all patients. The most common tumor type was metastatic colorectal cancer (n=32, 47%), followed by hepatocellular carcinoma (n=16, 24%), then neuroendocrine metastases (n=8, 12%) and intrahepatic or hilar cholangiocarcinoma (n=7, 10%). The FLR/TLV and FLR/BW (cubic centimeters per kilogram) ratios were highly correlated by regression analysis (Pearson correlation coefficient, 0.98; Fig. 1). Even in patients with BW, height, and BSA values beyond one standard deviation of the mean, the correlation was excellent (Pearson correlation coefficient, 0.89). Sixteen of the 68 patients developed postoperative hepatic dysfunction. ROC curve analysis was performed to determine the utility of FLR/TLV and FLR/BW in predicting postoperative hepatic dysfunction (Fig. 2). The areas under the ROC curve were 0.85 for FLR/TLV and 0.84 for FLR/BW (95% confidence interval, 0.71–0.97), indicating good discrimination for predicting postoperative hepatic dysfunction. Cutoff values were determined to yield the highest probability of a positive test while maintaining a low probability of a negative test. A cutoff FLR/BW value of ≤0.4 had a positive predictive value (PPV) of 78% and a negative predictive value (NPV) of 85%. The corresponding FLR/TLV cutoff value of ≤20% had a PPV of

Table 1 Clinicopathologic Factors of Patients Who Underwent Major Hepatectomy After Preoperative Portal Vein Embolization

Clinicopathologic factor	
Mean age (year)	60±11 (36–78)
Gender (*n*)	
Male	54
Female	14
Race (*n*)	
Caucasian	55
Hispanic	6
African–American	4
Asian	3
Tumor type (*n*)	
Colorectal metastases	32
Hepatocellular carcinoma	16
Neuroendocrine metastases	8
Intrahepatic or hilar cholangiocarcinoma	7
Gallbladder cancer	2
Thymoma metastases	1
Melanoma metastases	1
Endometrial metastases	1
Type of surgical resection (*n*)	
Extended right hepatectomy	54
Right hepatectomy	14
Body height (cm)	173 (140–208)[a]
Body weight (BW, kg)	83 (49–157)
Body surface area (BSA, m^2)	2.02 (1.45–2.67)
Body mass index	26.7 (19.8–48.4)
Future liver remnant volume (FLR, cm^3)	459 (261–1,098)
Estimated total liver volume (TLV, cm^3)	1,768 (1,037–2,593)
FLR/TLV (%)	27.7 (14.5–62.5)
FLR/BW (cm^3/kg)	0.58 (0.29–1.26)

[a] Data expressed as median (range)

80% and a NPV of 86%. The incidence of postoperative hepatic dysfunction was stratified according to cutoff values, and FLR/TLV and FLR/BW values of ≤20% and ≤0.4 emerged as most significant (Table 2). Six of the 18 patients with FLR/TLV between 20 and 25% and FLR/BW between 0.4 and 0.5 had postoperative hepatic dysfunction. Two of the 40 patients with FLR/TLV >25% or FLR/BW> 0.5 had postoperative hepatic dysfunction—one patient with

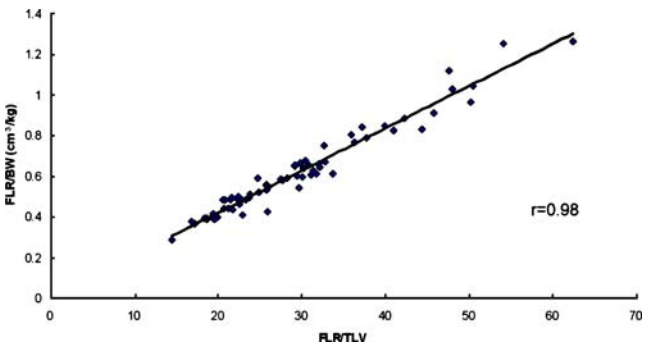

Figure 1 Correlation between FLR/TLV and FLR/BW.

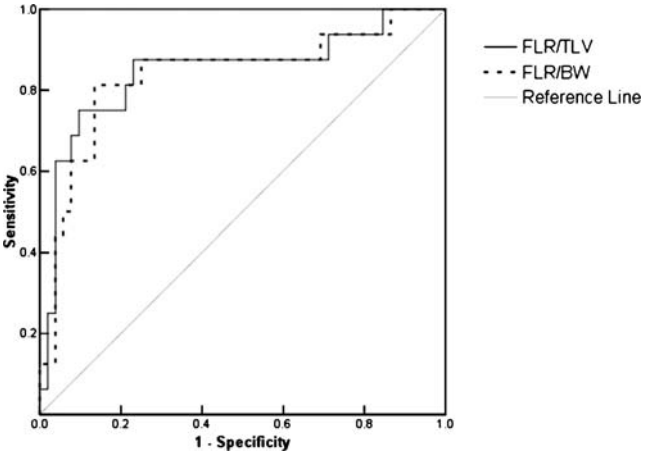

Figure 2 ROC curve of FLR/TLV and FLR/BW measurements in predicting postoperative hepatic dysfunction.

FLR/TLV of 33% and the other with FLR/TLV of 42%. Both patients had transient rises in bilirubin that normalized rapidly after postoperative days 5 and 9, respectively. The effects of risk factors for liver injury on postoperative hepatic dysfunction were assessed (Table 3). Preoperative systemic chemotherapy (median of 4 cycles, range 2–12), diabetes, obesity, and pre-existing liver injury (moderate steatosis, steatohepatitis, and/or fibrosis) were not significant risk factors. FLR/TLV≤20% and FLR/BW≤0.4 were significantly associated with postoperative hepatic dysfunction.

Discussion

The volume of the remnant liver or graft, which is measured preoperatively with three-dimensional CT, is critical for successful hepatic resection and LDLT. In adults without chronic liver disease, liver volume correlates linearly with body size.[11] Thus, the volume of the remnant liver after resection or graft to be transplanted can be normalized to patient BSA or BW. In LDLT, the graft volume is normalized to patient BW, which yields the GRWR. In hepatic resection, the FLR volume is standardized to TLV, which is calculated with a formula based on BSA. These measurements are used to determine the necessary volume of graft or FLR to minimize the likelihood of postoperative hepatic dysfunction.[4,9]

In this report, we examined the relationship between FLR standardized to BW versus BSA and the ability of these measurements to predict postoperative hepatic dysfunction in noncirrhotic patients undergoing major hepatectomy after PVE. We focused on postoperative hepatic dysfunction instead of overall postoperative morbidity as an endpoint to avoid confounding variables such as patient comorbidities and intraoperative factors. We found excellent correlation between FLR measurements standardized to

Table 2 Postoperative Hepatic Dysfunction Stratified by FLR/TLV and FLR/BW Values

	Number of patients	Postoperative hepatic dysfunction	PPV (%)	NPV (%)	*P* value
FLR/TLV≤20%	10	8	80	86	<0.05
FLR/BW≤0.4	9	7	78	85	<0.05
FLR/TLV≤25% or FLR/BW≤0.5	28	14	50	92	<0.05
FLR/TLV>25% or FLR/BW>0.5	40	2	–	–	–

PPV Positive predictive value, *NPV* negative predictive value

BW and BSA. Even in patients at the extremes of body height and weight, we found good correlation between FLR/TLV and FLR/BW ratios. Using ROC curve analysis, the areas under the curve for FLR/TLV and FLR/BW exceeded 0.8, demonstrating that both methods have high discrimination for predicting postoperative hepatic dysfunction.

Our results support previous studies showing the importance of volumetric analysis as a tool to predict postoperative outcome.[18,22] Previously, we showed that in non-cirrhotic patients, the ratio of FLR to TLV increased 9.2−15.6% after PVE.[19] This increase in FLR/TLV is caused by hypertrophy of the remnant liver and not by a change in TLV, which is calculated in relation to BSA, both pre- and post-PVE. Increased FLR volume is associated with improved hepatic metabolic function, as shown by increased indocyanine green excretion, improved biliary drainage, and faster normalization of postoperative liver function tests.[23–25] In LDLT, a GRWR of ≤0.8 (40% of TLV) results in increased postoperative morbidity and impaired graft survival.[3,9] In adult patients without underlying liver disease, a FLR/TLV ratio of ≤20% is associated with increased complications after major hepatectomy.[4,26] In LDLT, a higher liver graft volume is required because of ischemia-reperfusion injury to the graft and episodes of organ rejection.

We determined cutoff values of FLR/TLV≤20% and FLR/BW≤0.4 to predict the likelihood of postoperative hepatic dysfunction. In a recently published study by Truant et al.,[27] increased 30-day mortality and morbidity were associated with FLR/BW≤0.5, which corresponds to FLR/TLV of 25%. They also found FLR/BW to be more specific than FLR/TLV in predicting postoperative outcome. However, their calculation of TLV was based on the traditional method, in which TLV, liver to be resected, and tumor volume are measured separately with CT. This method is prone to multiplicative errors in patients with multiple tumors and cannot be used in patients with dilated bile ducts.[24,28,29] In our study, we estimated TLV using a formula based on the BSA, which has been validated in a meta-analysis as a precise and unbiased approach to estimating TLV.[14]

When the incidence of postoperative hepatic dysfunction was stratified according to FLR volumes, FLR/TLV and FLR/BW values of ≤20% and ≤0.4 had the highest PPV of 80 and 78%, respectively. Patients with measurements near these cutoff values, specifically FLR/TLV between 20–25% or FLR/BW between 0.4–0.5, were also prone to developing postoperative hepatic dysfunction. These patients may have had other risk factors for impaired liver regeneration, such as diabetes mellitus, obesity, hepatic steatosis, and chemotherapy-induced hepatotoxicity. We did not, however, find a significant association between such risk factors and postoperative hepatic dysfunction, but the number of patients was small. Thirty-four patients received preoperative chemotherapy for a median of four cycles, and none received >12 cycles, which has been associated with worse postoperative outcome.[30] In a previous report, we found that the presence of mild to moderate liver injury did not impair liver regeneration after PVE; similarly, response to PVE was comparable with or without neoadjuvant chemotherapy.[19] Until more data are available, patients with FLR/TLV between 20 and 30% and risk factors for liver injury, such as diabetes, obesity, and prolonged administration of chemotherapy, should be considered for preoperative PVE.[31]

A limitation of this study includes the restriction of the patient population to adults without underlying chronic liver disease. Although liver size bears a linear relationship to body size in patients with normal livers, this is not true with

Table 3 Risk Factors for Liver Injury

Risk factor	Number of patients	Number with liver injury	Number with postoperative hepatic dysfunction	Univariate analysis
Preoperative chemotherapy	34	5/34 (15%)	9/34 (26%)	*P*=NS
Diabetes	13	2/13 (15%)	2/13 (15%)	*P*=NS
BMI≥30	18	1/18 (5%)	5/18 (28%)	*P*=NS
Pre-existing liver injury	6	–	1/6 (17%)	*P*=NS
FLR/TLV≤20%	10	0	8/10 (80%)	*P*<0.05
FLR/BW≤0.4	9	0	7/9 (78%)	*P*<0.05

chronic liver disease, particularly in patients with shrunken, cirrhotic livers. Moreover, the estimation of TLV based on BW and BSA does not apply to children because the correlation between liver volume and body size is not constant during the growth period.[8]

In conclusion, we found excellent correlation between FLR standardized to BW versus BSA, with both methods similarly predictive of postoperative hepatic dysfunction. In noncirrhotic patients, a FLR/BW ratio of ≤0.4 and FLR/TLV of ≤20% provide equivalent thresholds for performing safe hepatic resection, confirming the importance of volumetric analysis in determining postoperative outcome after LDLT or major hepatectomy. Systematic measurement of the FLR and normalization to BW or BSA is an integral part of preoperative planning in patients with anticipated small remnant livers who will benefit from PVE.

References

1. Choti MA, Sitzmann JV, Tiburi MF, Sumetchotimetha W, Rangsin R, Schulick RD, Lillemoe KD, Yeo CJ, Cameron JL. Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann Surg 2002;235:759–766.
2. Todo S, Furukawa H. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. Ann Surg 2004;240:451–459 (discussion 459–461).
3. Kiuchi T, Kasahara M, Uryuhara K, Inomata Y, Uemoto S, Asonuma K, Egawa H, Fujita S, Hayashi M, Tanaka K. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. Transplantation 1999;67:321–327.
4. Abdalla EK, Barnett CC, Doherty D, Curley SA, Vauthey JN. Extended hepatectomy in patients with hepatobiliary malignancies with and without preoperative portal vein embolization. Arch Surg 2002;137:675–680 (discussion 680–681).
5. Makuuchi M, Thai BL, Takayasu K, Takayama T, Kosuge T, Gunven P, Yamazaki S, Hasegawa H, Ozaki H. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. Surgery 1990;107:521–527.
6. Heymsfield SB, Fulenwider T, Nordlinger B, Barlow R, Sones P, Kutner M. Accurate measurement of liver, kidney, and spleen volume and mass by computerized axial tomography. Ann Intern Med 1979;90:185–187.
7. Henderson JM, Heymsfield SB, Horowitz J, Kutner MH. Measurement of liver and spleen volume by computed tomography. Assessment of reproducibility and changes found following a selective distal splenorenal shunt. Radiology 1981;141:525–527.
8. Urata K, Kawasaki S, Matsunami H, Hashikura Y, Ikegami T, Ishizone S, Momose Y, Komiyama A, Makuuchi M. Calculation of child and adult standard liver volume for liver transplantation. Hepatology 1995;21:1317–1321.
9. Lo CM, Fan ST, Liu CL, Chan JK, Lam BK, Lau GK, Wei WI, Wong J. Minimum graft size for successful living donor liver transplantation. Transplantation 1999;68:1112–1116.
10. Broering DC, Hillert C, Krupski G, Fischer L, Mueller L, Achilles EG, Schulte am Esch J, Rogiers X. Portal vein embolization vs. portal vein ligation for induction of hypertrophy of the future liver remnant. J Gastrointest Surg 2002;6:905–913 (discussion 913).
11. Vauthey JN, Abdalla EK, Doherty DA, Gertsch P, Fenstermacher MJ, Loyer EM, Lerut J, Materne R, Wang X, Encarnacion A, Herron D, Mathey C, Ferrari G, Charnsangavej C, Do KA, Denys A. Body surface area and body weight predict total liver volume in Western adults. Liver Transpl 2002;8:233–240.
12. DeLand FH, North WA. Relationship between liver size and body size. Radiology 1968;91:1195–1198.
13. Madoff DC, Abdalla EK, Vauthey JN. Portal vein embolization in preparation for major hepatic resection: evolution of a new standard of care. J Vasc Interv Radiol 2005;16:779–790.
14. Johnson TN, Tucker GT, Tanner MS, Rostami–Hodjegan A. Changes in liver volume from birth to adulthood: a meta-analysis. Liver Transpl 2005;11:1481–1493.
15. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med 1987;317:1098.
16. Vauthey JN, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, Xiong HQ, Eng C, Lauwers GY, Mino-Kenudson M, Risio M, Muratore A, Capussotti L, Curley SA, Abdalla EK. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. J Clin Oncol 2006;24:2065–2072.
17. Little SA, Jarnagin WR, DeMatteo RP, Blumgart LH, Fong Y. Diabetes is associated with increased perioperative mortality but equivalent long-term outcome after hepatic resection for colorectal cancer. J Gastrointest Surg 2002;6:88–94.
18. Shoup M, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH, Tuorto S, Blumgart LH, Fong Y. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. J Gastrointest Surg 2003;7:325–330.
19. Ribero D, Abdalla EK, Madoff DC, Donadon M, Loyer EM, Vauthey JN. Portal vein embolization before major hepatectomy and its effects on regeneration, resectability and outcome. Br J Surg 2007 (in press).
20. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115:928–935.
21. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577.
22. Shirabe K, Shimada M, Gion T, Hasegawa H, Takenaka K, Utsunomiya T, Sugimachi K. Postoperative liver failure after major hepatic resection for hepatocellular carcinoma in the modern era with special reference to remnant liver volume. J Am Coll Surg 1999;188:304–309.
23. Ijichi M, Makuuchi M, Imamura H, Takayama T. Portal embolization relieves persistent jaundice after complete biliary drainage. Surgery 2001;130:116–118.
24. Vauthey JN, Chaoui A, Do KA, Bilimoria MM, Fenstermacher MJ, Charnsangavej C, Hicks M, Alsfasser G, Lauwers G, Hawkins IF, Caridi J. Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations. Surgery 2000;127:512–519.
25. Uesaka K, Nimura Y, Nagino M. Changes in hepatic lobar function after right portal vein embolization. An appraisal by biliary indocyanine green excretion. Ann Surg 1996;223:77–83.
26. Vauthey JN, Pawlik TM, Abdalla EK, Arens JF, Nemr RA, Wei SH, Kennamer DL, Ellis LM, Curley SA. Is extended hepatectomy for hepatobiliary malignancy justified? Ann Surg 2004;239:722–730 (discussion 730–732).
27. Truant S, Oberlin O, Sergent G, Lebuffe G, Gambiez L, Ernst O, Pruvot FR. Remnant liver volume to body weight ratio > or =0.5%: A new cut-off to estimate postoperative risks after extended resection in noncirrhotic liver. J Am Coll Surg 2007;204:22–33.
28. Yigitler C, Farges O, Kianmanesh R, Regimbeau JM, Abdalla EK, Belghiti J. The small remnant liver after major liver resection: how common and how relevant? Liver Transpl 2003;9:S18–S25.
29. Kubota K, Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, Harihara Y, Takayama T. Measurement of liver

volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. Hepatology 1997;26:1176–1181.
30. Aloia T, Sebagh M, Plasse M, Karam V, Levi F, Giacchetti S, Azoulay D, Bismuth H, Castaing D, Adam R. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. J Clin Oncol 2006;24:4983–4990.
31. Zorzi D, Laurent A, Pawlik TM, Lauwers GY, Vauthey JN, Abdalla EK. Chemotherapy-associated hepatotoxicity and surgery for colorectal liver metastases. Br J Surg 2007;94: 274–286.

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Treatment of Unresectable Cholangiocarcinoma with Gemcitabine-Based Transcatheter Arterial Chemoembolization (TACE): A Single-Institution Experience

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Abstract

Background Survival for patients with unresectable cholangiocarcinoma is reported to range from only 5–8 months without treatment. Systemic chemotherapy has not been shown to significantly improve survival, but newer regimens involving gemcitabine have shown increased response rates. Transcatheter arterial chemoembolization (TACE) has been shown to prolong survival in hepatocellular carcinoma patients, but experience using TACE in the treatment of cholangiocarcinoma is limited. We report our experience treating cholangiocarcinoma with TACE using chemotherapeutic regimens based on the well-tolerated drug gemcitabine.

Methods Forty-two patients with unresectable cholangiocarcinoma were treated with one or more cycles of gemcitabine-based TACE at our institution. Chemotherapy regimens used for TACE included: gemcitabine only (n=18), gemcitabine followed by cisplatin (n=2), gemcitabine followed by oxaliplatin (n=4), gemcitabine and cisplatin in combination (n=14), and gemcitabine and cisplatin followed by oxaliplatin (n=4).

Results Patients were 59 years of age (range 36–86) and received a median of 3.5 TACE treatments (range 1–16). Thirty-seven patients (88%) had central cholangiocarcinoma, and five (12%) had peripheral tumors. Nineteen patients (45%) had extrahepatic disease. Grade 3 adverse events (AEs) after TACE treatments were seen in five patients, whereas grade 4 AEs occurred in two patients. No patients died within 30 days of TACE. Median survival from time of first treatment was 9.1 months overall. Results did not vary by patient age, sex, size of largest initial tumor, or by the presence of extra-hepatic disease. Treatment with gemcitabine–cisplatin combination TACE resulted in significantly longer survival (13.8 months) compared to TACE with gemcitabine alone (6.3 months).

Conclusions Our report represents the largest series to date regarding hepatic-artery-directed therapy for unresectable cholangiocarcinoma and provides evidence in favor of TACE as a promising treatment modality in unresectable cholangiocarcinoma. Our results suggest that gemcitabine-based TACE is well tolerated and confers better survival when given in combination therapy (with cisplatin or oxaliplatin) for patients with unresectable cholangiocarcinoma.

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Introduction

Cholangiocarcinoma (CC) is a primary adenocarcinoma of the bile ducts. It is the second most common primary hepatic malignancy in the world, but is a rare neoplasm in the USA, with an incidence of only 0.5–2 per 100,000 population.[1] In other parts of the world, however, particularly in East and Southeast Asia, rates are much higher, and overall global incidence is increasing.[2–4]

Cholangiocarcinoma historically has an extremely poor prognosis. Patients who present symptomatically usually have advanced disease which is not amenable to surgical resection. Mortality results from progressive liver failure or biliary obstruction and resulting cholangitis and sepsis.[5]

Survival for patients with unresectable cholangiocarcinoma is reported to be only 5–8 months. Intrahepatic CC generally presents in a more advanced state than extrahepatic CC, so prognosis is often even worse.[6] Curative treatment is only possible with surgical resection, but less than 30% of patients are resectable at presentation.[7] Of those resected, 5-year survival is reported as only 20–40%.[8] Overall survival for all patients at 5 years from diagnosis is less than 5%.[9]

Systemic chemotherapy has been disappointing in regard to its efficacy, with most regimens resulting in a median survival of 6–12 months.[10] No randomized studies have shown a significant improvement in overall survival compared to observation alone.[11] Side effects are common and often limit patients' tolerance of the therapy and their quality of life. Because of the poor results with standard systemic therapy, there has been a great interest in other modalities for treatment of cholangiocarcinoma. These have included chemoradiation,[12] external beam radiation,[13] radiofrequency ablation,[14] photodynamic therapy (PDT),[15,16] brachytherapy,[17] hepatic artery infusion chemotherapy,[18] and transcatheter arterial chemoembolization.[6]

Transcatheter arterial chemoembolization (TACE) is a promising, minimally invasive treatment modality for unresectable liver tumors. TACE allows delivery of high doses of chemotherapeutic drug directly to the tumor with very little systemic drug exposure.

TACE has been shown to be a useful treatment modality in hepatocellular carcinoma (HCC), with two randomized trials showing an improved survival over supportive care alone.[19,20]

Experience with TACE for biliary malignancies is limited, but recently, several small series have shown promising survival in CC patients treated with TACE.[6,21,22] In addition, there have been numerous reports of intra-arterial delivery of chemotherapy drugs, in varied forms, in an attempt to maximize therapy to CC while limiting systemic side effects.[18,23–25]

Recently, patients receiving systemic chemotherapy for cholangiocarcinoma have benefited from the approval of gemcitabine, a nucleoside analogue drug which has shown promising response rates (up to 30–40%) in systemic treatment of CC.[26] Gemcitabine has become the most commonly recommended first-line drug for systemic treatment of CC. It is well tolerated, and combination therapy with drugs such as 5-FU, cisplatin, and oxaliplatin offers potential synergistic tumoricidal effects.[11] Because gemcitabine is rapidly deaminated to the inactive metabolite 2′2′ difluorodeoxyuridine (dFdU), it has an exceedingly high total body clearance (CL_{tb}). The liver serves as a primary source for deamination on first pass.[27] This should allow high intrahepatic gemcitabine concentrations to be achieved with little systemic exposure. Based on its promising antitumor activity and favorable pharmacokinetic and safety profiles, we selected gemcitabine as the basis for liver-directed TACE treatments in cholangiocarcinoma.

At the University of Pittsburgh Medical Center (UPMC) Liver Cancer Center, a significant experience with CC exists.[28] Multidisciplinary therapeutic options include: surgery, radiofrequency ablation, hepatic artery infusional chemotherapy, systemic chemotherapy, and TACE. In this report, we describe our experience treating unresectable cholangiocarcinoma with TACE using regimens based on the well-tolerated drug gemcitabine.

Methods

Study Design

We performed a retrospective review of medical records for all patients treated with gemcitabine-based TACE at the UPMC Liver Cancer Center between June 2001 and February 2007. Data for the current study were extracted from an institutional database designed specifically to track outcomes of liver cancer patients. All data evaluation was done under a protocol approved by the University of Pittsburgh Institutional Review Board.

Once qualifying patients were identified, all patient data were de-identified through the use of an approved Honest Broker. Data variables included demographic, pre-procedure, procedure, and post-procedure data as well as long-term follow-up of all patients. Data were retrospectively gathered from patient charts, electronic medical records, and review of available imaging and pathology. In addition, the Social Security Death Index (SSDI) was queried to determine date of death for any patients not captured by our records. Morbidity was graded on a 1–5 scale according to criteria established by the National Cancer Institute's Cancer Therapy Evaluation Program.[29]

Patients

During the study period (June 2001 to February 2007), 42 patients, all with unresectable intrahepatic cholangiocarcinoma, were treated with one or more cycles of gemcitabine-based TACE.

Initial Evaluation

Initial evaluation consisted of a complete history and physical at the UPMC Liver Cancer Center. All patients had biopsy-proven cholangiocarcinoma. Staging workup included a triphasic chest/abdomen/pelvis computed tomography (CT) scan or magnetic resonance imaging. Patients who were deemed to be unresectable but had acceptable laboratory values and performance status were considered for TACE. Contraindications to TACE included total bilirubin >3 mg/dl, serum creatinine >2× baseline, and ECOG performance status >1. Patients with biliary obstruction were treated with ERCP-based or percutaneous transhepatic biliary stents before administration of TACE.

TACE Procedures

All TACE procedures were performed by interventional radiologists at the University of Pittsburgh Medical Center using standard protocols. The day of the procedure, patients had screening laboratory studies and received intravenous fluid before treatment. Based on the results of the laboratory studies and recent imaging, a decision was made about the segment(s) of the liver to receive TACE and the drugs to be given.

After intravenous hydration was given for 2–3 h, patients were taken to the interventional radiology angiographic suite. Cannulation of the femoral artery was performed, and an arterial catheter was advanced under fluoroscopy to catheterize the celiac and superior mesenteric arteries. At the initial treatment session, complete diagnostic arteriography was performed to determine the hepatic arterial anatomy and to map all arterial vessels supplying the tumor. Therapeutic chemoinfusion was then performed, usually into the right or left hepatic artery, over a 30- to 45-min time period. If treatment included two drugs, each was given separately for a total infusion time of 60–90 min. After delivery of the drug(s) to the tumor, embolization was accomplished with Embospheres (Biosphere Medical, Inc., Rockland, MA, USA) until moderate to marked stasis of antegrade flow was seen in the artery.

Chemotherapeutic drug choice was made based on the patient's previous therapy history, laboratory profile, and functional status. Generally, patients were given one drug or combination of drugs until signs that the patient's tumor was progressing. At that point, if deemed clinically suitable, the patient's regimen was altered to add a drug or change drugs. In the case of bilateral liver tumors, therapy was given to the side of the dominant tumor(s) until progression occurred on the contralateral side. At this point, the contralateral side was treated. Intra-arterial gemcitabine treatments were conducted with dose escalation starting from 1,250 mg/m^2 up to 2,250 mg/m^2 as tolerated. Intra-arterial cisplatin and oxaliplatin doses were 100–125 mg/m^2 and 85–100 mg/m^2, respectively.

Patients were observed overnight after the procedure for control of pain and nausea, administration of intravenous fluids, and monitoring of systemic side effects. Patients were routinely discharged home the following morning after laboratory studies were evaluated. All patients were prescribed anti-emetic and pain medications for home management of post-embolization syndrome (low-grade fever, pain, nausea).

Follow-up

After discharge, laboratory values were checked weekly on an outpatient basis. Follow-up imaging was performed on all patients 6–8 weeks after each TACE procedure. Decisions regarding further TACE therapy were made based on the patients' tolerance of previous TACE as well as their tumor response to therapy.

Statistical Analysis

Overall survival was determined for all patients from the date of first TACE procedure to death. The Kaplan–Meier (product-limit) method was used to determine estimates of survival. The log-rank test was performed to assess overall survival and test differences in survival among TACE regimens. Survival plots and statistical analyses were performed using GraphPad Prism 5.0 software for Windows (GraphPad Software, Inc., San Diego, CA, USA). Statistical significance was assigned for a p value of ≤ 0.05.

Results

Patient and Tumor Details

During the study time period from June 2001 to February 2007, 42 patients underwent TACE with gemcitabine-based chemotherapy regimens for unresectable cholangiocarcinoma. The study included 21 men and 21 women. Patients' median age was 59 years (range 36–87). All patients had mass-forming intrahepatic cholangiocarcinoma proven by biopsy. All patients' ECOG performance status was 0 or 1. Thirty-seven patients (88%) had central cholangiocarcinoma, and five (12%) had peripheral tumors.

Median size of the largest single tumor nodule at presentation was 9.8 cm (range 1.3–17.0 cm). The median Ca 19-9 level for the group was 134 U/ml (<4–140,460). Nineteen of 42 patients (45%) had extrahepatic disease at time of presentation. Of these, four had disease confined to the porta hepatis lymph nodes, eight had intra-abdominal disease, and seven had distant metastasis (Table 1).

TACE Treatments

Forty-two patients underwent 199 TACE procedures, with a median of 3.5 TACE treatments per patient (range 1–16). Patients received one of five different chemotherapy-TACE regimens: gemcitabine alone, gemcitabine followed by cisplatin, gemcitabine followed by oxaliplatin, gemcitabine–cisplatin combination, and gemcitabine-cisplatin followed by oxaliplatin (Table 2).

Morbidity

TACE treatments were generally well tolerated. All patients were admitted overnight for observation after TACE. Ninety-six percent of patient admissions resulted in discharge on post-TACE day no. 1 (23-h admission), with the remaining 4% going home on post-TACE day no. 2.

Grade 4 adverse events (AEs) were seen in two patients. One patient developed crescendo angina 1 day after his first TACE and was diagnosed with an acute myocardial infarction. He underwent cardiac catheterization with angioplasty and coronary stent placement and made a full recovery. The patient went on to have nine further TACE cycles over the next 18 months. A second patient developed a hepatic abscess 1 week after his second TACE treatment. This patient required admission for drainage of the abscess and intravenous antibiotics. The patient developed grade 4 thrombocytopenia and sepsis associated with the abscess. This adverse event resulted in a hospitalization for 7 days with resolution of the sepsis, and the patient was discharged home with a percutaneous hepatic drain in place.

Five additional patients developed grade 3 AEs after TACE. One patient was over-sedated with narcotic pain medication and developed mild respiratory distress on the evening after TACE. When the patient's mental status changes were reported, naloxone was administered with immediate resolution. Two patients developed grade 3 hyperbilirubinemia, and two developed grade 3 thrombocytopenia.

Minor morbidity was seen in nine patients who developed grade 2 adverse events and seven who developed grade 1 AEs (Table 3). In addition, most patients did experience "post embolization syndrome" consisting of low-grade fever, nausea, and abdominal pain. These symptoms were easily managed with antiemetics and narcotic medications. As evidenced by the high discharge rate on post-TACE day no. 1, these symptoms were generally not prolonged.

Morbidity did not vary significantly by patient age, TACE regimen, or by the presence of extrahepatic disease.

Tumor Response to TACE

Tumor response to TACE was gauged using the response evaluation criteria in solid tumors (RECIST) guidelines.[30] Attempts were made to gauge response after three cycles of TACE if possible. Those receiving fewer TACE treatments were evaluated after the last TACE performed. Based on this evaluation, 20 patients were found to have stable disease, 15 patients had progressive disease, and seven were not evaluable.

Table 1 Patient and Tumor Characteristics

Variable	Value (range)	Percentage
Total number of patients	42	100
Sex		
Male	21	50
Female	21	50
Tumor location		
Central	37	88
Peripheral	5	12
Median age (range)	58.8 years (36.2–86.8)	
Median tumor Size (range)	9.8 cm (1.3–17.0)	
Median Ca 19-9 level	134 U/ml (<4–140,460)	
Extrahepatic disease	19	45.2
Porta hepatis lymphadenopathy	4	
Other intra-abdominal mets	8	
Distant mets	7	
All regimens	42	100

Table 2 TACE Regimens Used for Treatment of Unresectable Cholangiocarcinoma

TACE Regimen	*N*	Percentage	Age	Male	Central	EHD	# TACE
Gem alone	18	42.9	64.0	11	18	7	2.0
Gem then Cis	2	4.8	67.8	2	1	0	7.5
Gem then Ox	4	9.5	57.4	1	3	2	7.0
Gem/Cis combo	14	33.3	56.3	6	12	9	5.0
Gem/Cis then Ox	4	9.5	51.7	1	3	1	7.0
All regimens	42	100	58.8	21	37	19	3.5

Gem Gemcitabine, *Cis* cisplatin, *Ox* oxaliplatin, *EHD* extrahepatic disease

Survival

Median overall survival from the date of first TACE treatment was 9.1 months (Fig. 1). Survival did not vary by patient age, sex, size of largest initial tumor, or by presence of extrahepatic disease. Patients with peripheral cholangiocarcinoma, however, had better median survival (18.7 vs 8.2 months) than those with central tumors (p=0.012 by log-rank test).

Survival did vary by response to TACE as measured by RECIST criteria. Patients who had stable disease by RECIST criteria underwent a median of six TACE cycles and had median survival of 13.1 months post-TACE, whereas patients who had progressive disease were able to have three TACE cycles and had post-TACE median survival of 6.9 months (p=0.017).

Survival varied significantly by TACE regimen (Table 4). When comparing the survival curves for the two most frequently used TACE regimens, there was a statistically significant increase in survival for patients receiving TACE treatments with the gemcitabine-cisplatin combination over those receiving gemcitabine alone (13.8 vs 6.3 months, p=0.0005; Fig. 2).

Discussion

Cholangiocarcinoma is a deadly disease with a very poor prognosis. Most patients who are diagnosed with CC are not candidates for surgical resection, which is the only treatment with potential for cure. Currently, therapeutic options for unresectable cholangiocarcinoma afford little or no improvement in survival over supportive therapy alone. Thus, there is a strong incentive to explore newer therapeutic options in an attempt to improve the outcome in this difficult disease. In this paper, we present promising results for treatment of unresectable cholangiocarcinoma using gemcitabine-based TACE.

Treatment of cholangiocarcinoma with chemotherapy or chemoradiation has proven to have little benefit in improving survival. Most published studies using these modalities for cholangiocarcinoma are small, phase I or phase II single-institution trials with a wide variation in outcomes. The few larger scale studies published have involved multiple tumor types, often including both intrahepatic and extrahepatic CC as well as sometimes pancreatic cancer and hepatocellular cancer.[31] No randomized study has shown a significant benefit of systemic chemotherapy or chemoradiation over supportive care alone.[9]

The University of Pittsburgh Medical Center is a high volume center for the treatment of liver cancer.[28] Approximately 11% of our new liver cancer patients have cholangiocarcinoma. We favor an aggressive surgical approach to CC, with surgical resection as the goal.[32,33] Each patient is seen and discussed by a multidisciplinary team which offers the full range of treatment options from transplant and resection to systemic therapy, regional therapy, or palliative care. We offer special expertise in

Table 3 Minor (Grades 1 or 2) Morbidity Seen with Gemcitabine-Based TACE

Grades 1 and 2 AEs
Elevated total bilirubin
Elevated creatinine
Thrombocytopenia
Hyperglycemia
Hypertension
Pulmonary edema
Pancreatitis

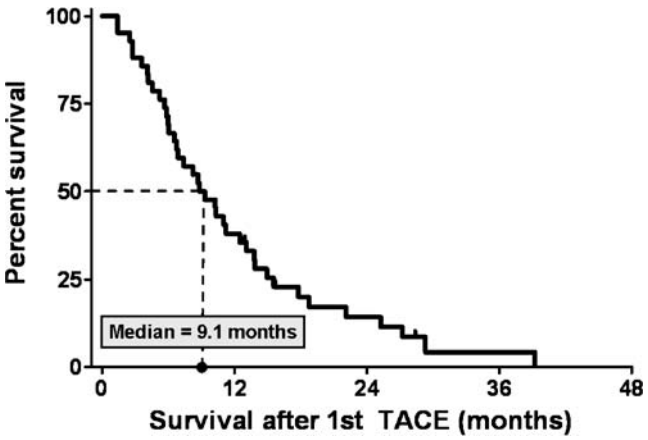

Figure 1 Overall survival for all gemcitabine-based TACE regimens (median survival 9.1 months).

Table 4 Survival by TACE Regimen

TACE regimen	N	# TACE	Alive	Median survival
Gem alone	18	2.0	0	6.3
Gem then Cis	2	7.5	0	18.8
Gem then Ox	4	7.0	1	13.0
Gem/Cis Combo	14	5.0	2	13.8
Gem/Cis then Ox	4	7.0	0	12.5
All regimens	42	3.5	3	9.1 months

Gem Gemcitabine, *Cis* cisplatin, *Ox* oxaliplatin

regional therapy strategies for treatment of liver tumors, performing a high volume of TACE (>400 treatments per year) and selective internal radiation with Yttrium-90 microspheres (>200 treatments to date) treatments in the setting of unresectable disease.

In the case of patients with unresectable disease, our primary goal of therapy is palliation: we aim to prolong survival while maintaining an acceptable quality of life. For these patients, we often choose a regional therapy as an attempt to maximize treatment delivered to the tumor while minimizing systemic side effects.

TACE is a modality widely used in patients with primary liver cancer (hepatoma). TACE is minimally invasive and can be performed on an outpatient or short-stay basis. Combining intra-arterial delivery of chemotherapy via the hepatic artery with embolization takes advantage of the fact that liver tumors receive 90–100% of their blood flow from the hepatic artery.[34] Thus, TACE provides targeted delivery of a chemotherapeutic agent directly to the tumor. Delivery of chemotherapy is followed by arterial embolization, which causes further necrosis of the tumor by eliminating its predominant blood supply. In addition, by reducing blood flow through the tumor, embolization may prevent washout of the chemotherapeutic agent, resulting in higher concentrations of the drug remaining in the tumor cells while also reducing systemic exposure.[35] TACE has been shown in two randomized controlled clinical trials,[19,20] to improve survival in patients with unresectable HCC, and these results have been confirmed in two meta-analyses.[36,37]

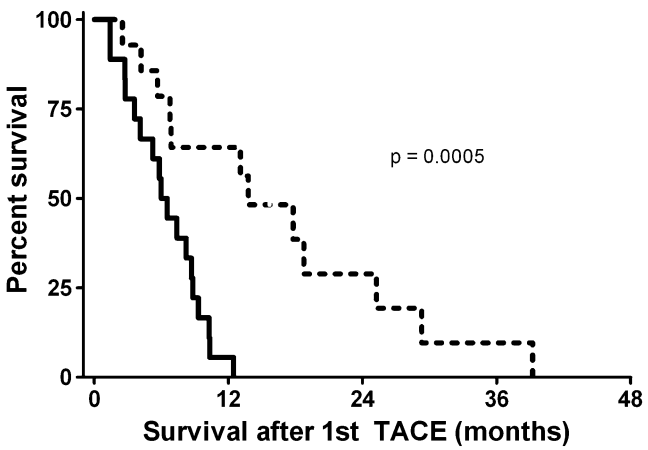

Figure 2 Overall survival for the most commonly used regimens for gemcitabine-based TACE: gemcitabine alone (*solid line*—median survival 6.3 months) vs gemcitabine–cisplatin combination (*dotted line*—median survival 13.8 months).

Our data reveal a median survival of 9.1 months after gemcitabine-based TACE. The survival provided by TACE is significant, especially in light of the fact that many of our patients had already failed other treatment modalities before undergoing TACE. More important, combination regimens utilizing gemcitabine as first-line therapy followed by cisplatin or oxaliplatin or using the gemcitabine–cisplatin combination showed significantly greater response and survival, reaching up to 13–18 months. Although some of the survival difference may be due to selection bias in our retrospective review, our results do imply a benefit from combination chemotherapy regimens.

Of note, median survival in our overall cohort of 42 patients was not significantly different in patients with liver-only disease as compared to those with extrahepatic disease. This supports the belief that in patients with liver-predominant disease, the liver tumors determine eventual survival rather than the extrahepatic disease, and therefore, we favor regional treatment in these patients.

Tumor response to TACE treatments, using RECIST criteria, predicted improved survival (median survival 13.1 months in patients with stable disease vs 6.9 months in patients with progressive disease), but many authors have questioned whether size-based criteria are the best measure of the effectiveness of TACE.[38,39] Although by RECIST criteria TACE treatments did not result in significant tumor shrinkage in any of our patients, several had evidence of tumor necrosis or decrease in tumor vascularity post-TACE (Fig. 3).

Gemcitabine-based TACE treatment proved to be well tolerated, with minimal significant side effects (only 17% of patients had grades 3 or 4 AEs). This tolerability profile fits with the goals of palliative therapy—to prolong life while maintaining quality of life. Although we did not specifically measure quality of life in this study, most patients were home 24 h post-TACE and did not suffer debilitating side effects. The limitations on their lifestyle consisted mainly of a post-embolization syndrome the few days after administration of TACE and the need for several outpatient laboratory studies obtained weekly after treatment. In contrast, systemic combination chemotherapy regimens

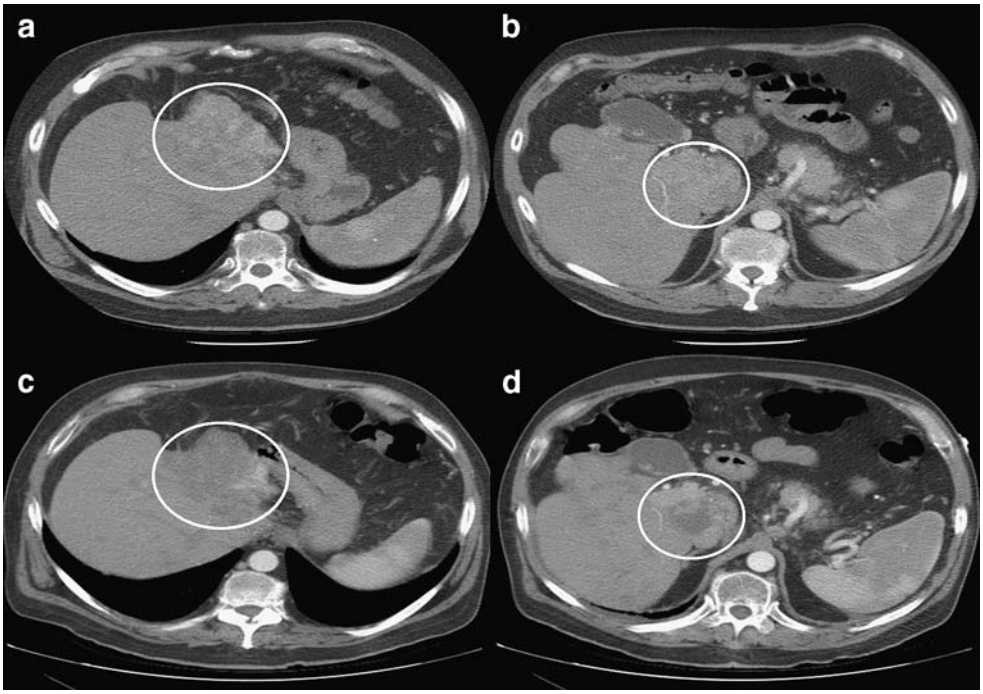

Figure 3 Arterial phase CT images of segment 4 (**a** and **c**) and segment 1 (**b** and **d**) cholangiocarcinoma tumors before (**a** and **b**) and after (**c** and **d**) TACE with gemcitabine/cisplatin combination. Post-TACE images show decreased tumor vascularity (C) and areas of necrosis (D).

using gemcitabine have grades 3–4 toxicities in over 40% of patients in recent phase II trials.[40,41]

In addition to our work, the results of two recent studies lend credence to the concept of intra-arterial treatment for unresectable cholangiocarcinoma. In a report from Burger et al.,[6] TACE was performed in 17 patients with unresectable intrahepatic cholangiocarcinoma. Their group utilized varying TACE chemotherapy regimens, mostly including cisplatin, doxorubicin, and mitomycin-C. Although they report a median survival of 23 months, their survival was measured from the time of diagnosis rather than initiation of TACE treatment, making direct comparison difficult. Also, there may be differences in the patient populations being treated with TACE, as ten of the patients in the Burger study had only stage II disease.

Tanaka et al.,[18] published promising results using a protocol of arterial chemoinfusion through an implanted port system in 11 patients. Their regimens of intra-arterial chemotherapy included various combinations of 5-FU, doxorubicin, cisplatin, and mitomycin-C and resulted in mean survival of 26 months in patients. Again, differences in reporting (mean survival rather than median) and patient characteristics (mean tumor size of 7 cm) make direct comparisons difficult.

As mentioned earlier, we selected gemcitabine as our drug of choice for liver-directed TACE therapy based on its in vivo and in vitro activity against CC, its pharmacokinetics, and its record of safety.[10,11,26,42] With similar considerations in mind, Vogl et al.[43] previously determined the maximum tolerated dose for intra-arterial treatment of cholangiocarcinoma and pancreatic cancer liver metastases with gemcitabine. In their study, 17 patients with intrahepatic cholangiocarcinoma were treated with transcatheter arterial gemcitabine (with or without starch microsphere embolization) in increasing doses from 200 to 2,000 mg/m^2. Chemotherapy infusions were given twice during an 8-day treatment cycle, with cycles repeated every 2 weeks. The maximum tolerated dose found in this study was 1,400 mg/m^2 without microspheres or 1,800 mg/m^2 with microspheres. This dose is similar to that used in our study and confirms that higher doses than those given systemically can be given intrahepatic with an acceptable toxicity profile.

Future investigations will be important to clarify the role of TACE in the treatment of unresectable cholangiocarcinoma. In particular, because of its excellent toxicity profile, TACE may be safely combined with other modalities of treatment to produce complementary or synergistic effects. Several authors have explored combinations of intra-arterial treatments with systemic chemotherapy,[21,24] radiation,[25,44] or biliary drainage[22] in small studies. Combinations of these therapies with gemcitabine-based TACE could potentially result in increased survival with minimal side effects.

In addition, as biliary obstruction is so often the eventual cause of mortality in patients with intrahepatic cholangiocarcinoma, it will be important to consider combinations of TACE with bile-duct-based therapeutic interventions such as PDT or brachytherapy. Brachytherapy or PDT lack adequate depth of tissue penetration to significantly affect large, mass-forming CC tumors, but the combination of these treatments with TACE may be beneficial.

The use of hepatic arterial infusion of Yttrium-90 microspheres for the treatment of inoperable CC is also a potential treatment modality. This technology is already approved by the United States Food and Drug Administration for selective internal radiation treatment in metastatic colorectal cancer (in combination with intra-arterial Floxuridine)[45] and hepatocellular cancer.[46] The treatment of other types of intrahepatic tumors is a current interest, and this treatment may represent a useful option for cholangiocarcinoma in the future.[47–50]

Conclusion

Our report represents the largest series to date describing hepatic-artery-directed therapy for unresectable cholangiocarcinoma. These results provide evidence in favor of gemcitabine-based TACE as a promising mode of therapy for palliation in unresectable cholangiocarcinoma. TACE regimens utilizing gemcitabine, especially in combination with cisplatin or oxaliplatin, may offer equivalent or improved survival when compared to traditional modalities such as systemic chemotherapy or chemoradiation.

We report gemcitabine-based TACE as a useful modality even in patients with minimal extrahepatic disease, offering similar survival outcomes to patients with liver-only disease. Our results demonstrate that gemcitabine-based TACE is well tolerated and suggest that it may offer acceptable quality of life. In a disease with limited treatment options and a dismal prognosis, we believe that this treatment modality warrants further investigation.

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References

1. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. Semin Liver Dis 2004;24(2):115–125.
2. Malhi H, Gores GJ. Cholangiocarcinoma: modern advances in understanding a deadly old disease. J Hepatol 2006;45(6):856–867.
3. Patel T. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. Hepatology 2001;33 (6):1353–1357.
4. Patel T. Worldwide trends in mortality from biliary tract malignancies. BMC Cancer 2002;2:10.
5. Farley DR, Weaver AL, Nagorney DM. "Natural history" of unresected cholangiocarcinoma: patient outcome after noncurative intervention. Mayo Clin Proc 1995;70(5):425–429.
6. Burger I, Hong K, Schulick R, et al. Transcatheter arterial chemoembolization in unresectable cholangiocarcinoma: initial experience in a single institution. J Vasc Interv Radiol 2005;16 (3):353–361.
7. Khan SA, Davidson BR, Goldin R, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. Gut 2002;51(Suppl 6):VI1–9.
8. Jarnagin WR, Shoup M. Surgical management of cholangiocarcinoma. Semin Liver Dis 2004;24(2):189–199.
9. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. Lancet 2005;366(9493):1303–1314.
10. Thongprasert S. The role of chemotherapy in cholangiocarcinoma. Ann Oncol 2005;16(Suppl 2):ii93–96.
11. Mazhar D, Stebbing J, Bower M. Chemotherapy for advanced cholangiocarcinoma: what is standard treatment? Future Oncol 2006;2(4):509–514.
12. Crane CH, Macdonald KO, Vauthey JN, et al. Limitations of conventional doses of chemoradiation for unresectable biliary cancer. Int J Radiat Oncol Biol Phys 2002;53(4):969–974.
13. Zeng Z-C, Tang Z-Y, Fan J, et al. Consideration of the role of radiotherapy for unresectable intrahepatic cholangiocarcinoma: a retrospective analysis of 75 patients. Cancer J 2006;12(2):113–122 (see comment).
14. Slakey DP. Radiofrequency ablation of recurrent cholangiocarcinoma. Am Surgeon 2002;68(4):395–397.
15. Berr F. Photodynamic therapy for cholangiocarcinoma. Semin Liver Dis 2004;24(2):177–187.
16. Wiedmann M, Berr F, Schiefke I, et al. Photodynamic therapy in patients with non-resectable hilar cholangiocarcinoma: 5-year follow-up of a prospective phase II study. Gastrointest Endosc 2004;60(1):68–75.
17. Chen Y, Wang X-L, Yan Z-P, et al. HDR-192Ir intraluminal brachytherapy in treatment of malignant obstructive jaundice. World J Gastroenterol 2004;10(23):3506–3510.
18. Tanaka N, Yamakado K, Nakatsuka A, et al. Arterial chemoinfusion therapy through an implanted port system for patients with unresectable intrahepatic cholangiocarcinoma—initial experience. Eur J Radiol 2002;41(1):42–48.
19. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002;359(9319):1734–1739.
20. Lo C-M, Ngan H, Tso W-K, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002;35(5):1164–1171.
21. Kirchhoff T, Zender L, Merkesdal S, et al. Initial experience from a combination of systemic and regional chemotherapy in the treatment of patients with nonresectable cholangiocellular carcinoma in the liver. World J Gastroenterol 2005;11(8):1091–1095.
22. Qian X-J, Zhai R-Y, Dai D-K, et al. Treatment of malignant biliary obstruction by combined percutaneous transhepatic biliary drainage with local tumor treatment. World J Gastroenterol 2006;12(2):331–335.
23. Melichar B, Cerman J, Jr., Dvorak J, et al. Regional chemotherapy in biliary tract cancers-a single institution experience. Hepato-Gastroenterology 2002;49(46):900–906.
24. Cantore M, Mambrini A, Fiorentini G, et al. Phase II study of hepatic intraarterial epirubicin and cisplatin, with systemic 5-fluorouracil in patients with unresectable biliary tract tumors. Cancer 2005;103(7):1402–1407.
25. Ben-Josef E, Normolle D, Ensminger WD, et al. Phase II trial of high-dose conformal radiation therapy with concurrent hepatic artery floxuridine for unresectable intrahepatic malignancies. J Clin Oncol 2005;23(34):8739–8747.
26. Dingle BH, Rumble RB, Brouwers MC, Cancer Care Ontario's Program in Evidence-Based Care's Gastrointestinal Cancer Disease Site G. The role of gemcitabine in the treatment of cholangiocarcinoma and gallbladder cancer: a systematic review. Can J Gastroenterol 2005;19(12):711–716.

27. Dedrick RL, Forrester DD, Ho DH. In vitro-in vivo correlation of drug metabolism—deamination of 1-β-D-arabinofuranosylcytosine. Biochem Pharmacol 1972;21(1):1–16.
28. Geller DA, Tsung A, Marsh JW, et al. Outcome of 1000 liver cancer patients evaluated at the UPMC Liver Cancer Center. J Gastrointest Surg 2006;10(1):63–68.
29. NCI-CTEP. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Available Online at http://ctep.cancer.gov. 2003.
30. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92(3):205–216.
31. Leonard GD, O'Reilly EM. Biliary tract cancers: current concepts and controversies. Exp Opin Pharmacother 2005;6(2):211–223.
32. Iwatsuki S, Todo S, Marsh JW, et al. Treatment of hilar cholangiocarcinoma (Klatskin tumors) with hepatic resection or transplantation. J Am Coll Surg 1998;187(4):358–364.
33. Casavilla FA, Marsh JW, Iwatsuki S, et al. Hepatic resection and transplantation for peripheral cholangiocarcinoma. J Am Coll Surg 1997;185(5):429–436.
34. Lau WY, Yu SCH, Lai ECH, Leung TWT. Transarterial chemoembolization for hepatocellular carcinoma. J Am Coll Surg 2006;202(1):155–168.
35. Ramsey DE, Kernagis LY, Soulen MC, Geschwind J-FH. Chemoembolization of hepatocellular carcinoma. J Vasc Interv Radiol 2002;13(9 Pt 2):S211–221.
36. Camma C, Schepis F, Orlando A, et al. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. Radiology 2002;224(1):47–54.
37. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology 2003;37(2):429–442.
38. Kamel IR, Bluemke DA, Ramsey D, et al. Role of diffusion-weighted imaging in estimating tumor necrosis after chemoembolization of hepatocellular carcinoma. AJR Am J Roentgenol 2003;181(3):708–710.
39. Tuma RS. Sometimes size doesn't matter: reevaluating RECIST and tumor response rate endpoints. J Natl Cancer Inst 2006;98 (18):1272–1274.
40. Lee G-W, Kang JH, Kim H-G, et al. Combination chemotherapy with gemcitabine and cisplatin as first-line treatment for immunohistochemically proven cholangiocarcinoma. Am J Clin Oncol 2006;29(2):127–131.
41. Alberts SR, Al-Khatib H, Mahoney MR, et al. Gemcitabine, 5-fluorouracil, and leucovorin in advanced biliary tract and gallbladder carcinoma: a North Central Cancer Treatment Group phase II trial. Cancer 2005;103(1):111–118.
42. Scheithauer W. Review of gemcitabine in biliary tract carcinoma. Semin Oncol 2002;29(6 Suppl 20):40–45.
43. Vogl TJ, Schwarz W, Eichler K, et al. Hepatic intraarterial chemotherapy with gemcitabine in patients with unresectable cholangiocarcinomas and liver metastases of pancreatic cancer: a clinical study on maximum tolerable dose and treatment efficacy. J Cancer Res Clin Oncol 2006;132(11):745–755.
44. Matsumoto S, Kiyosue H, Komatsu E, et al. Radiotherapy combined with transarterial infusion chemotherapy and concurrent infusion of a vasoconstrictor agent for nonresectable advanced hepatic hilar duct carcinoma. Cancer 2004;100 (11):2422–2429.
45. FDA. SIR-Spheres®-P990065. Available Online at http://www.fda.gov/cdrh/pdf/p990065.html. 2002.
46. FDA. H980006-TheraSphere®. Available Online at http://www.fda.gov/cdrh/ode/H980006sum.html. 1999.
47. Garrean S, Joseph Espat N. Yttrium-90 internal radiation therapy for hepatic malignancy. Surg Oncol 2005;14(4):179–193.
48. Lim L, Gibbs P, Yip D, et al. Prospective study of treatment with selective internal radiation therapy spheres in patients with unresectable primary or secondary hepatic malignancies. Intern Med J 2005;35(4):222–227.
49. Salem R, Thurston KG. Radioembolization with yttrium-90 microspheres: a state-of-the-art brachytherapy treatment for primary and secondary liver malignancies: part 3: comprehensive literature review and future direction. J Vasc Interv Radiol 2006;17(10):1571–1593.
50. Jakobs TF, Hoffmann R-T, Poepperl G, et al. Mid-term results in otherwise treatment refractory primary or secondary liver confined tumours treated with selective internal radiation therapy (SIRT) using (90)Yttrium resin-microspheres. Eur Radiol 2007;17 (5):1320–1330.

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The Effects of Physical Quality of Life, Time, and Gender on Change in Symptoms of Anxiety and Depression after Liver Transplantation

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Abstract Previous research demonstrated that physical health-related quality of life (HRQOL) improves after liver transplantation, but improvements in mental HRQOL are less dramatic. The aim of this study was to test the effects of physical HRQOL, time post-transplant, and gender on pre- to post-transplant change in anxiety and depression. Longitudinal HRQOL data were prospectively collected at specific times before and after liver transplantation using the SF-36® Health Survey (SF-36), Center for Epidemiologic Studies Depression Scale (CES-D), and Beck Anxiety Inventory (BAI). Within-subject change scores were computed to represent the longest follow-up interval for each patient. Multiple regression was used to test the effects of baseline score, time post-transplant, gender, and SF-36 physical component summary scores (PCS) on change in BAI and CES-D scores. About 107 patients (74% male, age=54±8 years) were included in the analysis. Time post-transplant ranged 1 to 39 months (mean=9±8). Improvement in symptoms of anxiety and depression was greatest in those patients with the most severe pre-transplant symptoms. Significant improvement in symptoms of depression occurred after liver transplant, but the magnitude of improvement was smaller with time suggesting possible relapse of symptoms. Better post-transplant physical HRQOL was associated with a greater reduction in symptoms of anxiety and depression after liver transplantation. This demonstrates clear improvements in post-transplant mental HRQOL and the significant relationships between physical and mental HRQOL.

Keywords Anxiety · Depression · Prevalence · Quality of life · Liver transplantation

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Introduction

Orthotopic liver transplantation is the treatment of choice for end-stage liver disease of various causes. With continued improvement in graft and patient survival, the importance of characterizing health-related quality of life (HRQOL) after liver transplantation has received increasing emphasis. The measurement of HRQOL can provide a more complete estimate of the overall health of liver transplant candidates and recipients. Previous research has demonstrated that functional performance and physical HRQOL improve after liver transplantation to levels close to those of the general population.[1–6] However, several of these studies, using the Medical Outcomes Study Short Form 36 Health Survey (SF-36), demonstrated only small

improvements in mental HRQOL after transplantation due to the fact that pre- and post-transplant scores averaged close to that of the general population.[1,4–6] Other reports, utilizing more specific survey instruments, have estimated the prevalence of moderate to severe symptoms of depression and anxiety before liver transplant to be as high as 25 and 30%, respectively. [7] Furthermore, there have been suggestions in the literature that the use of corticosteroids, which are an integral part of immunosuppresion regimens, may exacerbate symptoms of anxiety and depression. [8,9] With these substantial prevalence rates and biologic links between steroids and affective symptoms, it is important to characterize the impact of liver transplantation on mental health and also to determine the relationship between mental health measures and physical HRQOL. Moreover, the extent to which change in symptoms of anxiety and depression from pre- to post-transplant, and how this is related to post-transplant physical HRQOL, has not been characterized.

The aims of this study were (1) to estimate the prevalence of symptoms of anxiety and depression in liver transplant candidates and recipients and (2) to test the effects of pre-transplant symptom severity, time post-transplant, post-transplant physical HRQOL, and gender on pre- to post-transplant change in symptoms of anxiety and depression. We hypothesize that, after controlling for the effects of baseline symptom severity, time, and gender, patients who had poorer physical HRQOL would demonstrate less improvement in symptoms of anxiety and depression.

Materials and Methods

Beginning January 2002, liver transplant candidates and recipients have been asked to complete a battery of HRQOL surveys at defined pre- and post-transplant time points. This Institutional Review Board (IRB)-approved protocol involved the administration of a battery of surveys and collecting demographic and clinical data from the Vanderbilt Transplant Center and medical center databases and records. The HRQOL assessment battery included the Medical Outcomes Study Short Form 36 Health Survey (SF-36), the Center for Epidemiologic Studies Depression Scale (CES-D), the Beck Anxiety Inventory (BAI), a patient satisfaction inventory, a Visual Analogue Scale (VAS) measuring overall health, and an employment survey. Karnofsky functional performance status was also reported by transplant coordinators. Data collection occurred at specific time points: at initial evaluation, every 6 months while on the waiting list, and 1, 3, 6 months, and yearly post-transplant. A rolling enrollment system allowed for a patient to participate at any time point regardless of whether they had participated previously.

This study focused on three HRQOL measures: the SF-36, CES-D, and the BAI. The SF-36 was used to assess generic physical and mental HRQOL. This 36-item questionnaire measures eight areas of functioning and well being (role-physical, bodily pain, physical functioning, general health, vitality, social functioning, role-emotional, and mental health). A physical and mental component summary scale [physical (PCS) and mental component summary (MCS)] can then be computed as weighted composites of the eight subscales. The scales are scored from 0 to 100; a higher score indicates a better health state. The PCS and MCS scales are normed to the general population with a mean of 50 and standard deviation of 10. Thus, 68% of the general population would score 40–60 on the PCS and MCS scales.[10]

The CES-D scale is a 20-item self-report scale designed to identify symptoms of depression in both clinical and general populations.[11] The major symptoms of depression are addressed within this survey, and it describes how often the patient experienced these symptoms in the last week. Each question is scored from 0 to 3 with higher scores indicating more symptoms by frequency of occurrence. The CES-D is not intended to be a diagnostic tool but is designed to measure current level of depressive symptoms. The following cut-off scores have been suggested to approximate four severity stages of depressive symptoms (0–9=none, 10–16=mild, 17–24=moderate, and >24=moderate to severe).[11]

The BAI was employed to measure the severity of self-reported anxiety. This instrument consists of 21 items, each describing a common symptom of anxiety. The respondent is asked to rate how much, in the last week, he/she had been bothered by this symptom on a scale of 0 to 3. A score of 0 signifies that the patient was not bothered at all by the symptoms during the last week, whereas a score of 3 denotes that the patient was affected severely by the described symptoms in the last week. The items are summed to obtain a total score that can range from 0 to 63. The following cut-off scores have been suggested to approximate four severity stages of anxiety symptoms (0–7=minimal, 8–15=mild, 16–25=moderate, and >25=severe).[12]

Patients included in this study were liver transplant candidates listed after January 1, 2002 who received liver transplants through May 1, 2006. Each patient completed the HRQOL surveys pre- and postoperatively and had sufficiently complete SF-36 survey data on both measurement occasions to permit computation of the eight scales and two summary components. If pre-transplant HRQOL data had been reported on more than one occasion, the observation closest to the date of transplant was selected for

analysis. In instances where patients had multiple post-transplant HRQOL data, data from their last self-report were used. Within-subject change scores were computed for the four HRQOL measures by subtracting pre-transplant scores from post-transplant scores. A positive change on the SF-36 scales indicated improvement in HRQOL, whereas a negative change for the CES-D and BAI indicated improvement (i.e., reduction) in symptoms of anxiety and depression.

Paired *t* tests were used to evaluate pre- to post-transplant change in all HRQOL measures, and cross-classified categorical data were analyzed using the chi-square test. Multiple linear regression was used to test the effects of pre-transplant symptom severity, time post-transplant, gender, and post-transplant PCS on change in BAI and CES-D scores. Pre-transplant severity scores were included in the models to account for and quantify their expected effects on observed change post-transplant. All analyses were conducted using SPSS, version 15.0 (SPSS, Chicago, IL, USA). Summary data are presented throughout as percentages or means ± standard deviations.

Results

At the time of our investigation, 107 patients (predominantly non-veterans) returned at least one pre- and one post-transplant survey packets over the 43-month data collection period (September 2002 through February 2006). This represented 67% of our non-veteran transplant recipient population over this time period. There were 79 males (74%) and 28 females (26%), and an overwhelming majority were Caucasian (94%). The mean age at transplantation was 54±8 years, and the mean time post-transplant was 9±8 months, with a range from 1–39 months. Indications for liver transplantation included 59% for non-cholestatic cirrhosis (Hepatitis B, C, or alcoholic cirrhosis), 26% for metabolic liver disease, cryptogenic cirrhosis, non-alcoholic steatohepatitis, or autoimmune hepatitis, 13% for cholestatic cirrhosis (primary biliary cirrhosis or primary sclerosing cholangitis), and 2% for other indications (hepatocellular carcinoma and hepatic epitheliod hemangoendothelioma). The mean model of end-stage liver disease (MELD) score at transplant was 23.2±5.3 with a range from 12–40 (Table 1). In this study population, 22% of patients required antidepressant or anxiolytic therapy preoperatively and/or postoperatively. However, this did not represent the exact same set of patients, as some patients started and some discontinued therapy after transplantation. About 72% of patients were never on any therapy, 15% of patients were on therapy before and after liver transplantation, 6.5% of patients discontinued therapy after transplantation, and 6.5% started new therapy after transplantation.

Table 1 Demographic and Pre-transplant Clinical Measures

	Values
Age (years)	53.5±7.8
Gender (male)	74%
Race (Caucasian)	94%
Diagnosis–noncholestatic	59%
Metabolic/crypto/auto/NASH	26%
Cholestatic	13%
Other	2%
IDDM	12%
NIDDM	14%
MELD	23.2±5.3
AST (U/l)	88±83
ALT (U/l)	54±61
Alkaline phosphate (U/l)	166±120
Total bilirubin (mg/dl)	6.2±8.4
Creatinine (mg/dl)	1.3±0.7
INR	1.7±0.6
BMI	27.8±4.5
Albumin (g/dl)	3.1±0.65

Values expressed as mean ± standard deviation and percentages where appropriate.
Crypto Cryptogenic cirrhosis, *auto* autoimmune cirrhosis, *NASH* non-alcoholic steatohepatitis, *IDDM* insulin-dependent diabetes mellitus, *NIDDM* non-insulin-dependent diabetes mellitus, *MELD* Model of End Stage Liver Disease, *INR* international normalized ratio, and *BMI* body mass index (kg/m^2)

Pre-transplant HRQOL

On average, patients scored greater than two standard deviations below the general population on the SF-36 PCS before liver transplantation but were within a standard deviation of the general population on the MCS. In addition, pre-transplant scores averaged greater than a standard deviation below the general population on all of the individual SF-36 scales except for mental health. The greatest impairments were seen in the physical function and general health scales, which averaged greater than two standard deviations below the general population (Table 2). Before transplant, 50 and 62% of these patients had moderate to severe symptoms of anxiety and depression, respectively. The distribution of patients across severity categories for anxiety and depression is displayed in Table 3.

Post-transplant HRQOL

There was significant improvement in all measures of physical and mental HRQOL after liver transplantation (all $p<0.001$; Table 2). Although the PCS improved significantly, the average post-transplant score remained more than a standard deviation below that of the general

Table 2 Pre- and Post-transplant HRQOL Scores

Instruments	General Population	Pre-transplant Score	Post-transplant Score	Change Score	Number of patients	*p* value
SF-36 scales						
1. Physical Function	84±23	35±23	50±27	15	107	<0.001
2. Role Physical	81±34	16±31	34±39	18	104	<0.001
3. Bodily Pain	85±24	43±24	54±27	11	101	<0.001
4. General Health	72±20	22±17	55±20	33	102	<0.001
5. Vitality	61±21	20±18	43±26	23	103	<0.001
6. Social Functioning	83±23	43±25	63±30	20	107	<0.001
7. Role Emotional	81±33	42±44	66±42	24	101	<0.001
8. Mental Health	75±18	59±22	71±22	12	103	<0.001
SF-36 PCS	50±10	27±8	35±11	8	94	<0.001
SF-36 MCS	50±10	40±11	49±12	9	94	<0.001
BAI	0–7	17±10	10±7	−7	83	<0.001
CES-D	0–9	19±10	10±10	−9	75	<0.001

Values are expressed as means ± standard deviation.

population. The MCS improved to more closely approximate that of the general population. All of the SF-36 individual subscales improved enough to approximate scores for the general US population. Furthermore, there were significant improvements in CES-D and BAI scores. After transplantation, there was a statistically significant improvement in the percentage of patients with moderate to severe symptoms of depression and anxiety. Only 20 and 22% of patients had moderate to severe symptoms of anxiety and depression after liver transplantation, which was a statistically significant improvement in symptom prevalence compared to pre-transplant rates (Table 3).

Individual Change Scores

Individual (within-subject) change scores were positive and statistically significant (all $p<0.001$) for all SF-36 scales and summary components after liver transplantation. This positive change indicated improvement in HRQOL on all SF-36 measures (Table 2). Likewise, there was statistically improvement in symptoms of anxiety and depression from pre- to post-transplant. However, due to the way in which the BAI and CES-D are scored (with higher scores indicating greater symptom severity), improvement in these symptoms was represented by negative change score. Thus, the severity of symptoms of anxiety and depression were reduced after liver transplantation (Table 3).

Modeling Predictors of Change Scores

The first multiple linear regression model tested the effects of baseline anxiety severity score, time post-transplant, gender, and post-transplant PCS on change in BAI scores from pre- to post-transplant. Tests of overall model fit and effects due to individual covariates are displayed in Table 4. Statistically significant negative regression coefficients (B and β) reflect a predictor's association with symptom improvement (increasingly negative change scores). Those patients with the most severe pre-transplant symptoms of anxiety showed the greatest improvement after transplantation. Although time post-transplant did not have a significant effect on the reduction in symptoms of anxiety, the majority of patients reported symptom improvement. Thus, symptom improvement occurred early after liver transplantation and was sustained over time. Finally, those patients with higher post-transplant PCS scores (indicating better physical HRQOL) had the greatest improvement in symptoms of anxiety. Gender had no effect on the reduction in symptoms of anxiety. This model was statistically significant (model $p<0.001$, $R^2=0.71$) with 71% of the variance in change in symptoms of anxiety being attributed to the relevant predictors (pre-transplant symptom severity, time post-transplant, gender, and post-transplant PCS).

The second model tested the effects of baseline depression severity score, time post-transplant, gender,

Table 3 Prevalence and Symptom Severity of Anxiety and Depression

	Pre-transplant %	Post-transplant %	*p* value
Depression			0.033
Severe (>24)	25	14	
Moderate (17–24)	35	10	
Mild (10–16)	24	22	
Minimal (0–9)	16	54	
Anxiety			0.027
Severe (>25)	21	5	
Moderate (16–25)	31	16	
Mild (8–15)	33	41	
Minimal (0–7)	15	38	

Table 4 Factors Influencing Change in BAI Scores (Model 1)

Covariates	*B*	Standard Error	β	*t*	*p* value
Baseline BAI score	−0.90	0.07	−0.85	−13.20	<0.001
Time post txp (months)	−0.03	0.19	-0.02	−0.25	0.81
Gender (male)	1.50	1.60	0.06	0.97	0.34
Post-transplant PCS score	−0.23	0.07	−0.24	−3.52	0.001
Intercept	15.73	3.03		5.20	<0.001

Mean change=−9±12 points. Model R^2 =0.71, Model p=<0.001
B Unstandardized regression coefficient; β standardized regression coefficient; *t*=*t* statistic

and post-transplant PCS scores on the change in CES-D scores (Table 5). Like the previous model, those patients with the most severe pre-transplant symptoms improved the most after transplant. While improvement in pre-transplant symptoms of depression was evident throughout the entire post-transplant follow-up period, the statistically significant positive effect of time on change in CES-D scores indicates that the magnitude of symptom improvement was less as time post-transplant increased. This finding suggests that there may be a recurrence of symptoms of depression as patients become further removed from their transplant. Better post-transplant physical quality of life was associated with a greater improvement in symptoms of depression. Gender had no effect on the reduction in symptoms of depression. This model (model $p<0.001$, $R^2=0.53$), explains 53% of the variance in change in CES-D scores.

Discussion

The lifetime prevalence of generalized anxiety disorder and major depressive episodes within the US population has been estimated at 6 and 5.1%, with lifetime prevalence generally higher in women than men.[13,14] However, the prevalence of anxiety and depression in patients undergoing assessment for liver transplant has been estimated to be between 25–30%.[7,15] The prevalence of severe symptoms of anxiety and depression in our cohort is 21 and 25%, which is consistent with previously published estimates for transplant candidates. This increased prevalence in affective symptoms may be in response to their poor overall medical condition or related to the distress of awaiting a liver transplant. Although there is a high prevalence of affective symptoms, it is encouraging that the severity and total percentage of patients experiencing symptoms decreases after liver transplantation. In our cohort, the percentage of patients with severe symptoms of anxiety and depression post-transplant is 5 and 14%, respectively. It is important for clinicians to recognize that a significant percentage of patients undergoing assessment for liver transplant experience these symptoms and that they may go unrecognized. Few studies have examined changes in anxiety and depression after transplantation and the impact that other aspects of post-transplant HRQOL may have on improvement of these symptoms. Our present study prospectively examines the association between physical health-related quality of life, time, and gender differences and the change in symptoms of anxiety and depression after liver transplantation.

There are several important associations that can be gleaned from our prospective analysis of this cohort. Despite the high prevalence of symptoms of anxiety and depression, there is significant improvement in these symptoms after transplantation. A prospective study by O'Carroll et al.[7] had similar findings to ours. In their pre-transplant population of 164 patients, 31% had clinically significant anxiety, while 26% had clinical evidence of depression defined by the Hospital Anxiety and Depression Scale (HADS). After transplantation, only 10 and 2% of their cohort reported clinically significant symptoms of anxiety and depression, respectively. [7] In our analysis, the improvement in affective symptoms is greatest among those

Table 5 Factors Influencing Change in CES-D Scores (Model 2)

Covariates	*B*	Standard Error	β	*t*	*p* value
Baseline CES-D score	−0.73	0.10	-0.63	−7.14	<0.001
Time post txp (months)	0.38	0.15	0.22	2.46	0.02
Gender (male)	2.43	2.27	0.09	1.07	0.29
Post-transplant PCS score	−0.36	0.10	−0.31	−3.50	0.001
Intercept	29.22	4.98		5.86	<0.001

Mean change=-7±11 points. Model R^2 =0.53, Model p=<0.001
B Unstandardized regression coefficient; β standardized regression coefficient; *t*=*t* statistic

patients with the most severe preoperative symptoms. We can be encouraged not only by findings of general improvement in patients' symptoms, but those patients that are significantly affected have the most improvement after transplantation. This is also encouraging in that the corticosteroid anti-rejection therapy appeared not adversely influence reported symptoms of anxiety and depression.

Many of the prior studies characterizing HRQOL before and after liver transplantation have utilized the SF-36. Before liver transplantation, patients' physical health, as measured by the SF-36, is severely affected. In our cohort, the patients' PCS scores are greater than two standard deviations below the physical health of the general population. However, measurement of their mental HRQOL, using the SF-36 MCS, characterizes this population as being similar to that of the general population. Prior studies utilizing this instrument have had similar findings. Whiting et al.[3] also showed across the board improvements in SF-36 scores in 84 liver transplant recipients, with the exception of bodily pain. Like our findings, they showed lower scores on the physical scales as compared to the general population but relatively preserved mental health. Bravata et al.[2] published a meta-analysis of seven liver transplant studies utilizing SF-36 scores, which included 486 patients. This report also described better overall performance on the mental subscales with little change related to transplantation and significant improvement on several physical subscales after transplantation. Once again, the SF-36 mental subscales and the MCS portrayed the mental health of the liver transplant population similar to that of the general population.

Despite having mental health scores on the SF-36 similar to the general population, a significant proportion of our liver transplant candidates had notable symptoms of anxiety and depression as measured by the BAI and CES-D, respectively. There have been a cross-sectional and a prospective study utilizing a more sensitive instrument, the HADS, which also found a significant prevalence of pre-and post-transplant anxiety and depression.[7,15] These findings may suggest that the ability of the SF-36 to characterize affective disturbances is not as sensitive as the individual instruments that we and others have utilized in the liver transplant population.

The temporal course of change in symptoms of anxiety and depression in relation to pre-transplant levels differs. The improvement in symptoms of anxiety is early and sustained with time post-transplant. Improvement in symptoms of depression is also evident in the early post-transplant period. However, the magnitude of the improvement in relation to pre-transplant severity becomes less as time post-transplant increases suggesting that there may be signs of recurrence with time. Future research will investigate this finding in greater detail.

As there is an increased focus on HRQOL after transplantation, it is important to identify relationships between mental and physical HRQOL. A prospective study performed by O'Carroll et al.[7] and a cross-sectional study by Nickel et al.[15] show significant associations between mental HRQOL and physical HRQOL. O'Carroll et al. characterized the psychological status of liver transplant patients before transplant and defined pre-transplantation variables that were predictive of HRQOL outcome at 1 year. In their pre-transplant population of 164 patients, 31% had clinically significant anxiety, while 26% had clinical evidence of depression defined by the HADS. As previously mentioned, they showed marked improvement in their patients after transplant. In their multiple regression analysis, the best predictor of both psychological and physical HRQOL was pre-transplant HADS anxiety level.[7] In the study by Nickel et al.[15], they investigated the extent to which depression, anxiety, and coping affect post-transplant HRQOL in 186 patients. Twenty-three percent of their cohort had pathologically increased HADS scores. Their multiple regression analysis showed that increased symptoms of post-transplant depression had a negative influence on physical and mental HRQOL. Likewise, we demonstrate that physical HRQOL after transplant had a significant effect on improvement in symptoms of anxiety and depression. It is important to recognize that, due to these relationships between mental and physical HRQOL, improvement or deterioration in one realm may substantively affect the other.

Strengths of this study include its prospective design, which allowed us to follow a particular population and characterize significant changes in quality of life and affective status, and the diversity of physical and mental HRQOL measures used. The broad assessment of HRQOL with well-validated instruments in a relatively large cohort makes it more likely that findings will generalize to other liver transplant populations. In addition, the multivariate modeling of the change scores provides us with meaningful predictors of changes in affective status after transplantation. However, due to our design requiring that both pre- and post-transplant data be available for several HRQOL measures, a limitation of this study is that the cohort that met our inclusion criteria represents only two thirds of our total non-veteran population that was transplanted over this time period. With any study relying on self-report of quality of life, there may be responder bias depending on which population chooses to participate in the quality of life evaluations. Although the possibility of this bias cannot be completely eliminated, our cohort had a broad range of scores on all HRQOL instruments before and after transplantation, and scores were consistent with HRQOL studies reported in previous literature.[1,2,7,15]

Conclusion

This present study demonstrates that symptoms of anxiety and depression are present in a substantial number of liver transplant candidates and that these symptoms improve after transplantation. Those patients with the most severe symptoms of anxiety and depression improved the most after transplantation. Those patients with better post-transplant physical HRQOL as defined by their SF-36 PCS scores also had the greatest improvement in symptoms of anxiety and depression after transplant. These findings should make providers aware of the prevalence of anxiety and depression in liver transplant candidates and recipients and of the association between physical HRQOL and affective symptoms. Providers should consider that those patients who are at increased risk for poor post-transplant physical HRQOL for any reason (e.g., prolonged hospital course, rejection episodes, etc.) may have an increased risk of significant symptoms of post-transplant anxiety and depression.

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References

1. Pinson CW, Feurer ID, Payne JL, et al. Health-related quality of life after different types of solid organ transplantation. Ann Surg. 2000;232(4):597–607.
2. Bravata DM, Olkin I, Barnato AE, et al. Health-related quality of life after liver transplantation: a meta-analysis. Liver Transplant Surg. 1999;5(4):318–331.
3. Whiting J, Nabel JPG, et al. Clinical Determinants of Health-Related Quality of Life in Recipients of Solid Organ Transplants. J Surg Outcomes. 1999;2(1):21–26.
4. Bryan S, Ratcliffe J, Neuberger JM, et al. Health-related quality of life following liver transplantation. Qual Life Res. 1998;7(2):115–120.
5. Hellgren A, Berglund B, Gunnarsson U, et al. Health-related quality of life after liver transplantation. Liver Transplant Surg. 1998;4(3):215–221.
6. Hunt CM, Tart JS, Dowdy E, et al. Effect of orthotopic liver transplantation on employment and health status. Liver Transplant Surg. 1996;2(2):148–153.
7. O'Carroll RE, Couston M, Cossar J, et al. Psychological outcome and quality of life following liver transplantation: a prospective, national, single-center study. Liver Transplant. 2003;9(7):712–720.
8. Yehuda R, Boisoneau D, Mason JW, Giller EL. Glucocorticoid receptor number and cortisol excretion in mood, anxiety, and psychotic disorders. Biol Psychiatry. 1993;34(1–2):18–25.
9. MacNaughton KL, Rodrigue JR, Cicale M, Staples EM. Health-related quality of life and symptom frequency before and after lung transplantation. Clin Transplant. 1998;12(4):320–323.
10. Ware J, Kosinski M, Keller S. SF-36 Physical and Mental health Summary Scales: A Users' Manual. Boston: The Health Institute, New England Medical Center; 1994.
11. Radloff L. The CES-D Scale: A self-report depression scale for research in the general population. Appl Psychol Meas. 1977;1:385–401.
12. Beck A, Steer R. Beck anxiety inventory manual. San Antonio: The Psychological Corporation; 1993.
13. Kessler RC, Chiu WT, Demler O, et al. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005;62(6):617–627.
14. Cassem EH. Depressive disorders in the medically ill. An overview. Psychosomatics. 1995;36(2):S2–S10.
15. Nickel R, Wunsch A, Egle UT, et al. The relevance of anxiety, depression, and coping in patients after liver transplantation. Liver Transplant. 2002;8(1):63–71.

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Extrahepatic Portal Vein Aneurysm—Report of Six Patients and Review of the Literature

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Abstract Extrahepatic portal vein aneurysm is a rare condition. We report six patients with extrahepatic portal vein aneurysm, four of whom were surgically treated. In addition, a review of the literature was performed to examine natural history, management, and outcomes regarding portal vein aneurysm. Patients seen at our institution with extrahepatic portal vein aneurysm greater than 1.9 cm in diameter were reviewed (1998 to 2006). There were five females and one male; median age was 66.5 (30–77). Computed tomography (CT) scan was utilized for diagnosis in all cases. The median diameter of the aneurysm was 4.7 cm (2.7–6.0). Indications for surgery included gallstone pancreatitis, mass effect on the adjacent duodenum, a peripancreatic mass, and liver cirrhosis. Three patients underwent aneurysm resection, and one patient had an orthotropic liver transplant. Two patients were managed with observation. The median follow-up from first presentation and surgery was 50 months (9–181) and 5 months (2–73), respectively. At last follow-up, five patients were alive with radiologically proven portal vein patency. One patient died 2 months after liver transplantation. There was no case of aneurysmal rupture. One patient had intramural thrombus at presentation that resolved with conservative treatment. This report suggests that symptomatic aneurysms can be safely resected with excellent patency.

Keywords Portal vein · Aneurysm · Surgery

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Introduction

Extrahepatic portal vein aneurysm is a rare vascular anomaly of the portal venous system. Aneurysmal malformation is defined as a portal vein whose diameter exceeds 1.9 cm.[1] The etiology of such aneurysms is unclear, and it is hypothesized to be either a congenital malformation or a result of conditions such as portal hypertension, pancreatitis, or trauma.[2,3] Since first reported by Barzilai and Kleckner[4] in 1956, only 61 cases have been published in the English literature. These reports recognize rupture, thrombosis, and compression of adjacent organs as possible complications. However, because of the rarity of portal vein aneurysm, most are in the form of case reports, and there is a lack of published case series that specifically address indications for surgery and optimal surgical techniques to treat portal vein aneurysms. Recently, advances in imaging studies and their widespread use have led to an increasing number of extrahepatic portal vein aneurysms being discovered, and there is therefore a need to clarify management strategy. In this study, we present six patients with extrahepatic portal vein aneurysm, four of whom underwent

a surgical procedure. Review of the literature was performed examining the clinical course of patients who were managed with either surgical treatments or observation.

Materials and Methods

Approval for this report was obtained from the Institutional Review Board at the University of Pittsburgh Medical Center. From January 1998 to December 2006, six patients with extrahepatic portal vein diameter greater than 1.9 cm on CT scan were identified, and they form the basis of this retrospective study. Information regarding patient demographics, clinical presentation, associated medical conditions, treatment and follow-up data were extracted from hospital records.

Operative Techniques

The operative procedure chosen depended on the location and the shape of the portal vein aneurysm as well as comorbidities of the patients. A total of four patients underwent surgical procedures and two patients were observed. In one patient, a saccular venous aneurysm located at the main portal vein trunk was mobilized from the surrounding common bile duct and the pancreas, and a vascular stapler was used to perform aneurysmorrhaphy. In another patient, after entering the lesser sac through the gastrocolic omentum, a saccular portal vein aneurysm located at the confluence of the superior mesenteric vein and the splenic vein was dissected free from the surrounding structures. After obtaining proximal and distal vascular control, the saccular portion of the aneurysm was resected, and the remaining vessel wall was closed primarily.

In one patient with fusiform portal vein aneurysm, cholecystectomy and division of the common bile duct were first performed to gain access to the portal vein (Fig. 1). The aneurysm was then completely excised and replaced with an iliac venous interposition allograft. Both proximal and distal ends of the allograft were approximated to the remaining native portal vein in an end-to-end fashion, and the biliary tract was subsequently reconstructed using Roux-en-Y hepaticojejunostomy.

In these three cases, there were minimal adhesions and inflammation around the portal vein aneurysm, and it was dissected out without difficulty. One additional patient underwent orthotropic liver transplantation for end-stage liver disease, which addressed the left portal vein aneurysm at the same setting.

Review of the Literature

Medline search of English language articles was performed to search for cases with extrahepatic portal vein aneurysm.

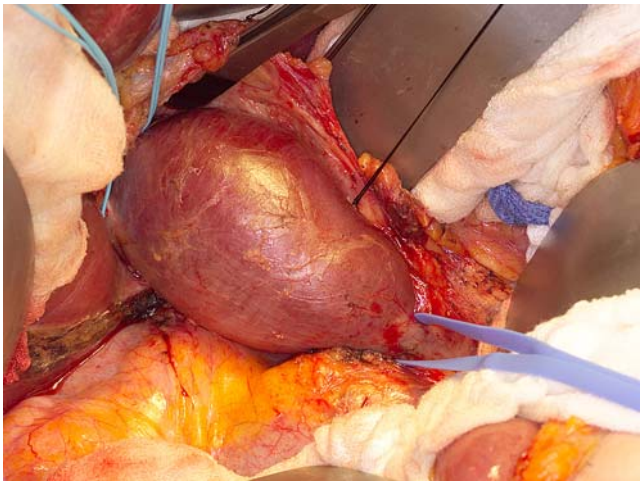

Figure 1 A portal vein aneurysm dissected from surrounding structures.

These inclusion criteria included extrahepatic location of the portal vein aneurysm and the maximum diameter of the aneurysm greater than 1.9 cm.

Results

Patient Characteristics and Presentation

Median age at presentation was 66.5 (range 30 to 77). There were five females and one male (Table 1). Five patients had symptoms of epigastric pain, nausea, and/or vomiting. Three patients had a normal preoperative upper endoscopy. A fourth patient had a stable portal vein aneurysm for 15 years and then developed gallstone pancreatitis. The fifth patient with non-alcoholic steatohepatitis (NASH) and evidence of portal hypertension was diagnosed with a left portal vein aneurysm during work-up for liver transplantation.

One patient had an incidental finding of portal vein aneurysm during a hospital stay for coronary artery bypass surgery and had a history of a right popliteal artery aneurysm resection. No other patients had previous vascular surgeries or additional aneurysms.

Aneurysmal Characteristics

The aneurysm was located at the confluence of the superior mesenteric vein and the splenic vein in three cases, at the main portal vein in two cases, and at the extrahepatic left portal vein in one case. The median diameter was 4.7 cm (range 2.7 to 6.0 cm). One patient had a partially thrombosed saccular portal vein aneurysm on CT scan, which was confirmed on a portal venogram (Fig. 2). In the remaining five patients, there was no intraaneurysmal thrombosis. A portal vein aneurysm caused a mass effect on the adjacent duodenum in one case (Fig. 3).

Table 1 Patient Demographics and Characteristics of the Portal Vein Aneurysm

Patient	Gender	Age at presentation	Presentation	Location of aneurysm	Diameter of aneurysm	Shape of aneurysm	Portal hypertension
1	F	71	Abdominal pain, n&v	MPV	5.0	Saccular	No
2	F	30	Abdom pain, n&v	SV-SMV C	4.4	Saccular	No
3	F	50	Gallstone pancreatitis	SV-SMV C	6.0	Fusiform	No
4	F	62	Liver cirrhosis	Left portal vein	2.7	Fusiform	Yes
5	M	76	Incidental finding	MPV	5.5	Saccular	No
6	F	77	Abdom pain, n&v	SV-SMV C	3.5	Fusiform	No

MPV = main portal vein; SV-SMV C = splenic vein and superior mesenteric vein confluence

Pathological Characteristics of Resected Portal Vein Aneurysms

Four specimens of resected portal vein aneurysms were examined grossly and microscopically with H&E (hematoxylin and eosin) staining. These revealed no pathological changes such as inflammation or necrosis to suggest possible etiology of the aneurysmal formation (Fig. 4). Specifically, one specimen (patient 1) was stained with Trichome, VVG (Verhoeff-Van Gieson) elastic stain, desmin, and CD31, and there was no disturbance of elastic tissue.

Morbidity and Mortality

Three patients who had aneurysmal resection did not require any blood transfusions, and postoperative course was uneventful. The length of stay for these patients was 3, 7, and 11 days. The patient who underwent orthotropic liver transplantation developed multiorgan failure 2 months after the surgery and subsequently died.

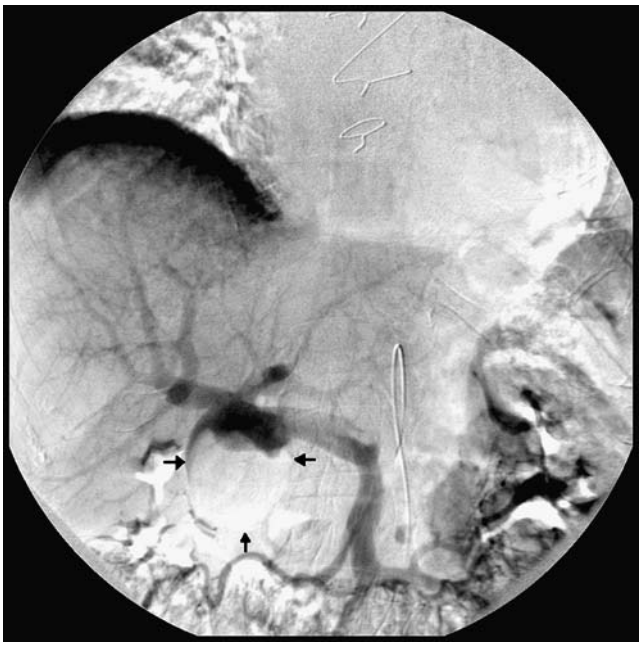

Figure 2 A portal venogram shows a saccular main portal vein aneurysm. The aneurysmal sac (*arrows*) is thrombosed with preserved flow in the portal vein.

Follow-up

The median follow-up from the time of first presentation was 50 months (range 9–181 months) and from surgery was 5 months (range 2–73) (Table 2). Three patients who underwent aneurysmal resection were alive without symptoms attributable to the extrahepatic portal vein aneurysm. Portal vein patency was demonstrated on follow-up surveillance CT scan or ultrasonography (Fig. 5a and Fig. 5b). The patient who had the iliac interposition allograft had initially widely patent portal vein on the first follow-up ultrasound scan. A 6-month follow-up CT scan, however, revealed moderate stenosis at the anastomotic sites, which has not required surgical intervention to date.

There was no case of aneurysm rupture. The patient with advanced cirrhosis had portal vein aneurysm for 42 months

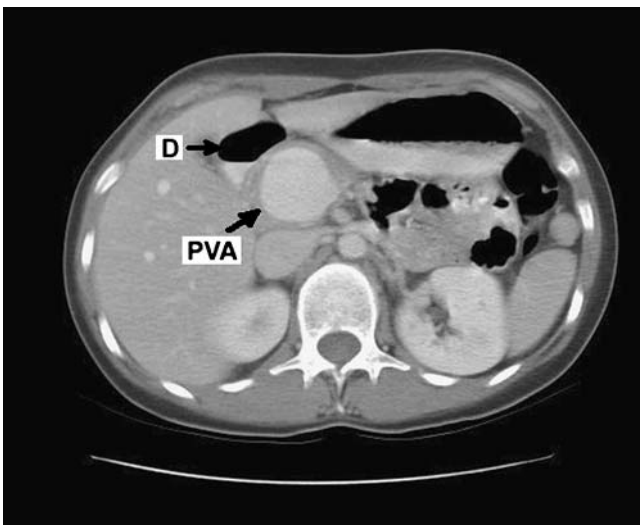

Figure 3 A CT scan shows a mass effect of the portal vein aneurysm (PVA) on the adjacent duodenum (D).

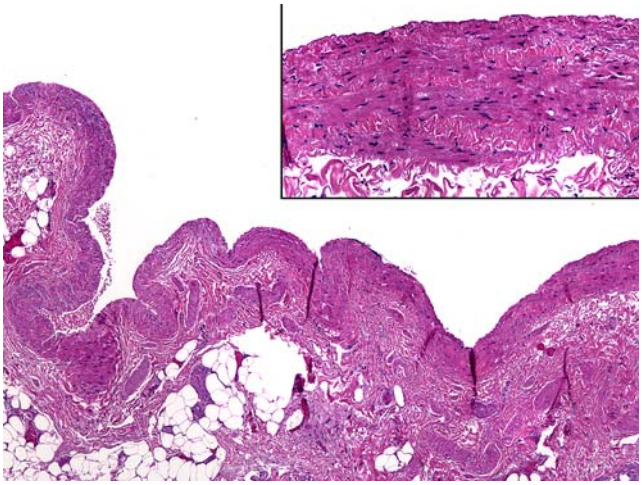

Figure 4 A hematoxylin and eosin stain 40× of the portal vein aneurysm wall. The vessel wall is intact without evidence of inflammation or necrosis.

without complications related to the aneurysm before she underwent liver transplantation.

The two patients who were expectantly observed have done well without any complications from the extrahepatic portal vein aneurysm. The intramural thrombus found at presentation in one patient resolved on a subsequent CT scan, and size of the aneurysm decreased from 5.5×5.0 cm to 4.6×4.5 cm (Fig. 6a,b). The patient was managed with daily 81 mg aspirin but no other anticoagulation.

Discussion

During the fourth and fifth weeks of embryonic development, three anastomoses form between right and left vitelline veins around the future duodenum.[5] A complex process of involution and interconnection of these vitelline veins results in the portal vein. Abnormal development of the portal venous system during this critical period may give rise to an extrahepatic portal vein aneurysm. Reports of portal vein diverticulum diagnosed in utero support this theory.[6] Other factors such as portal hypertension, pancreatitis, and trauma have been implicated as possible acquired causes, but causation remains speculative. Examination of four resected portal vein aneurysms in our series did not reveal any pathological abnormality, and etiology of the aneurysmal formation in our series is unknown.

The incidence of extrahepatic portal vein aneurysm is unclear, and review of the English literature from 1956 to 2006 produced a total of 61 cases. The demographic characteristics include female-to-male ratio of 2:1 and the median age at 52, with the range of 5–77. The size of the aneurysm ranged from 1.9 to 8.0 cm. The most common location of the aneurysm was at main portal vein trunk in 52.4% of cases, followed by 44.3% at the confluence of the superior mesenteric vein and the splenic vein, and 3.3% at the extrahepatic right or left portal vein.

As in our case series, the most frequently reported clinical presentation is nonspecific abdominal pain (54.1%), followed by incidental finding (24.6%), gastrointestinal bleeding (9.8%) and other miscellaneous symptoms such as fever, abdominal swelling, or jaundice (11.5%). One third of patients presented with complications. There were two patients who presented with spontaneous aneurysmal rupture, and one died as a result.[4,7] However, rupture is rarely reported, with last case published in 1967. Throm-

Table 2 Portal Vein Aneurysm Treatment, Outcome and Follow-up

Patient	Complications of portal vein aneurysm	Indication for surgery	Treatment	Length of stay after surgery	Morbidity of surgery	Follow-up from first presentation	Follow-up from surgery	Status
1	None	Abdo pain and abdominal mass	aneurysmorrhaphy, cholecystectomy	7 d	none	75	73	Alive
2	Mass effect on the duodenum	Mass effect on the duodenum	aneurysmorrhaphy	3 d	none	9	4	Alive
3	none	Choledocholithiasis and gallstone pancreatitis	CBD transsection, cholecystectomy, Aneurysmectomy, iliac venous interposition allograft, Roux-en-Y hepaticojejunostomy	11 d	none	181	6	alive
4	none	End-stage liver disease	liver transplantation	2 mo	sepsis	44	2	dead
5	Intramural non-occlusive thrombosis	N/A	Observation	N/A	N/A	56	N/A	Alive
6	none	N/A	Observation	N/A	N/A	12	N/A	Alive

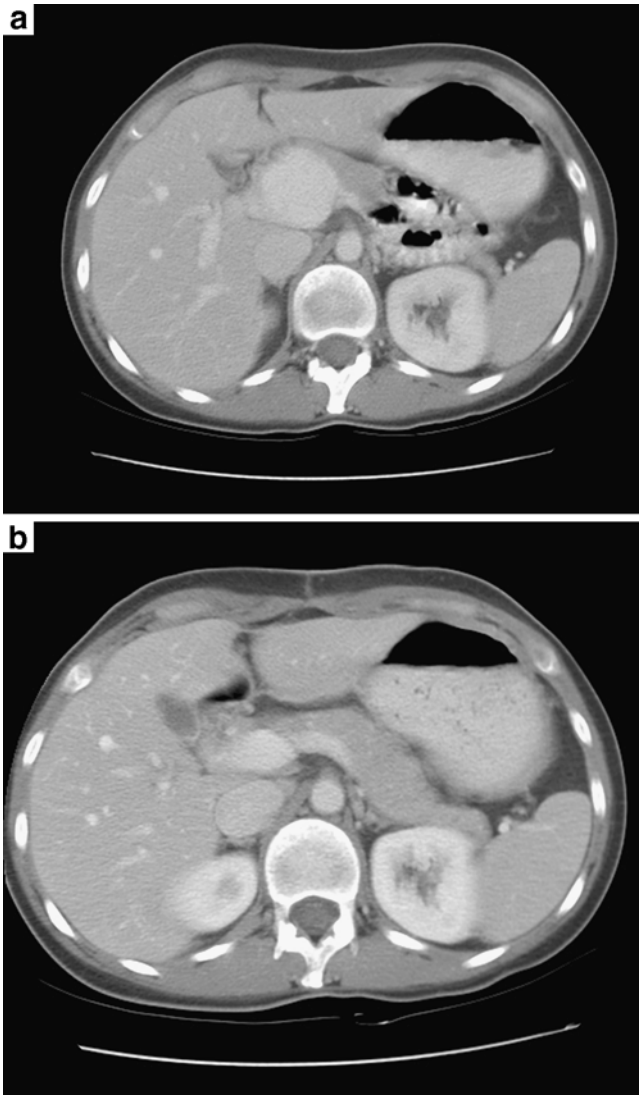

Figure 5 A CT scan before and after aneurysmorrhaphy (**a** and **b**, respectively). The normal diameter of the portal vein is restored.

bosis of the portal vein aneurysm was the most commonly noted complication, and it makes up 24.6% of cases reported. Compression of the adjacent organs by the portal vein aneurysm was found in 9.8% of cases reported, and it also occurred in one of our patients whose portal vein aneurysm caused a mass effect on the duodenum. In the literature, the common bile duct was the most frequent structure that was compressed or displaced by the aneurysm (n=5), followed by the duodenum and the inferior vena cava.[7–12]

In our search of the literature, 26.2% of reported cases underwent operation (Table 3), and several patients had more than one procedure performed.[3,4,7–9,13–22] For instance, there were eight cases of aneurysmorrhaphy, one case of aneurysmectomy, six cases of surgical shunt procedures, and seven cases of splenectomy. Of the nine patients who underwent aneurysmorrhaphy or aneurysmectomy, all were single-case reports with the exception of Jin et al.[21] who reported two cases. In our series, three different surgical procedures were utilized for portal vein aneurysm; two cases of aneurysmorrhaphy, one case of aneurysmectomy, and one case of liver transplantation.

The operation chosen depended on the shape and the location of the aneurysm, presence of complications, and associated comorbidities such as liver cirrhosis and portal hypertension. In our experience, two patients with saccular portal vein aneurysm were relatively easily excised, and aneurysmorrhaphy was performed to restore normal luminal diameter of the portal vein. Similarly, there have been eight reported cases of successful aneurysmorrhaphy in the literature and reported morbidity is low.[9,14,15,18–21] For fusiform portal vein aneurysms, if an aneurysmectomy is performed, a conduit to replace the resected portal vein

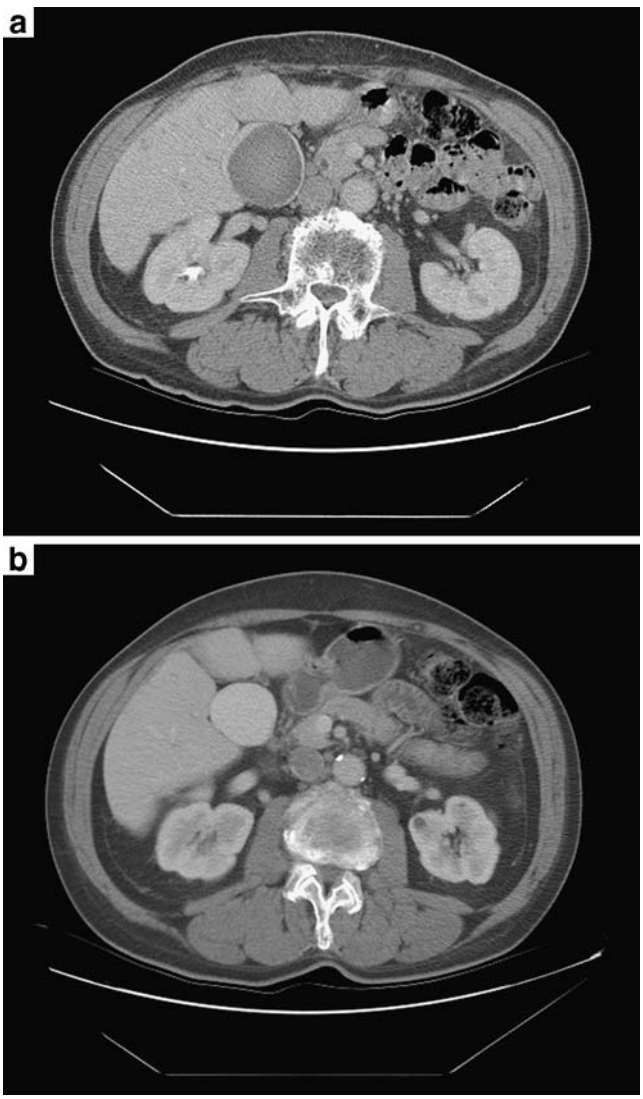

Figure 6 A CT scan of the thrombosed saccular portal vein aneurysm (**a**) and a 5-month follow-up CT scan (**b**). The thrombosis has disappeared and the aneurysmal size has decreased.

Table 3 Surgically Managed Portal Vein Aneurysms

Author	Year	Age	Gender	Size in cm	Location	Presentation	Portal Hypertension	Liver Disease	Complication of portal vein aneurysm	Indication for surgery	Operation	F/U in month	F/U Finding
Barzilai[4]	1956	21	F	2.0	R portal vein	GI bleed	Yes	Liver cirrhosis	Thrombosis, rupture	GI bleed	Splenectomy	11	Death
Leonsins[3]	1960	52	M	8.0	MPV	GI bleed	Yes	Liver cirrhosis	None	GI bleed	Splenectomy	1	Death
Sedgwick[8]	1960	25	F	5.0	SV-SMV C	Abdominal pain	Yes	Liver cirrhosis	Compression of CBD	Compression of CBD	Cholecystojejunostomy, splenorenal shunt	10	Good
Hermann[16]	1965	26	F	6.0	SV-SMV C	GI bleed	Yes	Portal fibrosis	None	Portal hypertension	Portocaval shunt	3	Good
Thomas[7]	1967	13	F	3.0	MPV	GI bleed	Yes	None	None	GI bleed	Portocaval shunt	48	Good
Liebowitz[17]	1967	55	F	8.0	SV-SMV C	Abdominal pain	No	None	None	Agnogenic myeloid metaplasia	Splenectomy	5	Death
Andoh[18]	1988	57	F	8.0	MPV	Abdominal pain	No	None	None	Symptom	Aneurysmorrhaphy, splenectomy	0	Good
Baker[13]	1990	34	F	8.0	SV-SMV C	Abdominal pain	No	None	Thrombosis	Acute thrombosis, no collateral vessels	Aneurysmectomy, splenectomy, shunt	0	Good
Glazer[14]	1992	26	F	7.0	SV-SMV C	Abdominal pain	No	None	Thrombosis	Thrombosis	Thrombectomy, aneurysmorrhaphy	120	Good
Brock[9]	1997	72	F	6.0	SV-SMV C	Abdominal pain	No	None	Compression of duodenum and IVC	Cecal cancer	Aneurysmorrhaphy	18	Good
Santana[19]	2002	68	F	7.8	MPV	Abdominal pain	No	None	Thrombosis	Acute thrombosis, collateral vessels present	Thrombectomy, aneurysmorrhaphy	6	Good
Flis[20]	2003	58	F	5.5	MPV	Abdominal pain	No	None	None	Symptom	Aneurysmorrhaphy	6	Good
Jin[21]	2005	45	F	4.0	MPV	Abdominal pain	No	None	None	Symptom	Aneurysmorrhaphy	6	Good
	2005	34	F	4.0	SV-SMV C	Incidental	No	None	None	Prophylactic surgery	Aneurysmorrhaphy, splenectomy	6	Good
Luo[22]	2006	54	M	4.5	MPV	Abdominal pain	Yes	Liver cirrhosis	None	Symptom	Splenectomy, spleno-renal shunt	6	Good
Wolff[15]	2006	32	F	8.0	MPV	Abdominal pain	No	None	Thrombosis	Acute thrombosis, no collateral vessels	Thrombectomy, aneurysmorrhaphy, portocaval shunt	0.5	Bleeding
Cho	2007	71	F	5.0	MPV	Abdominal pain	No	None	None	Abdominal pain, peri-pancreatic mass	Aneurysmorrhaphy, cholecystectomy	73	Good
	2007	30	F	4.4	SV-SMV C	Abdominal pain	No	None	Mass effect on the duodenum	Mass effect on the duodenum	Aneurysmorrhaphy	4	Good
	2007	50	F	6.0	SV-SMV C	Gallstone pancreatitis	No	None	None	Gallstone pancreatitis	Aneurysmectomy	6	Good
	2007	62	F	2.7	L portal vein	Incidental	Yes	Liver cirrhosis	None	Liver cirrhosis	Liver transplantation	2	Death

MPV = main portal vein; SV-SMV C = splenic vein and superior mesenteric vein confluence

segment is required and an allograft from a cadaveric donor, as in our case, or a synthetic graft can be utilized.

The presence of liver disease also influences the surgical procedure chosen for portal vein aneurysm. Among 11 patients identified in the literature who had liver disease and portal vein aneurysm, surgical shunt procedures and splenectomy were most commonly utilized operative procedures for the portal vein aneurysm.[3,4,7,8,16,22–24] These shunts were primarily to decompress portal hypertension, and they did not specifically treat the portal vein aneurysm. Among five patients with liver cirrhosis who underwent surgery, two patients died of gastrointestinal bleeding with postoperative follow-up between 1 and 11 months. This reflects a well-recognized complication of portal hypertension and the high risk of surgery in this patient group.[25] There are also reports of six patients with liver cirrhosis and portal vein aneurysm who were conservatively followed with no complication from portal vein aneurysm reported during follow-ups between 0 and 24 months. In view of the low reported rate of rupture and the risk of surgery in the presence of portal hypertension and liver cirrhosis, there is no strong evidence that prophylactic resection of the portal vein aneurysm in the setting of cirrhosis is beneficial.

Symptomatic portal vein aneurysms or the presence of associated complications such as rupture, complete thrombosis of portal vein aneurysm, or compression of adjacent structures are generally considered to be indications for surgical intervention. Previous reports of five patients who underwent surgery for thrombosis of portal vein aneurysm, and four patients had an excellent outcome postoperatively.[4,13–15,19] The most commonly performed procedures in this group were surgical thrombectomy and aneurysmorrhaphy. There is no report of long-term anticoagulation use for thrombosed portal vein aneurysms. In one patient previously reported, postoperative anticoagulation with warfarin resulted in a hemorrhagic complication requiring reoperation.[15] There is one report of use of aspirin therapy after thrombectomy and aneurysmorrhaphy, which maintained portal vein patency for 3 years postoperatively, and aspirin might reduce risk of recurrent thrombosis.[19]

There are also reports of successful conservative management of thrombosed portal vein aneurysm, as in one of our patients.[23,26–28] Aneurysmal thrombosis in the presence of collateral vessels and the absence of mesenteric thrombus extension can be managed with observation.[27] These patients, however, need to be closely monitored for signs of mesenteric vein thrombosis and portal hypertension, as natural history of aneurysmal thrombosis is not clear.

In the literature, two patients underwent surgery for a mass effect of the portal vein aneurysm on adjacent structures; one cholecystojejunostomy was performed for compression of the common bile duct and in the other case, aneurysmorrhaphy was used to relieve compression of the duodenum and the inferior vena cava.[8, 9] Both patients were well at 10 and 18 months of follow-up. There are also two cases of the common bile duct being displaced by the portal vein aneurysm, which was managed with observation.[11,12] In these two cases, however, there was no evidence of biliary obstruction on laboratory tests or on endoscopic retrograde cholangiopancreatography (ERCP).

In the 45 reported patients who were observed, the rate of subsequent complications or need for operative intervention during follow-up was low.[7,10–12,23,24,26–49] For instance, two patients died during follow-up, one with liver cirrhosis as a result of variceal bleeding and the other of gastric cancer.[7,42] However, many reports lack substantial follow up. In view of unclear natural history of portal vein aneurysm, it is important to maintain close follow-up if observation is chosen. CT scans or ultrasonography can be used to monitor change in aneurysmal size and/or luminal flow.

Conclusion

In this paper we report the largest series to date of patients with extrahepatic portal vein aneurysm addressed surgically. This provides further evidence that aneurysmorrhaphy can be performed safely in otherwise healthy patients. Use of iliac interposition graft after aneurysmectomy was associated with a clinically insignificant anastomotic stenosis on a follow-up CT scan in one of our cases. Our series, as well as previously published case reports, suggests that surgical intervention is typically used for portal vein aneurysms that are associated with symptoms or with complications. Our experience and published data suggest that aneurysmorrhaphy can be accomplished with relative technical ease and low morbidity. On the other hand, close follow-up with CT scan and/or ultrasonography may represent an alternative to surgery in otherwise healthy asymptomatic patients.

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References

1. Doust BD, Pearce JD. Gray-scale ultrasonic properties of the normal and inflamed pancreas. Radiology 1976;120:653–657.
2. Schild H, Schwedeb F, Braun B, Lang H. Aneurysm of the superior mesenteric vein. Radiology 1982;145:641–642.
3. Leonsins AJ, Siew S. Fusiform aneurysmal dilatation of the portal vein. Postgrad Med J 1960;36:570–574.
4. Barzilai R, Kleckner MS. Hemocholecyst following ruptured aneurysm of portal vein. Arch Surg 1956;72:725–727.

5. Sadler T. Cardiovascular system. In Sadler TW, ed. Langman's Medical Embryology, 8th edition. Philadelphia, Lippincott Williams & Wilkins, Baltimore, pp 246–248.
6. Gallagher DM, Leiman S, Hux C. In utero diagnosis of a portal vein aneurysm. J Clin Ultrasound 1993;21:147–151.
7. Thomas TV. Aneurysm of the portal vein: Report of two cases, one resulting in thrombosis and spontaneous rupture. Surgery 1967;61(4):550–555.
8. Sedgwick CE. Cisternal dilatation of portal vein associated with portal hypertension and partial biliary obstruction. Lahey Clin Bull 1960;11:234–237.
9. Brock PA, Jordan PH Jr, Barth MH, Rose AG. Portal vein aneurysm: A rare but important vascular condition. Surgery 1997;121:105–108.
10. Thompson PB, Oldham KT, Bedi DG, Guice KS, Davis M. Aneurysmal malformation of the extrahepatic portal vein. Am J Gastroenterol 1986;81(8):695–697.
11. Hagiwara H, Kasahara A, Kono M, Kashio S, Kaneko A, Okuno A, Hayashi N, Fusamoto H, Kamada T. Extrahepatic portal vein aneurysm associated with a tortuous portal vein. Gastroenterology 1991;100(3):818–821.
12. Fukui H, Kashiwagi T, Kimura K, Goto M, Takei Y, Kasahara A, Kawano S, Fusamoto H, Kozuka T, Kamada T. Portal vein aneurysm demonstrated by blood pool SPECT. Clin Nucl Med 1992;17(11):871–873.
13. Baker BK, Nepute JA. Computed tomography demonstration of acute thrombosis of a portal vein aneurysm. Mol Med 1990;87: 228–230.
14. Glazer S, Gaspar MR, Esposito V, Harrison L. Extrahepatic portal vein aneurysm: Report of a case treated by thrombectomy and aneurymorrhaphy. Ann Vasc Surg 1992;6:338–342.
15. Wolff M, Schaefer N, Schmidt J, Hirner A. Thrombosis of a large portal vein aneurysm: Treatment by thrombectomy, aneurysmorrhaphy and portocaval shunt. J Gastrointest Surg 2006;10:128–131.
16. Hermann RE, Shafer WH. Aneurysm of the portal vein and portal vein hypertension, first reported case. Ann Surg 1965;162: 1101–1104.
17. Liebowitz HR, Rousselot LM. Saccular aneurysm of portal vein with agnogenic myeloid metaplasia. N Y State J Med 1967;67 (11):1443–1447.
18. Andoh K, Tanohata K, Asakura K, Katsumata Y, Nagashima T, Kitoh F. CT demonstration of portal vein aneurysm. J Comput Assist Tomogr 1988;12:325–327.
19. Santana P, Jeffrey RB Jr, Bastidas A. Acute thrombosis of giant portal venous aneurysm: Value of color Doppler sonography. J Ultrasound Med 2002;21(6):701–704.
20. Flis V, Matela J, Gadzijev E. Portal vein aneurysm: When to operate? EJVES Extra 2003;5:31–33.
21. Jin B, Sun Y, Li YQ, Zhao YG, Lai CS, Feng XS, Wan CD. Extrahepatic portal vein aneurysm: Two case reports of surgical intervention. World J Gastroenterol 2005;11(14):2206–2209.
22. Luo HF, Wang HJ, Li B, Wang ZY. Diagnosis and management of extrahepatic portal vein aneurysm: A case report. Hepatobiliary Pancreat Dis Int 2006;5(2):311–313.
23. Lopez-Machado E, Mallorquin-Jimenez F, Medina-Benitez A, Ruiz-Carazo E, Cubero-Garcia M. Aneurysm of the portal venous system; ultrasonography and CT findings. Eur J Radiol 1998;26: 210–214.
24. So NM, Lam WWM. Calcified portal vein aneurysm and portohepatic venous shunt in a patient with liver cirrhosis. Clin Radiol 2003;58:742–744.
25. Rice HE, O'Keefe GE, Helton WS, Johansen K. Morbid prognostic features in patients with chronic liver failure undergoing nonhepatic surgery. Arch Surg 1997;132(8):880–884.
26. Kim J, Kim MJ, Song SY, Kim JH, Lim JS, Oh YT, Kim KW. Acute thrombosis of a portal vein aneurysm and development. Clin Radiol 2004;59:631–633.
27. De Gaetano AM, Andrisani MC, Gui B, Maresca G, Ionta R, Bonomo L. Thrombosed extrahepatic portal vein aneurysm: Report of two cases and review of the literature. Abdom Imaging 2006;31(5):545–548.
28. Tsukuda S, Sugimoto E, Watabe T, Amanuma M, Heshiki A. A case of extrahepatic portal vein aneurysm with massive thrombosis: Diagnosis with reconstruction images from helical CT scans. Radiat Med 1998;16(4):301–303.
29. Vine HS, Sequeira JC, Windrich WC, Sacks BA. Portal vein aneurysm. AJR 1979;132:557–560.
30. Boyez M, Fourcade Y, Sebag A, Valette M. Aneurysmal dilatation of the portal vein: A case diagnosed by real-time ultrasonography. Gastrointest Radiol 1986;11:319–321.
31. Dognini L, Carreri AL, Moscatelli G. Aneurysm of the portal vein: Ultrasound and computed tomography identification. J Clin Ultrasound 1991;19:178–182.
32. Lee HC, Yang YC, Shih SL, Chiang HJ. Aneurysmal dilatation of the portal vein. J Pediatr Gastroenterol Nutr 1989;8(3):387–389.
33. Savastano S, Feltrin GP, Morelli I, Miotto D, Chiesura-Corona M, El Khatib AB. Aneurysm of the extrahepatic portal vein associated with segmental portal hypertension and spontaneous porto-caval shunting through the inferior mesenteric vein. J Belge Radiol 1992;75:194–196.
34. Itoh Y, Kawasaki T, Nishikawa H, Ochi J, Miura K, Moriyasu F. A case of extrahepatic portal vein aneurysm accompanying lupoid hepatitis. J Clin Ultrasound 1995;23:374–378.
35. Feliciano PD, Cullen JJ, Corson JD. The management of extrahepatic portal vein aneurysms: Observe or treat? HPB Surg 1996;10:113–116.
36. Fulcher A, Turner M. Aneurysms of the portal vein and the superior mesenteric vein. Abdom Imaging 1997;22:287–292.
37. Ohnami Y, Ishida H, Konno K, Naganuma H, Hamashima Y, Zeniya A, Masamune O. Portal vein aneurysm: Report of six cases and review of the literature. Abdom Imaging 1997;22:281–286.
38. Atasoy KC, Fitoz S, Akyar G, Aytac S, Erden I. Aneurysms of the portal venous system Gray-scale and color Doppler ultrasonographic findings with CT and MRI correlation. Clin Imaging 1998;22:414–417.
39. Ozbek SS, Killi MR, Pourbagher A, Parildar M, Katranci N, Solak A. Portal venous system aneurysms: Report of five cases. J Ultrasound Med 1999;18:417–422.
40. Chen YY, Lin OS, Wu HK, Soon MS. A case of extrahepatic portal vein aneurysm: Evaluation by 3-dimensional computerized tomography angiogram. Hepatogastroenterology 1999;46:2965–2967.
41. Blasbalg R, Yamada RM, Tiferes DA. Extrahepatic portal vein aneurysms. AJR Am J Roentgenol 2000;174:877.
42. Yang DM, Yoon MH, Kim HS, Jin W, Hwang HY, Kim HS. CT findings of portal vein aneurysm caused by gastric adenocarcinoma invading the portal vein. Br J Radiol 2001;74:654–656.
43. Geubel AP, Maisse F, Boemer F. Images in hepatology. Aneurysm of the trunk of the portal vein. J Hepatol 2001;34:780.
44. Lau H, Chew DK, Belkin M. Extrahepatic portal vein aneurysm: A case report and review of the literature. Cardiovasc Surg 2002;10 (1):58–61.
45. Mhanna T, Bernard P, Pilleul F, Partensky C. Portal vein aneurysm: Report of two cases. Hepatogastroenterology 2004;51:1162–1164.
46. Oliver I, Lacueva J, Calpena R. A painful liver. Gut 2004;53(7): 937–943.
47. Onbas O, Kantarci M, Alper F, Erdogmus B, Altinkaynak M. Images of Interest. Hepatobiliary and pancreatic: Portal vein aneurysm 2004;19:1085.
48. Giavroglou C, Xinou E, Fotiadis N. Congenital extrahepatic portal vein aneurysm. Abdom Imaging 2006;31:241–244.
49. Alexopoulou A, Papanikolopoulos K, Thanos L, Dourakis SP. Aneurysmal dilatation of the portal vein: A rare cause of portal hypertension Scand J Gastroenterol 2005;40:233–235.

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The Challenges of Resident Training in Complex Hepatic, Pancreatic, and Biliary Procedures

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Abstract Operations on the liver and pancreas have fallen within the domain of the general surgeon and have been part of general surgery training. The more complex procedures involving these organs are limited in number in most general surgery residencies and do not afford an opportunity for vast experience. Moreover, fellowship programs in hepato-bilio-pancreatic (HPB) surgery and the development of laparoscopic techniques may have further limited the familiarity of general surgery residents with these operations. To determine the experience accrued by finishing general surgery residents, we accessed, through the Residency Review Committee of the Accreditation Council for Graduate Medical Education, the Resident Case Log System used by general surgery residents throughout their training to document operative cases. The number of operations on the gallbladder, bile ducts, pancreas, and liver was examined over the past 16 years (there were missing data for 3 years). Reference years 1995 and 2005 were compared to detect trends. Experience with laparoscopic cholecystectomy has steadily increased and averaged more than 100 cases in 2006. Experience in liver resection, distal pancreatectomy, and partial (Whipple) pancreatectomy has statistically improved from 1995 to 2005, but the numbers of cases are low, generally less than five per finishing resident. Experience in open common bile duct and choledocho-enteric anastomoses has statistically declined from 1995 to 2005, averaging less than four cases per finishing resident. The mode (most frequently performed number) for liver and pancreas resections was either 0 or 1. It is doubtful this experience in HPB surgery engenders confidence in many finishing residents. Attention should be focused on augmenting training in HPB surgery for general surgery residents perhaps through a combination of programmatic initiatives, ex vivo experiences, and minifellowships. Institutional initiatives might consist of defined HPB services with appropriate expertise, infrastructure, process, and outcome measures in which a resident-oriented, competency-based curriculum could be developed.

Keywords Hepatobiliary surgery · Pancreatic surgery · Graduate medical · Education

Introduction

> We need a system, and we shall surely have it, which will produce not only surgeons, but surgeons of the highest type, men who will stimulate the first youths of our country to study surgery and to devote their energies and their lives to raising the standard of surgical science—William Stewart Halsted, MD

William Halsted, in his treatise, *The Training of the Surgeon*, published in the *Bulletin of the Johns Hopkins Hospital* in 1904, advocated long hours and long years of apprenticeship for surgeons in training to encounter "any emergency that may arise and to perform any operation known to surgery..." Regarding the arduous nature of the residency period, Dr. Halsted commented "These positions are not for those who so soon weary of the study of their profession." Today, we have not the luxury of leisurely training surgeons until they are judged ready to practice their craft. Constraints of time, both in years and hours, even as the complexity of surgery has increased, have

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forced us to concentrate our efforts and provide fundamental skills to equip young surgeons for the multitude of patients and diseases they may see. If not the actual experience, we must teach the framework in which this can happen. However, in all our developing regulations, we must still strive to produce the system and achieve the standard Dr. Halsted advocated.

In essence, the goal today of a general surgical residency is to produce competent and compassionate surgeons. Competency is no longer judged at the whim of attending surgeons. The Accreditation Council for Graduate Medical Education (ACGME) has defined competency in terms of six categories to be addressed during the course of 5 years of training. Today's evolving educational paradigm includes proficiency in medical knowledge, patient care skills, self-reflection and assessment, interpersonal skills and communication, professionalism, and an ability to practice in the wide scope of health care systems. Still fundamental in these competencies for the general surgeon, just as in Dr. Halsted's day, is an ability to select the proper patient, operate skillfully, and render expert postoperative care. It is widely understood among surgeons that good outcomes, a recognized measure of quality, require these components.

For this reason, operative experience remains central to surgical training. There is no substitute for hands-on exposure to surgical anatomy and surgical disease and no substitute for conducting operations from start to finish, albeit with careful, graded supervision. A finishing surgical resident might, within days or weeks, be faced with complex procedures without the reassuring presence of a seasoned mentor. He or she must be prepared. For the most part, even with work hours limitations, the so-called "bread-and-butter" cases, such as laparoscopic cholecystectomy, breast excisions, appendectomies, and thyroidectomies, seem to be done in abundance. Proficiency should accompany such experiences.

The same may not hold true for hepatic, pancreatic, and complex biliary (HPB) surgery. How prepared are finishing general surgery residents to treat diseases of the liver, biliary system, and pancreas? We have sought to answer this question through examination of the operative experiences of general surgery residents in major hepatic, complex biliary, and major pancreatic resections using data submitted by residents to the ACGME Resident Case Log System database.

Materials and Methods

The ACGME Resident Case Log System for operative log reporting (operative log) is an Internet-based case log system using Current Procedural Terminology and International Classification of Diseases 9 codes to track operative experience during the years of residency training. The individual resident is responsible for entering procedures that accurately reflect their participation. The operative logs of finishing general surgery residents filed with the ACGME from 1990 to 2006 were examined for experience in the following procedures: distal pancreatectomy (DP), partial pancreatico-duodenectomy (Whipple-type pancreatectomy; PPD), total pancreatectomy (TP), laparoscopic cholecystectomy, open cholecystectomy, open and laparoscopic common bile duct exploration (CBDE), choledocho-enteric anastomoses, and major liver resections, including hemihepatectomy and segmentectomy but not wedge excisions. Values for each year in these categories were expressed as mean+ standard deviation (SD) or reflecting 95% confidence intervals around the mean and mode. The mode was defined as the most frequent number of cases performed. The total number of cases performed as operating surgeon was chosen. Those as surgeon assistant were excluded. There were 3 years, 1993, 1994, and 1999, for which data were not available through the ACGME. The reference years 1995 and 2005 were chosen to represent a 10-year span for trending purposes. The average number of cases in all HPB categories in 1995 and 2005 were compared for statistically significant variation in resident experience. All pairwise comparisons were done using Student's t test. p values were provided to allow the reader to assess significance. Significance was determined at $p<0.05$.

Results

In retrieving ACGME operative logs for HPB procedures, there were 3 missing years, 1993, 1994 (partial), and 1999. There has been a decline in the number of open cholecystectomies performed since 1990 with an eventual corresponding appearance and progressive increase in the numbers of laparoscopic cholecystectomies (Fig. 1). When comparing the number of laparoscopic cholecystectomies from reference years 1995 and 2005 (10-year span), there has been a statistically significant increase in the number of procedures in which the surgical residents were listed as the operating surgeon (52.7±28, 1995 vs 100.6±41, 2005, $p<0.001$). There has been a progressive decline in the numbers of open CBDE, achieving statistical significance from 1995 to 2005 (5.1±4, 1995 vs 1.7±2, 2005, $p<0.001$; Fig. 2). Corresponding to this, there has been a modest but statistically significant increase in the number of laparoscopic CBDEs performed during the 10-year span (0.6±2, 1995 vs 0.7±1.4, 2005, $p=0.025$). Lastly, for biliary procedures, there was a statistically significant decline in the number of choledocho-enteric anastomoses during the 10-year span (3.7±3, 1995 vs 2.6±2.6, 2005, $p<0.001$).

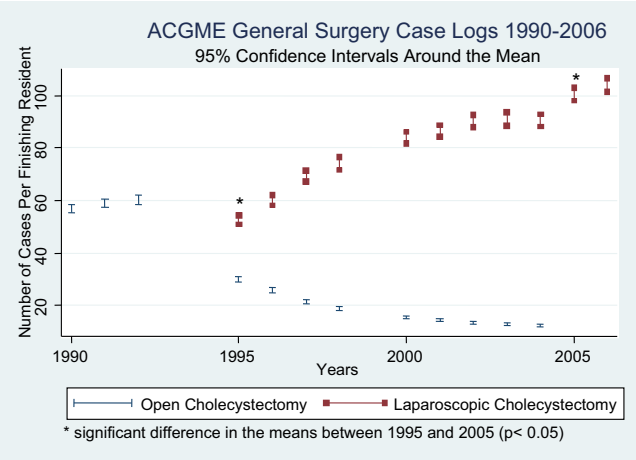

Figure 1 Experience with open and laparoscopic cholecystectomy from 1990 to 2006. In the reference period 1995 and 2005, there has been a statistically significant increase in the number of laparoscopic cholecystectomies done by finishing residents.

The number of major liver resections (hemihepatectomies and segmentectomies) performed by general surgery residents has risen over the 10-year span, achieving statistical significance (1.9±3, 1995 vs 3.9±4, 2005, $p<0.001$; Fig. 3). The maximum number of liver resections for any given program for 2005 was recorded as 38. Likewise, the number of pancreatectomies—PPDs and DPs—have increased over the 10-year span (PPD=2.1±3, 1995 vs 4.2±4.1, 2005, $p<0.001$; DP=1.4±2, 1995 vs 2.3±2.2, 2005, $p<0.001$; Fig. 4). The maximum number of PPDs for any given program for 2005 was recorded as 31. There have been a minimal number of TPs performed throughout the survey period and no appreciable increase in numbers during the reference period 1995–2005. Table 1 summarizes comparative HPB cases for the sentinel years 1995 and 2005.

The mode (most frequent number of cases) for all HPB procedures is listed in Table 2. There were 5 years in which complete information on the mode were not available, 1993, 1994, 1999, 2005, and 2006. While adequate numbers are recorded for both open and laparoscopic cholecystectomies, the mode for other hepatic, biliary, and pancreatic cases ranges from 0 to 3. In particular, the mode for major liver resection is most commonly zero or one case per finishing resident and for PPD and DP, most often one case per finishing resident.

Discussion

General surgery resident experience, as recorded in the ACGME operative case log system, has shown an approximate doubling in average number of liver and pancreas cases (liver resection, PD, DP) performed over the 16 years surveyed, from roughly two to four. For the reference years 1995 and 2005, the increase attained statistical significance. This may represent an actual enhancement of HPB experience or may simply reflect a better reporting system of operative cases developed over the years by the ACGME. However, many residents still report a meager experience, as judged by the mode, one or no cases, during their training. Even an average number of four PDs and major liver resections are of questionable value in preparing surgical residents for these procedures in practice. Both PD and major liver resections are complex procedures requiring not only familiarity with anatomy but also familiarity with the nuances of exposure, mobilization, and use of assistants during surgery. Our findings are reminiscent of those reported by Ong et al.[1] who concluded that surgical chief residents "have a widely variable experience in liver and pancreatic surgery." As well, our findings also are in agreement with the institutional experience from the University of Louisville furnished by Cheadle et al.[2] who indicated that their finishing residents had, on average, done less than five PDs and approximately five major liver resections. With a standard deviation for liver resections of approximately three and for PPDs from three to four and assuming a normal distribution, we can gather that about 68% of finishing residents (in 2005) have done from zero to eight liver resections and from zero to five PPDs. To look at it another way, about one half of finishing residents have done less than four liver resections and less than three PPDs.

We have also demonstrated a steady decline in the number of open CBDEs and a scanty experience with laparoscopic CBDE. In general, residents did less than half

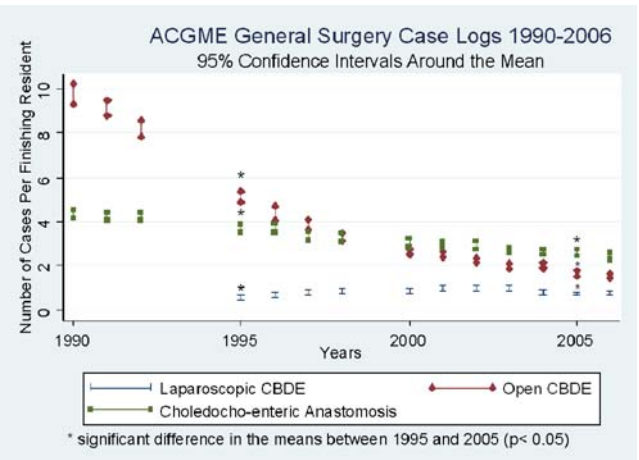

Figure 2 Experience of finishing general surgery residents in open and laparoscopic common bile duct explorations (*CBDE*) and in choledocho-enteric anastomoses. There has been a statistically significant decline in the number of open CBDE and the number of choledocho-enteric anastomoses performed in the reference period 1995 and 2005. There has been a significant, albeit unimpressive, increase in the number of laparoscopic CBDE during this same period.

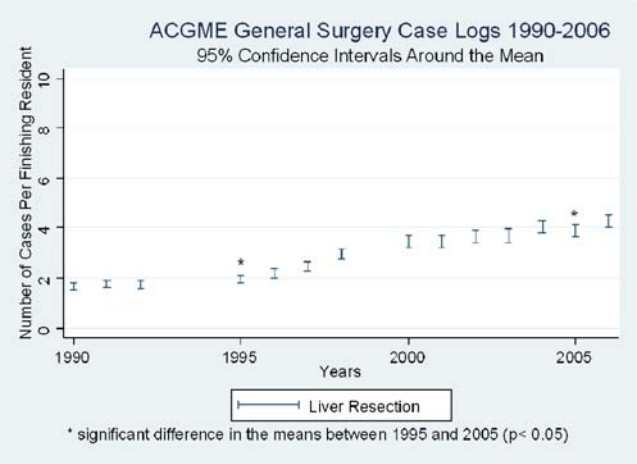

Figure 3 Experience in major liver resections reported by finishing residents in general surgery. There has been a statistically significant increase in the number of cases performed in the reference period 1995 and 2005.

the number of open CBDE in 2005 compared to 1995. Open CBDE has traditionally been an opportunity for surgical residents to become acquainted with the anatomy and operative exposure of the extrahepatic biliary tree and hilum of the liver. Principles involved in exposure of the common bile duct could be used for other, more emergent, situations such as trauma to the liver. No doubt, much of this decline has been due to the enhanced ability to remove choledochal stones with less invasive procedures namely, endoscopic retrograde cholangiopancreatography. Furthermore, the report by Livingston and Rege[3] described an increase in complications associated with open CBDE, which they linked to a dwindling surgeon experience. The introduction of laparoscopic CBDE has not seemed to compensate. While the numbers of laparoscopic CBDE have statistically increased from reference years 1995 to 2005, the average number of cases barely reaches one per finishing resident. Furthermore, the number of choledocho-enteric anastomoses has also statistically declined from 1995 to 2005 with residents, on average, doing only two or three of these procedures during their training, again reflecting diminishing opportunities to acquaint general surgery residents with extrahepatic biliary anatomy.

What implications does this have for the future of HPB surgical training—particularly for programs (perhaps half) in which there are relatively few HPB cases available? Is regionalization the answer so that HPB cases would be concentrated at designated centers? While there is some evidence to support attempts at regionalization,[4] it is doubtful that the relatively few high-volume centers could accommodate all patients in need of HPB surgery, even if they were willing to go. For example, Fong et al.,[5] using National Medicare databases, collected 3,734 patients who had hepatectomies and 2,592 patients who underwent pancreatectomies within a 2-year period. High volume was rather arbitrarily assigned as more than 25 such cases performed per institution per year. Using this definition, there were 10 of 1,101 (9%) surveyed hospitals classified as high volume. These hospitals cared for 291 of 2,592 patients (11%) who had a pancreatectomy. Similarly, for hepatic resection, 12 of 1,284 hospitals (9%) were classified as high-volume hospitals operating on 474 of the 3,734 patients (13%) who underwent an hepatectomy during the study period. Moreover, it is doubtful that there would be equal access to high-volume centers for all ethnic and socioeconomic strata. There has already been the observation that more Medicaid and uninsured patients go to low-volume hospitals.[6]

Let us assume, then, that regionalization in HPB surgery cannot and will not occur. How are we to provide adequate training with the number of cases currently being performed in many, if not most, programs? Is mandatory fellowship training in HPB the answer? Intensive training in high-volume centers should provide the exposure and experience necessary for competent HPB surgery. Currently, the International Hepato-Pancreato-Biliary Association recognizes 16 fellowships in HPB surgery in North America, training approximately 18 fellows per year—perhaps enough to staff the relatively few high-volume centers but unlikely enough to satisfy the large number of "low volume" programs. One thousand ten general surgery residents finished their training in 2006. While almost 70% will enter specialty fellowship training, almost one third will not and will presumably begin a practice in general surgery. Many of these practicing surgeons will want or need to operate on liver, biliary, or pancreatic problems. In fact, despite fellowship training, most surgeons who

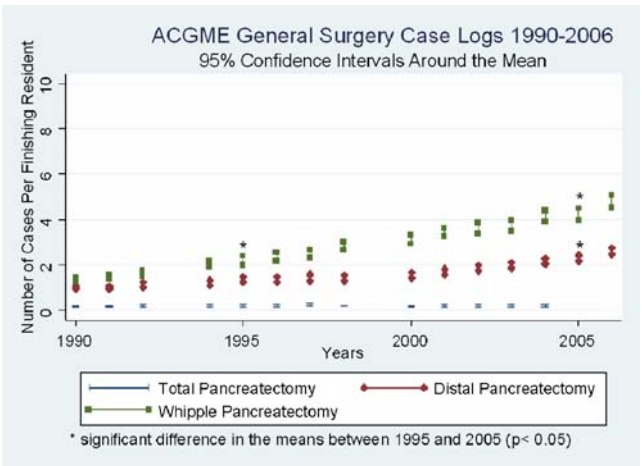

Figure 4 Experience in Whipple-type pancreatectomy, distal pancreatectomy, and total pancreatectomy reported by finishing general surgery residents. There has been a statistically significant increase in the number of Whipple-type and distal pancreatectomies performed during the reference period 1995 and 2005.

Table 1 Mean Number of HPB Procedures Completed in 1995 and 2005 ACGME General Surgery Case Logs

Procedure	1995	2005	*p* value
Major hepatic resection	1.948	3.886	<0.001
Distal pancreatectomy (DP)	1.379	2.291	<0.001
Whipple pancreatectomy (PPD)	2.185	4.238	<0.001
Total pancreatectomy (TP)	0.163	N/A	N/A
CBDE open	5.122	1.659	<0.001
CBDE laparoscopic	0.555	0.727	0.025
Lap cholecystectomy	52.665	100.599	<0.001
Open cholecystectomy	29.953	N/A	N/A
Choledocho-enterostomy	3.706	2.583	<0.001

TP cases were included in PPD cases for 2005; open cholecystectomies were no longer counted in 2005.
CBDE Common bile duct exploration

completed a general surgery residency maintain certification by the American Board of Surgery and conduct a broad general surgery practice.[2] There is currently not the capacity to formally train all of these individuals in HPB surgery, at least not in the conventional sense. How, then, do we teach these surgeons to safely perform major hepatic, biliary, and pancreatic operations? Are we to eventually subscribe to recommendations that general surgery training paradigm be changed into "specialist in general surgery" tracks after a period of core training[7]—so that all HPB cases are funneled to the "generalists" and not surgical "specialists"—or do we channel all of our surgical residents early on into superspecialization—HPB surgery included—much like the Swedish system?[8]

Within existing general surgery residencies, we would submit that programmatic development in HPB surgery, incorporating the pillars of structure, process, and outcomes, is necessary. Such programs require expertise, leadership, and infrastructure. Their primary focus should be excellence in the multidisciplinary care of patients with disorders of the liver and pancreas—much like the "center of excellence" concept. Within such programs residents could learn in a setting of comprehensive care. Components of patient selection, case review, and treatment planning are essential to instruct residents on the place of surgery in the care of these complex problems. Rarely is surgery standalone treatment but most often one element in a multifaceted approach. In fact, when such programs are established, the volume of HPB cases can dramatically increase.[9]

However, what about operative skills? Even with a programmatic concept, many residents, by ACGME operative logs, will continue to do only a handful of complex HPB cases. How many HPB cases confer proficiency? We cannot answer for sure but certainly more than one or two. Recent evidence indicates that continued improvement in outcome (blood loss, operative times, length of stay) occurs for individual surgeons even as experience climbs into the hundreds of cases.[10] Can surgical residents absorb and appreciate the complexities of hepatic or pancreatic surgery after only one or two exposures? It is doubtful. How can we augment that? Virtual training may be an option. For open operations, this can be done with ex vivo experiences such as cadaver dissection, preferably using fresh cadavers. Periodic cadaver laboratories, simulating the operating

Table 2 The Mode for HPB Cases Performed by Finishing General Surgery Residents from 1990 to 2006 ACGME General Surgery Case Logs

Year	Maj Hep	TP	DP	PPD	O CBDE	L CBDE	O Chole	L Chole	Chole-En
1990	0	0	0	0	7	N/A		N/A	3
1991	0	0	0	0	8	N/A	47	N/A	3
1992	0	0	0	1	6	N/A	27	N/A	2
1993		Information not available							
1994		1	1	0					
1995	0	0	1	1	5	0	22	39	3
1996	0	0	1	1	2	0	15	32	2
1997	1	0	1	1	3	0	15	43	2
1998	0	0	0	1	1	0	18	34	2
1999		Information not available							
2000	0	0	1	1	2	0	13	56	2
2001	2	0	1	1	1	0	11	76	2
2002	1	0	1	1	1	0	13	63	1
2003	1	0	1	1	1	0	10	73	1
2004	1	0	1	1	1	0	12	59	1
2005		Information not available							
2006		Information not available							

Maj Hep Major hepatic resection, *TP* total pancreatectomy, *DP* distal pancreatectomy, *PPD* partial pancreatico-duodenectomy, *O CBDE* open common bile duct exploration, *L CBDE* laparoscopic common bile duct exploration, *O Chole* open cholecystectomy, *L Chole* laparoscopic cholecystectomy, *Chole-En* choledocho-enterostomy

room, can allow senior-level residents the opportunity to leisurely work through hepatectomies and pancreaticoduodenectomies, with time to identify anatomic landmarks and even perform pancreatic anastomoses. There is some evidence that familiarity with cadaver dissection transfers well to the operating room and has been perceived by residents as a positive educational experience.[11,12]

Are there surrogates for actually performing operations? Is there any value to observing and assisting? Undoubtedly, there is. The use of short, intense visits to high-volume centers affords an opportunity to watch experienced HPB surgeons select and operate on patients. These "minifellowships" are not unknown in surgical practice and are sure to become commonplace in the future. Many bariatric surgeons, for example, have learned laparoscopic techniques through a few to several weeks of observation (apprenticeship) with an established laparoscopic surgeon.[13] In such settings, much information can be gleaned about the selection criteria, preoperative preparation, postoperative care, and adjuvant treatments to develop a quality HPB program. We are reminded, too, that "competence develops over time and is nurtured by reflections on experience"[14]—thus, the importance of outcomes assessment. The end of residency should not be the end of training but the beginning of lifelong learning.

In summary, operative experience for surgery residents in HPB surgery may continue to be highly variable and often meager. Our challenge as educators, then, is to provide a framework for success. Whether high volume or low volume, the resident should approach HPB surgery as a matter of lifelong learning, incorporating their experiences in graduate training with postgraduate education. Residents must view HPB surgery as a multidisciplinary endeavor involving their medical, radiology, and pathology colleagues in a programmatic setting of structure (expertise), process, and assessment of outcomes and not simply as procedures to master. Instrumental in this is the organizational leadership of committed HPB surgeon/educators who can function as mentors, much as in Dr. Halsted's days, to produce capable HPB surgeons for future generations.

References

1. Ong E, Helton WS, Espat NJ. HPB other 168: operative experience of U.S. general surgery residents: liver and pancreas 1989–2001. J Gastrointest Surg. 2003;7:311.
2. Cheadle WG, Franklin GA, Richardson JD, Polk HC Jr. Broad-based general surgery training is a model of continued utility for the future. Ann Surg. 2004;239:627–636.
3. Livingston EH, Rege RV. Technical complications are rising as common bile duct exploration is becoming rare. J Am Coll Surg. 2005;201:426–433.
4. Dixon E, Vollmer CM, Bathe O, Sutherland F. Training, practice, and referral patterns in hepatobiliary and pancreatic surgery: survey of general surgeons. J Gastrointest Surg. 2005;9:109–114.
5. Fong Y, Gonen M, Rubin D, Radzyner M, Brennan MF. Long-term survival is superior after resection for cancer in high-volume centers. Ann Surg. 2005;242:540–547.
6. Ko CY, Maggard M, Agustin M. Quality in surgery: current issues for the future. World J Surg. 2005;29:1204–1209.
7. Debas HT, Bass BL, Brennan MF, et al. American Surgical Association blue ribbon committee report on surgical education: 2004. Ann Surg. 2005;241:1–8.
8. Ihse I, Haglund U. The Swedish 40-hour work week: how does it affect surgical care? Surgery. 2003;134:17–18.
9. Granger SR, Glasgow RE, Battaglia J, et al. Development of a dedicated hepatopancreaticobiliary program in a university hospital system. J Gastrointest Surg. 2005;9:891–895.
10. Tseng JF, Pisters PWT, Lee JE, Wang H, Gomez HF, Sun CC, Evans DB. The learning curve in pancreatic surgery. Surgery. 2007;141:456–463.
11. Anastakis DJ, Regehr G, Reznick RK, Cusimano M, Murnaghan J, Brown M, Hutchison C. Assessment of technical skills transfer from the bench training model to the human model. Am J Surg. 1999;177:167–170.
12. Cundiff GW, Weidner AC, Visco AG. Effectiveness of laparoscopic cadaveric dissection in enhancing resident comprehension of pelvic anatomy. J Am Coll Surg. 2001;192:492–497.
13. Lord JL, Cottam DR, Dallal RM, Mattar SG, Watson AR, Glasscock JM, Ramanathan R, Eid GM, Shauer PR. The impact of laparoscopic bariatric workshops on the practice patterns of surgeons. Surg Endosc. 2006;20:929–933.
14. Leach DC. Competence is a habit. JAMA. 2002;287:243–244.

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The Outcome of Laparoscopic Heller Myotomy for Achalasia is Not Influenced by the Degree of Esophageal Dilatation

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Abstract In the past, a Heller myotomy was considered to be ineffective in patients with achalasia and a markedly dilated or sigmoid-shaped esophagus. Esophagectomy was the standard treatment. The aims of this study were (a) to evaluate the results of laparoscopic Heller myotomy and Dor fundoplication in patients with achalasia and various degrees of esophageal dilatation; and (b) to assess the role of endoscopic dilatation in patients with postoperative dysphagia. One hundred and thirteen patients with esophageal achalasia were separated into four groups based on the maximal diameter of the esophageal lumen and the shape of the esophagus: group A, diameter <4.0 cm, 46 patients; group B, esophageal diameter 4.0–6.0 cm, 32 patients; group C, diameter >6.0 cm and straight axis, 23 patients; and group D, diameter >6.0 cm and sigmoid-shaped esophagus, 12 patients. All had a laparoscopic Heller myotomy and Dor fundoplication. The median length of follow-up was 45 months (range 7 months to 12.5 years). The postoperative recovery was similar among the four groups. Twenty-three patients (20%) had postoperative dilatations for dysphagia, and five patients (4%) required a second myotomy. Excellent or good results were obtained in 89% of group A and 91% of groups B, C, and D. None required an esophagectomy to maintain clinically adequate swallowing. These data show that (a) a laparoscopic Heller myotomy relieved dysphagia in most patients with achalasia, even when the esophagus was dilated; (b) about 20% of patients required additional treatment; (c) in the end, swallowing was good in 90%.

Keywords Esophageal achalasia · Esophageal manometry · Botulinum toxin · Pneumatic dilatation · Laparoscopic Heller myotomy · Esophagectomy

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Over the past decade, a laparoscopic Heller myotomy and partial fundoplication has become the standard treatment for patients with achalasia.[1–5] The operation is safe, has a short recovery period, and is more effective than balloon dilatation or intra-sphincteric botulinum toxin injection.[6,7] Although the diagnosis and treatment of achalasia are usually established early in the course of the disease, some patients still present later with a dilated and sigmoid-shaped esophagus.[8] The treatment of such end-stage achalasia is controversial, since some believe that an esophagectomy is indicated,[9,10] while others recommend a myotomy as the first step.[11,12]

In this study, we (a) compared the results of a laparoscopic Heller myotomy and Dor fundoplication (LHMD) in patients with achalasia and various degrees of esophageal dilatation, and (b) assessed the role of endoscopic dilatation for patients with postoperative dysphagia.

Materials and Methods

Between October 1993 and March 2006, 201 patients with esophageal achalasia underwent LHMD at the University of California San Francisco (UCSF). Among the 141 patients who had a preoperative barium swallow at UCSF (i.e., the X-rays were available), 113 were followed-up longer than 6 months. They comprised the study population (Fig. 1). Each patient had the following evaluation:

Symptom Assessment Symptoms were graded using a five-point scale, ranging from 0 (no symptom) to 4 (disabling symptom). The duration of symptoms was based on the number of months the patient reported experiencing dysphagia. This symptom was chosen as every patient experienced it and remembered its onset. Swallowing status was graded as follows: excellent (no dysphagia), good (occasional dysphagia), fair (frequent dysphagia requiring dietary adjustments), and poor (severe dysphagia preventing the ingestion of solid food or unintentional weight loss). The duration of symptoms and the type of previous treatment (endoscopic and surgical) were also recorded.

Radiology A barium esophagram was performed in all patients. The degree of esophageal dilatation was assessed by measuring the maximal esophageal width on a postero-anterior projection esophagogram using eFilm Lite software (Merge eMed, Division of Merge Healthcare, Milwaukee, WI, USA). The shape of the esophagus (straight versus sigmoid) was recorded.

Endoscopy An upper endoscopy was performed in some patients preoperatively and in all patients with postoperative dysphagia.

Esophageal Manometry The patients were studied after an overnight fast as previously described.[13] Medications that might interfere with esophageal motor function were discontinued at least 48 h before the study. The following were assessed: (a) resting pressure and swallowing-induced relaxation of the lower esophageal sphincter (LES), and (b) amplitude, duration and velocity of esophageal peristaltic waves. Achalasia was diagnosed if the following were present: (a) incomplete or absent LES relaxation, and (b) absent primary esophageal peristalsis.

Evaluation of Patients with Persistent or Recurrent Dysphagia Persistent dysphagia was defined as the persistence of dysphagia after the operation without a symptom-free interval. Recurrent dysphagia was defined as the return of dysphagia after a symptom-free interval while taking an unrestricted diet. Patients were referred to a gastroenterologist (J.O.) for nonsurgical intervention. Savary dilatation was first offered to all patients, with a range in size between 44 and 56 French. Pneumatic dilatation was used in the event that Savary dilatation was ineffective. Pneumatic dilatation was performed using Rigiflex balloons with a range in size between 2.5 and 4.0 cm. The number of sessions was based on the patient's response, and balloons of increasing size were used for repeated sessions.

Surgical treatment The myotomy was 8 cm long and extended for 2 cm onto the gastric wall.[1] In patients with a dilated and sigmoid esophagus, a more extensive dissection was performed in the posterior mediastinum to straighten the esophageal axis. If the hiatus was enlarged or if a hiatal hernia was present, one or two stitches were placed behind the esophagus to approximate the crural pillars. In two patients who had a Nissen fundoplication

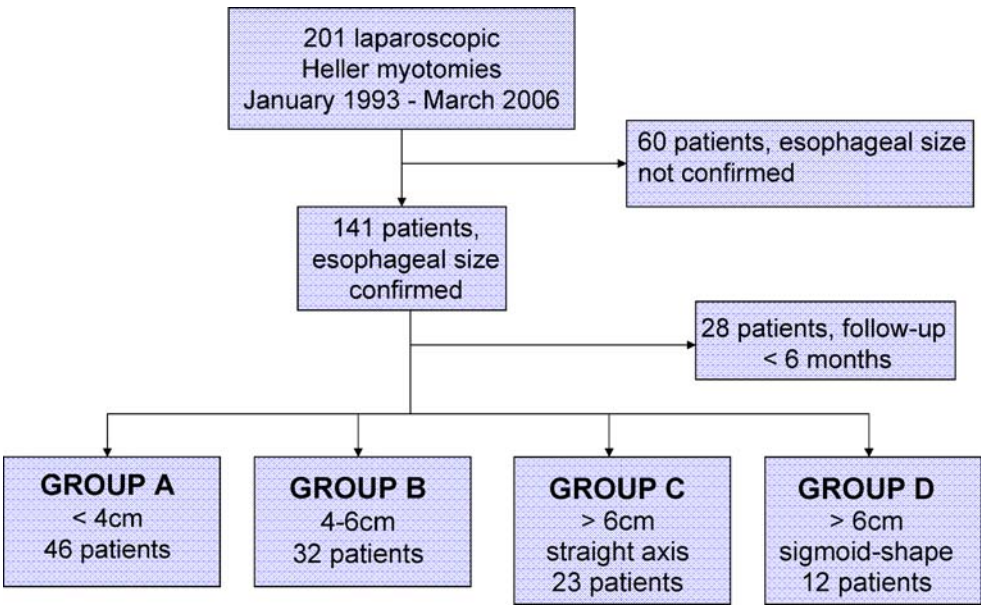

Figure 1 Study population and group assignment.

before coming under our care, the wrap was taken down before performing the myotomy. A more limited mediastinal dissection was performed in the five patients who had a previous left transthoracic Heller myotomy.

Follow-up The patients were seen 3 and 12 weeks postoperatively. Subsequent follow-up was through office visits or by telephone.

Statistical analysis Statistical analysis was performed using STATA Statistical Software: Release 9.1 (Stata Corporation, College Station, Texas, USA). Comparisons between groups used the Chi-square analysis of contingency table for dichotomous variables, the Kruskal-Wallis statistic for nonnormally distributed variables, and ANOVA followed by the *t* test for normally distributed data. The logistic regression model was used to examine covariates in a multivariate model of association with fair/poor outcome. Tests for interaction were performed and were not significant. The Hosmer-Lemeshow test with 10 groups was nonsignificant ($p=0.91$). Examination of model residuals identified no influential outliers.

The study protocol was reviewed and approved by the UCSF Committee on Human Subjects Research. All patients contacted by phone gave informed consent for study participation.

Results

A total of 113 patients were included, comprising 45 men and 68 women, whose mean age was 48 years. They had been symptomatic for a median of 54 months. The frequency of symptoms was as follows: dysphagia, 100%; regurgitation, 69%; chest pain, 51%; and heartburn, 48%. Seventy-eight patients (69%) had previous treatment: forty-five (40%) had pneumatic dilatations; seven (7%) had intra-sphincteric injections of Botulinum toxin; eighteen (16%) had both botulinum toxin and pneumatic dilatation; and eight (7%) had an operation: transthoracic Heller myotomy; 5; Nissen fundoplication, 2; and LHMD, 1 (Table 1).

Barium swallow The patients were placed into four groups based on the maximal diameter of the esophageal lumen and the shape of the esophagus: group A, diameter <4.0 cm, 46 patients; group B, diameter 4.0–6.0 cm, 32 patients; group C, diameter >6.0 cm and straight axis, 23 patients; and group D, diameter >6.0 cm and sigmoid-shaped esophagus, 12 patients.

Symptom Evaluation The preoperative symptoms were no different among the four groups (symptom score and the median duration of symptoms; Table 2).

Table 1 Demographics and Therapy Before Referral

	Group A	Group B	Group C	Group D
Total	46	32	23	12
Male	37%	47%	43%	25%
Mean age (range)	46 (14–78)	48 (20–76)	49 (24–77)	54 (25–84)
Pre-op dilation	48%	59%	59%	64%
Pre-op Botox[a]	15%	41%	18%	9%
Operations before referral	0	2	3	3

Group A: esophageal diameter <4 cm; Group B: esophageal diameter 4–6 cm; Group C: esophageal diameter >4 cm, straight esophageal axis; Group D: esophageal diameter >4 cm, sigmoid shaped esophagus

[a] Botox=Intra-sphincteric injection of Botulinum toxin.

Esophageal Manometry LES pressure and length did not differ among the 4 groups (Table 3). Peristalsis was absent in all patients.

Hospital Stay There was no difference among the groups (Table 4).

Perioperative Complications Five operations were converted to a laparotomy: one for a Verres needle puncture of the inferior vena cava, and four because of scar tissue from previous surgery. Major perioperative complications included: one patient had sepsis due to an intra-abdominal leak, which was successfully treated with percutaneous drainage and antibiotics; one patient had a splenic injury during a re-operation, which required a coincidental laparoscopic splenectomy; one patient had toxic shock syndrome due to tampons. Minor perioperative complications included pneumothorax, six patients; cardiac arrhythmia, one patient; and postoperative aspiration pneumonia, one patient.

Persistent Dysphagia Eight patients had persistent dysphagia (Table 5). Four (4%) had been initially treated at UCSF with a LHMD, and four had an operation elsewhere before coming to UCSF. At the conclusion of their treatment, swallowing was good in seven (Fig. 2).

Recurrent Dysphagia Twenty-three patients had recurrent dysphagia (Table 5). Four of them had transthoracic Heller myotomies elsewhere in the past. We performed a LHMD on the right side of the esophagus with good results (Fig. 3).

Nineteen patients (18%) had a LHMD at UCSF. Two underwent a second LHMD with good results. One patient had a herniated Dor fundoplication, and the other had a

Table 2 Preoperative Symptoms Score (Range 0–4; Median; Interquartile Range)

	Group A	Group B	Group C	Group D
Dysphagia	4 (3–4)	3 (3–4)	3 (3–4)	4 (3.5–4)
Regurgitation	2 (0–3)	2.5 (0–3)	3 (1–3.5)	3.5 (1.5–4)
Heartburn	1 (0–2)	0 (0–2)	0 (0–1)	0 (0–2.5)
Chest pain	1 (0–3)	1.5 (0–3)	0 (0–0.5)	2 (0–3)
Median duration of symptoms (years)	4 (1.5–8)	6 (1.5–10)	4 (2–10)	5 (2.3–8)

There were no statistically significant differences among the four groups. We excluded the 8 patients who had an operation prior to referral to our center. Group A: esophageal diameter <4 cm; Group B: esophageal diameter 4–6 cm; Group C: esophageal diameter >4 cm, straight esophageal axis; Group D: esophageal diameter >4 cm, sigmoid shaped esophagus

sigmoid-shaped esophagus that was straightened during a second operation.

Seventeen patients had dilatations as initial therapy. Three of six patients treated elsewhere had good outcomes with dilation alone. Of the other three, one had the Dor fundoplication taken down by another surgeon, which was complicated by vagal nerve injury, heartburn and regurgitation. One had another myotomy at UCSF with a good result. One patient has dysphagia despite several dilatations.

Seven of eleven patients treated with dilatation alone at UCSF had good results. In general, one or two Savary dilatations were done, and a pneumatic dilatation was performed if dysphagia persisted. One (group C) of the four patients who did not respond to pneumatic dilatation had a laparoscopic myotomy performed on the contralateral side of the esophagus. The dysphagia recurred and is presently managed with periodic dilations. He might eventually require an esophagectomy.

Overall, dysphagia was definitively relieved in 17 (74%) of the 23 patients who required dilatations.

Analysis Based on Maximum Esophageal Diameter When the data was analyzed based on maximum esophageal diameter and esophageal shape was not used to discriminate between groups C and D, then a trend towards an increased risk of a composite outcome of persistent or recurrent dysphagia was found. Nine of 46 (20%) of group A, 7 of 32 (22%) of group B, and 14 of 35 (40%) of groups C/D had either persistent or recurrent dysphagia (test of trend $p=0.046$). The odds-ratio of persistent or recurrent dysphagia among patients with esophageal diameter >6 cm compared to patients with an esophageal diameter <4 cm was 2.74 (95% CI 0.98–7.65). No statistically significant trend toward persistent or recurrent dysphagia were found when these two outcomes were analyzed separately ($p=0.23$ and 0.15, respectively).

Swallowing status At most recent follow-up, 102 (90%) of 113 patients are swallowing well: 89% of group A, and 91% of each of groups B, C and D ($p=0.99$; Table 6).

Logistic Regression Model A logistic regression model was created to examine factors associated with fair/poor outcome (Table 7). Given the small number of patients, group D was excluded from the analysis. The odds ratio of a fair/poor outcome if the patient had received botulinum toxin was 5.94 (95% CI 1.06–33.4; $p=0.043$) compared with other patients matched for age, sex, LES pressure and group. We examined the association between pneumatic dilatation and outcome in the same multivariate model (replacing the botulinum toxin variable with pneumatic dilatation). The use of pneumatic dilatation before to LHMD was not associated with a poor outcome, OR=

Table 3 Preoperative Esophageal Manometry (Mean±SD)

	Group A	Group B	Group C	Group D
LES pressure (mmHg)	23±13	24±12	20±12	21±12
LES total length (cm)	2.5±1.0	2.2±0.9	2.6±1.3	2.1±0.6
LES abdominal length (cm)	2.0±0.8	1.8±0.8	2.4±1.2	1.6±0.7

There were no statistically significant differences among the four groups. Group A: esophageal diameter <4 cm; Group B: esophageal diameter 4–6 cm; Group C: esophageal diameter >4 cm, straight esophageal axis; Group D: esophageal diameter >4 cm, sigmoid shaped esophagus

Table 4 Length of Hospital Stay

	Group A	Group B	Group C	Group D
Median LOS (days)	1.5	1	1	2
LOS≤48 hours	83%	87%	78%	73%

There were no statistically significant differences among the four groups; Group A: esophageal diameter <4 cm; Group B: esophageal diameter 4–6 cm; Group C: esophageal diameter >4 cm, straight esophageal axis; Group D: esophageal diameter >4 cm, sigmoid shaped esophagus

Table 5 Patients with Persistent or Recurrent Dysphagia

	Group A	Group B	Group C	Group D
Persistent dysphagia	2 (4%)	2 (6%)	2 (9%)	2 (17%)
Recurrent dysphagia	7 (15%)	6 (19%)	8 (35%)	2 (17%)
Either Persistent or Recurrent dysphagia	9 (20%)	7 (22%)	10 (44%)	4 (33%)

Group A: esophageal diameter <4 cm; Group B: esophageal diameter 4–6 cm; Group C: esophageal diameter >4 cm, straight esophageal axis; Group D: esophageal diameter >4 cm, sigmoid shaped esophagus

0.61 (95% CI 0.36–7.06, p=0.53). Other variables did not affect outcome. Given the large confidence interval, this model should be interpreted cautiously. Using three groups defined by maximal esophageal diameter, (A, B and C/D) did not alter the model.

Discussion

These findings show that (a) a laparoscopic myotomy was highly successful in treating achalasia even when the esophagus was markedly dilated; (b) dysphagia responded to endoscopic dilatation in most cases and rarely required long-term therapy.

Laparoscopic Heller Myotomy as Primary Treatment for Achalasia

Some surgeons recommend an esophagectomy as primary treatment for achalasia, on the assumption that a myotomy does not improve esophageal emptying and relieve dysphagia, especially if a sigmoid esophagus is present.[9,10] Others feel that a myotomy should be the first treatment and esophagectomy should be reserved for patients who have persistent symptoms.[11,12]

Among the 113 patients in this study, 3 (3%) might eventually require an esophagectomy. One patient (group D) had had an inappropriate laparoscopic Nissen fundoplication on the assumption that reflux caused her symptoms. Although the fundoplication was taken down and a Heller myotomy was performed, dysphagia persisted and did not respond to dilatation. The second patient (group C) had two myotomies, but dysphagia persisted, and swallowing is now marginally acceptable with periodic dilatations. The third patient (group C) had complications after a re-operation performed by other surgeons.

Myotomy proved to be technically feasible in every patient with a dilated and sigmoid esophagus (group D). A more extensive mediastinal dissection was required to straighten the distorted esophagus, but this did not lead to complications, and the improvement in swallowing was similar to what was seen in patients whose esophagus was of normal caliber. We also found that an esophagectomy could be avoided in most patients who had an unsuccessful previous transthoracic myotomy or multiple pneumatic

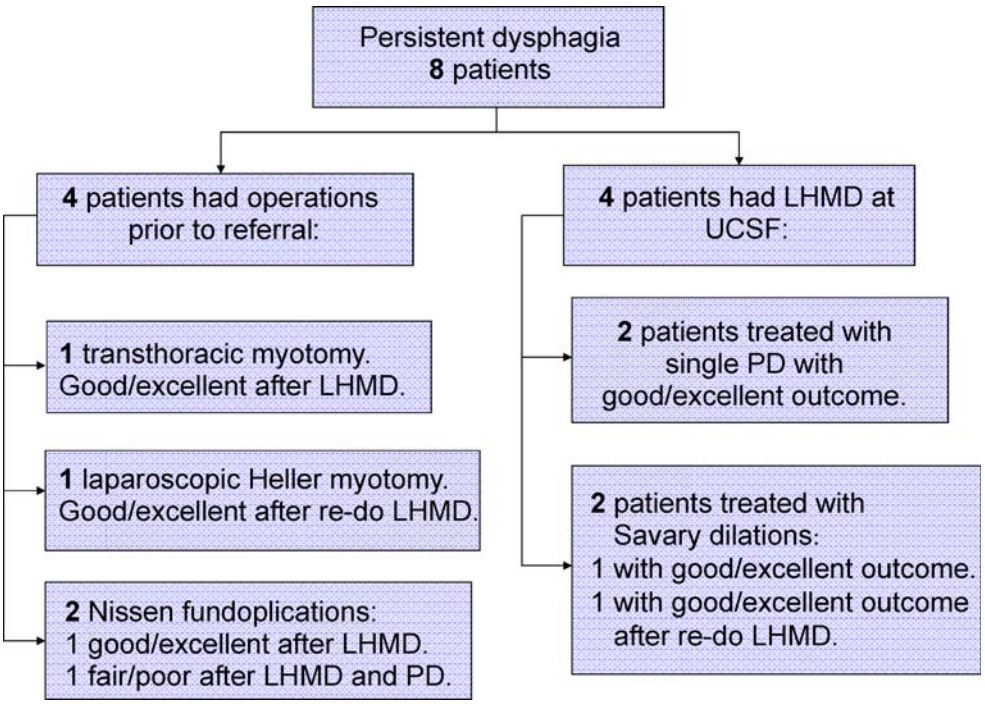

Figure 2 Outcome of patients with persistent dysphagia. *PD*, pneumatic dilatation. *LHMD*, laparoscopic Heller myotomy and Dor fundoplication.

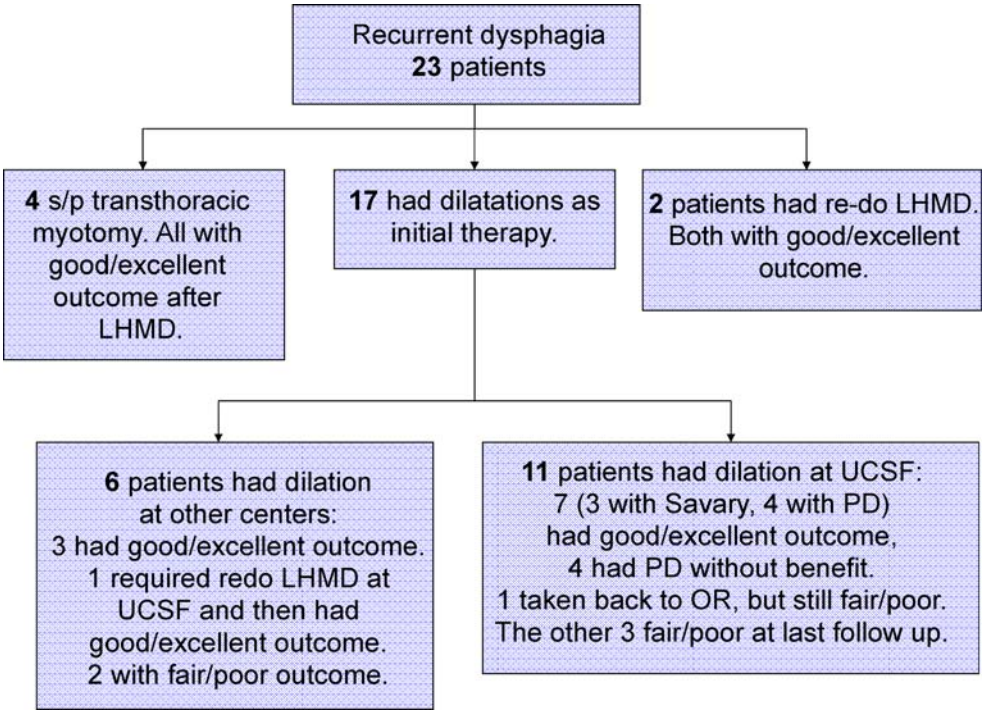

Figure 3 Outcome of patients with recurrent dysphagia. *PD*, pneumatic dilatation. *LHMD*, laparoscopic Heller myotomy and Dor fundoplication.

dilatations. In these cases, the laparoscopic myotomy was done on the right side of the esophagus. Although those patients with esophageal diameter >6 cm may be at increased risk for persistent or recurrent dysphagia when compared to those with less esophageal dilatation, this did not affect their ultimate swallowing status.

An esophagectomy should be avoided whenever possible because it is associated with a mortality rate of 3% even when done by the most experienced surgeons.[10,14] It also carries a high morbidity rate. For instance, Devaney and colleagues reported a 10% rate of anastomotic leak, 5% rate of hoarseness, and 2% rate of bleeding and chylothorax requiring a thoracotomy among 93 patients who had an esophagectomy for achalasia.[10] In addition, 46% of patients had dysphagia requiring dilatations, 42% had regurgitation, and 39% had dumping symptoms. The average hospital stay 12.5 days. The median hospital stay of our patients was less than 2 days, with 82% of patients leaving the hospital in under 48 h.

Table 6 Length of Follow-Up (Median) and Swallowing Status

	Group A	Group B	Group C	Group D
Months (range)	58 (7-150)	39 (9–124)	45 (9–128)	20 (12–98)
Excellent/ Good outcome	89%	91%	91%	91%

Group A: esophageal diameter <4 cm; Group B: esophageal diameter 4–6 cm; Group C: esophageal diameter >4 cm, straight esophageal axis; Group D: esophageal diameter >4 cm, sigmoid shaped esophagus

Previous Botox Treatment Lessens the Success of LHMD

Our results corroborate previous reports that treatment with botulinum toxin compromises the results of a Heller myotomy. Botulinum toxin too often produced inflammation and fibrosis, so the anatomic planes were less well defined and the dissection was more difficult. Others have confirmed these findings.[6,7,13,15,16] Since it is so marginally effective anyway, botulinum toxin treatment should probably be abandoned altogether.[16,17] On the other hand, pneumatic dilatation did not affect the results of LHMD.[13,18]

As an ancillary observation, preoperative LES pressure did not affect the outcome of the myotomy. The results in those with low LES pressure were the same as in those with a normal or a hypertensive LES. These findings contradict other reports that a good outcome is only achieved when the LES is hypertensive.[19] Thus, LHMD gave excellent relief in any achalasia patient, regardless of the preoperative manometric findings.

Table 7 Multivariate Logistic Model of Fair/Poor Outcome

	Odds ratio	95% CI	*P* value
Pre-op botox	5.94	1.06–33.4	0.04
Group B	0.60	0.11–3.36	0.56
Group C	0.70	0.11–4.55	0.70
Age	1.02	0.97–1.07	0.47
Male sex	1.93	0.41–9.03	0.40
LES pressure	0.94	0.86–1.02	0.14

Group D was excluded because of the insufficient number of patients
Botox, Intra-sphincteric injection of Botulinum toxin

Importance of a Multidisciplinary Approach in the Treatment of Achalasia

During the 1970s, 1980s, and 1990s, gastroenterologists used pneumatic dilatation only as the initial treatment for patients with achalasia. There was fear that a dilatation performed after a myotomy could result in a perforation. To the contrary, we found that postoperative dilatation was safe and effective. There were no perforations and the outcome was good in 59% of patients with postoperative dysphagia.

Conclusions

Our study has some limitations. This was not a series of consecutive patients, as they were selected based on the availability of the barium swallow for review. These 113 patients constitute 56% of the patients operated on during the same time frame. In addition, we did not have a comparison group of patients who had an esophagectomy for achalasia. Finally, our technique has somewhat evolved overtime as the myotomy we perform today is extended more onto the gastric wall as compared to the beginning of our laparoscopic experience.

Even considering these limitations, we feel that a laparoscopic Heller myotomy should be the primary treatment for achalasia regardless of the size and shape of the esophagus. Dilatation is very effective for the majority of patients who experience dysphagia after a myotomy. An esophagectomy should be reserved as a last resort for patients with dysphagia not amenable to other treatment.

References

1. Patti MG, Fisichella PM, Perretta S, Galvani C, Gorodner MV, Robinson T, Way LW. Impact of minimally invasive surgery on the treatment of achalasia. A decade of change. J Am Coll Surg 2003;196:698–705.
2. Oelschalager BK, Chang L, Pellegrini CA. Improved outcome after extended gastric myotomy for achalasia. Arch Surg 2003;138:490–497.
3. Richards WO, Torquati A, Holzman MD, Khaitan L, Byrne D, Lutfi R, Sharp KW. Heller myotomy versus Heller myotomy with Dor fundoplication. A prospective randomized double-blind clinical trial. Ann Surg 2004;240:405–415.
4. Khajanchee YS, Kanneganti S, Leatherwood AE, Hansen PD, Swanstrom LL. Laparoscopic Heller myotomy with Toupet fundoplication. Outcome predictors in 121 consecutive patients. Arch Surg 2005;140:827–834.
5. Bessell JR, Lally CJ, Schloithe A, Jamieson GG, Devitt PG, Watson DI. Laparoscopic cardiomyotomy for achalasia. Long-term outcomes. ANZ J Surg 2006;76:558–562.
6. Zaninotto G, Annese V, Costantini M, Del Genio A, Costantino M, Epifani M, Gatto G, D'Onofrio V, Benini L, Contini S, Molena D, Battaglia G, Tardio B, Andriulli A, Ancona E. Randomized controlled trial of botulinum toxin versus laparoscopic Heller myotomy for esophageal achalasia. Ann Surg 2004;239:364–370.
7. Portale G, Costantini M, Rizzetto C, Giurroli E, Ceolin M, Salvador R, Ancona E, Zaninotto G. Long-term outcome of laparoscopic Heller-Dor surgery for esophageal achalasia. Possible detrimental role of previous endoscopic treatment. J Gastrointest Surg 2005;9:1332–1339.
8. Shiino Y, Houghton SG, Filipi CJ, Awad ZT, Tomonaga T, Marsh RE. Manometric and radiographic verification of esophageal body decompensation for patients with achalasia. J Am Coll Surg 1999;189:158–163.
9. Peters JH, Kauer WKH, Crookes PF, Ireland AP, Bremner CG, DeMeester TR. Esophageal resection with colon interposition for end-stage achalasia. Arch Surg 1995;130:632–637.
10. Devaney EJ, Iannettoni MD, Orringer MB, Marshall B. Esophagectomy for achalasia. Patient selection and clinical experience. Ann Thorac Surg 2001;72:854–858.
11. Patti MG, Pellegrini CA, Horgan S, Arcerito M, Omelanczuk P, Tamburini A, Diener U, Eubanks TR, Way LW. Minimally invasive surgery for achalasia. An 8-year experience with 168 patients. Ann Surg 1999;230:587–594.
12. Mineo TC, Pompeo E. Long-term outcome of Heller myotomy in achalasic sigmoid esophagus. J Thorac Cardiovasc Surg 2004;128:402–407.
13. Patti MG, Feo CV, Arcerito M, De Pinto M, Tamburini A, Diener U, Gantert W, Way LW. Effects of previous treatment on results of laparoscopic Heller myotomy for achalasia. Dig Dis Sci 1999;11:2270–2276.
14. Pinotti HW, Cecconello I, Mariano da Rocha J, Zilberstein B. Resection for achalasia of the esophagus. Hepatogastroenterology 1991;38:470–473.
15. Eaker EY, Gordon JM, Vogel SB. Untoward effects of esophageal botulinum toxin injection in the treatment of achalasia. Dig Dis Sci 1997;42:724–727.
16. Smith CD, Stival A, Howell L, Swafford V. Endoscopic therapy for achalasia before Heller myotomy results in worse outcome than Heller myotomy alone. Ann Surg 2006;243:579–586.
17. Vaezi MF, Richter IE, Wilcox CM, Schroeder PL, Birgisson S, Slaughter RL, Koehler RE, Baker ME. Botulinum toxin versus pneumatic dilatation in the treatment of achalasia. A randomized trial. Gut 1999;44:231–239.
18. Gockel I, Junginger Th, Bernhard G, Eckardt V. Heller myotomy for failed dilatation in achalasia. How effective is it? Ann Surg 2004;239:371–377.
19. Arain MA, Peters JH, Tamhankar AP, Portale G, Almongy G, DeMeester SR, Crookes PF, Hagen JA, Bremner CG, DeMeester TR. Preoperative lower esophageal sphincter pressure affects outcome of laparoscopic esophageal myotomy for achalasia. J Gastrointest Surg 2004;8:328–334.

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The Evaluation of Esophageal Adenocarcinoma Using Dynamic Contrast-Enhanced Magnetic Resonance Imaging

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Abstract Although neoadjuvant chemoradiation eradicates esophageal adenocarcinoma in a substantial proportion of patients, conventional imaging techniques cannot accurately detect this response. Dynamic contrast-enhanced magnetic resonance imaging is an emerging approach that may be well suited to fill this role. This pilot study evaluates the ability of this method to discriminate adenocarcinoma from normal esophageal tissue. Patients with esophageal adenocarcinoma and control subjects underwent scanning. Patients treated with neoadjuvant therapy underwent pre- and postchemoradiation scans. Parameters were extracted for each pixel were K^{trans} (equilibrium rate for transfer of contrast reagent across the vascular wall), v_e (volume fraction of interstitial space), and τ_i (mean intracellular water lifetime). Five esophageal adenocarcinoma patients and two tumor-free control subjects underwent scanning. The mean K^{trans} value was 5.7 times greater in esophageal adenocarcinoma, and τ_i is 2.0 times smaller, than in the control subjects. K^{trans} decreased by 11.4-fold after chemoradiation. Parametric maps qualitatively demonstrate a difference in K^{trans}. DCE MRI of the esophagus is feasible. K^{trans}, a parameter that has demonstrated discriminative ability in other malignancies, also shows promise in differentiating esophageal adenocarcinoma from benign tissue. The determination of K^{trans} represents an in vivo assay for endothelial permeability and thus may serve as a quantitative measure of response to induction chemoradiation.

Keywords Esophageal adenocarcinoma · Dynamic contrast-enhanced magnetic resonance imaging · Neoadjuvant chemoradiation

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Introduction

Esophageal adenocarcinoma is a malignancy associated with a dismal prognosis, with an overall survival of less than 10% within 5 years.[1–3] The incidence rate of esophageal adenocarcinoma is rapidly rising, outpacing all other cancers. Since 1970, there has been a 350% increase in its incidence.[4–6] There are approximately 14,000 new cases of esophageal cancer per year in the United States, half of which are adenocarcinoma.[7]

There is a great need for strategies to improve the survival rate for patients with this disease, either by diagnosing the malignancy at an earlier stage when prognosis is more favorable, or by improving the efficacy of treatment. Under current standards, chemoradiation is administered preoperatively in the majority of patients with advanced locoregional disease.[8] One major problem hindering effective treatment in this context is that currently, there is no effective way to determine tumor response to chemoradiation, short of performing an esophagectomy.[9–13] An ideal tool to optimize esophageal adenocarcinoma

management would be able to detect the presence of adenocarcinoma with high sensitivity and specificity and assess the effect of chemoradiation on tumor burden. An imaging modality with these capabilities would serve to facilitate the "real-time" evaluation of therapeutic effectiveness with serial scanning, potentially eliminate the need for surgery in complete responders, improve the quality of palliation by stopping chemoradiation in those who progress with therapy, improve prognostication based on tumor response, facilitate the evaluation of new therapies, and enhance the quality of clinical trials.

Whereas early magnetic resonance imaging (MRI) studies using conventional clinical methods have demonstrated a limited ability to evaluate esophageal anatomy, dynamic contrast-enhanced MRI (DCE-MRI) identifies malignancies on the basis of their altered vascular integrity, which may result from pathologic angiogenesis.[14,15] This imaging approach entails the repeated scanning of an anatomic region as a bolus of intravenous contrast reagent (CR) is injected, and measuring the subsequent changes in signal intensity during bolus passage to detect areas of altered vascularity and/or vascular permeability.

Esophageal adenocarcinoma exhibits increased expression of proangiogenic factors including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).[16,17] This form of malignancy is associated with greater vascularization and has a higher microvascular density compared with normal esophageal tissue and precancerous lesions.[16–19] These properties suggest a valid pathologic basis upon which DCE MRI can detect esophageal adenocarcinoma and evaluate its response to chemoradiation.

We hypothesize that DCE MRI using the shutter-speed pharmacokinetic model ("shutter-speed MRI")[20–25] will be useful in the evaluation of esophageal adenocarcinoma. The purpose of this report is to demonstrate that anatomic and dynamic contrast-enhanced magnetic resonance images of the esophagus can be feasibly obtained and interpreted. It also serves as a pilot study to determine whether DCE can be used to discriminate benign esophageal tissue from esophageal adenocarcinoma.

Materials and Methods

Imaging Acquisition After intravenous access was achieved via a peripheral catheter, subjects were positioned in a 3-Tesla whole-body MRI instrument (Trio; Siemens, Malvern, PA, USA) and fitted with a cardiac RF transceiver coil. Pilot scanning was performed using a T_2-weighted half-Fourier acquisition single-shot turbo spin echo (HASTE) pulse sequence. A standard protocol for slice positioning was used. The esophagus was identified in coronal image slices taken with the HASTE localizer. The general axis of the esophagus can then be determined. Sagittal slices to be used for DCE-MRI were positioned parallel to this axis. A sufficient number of image slices were acquired to ensure visualization of the entire esophagus (typically four to seven slices) because it meanders through sagittal planes. For DCE-MRI imaging, we created a protocol based on a Turbo Flash pulse sequence, and adjusted it to yield the optimal image quality with the minimum image acquisition time. We used a 1.27 ms echo time, 250 ms repeat time, 17° flip angle, and 192×160 matrix size. At least 250 scans were performed sequentially. The initial scans provided the baseline (preinjection) images for the time courses. Gadoteridol (Prohance, Bracco Diagnostics, Inc, Princeton, NJ, USA), a gadolinium-based contrast agent, was dosed at 0.1 mmol/kg of body weight and delivered intravenously at 2 ml/s by a programmable power injector 20 s after scanning commences. This yielded a resolution of approximately 2 mm in the read-out and phase-encoding directions, and a temporal resolution of less than 2 s per scan. The image data were then transferred to off-line workstations for analysis.

Dynamic Contrast-Enhanced MRI A signal intensity time course for an anatomic region is generated from each pixel or region of interest (ROI). Such time courses exhibit reproducible shapes that appear to have the ability to differentiate benign from malignant tissue.[14,15,26,27]

To analyze time courses, pharmacokinetic models are used to generate predicted time courses that can be fitted to the observed shapes. A widely used method for analyzing the signal intensity time course relies on the "Tofts" model for pharmacokinetic analysis.[14] This is also referred to as the "standard model" (SM). A theoretical prediction is fitted to the observed time course by varying two parameters: K^{trans} (the equilibrium rate for the CR transfer across the vascular wall) and v_e (the interstitial space volume fraction).[28] The values for K^{trans} and v_e producing the best curve fitting can then be determined for the pixel or ROI data. K^{trans} is of particular interest since it has been used to discriminate benign from malignant breast tissue.[21,22,27] A map that displays the K^{trans} value for each pixel was produced to identify "hot spots", which characterize malignant tissues.

A more refined analysis, termed the "shutter-speed model" (SSM), was also used. This was developed at the Oregon Health & Science University Advanced Imaging Research Center[20–25] and can produce a better curve fitting, precisely for a malignant tumor, thereby more accurately discriminating malignant and benign tissue. The SSM takes into account the fact that equilibrium water exchange across the cell membrane is not infinitely fast, and introduces another parameter, the mean intracellular water lifetime (τ_i) (Table 1).

Table 1 Potential Parameters in the Standard and (First-generation) Shutter-speed Models [23]

	Potential parameters												
											Pharmacokinetic		
	NMR					Physiologic							
Homogeneous Models	r_{1p}	r_{1o}	R_{1b0}	R_{1o0}	R_{1i}	f_W	h_V	h	τ_b	τ_i	$v_p = v_b(1-h) = p_b(1-h)f_w$	$v_e = p_o f_w$	K^{trans}
Standard model	r_{1CF}	r_{1CF}	i.	i.	i.	0.8	0.5	0.3	i.	→0	→0	v.	v.
Shutter-speed model	r_{1CF}	r_{1CF}	e.	e.	R_{10}	0.8	0.5	0.3	i.	v.	→0	v.	v.

r_{1p} Contrast reagent (CR) relaxivity in plasma, r_{1o} CR relaxivity in interstitium, R_{1b0} relaxation rate constant of blood ^1H_2O before CR, R_{1o0} relaxation rate constant of interstitial space ^1H_2O before CR, R_{1i} relaxation rate constant of intracellular space ^1H_2O, R_{10} the measured, pre-CR value of tissue ^1H_2O R_1, f_w volume fraction accessible to mobile aqueous solutes, h_v macrovascular hematocrit, needed only if the arterial input function is directly measured, τ_i mean intracellular water lifetime, τ_b mean lifetime of a water molecule in the capillary, assuming no flow, v_p volume fraction of blood plasma, v_e volume fraction of the interstitial space, K^{trans} equilibrium rate for transfer of contrast agent from plasma to interstitial space, r_{1CF} cell free CR relaxivity, *i.* implied (treated as fixed value or not included in model), *h* microvascular hematocrit, *v.* varied (to fit measured time course), *e.* eliminated as a manipulated variable (calculated from other parameters)

The extraction of K^{trans}, v_e, and τ_i were performed with a Dell OptiPlex GX620 featuring 4 GB of RAM and a 2.8-GHz Pentium 4 processor, using an adaptation of BOLERO (BOLus Enhanced Relaxation Overview)[24] software—the first SSM generation.[23] This software package, implemented in MATLAB, was developed in-house. Allowing use of either the SM or SSM, this software analyzes DCE images and determines the optimal values of K^{trans}, v_e, and (if applicable) τ_i for fitting the observed signal time course of each pixel. These parameters are then mapped pixel by pixel to generate images based on each parameter.

Challenges with Esophageal MRI and Protocol Development Imaging the esophagus with magnetic resonance presents a unique set of challenges. Cardiac and respiratory motion, both of which affect the shape and position of the esophagus, may introduce motion artifacts. Motion is a particular issue in DCE-MRI because spatial registration between images taken at different time points is paramount. Breath holding for the entire DCE time course (several minutes) is not feasible. We therefore developed an imaging protocol in which images are acquired rapidly. The protocol minimizes the length of time for each scan, producing a higher sampling frequency that allows image fluctuations to at least partially "average out".

MRI anatomic planes containing the gastroesophageal junction are often indistinct, making it difficult to distinguish the distal esophagus from the surrounding tissues. Furthermore, the nearby presence of air-filled structures (such as the stomach and lungs) may affect the magnetic flux lines and introduce distortion in the images. To confirm the esophagus visualization and to structure the imaging protocol, commercially available blueberry juice (Brownwood Foods, Eastport, MI, USA) was used as a contrast reagent. Blueberry juice is suitable for this role because it contains a significant concentration of paramagnetic Mn^{2+}, which acts to reduce the ^1H_2O T_1 value, thereby providing a safe, inexpensive, and readily available MRI CR.[29–31] Under a protocol approved by the OHSU and Portland VAMC IRB, healthy subjects were positioned supine in the instrument with tubing, controlled by a handheld valve, through which they could ingest blueberry juice from a bottle. Anatomic images were acquired in three orientations using a HASTE sequence. After review of the images, a set of sagittal imaging planes was positioned over the putative location of the esophagus, and a series of images was repeatedly taken using a T_1-weighted protocol with the subject at rest and with ingestion of blueberry juice. Images were reviewed to determine whether this contrast reagent could be visualized. Dynamic contrast-enhanced MRI was conducted on separate days from scanning with oral contrast.

Evaluation of Regions of Interest Data in Normal and Malignant Tissue To assess the feasibility of using DCE-MRI to differentiate esophageal adenocarcinoma from normal esophageal tissue, data from ROIs in normal and malignant tissue were analyzed, with correction for changes in esophageal position because of motion. Two subjects participated in this portion of the study: one asymptomatic male who was endoscopically free of esophageal lesions and one male subject with a T4 esophageal adenocarcinoma easily identifiable with anatomic imaging. Subjects were positioned supine in the scanner and anatomic images were acquired in three orientations using the HASTE localizer. A set of four sagittal imaging planes was positioned over the putative esophagus position, and DCE MR images were repeatedly acquired. The CR dose was intravenously administered 20 s after scanning initiation. Scanning was continued until at least 250 image sets has been acquired. Images were then transferred to a postprocessing workstation. Regions of interest were manually outlined in

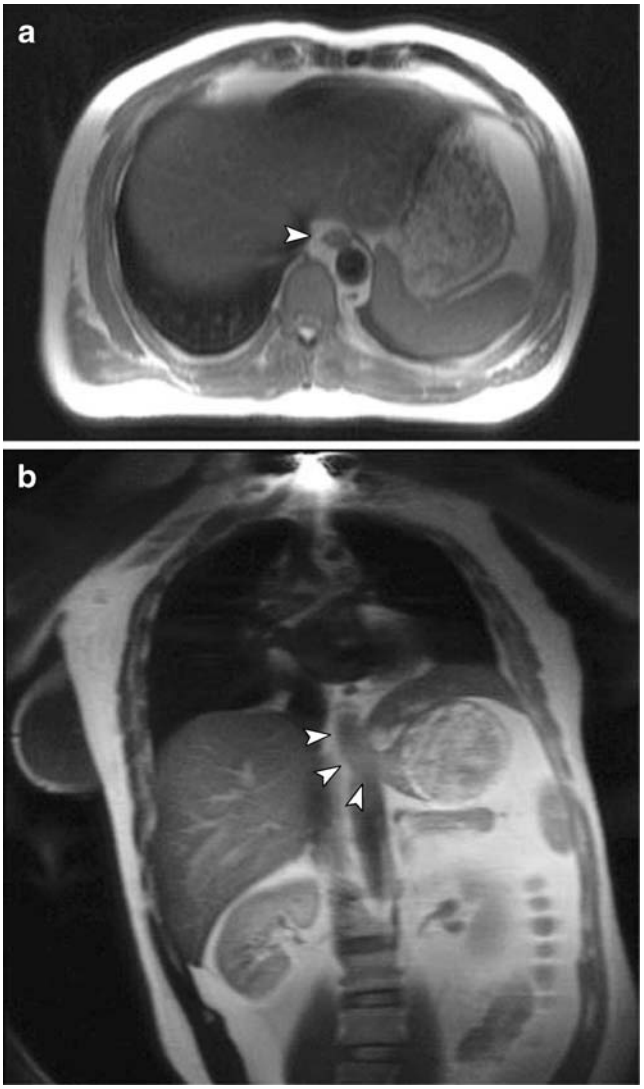

Figure 1 Anatomic images of the esophagus in **a** axial section and **b** coronal section, of a control subject taken with the T_2 weighted half-Fourier acquisition single-shot turbo spin echo (HASTE) sequence protocol. *Arrowheads* in the images indicate the location of the esophagus.

esophageal areas using Mass (Medis, Leesburg, VA, USA). These regions were redrawn for each time point to correct for esophageal movement because of cardiac activity and peristalsis. Regions of interest were also taken from the descending aorta for the purpose of determining arterial input functions. A signal intensity time course was plotted for each ROI. Curve fitting with the SM was used to extract two parameters (Table 1) from each time course: K^{trans} and v_e. The time courses were also analyzed with the SSM to extract K^{trans}, v_e, and τ_i. A signal intensity time course from an ROI in the aorta was used as the arterial input function for the pharmacokinetic analyses of data from each subject. K^{trans}, v_e, and τ_i values were compared between benign and esophageal tissues.

Evaluation of the Esophagus Using Parametric Maps For this portion of the study, patients with esophageal adenocarcinoma, and controls of similar ages, were included as subjects. Subjects underwent scans according to a study flow diagram. Patients with esophageal adenocarcinoma who underwent neoadjuvant chemoradiation underwent scanning before and after chemoradiation. Those who were treated with surgery alone underwent a single scan before esophagectomy. Sagittal images of the esophagus were obtained in the manner described above. To visualize the tumor while utilizing the maximal MRI spatial resolution, K^{trans}, v_e, and τ_i were evaluated for each pixel in the esophagus. Parametric maps were created by assigning each pixel a color according to the given parameter magnitude. These maps were coregistered with anatomic images and superimposed to facilitate interpretation. Images were reviewed by an observer blinded to the identities and diagnoses of the subjects. "Hotspots" were identified as areas with subjectively elevated K^{trans} values. Areas within these hotspots were outlined as regions of interest, and mean values for K^{trans}, v_e, and τ_i were determined for each subject. If no hotspots were subjectively apparent, a representative area of the esophagus was used as the region of interest. To avoid interpreting noise as a hotspot, each

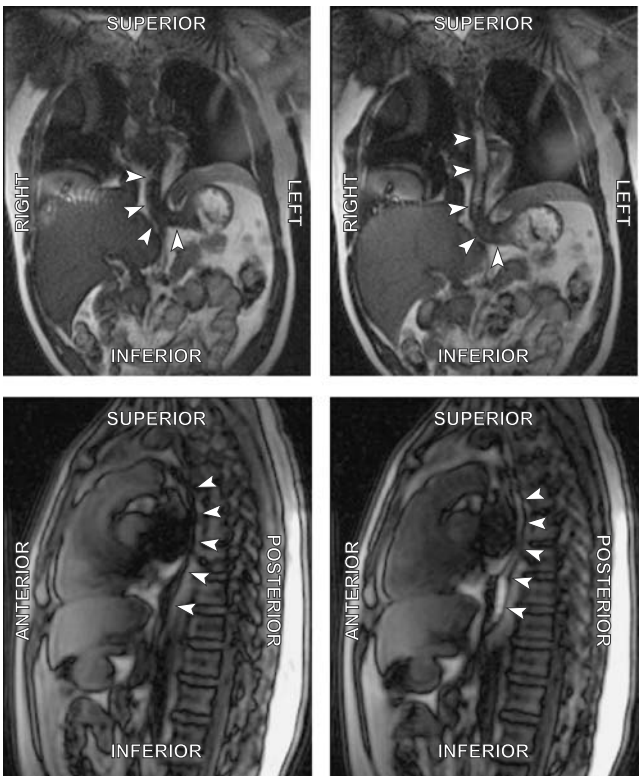

Figure 2 Confirmation of esophageal visualization with oral contrast reagent in another control subject. Coronal (*top*) and sagittal (*bottom*) images of the esophagus before (*left*) and during ingestion of blueberry juice (*right*). *Arrowheads* indicate the location of the esophagus.

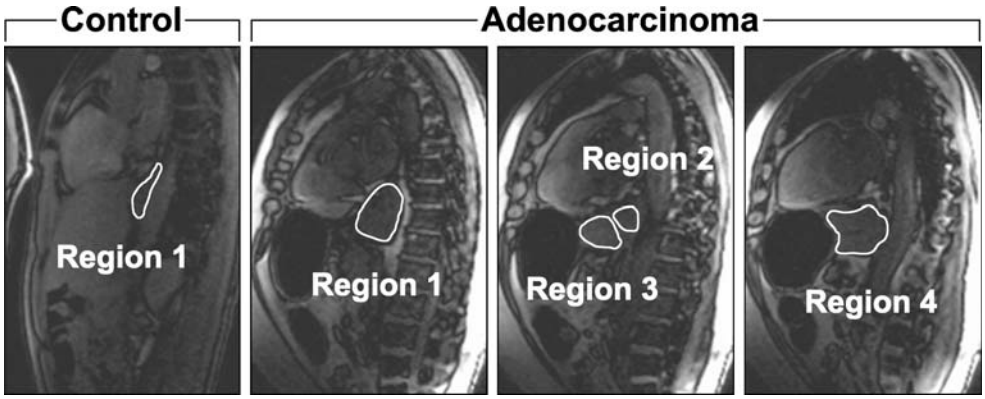

Figure 3 Regions of interest outlined in **a** a healthy control (shown in Figure 1) and **b** an esophageal adenocarcinoma patient. The mean signal intensity time courses from these regions of interest following Gd(III) monomeric chelate contrast reagent intraveneous injection were analyzed with the standard and shutter-speed pharmacokinetic models to evaluate K^{trans} (the equilibrium rate for contrast reagent transfer across the vascular wall), v_e (the volume fraction of interstitial space), and τ_i (mean intracellular water lifetime). The "foldover" seen in some of these images results from insufficiently large fields-of-view, but was deemed to not significantly affect the image intensities in the central portions of the slices, which were of interest.

region of interest had to include at least 15 pixels. K^{trans}, v_e, and τ_i were compared between patients with esophageal adenocarcinoma and normal controls. Map generation and image interpretation was performed using an in-house program written in MATLAB.

Results

Identification of the Esophagus Using a HASTE localizer, the esophagus was imaged in coronal, sagittal, and axial planes. Using this T_2-weighted protocol, the esophagus was readily identified in a control subject (arrowheads in Fig. 1). Cardiac motion and the presence of nearby air-filled structures generated only an acceptably low amount of distortion. Subjects and patients were then scanned with a T_1-based DCE-MRI protocol. Using this sequence, esophageal tissue was less distinct in the pre-CR images.

To ensure that T_1-weighted images included the esophagus, we performed earlier scans while administering an oral contrast reagent. Because we are aware of no product marketed as an oral CR for T_1-weighted ^1H_2O MRI, we used blueberry juice. Study subjects underwent coronal and sagittal imaging of the esophagus while ingesting the contrast material, as displayed in another control subject

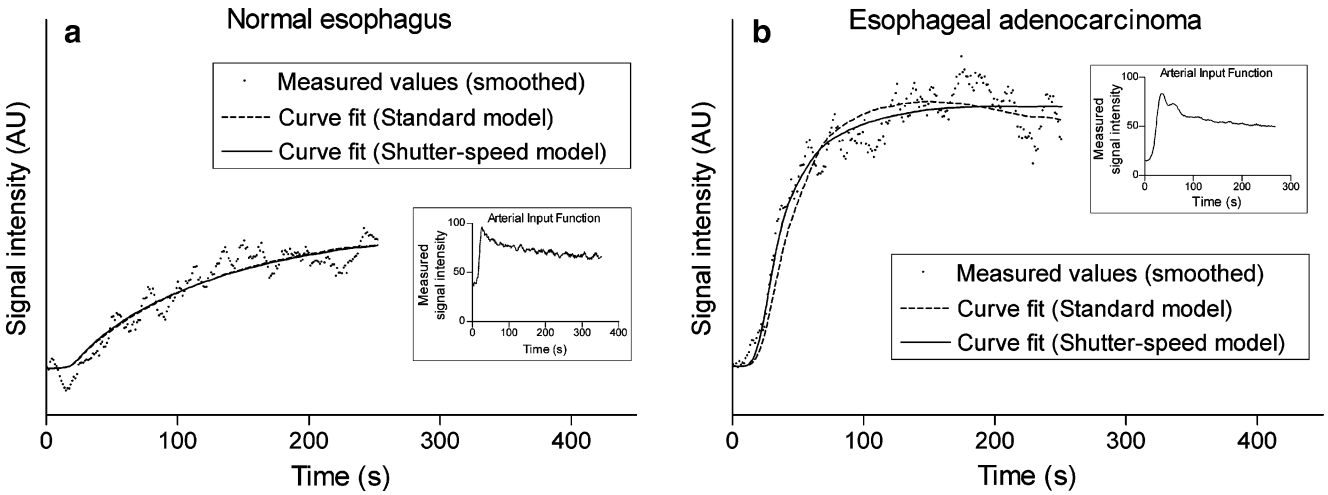

Figure 4 Signal intensity time courses from representative regions of interest (ROIs) taken from **a** the esophagus of a control subject (shown in Fig. 3a) and **b** the tumor in a patient with esophageal adenocarcinoma (shown in Fig. 3b). The *points* denote the measured values, which have been smoothed (by averaging adjacent values) to illustrate trends. *Periodic fluctuations* represent residual motional artifacts surviving smoothing. These periods are a few seconds. *Dashed curves* demonstrate the best-fittings generated using the standard model. Solid curves demonstrate the best-fittings generated using the shutter-speed model. (These are effectively indistinguishable in the normal esophagus.) Arterial input functions (*insets*) were measured from aortic ROIs. Note the more rapid initial signal intensity increase in the esophageal adenocarcinoma ROI, followed by a gradual decrease, suggesting greater vascular contrast reagent permeability in the tumor.

Table 2 K^{trans} (The Equilibrium Rate for CR Transfer Across the Vascular Wall), v_e (The Volume Fraction of Interstitial Space), and τ_i (Mean Intracellular Water Lifetime) Values Returned for Four ROIs (shown in Fig. 3) in a Subject with Esophageal adenocarcinoma, and one in a Healthy Control Subject by Shutter-speed Analyses

	K^{trans}	v_e	τ_i (s)
Adenocarcinoma			
Region 1	0.331	0.224	0.351
Region 2	0.295	0.794	0.516
Region 3	0.352	0.385	0.831
Region 4	0.121	0.167	0.165
Mean	0.275	0.393	0.466
Control			
Region 1	0.118	0.700	1.475

Regions of interest were drawn for each pharmacokinetic time point, to account for esophageal movement.

(Fig. 2). Before ingestion, the esophagus was identified only on the basis of its anatomic relationships to surrounding structures. Images taken during ingestion demonstrated the caudal passage of juice through the esophagus, confirming that the correct planes had been selected for esophageal imaging.

DCE MRI in Esophageal Tissue A subject with T4 esophageal adenocarcinoma and a control subject underwent DCE MRI. The study subject was a man with biopsy-proven esophageal adenocarcinoma, 7 cm in length. The control was a healthy man with normal esophageal mucosa demonstrated by endoscopy less than one month before the MRI, also shown in Fig. 1. Regions of interest were taken from a portion of normal esophageal tissue and from several portions of the adenocarcinoma (Fig. 3). These regions were adjusted from one pharmacokinetic image to another to correct for changes in esophageal position. Multiple ROIs were placed within the adenocarcinoma because the tumor was large and because portions of it demonstrated varying degrees of contrast enhancement. Signal intensity time courses from these ROIs qualitatively suggested that the CR uptake differs between esophageal adenocarcinoma and normal esophageal tissue (Fig. 4). Whereas the signal intensity increased slowly after administration of intravenous CR in normal esophageal tissue, in esophageal adenocarcinoma it initially increased more rapidly and then plateaued. These time courses were quantitatively analyzed using pharmacokinetic models that fitted predicted time courses to those observed, to determine K^{trans}, v_e, and (if applicable) τ_i. At first glance, it appears that the two models produce similar curves. However, there is a signature temporal mismatch by the standard model curve shape for the cancer that is exactly predicted by theory and simulation,[24] and found with all rodent[24,25] and human[20–22] malignant tumor data so far examined. This reflects a systematic error in the SM. It achieves its near fitting only at the expense of significantly underestimating the K^{trans} and v_e values, in some regions by factors of approximately two, compared with the SSM (not shown).

Parametric K^{trans} Maps Five patients and two control subjects underwent DCE MRI. Using the shutter-speed model, K^{trans}, v_e, and τ_i were evaluated for each pixel in the vicinity of the esophagus. "Hotspots" (area of increased K^{trans}, compared with the surrounding background) were identified as ROIs, and the mean K^{trans} was calculated in each of these hotspots (Fig. 5). On average, K^{trans} was 5.7-fold higher in esophageal adenocarcinoma patients than in controls (Table 3). A cutoff value of K^{trans} between 0.38 and 0.64 min^{-1} could be used to discriminate esophageal adenocarcinoma with 100% positive predictive value. This parametric range is sufficiently large to accommodate a larger patient population that would be more statically significant.

One patient with esophageal adenocarcinoma underwent shutter-speed MRI before and after neoadjuvant chemoradiation. A hotspot was seen in each scan. Measurements of K^{trans}, v_e, and τ_i in these hotspots demonstrated a substantial decrease in K^{trans} after chemoradiation (not shown). According to the tumor size determined by endoscopic ultrasound (EUS) (prechemoradiation) and the pathologic exam (postchemoradiation), this patient demonstrated a partial therapeutic response.

Discussion

Our initial DCE MRI experience demonstrates that this imaging technique is feasible and diagnostically informative in the detection of esophageal adenocarcinoma. Despite the difficulties of performing esophageal MRI, we were able to obtain interpretable anatomic images. Analysis of motion-corrected ROI data demonstrates that K^{trans} is greater in esophageal adenocarcinoma than in benign esophageal tissue. This study also demonstrates that pixel-by-pixel K^{trans} maps can be generated despite significant esophageal movement, suggesting that signal intensity fluctuations at any given pixel "average out" when enough time points are sampled.

Clinical Significance of K^{trans} In the DCE MRI analysis, K^{trans} is a pseudo first-order rate constant measuring the CR transfer between the intravascular space and the interstitial space within the tissue of interest. With cancer-related angiogenesis, the endothelium within tumor blood vessels may exhibit increased permeability thereby allowing a more

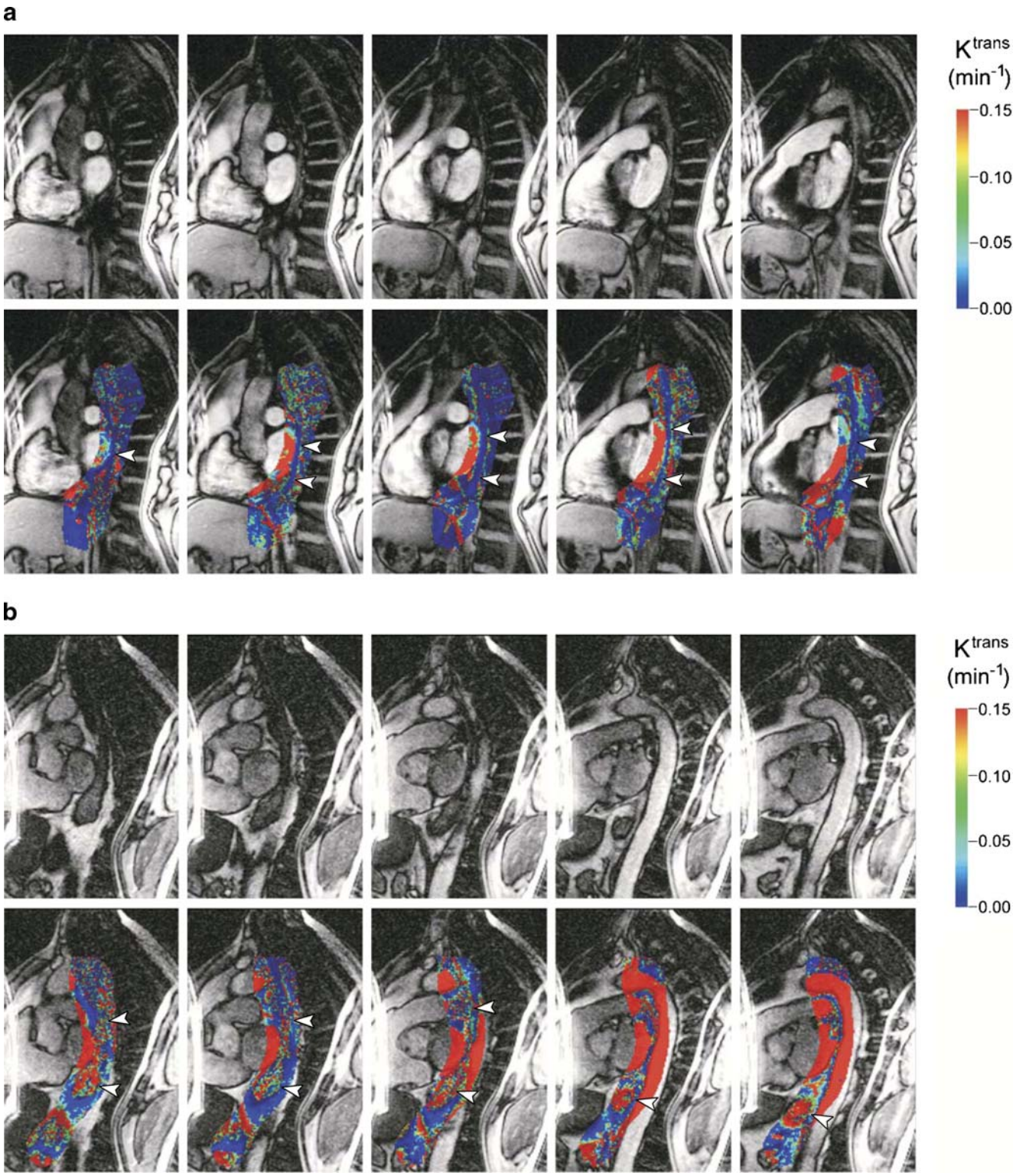

Figure 5 Parametic maps generated from K^{trans} values at each pixel, quantified with the color scales shown in **a** a healthy control and **b** a subject with esophageal adenocarcinoma. The parametric maps are coregistered and superimposed on anatomic images, which are also displayed in a row above the parametric maps. Each column shows a different sagittal image slice. The maps demonstrate larger K^{trans} values in the tumor as compared with normal esophageal tissue. *Arrowheads* indicate the location of the esophagus. The "foldover" seen in some of these images results from insufficiently large fields-of-view, but was deemed to not significantly affect the image intensities in the central portions of the slices, which were of interest.

Table 3 K^{trans} (The Equilibrium Rate for Transfer of Contrast Reagent Across the Vascular Wall), v_e (The Volume Fraction of Interstitial Space), and τ_i (Mean Intracellular Water Lifetime) Values Returned by Shutter-speed Analyses for Hotspots Identified on Parametric Maps of Five Subjects with Esophageal Adenocarcinoma and Two Healthy Control Subjects

	K^{trans} (min^{-1})	v_e	τ_i (s)
Adenocarcinoma			
Patient 1	0.69	0.40	0.66
Patient 2	0.64	0.46	0.87
Patient 3	0.91	0.62	0.78
Patient 4	2.41	0.68	0.68
Patient 5	0.78	0.71	1.03
Mean	1.09	0.57	0.80
Adenocarcinoma (postchemoradiation)			
Patient 4	0.21	0.54	0.40
Control			
Patient 6	0.00	0.49	2.19
Patient 7	0.38	0.42	1.00
Mean	0.19	0.45	1.59

One subject underwent scanning before and after neoadjuvant chemoradiation.

rapid transfer, when compared to benign tissue.[27] For the same reason the clearance of the contrast agent from the interstitium is usually also accelerated:[27] "fast in, fast out". Based on these characteristics, the K^{trans} parameter may prove useful for improved characterization of esophageal adenocarcinoma beyond currently available methods. K^{trans} may allow for a means of monitoring therapy and a gaining a more detailed understanding of individual tumor behavior, providing what is in essence an in vivo assay of endothelial permeability.

Assessing the Response to Chemoradiation Our findings suggest that shutter-speed MRI, particularly K^{trans}, shows promise in gauging response to chemoradiation, providing information that would be clinically very useful before committing a patient to esophagectomy. Patients with stage III disease typically undergo neoadjuvant chemoradiation, which has been demonstrated to offer a survival advantage.[32,33] Approximately 25% of patients who undergo preoperative chemoradiation develop a complete response as assessed by pathology analysis (an absence of tumor cells in the surgical specimen); these patients have an extended survival rate compared to nonresponders.[32,34–40] In patients with a partial response, the percentage of tumor reduction correlates directly with survival.[41] In fact, along with nodal status, the degree of response to preoperative chemoradiation independently predicts survival.[41] Along these lines, a number of patients will have progression of disease despite preoperative chemoradiation and are known to have very poor survival.[42]

Unfortunately, current diagnostic modalities, including EUS and combined CT-PET scanning, cannot reliably assess the degree of response to chemoradiation.[9–11,43–45] Endoscopic ultrasound lacks the specificity to differentiate post-therapy scarring from residual live tumor.[12,13] A number of studies have investigated the usefulness of CT-PET in assessing the response to chemoradiation in esophageal cancer, with varied results. While some studies demonstrate a correlation between CT-PET findings and response to chemoradiation,[46–49] others did not.[50,51] Furthermore, a negative CT-PET cannot distinguish small-volume residual disease from a complete response to chemoradiation.[50,52,53] As a result, complete response is discovered only at esophagectomy and subsequent histologic examination. Thus, even patients with a complete response to preoperative chemoradiation undergo a potentially unnecessary esophagectomy, which is accompanied by a significant morbidity rate arising from anastomotic strictures, leak, and conduit ischemia.[54]

Based on post-therapy changes in the vascular properties of tumors and adjacent lymph nodes, it is expected that shutter-speed MRI will enable clinicians to quantify the percentage of response to preoperative chemoradiation and identify nonresponders. This advance would enable physicians and researchers to individualize and improve treatment algorithms in several substantive ways: (1) facilitate a "real-time" evaluation of the effectiveness of therapy with serial scanning, (2) potentially eliminate the need for surgery in complete responders, (3) improve the quality of palliation by stopping chemoradiation in those who progress in response to therapy, (4) improve prognostication based on tumor response, (5) facilitate the study of new therapies, and (6) enhance the quality of clinical trials.

Conclusion

Shutter-speed MRI has great potential to impact the treatment of esophageal adenocarcinoma. It may improve the clinician's ability to stage patients to determine the most appropriate treatment. In patients who undergo neoadjuvant chemoradiation, it may provide a tool to gauge therapeutic response, a function that no other modality has been able to demonstrate. Given the promising results of this pilot study, a larger-scale study to evaluate the ability of shutter-speed MRI to determine the response to chemoradiation is warranted.

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References

1. Daly JM, Karnell LH, Menck HR. National Cancer Data Base report on esophageal carcinoma. Cancer 1996;78:1820–1828.
2. Ide H, Nakamura T, Hayashi K, Endo T, Kobayashi A, Eguchi R, Hanyu F. Esophageal squamous cell carcinoma: pathology and prognosis. World J Surg 1994;18:321–330.
3. Torres C, Turner JR, Wang HH, Richards W, Sugarbaker D, Shahsafaei A, Odze RD. Pathologic prognostic factors in Barrett's associated adenocarcinoma: a follow-up study of 96 patients. Cancer 1999;85:520–528.
4. Gopal DV, Powers J. Barrett's esophagus and esophageal adenocarcioma: a pratical approach to diagnosis and management. Ann Long Term Care 2002;10:27–31.
5. Spechler SJ. Screening and surveillance for complications related to gastroesophageal reflux disease. Am J Med 2001;111(Suppl 8A):130S–136S.
6. Spechler SJ. Clinical practice. Barrett's esophagus. N Engl J Med 2002;346:836–842.
7. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ. Cancer statistics, 2004. CA Cancer J Clin 2004;54:8–29.
8. Stahl M, Wilke H, Stuschke M, Walz MK, Fink U, Molls M, Siewert JR, Schroeder M, Makoski HB, Schmidt U, Seeber S, Vanhoefer U. Clinical response to induction chemotherapy predicts local control and long-term survival in multimodal treatment of patients with locally advanced esophageal cancer. J Cancer Res Clin Oncol 2005;131:67–72.
9. Zuccaro G Jr, Rice TW, Goldblum J, Medendorp SV, Becker M, Pimentel R, Gitlin L, Adelstein DJ. Endoscopic ultrasound cannot determine suitability for esophagectomy after aggressive chemoradiotherapy for esophageal cancer. Am J Gastroenterol 1999;94:906–912.
10. Melcher L, Wong W, Sanghera B, Bentzen SM, Hall M, Chambers J. Sequential FDG-PET scanning in the assessment of response to neoadjuvant chemotherapy in operable oesophageal cancer. J Clin Oncol 2004;22:327s.
11. Jones DR, Parker LA Jr, Detterbeck FC, Egan TM. Inadequacy of computed tomography in assessing patients with esophageal carcinoma after induction chemoradiotherapy. Cancer 1999;85:1026–1032.
12. Hordijk ML, Kok TC, Wilson JH, Mulder AH. Assessment of response of esophageal carcinoma to induction chemotherapy. Endoscopy 1993;25:592–596.
13. Laterza E, de Manzoni G, Guglielmi A, Rodella L, Tedesco P, Cordiano C. Endoscopic ultrasonography in the staging of esophageal carcinoma after preoperative radiotherapy and chemotherapy. Ann Thorac Surg 1999;67:1466–1469.
14. Tofts PS. Modeling tracer kinetics in dynamic Gd-DTPA MR imaging. J Magn Reson Imaging 1997;7:91–101.
15. Tofts PS, Berkowitz B, Schnall MD. Quantitative analysis of dynamic Gd-DTPA enhancement in breast tumors using a permeability model. Magn Reson Med 1995;33:564–568.
16. Couvelard A, Paraf F, Gratio V, Scoazec JY, Henin D, Degott C, Flejou JF. Angiogenesis in the neoplastic sequence of Barrett's oesophagus. Correlation with VEGF expression. J Pathol 2000;192:14–18.
17. Lord RV, Park JM, Wickramasinghe K, DeMeester SR, Oberg S, Salonga D, Singer J, Peters JH, Danenberg KD, Demeester TR, Danenberg PV. Vascular endothelial growth factor and basic fibroblast growth factor expression in esophageal adenocarcinoma and Barrett esophagus. J Thorac Cardiovasc Surg 2003;125:246–253.
18. Torres C, Wang H, Turner J, Shahsafaei A, Odze RD. Prognostic significance and effect of chemoradiotherapy on microvessel density (angiogenesis) in esophageal Barrett's esophagus-associated adenocarcinoma and squamous cell carcinoma. Human Pathol 1999;30:753–758.
19. Auvinen MI, Sihvo EI, Ruohtula T, Salminen JT, Koivistoinen A, Siivola P, Ronnholm R, Ramo JO, Bergman M, Salo JA. Incipient angiogenesis in Barrett's epithelium and lymphangiogenesis in Barrett's adenocarcinoma. J Clin Oncol 2002;20:2971–2979.
20. Yankeelov TE, Rooney WD, Huang W, Dyke JP, Li X, Tudorica A, Lee J-H, Koutcher JA, Springer CS. Evidence for shutter-speed variation in CR bolus-tracking studies of human pathology. NMR Biomed 2005;18:173–185.
21. Huang W, Li X, Morris EA, Tudorica LA, Venkatraman ES, Wang Y, Xu J, Springer CS. Shutter speed analysis of CR bolus-tracking data facilitates discrimination of benign and malignant breast disease. Proc Int Soc Magn Reson Med 2007;15:141.
22. Li X, Huang W, Yankeelov TE, Tudorica A, Rooney WD, Springer CS. Shutter-speed analysis of contrast reagent bolus-tracking data: Preliminary observations in benign and malignant breast disease. Magn Reson Med 2005;53:724–729.
23. Li X, Rooney WD, Springer CS. A unified MRI pharmacokinetic theory: intravascular and extracellular contrast reagents. Magn Reson Med 2005;54:1351–1359 (Erratum: Magn Reson Med 2006;55:1217).
24. Yankeelov TE, Rooney WD, Li X, Springer CS. Variation of the relaxographic "shutter-speed" for transcytolemmal water exchange affects the CR bolus-tracking curve shape. Magn Reson Med 2003;50:1151–1169.
25. Zhou R, Pickup S, Yankeelov TE, Springer CS, Glickson JD. Simultaneous measurement of arterial input function and tumor pharmacokinetics in mice by dynamic contrast enhanced imaging: effects of transcytolemmal water exchange. Magn Reson Med 2004;52:248–257.
26. Knopp MV, Weiss E, Sinn HP, Mattern J, Junkermann H, Radeleff J, Magener A, Brix G, Delorme S, Zuna I, van Kaick G. Pathophysiologic basis of contrast enhancement in breast tumors. J Magn Reson Imaging 1999;10:260–266.
27. Knopp MV, von Tengg-Kobligk H, Choyke PL. Functional magnetic resonance imaging in oncology for diagnosis and therapy monitoring. Mol Cancer Ther 2003;2:419–426.
28. Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV, Larsson HB, Lee TY, Mayr NA, Parker GJ, Port RE, Taylor J, Weisskoff RM. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. J Magn Reson Imaging 1999;10:223–232.
29. Hiraishi K, Narabayashi I, Fujita O, Yamamoto K, Sagami A, Hisada Y, Saika Y, Adachi I, Hasegawa H. Blueberry juice: preliminary evaluation as an oral contrast agent in gastrointestinal MR imaging. Radiology 1995;194:119–123.
30. Karantanas AH, Papanikolaou N, Kalef-Ezra J, Challa A, Gourtsoyiannis N. Blueberry juice used per os in upper abdominal MR imaging: composition and initial clinical data. Eur Radiol 2000;10:909–913.
31. Schmid MR, Hany TF, Knesplova L, Schlumpf R, Debatin JF. 3D MR gastrography: exoscopic and endoscopic analysis of the stomach. Eur Radiol 1999;9:73–77.
32. Walsh TN, Noonan N, Hollywood D, Kelly A, Keeling N, Hennessy TP. A comparison of multimodal therapy and surgery for esophageal adenocarcinoma. N Engl J Med 1996;335:462–467.
33. Mooney MM. Neoadjuvant and adjuvant chemotherapy for esophageal adenocarcinoma. J Surg Oncol 2005;92:230–238.
34. Donington JS, Miller DL, Allen MS, Deschamps C, Nichols FC 3rd, Pairolero PC. Tumor response to induction chemoradiation: influence on survival after esophagectomy. Eur J Cardiothorac Surg 2003;24:631–636; discussion 636–637.
35. Forastiere AA, Goepfert H, Maor M, Pajak TF, Weber R, Morrison W, Glisson B, Trotti A, Ridge JA, Chao C, Peters G,

Lee DJ, Leaf A, Ensley J, Cooper J. Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. N Engl J Med 2003;349:2091–2098.
36. Swisher SG, Ajani JA, Komaki R, Nesbitt JC, Correa AM, Cox JD, Lahoti S, Martin F, Putnam JB, Smythe WR, Vaporciyan AA, Walsh GL, Roth JA. Long-term outcome of phase II trial evaluating chemotherapy, chemoradiotherapy, and surgery for locoregionally advanced esophageal cancer. Int J Radiat Oncol Biol Phys 2003;57:120–127.
37. Berger AC, Farma J, Scott WJ, Freedman G, Weiner L, Cheng JD, Wang H, Goldberg M. Complete response to neoadjuvant chemoradiotherapy in esophageal carcinoma is associated with significantly improved survival. J Clin Oncol 2005;23:4330–4337.
38. Roth JA, Pass HI, Flanagan MM, Graeber GM, Rosenberg JC, Steinberg S. Randomized clinical trial of preoperative and postoperative adjuvant chemotherapy with cisplatin, vindesine, and bleomycin for carcinoma of the esophagus. J Thorac Cardiovasc Surg 1988;96:242–248.
39. Kelsen DP, Ginsberg R, Pajak TF, Sheahan DG, Gunderson L, Mortimer J, Estes N, Haller DG, Ajani J, Kocha W, Minsky BD, Roth JA. Chemotherapy followed by surgery compared with surgery alone for localized esophageal cancer. N Engl J Med 1998;339:1979–1984.
40. Urba SG, Orringer MB, Turrisi A, Iannettoni M, Forastiere A, Strawderman M. Randomized trial of preoperative chemoradiation versus surgery alone in patients with locoregional esophageal carcinoma. J Clin Oncol 2001;19:305–313.
41. Swisher SG, Hofstetter W, Wu TT, Correa AM, Ajani JA, Komaki RR, Chirieac L, Hunt KK, Liao Z, Phan A, Rice DC, Vaporciyan AA, Walsh GL, Roth JA. Proposed revision of the esophageal cancer staging system to accommodate pathologic response (pP) following preoperative chemoradiation (CRT). Ann Surg 2005;241:810–817; discussion 817–820.
42. Kim SB, Kim SH, Lee KH, Lee JW, Kim SW, Suh CW, Lee JS, Song HY, Chang HS, Choi EK, et al. Preoperative chemoradiotherapy for locoregional esophageal cancer: preliminary report. J Korean Med Sci 1995;10:111–120.
43. Yang Q, Cleary KR, Yao JC, Swisher SG, Roth JA, Lynch PM, Komaki R, Ajani JA, Rashid A, Hamilton SR, Wu TT. Significance of post-chemoradiation biopsy in predicting residual esophageal carcinoma in the surgical specimen. Dis Esophagus 2004;17:38–43.
44. Beseth BD, Bedford R, Isacoff WH, Holmes EC, Cameron RB. Endoscopic ultrasound does not accurately assess pathologic stage of esophageal cancer after neoadjuvant chemoradiotherapy. Am Surg 2000;66:827–831.
45. Cerfolio RJ, Bryant AS, Ohja B, Bartolucci AA, Eloubeidi MA. The accuracy of endoscopic ultrasonography with fine-needle aspiration, integrated positron emission tomography with computed tomography, and computed tomography in restaging patients with esophageal cancer after neoadjuvant chemoradiotherapy. J Thorac Cardiovasc Surg 2005;129:1232–1241.
46. Flamen P, Van Cutsem E, Lerut A, Cambier JP, Haustermans K, Bormans G, De Leyn P, Van Raemdonck D, De Wever W, Ectors N, Maes A, Mortelmans L. Positron emission tomography for assessment of the response to induction radiochemotherapy in locally advanced oesophageal cancer. Ann Oncol 2002;13:361–368.
47. Weber WA, Ott K, Becker K, Dittler HJ, Helmberger H, Avril NE, Meisetschlager G, Busch R, Siewert JR, Schwaiger M, Fink U. Prediction of response to preoperative chemotherapy in adenocarcinomas of the esophagogastric junction by metabolic imaging. J Clin Oncol 2001;19:3058–3065.
48. Song SY, Kim JH, Ryu JS, Lee GH, Kim SB, Park SI, Song HY, Cho KJ, Ahn SD, Lee SW, Shin SS, Choi EK. FDG-PET in the prediction of pathologic response after neoadjuvant chemoradiotherapy in locally advanced, resectable esophageal cancer. Int J Radiat Oncol Biol Phys 2005;63:1053–1059.
49. Kato H, Kuwano H, Nakajima M, Miyazaki T, Yoshikawa M, Masuda N, Fukuchi M, Manda R, Tsukada K, Oriuchi N, Endo K. Usefulness of positron emission tomography for assessing the response of neoadjuvant chemoradiotherapy in patients with esophageal cancer. Am J Surg 2002;184:279–283.
50. Brink I, Hentschel M, Bley TA, Walch A, Mix M, Kleimaier M, Moser E, Imdahl A. Effects of neoadjuvant radio-chemotherapy on 18F-FDG-PET in esophageal carcinoma. Eur J Surg Oncol 2004;30:544–550.
51. Downey RJ, Akhurst T, Ilson D, Ginsberg R, Bains MS, Gonen M, Koong H, Gollub M, Minsky BD, Zakowski M, Turnbull A, Larson SM, Rusch V. Whole body 18FDG-PET and the response of esophageal cancer to induction therapy: results of a prospective trial. J Clin Oncol 2003;21:428–432.
52. Brucher BL, Weber W, Bauer M, Fink U, Avril N, Stein HJ, Werner M, Zimmerman F, Siewert JR, Schwaiger M. Neoadjuvant therapy of esophageal squamous cell carcinoma: response evaluation by positron emission tomography. Ann Surg 2001;233:300–309.
53. Arslan N, Miller TR, Dehdashti F, Battafarano RJ, Siegel BA. Evaluation of response to neoadjuvant therapy by quantitative 2-deoxy-2-[18F]fluoro-D-glucose with positron emission tomography in patients with esophageal cancer. Mol Imaging Biol 2002;4:301–310.
54. Briel JW, Tamhankar AP, Hagen JA, DeMeester SR, Johansson J, Choustoulakis E, Peters JH, Bremner CG, DeMeester TR. Prevalence and risk factors for ischemia, leak, and stricture of esophageal anastomosis: gastric pull-up versus colon interposition. J Am Coll Surg 2004;198:536–541; discussion 541–532.

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Analysis of Anal Sphincter Preservation Rate According to Tumor Level and Neoadjuvant Chemoradiotherapy in Rectal Cancer Patients

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Abstract The anal sphincter preservation rate (ASPR) according to tumor level and neoadjuvant chemoradiotherpy (CRT) has not been fully evaluated. Therefore, the aim of this study was to evaluate the correlation between the tumor level, neoadjuvant CRT, and the ASPR in rectal cancer patients. We studied 544 patients (tumor level, 0–6 cm) who underwent curative resection for rectal cancer between 1991 and 2005. Patients were divided six into groups according to tumor level over 1-cm intervals, and the ASPR was evaluated in patients with and without neoadjuvant CRT according to tumor level. Sphincter preservation surgery was performed in 191 patients, and 86 patents underwent neoadjuvant CRT. The overall ASPR was 43.0% (37/86) in patients with neoadjuvant CRT and 33.6% (154/458) in patients without neoadjuvant CRT (P=0.094). In an analysis according to tumor level, the ASPR was 0.0 vs 0.0% in ≤1 cm, 0.0 vs 2.1% in 1≤2 cm (P=0.589), 11.8 vs 16.8% in 2≤3 cm (P=0.599), 55.6 vs 20.2% in 3≤4 cm (P=0.001), 57.7 vs 45.9% in 4≤5 cm (P=0.227), and 66.7 vs 69.5% in 5≤6 cm (P=0.827). Neoadjuvant CRT did not increase the ASPR in tumor level within ≤6 cm. However, for the tumor level (3≤4 cm), neoadjuvant CRT significantly increased the ASPR.

Keywords Anal sphincter preservation · Neoadjuvant chemoradiotherpy · Rectal cancer

Introduction

Since its first description by Miles[1] in 1908, abdominoperineal resection (APR) has been regarded as the standard surgery for patients with lower rectal cancer.[2] However, anal sphincter preservation has become an important issue for surgeons and patients with lower rectal cancer, and novel stapling instruments and innovative surgical techniques have allowed anal sphincter preservation surgery in many patients with lower rectal cancer.[3–5] Recently, the use of anal sphincter-preserving surgery has risen with increased knowledge of the pattern of tumor spread and the physiology of the anal sphincter mechanism. In addition, the use of neoadjuvant chemoradiotherapy (CRT) in patients with rectal cancer has increased, not only for the enhancement of local tumor control and survival but also for improvement of anal sphincter preserving surgery.[6–8] However, neoadjuvant CRT does not guarantee anal sphincter preservation in all rectal cancer patients because tumors of ≤1 cm are not amenable to sphincter preservation regardless of tumor responsiveness such as downsizing after neoadjuvant CRT.

Anal sphincter preserving surgery is influenced by tumor stage at the time of surgery, tumor distance from the anal verge, and responsiveness to neoadjuvant CRT.[9–13] Of these, tumor distance from the anal verge is the most important factor when selecting anal sphincter-preserving surgery for the treatment of low rectal cancer. However, the relationships among anal sphincter-preserving surgery, the distance from the anal verge, and the use of neoadjuvant CRT have not been fully addressed.

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Therefore, the purpose of this study was to evaluate the correlation between neoadjuvant CRT, the tumor distance from the anal verge, and the rate of sphincter preservation in rectal cancer patients.

Materials and Methods

Patients

Between January 1991 and December 2005, curative surgeries were performed for primary rectal cancer at the Severance Hospital, Yonsei University College of Medicine, Korea, in 2,296 patients who were histologically confirmed with adenocarcinoma and had no previous pelvis surgery, history of anal sphincter trauma, history of fecal incontinence, or pelvic radiotherapy. Other exclusion criteria were patients with tumor distance from anal verge >6 cm, patients who had undergone transanal excision and Hartmann's procedure, and patients with clinical stage T1 or T2 tumors. A final study population of 544 patients was selected, and their cases were reviewed retrospectively (Fig. 1). The concept for this study was approved by the institutional review board of Severance Hospital.

All patients underwent diagnostic workups consisting of computed tomography (CT) scans of the abdomen and pelvis, chest X-ray, routine blood work, proctoscopy, colonoscopy, and transrectal ultrasonography (TRUS). Pelvic magnetic resonance imaging (MRI) was used from 1994 to the present.

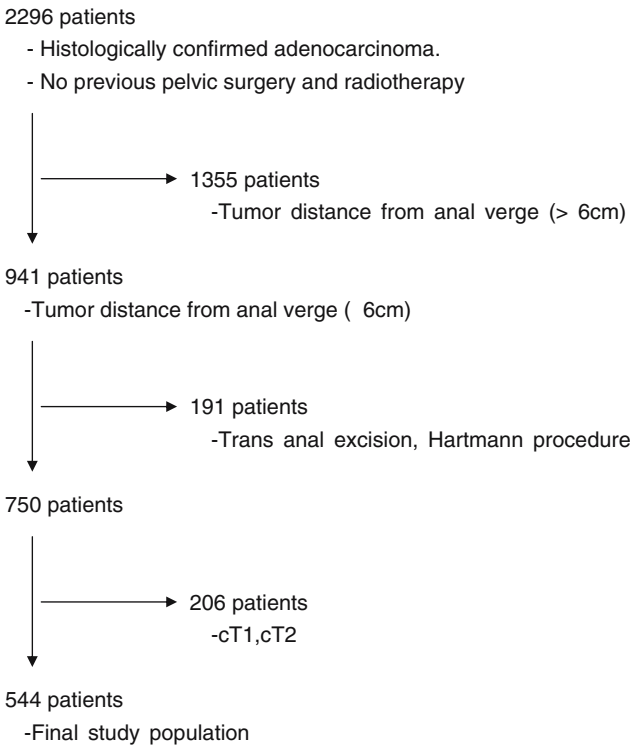

Figure 1 Flow chart of the selection process for the study group cT1 clinical T1 by pre-treatment imaging study; cT2 clinical T2 by pre-treatment imaging study.

Tumor distance from the anal verge was defined as the length between the distal border of the tumor and the anal verge and was measured in all patients at the time of diagnosis using rigid proctoscopy. The anal verge was defined as the border between the mucosa of the anal canal and the hair-bearing anal skin.

Surgical Resection

All surgery was performed by qualified colorectal surgeons at our institute, in accordance with the total mesorectal excision principle with curative intent. Anal sphincter preserving procedures were performed in patients who did not have direct tumor involvement of the levator or anal sphincter muscles. Procedures were performed four to 6 weeks after completion of neoadjuvant CRT or at the time of diagnosis in patients who did not receive neoadjuvant CRT. Low anterior resection (LAR) with colorectal anastomosis (CRA) and ultra low anterior resection (ULAR) with coloanal anastomosis (CAA) were used as anal sphincter-preserving procedures. CRA was performed using a double stapling method. If the distal rectal stump was insufficient for linear stapling, ULAR with CCA was performed instead. Details of these procedures have been described in a previous study.[14]

When a tumor was near the distal resection margin, frozen tissue sections were analyzed during the surgical procedure. If the tumor involved the resection margin, a completion APR was performed.

Neoadjuvant CRT

Neoadjuvant CRT was performed in patients with rectal cancer in stages cT3–cT4 or any N stage, as evaluated by pelvic MRI or abdomino-pelvic CT scan. However, as neoadjuvant CRT has not been routinely used in our institute, only 86 patients in this study received this treatment. Chemotherapy [5-fluorouracil (5-FU), 425 mg m^{-2} day^{-1} and leucovorin, 20 mg m^{-2} day^{-1}) was administered intravenously during weeks 1 and 5 of radiotherapy.

Radiation treatment was administered with a 6 MV/10 MV dual photon linear accelerator using a four-field box technique. The total radiation dosage was 5,040 cGY in 25 fractions delivered over 5 weeks. The radiation field was as follows: the upper margin was 1.5 cm above the sacral promontory (L5 level), the lateral margin was 1.5 cm laterally from the pelvic lateral wall, and the lower margin was 3 cm below the lower margin of the tumor.

Assessment

Patients were followed up at 3-month intervals during the first 3 years, at 6-month intervals during the fourth and fifth years, and annually thereafter for life. Medical histories, physical examinations, and blood tests with serum carcinoembryonic antigen were performed. Digital rectal examination was performed on all out-patients to detect local recurrences. CT and whole body bone scans were performed every year. Local tumor recurrences or systemic tumor recurrences were identified clinically by tissue diagnosis, where possible, or by CT or MRI. Positron emission tomography was used when possible.

Fecal incontinence was assessed for 45 patients who underwent ultra-low anterior resection and CAA using the Kirwan classification:[15] grade 1=perfect continence; grade II=flatus incontinence; grade III=minor soiling; grade IV=major soiling; and grade V=colostomy. Assessment of fecal incontinence was evaluated 6 months after closure of the diverting ileostomy.

Standardized pathology examinations were performed and the circumferential resection margin (CRM) was identified as described by Quirke et al.[16] Detailed technical procedures were described in our previous study.[17]

Statistical Analyses

Statistical analyses were performed using the statistical software package SPSS version 11.5 (SPSS, Chicago, IL, USA). Statistical comparisons were made using two-tailed X^2 tests or Fisher's exact test as appropriate for categorical variables and two-tailed Student's t tests for continuous variables. Recurrence and survival curves were calculated and compared using the Kaplan–Meier method and log-rank test, respectively. Values of $P<0.05$ were considered statistically significant.

Results

Patient Characteristics

Patient characteristics are summarized in Table 1. The study included 340 males and 204 females with a mean age of 56.8±12.1 years. Low anterior resection with CRA was performed in 146 patients (26.8%), ultralow anterior resection with CAA in 45 patients (8.2%), and APR in 353 patients (64.9%). Neoadjuvant CRT was given to 86 patients (15.8%), and complete response was noted in 7 patients (8.1%). Mean tumor distance from the anal verge was 4.2±1.4 cm, mean tumor size was 4.9±1.8 cm, and mean body mass index (BMI) was 22.6±2.9 kg/m^2. Clinical T stage was T3 in 510 patients (93.8%) and T4 in 34 patients (6.3%). Clinical N stage was N0 in 249 patients (45.8%) and N+ in 295 patients (54.2%).

Table 1 Patient Characteristics (n=544)

Characteristics	Number (%)
Mean age (years ± SD)	56.8±12.1
Sex	
Male	340 (62.5)
Female	204 (37.5)
Anal sphincter preserving surgery	
Yes	
LAR with CRA	146 (26.8)
ULAR with CAA	45 (8.2)
No	
APR	353 (64.9)
Neoadjuvant CRT	
Yes	86 (15.8)
No	458 (84.2)
Mean tumor distance from anal verge (cm ± SD)	4.2±1.4
Mean tumor size (cm ± SD)	4.9±1.8
Mean BMI (kg/m^2 ± SD)	22.6±2.9
Clinical T stage	
cT3	510(93.8)
cT4	34(6.3)
Clinical N stage	
cN(−)	249 (45.8)
cN(+)	295 (54.2)

SD Standard deviation; *LAR with CRA* low anterior resection with colorectal anastomosis; *uLAR with CAA* ultra low anterior resection with coloanal anastomosis; *APR* abdominoperineal resection; *CRT* chemoradiotherapy; *BMI* body mass index; *cT* clinical T stage by pre-treatment imaging study; *cN(−)*, clinical node-negative by pre-treatment imaging study; *cN(+)* clinical node-positive by pre-treatment imaging study

Factors Affecting Anal Sphincter Preservation Rate

Statistical analyses showed tumor distance from the anal verge and clinical T stage to be significant factors affecting the anal sphincter preservation rate. BMI and clinical N stage were not significant factors. Moreover, neoadjuvant CRT was not a significant factor affecting the anal sphincter preservation rate (P=0.094). Neoadjuvant CRT was performed in 19.4% of patients in the sphincter preservation group (n=191) and in 13.9% of patients in the group without sphincter preservation (n=353, Table 2).

Comparison of Anal Sphincter Preservation Rate with Respect Tumor Distance from Anal Verge in Patients with or without Neoadjuvant CRT

To further evaluate the effect of neoadjuvant CRT on the rate of anal sphincter preservation, results were re-analyzed with respect to tumor distance from the anal verge (Table 3). For tumors less than 1 cm from the anal verge, the sphincter

Table 2 Factors Affecting the Sphincter Preservation Rate in Rectal Cancer Patients

Factor	SP (n=191)	No SP (n=353)	P value
Tumor distance from anal verge (cm ± SD)	5.0±1.0	3.7±1.3	0.000
Tumor size (cm ± SD)	4.9±1.9	5.0±1.7	0.820
BMI (kg/m^2 ± SD)	22.8±2.9	22.5±3.0	0.247
T stage			0.000
3	190 (99.5%)	320 (90.7%)	
4	1 (0.5%)	33 (9.3%)	
N stage			0.917
0	88 (46.1%)	161 (45.6%)	
1	103 (53.9%)	192 (54.4%)	
Neoadjuvant CRT			0.094
Yes	37 (19.4%)	49 (13.9%)	
No	154 (80.6%)	304 (86.1%)	

SD Standard deviation; *SP* sphincter preservation; *BMI* body mass index; *CRT* chemo-radiotherpy

preservation rate was 0.0% in the CRT group vs 0.0% in the non-CRT group; for 1.1–2 cm tumors, the rate was 0.0 vs 2.9% (P=0.589); for 2.1–3 cm tumors, 11.8 vs 16.8% (P=0.599); for 3.1–4 cm tumors, 55.6 vs 20.2% (P=0.001); for 4.1–5 cm tumors, 57.7 vs 44.5% (P=0.227); and for 5.1–6 cm tumors, 66.7 vs 69.5% (P=0.827). In both CRT and non-CRT groups, tumors were evenly distributed over all distance intervals (Table 4).

Surgical Margins

For tumors less than 1 cm from the anal verge, the CRM involvement rate was 7.7% in the non anal sphincter preservation (SP) group. For tumors at 1.1–2 cm, the rate was 0.0% for the SP group vs 25.0% for no SP (P=1.000); at 2.1–3 cm, the rate was 5.3 vs 13.1% (P=0.463); at 3.1–4 cm, 9.7 vs 9.9% (P=1.000); at 4.1–5 cm, 7.8 vs 6.9% (P=1.000); and at 5.1–6 cm, 3.9 vs 8.8% (P=0.371). Overall, the CRM involvement rate was 6.3% in SP group and 11.9% in no SP group (Table 5).

In the SP group, the mean distal margin, assessed in fresh specimens without traction, was 2.6±0.92 cm (range 1–4 cm) in patients who underwent neoadjuvant CRT and 2.1±0.97 cm (range 1–4 cm) in patients who did not undergo neoadjuvant CRT.

Table 3 Comparison of Anal Sphincter Preservation Rate with Respect to Tumor Distance from the Anal Verge in Patients with or without Neoadjuvant CRT

Distance from the anal verge (cm)	No. of anal sphincter preserving procedures (%)				P value
	Neoadjuvant CRT (+)		Neoadjuvant CRT (−)		
≤1	0/0		0/13		
1.1–2	0/10	(0)	1/35	(2.9)	0.589
2.1–3	2/17	(11.8)	17/101	(16.8)	0.599
3.1–4	10/18	(55.6)	21/104	(20.2)	0.001
4.1–5	15/26	(57.7)	49/110	(44.5)	0.227
5.1–6	5/15	(66.7)	66/95	(69.5)	0.827
Total	37/86	(43.0)	154/458	(33.6)	0.094

CRT Chemoradiotherapy

Functional and Oncologic Outcomes

Forty-five patients who underwent CAA were evaluated 6 months after closure of the diverting ileostomy. Fifteen patients were treated with neoadjuvant, CRT and 30 patients did not receive with neoadjuvant CRT.

Perfect continence was noted in 10 patients (10/15, 66.7%) and 17 patients (17/30, 56.7%), respectively. Flatus or mucus leakage was noted in one patient (1/15, 6.7%) and three patients (3/30, 10.0%), respectively. Minor soling was noted in four patients (4/15, 26.7%) and ten patients (10/30, 33.3%), respectively (P=0.805). Major incontinence was not noted in either group.

The 5-year disease-free survival rate was 59.0% in the anal sphincter preservation group and 49.9% in the group with no anal sphincter preservation (P=0.017; Fig. 2). The overall local recurrence rate was 9.8%. The 5-year local recurrence rate was 6.0% in the anal sphincter preservation group and 10.7% in the anal sphincter non-preservation group (P=0.066; Fig. 3).

Discussion

Tumor size, stage, and distance from the anal verge have been reported to be important factors influencing the use of sphincter-preserving surgery in low-rectal cancer patients.[9–13] In addition, the surgical techniques and instruments used can influence the final outcome.[3–5]

Table 4 Distribution of Tumor Distance from Anal Verge in Patients with or without Neoadjuvant CRT

Distance from the anal verge (cm)	Neoadjuvant CRT (+)		Neoadjuvant CRT (−)		*P* value
	Number	Percent	Number	Percent	
≤1	0	(0)	13	(2.8)	0.345
1.1–2	10	(11.6)	35	(7.6)	
2.1–3	17	(19.8)	101	(22.1)	
3.1–4	18	(20.9)	104	(22.7)	
4.1–5	26	(30.2)	110	(24.0)	
5.1–6	15	(17.4)	95	(20.7)	
Total	86	(100)	458	(100)	

CRT Chemoradiotherapy

Neoadjuvant CRT in rectal cancer patients results in tumor size reduction, downstaging, especially T downstaging, and changes in the tumor distance from the anal verge due to tumor shrinkage. These tumor responsiveness after neoadjuvant CRT may allow complete resection of the rectal cancer and with an anal sphincter preserving procedure in circumstances where an APR was originally required for curative resection.[6–8,11,12] The mechanism of facilitating the performance of an anal sphincter preserving procedure after neoadjuvant CRT is that tumor responsiveness after neoadjuvant CRT may help to obtain safe distal and CRM regardless of anal sphincter preserving procedure.

However, it should be noted that the measurement methods or the definition of responsiveness to neoadjuvant CRT varies among studies. Depending on the study, different combinations of digital rectal examination, volumetric analysis for the measurement of tumor shrinkage, pathologic T or N downstaging, and tumor regression grading were used, and therefore the response to neoadjuvant CRT in specific cases was expressed differently, depending on the measurements used. In addition, responses ranged from non-response to complete response, and complete pathologic response rates varied from 8 to 27%.[18–20]

As an expression of tumor responsiveness after neoadjuvant CRT, downstaging only decreases the depth of invasion or nodal metastasis. Because of this, the tumor distance from the anal verge may not be increased sufficiently to allow anal sphincter preserving surgery regardless of downstaging, whereas tumor shrinkage after neoadjuvant CRT may be related to an increase in the tumor distance from the anal verge and may facilitate a sphincter preserving procedure. However, tumor shrinkage does not necessarily associate with downstaging because the depth of invasion and nodal involvement may not be decreased in patients who showed tumor shrinkage after neoadjuvant CRT. Moreover, tumor shrinkage does not always increase the rate of anal sphincter preservation, and anal sphincter preservation surgery will be impossible if the absolute tumor distance is ≤1 cm from the anal verge regardless of shrinkage after neoadjuvant CRT.

Thus, we hypothesized that it might not be possible to reliably predict when anal sphincter preserving surgery would be effective after neoadjuvant CRT.

Kim et al.[11] reported that neoadjuvant CRT improved sphincter preservation in rectal cancer within 3 cm of the anal verge. However, changes in chemotherapy protocols throughout the study period and the relatively small number of enrolled patients may have biased for their results, despite the fact that they analyzed the total effect of neoadjuvant CRT on sphincter preservation regardless of response status. Whereas, Crane et al.[12] showed that histological complete response to neoadjuvant CRT contributed to sphincter preserving surgery in patients with low rectal cancer. However, this result was confined to the group that responded completely to neoadjuvant CRT and not to all patients treated.

Interestingly, in our study, neoadjuvant CRT was not a significant factor affecting the rate of sphincter preservation except in patients with tumors between 3.1 and 4 cm from the anal verge. This result indicates that anal sphincter preserving surgery is technically difficult in patients with tumors less than 3 cm from the anal verge, regardless of tumor response after neoadjuvant CRT. Conversely, sphinc-

Table 5 Circumferential Resection Margin Status According to Anal Sphincter Preservation and Tumor Distance from Anal Verge

Distance from the anal verge (cm)	SP group			No SP group			P value
	CRM(+)	CRM(−)	Percent	CRM(+)	CRM(−)	Percent	
≤1	0	0	–	1	12	(7.7)	–
1.1–2	0	1	(0.0)	11	33	(25.0)	1.000
2.1–3	1	18	(5.3)	13	86	(13.1)	0.463
3.1–4	3	28	(9.7)	9	82	(9.9)	1.000
4.1–5	5	59	(7.8)	5	67	(6.9)	1.000
5.1–6	3	73	(3.9)	3	31	(8.8)	0.371
Total	12	179	(6.3)	42	311	(11.9)	0.037

SP Sphincter preservation; *CRM* circumferential resection margin

ter preserving surgery is possible in patients with tumor distances more than 4 cm from the anal verge, even if the patient did not receive neoadjuvant CRT or did not respond to it.

In our study, the enrolled population was treated with the same concept of curative surgical resection, the same selection criteria for anal sphincter preservation surgery, and the same dose and schedule of neoadjuvant CRT throughout the entire study period. Therefore, our results can reliably show the correlations between the effects of neoadjuvant CRT and tumor distance from the anal verge on the rate of sphincter preserving surgery. In addition, the large number of enrolled patients makes it possible to compensate for potential biases, which cannot be evaluated statistically, such as changes in surgical and radiotherapy techniques, instruments, and technology, or the surgeon's treatment philosophy. Furthermore, the number of patients who underwent neoadjuvant CRT was evenly distributed over the range of tumor distances from the anal verge, which allowed us to interpret our results more objectively.

The CRM is the most important factor determining local recurrence after rectal cancer surgery.[16] Achieving an adequate CRM is difficult in lower rectal cancer, especially during anal sphincter preserving surgery, because the mesorectum is either thin or lacking at this level. In our study, the CRM involvement rate of the anal sphincter preserving group was not higher than that in the group without anal sphincter preservation. Moreover, local recurrence rate and survival rate between the two groups were not significantly different. With respect to functional outcomes, our results showed acceptable results, and no statistical difference was observed between patients who underwent neoadjuvant CRT and those who did not.

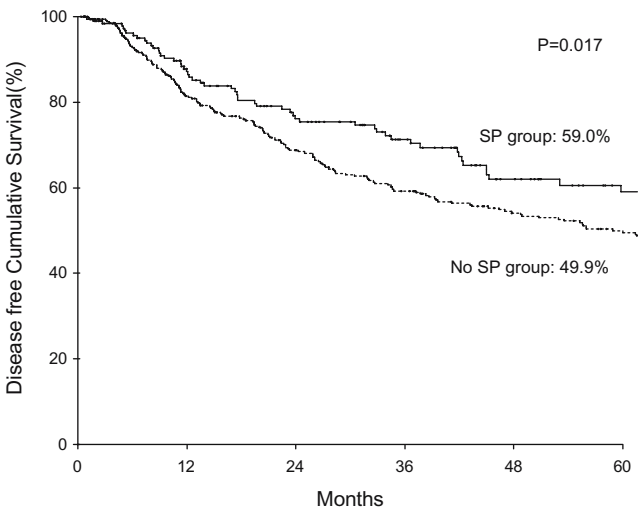

Figure 2 Five-year disease-free survival rate according to anal sphincter preservation rate SP group, anal sphincter preservation group; No SP group, no anal sphincter preservation group.

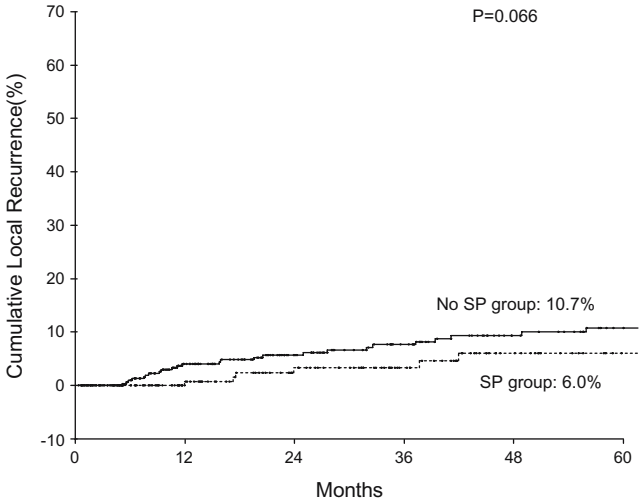

Figure 3 Five-year local recurrence rate according to anal sphincter preservation SP group, anal sphincter preservation group; No SP group, no anal sphincter preservation group.

The most widely used chemotherapeutic agent in colorectal cancer is 5-FU, which was used in the present study; however, numerous attempts have been made to improve oncologic outcomes, including biomodulation and protracted infusion.[21] Kim et al.[22] reported that neoadjuvant CRT using capecitabine achieved encouraging rates of tumor downstaging with a low toxicity. Moreover, preliminary results showed that neoadjuvant CRT with cetuximab and capecitabine was effective and that new preoperative radiation treatments such as high-dose neoadjuvant radiotherapy could provide a greater beneficial effect on tumor response and sphincter preservation.[23,24]

Recently, intersphincteric resection with additional partial external sphincteric resection was introduced as an alternative procedure that could be recommended for rectal cancer patients who are candidates for abdomoperineal resection.[25] These new therapeutic attempts may make it possible to perform anal sphincter-preserving surgery in patients with tumors less than 3 cm from the anal verge.

Conclusion

Neoadjuvant CRT was not a significant factor affecting the rate of anal sphincter preservation in low rectal cancer patients with tumor ≤6 cm from the anal verge. However, our analysis showed that neoadjuvant CRT significantly affected the rate of anal sphincter preservation when tumors were between 3.1 and 4 cm from anal verge.

This information will help to predict the possibility of anal sphincter preservation in low rectal cancer patients and will be a valuable aid to patient counseling.

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References

1. Miles WE. A method of performing abdomino-perineal excision for carcinoma of the rectum and the terminal portion of the pelvic colon. Lancet 1908;2:1812.
2. Rothengerger DA, Wong WD. Abdominoperineal resection for adenocarcinoma of the low rectum. World J Surg 1992;16:478–485.
3. Karanjiia ND, Schache DJ, North WR, Heald RJ. "Close shave" in anterior resection. Br J Surg 1990;77:510–512.
4. Lavery IC, Lopez-Kostner F, Fazio VW, Fernandez-Martin M, Milsom JW, Church JM. Chances of cure are not compromised with sphincter-saving procedures for cancer of the lower third of the rectum. Surgery 1997;122:779–785.
5. Cohen Z, Myers E, Langer B, Taylor B, Railton RH, Jamieson C. Double stapling technique for low anterior resection. Dis Colon Rectum 1983;26:231–235.
6. Marks G, Mohiuddin M, Masoni L. The reality of radical sphincter preservation surgery for cancer of the distal 3 cm rectum following high-dose radiation. Int J Radiat Oncol Biol Phys 1993;27:779–783.
7. Rouanet P, Fabre JM, Dubois JB, et al. Conservative surgery for low rectal carcinoma after high-dose radiation. Functional and oncologic results. Ann Surg 1995;221:67–73.
8. Wagman R, Minsky BD, Cohen AM, Saltz L, Paty PB, Guillem JG. Sphincter preservation in rectal cancer with preoperative radiation therapy and coloanal anastomosis: long-term follow-up. Int J Radiat Oncol Biol Phys 1998;40:569–574.
9. Tytherleight MG, McC Mortensen NJ. Options for sphincter preservation in surgery for low rectal cancer. Br J Surg 2003;90:922–933.
10. Swedish Rectal Cancer Trial. Improved survival with preoperative radiotherapy in respectable rectal cancer. N Engl J Med 1997;336:980–987.
11. Kim DW, Lim SB, Kim DY, et al. Pre-operative chemo-radiotherapy improves the sphincter preservation rate in patients with rectal cancer located within 3 cm of the anal verge. Eur J Surg Oncol 2006;32:162–167.
12. Crane CH, Skibber JM, Feig BW, et al. Response to preoperative chemoradiation increase the use of sphincter-preserving surgery in patients with locally advanced low rectal carcinoma. Cancer 2003;97:517–523.
13. Sauer R, Becker H, Hohenberger W, Rodel C, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 2004;351:1731–1740.
14. Baik SH, Kim NK, Lee KY, Sohn SK, Cho CH. Hand-sewn coloanal anastomosis for distal rectal cancer: long-term clinical outcomes. J Gastrointest Surg 2005;9:775–780.
15. Kirwan WO, Turnbull RB Jr, Fazio VW, Weakley FL. Pullthrough operation with delayed anastomosis for rectal cancer. Br J Surg 1978;65:695–698.
16. Quirke P, Durdey P, Dixon MF, Williams NS. Local recurrence of rectal adenocarcinoma due to inadequate surgical resection: histopathological study of lateral tumor spread and surgical excision. Lancet 1986;2:996–999.
17. Baik SH, Kim NK, Lee YC, et al. Prognostic significance of circumferential resection margin following total mesorectal excision and adjuvant chemoradiotherapy in patients with rectal cancer. Ann Surg Oncol 2007;14:462–469.
18. Grann A, Minsky BD, Cohen AM, et al. Preliminary results of preoperative 5 fluorouracil, low dose leucovorin and concurrent radiation therapy for clinically resectable T3 rectal cancer. Dis Colon Rectum 1997;40:515–522.
19. Medich D, McGinty J, Parda D, et al. Preoperative chemo-radiotherapy and radical surgery for locally advanced distal rectal adenocarcinoma: Pathologic findings and clinical implications. Dis Colon Rectum 2001;44:1123–1128.
20. Janjan NA, Khoo VS, Abbruzzese J, et al. Tumor downstaging and sphincter preservation with preoperative chemoradiation in locally advanced rectal cancer: The M.D. Anderson Cancer Center Experience. Int J Radiat Oncol Biol Phys 1999;44:1027–1038.
21. Tepper JE, O'Connell M, Niedzwiecki D, et al. Adjuvant therapy in rectal cancer: Analysis of stage, sex, and local control-final report of intergroup 0114. J Clin Oncol 2002;20:1744–1750.
22. Kim JC, Kim TW, Kim JH, et al. Preoperative concurrent radiotherapy with capcitabine before total mesorectal excision in locally advanced rectal cancer. Int J Radiat Oncol Biol Phys 2005;63:346–353.
23. Hofheinz RD, Horisberger K, Woernle C, et al. Phase I trial of cetuximab in combination with capecitabine, weekly irinotecan, and radiotherapy as neoadjuvant therapy for rectal cancer. Int J Radiat Oncol Biol Phys 2006;65:1384–1390.
24. Gerard JP, Chapet O, Nemoz C, et al. Improved sphincter preservation in low rectal cancer with high-dose preoperative radiotherapy: The lyon R96-02 randomized trial. J Clin Oncol 2004;22:2404–2409.
25. Saito N, Ono M, Sugito M, Ito M, et al. Early results of intersphincteric resection for patients with very low rectal cancer: an active approach to avoid a permanent colostomy. Dis Colon Rectum 2004;47:459–466.

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Analysis of Recurrence Pattern and Its Influence on Survival Outcome After Radiofrequency Ablation of Hepatocellular Carcinoma

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Abstract
Background Radiofrequency ablation (RFA) is an effective local ablation therapy for hepatocellular carcinoma (HCC) with favorable long-term outcome. There is no data on the analysis of recurrence pattern and its influence on long-term survival outcome after RFA in HCC patients.
Aim of Study To evaluate the tumor recurrence pattern and its influence on long-term survival in patients with HCC treated with RFA.
Patients and Methods From April 2001 to January 2005, 209 patients received RFA using internally cooled electrode as the sole treatment modality for HCC. Among them, 117 patients (56%) had unresectable HCC because of bilobar disease, poor liver function, and/or high medical risk for resection; whereas 92 patients (44%) underwent RFA as the primary treatment for small resectable HCC. The ablation procedure was performed through percutaneous (n=101), laparoscopic (n=17), or open approaches (n=91). The tumor recurrence pattern and long-term survival were analyzed. Multivariate analysis was carried out to identify independent prognostic factors affecting the overall survival of patients.
Results The mortality and morbidity rates were 0.9 and 15.7%, respectively. Complete tumor ablation was achieved in 192 patients (92.7%). With a median follow-up period of 26 months, local recurrence occurred in 28 patients (14.5%). Same segment and different segment intrahepatic recurrence occurred in 30 patients (15.6%) and 78 patients (40.6%), respectively. Twenty patients (10.4%) developed distant extrahepatic metastases. The overall 1-, 3-, and 5-year survival rates were 87.2, 66.6, and 42%, respectively. Different segment intrahepatic recurrence and distant recurrence after RFA carried significant poor prognostic influence on overall survival outcome. Using multivariate analysis, Child–Pugh grade (risk ratio [RR]=2.918, 95% confident interval [CI] 1.704–4.998, p=0.000), tumor size (RR=1.231, 95% CI 1.031–1.469, p=0.021), and pattern of recurrence (risk ratio [RR]=1.464, 95% CI 1.156–1.987, P=0.020) were identified as independent prognostic factors for overall survival.
Conclusion The tumor recurrence pattern after RFA carries significant prognostic value in relation to overall survival. Long-term regular surveillance and aggressive treatment strategy are required for patients with different segment intrahepatic recurrence to optimize the benefits of RFA.

Keywords Recurrence · Pattern · Radiofrequency · Ablation · Hepatocellular · Carcinoma

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Introduction

Management of hepatocellular carcinoma (HCC) has long been a great challenge to clinicians because of the aggressive tumor and its high propensity of vascular invasion. Moreover, high prevalence of cirrhosis among patients with HCC has further complicated the treatment strategy. Hepatic resection is regarded as the curative treatment option for HCC patients with low mortality.[1,2] Nevertheless, the associated cirrhosis limits the extent of surgery and thus increases the risk of postoperative liver failure. With advancement in the technology of local ablation therapy, radiofrequency ablation (RFA) has widely been used to treat

patients with unresectable HCC with a curative intent.[3,4] This localized thermal ablation technique utilizes high frequency alternating current to induce ionic vibration in targeted tumor. The resulting in situ lethal temperature (>60°C) leads to irreversible cellular changes, including intracellular protein denaturation, melting of membrane lipid bilayers, and coagulative necrosis of individual tumor cells. Early series of RFA have shown its safety and short-term efficacy for HCC patients.[5–7]

Over the past years, the rapid development and refinement of RFA technology has led to an increasing applicability of this treatment modality in HCC patients. At present, significant long-term survival results have been reported in a few studies.[8–13] Favorable overall 5-year survival rates can be achieved, ranging from 33 to 55.4%. Nevertheless, the tumor recurrence rates were inevitably high, up to 83%. Intrahepatic recurrences, including local recurrence and distant intrahepatic recurrence, accounted for the majority of tumor recurrence after RFA in these series. Whereas local recurrence at RFA site is caused by the untreated viable tumor cells, distant intrahepatic recurrence is either related to new tumor occurrence from the underlying cirrhosis or resulted from tumor metastases via intrahepatic portal venous system. Theoretically, intrahepatic tumor recurrence confined to the same liver segment as RFA site may have different pathogenesis from those occurred in different liver segments. It is postulated that the former is more likely caused by tumor spread along the same segmental portal vein, whereas the latter is the result of multicentric tumor occurrence in cirrhotic liver. Therefore, the biological characteristics of recurrent tumor between same segment intrahepatic recurrence and different segment intrahepatic recurrence may differ, and hence, the associated prognosis may vary. The influence of such recurrence pattern on survival outcome of HCC patients after RFA carries important clinical implication and has not been mentioned in previous studies. With the prognostic values of RFA recurrence pattern, the treatment strategy of RFA for HCC patients can be optimized to improve patients' overall outcome. Thus, the present retrospective study aimed to evaluate the recurrence pattern and its influence on survival outcome after RFA for HCC patients in a single tertiary referral center.

Patients and Methods

Selection of Patients

From April 2001 to January 2005, 209 patients received RFA as the sole treatment for HCC at Queen Mary Hospital, the University of Hong Kong. Patients with tumor invasion to major intrahepatic blood vessels or with extrahepatic metastases were excluded from the study. In 130 patients (62.2%), diagnosis of HCC was made by fine-needle (21-gauge) aspiration cytology in patients undergoing percutaneous RFA or by intraoperative core biopsy using an 18-gauge biopsy needle in patients undergoing laparoscopic, thoracoscopic, or open RFA. For the remaining 79 patients (37.8%), the diagnosis of HCC was based on the radiological features in computed tomography (CT) scan or magnetic resonance imaging (MRI) and/or raised serum α-fetoprotein concentration (over 400 ηg/ml). One hundred and seven patients (51.2%) had unresectable HCC because of bilobar disease, poor liver function, or high medical risk for hepatic resection; whereas 92 patients (44.0%) received RFA as primary treatment for potentially resectable small HCC (up to 5 cm for a solitary tumor or up to three nodules, each up to 3 cm in diameter). Patients with tumors within the size and number limit over both lobes of liver were considered to have potentially resectable small HCC. Fifty patients (23.9%) had previous hepatic resection and received RFA for intrahepatic recurrent tumors. Fifty patients (23.9%) had previous transarterial chemoembolization (TACE) for unresectable HCC, and RFA was performed for tumors that did not respond to TACE, the last dose of which was given more than 2 months before RFA. RFA was performed for solitary tumor in 160 patients (76.5%), 2 tumors in 30 patients (14.3%), 3 tumors in 10 patients (4.7%), and 4 tumors in 9 patients (4.3%). Each treatment underwent a single session of RFA, and a total of 286 tumor nodules were ablated in a single session of RFA.

Approaches of Radiofrequency Ablation

The guideline to choose different approaches for RFA in our center has been mentioned in our previous study.[14] Patients with small HCC (up to 5 cm) at favorable location for percutaneous RFA were treated with this approach. Under local anesthesia and intravenous sedation using midazolam and merperidine, RFA was carried out using transcutaneous ultrasonographic or CT scan guidance. RFA through open approach was considered in the presence of the following: (1) large tumors (over 5 cm in diameter) that required multiple overlapping ablation zones to cover the entire tumor with adequate ablation margin of 0.5 cm; (2) tumors located near the liver dome, for which percutaneous RFA might cause lung injury; and (3) tumors close to visceral organs, such as gallbladder, small and large bowels, and stomach. Laparotomy under general anesthesia and mobilization of the liver lobes were required in open RFA. In selected patients without previous abdominal surgery, the laparoscopic approach was adopted if the location of tumor was favorable. Moreover, a thoracoscopic approach was used in patients with HCC in the dome of the liver. Intraoperative or laparoscopic ultrasonography was used to guide the ablation process in these situations. Hepatic inflow occlusion (Pringle maneuver) was not applied in any patient during RFA.

Techniques of Radiofrequency Ablation

The Cool-tip® radiofrequency system (Radionics, Burlington, MA, USA) was used in all patients. A single radiofrequency (RF) electrode with an exposed length of 3 cm was used for tumors less than 3 cm in diameter, whereas a clustered electrode (three parallel single electrodes close to each other) with an exposed length of 2.5 cm was used to treat large tumors (over 3 cm in diameter). Each ablation cycle lasted for 8–12 min, and multiple overlapping ablation zones were required in large tumors. In selected patients with tumors close to the portal structures at liver hilum, bile duct cooling by cold saline was performed through a catheter, which was inserted via cystic duct into the common bile duct after cholecystectomy. In all patients, the aim of RFA was to ablate all detected liver tumors with curative intent and an ablation margin of at least 0.5 cm in a single session.

Data Collection and Outcome Measures

Clinical details of all patients were prospectively collected in a database. The treatment protocol and data collection were approved by the Institutional Review Board of the hospital. The clinical details, short-term, and long-term outcome measures were evaluated in all 207 patients. The short-term outcome measures include post-RFA complication rate, treatment-related mortality, and complete ablation rate. The long-term outcome measures include tumor recurrence pattern and survival after RFA. A complication was defined as any adverse event after RFA, excluding pain or transient febrile reaction to the ablation. Treatment-related mortality was defined as any death within 30 days of the RFA procedure. Tumor response to RFA was assessed by CT scan 1 month after ablation. Complete ablation was defined as the absence of contrast enhancement within the original tumor. Any contrast-enhancing areas within the targeted tumor on postablation CT scan indicated incomplete tumor ablation. All patients had monitoring of serum α-fetoprotein concentration, chest radiograph, and CT scan every 3 months to detect tumor recurrence. All images of CT scan were evaluated by two designated radiologists (YJ and WKT). Local recurrence was defined as tumor recurrence within or at the periphery of the original ablated lesion in subsequent CT scans after complete ablation was confirmed on the first postablation CT scan. Same segment intrahepatic recurrence was defined as any new tumor that occurred in the liver separate from the ablated area but within the same liver segment as RFA site according to Couinaud's classification.[15] Different segment intrahepatic recurrence was defined as recurrent tumor that occurred in different Couinaud's segments[15] from RFA site. Extrahepatic recurrence referred to any recurrence outside the liver.

Statistical Analysis

All epidemiologic and clinicopathologic data were prospectively collected in a computerized database. Continuous data were expressed as median with ranges and were compared using Mann–Whitney *U* test. Categorical data were compared using the χ^2test with Yates' correction or the Fisher's exact test where appropriate. The primary end-point of this study was survival time after RFA. The overall and disease-free survival rates were calculated by the Kaplan–Meier method and compared using the log-rank test. Hospital deaths were included in the overall survival analysis but were excluded from the disease-free survival analysis. Fifteen clinicopathologic variables of potential prognostic value were analyzed for their effects on overall survival. Host factors included age (<60 vs ≥60 years), gender, hepatitis B surface antigen status, antihepatitis C antibody status, Child–Pugh grading,[16] indocyanine green (ICG) retention at 15 min (≤20 vs >20%), and histologic status of the liver. Tumor factors included maximum tumor size (≤5 vs >5 cm), number of tumors (solitary vs multiple), tumor staging according to the Cancer of the Liver Italian Program (CLIP) scoring system,[17] subcapsular tumor location, perivascular tumor location (definition of perivascular HCC was mentioned in our previous study[14]), and serum α-fetoprotein concentration (≤400 vs >400 ηg/ml). Finally, the approaches of RFA and tumor recurrence pattern were included in the analysis. Multivariate analysis was performed using the Cox's proportional hazards model to identify independent prognostic factors of overall survival. All statistical analyses were performed using a statistical software (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL). A *P* value of less than 0.05 was considered statistically significant.

Results

Patient Characteristics

The demographic and clinicopathologic data of 209 patients with hepatocellular carcinoma treated with RFA were summarized in Table 1. The median age was 60, and there was male predominance. The majority of patients had chronic viral infection because of hepatitis B (85.6%) or C (10.5%), and liver cirrhosis was present in 88.5% of patients, as evidenced by histological and radiological examinations. One hundred and ninety-one patients (91.3%) had well-preserved liver function (Child–Pugh class A[16]), whereas 15 patients (7.1%) and three patients (1.4%) had Child–Pugh class B and C functional status,[16] respectively. In terms of dynamic liver function, the median ICG retention at 15 min was 16.6%, ranging from 2.7 to

Table 1 Demographic and Clinicopathologic Data of Patients with Hepatocellular Carcinoma Treated by Radiofrequency Ablation

Characteristics	Values
No. of patients	209
Age (years)[a]	60 (18–85)
Gender (M/F)	162/47
Hepatitis B surface antigen	179 (85.6)
Hepatitis C antibody	22 (10.5)
Child–Pugh grading	
Class A	191 (91.3)
Class B	15 (7.1)
Class C	3 (1.4)
Presence of cirrhosis	185 (88.5)
Serum bilirubin (μmol/l)[a]	18 (6–66)
Serum albumin (g/l)[a]	38 (18–48)
Platelet count ($\times 10^9$/l)[a]	110 (29–272)
Indocyanine green retention rate at 15 min (%)[a]	16.6 (2.7–78.4)
Serum α-fetoprotein (ηg/ml)[a]	400 (1–97200)
Size of largest tumor (cm)[a]	2.8 (0.5–8)
Largest tumor (≤5 cm/>5 cm)	198/11
No. of tumors treated (solitary/multiple)	160/49
One or more subcapsular tumors	107 (51.2)
One or more perivascular tumors	78 (38.7)
CLIP scoring system	
Score 0	100 (47.8)
Score 1	89 (42.5)
Score 2	20 (9.6)
Approaches of radiofrequency ablation	
Percutaneous	101 (48.3)
Laparoscopic	14 (6.6)
Thoracoscopic	3 (1.4)
Open	91 (43.5)

Values are number of patients (percentage) unless stated otherwise.
CLIP Cancer of the liver Italian program
[a] Values are median (range).

78.4%. One hundred and twenty-three patients (58%) had ICG retention at 15 min ≤20%, and 86 patients (41.2%) had ICG retention at 15 min >20%. Such high proportion of patients with satisfactory liver function can be explained by our patient selection criteria, in which 44% of patients received RFA as primary treatment for potentially resectable small HCC. The majority of patients had early stage HCC, i.e., small tumor size of ≤5 cm (n=198, 94.7%) and solitary tumor nodule (n=160, 76.5%). Using CLIP scoring system,[17] 47.8, 42.5, and 9.6% of patients had scores 0, 1, and 2, respectively. Most of the ablation procedures were carried out through percutaneous (n=101, 48.3%) and open (n=91, 43.5%) approaches, whereas 14 patients and three patients underwent laparoscopic and thoracoscopic RFA, respectively.

Treatment Mortality and Morbidity

Table 2 described the details of treatment-related mortality and morbidity. Two patients (0.9%) died of multiorgan failure during postoperative period after open RFA. The overall complication rate after RFA was 15.7%. Liver abscess was the commonest complication after RFA (3.3%). Among seven patients with liver abscess after RFA, six patients had underlying diabetes mellitus, and four patients had previous biliary-enteric anastomosis. Three patients had bile duct injury after RFA for tumors close to segmental bile ducts. Because we adopt cold saline irrigation of bile duct during RFA procedure for such tumor location in subsequent 15 patients, none of them developed bile duct injury during the follow-up period. Liver decompensation after RFA occurred in four patients, and all of them had Child–Pugh class C[16] liver functional status.

Short-term Treatment Outcome

The median hospital stay was 4 days (range: 2–21 days). Patients who underwent percutaneous RFA had significantly shorter median hospital stay than those who received open RFA (2 vs 6 days, P=0.035). Similarly, median hospital stay for patients with laparoscopic or thoracoscopic RFA was significantly shorter than those with open RFA (3 vs 6 days, P=0.042). The completeness of ablation could not be assessed in two patients, who had hospital mortality after RFA. The overall complete tumor ablation was achieved in 192 patients (92.7%). Taking into consideration of the total number of ablated tumor nodules, the overall complete ablation rate was 96.1% (275 of 286 tumor nodules). One hundred and eighty-six patients (88.9%) had complete tumor ablation after a single session of RFA, whereas six patients (4.3%) required a second ablation for residual tumor. Other treatment modalities was given to 15 patients with incomplete tumor ablation after RFA as salvage treatment, including TACE (n=8), percutaneous ethanol

Table 2 Treatment-related Mortality and Morbidity in 209 Patients Receiving Radiofrequency Ablation for Hepatocellular Carcinoma

	No. of patients
RFA-related mortality	2 (0.9)
RFA-related morbidity	33 (15.7)
Liver abscess	7 (3.3)
Bile duct injury	3 (1.4)
Intrahepatic pseudoaneurysm	1 (0.4)
Liver failure	4 (1.9)
Symptomatic ascites	4 (1.9)
Chest infection	3 (1.4)
Cardiac arrhythmia	4 (1.9)
Symptomatic pleural effusion	3 (1.4)
Duodenopleural fistula	1 (0.4)
Renal failure	3 (1.4)

Values are number of patients (percentage).
RFA Radiofrequency ablation

injection (n=2), and hepatic resection (n=5). Two patients were offered conservative treatment for malignancy because of poor liver functional reserve.

Tumor Recurrence Pattern

All surviving patients were followed up for at least 16 months after RFA. Excluding two patients with hospital mortality, the median (range) follow-up of the remaining 207 patients was 26 months (16–65 months). Among 192 patients with overall complete tumor ablation, the pattern of first tumor recurrence was evaluated. Thirty-six patients (18.7%) remained recurrence-free during the study period. Meanwhile, 136 patients (70.8%) developed intrahepatic recurrence, and 20 patients (10.4%) developed extrahepatic metastases. The sites of distant metastases included the lung (n=13), bone, (n=6) and brain (n=1). Among patients with intrahepatic recurrence, 28 patients (14.5%) had local recurrence at RFA sites; whereas same segment and different segment intrahepatic recurrences occurred in 30 patients (15.6%) and 78 patients (40.6%), respectively. Time to develop local recurrence in 28 patients ranged from 9 to 48 months. Patients with early local recurrence (within 12 months from RFA) have larger tumors (median size, 5.5 vs 3.5 cm) when compared with those with late local recurrence. All patients with local recurrence received further treatment, including RFA (n=20), hepatic resection (n=5), and TACE (n=3). Meanwhile, 28 of 30 patients (93.3%) with same segment intrahepatic recurrence underwent further treatment, including RFA (n=16), TACE (n=10), and hepatic resection (n=2). On the other hand, liver-directed treatment can only be offered to 50 of 78 patients (64.1%) with different segment intrahepatic recurrence, including RFA (n=25) and TACE (n=25). Conservative treatment was adapted to two patients with same segment recurrence and 28 patients with different segment recurrence because of advance tumor status and poor liver reserve.

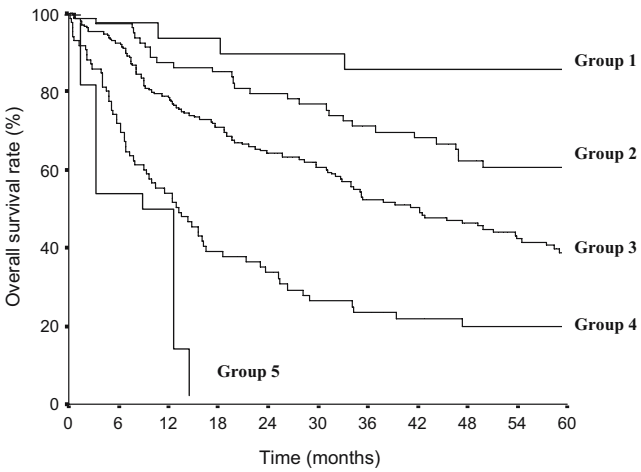

Figure 1 Overall survival results of 192 patients with complete tumor ablation after RFA. *Group 1*: patients with no recurrence during follow-up. *Group 2*: patients with initial local recurrence at RFA sites. *Group 3*: patients with same segment intrahepatic recurrence. *Group 4*: patients with different segment intrahepatic recurrence. *Group 5*: patients with extrahepatic metastases (Groups 1 vs 2: P=0.031; Groups 2 vs 3: P=0.015; Groups 3 vs 4: P=0.022; Groups 4 vs 5: P=0.016).

Subgroup analysis was performed for 108 patients with intrahepatic recurrence excluding those with local recurrence. The clinicopathologic characteristics of patients with same segment and different segment intrahepatic recurrence were summarized in Table 3. There was no significant difference between two groups in age, gender, proportion of patients with chronic hepatitis B infection, Child–Pugh grading[16] of liver function, and proportion of patients with cirrhosis. Nevertheless, the proportion of patients with chronic hepatitis C infection was significantly higher in different segment recurrence group than same segment recurrence group (12.8 vs 6.6%; P=0.046). Moreover, patients in different segment recurrence group had more advanced initial tumor status than those in same segment recurrence group, in terms of higher medium serum α-fetoprotein concentration (685 vs 150 ηg/ml; P=0.021), larger median tumor diameter (4 vs 2.2 cm; P=0.020), higher proportion of patients with multiple tumors (44.8 vs 6.6%; P=0.000), and more advanced CLIP scoring system (P=0.000). There was no difference between the two groups in the RFA approaches adapted. The median time to tumor recurrence was significantly shorter in same segment recurrence group than different segment recurrence group (9 vs 15 months; P=0.044).

Long-term Survival Outcome

Among a total of 209 patients with RFA for HCC, the cumulative overall survival rates were 87.8% at 1 year, 60% at 3 years, and 42.3% at 5 years; whereas the cumulative disease-free survival rates were 57.8% at 1 year, 30% at 3 years, and 28.4% at 5 years. The overall survival outcome of 192 patients with complete tumor ablation after initial RFA was further analyzed with respect to the pattern of tumor recurrence (Fig. 1). Patients without recurrence had the best overall survival among the whole group. The overall survival rates were significantly better in patients with no recurrence (n=36) than those with local recurrence (n=28; 1 year, 94.7 vs 86.3%; 3 years, 85.6 vs 74.8%; 5 years, 83 vs 63.6%; P=0.031). Patients with initial local recurrence had better overall survival outcome than those with distant intrahepatic recurrence. Among 108 patients with distant intrahepatic recurrence excluding local recurrence, the overall survival rates were significantly better in same segment recurrence group than different segment recurrence group (1 year, 78.6 vs 57.2%; 3 years, 57.0 vs 27.3%; 5 years, 42.0 vs 23.0%; P=0.022). Patients with extrahepatic metastases had the worse overall survival with 1-year survival rate of 18.3%, and all patients died within 14 months from RFA.

Table 3 Clinicopathologic Characteristics of 108 Patients with Same Segment or Different Segment Intrahepatic Recurrence After Radiofrequency Ablation for Hepatocellular Carcinoma

Characteristics	Same segment intrahepatic recurrence (n=30)	Different segment intrahepatic recurrence (n=78)	P value
Age (years)*	52 (20–75)	54 (25–80)	0.655
Gender (M/F)	25/5	68/10	0.757
Hepatitis B surface antigen	26 (86.6)	67 (85.8)	0.846
Hepatitis C antibody	2 (6.6)	10 (12.8)	0.046**
Child–Pugh grading			0.955
Class A	24 (80)	69 (88.4)	
Class B	6 (20)	8 (10.2)	
Class C	–	1 (1.2)	
Presence of cirrhosis	27 (90)	72 (92.3)	1.000
Serum α-fetoprotein (ηg/ml)*	150 (20–13350)	685 (120–97200)	0.021**
Size of initial largest tumor (cm)*	2.2 (0.6–4)	4 (2.5–6.5)	0.020**
Initial largest tumor (≤5cm/>5cm)	30/0	69/9	0.047**
No. of initial tumors treated (solitary/multiple)	28/2	43/35	0.000**
One or more subcapsular tumors	19 (63.3)	55 (70.5)	0.388
One or more perivascular tumors	13 (43.3)	26 (33.3)	0.090
CLIP scoring system			0.000**
Score 0	18 (60)	9 (11.5)	
Score 1	10 (33.3)	54 (69.2)	
Score 2	2 (6.6)	15 (19.2)	
Approaches of RFA			0.549
Percutaneous	15 (50)	40 (51.2)	
Laparoscopic	2 (6.6)	10 (12.8)	
Thoracoscopic	–	2 (2.5)	
Open	13 (43.3)	26 (33.3)	
Time interval between RFA and recurrence (months)*	9 (6–15)	15 (12–24)	0.044**

Values are number of patients (percentage) unless stated otherwise.
RFA Radiofrequency ablation
*Values are median (range).
**Statistically significant

Univariate analysis revealed that 5 of the 15 evaluated factors had a significant prognostic influence on overall survival among 209 patients with RFA for HCC (Table 4). Child–Pugh class B or C[16], large tumor size, high serum α-fetoprotein concentration, and different segment intrahepatic recurrence or extrahepatic metastasis were associated with worse overall survival. On multivariate analysis, only Child–Pugh grading[16] (risk ratio [RR]=2.918, 95% CI 1.704–4.998, P=0.000), tumor size (risk ratio [RR]=1.231, 95% CI 1.031–1.469, P=0.021) and pattern of recurrence (risk ratio [RR]=1.464, 95% CI 1.156–1.987, P=0.020) were independent predictors of overall survival.

Discussion

This study has evaluated the long-term overall survival outcome after RFA for HCC. At present, clinical evidences have proven the efficacy of RFA for HCC patients. Randomized controlled studies[18–20] have shown that RFA can achieve a more complete ablation response (lower recurrence and longer overall survival rates) than percutaneous ethanol injection (PEI), which is another effective local ablation therapy for HCC. Moreover, optimistic long-term survival results have been reported in recent retrospective studies.[9–13] Tateishi et al.[9] have reported the largest series of 664 patients with HCC treated with percutaneous RFA. A major complication rate of 4% was observed, and there was no treatment-related death. The cumulative survival rates at 1, 3, and 5 years were 94.7, 77.7, and 54.3% for primary HCC and 91.8, 62.4, and 38.2% for recurrent HCC, respectively. Compared with these studies,[9–13] we have observed similar long-term survival rates (5-year overall survival rate of 42.3% and 5-year disease-free survival rate of 28.4%).

This study is unique in the analysis of tumor recurrence pattern and its association with overall survival in HCC patients after RFA. Different prognostic factors affecting the overall survival after RFA has been described in the previous studies. Poor liver function in terms of advanced Child–Pugh grading,[9,11,12] large tumor,[9,12,13] multiple tumor nodules,[11] high serum α-fetoprotein concentration,[9] and advanced tumor-node-metastasis cancer staging[10] were associated

Table 4 Significant Prognostic Factors for Overall Survival in 209 Patients with Radiofrequency Ablation for Hepatocellular Carcinoma

Prognostic factors	Median survival (months)	*P* value
Child–Pugh grading		
Class A (*n*=191)	49.2	0.000
Class B (*n*=15)	18.6	
Class C (*n*=3)	8	
Largest tumor size		
≤5 cm (*n*=198)	49.7	0.006
>5 cm (*n*=11)	23.3	
Serum alpha fetoprotein (ηg/ml)		
≤400 (*n*=171)	29.7	0.015
>400 (*n*=38)	18	
Indocyanine green retention at 15 min (%)		
≤20 (*n*=123)	52	0.014
>20 (*n*=86)	32.6	
Pattern of recurrence[a]		
No recurrence (*n*=36)	>60	0.013
Local recurrence (*n*=28)	>60	
Same segment recurrence (*n*=30)	43	
Different segment recurrence (*n*=78)	15	
Extrahepatic recurrence (*n*=20)	12	

[a] Pattern of recurrence can only be assessed in 192 patients with complete tumor ablation after RFA.

with poor overall survival outcome after RFA. These factors only indicate the tumor aggressiveness and the degree of liver damage by the underlying chronic hepatitis infection. From the practical point of view, they have limited application in the overall management of patients after RFA. In contrast, our findings of significant prognostic value in tumor recurrence pattern after RFA give new insights in the treatment strategy of HCC patients. We have found that majority of patients (70.8%) would develop intrahepatic recurrence after complete ablation of HCC by RFA. Among them, patients presented with initial local recurrence at RFA site had the best 5-year overall survival rate of 63.6%. Although distant intrahepatic recurrence was associated with poor survival outcome, patients with same segment recurrence had significant better survival than those with different segment recurrence (5-year survival rate, 42 vs 23%). Hepatitis C carrier, large tumor size, multiple tumor nodules, high serum α-fetoprotein concentration, and high CLIP scores[17] were associated with higher chance of developing different segment intrahepatic recurrence after RFA treatment. The main reason accounting for better survival outcome of local recurrence and same segment recurrence groups is that liver-directed therapies can be carried out in the majority of these patients (100% in local recurrence group and 93.3% in same segment recurrence group). Within these groups, the recurrent tumors were still confined within the same liver segments as RFA, such that effective treatment (hepatic resection, RFA, and TACE) can be performed. On the other hand, only 64.1% of patients in different segment recurrence group can receive further treatment for recurrence. Thus, a more aggressive approach for the surveillance and treatment of recurrence is necessary in this latter poor-risk group to optimize the overall survival outcome of patients.

One drawback in the suggestion of aggressive approach for different segment recurrence group is the difficulty in the patient selection and prediction of this recurrence pattern in the early postoperative period. By the time the tumor recurs in this group, the tumor status may be too advanced for the effective salvage treatment. Nonetheless, patients with risk factors (hepatitis C carrier, large tumor size, multiple tumor nodules, high serum α-fetoprotein concentration, and high CLIP score) for development of different segment recurrence should be closely monitored. Our policy is to perform surveillance CT or MRI scan and check serum α-fetoprotein concentration every 3 months after RFA in both early and late period of follow-up. Hence, effective treatment modalities (hepatic resection, repeated RFA, or TACE) can be considered as early as possible before the recurrent tumor progresses. Theoretically, preemptive liver transplantation may be considered at the stage when tumor clearance is confirmed immediately after RFA. This could be the most efficient way to prevent further recurrence in the remnant liver. However, such treatment strategy should be evaluated carefully by large-scale prospective study.

The time to tumor recurrence among patients with either same segment or different segment intrahepatic recurrence have provided some clues on the underlying pathogenesis of recurrent tumor. HCC is characterized by its high propensity for vascular invasion, and the presence of microscopic venous invasion is the most consistently reported risk factor for recurrence after hepatic resection.[21, 22] It is widely accepted that intrahepatic metastasis via the portal venous system is an important mechanism for intrahepatic recur-

rence.[21] On the other hand, cirrhosis caused by chronic hepatitis infection is a well-established precancerous state. High hepatocellular proliferation, in terms of random mutations and promotion, may lead to an increased risk of development of HCC in cirrhotic liver. Hence, multicentric occurrence is another mechanism responsible for intrahepatic recurrence.[23] From our previous study on early and later intrahepatic recurrence after hepatic resection for HCC, early recurrence appeared to arise mainly from intrahepatic metastases, whereas later recurrence was more likely to be multicentric in origin.[24] In fact, we have identified a similar pattern in the timing of recurrence between same segment and different segment recurrence groups. We postulate that the recurrence in the former group is more likely caused by intrahepatic metastasis, whereas multicentric recurrence may account for the recurrence in the latter group. Because RFA often causes vaporization of intracellular water and formation of microbubbles within the ablation zone, the resulting high intralesional pressure may facilitate tumor dissemination along portal venous system within the same segment as RFA, thus accounting for early same segment intrahepatic recurrence. As different segment recurrence has a tendency to occur late in the follow-up period (median=15 months), regular long-term surveillance program is thus essential. The urge for the development of novel preventive measures is another important strategy to alleviate the occurrence of different segment intrahepatic recurrence according to its pathogenesis. In this context, Muto et al.[25] have described the use of polyprenoic acid to suppress multicentric tumor recurrence in cirrhotic liver.

The retrospective data analysis is the main limitation of the present study. It would be desirable if the prospective study can be performed to evaluate the efficacy of different treatment modalities in patients with different recurrence patterns. In this study, we use the pattern of first recurrence after RFA to stratify different groups of patients and study the survival outcome. The issue of treatment strategy of further recurrence after initial control of first recurrence deserves future large-scale study.

In conclusion, a durable long-term survival was observed in HCC patients after radiofrequency ablation. The tumor recurrence pattern after RFA carries significant prognostic value in relation to overall survival. Patients with local or same segment intrahepatic recurrence had better overall survival than those with different segment intrahepatic recurrence. The latter group may therefore require long-term regular surveillance and aggressive treatment for recurrence such as liver transplantation to optimize the benefits of RFA.

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References

1. Poon RT, Fan ST, Lo CM, Ng IO, Liu CL, Lam CM, Wong J. Improving survival results after resection of hepatocellular carcinoma: a prospective study of 377 patients over 10 years. Ann Surg 2001;234:63–70.
2. Fong Y, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a Western center. Ann Surg 1999;229:790–799.
3. Ng KK, Poon RT. Radiofrequency ablation for malignant liver tumor. Surg Oncol 2005;14:41–52.
4. Sutherland LM, Williams JA, Padbury RT, Gotley DC, Stokes B, Maddern GJ. Radiofrequency ablation of liver tumors: a systematic review. Arch Surg 2006;141:181–190.
5. Rossi S, Di Stasi M, Buscarini E, Quaretti P, Garbagnati F, Squassante L, Paties CT, Silverman DE, Buscarini L. Percutaneous RF interstitial thermal ablation in the treatment of hepatic cancer. AJR Am J Roentgenol 1996;167:759–768.
6. Curley SA, Izzo F, Ellis LM, Nicolas Vauthey J, Vallone P. Radiofrequency ablation of hepatocellular cancer in 110 patients with cirrhosis. Ann Surg 2000;232:381–391.
7. Giovannini M, Moutardier V, Danisi C, Bories E, Pesenti C, Delpero JR. Treatment of hepatocellular carcinoma using percutaneous radiofrequency thermoablation: results and outcomes in 56 patients. J Gastrointest Surg 2003;7:791–796.
8. Buscarini L, Buscarini E, Di Stasi M, Vallisa D, Quaretti P, Rocca A. Percutaneous radiofrequency ablation of small hepatocellular carcinoma: long-term results. Eur Radiol 2001;11:914–921.
9. Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 2005;103:1201–1209.
10. Machi J, Bueno RS, Wong LL. Long-term follow-up outcome of patients undergoing radiofrequency ablation for unresectable hepatocellular carcinoma. World J Surg 2005;29:1364–1373.
11. Lencioni R, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. Radiology 2005;234:961–967.
12. Raut CP, Izzo F, Marra P, Ellis LM, Vauthey JN, Cremona F, Vallone P, Mastro A, Fornage BD, Curley SA. Significant long-term survival after radiofrequency ablation of unresectable hepatocellular carcinoma in patients with cirrhosis. Ann Surg Oncol 2005;12:616–628.
13. Cabassa P, Donato F, Simeone F, Grazioli L, Romanini L. Radiofrequency ablation of hepatocellular carcinoma: long-term experience with expandable needle electrodes. AJR Am J Roentgenol 2006;186:S316–S321.
14. Ng KK, Poon RT, Lam CM, Yuen J, Tso WK, Fan ST. Efficacy and safety of radiofrequency ablation for perivascular hepatocellular carcinoma without hepatic inflow occlusion. Br J Surg 2006; 93:440–447.
15. Couinaud C. Liver anatomy: portal (and suprahepatic) or biliary segmentation. Dig Surg 1999;16:459–467.
16. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646–649.
17. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology 1998;28:751–755.
18. Lencioni RA, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, Bartolozzi C. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235–240.

19. Shiina S, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005;129:122–130.
20. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. Gut 2005;54:1151–1156.
21. Yamamoto J, Kosuge T, Takayama T, Shimada K, Yamasaki S, Ozaki H, Yamaguchi N, Makuuchi M. Recurrence of hepatocellular carcinoma after surgery. Br J Surg 1996;83:1219–1222.
22. Okada S, Shimada K, Yamamoto J, Takayama T, Kosuge T, Yamasaki S, Sakamoto M, Hirohashi S. Predictive factors for postoperative recurrence of hepatocellular carcinoma. Gastroenterology 1994;106:1618–1624.
23. Rua S, Comino A, Fruttero A, Torchio P, Bouzari H, Taraglio S, Torchio B, Capussotti L. Flow cytometric DNA analysis of cirrhotic liver cells in patients with hepatocellular carcinoma can provide a new prognostic factor. Cancer 1996;78:1195–1202.
24. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. Cancer 2000;89:500–507.
25. Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, Tsurumi K, Okuno M, Tomita E, Nakamura T, Kojima T. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. N Engl J Med 1996;334:1561–1567.

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Radiofrequency Ablation Versus Surgical Resection for the Treatment of Hepatocellular Carcinoma in Cirrhosis

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Abstract
Background and Aims Percutaneous radiofrequency ablation (RFA) demonstrated good results for the treatment of hepatocellular carcinoma (HCC) in cirrhotic patients; it is still not clear whether the overall survival and disease-free survival after RFA are comparable with surgical resection. The aims of this study are to compare the overall survival and disease-free survival in two groups of cirrhotic patients with HCC submitted to surgery or RFA.
Methods Two hundred cirrhotic patients with HCCs smaller than 6 cm were included in this retrospective study: 109 underwent RFA and 91 underwent surgical resection at a single Division of Surgery of University of Verona.
Results Median follow-up time was 27 months. Overall survival was significantly longer in the resection group in comparison with the RFA group with a median survival of 57 and 28 months, respectively (P=0.01). In Child–Pugh class B patients and in patients with multiple HCC, survival was not significantly different between the two groups. In patients with HCC smaller than 3 cm, the overall survival and disease-free survival for RFA and resection were not significantly different in univariate and multivariate analysis. Whereas in patients with HCC greater than 3 cm, surgery showed improvement in outcome in both univariate and multivariate analysis.
Conclusions Surgical resection significantly improves the overall survival and disease-free survival in comparison with RFA. In a selected group of patients (Child–Pugh class B, multiple HCC, or in HCC ≤3 cm), the results between the two treatments did not show significant differences.

Keywords Hepatocellular carcinoma · Surgery · Radiofrequency ablation · Liver cirrhosis

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Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary liver neoplasm, and its incidence is increasing worldwide.[1] Because of the underlying cirrhosis, the treatment of this malignancy requires a multimodality approach: although surgical resection has the best results in terms of overall survival and disease-free survival, other treatments demonstrated to be successful in improving survival.[2]

Percutaneous ethanol injection (PEI) and percutaneous radiofrequency ablation (RFA) proved their efficacy and safety in patients with small HCC not eligible for surgery.[3] RFA has shown a greater effectiveness than PEI in obtaining complete tumor necrosis with fewer number of treatments.[4]

More recently, RFA has been also successfully offered in patients eligible for liver resection or transplantation.[5,6] Few studies in literature evaluate the outcomes of percutaneous treatments in comparison with surgical treatment.[7–9]

The aim of this study is to compare the prognosis of cirrhotic patients with HCC who were submitted to surgical resection or RFA.

Material and Methods

From January 1996 to August 2006, 260 patients with chronic liver disease and HCC were submitted to liver resection or RFA at a single Division of Surgery of the Department of Surgery and Gastroenterology of the University of Verona, Italy. Two hundred patients with single or multiple (≤3 nodules) HCC measuring ≤6 cm were included in this retrospective study.

Diagnosis of HCC was based on accordance two imaging techniques (US, CT, or MRI) showing an arterial enhancement in a focal lesion ≥2 cm or with a combined criteria of an imaging technique and serum alpha-fetoprotein (AFP) level greater than 400 ng/dl, according to the European Association for Study of the Liver (EASL) consensus conference criteria. A fine-needle biopsy was performed in patients with uncertain diagnosis.[10]

Before surgery or RFA, all patients were submitted to complete liver function tests (bilirubin, alkaline phosphatase, AST, ALT, GGT, albumin, prothrombin time), blood count, creatinine level, chest x-rays, liver ultrasound, and abdominal triple phase computed tomography (CT) and/or contrast-enhanced magnetic resonance (MR).

Resection Group

During the study period, 91 patients were submitted to surgical resection of HCC. The characteristics of the patients submitted to surgery are reported in Table 1.

Surgical resection was considered the treatment of choice for patients with Child–Pugh class A cirrhosis and single HCC. Resection was also performed in selected patients with multiple HCC or with Child–Pugh class B cirrhosis. All surgical resections had negative resection margins confirmed with histology. Surgical specimen examination confirmed the presence of liver fibrosis in all patients. The type of resection included 28 wedge resections, 51 segmentectomies, 8 bisegmentectomies, and 2 major resections (≥3 segments).

RFA Group

During the study period, 109 patients submitted to RFA were included in this study. The characteristics of the patients submitted to RFA are reported in Table 1.

Table 1 Characteristics of the Patients Included in the Study

Variables	Resection, *N* (%)	RFA, *N* (%)	*P*
Gender			0.92
M	73 (80)	88 (81)	
F	18 (20)	21 (19)	
Age			0.01
≤65 years	47 (52)	38 (35)	
>65 years	44 (48)	71 (65)	
Chronic liver disease			0.36
Viral HBV	10 (11)	14 (13)	
Viral HCV	55 (61)	58 (53)	
Not viral	26 (28)	37 (34)	
Child–Pugh class			0.01
A	69 (76)	64 (59)	
B	22 (24)	45 (41)	
Tumor			0.01
Single	69 (76)	65 (60)	
Multiple	22 (24)	44 (40)	
Size			0.4
≤3 cm	31 (34)	32 (30)	
>3, ≤6 cm	60 (66)	77 (70)	
Serum alpha-fetoprotein level			0.8
≤20 ng/dl	57 (63)	66 (61)	
> 20 ng/dl	34 (37)	43 (39)	

Percutaneous RFA was considered the treatment of choice for patients with Child–Pugh class B cirrhosis or with multiple tumors. A small number of patients with Child–Pugh class A cirrhosis and single tumor was treated with RFA. In these patients, ablative therapy was indicated because tumors were ill-located requiring major hepatic resection or refusal of surgery. All patients included in the study did not have general contraindication to surgery.

All the patients underwent RFA with a percutaneous approach under real-time ultrasonographic guidance in an operative room setting under conscious sedation or general anesthesia. An expandable, electrode-needle type probe, connected to a radiofrequencies generator (RITA Medical System, CA, USA) was utilized in all patients. In the first period from 1998 to 2000, we utilized a four-hook electrode-needle connected to a 50-Watt radiofrequency generator (RITA model 500, RITA Medical System, CA, USA). After 2000, a new model, expansible nine-hook needle linked to a 150-Watt generator was utilized (RITA model 1500, RITA Medical System, CA, USA).

Evaluation of treatment response was performed with CT or MR after 30 days. Evaluation of tumor response after RFA was based on the World Health Organization (WHO) criteria in which complete tumor response is defined as the absence of arterial enhancement within or at the periphery of all treated tumors determined by imaging observation

(CT or MRI).[11] HCC with incomplete response were reevaluated for a new RFA session.

Post Treatment Follow-up

Patients were monitored for recurrences every 3 months by physical examination, serum AFP level and imaging studies (CT or MR) were performed every 6 months. All patients with intrahepatic recurrence were evaluated for new treatment with ablative therapies (PEI or RFA), transarterial chemoembolization, or surgery in relation to the severity of liver dysfunction and tumor stage.

Statistical Analysis

All data were collected and analyzed with a statistical computer software (SPSS 14.0, SPSS, IL, USA). Categorical variables were compared using the chi-square test and continuous variables using the Student's *t* test. Overall survival and disease-free survival analyses were carried out using the Kaplan–Meier methods; comparisons between different groups were carried out using the log rank test.

Multivariate analyses for survival and disease free survival were carried out using the Cox's regression model.

Table 2 Univariate Analysis of Survival for Patients with Different Child–Pugh Class and for Patients with HCC Smaller and Larger than 3 cm

Variable	*N*	Median	95%CI	1 year %	3 year %	5 year %	*P*
Overall							*0.01*
RFA	109	28	24–32	83	42	20	
Resection	91	57	36–77	84	64	48	
Child–Pugh class A							*0.01*
RFA	64	33	22–43	86	44	28	
Resection	69	64	51–76	90	72	57	
Child–Pugh class B							*0.90*
RFA	45	21	10–32	70	29	9	
Resection	22	32	11–52	70	42	14	
HCCs ≤3 cm							*0.12*
RFA	32	37	30–44	91	50	29	
Resection	31	65	46–83	89	78	54	
Single HCCs ≤3 cm and Child–Pugh class A							*0.06*
RFA	11	33	23–42	100	50	–	
Resection	20	106	–	100	93	71	
Multiple HCCs ≤3 cm and Child–Pugh class A							*0.7*
RFA	6	36	2–70	100	75	37	
Resection	7	30	–	100	50	–	
Single HCCs ≤3 cm and Child–Pugh class B							*0.08*
RFA	10	28	13–42	75	45	30	
Resection	4	3	0–7	25	0	–	
Multiple HCCs ≤3 cm and Child–Pugh class B							–
RFA	5	43	–	80	60	0	
Resection	0	–	–	–	–	–	
HCCs >3 cm							*0.01*
RFA	77	24	20–30	77	33	14	
Resection	60	40	29–51	81	56	44	
Single HCCs >3 cm and Child–Pugh class A							*0.7*
RFA	23	38	19–36	84	63	45	
Resection	33	64	17–111	80	64	55	
Multiple HCCs >3 cm and Child–Pugh class A							*0.13*
RFA	24	25	14–36	77	22	0	
Resection	9	–	–	100	–	–	
Single HCCs >3 cm and Child–Pugh class B							*0.16*
RFA	21	15	14–16	66	15	–	
Resection	12	24	9–39	82	47	23	
Multiple HCCs >3 cm and Child–Pugh class B							*0.7*
RFA	9	29	0–68	75	45	15	
Resection	6	32	0–72	67	33	33	

Results

Resection Group

No operative mortality was observed in the patients included in this group; 33 (36.2%) patients suffered from postoperative complications. The mean follow-up for patients submitted to resection was 32 months (range 3–120 months).

Median survival time was 57 months (95%CI=36–77 months) with a 3-year and 5-year survival rate of 64% and 48%, respectively.

Median disease-free survival was 36 months (95%CI=27–44 months) with a 3-year and 5-year disease-free survival rate of 56% and 27%, respectively.

RFA Group

There were no mortality after the procedure, and 11 (10%) patients suffered of minor complications. The mean follow-up in the RFA group was 23 months (range 3–92 months).

After single or multiple treatment (range 1–4 RFA sessions), complete necrosis of the tumor was obtained in 89 patients, the rate of complete necrosis was related to HCC size, 93.3% in patients with tumors ≤3 cm and 80.3% in patients with tumors larger than 3 cm (P=0.05).

Median survival was 28 months (95%CI=24–32 months) with 3-year and 5-year survival rate of 42% and 20%, respectively.

Median disease-free survival for the RFA group was 16 months (95%CI=11–20) with a 3-year and 5-year disease-free survival rate of 22% and 22%, respectively.

Comparison Between the Two Groups

Overall survival was significantly longer in the resection group in comparison with the RFA group (P=0.001) (Table 2). In Child–Pugh class A patients, surgery showed better results with a median survival of 64 months in comparison to 33 months in the RFA group (P=0.01). Survival for patients with Child–Pugh class A cirrhosis and single HCC was significantly longer in the resection group in comparison to the RFA group with a median survival of 65 and 38 months, respectively (P=0.05). In patients with multiple HCCs or Child–Pugh class B cirrhosis, we did not observe differences in survival between the two groups. (Table 2).

Multivariate analysis identified that Child–Pugh class B, AFP level grater than 20 ng/dl, and RFA treatment were significantly related with survival, the relative hazards were 2.1, 2.7, and 3.2, respectively (Table 3).

Comparison Between the Two Groups in HCCs ≤3 cm

Survival analysis in patients with tumors ≤3 cm identified that survival was longer in patients in the resection group in comparison to the RFA group with a median survival time of 65 and 37 months, respectively; the difference did not reach statistical significance. Subgroup survival analyses of patients with single or multiple HCC in Child–Pugh A and B patients did not show significantly different survival rates between the two groups (Table 2). Multivariate analysis in patients with HCC ≤3 cm identified that only Child–Pugh class was significantly related with survival (Table 3).

Table 3 Multivariate Cox's Regression Model for Survival in All Patients, Cox's Regression Model for Patients with HCCs Smaller than 3 cm, and Cox Regression Model for HCCs Larger than 3 cm

	N	Coefficient	Relative hazard	95%CI	*P*
Overall survival					
RFA vs resection	109 vs 91	1.15	3.18	1.70–5.93	0.01
Child–Pugh class B vs A	67 vs 133	0.72	2.06	1.27–3.35	0.01
Serum AFP >20 vs ≤20 ng/dl	77 vs 123	0.97	2.64	1.64–4.24	0.01
Tumor size >3 vs ≤3 cm	137 vs 63	–	–	–	0.12
Multiple vs single tumor	66 vs 134	–	–	–	0.74
HCCs ≤3 cm					
RFA vs resection	32 vs 31	–	–	–	0.15
Child–Pugh class B vs A	19 vs 44	1.44	4.24	1.50–11.97	0.01
Serum AFP >20 vs <20 ng/dl	31 vs 74	–	–	–	0.29
Multiple vs single tumor	18 vs 45	–	–	–	0.90
HCCs >3 cm					
RFA vs resection	77 vs 60	0.95	2.58	1.23–5.41	0.01
Child–Pugh class B vs A	48 vs 89	0.56	1.75	1.01–3.01	0.04
Serum AFP >20 vs <20 ng/dl	46 vs 69	0.94	2.56	1.47–4.43	0.01
Multiple vs single tumor	48 vs 89	–	–	–	0.56

Table 4 Univariate Analysis of Disease Free Survival for All Patients, for Patients with HCC Smaller than 3 cm and for Patients with HCC Larger than 3 cm

	N	Median	95%CI	1 year %	3 year %	5 year %	*P*
All patients							
RFA[a]	89	16	11–20	60	22	22	0.001
Resection	91	36	27–44	83	56	27	
HCCs ≤3 cm							
RFA[a]	28	25	14–35	72	36	36	0.70
Resection	31	36	26–45	80	58	19	
HCCs >3 cm							
RFA[a]	61	12	6–17	58	12	–	0.001
Resection	60	36	21–50	82	54	34	

[a] In this group, only patients with complete necrosis were included in the statistical analysis.

Comparison Between the Two Groups in HCCs >3 cm

In patients with tumors greater than 3 cm, the overall survival was significantly longer in the resection group with a median survival of 40 months compared to 24 months for the RFA group (P=0.01; Table 2). Survival for patients with HCC larger than 3 cm and Child–Pugh class A cirrhosis was significantly longer in the resection group compared with the RFA group with a median survival time of 64 and 27 months, respectively (P=0.05). Subgroup analysis in patients with Child–Pugh class A cirrhosis and single HCC confirmed longer median survival time for the resection group but the difference did not reach statistical significance (Table 2). Whereas surgical resection did not show superior results compared to the RFA group in Child–Pugh class B patients or in multiple HCC. Multivariate analysis in patients with HCC greater than 3 cm identified that Child-Pugh class B, serum AFP level grater than 20 ng/dl, and RFA were significantly related with worse prognosis with relative hazards of 1.7, 2.6, and 2.6, respectively (Table 3).

Disease-free Survival

Disease-free survival for the resection group was significantly longer in comparison to the RFA group with a median recurrence-free time of 36 and 16 months, respectively (P=0.001).

Disease free survival analysis in patients with tumors ≤3 cm showed similar results between the two groups with 5-year disease-free survival of 36% in the RFA group and 19% in the resection group (P=0.70; Table 4). Multivariate analysis confirms that the type of treatment was not significantly related with recurrence (Table 5).

In patients with HCCs >3 cm, median disease-free survival was significantly longer in the resection group compared with the RFA group, 36 and 12 months respectively (P=0.001; Table 4). Multivariate analysis for HCCs grater than 3 cm identified that RFA treatment and multiple tumors were significantly related with higher recurrence rate with relative hazards of 2.5 and 2.1, respectively (Table 5).

Table 5 Multivariate Cox's Regression Model for Disease-free Survival in All Patients, Cox's Regression Model for Patients with HCCs Smaller than 3 cm, and Cox Regression Model for HCCs Larger than 3 cm

	N	Coefficient	Relative hazard	95%CI	*P*
Overall disease-free survival					
RFA[a] vs resection	89 vs 91	0.64	1.89	1.15–3.13	0.01
Child–Pugh class B vs A	60 vs 120	–	–	–	0.64
Multiple vs single tumor	68 vs 112	0.58	1.78	1.12–2.83	0.01
Size >3 vs ≤3 cm	119 vs 61	–	–	–	0.25
AFP >20 vs ≤20 ng/dl	60 vs 120	–	–	–	0.11
HCCs ≤3 cm					
RFA[a] vs resection	28 vs 31	–	–	–	0.46
Child–Pugh class B vs A	17 vs 42	–	–	–	0.16
Multiple vs single tumor	30 vs 71	–	–	–	0.38
AFP >20 vs ≤20 ng/dl	15 vs 44	–	–	–	0.93
HCCs >3 cm					
RFA[a] vs resection	61 vs 60	0.91	2.50	1.24–5.07	0.01
Child–Pugh class B vs A	40 vs 81	–	–	–	0.42
Multiple vs single tumor	40 vs 59	0.73	2.09	1.18–3.69	0.01
AFP >20 vs ≤20 ng/dl	43 vs 78	–	–	–	0.14

[a] In this group, only patients with complete necrosis were included in the statistical analysis.

Discussion

The management of hepatocellular carcinoma on cirrhosis involves nowadays many treatment options in relation to the tumor stage and the severity of underlying chronic liver disease.[10,12,13] Among these, liver transplantation has the best results in terms of overall survival and disease-free survival, but only few patients can be submitted to this treatment because of organ shortage.[14,15]

Liver surgery for hepatocellular carcinoma has improved its results in patients with and without chronic liver disease during the last decades with mortality lower than 5% in most series.[6,16] Long-term outcome after surgery is good with a 5-year survival rate of 50%, but recurrence of the disease is still a major issue with more than 70% of patients who suffer recurrent disease.[17]

Local ablative techniques, in particular RFA, gained much consent in the last years for its low complications rate and for its efficacy in local necrosis of the tumor.[3,18] The indications for these treatments, the real impact in HCC natural history, and the long-term survival are still matter of debate. Moreover, RFA has been successfully applied in the setting of liver transplantation as a bridge procedure to control the tumor progression during the waiting list period.[15]

Recent studies in literature compared local ablative therapies with surgical resection. In a previous retrospective multicenter study, the authors identified that surgical resection has still the best results in terms of overall survival and disease-free survival.[7] On the contrary Wakai et al., in a retrospective study in HCCs smaller than 4 cm, identified that results of local ablative therapy are comparable with surgery in HCCs smaller than 2 cm with a 10-year actuarial survival rate of 45% for ablative therapy and 58% for surgical resection (P=0.08), whereas surgery showed superior results in larger HCCs.[9] More recently, Hong et al. found that RFA is as effective as surgery in small HCC (≤4 cm), also with preserved liver function (Child–Pugh class A) without differences in overall survival and disease-free survival.[8]

In literature, only one prospective randomized trial had been recently published. In this study, the authors identified that surgery and RFA have similar results for single HCCs smaller than 5 cm in terms of overall survival and disease-free survival.[19]

The results of the current study confirm that surgery has still superior results in comparison to RFA and that these differences are more clearly demonstrated in HCCs larger than 3 cm. The current study has some limitations because it is retrospective with a relatively small sample and because the two groups of patients have differences in severity of chronic liver disease and in tumor stage. However, we think that these biases did not influence the statistical analysis in the subgroups and in the multivariate analyses.

In the current study, we included in the analysis only patients submitted to surgical or ablative treatment with curative intent because the strong prognostic value of complete response of treatment both in surgical therapies and in RFA has been clearly demonstrated.[20,21] With regard to tumor size, we choose the 3-cm cutoff value because RFA showed higher tumor necrosis rate in HCCs smaller than 3 cm.[18]

In tumor smaller than 3 cm, we observed a longer median survival time for patients submitted to surgical resection, 65 vs 37 months, respectively, but the difference did not reach statistical significance (P=0.12). Also, multivariate analysis showed that type of treatment did not influence the survival in this group of patients.

Although these data emphasized the efficacy of RFA in small HCCs, surgical resection still has better results also in small HCCs. The role of RFA in these patients should be evaluated in relation with the severity of chronic liver disease where the lower risk of the procedure without impairment of liver function could give some advantages in these patients. Moreover, RFA could be preferred to surgical resection in patients candidate for liver transplantation.

Surgical resection proved its efficacy in HCCs larger than 3 cm in which hepatectomy can remove small peritumoral satellites and microvascular invasion that are frequently observed in larger tumors.[22] In these patients, the results of surgery was clearly superior to RFA in both univariate and multivariate analyses.

In conclusion, this retrospective study shows that RFA have comparable results with surgical resection in patients with more severe liver dysfunction (Child–Pugh class B) or multiple nodules. In single small HCCs (≤3 cm), surgery has still better results but the difference with RFA did not reach statistical significance in univariate and multivariate analysis.

In larger HCCs (>3 cm and <6 cm), surgical resection has still demonstrated its efficacy in terms of long-term survival and disease-free survival. More studies are necessary to demonstrate if RFA can be a curative therapeutic option in patients candidate for surgical resection for HCCs smaller than 3 cm.

References

1. Bosch FX, Ribes J, Cleries R, Diaz M. Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2005;9(2):191–211.
2. Carr B. Hepatocellular carcinoma: current management and future trends. Gastroenterology 2004;127(5 Suppl 1):S218–S224.
3. Poon RT, Fan ST, Tsang FH, Wong. Locoregional therapies for hepatocellular carcinoma: a critical review from the surgeon's perspective. Ann Surg 2002;235(4):466–486.

4. Sutherland LM, Williams JA, Padbury RT, Gotley DC, Stokes B, Maddern GJ. Radiofrequency ablation of liver tumors: a systematic review. Arch Surg 2006;141(2):181–190.
5. Ng KKC, Lam CM, Poon RT, et al. Thermal ablative therapy for malignant liver tumors: a critical appraisal. J Gastroenterol Hepatol 2003;18:616–629.
6. Curley S. Radiofrequency ablation of malignant liver tumors. Ann Surg Oncol 2003;10:338–347.
7. Vivarelli M, Guglielmi A, Ruzzenente A, Cucchetti A, Bellusci R, Cordiano C, Cavallari A. Surgical resection versus percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma on cirrhotic liver. Ann Surg 2004;240(1):102–107.
8. Hong SN, Lee SY, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC, Rhee JC, Choi D, Lim HK, Lee KW, Joh JW. Comparing the outcomes of radiofrequency ablation and surgery in patients with a single small hepatocellular carcinoma and well-preserved hepatic function. J Clin Gastroenterol 2005;39(3):247–252.
9. Wakai T, Shirai Y, Suda T, Yokoyama N, Sakata J, Cruz P, Kawai H, Matsuda Y, Watanabe M, Aoyagi Y, Hatakeyama K. Long-term outcomes of hepatectomy vs percutaneous ablation for treatment of hepatocellular carcinoma < or =4 cm. World J Gastroenterol 2006;12(4):546–552.
10. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.
11. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207–214.
12. El-Serag HB, Mallat DB, Rabeneck L. Management of the single liver nodule in a cirrhotic patient: a decision analysis model. J Gastroenterol 2005;39(2):152–159.
13. Taura K, Ikai I, Hatano E, Fujii H, Uyama N, Shimahara Y. Implication of frequent local ablation therapy for intrahepatic recurrence in prolonged survival of patients with hepatocellular carcinoma undergoing hepatic resection: an analysis of 610 patients over 16 years old. Ann Surg 2006;244(2):265–273.
14. Hashikura Y, Kawasaki S, Terada M, et al. Long-term results of living-related donor liver graft transplantation: a single center analysis of 110 patients. Transplantation 2001;72:95–99.
15. Mazzaferro V, Battiston C, Perrone S, Pulvirenti A, Regalia E, Romito R, Sarli D, Schiavo M, Garbagnati F, Marchiano A, Spreafico C, Camerini T, Mariani L, Miceli R, Andreola S. Radiofrequency ablation of small hepatocellular carcinoma in cirrhotic patients awaiting liver transplantation: a prospective study. Ann Surg 2004;240(5):900–909.
16. Grazi GL, Ercolani G, Pierangeli F, Del Gaudio M, Cescon M, Cavallari A, Mazziotti A. Improved results of liver resection for hepatocellular carcinoma on cirrhosis give the procedure added value. Ann Surg 2001;234(1):71–78.
17. Poon RT, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Extended hepatic resection for hepatocellular carcinoma in patients with cirrhosis: is it justified? Ann Surg 2002;236(5):602–611.
18. Guglielmi A, Ruzzenente A, Battocchia A, Tonon A, Fracastoro G, Cordiano C. Radiofrequency ablation of hepatocellular carcinoma in cirrhotic patients. Hepatogastroenterology 2003; 50:480–484.
19. Chen MS, et al. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. Ann Surg 2006;243(3):321–328.
20. Sala M, Llovet JM, Vilana R, et al. Initial response to percutaneous ablation predicts survival in patients with hepatocellular carcinoma. Hepatology 2004;40(6):1352–1360.
21. Guglielmi A, Ruzzenente A, Sandri M, Pachera S, Pedrazzani C, Tasselli S, Iacono C. Radiofrequency ablation for HCC in cirrhotic patients: prognostic factors for survival. J Gastrointest Surg 2007;11(2):143–149, Feb.
22. Toyosaka A, Okamoto E, Mitsunobu M, Oriyama T, Nakao N, Miura K. Intrahepatic metastases in hepatocellular carcinoma: evidence for spread via the portal vein as efferent vessel. Am J Gastroenterol 1996;91(8):1610–1615.

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Unusual Segmental Stricture of the Lower Common Bile Duct Mimicking Bile Duct Cancer

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Abstract In some cases of bile duct stricture, malignancy cannot be diagnosed preoperatively even with the use of various diagnostic imaging modalities and histologic examination. As long as malignancy cannot be ruled out completely, surgery can be undertaken for the purposes of diagnosis and treatment. We report a case of unusual segmental stricture of the lower common bile duct mimicking bile duct cancer and discuss the differential diagnosis.

Keywords Common bile duct · Segmental stricture · Benign stricture

Case Report

An 82-year-old man presented with high fever, vomiting, and epigastralgia, and laboratory findings were consistent with obstructive jaundice and liver dysfunction. Percutaneous transhepatic biliary drainage was performed at a nearby hospital, and the patient was admitted to our hospital for further examination and treatment. Laboratory values upon admission were as follows: total bilirubin 7.6 mg/dl (normal 0.2–1.2 mg/dl), direct bilirubin 5.7 mg/dl (normal 0.1–0.7 mg/dl), aspartate aminotransferase 75 IU/l (normal 13–33 IU/l), alanine aminotransferase 66 IU/l (normal 8–42 IU/l), gamma-glutamyl transpeptidase 68 IU/l (normal 10–47 IU/l), and alkaline phosphatase 408 IU/l (normal 115–359 IU/l). Carcinoembryonic antigen and carbohydrate antigen 19-9 levels were within normal limits. Endoscopic retrograde cholangiopancreatography (ERCP) showed severe stricture of the lower common bile duct (CBD), and both endoscopic retrograde and percutaneous transhepatic cholangiography revealed a limited segmental stricture, about 15 mm in length, of the lower CBD (Fig. 1). There were no abnormalities suggestive of autoimmune pancreatitis (AIP) or primary sclerosing cholangitis (PSC) in the pancreatic duct or the intrahepatic bile ducts, respectively, and neither brushing cytology nor histologic examination of the stricture revealed any evidence of malignancy. Computed tomography (CT) and endoscopic ultrasonography (EUS) revealed no apparent tumorous lesion at the corresponding bile duct, and there were no angiographic abnormalities with the celiac angiography. Magnetic resonance cholangiopancreatography (MRCP) could not be performed because the patient had pacemaker implanted for sick sinus syndrome. Percutaneous transhepatic cholangioscopy revealed the existence of a papillary tumorous lesion, but biopsy showed only hyperplastic change of the lining epithelium with no malignant component. Because the cause of the stricture was unclear and malignancy could not be ruled out completely, we decided to perform pylorus-preserving pancreaticoduodenectomy with lymph node dissection for the purposes of accurate diagnosis and treatment. At laparotomy, no ascites was found, and there was no evidence of lymph node metastasis. The resected specimen showed limited stricture, 17×12 mm in size, of the lower

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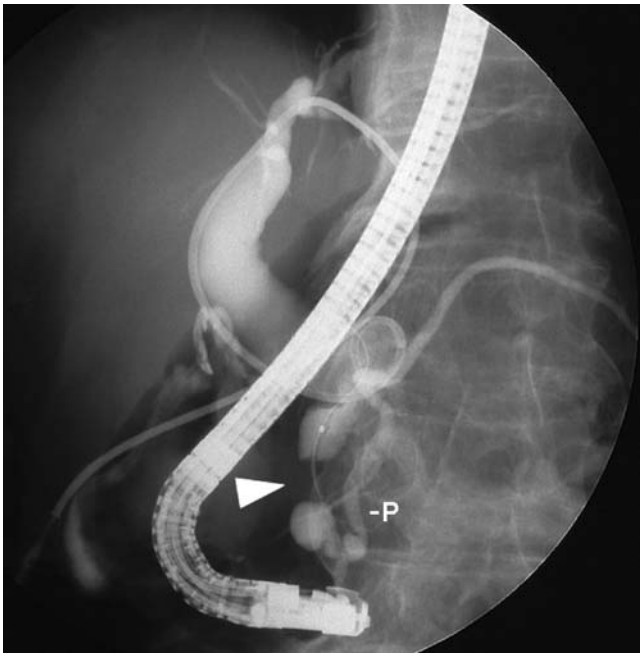

Figure 1 Cholangiopancreatography shows a limited segmental stricture (white arrowhead), about 15 mm in length, of the lower CBD. No abnormalities are seen in the pancreatic duct or the intrahepatic bile ducts. P = Pancreatic duct.

CBD accompanying flattened papillary appearance (Fig. 2). Histologically, the lining epithelium of the CBD showed marked micropapillary projection of the mucosal surface (Fig. 3a). Erosion of the mucosal surface, with fibrinopurulent exudates, was also found. Marked fibrosis and capillary proliferation were seen around the bile duct and continued into the pancreatic tissue (Fig. 3b). Inflammatory infiltration was also seen, but infiltration of plasma cells was not so striking and plasma cells were negative for IgG4 immunohistochemically. No findings suggestive of chronic pancreatitis were observed in the adjacent pancreatic tissue, and the characteristic pathologic feature of PSC, concentric periductal fibrosis ("onion skinning"), was also absent. Immunohistochemically, there were no epithelial cells showing immunoreactivity for p53, and the MIB-1 index was very low at the proliferative sites. Although some nuclear enlargement was noted, no neoplastic overgrowth was found, and the possibility of reactive hyperplasia because of obstruction was considered. From these findings, the localized type of sclerosing cholangitis (SC) was most likely.

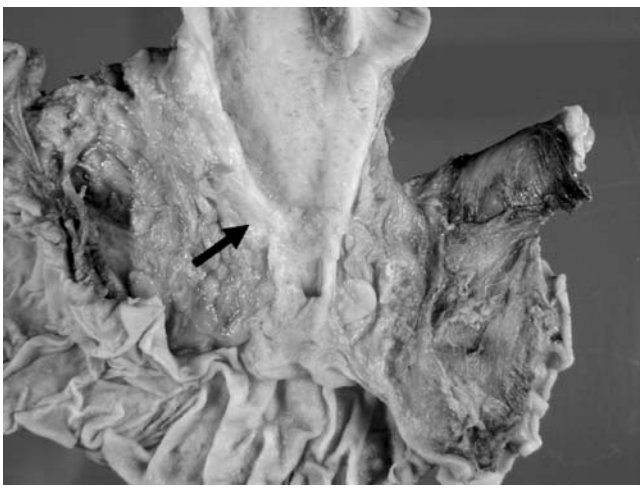

Figure 2 Photograph of the resected specimen. Limited stricture of the lower CBD with flattened papillary appearance is shown (black arrow = proximal stricture site).

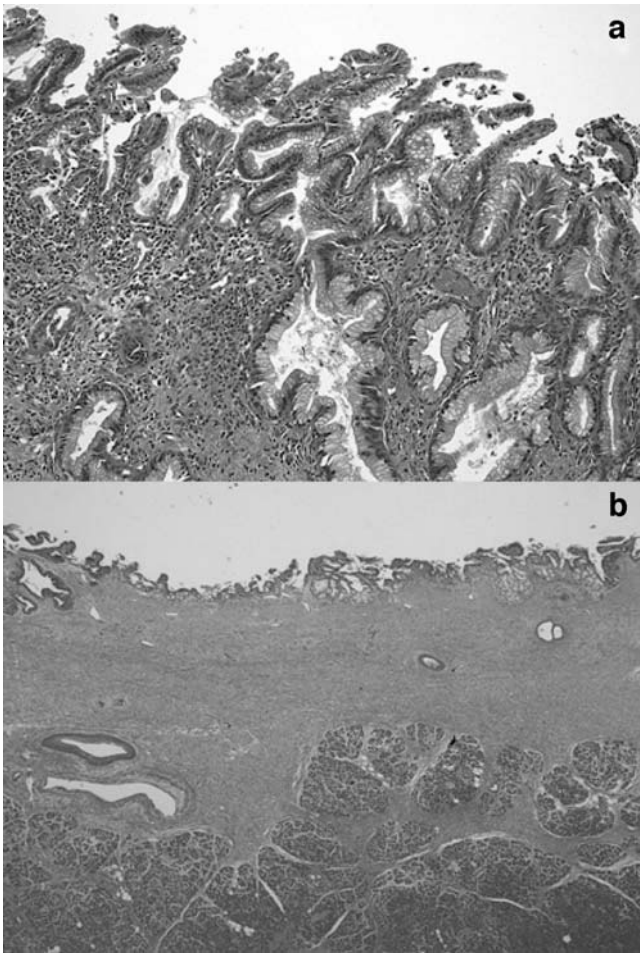

Figure 3 Histologic findings (**a** hematoxylin and eosin stain, original magnification ×40, **b** hematoxylin and eosin stain, original magnification ×12.5). **a** The lining epithelium of the CBD showed reactive hyperplasia. **b** Marked fibrosis around the bile duct and pancreas are seen.

Discussion

Benign bile duct stricture results from various conditions, including primary and secondary SC, infection, toxins, and ischemia.[1] Biliary tract cancer is the second most common primary hepatobiliary cancer, after hepatocellular cancer, with approximately 7,500 new cases diagnosed per year in the United States. Biliary tract cancer may spread directly

into adjacent organs or the abdominal cavity, where it can be detected by ultrasonography, CT, magnetic resonance imaging, or EUS, but it may be impossible to determine the exact extent of invasion.[2] The diagnostic accuracy of MRCP is considered to be equivalent to that of ERCP for a broad spectrum of benign and malignant pancreatic and biliary ductal diseases.[3] Magnetic resonance cholangiopancreatography is optimal for visualization of both intrahepatic and extrahepatic cholangiocarcinomas, which appear as hypointense lesions on T1-weighted images and hyperintense lesions on T2-weighted images.[4] Choi et al.[5] analyzed the use of multiphasic helical CT in 50 patients with only a focal CBD stricture, 32 with a malignant and 18 with a benign stricture. The results revealed that stricture length and ductal diameter proximal to the stricture were significantly greater in cases of malignant stricture than in cases of benign stricture and that wall thickness of more than 1.5 mm was observed in 81% of malignant cases but only 16% of benign cases. The authors concluded that hyperenhancement of the involved CBD during the portal venous phase is the main factor distinguishing malignant from benign CBD stricture.[5] In our case, wall thickness was less than 1.5 mm, stricture length and ductal diameter proximal to the stricture were less than those usually seen in cases of malignancy, and there was no hyperenhancement during the portal venous phase.

To distinguish benign stricture from malignant stricture, histologic evaluation is essential. Stewart et al.[6] reviewed the results of brush cytology in 406 cases of pancreaticobiliary stricture and reported overall diagnostic sensitivity and specificity of 59.8% (147/246) and 98.1% (157/160), respectively. There were 30 of 48 (62.5%) primary bile duct neoplasia specimens also diagnosed as malignant by brush cytology.[6] A similar analysis was made by Elek et al.,[7] who reported the diagnostic accuracy of cytology and biopsy for diagnosis of malignancy of the CBD to be 54 and 55%, respectively, and that combined application of intraductal biopsy and cytology led to more accurate diagnosis than the use of either diagnostic method alone.[7] In our case, preoperative intraductal cytology and biopsy did not yield convincing evidence of malignancy. However, because the cause of stricture was unclear and malignancy could not be completely ruled out, surgery was undertaken for the purposes of diagnosis and treatment.

In patients without previous biliary tract surgery, choledocholithiasis, congenital abnormality of the biliary tract, bile duct carcinoma, or pancreatic disease, some cases of SC has been reported to affect the extrahepatic biliary tree, and dilatation of the proximal bile ducts and short, ring-like stenosis were associated. Nakanuma et al.[8] mentioned that PSC may be diverse in terms of etiology and pathogenesis and that it may include some variants as well as the classic PSC.

Autoimmune pancreatitis accompanied by serum IgG4 elevation has recently been proposed as a new clinical entity.[9] This condition is sometimes associated with other autoimmune diseases such as Sjögren syndrome or SC. In the study by Nakazawa et al.,[10] cholangiographic findings such as band-like stricture, beaded or pruned-tree appearance, and diverticulum-like formation were characteristic and specific for PSC, whereas segmental stricture, long stricture with prestenotic dilatation, and stricture of the distal CBD were characteristic of AIP-SC. The difference in pathophysiology between AIP-SC and PSC was analyzed by Uehara et al.,[9] and the results showed that the IgG4-positive plasma cell/mononuclear cell ratio was significantly higher in AIP-SC than in PSC.[10] In some cases in the AIP category reported so far, only biliary strictures were present, with no changes in the pancreas itself. If the histopathologic findings are consistent with the biliary changes of lymphoplasmacytic sclerosing pancreatitis that are characteristic of AIP, and if plasma cells that stain positively for anti-IgG4 antibody are present, the case may represent a new clinical entity within the AIP category.[10] Although AIP-SC was excluded because of normal IgG4 level and a lack of infiltration of IgG4-positive plasma cells, as in our case reported here, the differential diagnosis from AIP-SC would be still required.

References

1. Adsay NV. Gallbladder, extrahepatic biliary tree, and ampulla. In Mills SE, Carter D, Greenson JK, Oberman HA, Reuter VE, Stoler MH, eds. Sternberg's Diagnostic Surgical Pathology, 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2004, pp 1775–1828.
2. de Groen PC, Gores GJ, LaRusso NF, Gunderson LL, Nagorney DM. Biliary tract cancers. N Engl J Med 1999;341:1368–1378.
3. Barish MA, Yucel EK, Ferrucci JT. Magnetic resonance cholangiopancreatography. N Engl J Med 1999;341:258–264.
4. Malhi H, Gores GJ. Review article: The modern diagnosis and therapy of cholangiocarcinoma. Aliment Pharmacol Ther 2006;23:1287–1296.
5. Choi SH, Han JK, Lee JM, Lee KH, Kim SH, Lee JY, Choi BI. Differentiating malignant from benign common bile duct stricture with multiphasic helical CT. Radiology 2005;236:178–183.
6. Stewart CJ, Mills PR, Carter R, O'Donohue J, Fullarton G, Imrie CW, Murray WR. Brush cytology in the assessment of pancreatico-biliary strictures: A review of 406 cases. J Clin Pathol 2001;54:449–455.
7. Elek G, Gyokeres T, Schafer E, Burai M, Pinter F, Pap A. Early diagnosis of pancreatobiliary duct malignancies by brush cytology and biopsy. Pathol Oncol Res 2005;11:145–155.
8. Nakanuma Y, Harada K, Katayanagi K, Tsuneyama K, Sasaki M. Definition and pathology of primary sclerosing cholangitis. J Hepatobiliary Pancreat Surg 1999;6:333–342.
9. Uehara T, Hamano H, Kawa S, Sano K, Honda T, Ota H. Distinct clinicopathological entity 'autoimmune pancreatitis-associated sclerosing cholangitis'. Pathol Int 2005;55:405–411.
10. Nakazawa T, Ohara H, Sano H, Aoki S, Kobayashi S, Okamoto T, Imai H, Nomura T, Joh T, Itoh M. Cholangiography can discriminate sclerosing cholangitis with autoimmune pancreatitis from primary sclerosing cholangitis. Gastrointest Endosc 2004;60:937–944.

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Successful Preoperative Diagnosis of a Rare Bowel Obstruction: Cecal Volvulus

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Abstract Cecal volvulus is an uncommon cause of intestinal obstruction and is rarely diagnosed correctly at the time of presentation. We report a case in an 86-year-old man presented with an abrupt onset of lower abdominal distension, preoperatively diagnosed as cecal volvulus by abdominal CT. Surgery revealed a 20 cm length of the closed-loop that had rotated in a counter-clockwise rotation. A right hemicolectomy with colonic fixation was subsequently performed; early diagnosis enabled this procedure to be performed in a timely fashion. This case demonstrates the need for clinicians to consider cecal volvulus as a possible cause of acute abdomen. Performing abdominal CT studies in such patients may enable early diagnosis and prompt surgical intervention.

Keywords Cecal volvulus · Bowel obstruction · Computed tomography · Preoperative diagnosis

Abbreviations
CT Computed tomography
US Ultrasonography

Case Report

An 86-year-old man presented with an abrupt onset of lower abdominal distension while he was hospitalized for pneumonia. His medical profile included Parkinson disease diagnosed 15 years previously and a history of chronic intermittent abdominal pain along with intermittent constipation for several years. Physical examination revealed diffuse abdominal distention with mild tenderness and a palpable firm mass in the lower abdomen. The plain abdominal radiographs showed a markedly dilated bowel segment with haustra in the right lower abdomen and laterally dilated small intestine (Fig. 1), but this finding was not specific enough to lead to a diagnosis. A subsequent abdominal computed tomography (CT) showed extremely dilatated bowel with haustra in the right abdomen that resembled a "coffee bean" appearance. Contiguous CT sections identified progressive tapering of ascending colon, terminating at a site of torsion in a "bird's beak" equivalent (Fig. 2a), and also confirmed that the descending colon was clearly separated from the lesions (Fig. 2b, c). Together, these findings led to the diagnosis of a cecal volvulus with an associated bowel obstruction and the patient was immediately taken into surgery. Surgery revealed a marked distension of the cecum and a 20-cm length of the closed-loop that had rotated in a counterclockwise rotation around its long axis, in agreement with the preoperative diagnosis made by CT (Fig. 3). The patient had no intestinal nonrotation but rather a mobile cecum, making it susceptible to volvulus. A right hemicolectomy with colonic fixation was subsequently performed. The resected tissue was found to be massively distended, thin, necrotic, and close to perforation. No abnormalities such as an adhesion, tumors, or an intestinal rotation abnormality were identified that could have acted as a leading point for the volvulus.

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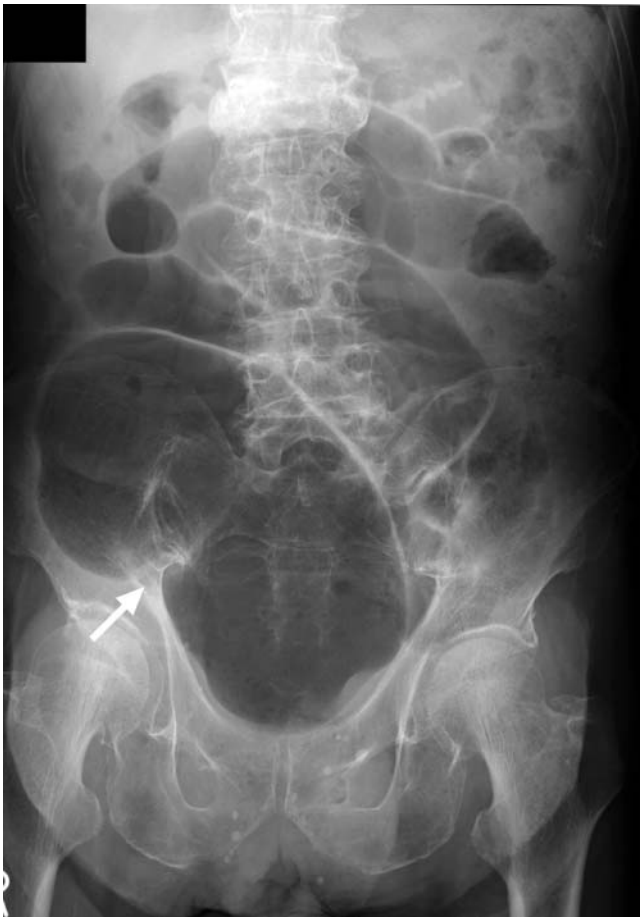

Figure 1 Abdominal radiograph showing the markedly air-distended colon in the shape of a "coffee bean" with an associated small bowel obstruction. The tapered end is directed toward the right lower quadrant (*arrow*).

The patient had an excellent recovery and no further recurrence of abdominal pain at 1 year after the operation. Although cecal volvulus is only a rare form of colonic volvulus, it should be considered, especially if the patient is known to have predisposing anatomical abnormalities including mobile cecum or other risk factors for cecal volvulus.

Discussion

Colonic volvulus is an uncommon cause of intestinal obstruction, accounting for approximately 1–1.5% of all bowel obstructions in adults; it occurs in 10–15% of all large bowel obstructions, and rarely is the cecum involved.[1] Cecal volvulus is rarely diagnosed correctly at the time of presentation because the symptoms are often nonspecific.[1,2] Clinically, cecal volvulus, resulting in colonic ischemia and perforation with resultant large bowel obstruction and strangulation, requires immediate surgical exploration because of the significant risk of severe complications.

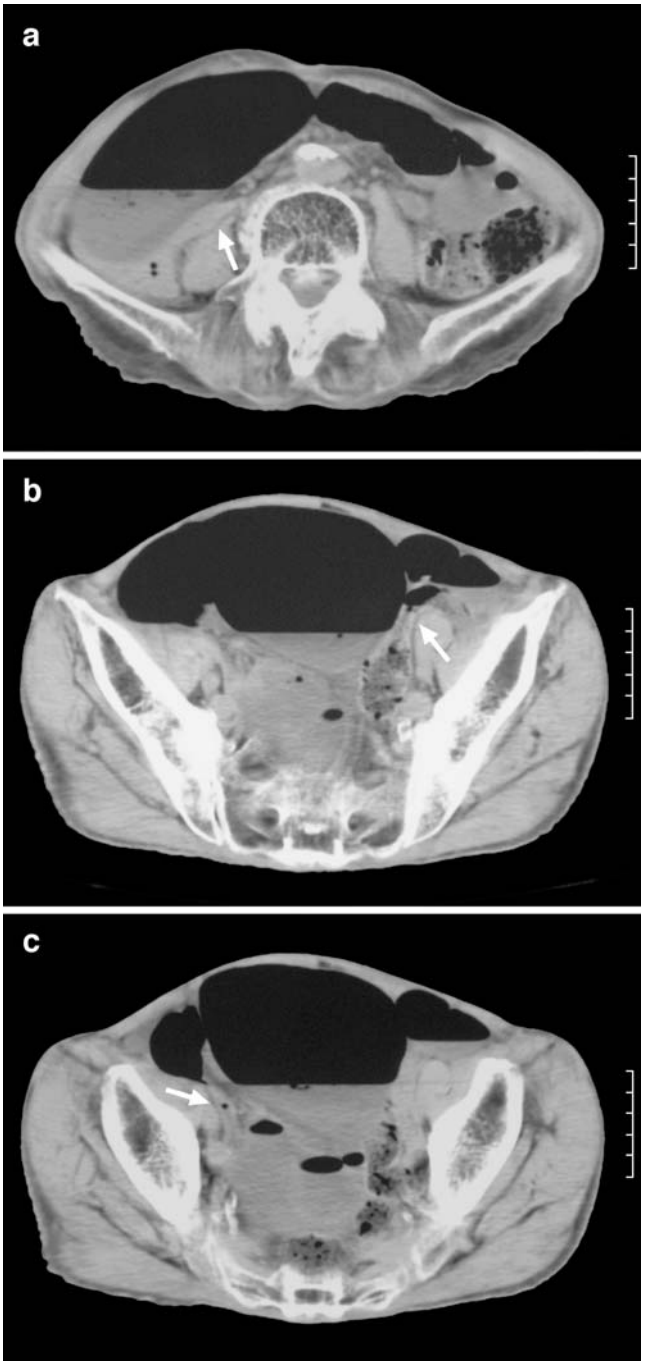

Figure 2 Abdominal CT scan showing a "coffee bean" appearance in the right abdomen: **a** The ascending colon gradually tapers and converges at the site of the torsion, resulting in the "bird's beak" appearance (*arrow*). **b** The CT section, contiguous with subpanel **a**, reveals that the descending colon is clearly separated from the distended bowel (*arrow*), confirming the diagnosis of cecal volvulus. **c** The CT section shows the gas-filled appendix, which is involved with the cecal volvulus (*arrow*).

Immediate surgical reduction of the twisted segment is the most effective treatment. This eliminates the possibility of recurrence and also has low morbidity and mortality rates.[1,3] In this case, we observed the nonviable segment

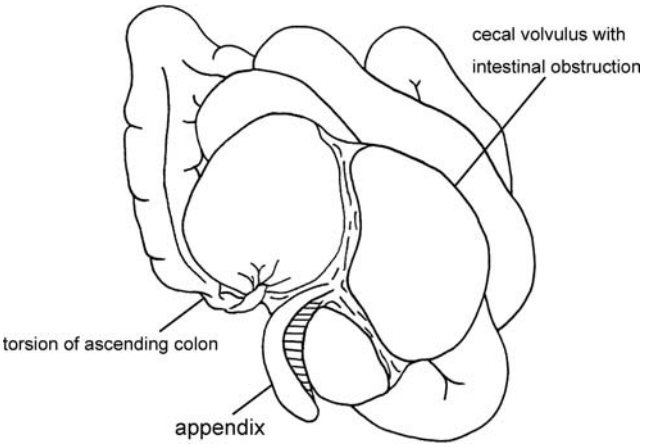

Figure 3 Schematic diagram of the cecal volvulus in this case showing a counterclockwise twisting of the ascending colon and terminal ileum resulting in closed loop obstruction of the cecum with an associated small bowel obstruction.

of twisted terminal ileum at the laparotomy, so early diagnosis enabled this procedure to be performed in a timely fashion.

The underlying etiology and predisposing factors for cecal volvulus include conditions such as chronic constipation, abdominal masses, prior abdominal surgery, and colonoscopy and presumably develops through cecal displacement and colonic distention.[4-6] Acute colonic volvulus, reported to be more frequently seen in patients in hospital for other illness at the time of diagnosis, is associated with the increased occurrence of colon distention and intestinal dysmotility.[3] However, a mobile cecum, the absence of normal fixation of the terminal ileum, is primarily required for this twisting to occur, as it was in this case. Autopsy series indicate that 0.5% of the population has a clinically silent abnormality of intestinal fixation.[2] In our case, the patient had anatomical susceptibility with mobile cecum under conditions of severe constipation. Discovery of anomalous intestinal rotation or cecal displacement in an asymptomatic adult does not warrant any surgical intervention. However, both patient and physician should be aware of the abnormality so that abdominal symptoms are interpreted correctly and managed properly.

Studies have indicated that contrast study with water-soluble contrast medium and colonoscopy can establish the diagnosis of intestinal volvulus preoperatively by identifying a "bird's beak" appearance or a smooth tapering cut off indicating a point of obstruction from the colonic twist and a dilated malpositioned cecum.[2,3] These studies are associated with minimal complications in the absence of clinical peritonitis and severe constitutional symptoms[2] but are not recommended in the management of acute volvulus patients with advanced obstruction, suspected perforation, and gangrenous bowel.[3] In addition, these would have increased the potential risk of obscuring visualization for a subsequent CT examination.[7] On the other hand, abdominal ultrasonography (US), a widely used and less invasive method for diagnosing abdominal disease, could not detect the dilation and obstruction of the intestine in the present case because of profuse intestinal gas. Indeed, it is unlikely that US can play a major role in the diagnosis of colonic volvulus because massive intestinal gas is frequently seen in such cases.[3]

Because of its recent advent as an imaging technique, abdominal CT is being increasingly used for the evaluation of acute abdomen as the preferred imaging modality and can be utilized to confirm this rare diagnosis.[7] The "coffee bean" and "bird's beak" signs are common CT findings associated with acute colonic volvulus,[7] as seen in this case, preoperatively confirming the diagnosis of cecal volvulus by CT. We believe that abdominal CT is the most useful procedure for identifying cecal volvulus when radiographic findings are obscured, such as with a fluid-filled, as opposed to an air-filled and distended, closed loop cecal segment. Prompt abdominal CT in advance of exploratory laparotomy should be considered in all patients with intestinal obstruction of unclear etiology. In conclusion, this case, though rare, demonstrates the need for clinicians to consider cecal volvulus as a possible cause of abdominal pain and that performing abdominal CT studies may enable early diagnosis and allow for prompt surgical intervention.

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References

1. Hoeffel C, Crema MD, Belkacem A, Azizi L, Lewin M, Arrive L, Tubiana JM. Multi-detector row CT: spectrum of diseases involving the ileocecal area. Radiographics 2006;26:1373–1390.
2. Moore CJ, Corl FM, Fishman EK. CT of cecal volvulus: unraveling the image. AJR Am J Roentgenol 2001;177:95–98.
3. Vo NJ, O'Hara SM, Alonso MH. Cecal volvulus: a rare cause of bowel obstruction in a pediatric patient diagnosed pre-operatively by conventional imaging studies. Pediatr Radiol 2005;35:1128–1131.
4. Renzulli P, Maurer CA, Netzer P, Buchler MW. Preoperative colonoscopic derotation is beneficial in acute colonic volvulus. Dig Surg 2002;19:223–229.
5. Wales L, Tysome J, Menon R, Habib N, Navarra G. Caecal volvulus following laparoscopy-assisted sigmoid colectomy for sigmoid volvulus. Int J Colorectal Dis 2003;18:529–532.
6. Viney R, Fordan SV, Fisher WE, Ergun G. Cecal volvulus after colonoscopy. Am J Gastroenterol 2002;97:3211–3212.
7. Amidon PB, Story RK, Jr. Cecal volvulus after colonoscopy. Gastrointest Endosc 1993;39:105.