Presidential Address: The One Thing of Which I Am Sure

Robert W. Beart, Jr., M.D.

As were my predecessors, I was very honored and pleased to have had this opportunity to serve as your president for the past year. This is a unique, singular honor of which I am very proud, and I recognize the significance of your bestowing it on me. It has been a wonderful experience—both serving in this capacity and having the opportunity to work with such wonderful people of high caliber and dedication. Over the course of this year, we have had an opportunity to bring a number of issues to fruition and to initiate a number of others. I look forward to continuing to have the opportunity to work with you over the coming few years.

Everyone in this room has had an interest in academics and realizes the extra commitment that it requires not only on the part of the surgeon but also by the family and others who surround the surgeon. I have been blessed with a wonderful wife, Cindy, who has been very supportive over these decades, and I am pleased that she can be here today. I want to express to her not only my love but also my appreciation for all that she has done and that she has meant to me through these years. I am also pleased that my mother and sister could be here. Obviously, the foundations were laid many years ago for my interests and personal traits, and these are the people who have meant so much to me as I have developed my interests. Thank you very much for all that you have done to make this possible. I am a bit sad that our three girls could not be here, but I am pleased that two of them are expecting a child in the near future, which has made travel difficult. Our third daughter is to be married soon and is working her way through those intricacies at this point. They are very special to us, and they are certainly with us in spirit.

In preparation for this talk, I had the opportunity to review the talks of past presidents. It is interesting that they tend to fall into categories. A number have reviewed the status of the Society and the progress and issues that had evolved the previous year. Others tended to focus on areas of personal clinical expertise and interests. A third group tended to allow an opportunity for personal reflection and sharing of what I will call A Wisdom of the Ages.

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I have had the opportunity to give a talk of this nature before, and it is very threatening to sit back and think of the various ways you could approach a free talk like this. We all frequently give talks in our areas of special expertise, and in some ways, that is an easier way out at this point. At this time in my career, however, I would like to take on the challenge of trying to share some thoughts and perspectives that are the products of my experiences and unique environments in which I have worked. I also find

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these to be rapidly evolving times, which may make my past experience either exceptionally relevant or perhaps totally irrelevant. I do, however, have some thoughts that I would like to share with you in this area of my evolution of thoughts and experiences, and to some degree, I consider this a call to arms.

Dr. Fischer in his Presidential Address last year pointed out that things just are not the way they used to be. He lamented the fact that the changes that are taking place may not allow for surgeons to be of the same caliber in the future. I think it is difficult to know how these changes will evolve and what impact they will have on the quality and interests of our successors. It is not completely obvious that we cannot train people in a little more humane way. On the other hand, we all recognize that there is no substitute for seeing certain problems and, as I will emphasize, there seems to be a correlation between the quality of the surgeon and the volume of work that they do. I think this presents unique opportunities, and I am sure our profession will respond over the coming years to create more efficient and effective ways of training our successors.

As one approaches the end of a career, you cannot help but look back and question, "What have I learned?" It has been a changing environment. There have been a lot of different exposures, and a number of different venues, and clearly there must be something substantive that I can share with you. I am reminded of the words of Conrad Hilton as he retired from 50 years in the hotel business. He pointed out that the only thing of which he was certain at the end of 50 years was that when you take a shower, the curtain goes on the inside of the bathtub. I initially thought this was sort of tragic—that after 50 years, this was the most substantive lesson that he had learned. As I have gotten older, however, I realize that there is a degree of profundity in that statement. As we looked at things in the earlier years, we were often quite sure and certain how we could address and fix them, but as we become older and we see the complexity of our environments, we become less secure in the facts and knowledge that seemed so evident just a decade ago. When I started out in medicine, it was clear to me that as a physician I would be respected, I would have an opportunity to make a substantive income, and I would make some contributions to society. I think all of us recognize that the medical profession has undergone dramatic changes. We are frequently criticized, and certainly the respect in the community is not guaranteed. Finances have become a major issue through the years. With increasing expenses but decreasing reimbursement, many physicians are frustrated and are leaving practice at younger ages. The surgical profession is increasingly unattractive to physicians; in fact, the whole area of medicine is increasingly less attractive to the highest-caliber students in our colleges. I am not going to pretend to address this issue today, but it is clear that things have evolved, and the issues that were clear when I was younger are not as clear today.

The issues of patient care, which in retrospect seemed pretty simple, have become increasingly complex as we have become more knowledgeable about physiology and other aspects of the controlled trauma that we induce. What to me was a relatively straightforward and perhaps technical area of medicine has clearly become a very cognitive and complex area of medicine. Physicians who perhaps at one time were able to handle a large volume of relatively simple cases find it increasingly complex to manage a much smaller volume of work.

I often viewed medicine as an area in which I would be my own boss. I would have the opportunity to create my own environment and be in control. Increasingly, however, I recognize that third-party payers are in control of what I do and how I do it. The hospital environment no longer has the luxury of excess space, time, and personnel. We are increasingly constrained at all levels whether it be hospital beds that are available or operating time that must be filled. Finally, the patients are more knowledgeable and more demanding than ever, making us less in control and more reactionary in the management of their problems.

Dr. Fischer has made a major effort this year for us to focus on the purpose of our Society. By virtually every measure it is quite successful. If you look at the financial situation, the increasing membership, the quality of the program, and the wonderful reception that *The Journal of Gastrointestinal Surgery* has received, we are all very pleased with the progress we have made. Nevertheless, it seems that an educational mission in and of itself may no longer be sufficient to sustain the interest of the membership and the vitality of the organization. The Executive Committee of our Society has been searching for a more vibrant mission for the Society. This discussion that we have been having over the last year has challenged my thoughts and my thinking a bit, and it is with this in mind that I have put together these thoughts.

When I was at the Mayo Clinic in Rochester, Minnesota, there was a lot of concern about the evolution of managed care. It became clear that patients were going to be denied the opportunity to move about to seek their care in other locations. Furthermore, the growth of the country was not in the upper Midwest, and it was clear that if the Mayo Clinic was going to have access to patients, it needed to have a presence in the Sun Belt. As a result, the Mayo Clinic built clinics in Scottsdale, Arizona, and

Jacksonville, Florida. Both of these have been struggling enterprises but have, over time, done well. However, the clinic in Rochester seems to have done no less well. It seems that the predictions that one needed to be in the Sun Belt were untrue and that things were not as the prognosticators would have us believe. The quality of the Mayo Clinic has sustained itself in Rochester and that remote location has not been a disadvantage over the years. When I moved to California, everyone was convinced that not only was managed care going to be an increasing emphasis as the Clinton administration was revving up their attack on the medical system but also one needed a presence of primary care. If one had not secured a large primary care base, then there was no way that a referral tertiary institution would be able to maintain itself. Over the subsequent decade, it was clear that a large primary base has not been necessary and that quality tertiary care institutions have sustained themselves quite well. In fact, it is the more primary care–based, smaller hospitals that have seen trouble. Many in the Los Angeles area have closed, and there are many more for sale even as we speak today. What then, in this changing climate, in this changing environment, this evolution of our profession becomes a common denominator? What can we grab onto that will sustain us through these changes?

In the late 1980s and early 1990s when health maintenance organizations were evolving, I remember Mike Zinner of Peter Bent Brigham Hospital in Boston, Massachusetts, making presentations about new models for medical care and how, if we didn't train another surgeon for 20 years, we would still have a surplus of surgeons. Similar predictions were made for most medical and surgical specialties. Yet now, a decade later as we look at what is happening, we find that we are projecting shortages of surgeons, and that in the near future we may have crises in the availability of the specialties for which we thought we had a 20- or 30-year supply.

It is clear that times are going to change. It is clear that models will evolve and algorithms will be created that will change the emphasis, and that people will come up with new solutions for problems as they evolve. Yet, can we point to any one thing that is a constant? Can we find any anchor to which we can secure our boat in an ebbing and flowing tide that will allow us to sustain ourselves through these turbulent times? I believe there is. If there is any one thing of which I am sure it is that quality patient care depends on the commitment that whatever is in the best interest of the patient always wins. In every model that I have seen, in every environment in which I have worked, the individual, the practice, the group that commits itself to quality patient care will always do well. It will always rise to the top no matter what constraints may be put on the system. Regardless of what obstacles may be placed in the path of patients, they will always seek excellence, and they will always demand and try to find the highest quality care that can be found.

If this is true, how do we relay it to The Society for Surgery of the Alimentary Tract (SSAT)? If we are looking for a principle on which we can hang our hats, if we are looking for a more vibrant mission, perhaps we should look to this one constant in medical care. It is important that we emphasize the education of our colleagues, and it is important that we provide a venue to share thoughts and experiences. But, perhaps the common denominator that we need to emphasize is excellence in patient care. Above all, this is a common and sustaining element in our relationships, not only with our patients but also with each other. By virtue of our interests in academic surgery and by virtue of our interests in the Society, we are interested in improving ourselves and we are interested in providing the highest quality of care to our patients. How does this translate into action? It has already started to translate in action. There is recognition on the part of the Program Committee that we should be more clinically relevant. Members of the Society have reflected, in recent surveys taken earlier this year and taken several years ago, that they want to see more clinically relevant issues as the foundation of the annual SSAT program. The Program Committee has responded to that and I think that, if you look at the program, you will see that the vast majority of the things presented are of clinical relevance. To be sure, the membership also wants to hear about the newer science, particularly science that is in a translational phase.

I propose, however, that we need to go a step beyond this. Historically, we have all been well trained; we have done the best we can to create training programs; we are well founded and supervised through the residency review committees and, subsequently, the American Board of Surgery. The various subspecialty boards and certification processes have put in place systems that help to ensure the individuals in this country are offered a good training environment and have achieved at least a base level of quality training. I would submit, however, that some specialty training is an excellent start, but with the rampant evolution and changing environment in which we find ourselves, one is rapidly outdated perhaps in as soon as 5 years if an effort is not made to keep up. A Society such as ours helps to provide an environment to keep people updated. Because we have all had the same training and basic experience, for many

decades we have assumed that we all provide a common product, we all provide a common quality of product of service, to our patients. However, over the last decade, perhaps stimulated by the health maintenance organization experience, the resulting decreasing reimbursement, and the resulting competition we all feel in our practices, it has become increasingly clear, and we have been inspired to look more carefully at outcomes and to judge analyses. In a unique way it was evident in the cardiac surgical experience in New York City with their statewide registry, and I believe at Johns Hopkins Medical Institutions in Baltimore, Maryland, with Dr. John Cameron's and Dr. Keith Lillemoe's experience with pancreatic cancer. The excellence that they achieved did much to perhaps sensitize us all to the fact that we are not all equal; we do not all provide the same high-quality surgical experience for our patients. In my field of colorectal surgery, there have been many articles written in the past decade that addressed the issue of quality of care. Every article points in basically the same direction, and that is to say that there is a measurable difference in the quality of care provided by surgeons to patients who have colorectal cancer, that this difference is pretty clearly predicated to some degree on training, but as time goes on, the volume of surgery and the experience seem to be even more significant constants in predicting who will provide high-quality outcomes, as measured by postoperative complications and long-term, cancer-free survival. These data are accumulating with such frequency and with such clarity and force that it has become undeniable. This isn't to say that some individuals who have not had the training, or perhaps do not do the volume, do not perform equally well. The capacity exists today to measure how we do as individuals, and in the absence of performance analysis, treating in volume has become a surrogate for quality. These data have been produced in a number of fields, but is particularly strong in colorectal surgery. It is sitting there and nobody is doing anything with it. If we are to sustain ourselves as a quality organization, if we are to identify a vibrant mission that we can approach, I believe it should be a mission, which focuses on the well-being of our patients. I believe that we should recognize that we do not all perform surgical procedures with the same degree of expertise. I would not pretend to say that I could do a pancreatic resection with the skill of Dr. Lillemoe. I cannot treat surgical infections with the skill of Dr. Fischer. As a Society and surgeons committed to excellence, we should recognize and embody these truths in our practices, and begin to think about ways that (1) we can help to bring the skills of those who are not performing as well up to a higher, measurable standard; (2) we can provide a forum through which they can measure their outcomes and confirm that they are doing as well and, if they are not doing as well, take steps to improve themselves; and (3) we can embrace the concepts that are evolving in the literature that substantiate that training and surgical volume are surrogates for quality. We should emphasize this approach among our group and stand up and recognize that perhaps, as other countries have done, we should limit ourselves in what we do so that whatever is being done and whoever is doing it is consistent with the patients' best interests. Is this controversial? Of course it is. Is it going to be difficult? Of course it is. Most things in our lives that have been worthwhile are difficult and frequently controversial, but they are right. Standing for what is in the patients' best interest in my mind has proved to be the one constant, the one anchor to which we can attach ourselves with a high-end guarantee success. Success in an ever-changing environment, success amid confusion, success amidst changing priorities—these are the performance indicators that will predict success and relevance of our Society in the coming years. I know of no other societies that have fundamentally committed themselves to this principle, and it seems to me to be one in which all of us can rally.

As I look at the surgical experience, the vast majority of what we do is relatively straightforward, the outcomes are predictable, and they do not vary much between surgeons; but, there are procedures that do, and I think it is time that we take this fact out of the closet, acknowledge the body of literature and evidence that is evolving, and make an even greater effort to understand it and to take advantage of it to the patients' well-being. The principle here is not to constrain people, not to prevent them from performing in their areas of interest. In contrast, it is to embrace the concepts of excellence, embrace the concept of doing what is in the patients' best interest, and then to take a stand to have our Society recognized for adhering to this basic principle.

How do we implement this? As a Society I think we first have to provide vehicles for people to record and analyze what they do. Many large institutions already do this, but for the average member of the Society and for those in private practice, this is a more daunting enterprise. Cardiovascular and thoracic surgeons have done this nationally. They have the computerized programs available. We can piggyback on their experience, avoid their mistakes, and create a model that will help us to identify excellence.

I think we need to come to some consensus about which procedures fall outside the realm of those that I identified as being common with predictable outcomes and where the variability between physicians

is relatively little. After we identify the selected operations, we should as a Society help to educate the membership and the public about the importance of identifying a surgeon who has the necessary credentials to do this work well.

I would like to see our Society take the leadership role in promoting excellence in patient care and develop an ethical framework of always doing what is in the patients' best interests. After 40 years in the medical profession, things have changed a lot. Increasingly, I am uncertain about how things will go in the future and I am uncertain about how to prepare myself. However, of this I am sure—quality and commitment to excellence in patient care will always win. If we as a Society evolve to this commitment, we will be unique in the medical profession. If we are willing to take the necessary steps to promote excellence above anything else, I believe we will win and I believe we will provide a vibrant and exciting mission for the Society.

This is obviously a difficult concept from the standpoint of socially implementing it. It is not impossible, however, and in fact there are several countries, such as Sweden, that now license physicians to perform specific types of surgery, such as rectal cancer surgery. It is clear that this is an area that can be extremely variable among physicians, and standardization is clearly in the patients' best interests.

I have had the good fortune to practice in several environments where I think the quality of surgical care was generally quite good. At the Mayo Clinic, we had areas of surgical specialization, although there were certain cases that were handled by everyone, such as hernias and gallbladder procedures and, in the early days, breast biopsies. Breast biopsies, however, have become a more sophisticated alternative and no longer spread as equally among our practices as they once were. By the same token, we also had areas of focused excellence and cases would be referred into those practices. I believe this is common in most academic centers, and I think it represents one of the reasons that academic centers tend to do quite well with these otherwise relatively complicated problems. At the University of Southern California, Dr. Thomas DeMeester created what he called *focused services* with much the same concept. Many would do gallbladders and hernias, whereas "lumps and bumps" were done on nearly all of the services, but for the most part, the more complicated procedures were relegated to the hands of relatively few. Based on outcome data that were produced in the state of California, this has resulted in not only large volumes of surgery but also low morbidity rates compared with published data in California and other states, and in other institutions within California.

Have I stepped on a few toes today? I suspect I have. I suspect a few would find it offensive that we might stand up and say, "Brother, you haven't had the training for this," or "Brother, you don't do this with the same expertise as your neighbor does, and therefore you should not be doing this." Our Society stands up and acknowledges that we do not all do things equally well. We should respect this fact as we stand up for what is most important in our profession, and that is what is in the best interest of the patient. After all, this is going to be controversial. This is going to be difficult. I still think that does not make it wrong. I think that this principle is generally true and is something that a society such as the SSAT needs to be willing to stand up for and support.

Does Fibrin Glue Sealant Decrease the Rate of Pancreatic Fistula After Pancreaticoduodenectomy? Results of a Prospective Randomized Trial

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Despite substantial improvements in perioperative mortality, complications, and specifically the development of a pancreatic fistula, remain a common occurrence after pancreaticoduodenectomy. It was the objective of this study to evaluate the role of fibrin glue sealant as an adjunct to decrease the rate of pancreatic fistula after pancreaticoduodenectomy. One hundred twenty-five patients were randomized after pancreaticoduodenal resection only if, in the opinion of the surgeon, the pancreaticojejunal anastomosis was at high risk for development of a pancreatic anastomotic leak. After completion of the pancreaticojejunal anastomosis, the patients were randomized to topical application of fibrin glue sealant to the surface of the anastomosis or no such application. The primary postoperative end points in this study were pancreatic fistula, total complications, death, and length of hospital stay. A total of 59 patients were randomized to the fibrin glue arm, whereas 66 patients were randomized to the control arm and did not receive fibrin glue application. The pancreatic fistula rate in the fibrin glue arm of the study was 26% vs. 30% in the control group (p = not significant [NS]). The mean length of postoperative stay for all patients randomized was similar (fibrin glue = 12.2 days, control = 13.6 days) and the mean length of stay for patients in whom pancreatic fistula developed was also not different (fibrin glue = 18.9 days, control = 21.7 days). There were no differences with respect to total complications or specific complications such as postoperative bleeding, infection, or delayed gastric emptying. These data demonstrate that the topical application of fibrin glue sealant to the surface of the pancreatic anastomosis in this patient population undergoing high-risk pancreaticojejunal anastomosis did not reduce the incidence of pancreatic fistula or total complications after pancreaticodudodenectomy. There seems to be no benefit regarding the use of this substance in this setting. (J GASTROINTEST SURG 2004;8:766–774)

KEY WORDS: Pancreaticoduodenectomy, pancreatic fistula, fibrin glue sealant

Although perioperative mortality associated with pancreaticoduodenectomy has decreased significantly over the years to less than 5%, the postoperative complication rate associated with this procedure still approaches 40%–50%.[1–3] Common postoperative complications include delayed gastric emptying, wound infection, and pancreatic fistula. The most problematic complication of these is pancreatic anastomotic leak with an incidence that varies from 5%–40% in different series.[1–6] Historically, pancreatic fistula accounted for a considerable proportion of postoperative deaths. In recent series this complication is not a common cause of mortality, but contributes considerably to an increased length of hospital stay, hospital costs, and the necessity for reoperation or nonoperative invasive procedures.

Because pancreatic anastomotic leak has remained a frequent complication associated with pancreaticoduodenectomy, different maneuvers have been evaluated in an attempt to decrease its incidence. These

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maneuvers have included avoidance of pancreatic anastomosis by pancreatic duct occlusion with either polymers, suture ligation of external drainage,[7–10] modification of anastomotic techniques,[11–17] or administration of somatostatin analog.[18–23] Prospective randomized trials have indicated that neither the type of pancreatic-enteric anastomosis (pancreaticogastrostomy vs. pancreaticojejunostomy)[11] nor the administration of the somatostatin analog octreotide[18–23] are clearly effective in reducing the level of pancreatic fistula after pancreaticoduodenectomy.

Fibrin glue sealants are tissue adhesives that are composed of human fibrinogen and thrombin that are used for hemostasis, wound closure, and tissue sealing. A number of nonrandomized series have suggested that application of fibrin glue sealant decreases the incidence of pancreatic fistula after pancreatic resection.[24–27] Furthermore, a prospective randomized trial including 56 patients has demonstrated that topical application of fibrin glue to the pancreatic stump after distal pancreatectomy significantly reduced pancreatic fistula rate.[28] In contrast, a prospective randomized trial of 96 patients undergoing both pancreaticoduodenectomy and distal pancreatectomy did not indicate a benefit regarding the application of fibrin glue.[29] Finally, a prospective randomized trial of pancreatic duct occlusion with fibrin glue during both pancreaticoduodenectomy and distal pancreatectomy indicated no difference in the incidence of pancreatic fistula or other intraabdominal complications.[10]

A criticism of these studies is that they did not limit randomization to only patients with a soft pancreatic texture—the group at major risk of pancreatic leak. The rate of pancreatic fistula has been strongly correlated to pancreatic texture with the incidence of pancreatic fistula in patients with hard texture being 0%, intermediate texture being less than 5%, and soft texture glands being greater than 20%.[10,11] Therefore, an inadequate number of high-risk anastomoses may not have been included in these studies.

The current prospective, randomized, single-institution trial was designed to evaluate the effect of topical fibrin glue sealant on the primary postoperative end points of pancreatic fistula, total complications, death, and length of hospital stay in patients undergoing pancreaticoduodenectomy with pancreatico-enteric anastomosis in the setting of a high-risk soft pancreatic gland.

MATERIAL AND METHODS

This study was approved by the Joint Committee on Clinical Investigation of the Johns Hopkins University School of Medicine. Patients were recruited into the study before surgery on the basis of anticipated elective pancreaticoduodenal resection and appropriate informed consent was obtained. The study was carried out between August 2000 and May 2002.

Surgical Technique

Patients underwent pancreaticoduodenal resection as a partial pancreatectomy with either pylorus-preservation or distal gastrectomy as described previously in detail.[3,11,20] Vagotomy, tube gastrostomy, and feeding jejunostomy were not used. All pancreatic anastomoses were hand-sewn in two layers after mobilizing the pancreatic remnant for 2–4 cm. Silk (3-0) was used for the outer layer and polyglactin (3-0 or 4-0) was used for the inner layer. Pancreaticojejunostomy was performed in either an end-to-side or end-to-end fashion at the surgeon's discretion. Pancreaticogastrostomy was used in only 1 patient in the study, as previously described.[11] The use of a short non-externalized pancreatic stent was used, again at the surgeon's discretion. At the conclusion of the pancreaticoduodenal reconstruction, one or two 3/16 or 1/4 in. round silicone closed suction drains (Relia Vac, Davol, Cranston, RI) were introduced through separate abdominal stab incisions and placed in the vicinity of the pancreatic anastomosis.

Randomization Criteria

Entry into the study was allowed only when, in the opinion of the operating surgeon, the pancreaticoenteric anastomosis was considered to be at high risk for the development for pancreatic leak. A soft normal texture gland and a nondilated pancreatic duct were considered as the essential criteria for inclusion. After determination of eligibility, the patients were randomized using a randomly generated number pattern to either the topical application of fibrin glue sealant (Hemaseel APR) (Hemacure Corp., Sarasota, FL; Baxter Health Care Corp., Glendale, CA) or no application. Those patients randomized to the treatment arm received 8 ml of rapid-acting fibrin glue sealant applied topically through a double-barrel syringe connected to a Y-shaped catheter. The sealant was applied circumferentially to the entire anastomosis after completion of all three anastomoses before closure of the abdomen. Those patients randomized to the control arm received no such application. Fibrin glue was not administered into the pancreatic duct.

Postoperative Management

All patients received H_2 receptor antagonists during the postoperative period as a prophylaxis against

stress and marginal ulceration. Most patients received erythromycin lactobionate (200 ml intravenously every 6 hours from postoperative day 2 until discharge) as prophylaxis against delayed gastric emptying. The use of prophylactic antibiotics and venous thrombosis prophylaxis were as directed by the individual surgeon. Prophylactic administration of octreotide was not used.

Surgically placed drains in the vicinity of the pancreatic anastomosis were left undisturbed with their outputs recorded daily for at least 4 postoperative days. Samples of the drain fluid were sent for amylase determination between postoperative days 3 and 7 if the outputs were greater than 30 cc per day or if the effluent seemed abnormal. In the absence of a pancreatic fistula (defined below), the drains were removed. In the presence of a pancreatic fistula, management was left to the discretion of the primary surgeon that for most patients included conservative management with nothing by mouth, total parenteral nutrition, and subcutaneous administration of octreotide.

Data Collection

Data was collected prospectively for all patients. History, details of the surgical procedure (including the type of resection performed, pancreatic texture, and duct size), number of drains used, and the pathologic analysis of the resected specimen were recorded. Clinical information regarding the postoperative course and complications were collected by study nurses without knowledge of the treatment arm (fibrin glue vs. control). Follow-up was complete through July 2002.

Study End Points

The primary study end points included pancreatic fistula, complications, length of stay, and death. Pancreatic fistula was defined as drainage of greater than 50 ml of amylase rich fluid (greater than threefold elevation above the level in serum) per day through the surgically placed drains on or after postoperative day 10 or pancreatic anastomotic disruption demonstrated radiographically. Other complications were defined in standard fashion, as previously described.[3,11,20]

Statistical Analyses

The study design predicted the number of patients necessary for statistical validity (one-sided). This was based on the premise of improving the pancreatic fistula rate from 25% to 10%, with alpha set at 0.05 and beta set at 0.2 yielding a power of 80%. One hundred twelve patients were calculated to be required in each arm of the study for a total study population of 224 patients. The study was reviewed annually by an informal Data Safety Monitoring Board who assessed for adverse events and end points. At the time of the second annual review (n = 124), the pancreatic fistula rate was 26% in the fibrin glue arm of the study vs. 30% in the control group (p = NS). Analysis of these data failed to reveal any benefit from the topical administration of fibrin glue. After careful evaluation of the entire study and an additional subgroup analysis, it was determined that the fibrin glue application provided no benefit in any subgroup nor would a benefit be possible with continued accrual and thus the study was closed ceasing further accrual.

Comparability between the fibrin glue and control groups was verified using the Student t test and χ^2 statistics. Results are reported as mean ± standard error of the mean. Significance was accepted at the 5% level. Data regarding the cost of fibrin glue application were based on hospital product cost provided by the operating room administration.

RESULTS

Patient Population

During the time period of this study, 404 patients underwent pancreaticoduodenectomy. A high-risk pancreatic gland (soft texture with normal duct) was present, as determined by the surgeon, in 124 patients (31%). These patients were randomized with 58 patients in the fibrin glue group and 66 patients in the control group (Table 1). The mean patient age was 64 ± 2.0 years and was similar in both groups. There were no differences between the groups in terms of gender, race, or multiple preoperative factors.

No marked differences between the groups were observed in a number of intraoperative parameters (Table 2). Most resections involved pylorus-preservation. Most pancreatic-enteric anastomoses were performed as an end-to-side pancreaticojejunostomy. Radical or extended pancreaticoduodenectomy was not performed in any of the patients. The texture of the pancreas at the neck transection site was judged by the surgeon to be soft in 100% of patients. Table 3 depicts the final pathologic analysis of the resected specimens. The two groups were comparable in terms of pathology, with the most common tumor being ampullary adenocarcinoma.

Complications

The postoperative complications and course are illustrated in Table 4. There was one death in the

Table 1. Patient characteristics and preoperative factors

Patient Characteristics	Fibrin Glue n = 58	No Fibrin Glue n = 66
Age (year)	64 ± 2	64 ± 2
Mean	66	66
Median	37 (64%)	40 (61%)
Gender (male)		
Race		
White	51 (88%)	53 (80%)
Black	2 (3%)	4 (6%)
Other	5 (9%)	9 (14%)
Preoperative factors		
Jaundice	30 (52%)	32 (48%)
Abdominal pain	21(36%)	25 (38%)
Weight loss	18 (31%)	21 (32%)
Nausea/vomiting	7 (12%)	7 (11%)
GI blood loss	2 (3%)	2 (3%)
Fever/chills	0 (0%)	3 (5%)
Hypertension	18 (31%)	22 (33%)
Coronary artery disease	6 (10%)	8 (12%)
Diabetes mellitus	4 (7%)	6 (9%)
Chronic lung disease	1 (2%)	2 (3%)
Peripheral vascular disease	2 (3%)	4 (6%)
Pancreatitis	3 (5%)	4 (6%)
Endoprosthesis	21 (36%)	22 (33%)
PTC	12 (21%)	8 (12%)

GI = gastrointestinal; PTC = percutaneous transhepatic catheter.

control group (a patient suffering a myocardial infarction on postoperative day 7). One patient in each group required reoperation during their index admission. Overall, 56% of the patients exhibited one or more postoperative complications distributed as 52% in the fibrin glue arm and 61% in the control arm. The most common postoperative complications were pancreatic fistula, delayed gastric emptying, and wound infection. There were no differences with respect to any specific complications between the two groups. The complication rates were similar between the two groups, as was the length of postoperative hospital stay (fibrin glue group: mean = 12.2 ± 0.8 days, median = 10 days; control group: mean = 13.6 ± 1.0 days, median = 11 days). None of the complications were believed to be directly associated with fibrin glue application.

Table 2. Intraoperative parameters

Intraoperative Parameters	Fibrin Glue n = 58	No Fibrin Glue n = 66
Type of resection		
Pylorus-preserving	45 (78%)	48 (73%)
Classic	13 (22%)	18 (27%)
Type of pancreatic anastomotis		
PJ	58 (100%)	65 (98%)
PG	0 (0%)	1 (2%)
Type of PJ		
End-to-end	0 (0%)	2 (3%)
End-to-side	58 (100%)	63 (97%)
Median operative time (hr:min)	5:48	5:34
Median blood loss (ml)	800	650
Median red cell transfusion (units)	0	0

PG = pancreaticogastrostomy; PJ = pancreaticojejunostomy.

Pancreatic Fistula

The overall incidence of pancreatic fistula was 28% (35 of 124 patients) and was similar between the fibrin glue group at 26% (15 of 58 patients) and the control group at 30% (20 of 61 patients). The mean postoperative length of hospital stay for patients with a pancreatic fistula in the fibrin glue arm was 18.9 ± 0.7 days (median = 19 days), whereas the mean postoperative stay in control patients with pancreatic fistula was 21.7 ± 1.3 days (median = 19 days).

Cost Issues

This study was performed with the expert assistance of the Johns Hopkins Blood Bank and the surgical operating room nurses. The cost of the fibrin glue product was determined from hospital cost data with a cost per application of $164. Typical application included two package administrations; therefore, the saving per patient by not using fibrin glue was $328. The cost savings for the Johns Hopkins Hospital by not using fibrin glue during a typical year at this institution (250 Whipples per year) would be $82,000 per year. If application was limited only to patients with a soft gland as was performed in this study (estimated to be 31% of patients undergoing pancreaticoduodenectomy), a cost savings of $25,420 would be observed.

DISCUSSION

This prospective randomized single institution trial was designed to evaluate the efficacy of topical administration of a fibrin glue sealant to prevent pancreatic anastomotic leak after pancreaticoduodenectomy. The primary end points of this study included pancreatic fistula and other intraabdominal complications, death, and the length of postoperative hospital stay. During this period, we recruited, randomized, and analyzed 124 patients. Recognizing the infrequent occurrence of pancreatic anastomotic leak associated with glands of hard or intermediate texture, only soft textured glands with a high risk of pancreatic leak were included in this study. As illustrated in

Table 3. Pathologic findings

Pathologic Findings	Fibrin Glue n = 58	No Fibrin Glue n = 66
Ampullary adenocarcinoma	16 (28%)	15 (23%)
Pancreatic adenocarcinoma	13 (22%)	15 (23%)
IPMN	8 (14%)	6 (9%)
Pancreatic cystadenoma	5 (9%)	1 (2%)
Malignant islet cell tumor	5 (9%)	0 (0%)
Distal bile duct adenocarcinoma	4 (7%)	6 (9%)
Duodenal adenocarcnoma	1 (2%)	3 (5%)
Tubular/villous adenoma	1 (2%)	4 (6%)
Cystadenocarcinoma	0 (0%)	1 (2%)
Benign islet cell tumor	0 (0%)	3 (5%)
Chronic pancreatitis	0 (0%)	2 (3%)
IPMN with invasive cancer	0 (0%)	2 (3%)
Other	5 (9%)	8 (12%)

IPMN = intraductual papillary mucinous neoplasm.

Tables 1–3, the fibrin glue and control groups were comparable with respect to patient characteristics and preoperative parameters, intraoperative parameters, and pathologic findings.

The results of this study indicate that topical application of fibrin glue sealant to a completed pancreatic-enteric anastomosis does not decrease the rate of pancreatic fistula, total complications, or death nor does it decrease the overall length of postoperative hospital stay or the length of stay for patients in whom a fistula develops. There were no adverse effects associated with fibrin glue. Experience with this product would suggest that such treatment would be unlikely to be associated with substantial short-term or long-term complications. Yet, based on the frequency of product application, avoidance regarding the routine use of this product will decrease the hospital cost by $25,000–$80,000 per year at this high-volume hospital.

Table 4. Postoperative complications and course

Postoperative Complications and Course	Fibrin Glue n = 58	No Fibrin Glue n = 66
Death	0 (0%)	1 (2%)
Reoperation	1 (2%)	1 (2%)
Percutaneous radiologic intervention	3 (5%)	6 (9%)
Any complication	15 (26%)	20 (30%)
Pancreatic fistula	8 (14%)	16 (24%)
Early delayed gastric emptying	5 (9%)	6 (9%)
Wound infection	3 (5%)	3 (5%)
Bile leak	1 (2%)	3 (5%)
Pancreatitis	1 (2%)	3 (5%)
Cardiac arrythmias	1 (2%)	1 (2%)
Intraabdominal abscess	0 (0%)	2 (3%)
Cholangitis	0 (0%)	1 (2%)
Postoperative hospital stay (days)		
Mean	12.2 ± 0.8	13.6 ± 1.0
Median	19	19
Postoperative hospital stay (days) without pancreatic fistula		
Mean	9.8 ± 0.6	10.1 ± 0.4
Median	8	9

For the last 20 years, dramatic improvements have been observed in the operative results of pancreaticoduodenectomy. Perioperative mortality rates were consistently reported in excess of 20% in series published before 1985. Yet, numerous series published in the last 20 years have reported perioperative mortality to be less than 5%,[1–3] with numerous series reporting no deaths in consecutive resections numbering in excess of 100 patients.[1,3,30,31] Despite this dramatic decrease in perioperative deaths, the complication rate after pancreaticoduodenectomy remains high. The incidence of major postoperative complications approaches 40%–50%.[1–6] In most series, the three most frequent complications include early delayed gastric emptying, wound infection, and pancreatic fistula resulting from pancreatic-enteric anastomotic leak. Although historically the latter complication was a cause of postoperative death and a necessity for reoperation, current management has markedly diminished the severity of the complication. It does, however, remain a frequent reason for extending hospital stay therefore adding to hospital costs and delaying patient recovery.

Because pancreatic anastomotic leak has remained the "Achilles heel" of this operation, considerable attention has been focused on trying to decrease its incidence. These efforts have encompassed various modifications of the pancreatic-enteric anastomosis including differences with regard to operative technique, the use of anastomotic ductal stents, and routine drainage into the stomach (pancreaticogastrostomy)[11–17] as opposed to the small intestine (pancreaticojejunostomy). A randomized prospective trial that addresses these technical issues was published from our institution in which pancreaticogastrostomy and pancreaticojejunostomy were compared.[11] In this study, no differences in anastomotic leak rates or other complications were observed.

One of the more controversial adjuncts appropriated to decrease the risk of pancreatic anastomosis has been the use of prophylactic octreotide. A number of randomized control studies have been reported. Four of these studies[18–21] (from Europe) have indicated a benefit whereas two prospective randomized single institution trials (from the United States)[22,23] have indicated no benefit. It is likely that differences regarding randomization criteria and type of operation as well as analysis techniques may have

contributed to this controversy. At present, no consensus exists with respect to the use of octreotide as an adjunct to promote pancreatic anastomotic healing, but it is generally not used in the United States.

Finally, to diminish the rate of pancreatic anastomotic leak, some surgeons have avoided pancreatic anastomosis choosing to either suture ligate or occlude the pancreatic duct with permanent organic polymers or to simply externally drain the pancreatic remnant.[7–10] The risk of such treatment, however, includes not only short-term complications, but also the induction of pancreatic atrophy with complete loss of exocrine function and almost certain loss of pancreatic endocrine function.

Recently, attention has focused on the role of fibrin glue sealant as an adjunct in the healing of pancreatic anastomoses including its use as either a topical application or as an intraductal injection for temporary pancreatic ductal occlusion during pancreaticoduodenectomy or distal pancreatectomy. Although numerous authors have demonstrated a considerable advantage regarding the use of fibrin glue in uncontrolled trials,[24–27] the treatment has revealed mixed results from three reported prospective randomized comparisons. In the first trial, 56 patients undergoing distal pancreatectomy for either gastric cancer or pancreatic disease were randomly assigned to fibrin glue application to the suture line of the pancreatic stump vs. no treatment.[28] The overall incidence of pancreatic fistula in the treatment group was 15.4% vs. 40% in the control group ($p = 0.04$). In the second prospective randomized study, 96 patients either undergoing pancreaticoduodenectomy (n = 30), pancreaticojejunostomy (40 patients), distal pancreatectomy (23 patients), or tumor excision (4 patients) were randomized to either topical application of fibrin sealant on the pancreatic anastomosis or stump vs. no treatment. In this study, the rate of pancreatic fistula in the treatment arm was 13.9% vs. 11.1% in the control arm (NS). In the final prospective randomized trial, fibrin glue was injected into the pancreatic duct during either distal pancreatectomy or pancreaticoduodenectomy to provide temporary occlusion of the duct.[10] In this study, the fibrin glue was not applied to the surface of the anastomosis or pancreatic stump. One hundred two patients received the fibrin glue ductal injection and 80 patients served as controls. There were, again, no considerable differences with respect to intraabdominal complications and pancreatic fistula; however, the authors clearly noted that the presence of a normal nonfibrotic pancreatic stump and a main pancreatic duct diameter of less than 3 mm were notable risk factors for the development of intraabdominal complications and pancreatic leak. This observation suggests that a flaw exists with regard to all of these studies in that the randomizations did not distinguish between high-risk pancreatic anastomoses (soft gland texture, normal duct) vs. low risk anastomoses (hard gland texture, dilated pancreatic duct). It is possible that if a treatment difference were actually present, the advantage may only exist in those patients in which a high rate of anastomotic failure could be predicted.

In light of the controversial and inconsistent results from both nonrandomized and randomized controlled studies, our group believes that a prospective trial would be appropriate with inclusion limited only to patients undergoing pancreaticoduodenectomy with an anastomosis perceived to be a high risk for failure. Despite this strategy, this study has observed no considerable decrease in the incidence of pancreatic fistula with the application of fibrin glue. The clinical significance of the pancreatic leak was evident with a considerable increase in the length of hospital stay associated with the presence of a fistula. Postoperative hospital stay for patients in whom pancreatic fistulas developed in either group was associated with a median length of stay of 19 days, whereas patients whose course was not complicated by pancreatic fistula indicated a median length of stay of 9 days.

Finally, although the results of this study would seem evident, one final point of discussion remains. This study used the commercially available fibrin glue product (containing 3000 KIU/ml of aprotinin for application) obtainable in the United States. It seems that a higher aprotinin concentration may protect against degradation of the product by pancreatic enzymes. This advantage would seem most valuable with regard to protocols involving pancreatic ductal injection rather than topical application, as was used in this study. Furthermore, the single prospective trial of topical application of fibrin glue, which indicated an advantage after distal pancreatectomy, employed fibrin glue with 3000 KIU/ml of aprotinin.[28]

In conclusion, this data demonstrates that the topical application of fibrin glue sealant to a high-risk pancreatic enteric anastomosis after pancreaticoduodenectomy does not reduce the incidence of pancreatic fistula, total complication, death, or hospital length of stay. There seems to be no role for the use of this treatment with regard to patients undergoing pancreatic resection.

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Discussion

Dr. L. W. Traverso (Seattle, WA): Thank you for providing this paper, Dr. Lillemoe. To improve, one must measure one's outcomes. In the case of pancreatic leak after an anastomosis, the Hopkins group has achieved this on two prior occasions with regard to prospective randomized trials. The first one was a prophylactic octreotide study, which revealed no difference in leak rate, and the second one dealt with

connecting the pancreatic duct to the jejunum versus the stomach, which also indicated no difference. Today, they report a third trial regarding the use of fibrin glue to produce more favorable outcomes after a pancreaticojejunostomy.

What are the pros of this study regarding the way in which it was designed? Well, this is the first trial to limit the entrance criteria to just pancreaticoduodenectomy with pancreaticojejunostomy (except for one case of pancreaticogastrostomy). Distal pancreatectomy was excluded. Because the study involved just a few surgeons, the variability of the surgeon was decreased and these are high-volume surgeons. Except for the partial use of internalized stents, in general, everyone performed the operation the same way. It was limited only to patients at risk; that is, of the 404 patient cases handled during this time, only 124 were entered because the surgeon subjectively believed that the pancreatic remnant was at risk to leak.

According to the paper, the study was stopped early because they observed no difference using their power analysis and the expected fistula rate. My first question is, why stop the study regarding leak rates based on amylase in drains? Why not stop it because of observations that might suggest the quantity or the severity of leak such as delayed gastric emptying or the use of external percutaneous drainage?

The cons of this study, as I see it, are that the drains may fail to indicate an increased amylase when for some patients there is a leak. The only way you can tell if a leak or fistula is present is if delayed gastric emptying occurs, if percutaneous drainage is necessary, or both. Did you find that any of the patients who did not exhibit elevated amylase in their drains actually resulted in the development of a pancreatic fistula that required drainage?

One more question. I find fibrin glue to exhibit very poor adherence. When you apply the liquid around the anastomosis, allow it to dry, and then pass a sucker by, the film of dried glue comes off easily. I have often wondered how it is even remotely fathomable that this glue could stop any leakage.

Thank you, Keith, for a fine study that allows us to examine other variables, besides fibrin glue, that could potentially improve pancreaticojejunostomy leak rates.

Dr. Lillemoe: Thank you, Dr. Traverso. Bill noted that we did stop this study earlier than we anticipated. We had predicted that if fibrin glue was to indicate an advantage, it would lower the fistula rate from 25% to 10% and that we would require approximately 115 patients in each arm of the study. At the time of analysis, which was about 2 years into the study, as you could see, the fistula rate was quite high and there was really no difference.

Meeting with our statisticians, they told me that if we were planning to complete the study and add the full 115 patients, every one of them in the control arm would have to leak and every patient in the fibrin glue would not leak so perhaps we would possibly have a chance of reaching statistical significance. However, your point is well taken regarding the fact that this was the single end point we examined. We did not examine other complications nor did we assess the need for intervention as a difference, although, as could be conceived, there was not much of a difference between the two.

You spoke briefly regarding the nature of our leaks. As you are aware, we have experienced a fairly liberal acceptance with regard to the definition of pancreatic leak. I know the group just got together in Greece and redefined what a pancreatic leak is. This will probably add to their clinical significance.

There was no difference regarding the number of interventions that were necessary between the two groups. I would add that some of these were not just based on serum amylase. They were based on fistulograms. They were somewhat contingent upon the clinical appearance. However, I think that it was rather evident based on all of the analysis, even sequentially and not just with respect to the measurement of amylase in the drains, that there was no advantage.

Finally, you commented on the fibrin glue and I would agree with you that vigorous irrigation and suction in proximity to this product would probably make it disappear. The lack of confidence you have that the glue will actually initiate an effect does raise some concern. The company itself told us that we were actually using the American product with respect to the percentage of aprotinin in the fibrin glue and that if we had used the European product, it would most likely have been successful. I would add, however, that the one prospective randomized trial that indicated a difference actually used the same concentration that we did. Whether there is a different bioadhesive—another substance that might work—I do not know. We may investigate that as another option for preventing leaks in this setting, as well as in distal pancreatectomies, which can also be problematic.

Dr. A. Warshaw (Boston, MA): Keith, I would like to congratulate you not only for conducting another randomized trial, but for the honesty with which you have reported its results. As you point out, the leak problem continues to plague pancreatic surgery: the leak rate after distal pancreatectomy is even higher than after a Whipple resection.

Addressing the issue of adhesive types, we do not actually know what the antiprotease levels would be

that might prevent pancreatic juice dissolution of fibrin glue, but I would suggest that we convene to figure out an appropriate in vitro analysis of what would be an effective level. We have used a nonbiologic material, Focal Seal (Genzyme Corp., Cambridge, MA), which is a light-activated adhesive, for distal pancreatectomies and it has not worked either.

I have several questions for you. Was there some variation among the participating surgeons in your study and their individual preferences regarding how the pancreas was drained? One could argue that drains exhibit two aspects—one is preventative of collections and the other can be causative of anastomotic breakdown. The presence of a drain near an anastomosis may actually promote leakage from it by interfering with tissue apposition. That has been illustrated, for example, with colon anastomoses. Because the surgeons in your study either used one or two drains, is it possible to perform a subset analysis that relates to the actual drainage method in these cases? Also, the stent versus no stent question might be asked. Do you have enough cases to perform a subset analysis with regard to stenting the pancreatic anastomosis created a difference?

Finally, I ask for your help. When you observe one of these leaks, as you point out in your paper, most of them turn out to be benign. That is, most are eventually self-limited and one can safely remove the drains after the leakage ceases. Occasionally, however, trouble ensues, especially with regard to bleeding from an eroded gastroduodenal artery. What criteria do you use regarding the need to reexplore a patient in whom we fail to prevent a leak?

Dr. Lillemoe: Thank you, Andy. I suppose when you are talking about honesty, you mean someone who will stand up and admit to having a 30% pancreatic anastomotic leak as either being honest or stupid, I assume. Also, I would agree with you that the distals are most likely more problematic, particularly distals that are performed with, in actuality, a normal gland on the other side as well.

You spoke about differences regarding perioperative or operative techniques among the surgeons. That would have been a good point, that is, to analyze the role of drains, because we do possess that information, whether or not there was one or two drains placed. Actually, that could probably still be accomplished. I would say, however, that there was no actual difference between the three primary surgeons who handled over 90% of this in terms of the fistula rate. Also, I do not think we consistently used—I almost always use two and John will occasionally use just one, but every patient did have a drain. I would acknowledge that.

With respect to stenting, I am the only surgeon who used a stent for that anastomosis at that institution. The others did not use stents. Again, there was no difference between my rate and the other individual's rates.

Regarding your question concerning the criteria to reoperate, I think the number of patients who actually need to be reoperated on to control sepsis or bleeding in all of our institutions has decreased dramatically and, in general, we attempt whatever manipulations can be accomplished with the interventional radiologist to control sepsis and to control the collections that may be there.

I think a patient has to undoubtedly exhibit uncontrolled sepsis associated with a collection before we would intervene or, obviously, if bleeding occurs. If we did have to operate for bleeding, in most cases it would be after arterial embolization, because I think that is the best way to, at least, temporize until you can get to the operating room so the patient is not exsanguinating on the table. It is a very rare occurrence; in fact, seldom does it occur that our interventional radiologists fail us.

Dr. G. Aranha (Maywood, IL): Keith, that was a very interesting presentation and welcome to the midwest. I would like to ask you, because I like most pancreatic surgeons, have noted that a soft pancreas is observed in ampullary carcinomas and cystic tumors, and adenomas. I suspect that when you speak of adenomas you mean the villous adenomas of the ampulla and the duodenum. I want to concentrate on that group and ask you if are you selective. Do you use endoscopic ultrasound to decide which is a T1 lesion, resect those transduodenally, or do you perform Whipples on all adenomas?

Dr. Lillemoe: There are some cyst adenomas of the pancreas that were included in there, but there were, as you noted, some periampullary villous adenomas that were also included. We do use endoscopic ultrasound to try to help. I think Dr. Warshaw's group reported that endoscopic biopsies are incorrect about 50% of the time with regard to assessing the determination of cancer versus noncancer and I think EUS has aided us to assess more thoroughly. It has also helped us to understand the relationship of the villous adenoma with regard to the pancreatic duct and the bile duct. To answer to your question, then, if we observe a lesion that seems to be benign with respect to all the endoscopic criteria and benign regarding biopsies, we will attempt a transduodenal excision. If it can be accomplished with negative margins and allow for reconstruction, we will do that, although I think more cases than not will end up performing pancreatoduodenectomy in that setting.

Recurrent Disease After Microscopically Radical (R0) Resection of Periampullary Adenocarcinoma in Patients Without Adjuvant Therapy

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The survival rate after microscopically radical resection of pancreatic duct adenocarcinoma is still poor. Patients with ampulla of Vater and distal common bile duct adenocarcinoma indicate a much more favorable prognosis. Controversy exists as to whether adjuvant therapy could improve the outcome in these patients after resection. The aim of the present study was to analyze the pattern of recurrence in patients with periampullary adenocarcinoma after pancreatoduodenectomy. Between January 1992 and December 2002, all patients with an R0 resection were identified and used for this analysis. A total of 190 patients underwent a microscopically radical resection and received no adjuvant therapy. Of those, 72 patients were diagnosed with pancreatic duct adenocarcinoma, 86 patients were diagnosed with ampulla of Vater adenocarcinoma, and 31 patients were diagnosed with distal common bile duct adenocarcinoma. Recurrent disease was indicated in 81% of the patients with pancreatic duct adenocarcinoma, 50% of the patients with ampulla of Vater adenocarcinoma, and in 74% of the patients with bile duct adenocarcinoma. Multivariate analysis revealed that lymph node metastases were prognostic for recurrent disease in patients with pancreatic duct adenocarcinoma ($P = 0.038$). The depth of invasion (T4, $P < 0.032$) and lymph node metastases ($P < 0.001$) were prognostic in patients with ampulla of Vater adenocarcinoma. Poor tumor differentiation ($P < 0.001$) was prognostic in patients with distal bile duct adenocarcinoma. Selected patients with periampullary malignancies exhibited a high recurrence rate and should be encouraged to enroll in clinical trials for adjuvant treatment including local therapy (radiotherapy) according to the identified prognostic factors. (J GASTROINTEST SURG 2004;8:775–784)

KEY WORDS: Carcinoma, pancreatic ductile; bile duct neoplasms; ampulla of Vater; chemotherapy; adjuvant; radiotherapy, adjuvant

Pancreatic duct adenocarcinoma accounts for 5% of cancer deaths and radical resection offers the only hope for cure.[1] Although considerable improvements have been achieved concerning the safety of pancreatic resection, as illustrated by the decreasing mortality and morbidity rates, the overall 5-year survival rate remains disappointing.[2–5] Recent studies examining the prognostic factors with regard to the survival rate indicated that a microscopically radical resection margin is the most powerful independent prognostic factor for long-term survival rate.[3,6] However, the survival rate still remains poor even after a microscopically radical resection.

A possible benefit of adjuvant treatment for pancreatic duct adenocarcinoma was already recognized by the Gastrointestinal Tumor Study Group (GISTS) almost two decades ago.[7] Since then numerous studies have indicated modest results in terms of the survival benefit after adjuvant chemotherapy.[8–10] Unfortunately, most of these studies were statistically underpowered or did not include an observation-only arm. Recently, a large randomized trial indicated a substantial survival benefit in patients who received adjuvant chemotherapy after resection as compared with patients who were observed or who underwent

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chemoradiation after resection for pancreatic duct carcinoma.[11]

Compared with pancreatic cancer, patients with ampulla of Vater adenocarcinoma exhibit a relatively favorable outcome.[12,13] Consequently, controversy exists regarding the benefit of adjuvant treatment for ampulla of Vater adenocarcinoma. A multicenter EORTC (European Organization for Research and Treatment of Cancer) study indicated no substantial benefit in the survival rate in patients with periampullary adenocarcinoma (pancreatic duct, ampulla of Vater, and distal bile duct adenocarcinoma).[14] The latter study resulted in a consensus to not provide adjuvant therapy routinely in our center.

Adjuvant treatment modalities are continuously being developed. Modalities such as gene therapy are in phase I trials and will hopefully substantiate an important role in the future.[15] To develop optimal adjuvant treatment strategies, it is important to understand the pattern of recurrence after pancreatoduodenectomy. The present study represents a single-center experience in which patients underwent a microscopically radical resection for periampullary adenocarcinoma and did not receive adjuvant therapy. The aim of the present study is to analyze the pattern of recurrence and evaluate prognostic factors for recurrence to identify patients suitable for adjuvant treatment.

MATERIAL AND METHODS

Patients

Between 1992 and 2002, 459 patients underwent a pancreatoduodenectomy in the Academic Medical Center (Amsterdam, The Netherlands). Of these, 173 patients were diagnosed with pancreatic duct adenocarcinoma (38%), 115 patients were diagnosed with ampulla of Vater adenocarcinoma (25%), and 60 patients were diagnosed with distal common bile duct adenocarcinoma (13%). Forty-seven patients underwent a pancreatoduodenectomy for chronic pancreatitis (10%) and 64 patients underwent surgery for cystic, endocrine, or other rare tumors (14%). Only the patients with microscopically tumor-free resection margins and periampullary adenocarcinoma were selected from our prospectively collected database for this retrospective analysis. Patients with duodenal adenocarcinoma were not included in this analysis.

Surgical Technique

A pancreatoduodenectomy was performed as previously described.[16–18] To state it briefly, an en bloc resection of the duodenum, pancreatic head, bile duct, and gallbladder were performed, whereas the pylorus was preferably preserved. Only lymph nodes surrounding the pancreas anteriorly and posteriorly, in the hepatoduodenal ligament, and right of the common hepatic artery and superior mesenteric vein were removed.[19] Paraaortal nodes were sampled preoperatively for staging studies in selected patients and the results indicated no therapeutic consequences. If limited involvement of the portal vein or superior mesenteric vein was indicated, a vascular wedge resection would be performed with curative intent. Segmental superior mesenteric or portal vein resections were not performed in the present study. Reconstruction was performed using a retrocolic jejunal loop with an end-to-side pancreaticojejunostomy, hepaticojejunostomy, and gastrojejunostomy or duodenojejunostomy.

Histological Assessment of Specimen and Recurrence Identification

Patients were classified according to the pathological tumor-node-metastasis (pTNM) stages (Union Internationale Contre le Adenocarcinoma, 1997). Only patients with resections classified as R0, defined as microscopically complete removal of the tumor, were analyzed. Patient follow-up occurred according to a standardized schedule of visits at 3, 6, and 12 months after discharge from the hospital, followed by every 6 months for 5 years, and then yearly after 5 years at our outpatient clinic. The follow-up consisted of a discussion with regard to the history of symptoms and a physical examination including weight measurements. Further examinations were only performed if recurrence was suspected and generally consisted of ultrasonography and computed tomography. The pattern of recurrence was divided into local recurrence, defined as anastomotic recurrence or recurrence along the superior mesenteric artery and vein, and distant metastases. Metastases were divided into intraabdominal metastases, defined as metastases occurring in any other organ in the abdominal compartment and extraabdominal metastases, defined as involvement of any extraabdominal organ. Histological or radiological evidence was required for the diagnosis of recurrent disease.

The following prognostic factors for recurrence were analyzed: patient characteristics (age, gender, comorbidity, diabetes, and the American Society of Anesthesiologists classification); type of resection (classical Whipple or pylorus-preserving pancreatoduodenectomy); characteristics of procedure (pancreatic transsection with surgical knife or linear stapler, diameter of pancreatic duct, single jejunal loop or Roux-y, or one- or two-layer anastomosis); drainage of pancreatic or hepatic duct; operative time; packed cells transfused within 24 hours postoperatively; preoperative blood loss; use of octreotide prophylaxis;

pathology of the tumor (tumor infiltration depth [T-stage], lymph node involvement [N-stage], portal or mesenteric vein ingrowth and microscopic vascular or neural ingrowth, defined as tumor ingrowth observed in vascular or neural tissue during microscopic evaluation).

Statistical Analysis

Statistical analysis was performed using SPSS statistical software (version 11.5; SPSS Inc., Chicago, IL). A P value of <0.05 was considered statistically significant. Continuous variables are depicted as mean with standard deviation (SD) if the distribution was Gaussian or median and range if the distribution was not Gaussian. The Wilcoxon–Mann–Whitney and Kruskal–Wallis tests were used accordingly to analyze differences between continuous data. Pearson's test was used to assess if differences between dichotomous groups were significant. Fisher's exact test was used when a table exhibited a cell with an expected frequency of less than 5. The recurrence rate was computed using the Kaplan–Meier estimation of recurrence at 5 years, omitting all proven instances of death due to other causes. These patients were censored at the point that they died from some other cause. The date of the radiological or pathological examination was used as recurrence date. The recurrence rate and survival rate are depicted as mean with 95% confidence interval. A univariate analysis of specific prognostic factors for recurrence was performed using the log-rank test. A multivariate analysis of all the significant prognostic factors was performed using the Cox proportional hazards model with simultaneous inclusion of the significant prognostic factors indicated after univariate analysis.

RESULTS

A microscopically radical resection (R0) was achieved in 87 of the 173 patients (50%) with pancreatic duct adenocarcinoma, 98 of the 115 patients (85%) with adenocarcinoma of the ampulla of Vater, and 35 of the 60 patients (58%) with adenocarcinoma of the distal common bile duct. Eleven patients (5%) with pancreatic duct adenocarcinoma and 2 patients (6%) with distal bile duct adenocarcinoma received adjuvant treatment in the early years (EORTC trial) and were subsequently excluded.[14]

Patient Characteristics

Patient characteristics were comparable for all groups with the exception of the surgery-related complication rate (Table 1). Patients with pancreatic duct adenocarcinoma indicated a lower surgery-related complication rate compared with patients with ampulla of Vater adenocarcinoma (37% vs. 52%, respectively, $P < 0.05$). The in-hospital mortality rate was 0%, 4%, and 3% for pancreatic duct, ampulla of Vater, and distal common bile duct, respectively. Mortality during follow-up due to other causes (n = 13) were comparable in all three groups and consisted of myocardial infarction (n = 6), stroke (n = 3), septicemia (n = 2), histologically proven unresectable lung adenocarcinoma (n = 1), and multiple liver abscesses confirmed surgically (n = 1) without signs of recurrence. These patients exhibited a mean survival rate of 34 months (95% confidence interval [CI] = 13–55) and were excluded from further analysis. Overall, 72 patients with pancreatic duct adenocarcinoma, 86 patients with ampulla of Vater adenocarcinoma, and 31 patients with distal bile duct adenocarcinoma were eligible for analysis.

Table 1. Patient characteristics

	Pancreatic duct (n = 76)	Ampulla of Vater (n = 98)	Distal bile duct (n = 33)
Mean age in years (SD)	64 (10)	64 (9)	63 (12)
Gender ratio, male/female	1.05	1.39	1.75
Median duration of symptoms in weeks (range)	11.5 (2–104)	12 (1–75)	10.5 (4–30)
Pain	31 (41%)	36 (37%)	16 (47%)
Jaundice	73 (96%)	84 (86%)	32 (97%)
Preoperative drainage (% of patients with jaundice)	70 (96%)	84 (100%)	31 (97%)
Surgery related complications[‡]	28 (37%)[†]	51 (52%)*	12 (36%)
General postoperative complications[§]	25 (33%)	29 (30%)	11 (33%)
In-hospital mortality rate	0	4 (4%)	1 (3%)
Mortality due to other causes during follow-up	4 (5%)	8 (8%)	1 (3%)
No. of patients eligible for analysis	72	86	31

$P < 0.05$ compared to *pancreatic duct and [†]ampulla of vater.
[‡]Surgery related complications included: pancreatic leakage, biliary leakage, intra-abdominal abscesses, hemorrhage, woundinfection and sepsis.
[§]General postoperative complications included: cardiac, urological and pulmonary complication, and embolisms.

Pattern and Time of Recurrence

Complete follow-up, defined as follow-up until death, was obtained in 57 patients (75%) with pancreatic duct adenocarcinoma, 54 patients (55%) with ampulla of Vater adenocarcinoma, and 24 patients (73%) with distal bile duct adenocarcinoma. The median follow-up for the remaining patients who were still alive was 47 months (16–137). No patients were lost to follow-up in the present study. Recurrent disease was indicated in 58 out of 72 patients (81%) with pancreatic duct adenocarcinoma, 43 out of 86 patients (50%) with ampulla of Vater adenocarcinoma, and 23 out of 31 patients (74%) with distal bile duct adenocarcinoma and was lower in patients with an ampulla of Vater adenocarcinoma compared with the other groups ($P < 0.05$). The mean time to recurrence was longer for patients with ampulla of Vater adenocarcinoma (42 months, 95% CI 34–50, $P < 0.05$) compared with pancreatic duct adenocarcinoma (21 months, 95% CI 14–27) and distal bile duct adenocarcinoma (20 months, 95% CI 14–25).

The overall pattern of recurrent disease was not significantly different between the groups with recurrent disease (Table 2). Local recurrence alone was indicated in 19 patients (33%), 13 patients (30%), and 8 patients (35%) with pancreatic duct, ampulla of Vater, and distal bile duct adenocarcinoma, respectively. Metastases were indicated in 39 patients (66%), 30 patients (66%), and 15 patients (65%), respectively. The mean time to extraabdominal metastases was 20 months (95% CI = 13–26) and was longer compared with local recurrence (12 months, 95% CI = 9–14) and intraabdominal metastases (10 months, 95% CI = 8–12, $P = 0.005$). The mean survival rate after detection of recurrence was comparable for all groups: 4.2 months (95% CI = 3.0–5.5), 4.7 months (95% CI = 0–6.3), and 5.5 months (95% CI = 3.0–8.0), respectively.

Prognostic Factors for Recurrence

A univariate analysis revealed that lymph node involvement ($P = 0.035$) was the only prognostic factor for recurrence in patients with R0 resected pancreatic duct adenocarcinoma (Table 3). Prognostic factors for recurrence in patients with ampulla of Vater adenocarcinoma included pain in history ($P < 0.001$), T2 (tumor invades duodenal wall) vs. T3 (tumor invades 2 cm or less into pancreas invasion depth, $P = 0.036$), T3 vs. T4 (tumor invades more than 2 cm into pancreas and/or into adjacent organs invasion depth, $P < 0.001$), lymph node involvement ($P < 0.001$), and microscopic neural ingrowth ($P = 0.012$). Prognostic factors for recurrence in patients with distal bile duct adenocarcinoma included T1 vs. T2 or more advanced invasion depth (tumor invades beyond the wall of the bile duct, $P = 0.008$) and good vs. moderate ($P = 0.015$) and moderate vs. poor ($P < 0.001$) differentiation grades of the tumor. A multivariate analysis revealed the following independent prognostic factors for recurrent disease: lymph node involvement for patients with pancreatic duct adenocarcinoma, lymph node involvement and T4 invasion for patients with ampulla of Vater adenocarcinoma, and good vs. moderate and moderate vs. poor differentiation for patients with bile duct adenocarcinoma. The recurrence rate after 5 years for patients with pancreatic adenocarcinoma was 67% if there was no lymph node involvement compared with 93% if lymph nodes were involved (Fig. 1, *top*). The

Table 2. Characteristics of recurrence after R0 resection

	Pancreatic duct	Ampulla of Vater	Distal bile duct
No. of eligible patients	(n = 72)	(n = 86)	(n = 31)
No. of patients with recurrent disease	58 (81%)[†]	43 (50%)[*‡]	23 (74%)[†]
Mean recurrence free survival, in months (95%, CI)	21 (14–27)	42 (34–50)	20 (14–25)
No. of patients with recurrent disease	(n = 58)	(n = 43)	(n = 23)
Local recurrence	19 (33%)	13 (30%)	8 (35%)
Metastasis	39 (66%)	30 (70%)	15 (65%)
Intra-abdominal	36 (62%)	26 (61%)	14 (61%)
Proportion in liver	30 (83%)	23 (89%)	13 (93%)
Extra-abdominal	3 (5%)	4 (9%)	1 (4%)
No. of patients with local recurrence ≤6 months	6 (10%)	3 (7%)	3 (13%)
No. of patients with distant metastasis ≤6 months	24 (41%)	11 (26%)	5 (22%)
Mean survival after detection of recurrence, in months (95%, CI)	4.2 (3.0–5.5)	4.7 (3.0–6.3)	5.5 (3.0–8.0)

$P < 0.05$ compared to *Pancreatic duct, [†]Ampulla of Vater and [‡]Distal bile duct.

Table 3. Prognostic factors for recurrence after R0 resection in univariate analysis (Kaplan-Meyer 5-year)

	Pancreatic duct (n = 72)			Ampulla of Vater (n = 86)			Distal bile duct (n = 31)		
	n	Percentage	*P*-value	n	Percentage	*P*-value	n	Percentage	*P*-value
Pain									
No	41	87	0.353	55	47	<0.001	17	76	0.831
Yes	31	84		31	76		14	89	
Depth of invasion									
T1	17	100	0.697	22	33	0.510	6	58	0.008
T2	28	81	0.853	27	50	0.036	10	100	0.920
T3	27	80		31	78	<0.001	15	86	
T4	0	-		6	100		0	-	
Lymph node involvement									
N0	21	70	0.035	50	32	<0.001	22	81	0.979
N1	51	93		36	92		9	100	
Microscopic neural invasion									
No	43	87	0.738	72	53	0.008	16	81	0.514
Yes	29	83		14	83		15	91	
Differentiation grade									
Good	8	63	0.084	11	46	0.914	5	20	<0.001
Moderate	29	93	0.308	43	59	0.743	19	100	0.015
Poor	35	85		32	60		7	100	

recurrence rate after 5 years for patients with ampulla of Vater adenocarcinoma was 32% for patients with a T1–T3 and N0 tumor, 90% for patients with a T1–T3 and N1 tumor, and 100% for patients with a T4 and N1 tumor (Fig. 1, *middle*). For patients with distal bile duct adenocarcinoma, a recurrence rate of 100% was observed in patients with a poor and moderate differentiation and a recurrence rate of 20% was observed for patients with good differentiation (Fig. 1, *bottom*). The overall actuarial 5-year survival rate was 17%, 41%, and 12% for pancreatic duct, ampullary, and distal common bile duct adenocarcinoma, respectively (Fig. 2). The overall survival rate was higher for patients with ampulla of Vater adenocarcinoma compared with pancreatic and distal bile duct adenocarcinoma ($P = 0.01$).

DISCUSSION

In patients undergoing (pylorus-preserving) pancreatoduodenectomy for periampullary adenocarcinoma with R0 resection, tumor recurrence was 81% for pancreatic adenocarcinoma, 74% for distal bile duct adenocarcinoma, and 50% for ampullary adenocarcinoma with a median follow-up of 47 months. With regard to the patients with metastases, this occurred within 6 months of surgery in 41% of the patients with pancreatic duct adenocarcinoma compared with approximately a quarter of the patients with ampulla of Vater and distal bile duct adenocarcinoma. The overall 5-year survival rates were 17%, 12%, and 41%, respectively.

Various methods have been applied with the intent of reducing the local recurrence rate, especially in patients with pancreatic duct adenocarcinoma. Firstly, this was addressed by performing a more extended surgical resection. These resections comprised radical peripancreatic soft tissue clearance combined with resection of major vascular structures, but did not yield a more favorable outcome.[20] Later, more extensive nodal dissection was proposed by many authors with the hope of achieving a more encouraging outcome, but recent controlled studies clearly indicate that extensive lymphadenectomy confers no survival benefit.[19,21–24] Secondly, most studies have focused on radiotherapy with or without chemotherapy to reduce the local recurrence rate, but so far have met with limited success.[11,25]

Strategies to decrease the distant metastases rate and improve the survival rate have concentrated on a more systemic approach. The GITSG trial indicated an improved survival rate using a combination of radiotherapy and concurrent chemotherapy (5-Fluorouracil) in patients with R0 resection. Adjuvant treatment was associated with a significantly improved survival rate (20 vs. 10% at 2 years), but the study was criticized for its long inclusion time, early termination, low radiation dose, extended interval between surgery and treatment initiation, and, finally, the limited number of patients involved in the analysis. Preliminary reports with regard to adding interferon-α to modified GISTG (Gastrointestinal Tumor Study Group) adjuvant chemoradiation protocol seems promising given a 2-year survival rate of 84%.[26,27]

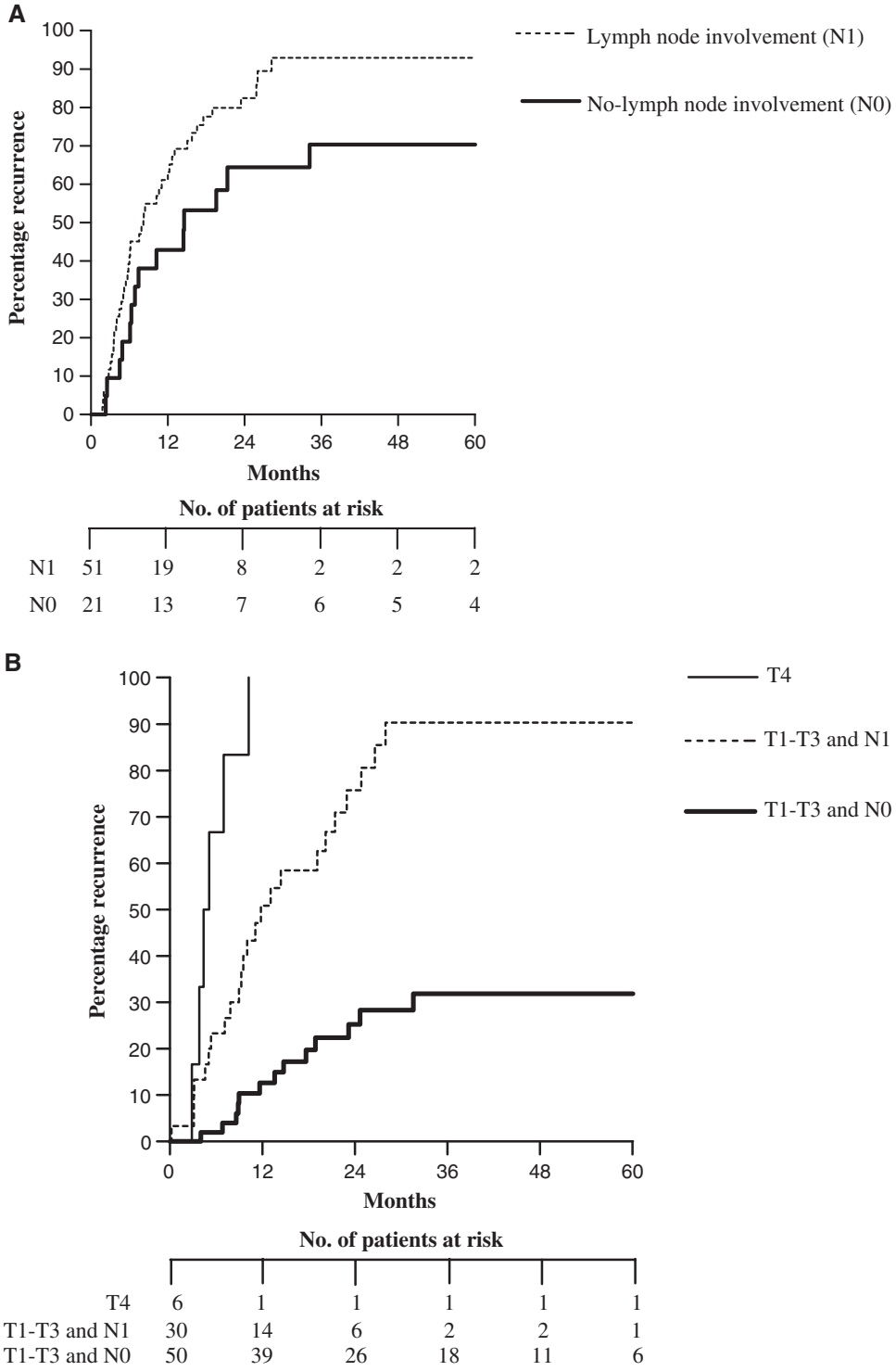

Fig. 1. Recurrence rate according to the prognostic factors indicated in multivariate analysis in patients with pancreatic adenocarcinoma (**A**) (N0 = no lymph node involvement, *solid line*; N1 = lymph node involvement, *dashed line*), ampulla of Vater (**B**) (T1−T3 [T1 = tumor limited to ampulla of Vater or sphincter of Oddi, T2 = tumor invades duodenal wall, T3 = tumor invades pancreas] and N0 [N0 = no lymph node involvement], *thick solid line*; T1−T3 and N1, *dashed line*; T4 [T4 = tumor invades peripancreatic soft tissues or other adjacent organs or structures], *thin solid line*), and distal bile duct adenocarcinoma (**C**) (poor differentiation = *thick solid line*; moderate differentiation = *dashed line*; good differentiation = *thin solid line*).

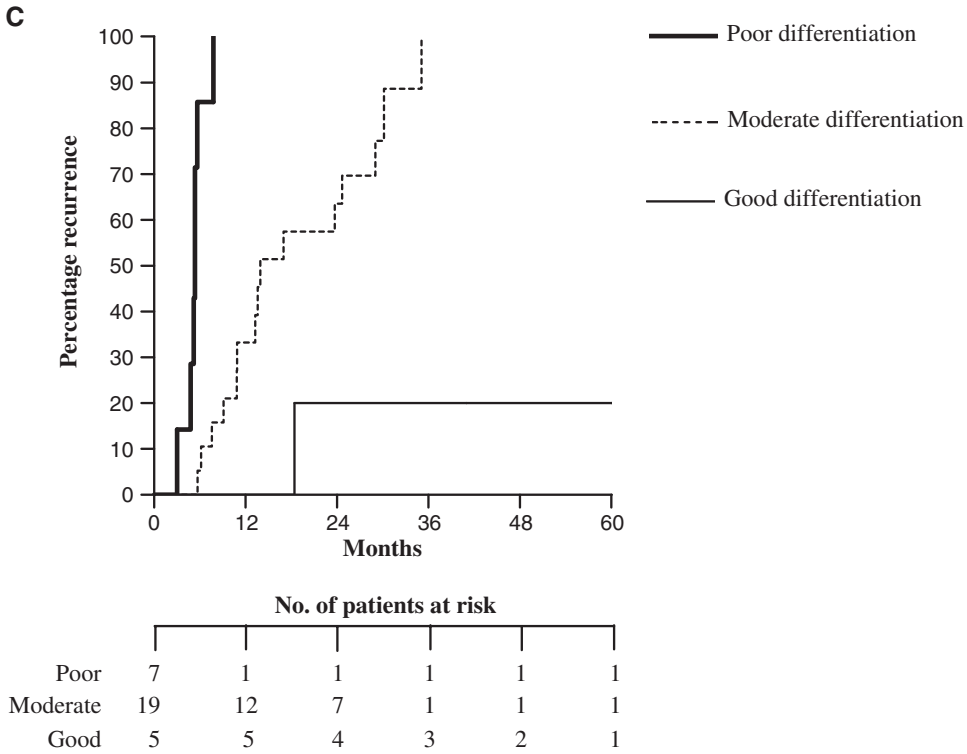

Fig. 1. (*Continued*)

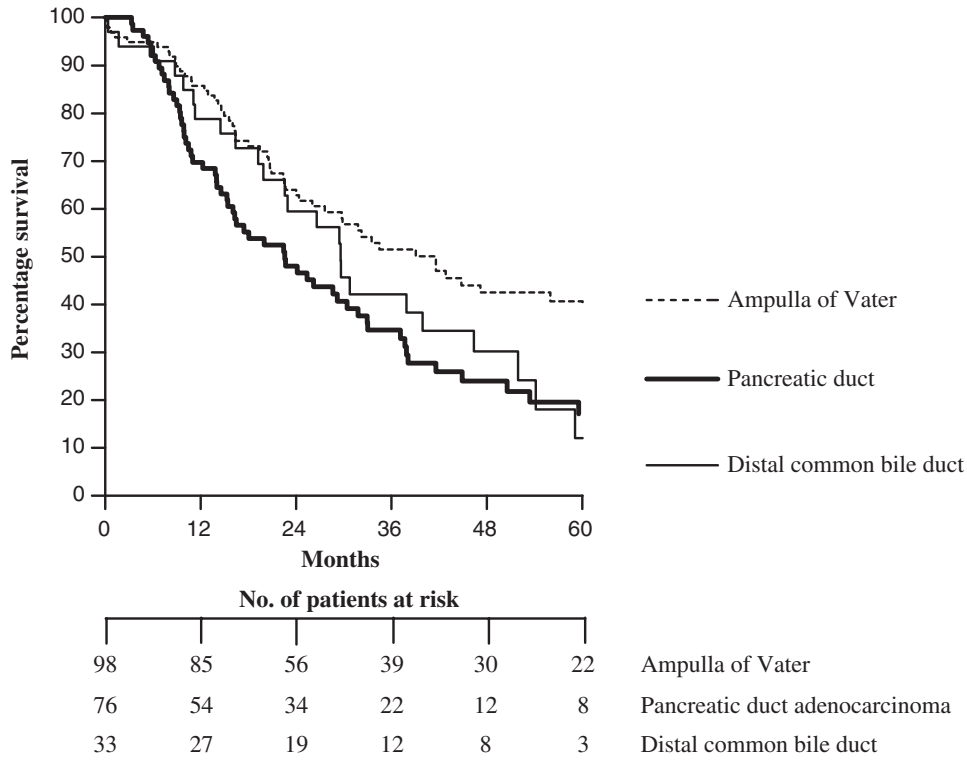

Fig. 2. Overall survival rate for patients with periampullary carcinoma and R0 resection (ampulla of Vater = *dashed line*; pancreatic duct = *thick solid line*, distal bile duct = *thin solid line*).

The ESPAC-1 (European Study Group for Pancreatic Cancer—Trial 1) trial indicated no survival benefit in patients who received chemoradiation (median survival rate of 15.5 months with chemoradiation vs. 16.7 months without chemoradiation). In contrast, chemotherapy alone revealed a significant benefit (median survival rate of 21.6 months with chemotherapy vs. 14.8 months without chemoradiation, $P < 0.001$).[11,25] Although this trial provides the best evidence to date, several drawbacks should be taken into account. Patients and clinicians were allowed to select which trial to enter, introducing the possibility of bias. Furthermore, the different treatment arms were pooled together by treatment received rather than according to the intention to treat principle.

The EORTC trial indicated no significant difference in survival rate in patients with pancreatic adenocarcinoma (26% vs. 34% for control and treated patients, respectively) and also no difference in patients with ampulla of Vater and distal bile duct adenocarcinoma (67% vs. 63% for control and treated patients, respectively).[14] In contrast, other relatively minimal series do suggest a benefit with regard to postoperative adjuvant therapy for ampullary adenocarcinoma in selected patients.[28,29] Unfortunately, these studies were underpowered and, in the latter, an observational arm was lacking.

The optimal timing of adjuvant therapy is also not clear. Published trials that compare preoperative with postoperative chemotherapy are not currently available.[30–33] A few possible benefits regarding preoperative chemotherapy are conceivable. Firstly, as the present study suggests, 22%–40% of recurrences are detected within 6 months of surgery suggesting that these recurrences were already present at the time of resection. Secondly, delayed delivery of adjuvant therapy in patients with prolonged recovery after pancreatoduodenectomy is overcome.[31,34] On the other hand, a major drawback of neoadjuvant treatment is that staging is dependent upon preoperative imaging with the possible consequence of overtreating or undertreating patients. In addition, a biopsy-proven diagnosis of cancer is not always obtained and approximately 8% of the patients in our center do not exhibit cancer after surgery . Initial reports of neoadjuvant chemotherapy with 5-Fluorouracil demonstrated that it could be given without influencing postoperative mortality and morbidity.[35–38] With regard to preoperative radiotherapy, some practitioners even postulate that pancreatic leakage can be reduced by inducing pancreatic fibrosis, which is a protective factor for anastomotic leakage.

Tumors originating at the ampulla of Vater are generally known for their relatively encouraging prognosis. The high incidence of recurrence and the minimal median time to recurrence of pancreatic and biliary tract adenocarcinomas together with the comparable duration of symptoms and incidence of jaundice in all three groups suggests that there is an inherent biological difference between these adenocarcinomas. It also suggests that because of the anatomical location (i.e., ampullary tumors cause jaundice at an earlier and thus less advanced stage), earlier presentations most likely attribute less to the optimistic prognosis.

The use of adjuvant therapy for periampullary adenocarcinoma is still in the experimental stage in Europe and the recurrence pattern should be taken into account when strategies are considered to improve results. The present study reveals that all patients with pancreatic and distal bile duct adenocarcinomas exhibit a high recurrence rate after microscopically radical resection and could potentially benefit from adjuvant treatment. When considering the use of adjuvant treatment modalities in patients with ampulla of Vater adenocarcinomas, possible harm ought to be carefully discerned, because not all patients exhibit a high recurrence rate. However, selected patients (T1–T3 and N1) should receive adjuvant treatment, because they exhibit a high recurrence rate without adjuvant treatment. These patients ought to be encouraged to enroll in clinical trials so that evaluations regarding the effectiveness of potential adjuvant regimens can be assessed.

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Discussion

Dr. M. Sarr (Rochester, MN): We might say that we have not heard anything new regarding this study, because it is just another prognostic analysis, but I think we have. We have learned that recurrences occur early and that R0 resection is achievable in approximately 50% of the patients with pancreatic cancer vs. the rate of 20% indicated by everyone else. It is a believable survival.

In essence, I have two questions for you. When I think of periampullary cancers, I think of four types and I also include duodenal cancer here. You could have changed this from a triple, that is, a three-base hit, to a home run by including duodenal cancer. This is America, after all. Why did you chose not to include duodenal cancers?Also, you seem to be suggesting that only some patients received adjuvant therapy. Why did only some patients receive adjuvant therapy and not all of them and what adjuvant therapy are you using?

Dr. J. Kral (Brooklyn, NY): Could you explain why one-third of your patients did not return for follow-up? You indicated a 65% follow-up rate.

Dr. S. de Castro: Thank you, Professor Sarr. Concerning the first question, the proportion of patients with duodenal cancer was very limited in our group—approximately 20 patients during the same time frame—which made analysis difficult. Another reason why we excluded these patients was because they underwent different types of surgery. The polyposis and limited adenocarcinomas underwent a pancreatic head-sparing duodenectomy or segmentectomy, which made it difficult to compare outcomes. Concerning the second question, until the New England study, adjuvant therapy was not administered to any of these patients. We do occasionally administer adjuvant treatment to patients with microscopically irradical resection margins, but this is considered a palliative treatment. Currently, we are participating in an upcoming European study to analyze and complement the results of the New England study. Finally, concerning the third question from Dr. John Kral, by complete follow-up we meant follow-up until death. I want to clearly state that 65% of these patients were followed-up until death. The remainder were still alive and involved in the follow-up and indicated a median follow-up of 47 months.

Hepatic Resection for Incidentaloma

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The study goal was to review a single-center experience in hepatic resection for patients who presented with incidental liver tumors. With recent advances in diagnostic imaging techniques, incidental finding of liver tumors, or "incidentalomas," is increasing in asymptomatic and healthy individuals. However, little information is available in the literature regarding the underlying pathology and operative outcomes after hepatic resection. Between January 1989 and December 2002, 1011 patients underwent hepatic resection for liver tumors; of these patients, 107 (11%) were asymptomatic individuals who presented with incidentalomas. Incidentalomas were first detected on percutaneous ultrasonography (n = 83), computed tomography (n = 23), or magnetic resonance imaging (n = 1). Fifteen (14%) patients had preoperative aspiration for cytology or biopsy for histology, and the results correlated with the final pathology in 12 patients. Fifty-six (52%) patients underwent major hepatic resection with resection of three or more Coiunaud's segments. Median postoperative hospital stay was 8 days (range, 3–66 days). The operative mortality rate was 1%, and the operative morbidity rate was 21%. Histologic examination of the resected specimen revealed malignant liver tumors in 62 (58%) patients, including hepatocellular carcinoma (HCC) (n = 48), cholangiocarcinoma (n = 8), lymphoma (n = 2), cystadenocarcinoma (n = 2), carcinoid tumor (n = 1), and malignant fibrous histiocytoma (n = 1). Benign pathologies were found in 45 (42%) patients, including focal nodular hyperplasia (n = 17), hemangioma (n = 12), angiomyolipoma (n = 5), cirrhotic regenerative nodule (n = 4), hepatic adenoma (n = 2), and others (n = 5). On multivariate analysis, male sex, age of greater than 50 years, and tumor size of greater than 4 cm were the independent predictive factors for malignant diseases. On retrospective analysis, 48 patients with HCC who presented with incidentalomas had significantly better survival outcomes after hepatic resection than did 646 patients with HCC who presented otherwise during the same study period. Hepatic resection for patients with incidentalomas is associated with a low operative mortality and acceptable morbidity. The diagnosis of malignant disease, especially HCC, should be considered in male patients older than 50 years who present with large hepatic lesions. (J GASTROINTEST SURG 2004;8:785–793)

KEY WORDS: Hepatic resection, liver tumors, hepatocellular carcinoma, survival outcomes

With recent advances in diagnostic imaging techniques, incidental finding of liver tumors, or hepatic incidentalomas, is increasing in asymptomatic and healthy individuals.[1–3] Although the term has not gained wide acceptance,[4] patients who presented with hepatic incidentalomas represent a potential medical problem concerning their management. Asymptomatic hepatic lesions have been reported to be common findings in healthy individuals.[5] Although most of the lesions are benign in nature and do not cause any symptoms, incidentalomas detected on imaging may pose a significant clinical decision-making dilemma,

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because differentiation between malignant nature and benign disease may not be obvious in some of the patients.[6] Most surgeons recommend hepatic resection for patients harboring incidentalomas with radiologic or clinical features suggestive of malignant diseases.[6,7] However, because the incidentalomas are asymptomatic and there has been no study reporting on the management of a large number of patients, information regarding the underlying pathology and operative outcomes after hepatic resection is lacking. There also has not been any guidelines for clinicians to counsel the patients and to outline the treatment regimen for the patients. Therefore, there is a need to accumulate better and more detailed information related to hepatic resection for incidentalomas to establish more reliable guidelines for patient management. Our study goal was to review a single-center experience in the operative outcome of hepatic resection for 107 patients who presented with incidentalomas during a 14-year period. An analysis of the correlation between various clinical parameters and malignancy was also performed, in an attempt to define the predictive criteria that would help establish a diagnosis and select the patients for surgery.

PATIENTS AND METHODS

Between January 1989 and December 2002, 1011 patients underwent hepatic resection for liver tumors at the Department of Surgery, Queen Mary Hospital, Hong Kong, among whom 107 (11%) patients presented with hepatic incidentalomas. *Hepatic incidentaloma* was defined as the incidental finding of a liver tumor on radiologic examinations in healthy and asymptomatic individuals. The following groups of patients were excluded: (1) patients presenting with symptoms related to the liver tumor; (2) patients with known liver cirrhosis and new liver tumors detected with routine interval screening; (3) chronic hepatitis B carriers with raised preoperative α-fetoprotein of greater than 200 mg/ml, because the probable diagnosis of hepatocellular carcinoma (HCC) was made before liver resection; and (4) patients with liver lesions discovered during the staging or surveillance for recurrence of known malignant diseases (e.g., colorectal cancers) and thought to be metastatic lesions. Incidentalomas with uncertain preoperative diagnosis in these 107 patients were first detected on percutaneous ultrasonography (n = 83), computed tomography (CT) (n = 23), and magnetic resonance imaging (n = 1). Before referral to us, fine-needle aspiration of the incidentalomas for cytology was performed on 8 patients and showed suspicious malignant cells in 6 patients. A percutaneous biopsy of the incidentalomas for histology was performed before referral on 7 patients and showed malignant disease in 5 patients.

Preoperative evaluation for hepatic resection of these 107 patients followed a standard protocol. Preoperative investigation of the patients included blood biochemistry, serum α-fetoprotein level, chest radiography, percutaneous ultrasonography, CT scan of the abdomen, indocyanine green clearance test, and hepatic arteriography in selected patients. All CT examinations of the patients were performed and interpreted by an expert radiologist and reported as probable malignant disease before hepatic resection. In view of the risks of peritoneal seeding of malignant cells, tumor bleeding, and tumor rupture, it was the policy of our institution not to perform any fine-needle aspiration or Tru'cut biopsy for potentially resectable liver tumors. Hepatic resection was performed using the standard technique described previously,[8,9] and an ultrasonic dissector was used for parenchymal transection.[10,11] All patients received the same perioperative care by the same team of surgeons and were cared for in the intensive care unit during the early postoperative period after hepatic resection. All intraoperative complications and postoperative morbidities were recorded prospectively. *Hospital mortality* was defined as death during the same period of hospitalization for the hepatic resection.

The preoperative, intraoperative, and postoperative data were collected prospectively by a single research assistant. Continuous variables were expressed as median (range) and compared using the Mann-Whitney *U* test. Categorical variables were compared using the χ^2 test or Fisher exact test where appropriate. Discriminant analysis was performed on continuous variables to identify the best numeric values to distinguish patients with malignant diseases from those with benign pathologies. Multivariate analysis was performed using a stepwise logistic regression analysis to identify independent predictive factors for malignant disease. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using SPSS for Windows 11.0 computer software (SPSS Inc., Chicago, IL).

RESULTS

Among the 107 patients who underwent hepatic resection for incidentaloma during the study period, there were 73 men and 34 women with a median age of 50 years. Fifty-six (52%) patients were found to be hepatitis B surface antigen serology positive, and the majority of them had normal liver blood biochemistry on presentation. The clinical parameters of the

patients are listed in Table 1. The median size of the tumors on presentation ranged from 0.5 to 17 cm with a median of 3.7 cm. Fifty-six (52%) patients underwent major hepatic resection with resection of three or more Coiunaud's segments.[12] The extent of hepatic resection is listed in Table 2.[9] The median intraoperative blood loss was 700 ml, and 94 (88%) patients did not require blood transfusion (Table 3). The median postoperative hospital stay was 8 days (range, 3–66 days). The operative morbidity rate was 21%. Hospital mortality occurred in 1 patient (1%), who presented with an incidental finding of a 2.5-cm HCC in segment 8 of the liver with underlying cirrhosis from chronic hepatitis B infection. He underwent a segmentectomy but subsequently died of bronchopneumonia and liver failure.

Histologic examination of the resected specimen revealed malignant liver tumors in 62 (58%) patients (group A). These tumors included HCC (n = 48), cholangiocarcinoma (n = 8), lymphoma (n = 2), cystadenocarcinoma (n = 2), carcinoid tumor (n = 1), and malignant fibrous histiocytoma (n = 1). Benign pathologies were found in 45 (42%) patients (group B); these included focal nodular hyperplasia (n = 17), hemangioma (n = 12), angiomyolipoma (n = 5), cirrhotic regenerative nodule (n = 4), hepatic adenoma (n = 2), focal fatty change (n = 2), arteriovenous malformation (n = 1), ductal plate malformation (n = 1), and inflammatory pseudotumor (n = 1).

Table 1. Clinical and laboratory data of 107 patients who presented with an incidentaloma and underwent hepatic resection

Clinical parameter	
No. of patients	107
Male/female (n)	73:34
Age* (yr)	50 (19–86)
Hepatitis B carrier (n)	56 (52%)
Chronic alcoholism (n)	24 (22%)
Serum α-fetoprotein* (ng/ml)	4 (1–402)
Serum carcinoembryonic antigen* (ng/ml)	2 (0–25)
Serum albumin* (g/L)	43 (30–50)
Serum total bilirubin* (μmol/L)	11 (3–42)
Alkaline phosphatase* (U/L)	79 (23–224)
Aspartate aminotransferase* (U/L)	26 (10–219)
Alanine aminotransferase* (U/L)	29 (8-418)
Hemoglobin* (g/dl)	13.9 (10.5–16.3)
Prothrombin time* (secs)	12.0 (9.6–16.7)
Tumor size* (cm)	3.7 (0.5–17)
Indocyanine green retention at 15 minutes* (%)	9 (1.7—38.1)

*Value expressed in median with range in parentheses.

Table 2. Extent of hepatic resection in 107 patients who presented with an incidentaloma and underwent hepatic resection

Hepatic resection	No. of patients
Right hepatectomy	37
Right trisectionectomy	2
Right hepatectomy with extension to segment 4	4
Right hepatectomy with extension to segment 4 + segment 1 resection	1
Left hepatectomy	7
Left hepatectomy + segment 1 resection	1
Left hepatectomy with extension to segment 4	3
Left leteral sectionectomy	10
Segment 1 + 4 + 5+ 8 resection	1
Segmentectomy	18
Wedge resection	23
Total	107

*The nomenclature of types of hepatic resection was based on the Brisbane 2000 Terminology of Liver Anatomy and Resections.[9]

Clinical parameters of groups A and B are listed in Table 4. Group A patients were of a significantly older age group (median, 58.5 years versus 43 years, $P < 0.0001$), with a male predominance and significantly higher incidence of hepatitis B surface antigen serology positivity. The preoperative serum α-fetoprotein level was significantly higher in group A (median, 6 ng/ml versus 3 ng/ml, $P = 0.0118$). Preoperative liver function was significantly worse in group A in terms of liver enzymes and indocyanine green clearance test (median retention at 15 minutes, 9.9% versus 7.0%, $P = 0.011$). The median size of the tumors was 4.5 cm (range, 1–17 cm) in group A

Table 3. Intraoperative and postoperative data of 107 patients who presented with an incidentaloma and underwent hepatic resection

Intraoperative or postoperative parameter	
Intraoperative blood loss* (L)	0.7 (0–9)
Intraoperative blood transfusion* (L)	0 (0-8.2)
Patients without transfusion (n)	94 (88%)
Operating time* (min)	315 (75–755)
Indocyanine green retention at 15 minutes on postoperative day 7* (%)	14.5 (1.2–38.8)
Postoperative hospital stay (days)	8 (3–66)
Operative morbidity (n)	23 (21%)
Hospital mortality (n)	1 (1%)

*Value expressed in median with range in parentheses.

Table 4. Clinical and laboratory data of patients who presented with an incidentaloma, underwent hepatic resection, and had a final diagnosis of malignant tumors (group A) and benign conditions (group B)

Clinical parameter	Group A (malignant disease) (n = 62)	Group B (benign disease) (n = 45)	*P* value
No. of patients	62	45	—
Male/female (n)	52:10	21:23	<0.001†
Age* (yr)	58.5 (19–86)	43 (24–72)	<0.001†
Hepatitis B carrier (n)	40 (65%)	16 (36%)	0.009†
Chronic alcoholism (n)	18 (29%)	6 (13%)	0.055
Serum α-fetoprotein* (ng/ml)	6 (1–189)	3 (1–402)	0.012†
Serum carcinoembryonic antigen* (ng/ml)	2 (0–25)	1.3 (0–6.6)	0.55
Serum albumin* (g/L)	42.5 (31–50)	44 (30–50)	0.11
Serum total bilirubin* (μmol/L)	11 (3–28)	11 (4–42)	0.81
Alkaline phosphatase* (U/L)	90 (23–224)	72 (40–216)	<0.001†
Aspartate aminotransferse* (U/L)	32 (13–219)	22 (10–172)	<0.001†
Alanine aminotransferase* (U/L)	34 (8–418)	19 (10–219)	<0.001†
Hemoglobin* (g/dl)	14.1 (10.6–16.3)	13.7 (10.5–15.8)	0.212
Prothrombin time* (sec)	12.2 (9.6–14.7)	11.8 (9.8–16.7)	0.117
Tumor size* (cm)	4.5 (1–17)	2.8 (0.5–10.5)	0.003†
Indocyanine green retention at 15 minutes* (%)	9.9 (2.1–36.6)	7 (1.7–38.1)	0.011†

*Value expressed in median with range in parentheses.
†Statistically significant difference.

and was significantly larger than that of 2.8 cm (range, 0.5–10.5 cm) in group B ($P = 0.003$). Thirty-seven (60%) patients in group A underwent major hepatic resection, and 19 (42%) patients in group B underwent major hepatic resection ($P = 0.074$, Table 5). The intraoperative and postoperative data of both groups of patients are listed in Table 6. The operating time in group A was significantly longer with significantly higher intraoperative blood loss and longer postoperative hospital stay.

Statistical analysis was performed on all of the 107 patients who presented with hepatic incidentalomas and underwent hepatic resection to identify independent factors predicting malignant disease. Twelve preoperative clinical factors were examined: age, gender, tumor size, hepatitis B surface antigen positivity, serum α-fetoprotein level, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, serum bilirubin level, hemoglobin, prothrombin time, and indocyanine green clearance. On multivariate analysis, male sex, age greater than 50 years, and tumor size greater than 4 cm were independent predictive factors for malignant diseases (Table 7).

The clinical details of the 48 patients who presented initially with hepatic incidentaloma and were diagnosed with HCC (group C) were further analyzed and compared with the clinical features and operative outcomes of 646 patients with HCC and presented otherwise (group D) during the same study period. The clinicopathologic data for both groups of patients are provided in Table 8. Group C had a significantly lower α-fetoprotein level and better preserved liver function in terms of lower serum alkaline phosphatase and aspartate aminotransferase levels on presentation. The tumor size in group C was significantly

Table 5. Extent of hepatic resection of patients who presented with an incidentaloma, and underwent hepatic resection, and had a final diagnosis of malignant tumors (group A) and benign conditions (group B)

Hepatic resection	Group A (malignant disease) (n = 62)	Group B (benign disease) (n = 45)
Right hepatectomy (n)	25	12
Right trisectionectomy (n)	1	1
Right hepatectomy with extension to segment 4 (n)	3	1
Right hepatectomy with extension to segment 4 + segment 1 resection (n)	1	0
Left hepatectomy (n)	4	3
Left hepatectomy + segment 1 resection (n)	1	0
Left hepatectomy with extension to segment 4 (n)	1	2
Left leteral sectionectomy (n)	3	7
Segment 1 + 4 + 5+ 8 resection (n)	1	0
Segmentectomy (n)	11	7
Wedge resection (n)	11	12
Total	62	45

*The nomenclature of types of hepatic resection was based on the Brisbane 2000 Terminology of Liver Anatomy and Resections.[9]

Table 6. Intraoperative and postoperative data of patients who presented with an incidentaloma, underwent hepatic resection, and had a final diagnosis of malignant tumors (group A) and benign conditions (group B)

Intraoperative or postoperative parameter	Group A (malignant disease) (n = 62)	Group B (benign disease) (n = 45)	*P* value
Intraoperative blood loss* (L)	0.88 (0.1–9)	0.55 (0–2.5)	<0.001†
Intraoperative blood transfusion* (L)	0 (0–8.2)	0 (0–1.3)	0.187
Patients without transfusion (n)	52 (84%)	42 (93%)	0.194
Operating time* (min)	335 (120–755)	270 (75–585)	<0.001†
Indocyanine green retention at 15 minutes on postoperative day 7* (%)	15.8 (8.3–38.8)	12.4 (1.2–20.6)	0.035
Postoperative hospital stay (days)	10 (4–66)	7 (3–56)	<0.001†
Operative morbidity (n)	16 (26%)	7 (16%)	0.203
Hospital mortality (n)	1 (2%)	0	1

*Value expressed in median with range in parentheses.
†Statistically significant difference.

smaller than that of group D (median, 3.5 cm versus 7 cm, $P < 0.0001$), and the tumors in group C were of significantly earlier staging[13] compared with those in group D (Table 9). The extent of hepatic resection in both groups of patients is listed in Table 10. Twenty-seven (56%) patients in group C and 425 (66%) patients in group D underwent major hepatic resection ($P = 0.181$). The intraoperative and postoperative data of both groups of patients are listed in Table 11. On follow-up, the median disease-free survival of group C was 45.3 months, which was significantly longer than that of 15.0 months in group D ($P = 0.0011$, Fig. 1). The overall median survival of group C was 69.3 months, which was also significantly longer than that of 44.6 months in group D ($P = 0.0023$, Fig. 2).

DISCUSSION

Based on autopsy findings, benign hepatic lesions have been reported in up to 52% of the general population.[5] Refinement in imaging techniques has resulted in increased number of small liver lesions detected as incidentalomas in healthy individuals. Volk et al.[14] reported a total of 108 hepatic incidentalomas in 33 of 100 healthy individuals on contrast-enhanced spiral CT examinations during a 13-month period. Nevertheless, none of the lesions detected caused management problems, because all of the lesions were considered benign in nature, including four "nonclassified lesions" in two patients. Seventy (65%) of the 108 lesions were smaller than 1 cm. Others also reported an incidence of small benign incidentalomas detected on CT ranging from 10.2% to 14.3%.[15,16] Although most of hepatic incidentalomas can be confidently classified as benign lesions based on various imaging characteristics and can be safely put under observation, a small proportion of the lesions pose a management dilemma to the clinicians. One would not like to miss the diagnosis of malignant tumors, resulting in delayed treatment and adverse survival outcome, yet a major surgical resection for benign lesions rendering morbidity and mortality should be cautioned.[17]

Table 7. Multivariate analysis on preoperative factors predicting malignant disease of incidentaloma

Factor	Relative risk	95% Confidence interval	*P* value
Male sex	10.702	3.525–32.492	<0.001
Age >50 yr	5.445	1.969–15.055	0.001
Tumor >4 cm	3.356	1.180–9.546	0.023

The 107 hepatic incidentalomas of the present study illustrate the various differential diagnoses of malignant and benign lesions that were incidentally discovered on abdominal imaging and received surgical resection. These incidentalomas were diagnosed as malignant diseases in 58% and as benign diseases in 42% of the patients. The relatively high incidence of malignant diseases could be explained by the selection procedure before surgery. Little et al.[3] reported 81% of patients with benign lesions among the 36 patients who presented with hepatic incidentalomas over a period of 36 months. In addition, 14% of their patients had metastatic cancer from colorectal cancers. The differences in observation between the two reports could be explained by the differences in the selection criteria. Most of the patients with colorectal liver metastasis did not meet the diagnostic criteria of the present study, as liver lesions detected during staging or surveillance of recurrent disease, either after hepatic resection for HCC or colorectal surgery for primary colorectal cancers were excluded, because

Table 8. Clinical and laboratory data of patients with hepatocellular carcinoma (HCC) who presented with an incidentaloma (group C) and those who presented otherwise (group D) during the study period

Clinical parameter	Group C (incidental HCC) (n = 48)	Group D (other HCC) (n = 646)	*P* value
No. of patients	48	646	—
Male/female (n)	43:5	526:120	0.156
Age* (yr)	56.5 (19–79)	54 (5–86)	0.502
Hepatitis B carrier (n)	39	543	0.61
Chronic alcoholism (n)	14	185	0.959
Serum α-fetoprotein* (ng/ml)	6 (2–19)	238 (2–1,335,900)	<0.001†
Serum albumin* (g/L)	41 (31–50)	40 (23–56)	0.038†
Serum total bilirubin* (μmol/L)	13 (5–28)	12 (2–70)	0.658
Alkaline phosphatase* (U/L)	88 (23–136)	96.5 (35–1940)	0.021†
Aspartate aminotransferse* (U/L)	35.5 (15–219)	46 (14–768)	0.021†
Alanine aminotransferase* (U/L)	42 (13–418)	46.5 (6–408)	0.931
Hemoglobin* (g/dl)	14.1 (11.5–16.3)	13.7 (5.9–21.3)	0.103
Prothrombin time* (sec)	12.3 (10.6–14.7)	12.3 (8.7–21.5)	0.618
Indocyanine green retention at 15 minutes* (%)	9.9 (2.3–36.6)	10.85 (1.2–66.9)	0.646

*Value expressed in median with range in parentheses.
†Statistically significant difference.

these subjects were not previously healthy and asymptomatic individuals.

The inclusion of 45 patients who presented with incidentalomas and were subsequently diagnosed with benign diseases for hepatic resection might appear to be diagnostic errors.[7,17] However, all of these patients were diagnosed with probable malignant disease based on radiologic investigations. Although preoperative fine-needle aspiration may minimize the diagnostic error, most clinicians would not recommend performing such a procedure for all patients with a preoperative diagnosis of malignant disease. Fine-needle aspiration cytology has been reported to have a high specificity and a positive predictive value in patients with hepatic lesions, but it was associated with a nontrivial morbidity of 0.5% and mortality of 0.05%.[18–20] The risks of complications are expected to be higher in patients with large bulging and vascular tumors such as HCC. The risk of tumor implantation along the fine-needle tract is

Table 9. Pathologic data of patients with hepatocellular carcinoma (HCC) who presented with an incidentaloma (group C) and those who presented otherwise (group D) during the study period

Pathologic data	Group C (incidental HCC) (n = 48)	Group D (other HCC) (n = 646)	*P* value
Tumor size* (cm)	3.5 (1–16)	7 (0.5–27)	<0.001†
Tumor-free resection margin* (cm)	1.1 (0–5.5)	1 (0–10)	0.078
Resection margin involved by tumor (n)	1	52	0.133
Venous infiltration of tumor (n)	9	313	<0.001†
TNM tumor Stage[13] (n)			<0.001†
I	36	234	
II	8	184	
IIIA	3	116	
IIIB	1	100	
IIIC		3	
IV		9	
Nontumorous liver (n)			0.662
Normal	4	81	
Active hepatitis	20	244	
Cirrhosis	24	321	

*Value expressed in median with range in parentheses.
†Statistically significant difference.

Table 10. Extent of hepatic resection of patients with hepatocellular carcinoma (HCC) who presented with an incidentaloma (group C) and those who presented otherwise (group D) during the study period

Hepatic resection	Group C (incidental HCC) (n = 48)	Group D (other HCC) (n = 646)
Right hepatectomy (n)	22	196
Right hepatectomy + segment 1 resection (n)	0	15
Right trisectionectomy (n)	0	46
Right trisectionectomy + segment 1 resection (n)	0	13
Right hepatectomy with extension to segment 4 (n)	2	75
Right hepatectomy with extension to segment 4 + segment 1 resection (n)	1	6
Left hepatectomy (n)	1	36
Left hepatectomy + segment 1 resection (n)	0	2
Left hepatectomy with extension to segment 4 (n)	1	25
Left hepatectomy with extension to segment 4 + segment 1 resection (n)	0	4
Left hepatectomy + segment 5 + 8 resection (n)	0	4
Left leteral sectionectomy (n)	1	43
Segment 4 + 5+ 8 resection (n)		3
Segmentectomy (n)	10	81
Wedge resection (n)	10	97
Total	48	646

also a concern. In an animal study, fine-needle aspiration of a highly cellular tumor produced a spread of 10^3 to 10^5 tumor cells in the needle tract.[21] For fine-needle aspiration in patients with HCC, the risk of tumor cell seeding has been reported to be as high as 2%.[20,22] With the potential risk involved in fine-needle aspiration, most clinicians advocate avoiding the procedure in patients with resectable liver tumors or in candidates for liver transplantation.

In addition, preoperative radiologic examinations and fine-needle aspiration for cytology may not be always helpful in distinguishing a malignant disease from a benign nature.[7] In the present study, among the 15 patients who underwent fine-needle aspiration or Tru'cut biopsy before referral to us, the diagnosis of malignancy was missed in 4 patients. Belghiti et al.[6] reported routine hepatic resection for 51 women younger than 50 years who presented with presumed benign liver tumors and without chronic liver disease during a 7-year period. Three (6%) patients were found to have HCC on histologic examination of the resected specimen. Several studies have documented the cause, differential diagnosis, and treatment of benign hepatic tumors, including focal nodular hyperplasia, hepatic adenoma, and hemangioma.[23–25] Although findings in these studies provide a framework for the management of benign hepatic tumors, the most appropriate treatment remains controversial. Hepatic adenoma has the risk of rupture and malignant change. Large hemangiomata has the risk of intralesional or peritoneal bleeding. Focal nodular hyperplasia was also shown to associate with primary hepatic neoplasm.[24] In the present study, the operative morbidity of the patients with benign hepatic lesions was 16% without any hospital

Table 11. Intraoperative and postoperative data of patients with hepatocellular carcinoma (HCC) who presented with an incidentaloma (group C) and those who presented otherwise (group D) during the study period

Intraoperative or postoperative data	Group C (incidental HCC) (n = 48)	Group D (other HCC) (n = 646)	*P* value
Intraoperative blood loss* (L)	0.8 (0.1–3.7)	1.3 (0.01–20)	<0.001†
Intraoperative blood transfusion* (L)	0 (0–0.86)	0 (0–9.9)	<0.001†
Patients without transfusion (n)	44 (92%)	379 (59%)	<0.001†
Operating time* (min.)	345 (120–755)	360 (70–1080)	0.555
Indocyanine green retention at 15 mintues on postoperative day 7* (%)	17.9 (8.3–38.8)	17.2 (0–74)	0.847
Postoperative hospital stay (days)	10 (4–66)	12 (1–130)	0.001†
Operative morbidity (n)	14 (29%)	237 (37%)	0.296
Hospital mortality (n)	1 (2%)	39 (6%)	0.257
Median disease-free survival (mo)	45.3	15.0	0.001†
Median survival (mo)	69.3	44.6	0.002†

*Value expressed in median with range in parentheses.
†Statistically significant difference.

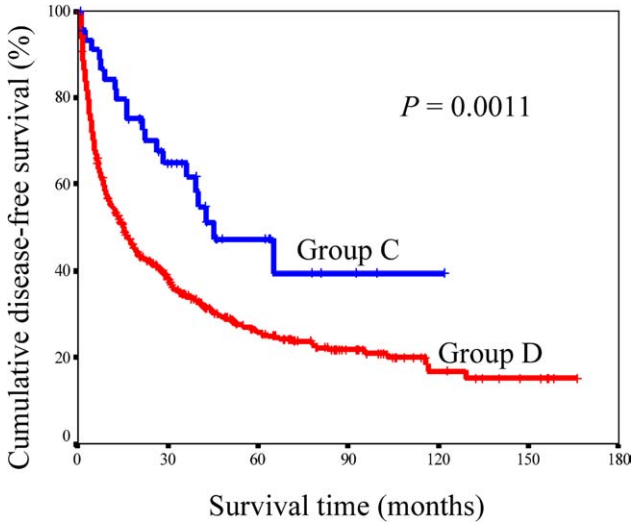

Fig. 1. Cumulative disease-free survival after hepatic resection for hepatocellular carcinoma (HCC) in patients who presented with incidentaloma (Group C, n = 48) and who did not present with incidentaloma (Group D, n = 646) from 1989 to 2002.

mortality. The operative risks appeared justified, especially when preoperative investigations suggested hepatic malignancy and the potential risks of complications from the benign lesions existed.

On multivariate analysis, male sex, age greater than 50 years, and tumor size greater than 4 cm were the independent predictive factors for malignant diseases in the present cohort of 107 patients with hepatic incidentalomas who underwent hepatic resection. With these three independent risk factors used for the prediction of malignant diseases, the negative predictive value of the 10 patients without any of the risk factors was 100%. All of the female patients younger than 50 years with an incidentaloma of 4 cm or smaller were diagnosed with benign lesions. The findings may help in the counseling of the patients and serve as a reference in the management regimen. One may consider further investigations, including percutaneous tissue diagnosis, before decision for hepatic resection in patients with low risk for malignancy.

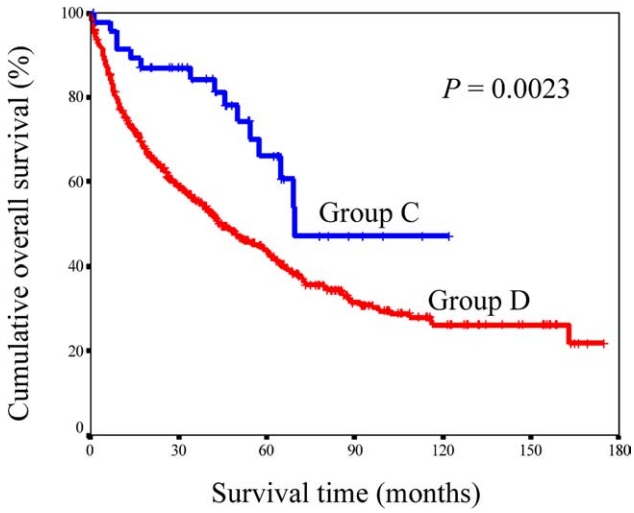

Fig 2. Cumulative overall survival after hepatic resection for hepatocellular carcinoma (HCC) in patients who presented with incidentaloma (Group C, n = 48) and who did not present with incidentaloma (Group D, n = 646) from 1989 to 2002.

Patients with HCC who presented with incidentalomas were shown to have significantly better survival outcomes after hepatic resection compared with patients who presented otherwise during the same study period in the present study. This was probably related to an earlier tumor staging with smaller size of the tumors on presentation.[26] The extent of hepatic resection was significantly less with less intraoperative blood loss. In patients with HCC who were undergoing hepatic resection, early tumor stage, asymptomatic disease, and little intraoperative blood loss have been reported to be associated with better postoperative prognosis. The findings of the present study may support the potential benefits of screening of patients with asymptomatic HCC for early diagnosis and better survival outcomes.

In conclusion, this study of 107 hepatic incidentalomas evaluated and operated on at a single center illustrates the relatively high incidence of malignant tumors. Malignancy should be strongly suspected in male patients older than 50 years with tumor mass of greater than 4 cm. In view of a relatively low operative morbidity in patients with well-preserved liver function and small tumors, a more radical approach can be considered in patients with high suspicion of malignant disease on clinical parameters and investigations. For patients with low relative risks for malignancy, the treatment regimen can be modified, including whether a preoperative percutaneous tissue diagnosis should be obtained, after detailed counseling with the patients.

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Hepatitis Serology Predicts Tumor and Liver-Disease Characteristics But Not Prognosis After Resection of Hepatocellular Carcinoma

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The impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection on survival rates after resection of hepatocellular carcinoma (HCC) is controversial. The objective of this study was to determine whether serologic evidence of HBV or HCV infection ("hepatitis serology") can predict underlying liver disease, tumor factors, and survival rates in patients with HCC. Using a multicenter international database, we identified 446 patients with complete HBV and HCV serology. One hundred twenty-six patients were negative for HBV and HCV, 163 patients had HBV infection only, 79 patients had HCV infection only, and 78 patients had coinfection with HBV and HCV. Patients with hepatitis were more likely to have tumors smaller than 5 cm and bilateral HCC involvement. Hepatitis status (negative vs. HBV vs. HCV vs. coinfection with HBV and HCV) did not predict tumor grade or the presence of multiple tumor nodules. Patients with HCV or coinfection with HBV and HCV exhibited a lower incidence of vascular invasion, but worse fibrosis than patients with negative serology or HBV. The median survival rate was 47.9 months. The presence of hepatitis did not significantly affect the survival rate, but hepatic fibrosis and vascular invasion predicted a decreased survival rate. The prognosis after resection of HCC is influenced by tumor factors and liver disease, but not by HBV or HCV infection. The treatment for HCC should be dictated by the extent of underlying liver disease rather than by hepatitis serology.
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KEY WORDS: Hepatitis, hepatocellular carcinoma, resection

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in men and the ninth most common malignancy in women, accounting for 500,000 to 1 million cancer cases annually worldwide.[1] Most cases of HCC occur in areas where viral hepatitis is endemic; hepatitis B virus (HBV) and hepatitis C virus (HCV) are known to be important etiologic factors in HCC.[2,3] Previous studies[4,5] have reported rates of HBV infection ranging from 13%–73% and rates of HCV infection ranging from 11%–88% in patients diagnosed with HCC.

Although the association between viral hepatitis and HCC is well established, the effect of viral infection on tumor characteristics, underlying liver disease, and prognosis after resection of HCC remains controversial. Studies attempting to correlate serologic evidence of HBV or HCV infection ("hepatitis serology") with clinicopathologic features and prognosis in patients with HCC have produced conflicting and inconsistent findings. For example, Haratake and associates[6] determined that patients with HCV exhibited improved survival rates compared with

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patients with HBV, whereas Yamanaka and associates[7] reported that patients with HCV exhibited worse 5-year survival rates compared with patients with HBV (42% vs. 54%). Yamanaka and associates[7] also reported that patients with HCV were more likely to exhibit advanced underlying liver disease and advanced tumor stage, but other authors[8] have not noted this association. On the basis of data indicating that patients with HCV have a worse prognosis after resection, some authors[9] have advocated that HCC patients with serologic evidence of HCV infection and tumors smaller than 5 cm be considered for early liver transplantation rather than hepatic resection. Other investigators,[10] however, have questioned these recommendations, noting that the data supporting treatment based on hepatitis serology are conflicting.

Studies examining hepatitis serology and HCC have come from single-institution experiences exclusively from the East or West. We herein report the results from a multicenter study with regard to surgical resection for HCC among patients with different hepatitis serology status from both the East and the West. The objective of this study was to determine whether differences in hepatitis serology predict underlying liver disease, tumor characteristics, and survival rates after resection in patients with HCC.

MATERIAL AND METHODS

Using a multicenter international database, we identified 446 patients with complete HBV and HCV serology who underwent hepatic resection for HCC between 1990 and 2000 at five major hepatobiliary centers: The University of Texas M. D. Anderson Cancer Center (Houston, TX), Mayo Clinic (Rochester, MN), Beaujon Hospital (Paris, France), Kyoto University Graduate School of Medicine (Kyoto, Japan), and Queen Mary Hospital (Hong Kong, China). All patients with HCC and no clinical, radiographic, or intraoperative evidence of extrahepatic disease were eligible for resection. Patients were deemed to have surgically resectable disease on the basis of the distribution and extent of tumors and the presence of a functional hepatic reserve adequate to tolerate hepatic resection. In all patients, the intent of the surgical procedure was curative.

For purposes of this study, patient characteristics, underlying liver and tumor characteristics, and survival data were examined. Specifically, the following data were collected for all patients: patient age and sex; tumor histologic subtype, number, location, and size; presence of vascular invasion; degree of underlying hepatic fibrosis; extent of hepatic resection (less than a hemi-hepatectomy, hemi-hepatectomy, or extended hepatic resection [five or more liver segments]);[11] operative details; vital status (living vs. deceased); most recent follow-up date; deceased date; serum alpha-fetoprotein (AFP) level; and hepatitis serology. The serologic presence of HBV surface antigen or HBV core antibody was considered evidence of HBV exposure because both increase the risk of HCC.[12] The serologic presence of HCV antibody was considered evidence of HCV infection. Tumor size was defined as the largest diameter of the tumor specimen. Microscopic vascular invasion was defined as the presence of tumor emboli within the central vein, the portal vein, or the large capsular vessels or the involvement of the segmental or sectoral branches of the portal vein or the hepatic veins.[13,14] Major vascular invasion was defined as gross invasion of the right or left main branches of the portal vein or the hepatic veins.[15] Tumor grade was assessed using the scheme outlined by Edmondson and Steiner[16] and the degree of fibrosis was graded according to the classification of Ishak and associates.[17]

Clinical data were reviewed on site at each of the five study centers by three of the investigators (J.N.V., D.M.N., R.T.P.). The pathologic resection specimens from each patient were similarly reviewed on site by two pathologists (G.Y.L., I.O.N.). Pathologic specimens were prepared at each center using hematoxylin-eosin staining.

Statistical analyses were performed to investigate possible associations between hepatitis status and patient characteristics, underlying liver and tumor characteristics, and mortality outcomes. Univariate tests (χ^2) were used to test for differences in these distributions with regard to hepatitis status. Factors that seemed to be significantly associated with hepatitis status were entered into a Cox proportional hazards model to test for significant effects while adjusting for multiple factors simultaneously. Actuarial survival rates were calculated using the Kaplan–Meier method. Differences in survival rates were examined using the log-rank test. A p value of less than 0.05 was considered significant.

RESULTS

Of the 446 patients with HCC who underwent hepatic resection between 1990 and 2000, 126 patients (28.3%) lacked evidence of HBV or HCV infection and 320 patients (71.7%) exhibited evidence of HBV, HCV, or coinfection with HBV and HCV. In general, patients with positive hepatitis serologies were older ($p = 0.01$), exhibited smaller tumors ($p = 0.0003$), and were more likely to exhibit bilateral

disease ($p = 0.008$). The male-to-female ratio was not significantly different between the two groups ($p = 0.24$).

Patients were divided into four groups for the purposes of analysis. One hundred twenty-six patients (28.3%) were negative for HBV and HCV infection, 163 patients (36.5%) exhibited HBV infection only, 79 patients (17.7%) exhibited HCV infection only, and 78 patients (17.5%) exhibited coinfection with HBV and HCV.

The clinical features of the patients are summarized in Table 1. Although patients negative for HBV and HCV had a median age almost one decade younger than patients with hepatitis, there was no difference in median age among the three subgroups of patients with hepatitis. The male-to-female ratio was higher in patients with HBV infection only than in patients in the other three groups ($p < 0.05$). In contrast, there was no difference in Child's class according to hepatitis status. Patients in China were more likely to have HBV infection only ($p < 0.0001$) and patients in Japan were more likely to exhibit coinfection with HBV and HCV ($p < 0.0001$) (Table 1). Hepatitis status was strongly associated with AFP level at presentation: patients with HBV infection only exhibited a significantly higher median AFP level than patients in the other groups ($p < 0.0001$).

Tumor characteristics and underlying liver disease (degree of fibrosis) are summarized in Table 2. Although the percentage of patients with multiple HCC nodules was similar in the four groups, patients with negative hepatitis serology were less likely to have bilateral disease ($p < 0.05$). The distribution of histologic grade was not significantly different in the four groups ($p = 0.99$). However, median tumor size was significantly larger in patients with negative hepatitis serology and patients with HBV infection only than in patients with HCV infection only and patients with coinfection ($p < 0.0001$).

Hepatitis-negative patients and patients with HBV infection only were also noted to have significantly higher incidences of both major ($p = 0.01$) and microscopic ($p < 0.001$) vascular invasion. Both major and microscopic vascular invasion were more frequent in patients with either negative hepatitis serology or positive serology for HBV only. Whereas the incidence of major vascular invasion in patients who were hepatitis-negative or positive for HBV only was approximately 9%–10%, patients who were either HCV positive or coinfected with HBV and HCV exhibited major vascular invasion approximately half as often ($p = 0.01$). The same overall pattern was noted for microscopic vascular invasion. In contrast, the incidence of coexisting severe fibrosis/cirrhosis (Ishak grade 5–6) was highest in patients with HCV infection only or coinfection with HBV and HCV and lowest in patients with negative hepatitis serology ($p < 0.0001$). This was true even though patients with HCV infection only or coinfection with HBV and HCV tended to exhibit smaller tumors with less associated vascular involvement (Fig. 1).

The extent of hepatic resection is illustrated in Table 3. Approximately half of the patients (n = 221, 49.6%) underwent less than a hemi-hepatectomy; a

Table 1. Clinical features*

Feature	Hepatitis-Negative	HBV Infection Only	HCV Infection Only	Coinfection
Number of patients	126	163	79	78
Median age (years)	51†	60	60	61
Sex ratio (male:female)	2.5:1	5.3:1‡	1.6:1	2.7:1
Child's class				
A	105 (83.3)	148 (90.8)	58 (73.4)	58 (74.4)
B	21 (16.7)	15 (9.2)	21 (26.6)	20 (25.6)
Country of origin				
China	26 (20.6)	113 (69.3)‡	10 (12.7)	3 (3.8)
Japan	18 (14.3)	12 (7.4)	31 (39.2)	53 (67.9)§
France	52 (41.3)	28 (17.2)	30 (38.0)	17 (21.8)
United States	30 (23.8)	10 (6.1)	8 (10.1)	5 (6.5)
Median AFP level (ng/ml)	11.5	267.0†	32.0	26.0

AFP = alpha-fetoprotein; HBV = hepatitis B virus; HCV = hepatitis C virus.
*Values indicate the numbers of patients (percentages) unless otherwise denoted.
†$p < 0.05$ (hepatitis-negative patients vs. other groups).
‡$p < 0.05$ (patients with HBV infection only vs. other groups).
§$p < 0.05$ (patients with coinfection vs. other groups).

Table 2. Tumor characteristics*

Characteristics	Hepatitis-Negative (n = 126)	HBV Infection Only (n = 163)	HCV Infection Only (n = 79)	Coinfection (n = 78)
Median tumor size (cm)	7.0	7.0	3.0†	3.2†
Bilateral disease	11 (8.7)	33 (20.2)‡	12 (15.2)‡	11 (14.1)‡
Multiple (> 1) tumors	20 (15.8)	29 (17.8)	13 (16.9)	12 (15.2)
Microscopic vascular invasion	69 (54.8)	85 (52.1)	32 (40.5)†	25 (32.1)†
Major vascular invasion	12 (9.5)	16 (9.8)	3 (3.8)†	4 (5.1)†
Severe fibrosis/cirrhosis	45 (35.7)	82 (50.3)	49 (62.0)†	50 (64.1)†

HBV = hepatitis B virus; HCV = hepatitis C virus.
*Values indicate the numbers of patients (percentages) unless otherwise denoted.
†$p < 0.05$ (patients with HCV infection only or coinfection vs. other groups).
‡$p < 0.05$ (patients with HBV, HCV, or coinfection vs. hepatitis-negative patients).

minority (n = 64, 14.3%) underwent an extended resection.

The 30-day mortality rate was low in all groups (negative hepatitis serology, 1.6%; HBV infection only, 0.6%; HCV infection only, 0%; coinfection, 0%) ($p = 0.45$). At a median follow-up time of 33 months (range 0.2–143 months), the median actuarial survival rate was 47.9 months (95% confidence interval [CI] 39.1–55.6 months) (Fig. 2). There was no significant difference in the median survival rate among the four patient groups (negative hepatitis serology, 48.7 months; HBV infection only, 40.7 months; HCV infection only, 50.5 months; coinfection, 60.6 months) ($p = 0.39$) (Fig. 3). Furthermore, there was no difference in survival rates based on hepatitis status when the subsets of patients with tumors smaller than 5 cm and 5 cm or larger were analyzed separately ($p > 0.05$) (Fig. 4).

Univariate analysis revealed that tumors 5 cm or larger, AFP levels greater than 30 ng/ml, Child's class B disease, the presence of vascular invasion, and the presence of severe fibrosis were all significant predictors of diminished overall survival. Patients with tumors 5 cm or larger exhibited a median survival of 33.7 months compared with 57.0 months for patients with tumors smaller than 5 cm ($p = 0.003$). Whereas patients with AFP levels 30 ng/ml or less exhibited a median survival of 70.9 months, patients with higher AFP levels exhibited a median survival of 31.1 months ($p < 0.0001$). Patients with Child's class B disease similarly fared poorly. Patients with Child's class B disease exhibited a median survival of 18.0 months compared with 50.5 months for patients with Child's class A disease ($p < 0.0001$). Patients with major vascular invasion exhibited a median survival of only 12.5 months vs. 50.5 months for patients without major vascular invasion ($p < 0.0001$). Although not as ominous, microscopic vascular invasion similarly predicted a poor long-term outcome. Whereas patients with no vascular invasion exhibited a median survival of 65.1 months, those with microscopic vascular invasion exhibited a median

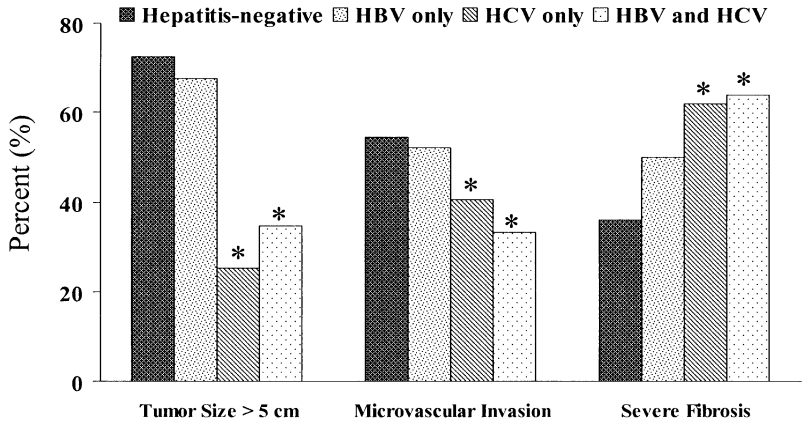

Fig. 1. Hepatitis-negative patients and hepatitis B virus (HBV)-positive patients exhibited less fibrosis, but larger tumors with more vascular invasion compared with hepatitis C virus (HCV)-positive patients and patients with coinfection with HBV and HCV (* = HCV-positive or coinfection patients vs. hepatitis-negative or HBV-positive patients; $p < 0.001$).

Table 3. Extent of hepatectomy*

Characteristics	Hepatitis-Nagative (n = 126)	HBV Infection Only (n = 163)	HCV Infection Only (n = 79)	Coinfection (n = 78)
Less than a hemi-hepatectomy	53 (42)	63 (38)	66 (84)†	39 (52)†
Hemi-hepatectomy	48 (38)	68 (42)	12 (15)	30 (40)
Extended hepatic resection	25 (20)	32 (20)	1 (1)†	6 (8)†

HBV = hepatitis B virus; HCV = hepatitis C virus.
*Values indicate the numbers of patients (percentages) unless otherwise denoted.
†$p < 0.0001$ (patients with HCV infection only or coinfection vs. other groups).

survival of 23.2 months ($p < 0.0001$) (Fig. 5, *A*). Patients with moderate-to-severe fibrosis/cirrhosis (Ishak grade 3–6) had a median survival rate of only 39.4 months compared with 88.5 months for patients with no or minimal fibrosis (Ishak grade 0–2) ($p < 0.0001$) (Fig. 5, *B*). Further analysis indicated a trend toward shorter survival rates for patients with severe fibrosis/cirrhosis (Ishak grade 5–6) (median survival = 36.0 months) than for patients with moderate fibrosis/cirrhosis (Ishak grade 3–4) (median survival = 43.8 months) ($p = 0.09$).

On multivariate survival analysis, both the presence of severe fibrosis and the presence of vascular invasion remained independent predictors of poor overall survival. Patients with moderate-to-severe fibrosis (Ishak grade 3–6) exhibited a higher likelihood of death than those with less severe underlying liver fibrosis (Ishak grade 0–2) (hazard ratio [HR] = 2.16, 95% CI = 1.48–3.15, $p < 0.0001$). Similarly, vascular invasion was associated with an increased risk of death. Patients with major vascular invasion indicated a greater than doubled risk of death (HR = 2.36, 95% CI = 1.50–3.72, $p < 0.0001$). The presence of microscopic vascular invasion conferred a similar, although slightly less, risk of death (HR = 1.88, 95% CI = 1.44–2.46, $p < 0.0001$).

DISCUSSION

HCC occurs mostly in areas where viral hepatitis is endemic and there is considerable variation in the prevalence of HBV and HCV depending on the patient's country of origin.[18] It is estimated that 25% of patients with HCC in the United States exhibit evidence of HCV infection and HBV and HCV together account for no greater than 40% of HCC cases in the United States.[19,20] In contrast, both HBV and HCV infection are considered to be endemic in many Eastern countries, where the vast majority of HCC patients are positive for HBV or HCV. A survey conducted by the Liver Cancer Study Group of Japan

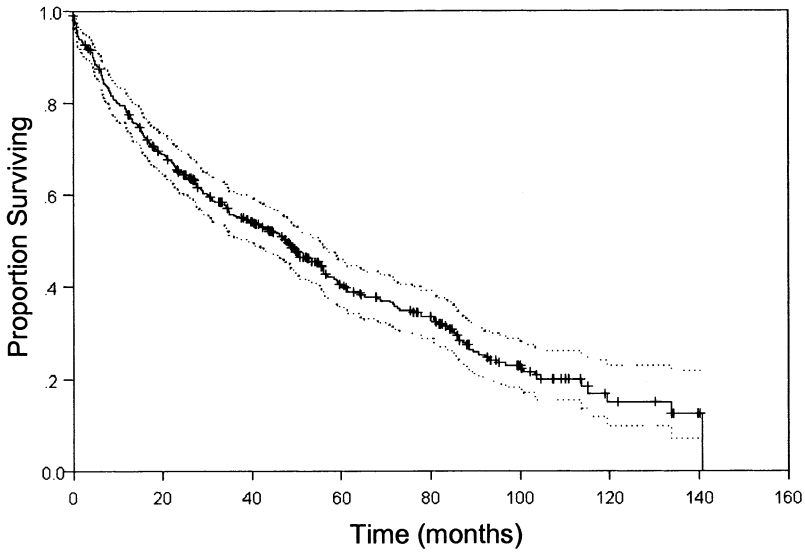

Fig. 2. At a median follow-up time of 33 months (range 0.2–143 months), the median actuarial survival rate for all hepatocellular carcinoma (HCC) patients regardless of hepatitis serologic status was 47.9 months (95% confidence interval [CI] 39.1–55.6 months).

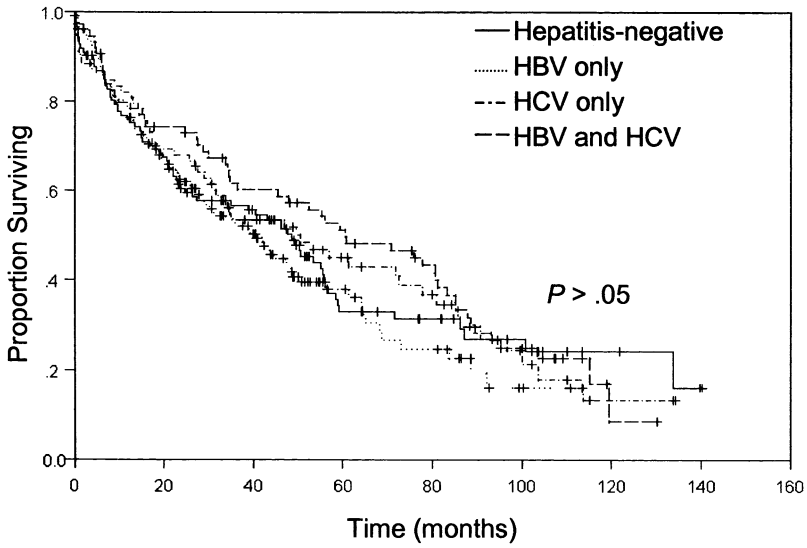

Fig. 3. Median survival rates did not differ by hepatitis serology. Hepatitis-negative patients exhibited a median survival rate (48.7 months) similar to that of hepatitis B virus (HBV)-positive patients (40.7 months), hepatitis B virus (HCV)-positive patients (50.5 months), and coinfected patients (60.6 months) ($p = 0.39$).

revealed that 72% of Japanese patients with HCC were positive for HCV and 26% were positive for HBV.[21] In the current study, we identified a similar incidence of HCV in Japanese patients (73.7%), however, the incidence of HBV was higher (57.0%). One possible explanation for the increased rate of HBV noted in the current study is that the serologic presence of HBV surface antigen or HBV core antibody was considered evidence of HBV exposure. The presence of HBV core antibody has previously been associated with an increase in the risk of HCC even after the seroconversion of HBV surface antigen (HBsAg).[12] In fact, HBV DNA can still be present after the seroconversion of HBsAg in patients[22,23] and HBV sequences are often found in HCC tissues in patients without HBsAg.[24,25] These data suggest that HBV genes play a role in the development of HCC in patients who are HBV core antibody positive, but HBsAg negative.[25,26] In support of this, the presence of HBV core antibody alone was previously shown to be an excess risk factor for HCC.[27,28]

Despite the endemic nature of hepatitis, there seems to be geographic variations even in Eastern countries. For example, in contrast to the situation in Japan, in Taiwan the HBV infection rate among patients with HCC is 80%–85%, whereas the HCV infection rate is low.[29,30] Similar to findings in previous epidemiologic reports, in the current study, more patients from the West (France and United States) than from the East (Hong Kong and Japan) exhibited negative hepatitis serology (65.1% vs. 34.9%). Furthermore, we identified regional differences in hepatitis infection rates among Eastern patients: Hong Kong patients were most likely to exhibit HBV infection only, whereas Japanese patients were most likely to exhibit coinfection with HBV and HCV. Geographical variations in the seroprevalence of hepatitis can make comparative studies of HBV and HCV infection and HCC difficult. However, international cooperative studies, such as the current one, may be more informative as they allow for meaningful comparison of results across hepatobiliary centers.

Several studies have investigated differences in the mechanisms of carcinogenesis between HBV-related and HCV-related HCC.[31] HCC carcinogenesis in patients with HBV infection is believed to be initiated by integration of HBV proviral DNA into the host DNA.[32] The integration of HBV double-stranded DNA into the host genome has been shown to enhance expression of the C-myc and N-myc oncogenes and to inactivate the tumor suppressor gene p53.[33,34] Such alterations can adversely affect cell cycle control, signal pathways, and apoptosis, thereby leading to an increased risk of carcinogenesis.[35] In contrast, HCC carcinogenesis in patients with HCV infection is unrelated to insertional mutagenesis, as HCV is an RNA virus that is not integrated. Rather, HCV most likely leads to carcinogenesis by inducing fibrosis and subsequent cirrhosis, thereby creating a "field of cancerization."[36,37] Whereas HBV-related chronic liver diseases tend to subside with seroconversion in approximately 90% of patients, HCV-related chronic liver diseases are characterized by persistent inflammatory activity with little decrease in their carcinogenic potential.[31,38] This fact supports

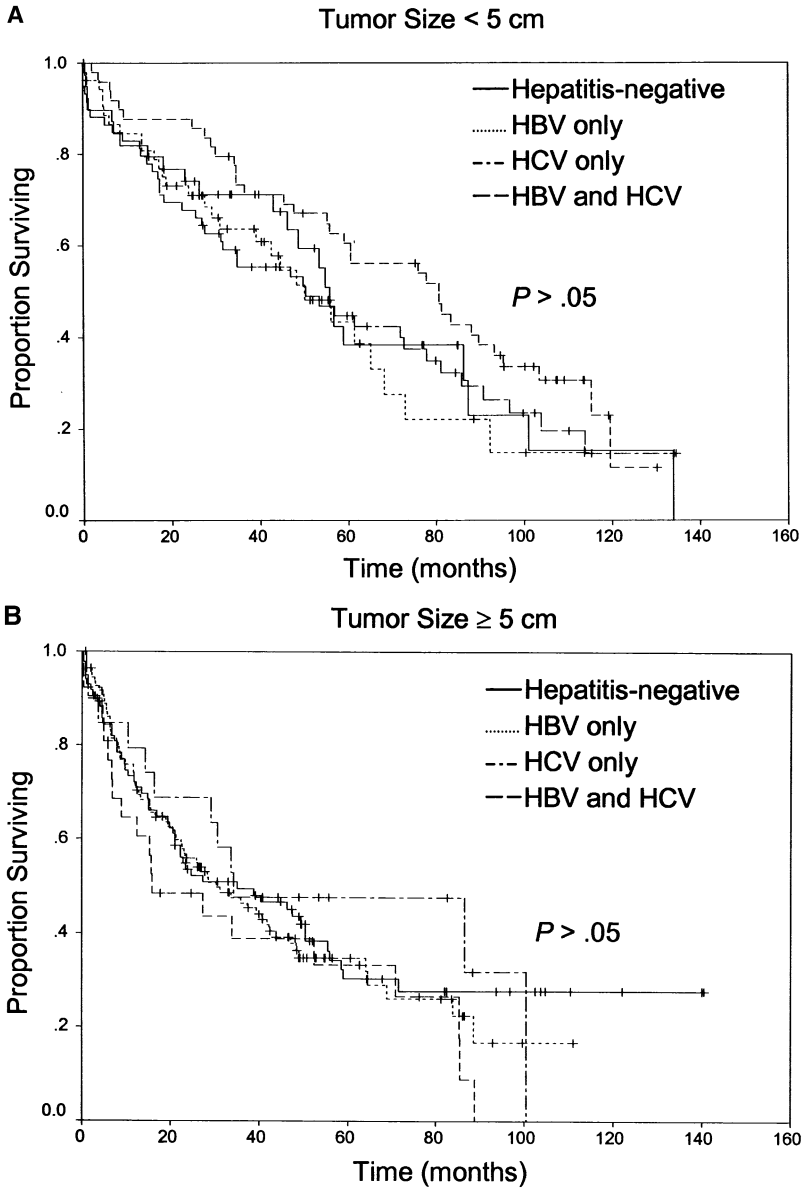

Fig. 4. In both patients with tumors smaller than 5 cm (**A**) and patients with tumors 5 cm or larger (**B**), survival rates did not differ by hepatitis status ($p > 0.05$). HBV = hepatitis B virus; HCV = hepatitis C virus.

the theory that HCV probably leads to HCC through chronic inflammatory stimulation.[31] Coinfection with HBV and HCV has been reported to cause much more severe liver disease in terms of histologic findings and clinical decompensation.[39,40] In fact, dual HBV and HCV positivity is an independent and significant risk factor for the development of HCC.[41] Taken together, these distinct viral mechanisms of carcinogenesis may explain the clinically relevant differences in the clinicopathologic features of patients with HCC with different hepatitis profiles observed in the current study.[9]

In this series, patient and tumor characteristics varied dramatically according to hepatitis serologic status. For example, the median age of patients with positive hepatitis serology was significantly higher than that of patients in the hepatitis-negative group. This is consistent with previous reports[30,42,43] and may be caused by the delayed carcinogenesis observed in patients with long-term inflammation of the liver induced by viral hepatitis. Although there was no significant difference between the groups with and without evidence of viral hepatitis in the overall number of tumors resected, patients with HBV,

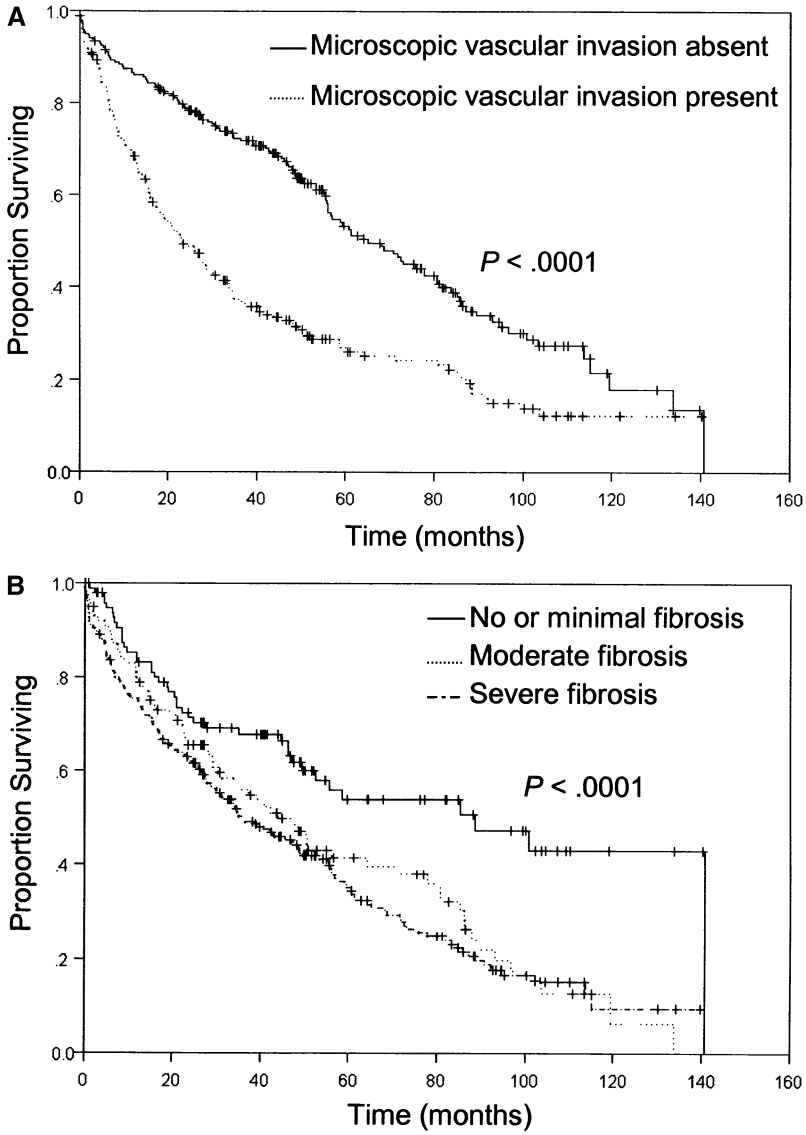

Fig. 5. The presence of severe fibrosis or vascular invasion adversely affected overall survival rates. (**A**) Patients with microscopic vascular invasion exhibited a significantly shorter median survival rate (23.2 months) than patients with no vascular invasion (65.1 months) ($p < 0.0001$). (**B**) Similarly, patients with moderate-to-severe fibrosis exhibited a significantly worse survival rate than patients with no or minimal fibrosis ($p < 0.0001$).

HCV, or coinfection with HBV and HCV were more likely to exhibit multicentric or bilateral disease. Other studies[35,43] have also indicated higher rates of multicentricity in patients with HBV or HCV infection.

Hepatitis-negative and HBV-positive patients exhibited significantly larger tumors than patients with HCV or coinfection with HBV and HCV. Both Takenaka and associates[43] and Yamanaka and associates[7] have previously reported that HBV-positive Japanese patients exhibit larger tumors, whereas Chen and associates[44] reported smaller tumors in Taiwanese patients infected with HCV. HBV-positive patients were also noted to exhibit significantly higher preoperative AFP levels. Although the higher AFP levels in HBV-positive patients may in part be explained by the larger median tumor size in this cohort, tumor burden cannot completely account for the differences. AFP levels were significantly higher in HBV-positive patients (267.0 ng/ml) than in hepatitis-negative patients (11.5 ng/ml) despite the fact that the two groups exhibited the same median tumor size. Others[7,45] have also reported elevated AFP levels in HBV-positive patients and have postulated

that the AFP value may reflect both tumor burden and the degree of acute inflammatory activity of the hepatitis infection.

Some have suggested that HCV-positive patients with small (< 5 cm) resectable tumors be given special consideration for early transplantation because of allegedly worse overall survival after resection.[9] In the current study, when patients were analyzed separately, there was no difference in survival rates based on hepatitis status in patients with tumors smaller than 5 cm ($p = 0.26$) or in patients with tumors 5 cm or larger ($p = 0.50$) (Fig. 4). Given these findings, we advocate that the mere presence of positive HBV or HCV infection should not be used to exclude patients from consideration for resection. Rather, individual tumor characteristics and underlying liver function should determine whether resection or transplantation is most appropriate.

Histopathologically detected vascular invasion and histopathologically detected adjacent severe fibrosis/cirrhosis of the nontumorous liver are known prognostic factors after resection of HCC.[13] In the current series, patients with negative hepatitis serology and patients with HBV infection only were significantly more likely to exhibit both microscopic and major vascular invasion. Tsai and associates[46] noted an association between increasing tumor size and increasing rates of both microscopic and macroscopic vascular invasion. Our finding that hepatitis-negative and HBV-positive patients—the two groups with the largest median tumor size—also exhibited the highest incidence of vascular invasion is consistent with earlier investigations correlating tumor size with vascular invasion.

Several studies[47,48] have documented an association between cirrhosis and recurrence of HCC that is presumably caused by continued carcinogenesis in the affected liver remnant. In the current series, patients with HCV infection only and patients with coinfection with HBV and HCV were significantly more likely to exhibit severe fibrosis/cirrhosis of the adjacent nontumorous liver. Given the distinct viral mechanisms of carcinogenesis, it is not surprising that a significantly higher proportion of patients with HCV than of patients with HBV exhibited cirrhosis in the surrounding parenchyma. Although two Japanese studies[43,49] examining the same topic indicated no significant difference in the incidence of cirrhosis between HCC patients infected with HCV and HBV, other studies[9,35] have demonstrated a significantly increased risk of severe fibrosis/cirrhosis with HCV infection. Scheuer and associates[38] and Takenaka and associates[43] noted that hepatitis activity was more serious and that liver function was generally more depressed in patients with HCV infection than in patients with HBV infection. Earlier studies[39,40] have also revealed that patients with coinfection with HBV and HCV tend to exhibit more severe and progressive liver disease. Our study confirms these earlier findings that implicate HCV and coinfection with HBV and HCV as significant risk factors for severe fibrosis/cirrhosis.

Given that severe fibrosis/cirrhosis is a strong predictor of poor overall survival, shorter survival rates after resection might have been predicted for patients with HCV infection or coinfection with HBV and HCV. However, in the current study, the survival rate curves for all four groups of patients, regardless of hepatitis status, were similar (Fig. 3). Others have reported inconsistent long-term results after resection of HCC in patients with different hepatitis serologic status. Yamanaka and associates[7] reported that patients with HBV-associated HCC had higher 5-year survival rates than patients with HCV-associated HCC (54% vs. 42%). In contrast, Haratake and associates[6] determined that patients with HCV-associated HCC had higher 1-, 2-, and 3-year survival rates compared with patients with HBV-associated HCC. However, the majority of series[43,49,50] have not shown a difference in overall survival rates between HBV-positive and HCV-positive patients. The current trial provides a multicenter international validation of these findings.

The current study may provide insight into why long-term survival rates among the four groups of patients is similar. We believe that the similar long-term survival rates may be related to differences in the characteristics of the tumors and the adjacent liver (Fig. 1). Whereas hepatitis-negative and HBV-positive patients exhibited larger tumors and a higher incidence of vascular invasion, HCV-positive patients and patients with HBV and HCV coinfection had a higher incidence of severe fibrosis/cirrhosis. On multivariate analysis, the relative risk of death for these two factors was similar (for vascular invasion, HR = 2.36; for severe fibrosis/cirrhosis, HR = 2.16). Therefore, although the profile of poor prognostic factors differed according to hepatitis status, the cumulative risk of death after resection seemed to be the same when all risk factors were considered.

CONCLUSION

Data from a large international cohort of patients with HCC revealed that hepatitis-negative and HBV-positive patients had larger tumors with a higher frequency of both microscopic and major vascular invasion, whereas HCV-positive patients and patients with HBV and HCV coinfection were more

likely to have severe fibrosis/cirrhosis. Differences in tumor characteristics and underlying liver disease may relate to different mechanisms of viral infection and viral oncogenesis. The long-term surgical outcomes in hepatitis-negative, HBV-positive, HCV-positive, and coinfected patients were almost identical. Our data suggest that this finding may be related to the distinct, but off-setting, adverse prognostic factors particular to each hepatitis subgroup. Because hepatitis status does not, per se, dictate overall prognosis, we advocate against the use of serologic evidence of HBV or HCV infection as a surgical selection criteria. Rather, tumor and underlying liver characteristics such as the presence of vascular invasion or severe fibrosis/cirrhosis should take priority in formulating the treatment plan for patients with HCC.

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Discussion

Dr. M. Choti (Baltimore, MD): Thank you, Dr. Pawlik, for an excellent talk and an opportunity to review the manuscript in advance. I would like to congratulate this group of investigators on another excellent study using this international cooperative retrospective database. It appears that what you found is that the nonhepatitis and the hepatitis B patient exhibit poor oncologic prognostic factors such as vascular invasion and size and the hepatitis C patient experiences earlier stage cancers, but a higher degree of cirrhosis or severe fibrosis.

First of all, as implied from the title, the real question is does the hepatitis itself contribute to a poorer prognosis? Did you compare the patients with hepatitis

versus nonhepatitis with the same degree of cirrhosis? How do patients with severe fibrosis or cirrhosis from alcoholic or idiopathic cirrhosis compare with those patients with hepatitis C?

The second question relates to the pattern of recurrence. We often grapple regarding patients with cirrhosis, in particular those with hepatitis C, as to whether a recurrence is related to a new multifocal cancer or a true intrahepatic recurrence. Did you analyze the recurrence pattern regarding the patients with hepatitis C? Did they die of liver failure and did recurrence within the liver occur more frequently compared with patients with nonhepatitis or with hepatitis B in which one might expect the recurrence to more likely be extrahepatic?

Again, thank you for an excellent presentation.

Dr. T. M. Pawlik: With regard to the first question, I agree with you. I think hepatitis serologic status may be acting as a surrogate. As you know, the two most important prognostic factors for long-term survival after resection for hepatocellular carcinoma are vascular invasion, whether it is major or microscopic, and fibrosis. So, when looking at patients who are hepatitis negative but who also have severe fibrosis, that is, Ishak grade V or VI, compared with patients who are hepatitis positive and also have severe fibrosis, they have a similar long-term outcome. In the current study, we are recognizing that patients who have hepatitis C, or B and C, are much more likely to develop severe fibrosis. However, if you have severe fibrosis, regardless of how you acquired it, it is a poor prognostic factor and these patients have a similar long-term outcome.

With regard to your second question, I think that patients with hepatitis C or coinfection with B and C suffer from a field cancerization effect. Both hepatitis C and coinfected patients are more likely to exhibit bilobar disease. Additionally, in the literature, these two cohorts of patients have been determined to be much more likely to recur intrahepatically. So, although we did not look specifically at the pattern of recurrence in our study, I think that you are correct in stating that patients who have hepatitis C or coinfection are much more likely to develop intrahepatic recurrence and therefore need to be followed closely with regard to this.

Dr. K. Kelly (Scottsdale, AZ): Is it possible that severe hepatic fibrosis identified in patients with hepatitis C could prevent or impair vascular invasion by hepatic tumors indicated in patients who also exhibit hepatitis B?

Dr. Pawlik: I think vascular invasion is related more to the tumor size. It is well established in both the published literature, as well as work that we are in the process of publishing, that as tumors become larger, there is an incremental increase in the amount of vascular invasion. So, in patients with hepatitis B who exhibit larger tumors, vascular invasion is more likely. If you are implying that the actual fibrosis may act as insulation for the liver thereby preventing vascular invasion, I would say that this may be possible, but is purely speculative. Our data has more likely indicated that large tumor size associated with hepatitis B is probably more of a direct effect and is also why we are seeing greater vascular invasion in this cohort of patients. It is not surprising that we do not observe as much vascular invasion in the group of patients with hepatitis C or coinfection because their tumors had a median size of only 3 cm, whereas hepatitis B patients had a median tumor size over twice that (7 cm). Thus, I really think the main reason for the vast difference regarding the incidence of vascular invasion relates more to tumor size.

Magnetic Resonance Imaging Provides Accurate and Precise Volume Determination of the Regenerating Mouse Liver

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Direct and repetitive noninvasive determination of the time course and the strain-specific hepatic regenerative capacity after partial hepatectomy can extend our knowledge about the basic mechanisms of liver regeneration and repair. The aim of this study was to develop a magnetic resonance (MR)-based volumetric procedure to measure the hepatic volume in the regenerating mouse liver. In Balb-C mice (n = 14), varying amounts of liver tissue were resected and MR imaging was performed 24 hours later in a 1.5 Tesla Magnet Unit. Three dimensional (3D) T1- (volumetric interpolated breath-hold examination [VIBE] sequence) and T2-weighted images were acquired with continuous 1-mm thin slices. Animals with and without intravenous administration of paramagnetic contrast agents were compared. Immediately after MR examination, mice were euthanized and livers were weighted. The liver volume was determined on MR images using Cavalieri's method and linear regression analysis was performed from the data obtained. Correlation coefficients between the liver volume measured by MR and the liver weight were 0.98 (T1) and 0.94 (T2) in the group without paramagnetic contrast injection and 0.70 (T1) and 0.96 (T2) after paramagnetic contrast application. We conclude that MR-based liver volumetry allows precise liver volume measurement during hepatic regeneration after partial hepatectomy in mice and can be a valuable tool with regard to experimental hepatology. (J GASTROINTEST SURG 2004;8:806–811)

KEY WORDS: Animal model, liver regeneration, mouse, MR imaging, volumetry

Intense liver regeneration and close to 100% survival rate was indicated after partial hepatectomy in rodents.[1,2] Variations in hepatic regeneration were determined to be dependent upon the amount of liver mass resected, age, diet, sex, and the individual animal strain.[3–7] Transgenic and knock-out mouse strains provide powerful molecular tools to extend our understanding of the concerted action and molecular mechanisms involved in liver regeneration and repair. These mouse strains are based on a variety of genetic backgrounds resulting in significant differences with regard to liver architecture. Successful noninvasive protocols to monitor liver regeneration after partial hepatectomy have thus far been developed in the rat,[8–11] but not in the mouse.

The traditional approach to standardize the surgical procedure of partial hepatectomy consists in an a priori liver lobe weight measurement. After determination of the relative liver lobe mass, partial hepatectomies of varying degrees can then be performed by an experienced microsurgeon. Resection of a single or any combination of the five mouse liver lobes results in a highly reproducible strain-specific liver regeneration model.[12]

A noninvasive volumetric imaging technique would represent a valuable alternative approach to

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standardize the surgical procedure of partial hepatectomy. Rather than defining the amount of liver to be resected, a volumetric imaging procedure could determine the liver remnant in each individual animal after partial hepatectomy. A noninvasive procedure would allow repetitive and consistent monitoring of the hepatic regenerative capacity in each animal over time. Even animals with different genetic backgrounds and therefore different liver lobe sizes could then be directly compared during liver regeneration and repair.

We hypothesized that magnetic resonance (MR) imaging-based volumetry would allow accurate and precise liver volume measurement during liver regeneration after partial hepatectomy in mice. Our aim was, therefore, to develop a reliable, reproducible, safe, and expeditious method for liver volume determination in this prospective experimental animal study, correlating imaging findings with real liver mass measurements at autopsy.

MATERIAL AND METHODS

Animals

Adult mice (20–25 g, 6–8 weeks) were kept under standard conditions for laboratory animals. All animal experimentation was approved by the local committee for animal welfare in accordance with the European Convention on Animal Care. Balb C mice (n = 14) were used for MR-based volumetry. In a second experiment, the liver lobe size was surgically assessed in Balb C (n = 32) and C57/B6 (n = 7) mice. Operative procedures were performed under general ether inhalation anesthesia (Sigma, Buchs, Switzerland).

Surgical Procedures

Animals were immobilized in a supine position, the abdomen entered through an oblique incision, and the liver lobes exposed. Varying amounts of liver tissue (i.e., left, median, and caudate lobes = 70%, n = 2; left and median lobes = 62%, n = 2; left and caudate lobes = 43%, n = 3; left lobe = 35%, n = 2; median lobe = 27%, n = 2; caudate lobe = 8%, n = 3) were resected and the animals were allowed to recover from surgery. Animals were blindly stratified for the upcoming MR imaging examination in two groups: with or without intravenous contrast agent application (Gadobutrolum, Gadovist, Schering AG, Berlin, Germany).

MR Imaging

Under intraperitoneal combination anesthesia with a combination of ketamin (0.065 mg/g body weight, Ketalar, Parke Davis, Baar, Switzerland), xylazine (0.013 mg/g body weight, Rompun; Bayer AG, Leverkusen, Germany), and acepromazine (0.002 mg/g body weight, Sedalin; Chassot GmbH, Ravensburg, Germany) MR imaging was performed 24 hours after partial hepatectomy in a 1.5 Tesla Magnet Unit (MAGNETOM Sonata; Siemens Medical Solutions Health Services Corp., Maivern, DA) using a phased-array coil for small parts (dedicated wrist coil). T1-and T2-weighted images (T1-Volumetric interpolated breath-hold examination [VIBE]-3-dimensional [3D] sequence, repetition time [TR] 11, 3-mm slices TR 5.51 ms, imaging time 7:13 minutes; T2 turbo spin echo [TSE], 3D sequence, TR 3000, echo time [TE] 113 ms, imaging time 6:32 minutes) in axial plane were acquired with continuous 1-mm thin slices to permit accurate volumetric measurement. Two groups of animals were examined (in the contrast group, 0.2 mmol/kg body weight of Gadobutrolum was injected intravenously before imaging). Immediately after MR examination, animals were euthanized and the liver was resected and weighted (Sartorius BL 150S; Sartorius AG Goettingen, Germany).

MR Volumetry and Statistical Analysis

The liver borders were electronically drawn in every sequence performed (T1, T2, and postcontrast images) in each 1-mm thick MR slice by two independent blind examiners and the hepatic "cut surface area" was measured automatically by 2 computers (standard software, Advantage Workstation; GE Healthcare, Waukesha, WI). According to Cavalieri's method,[13] addition of all measured liver section surfaces results in total liver volume (area [mm^2] × slice thickness [mm]). Linear regression analysis was performed from the data obtained to correlate MR volumetry findings with the real liver mass determined at autopsy.

RESULTS

All animals (n = 14) survived 30 minutes of data acquisition using MR imaging. No technical difficulties during anesthesia or the MR procedure were encountered. Representative MR images obtained in T1 and T2 series with and without paramagnetic contrast application are illustrated in Fig. 1. Liver delineation and volume calculation for a single animal in each T1- and T2-weighted series required an average of 15 minutes duration (Fig. 1).

Acquired hepatic volume and liver weight data are indicated in Figs. 2 and 3. In a linear regression analysis, correlation coefficients determined were 0.98 in

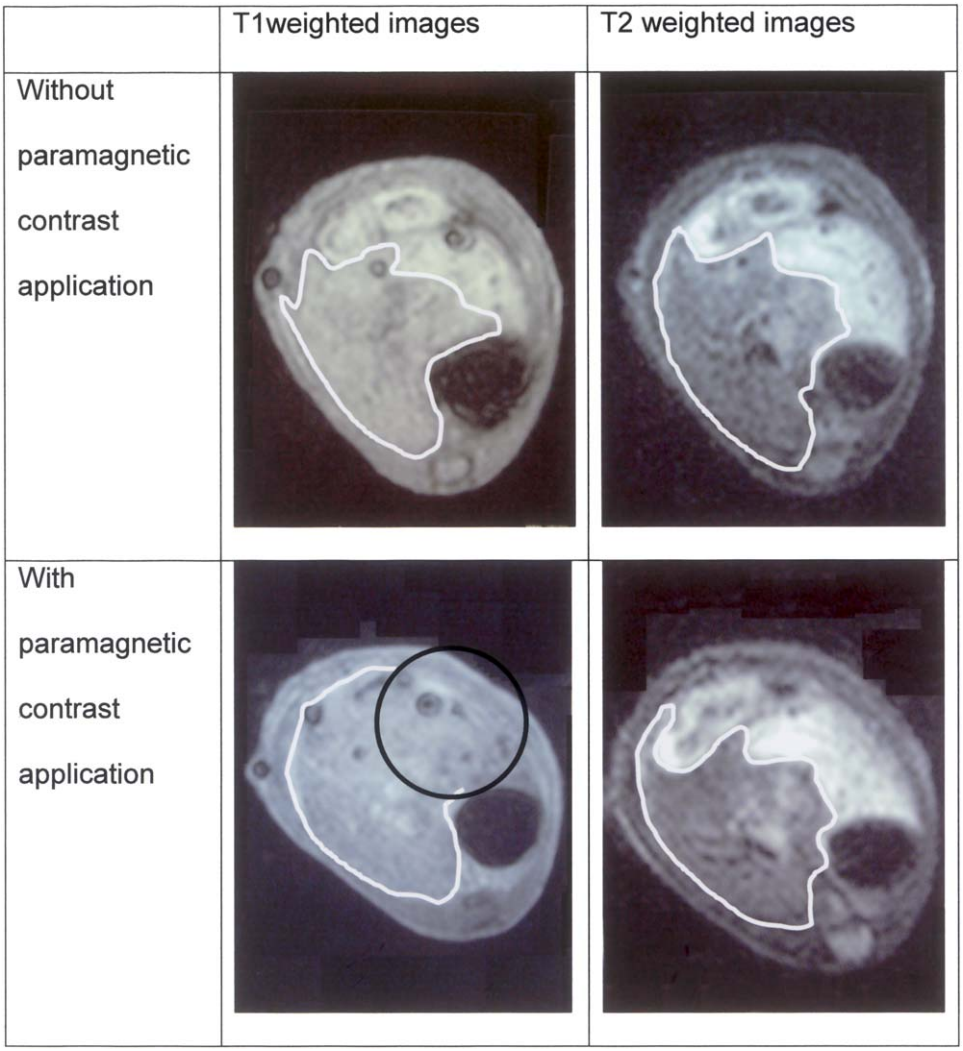

Fig. 1. Representative magnetic resonance (MR) images of the upper mouse abdomen 24 hours after microsurgical partial hepatectomy. T1- and T2-weighted images with and without paramagnetic contrast application are displayed. The liver borders were electronically delineated on each 1 mm thin MR slice in every sequence performed. The hepatic "cut surface area" was determined automatically using standard software and, according to Cavalieri's method, addition of all measured liver section-surfaces resulted in total liver volume.

T1-weighted images (Fig. 2, *top*) in the group without paramagnetic contrast injection and 0.94 in the corresponding T2-weighted images (Fig. 2, *bottom*).

Paramagnetic contrast agents accumulated in postoperative liver adjacent tissue and mimicked enhancing hepatic parenchyma precluding precise liver delineation in T1 images (Fig. 1). Accordingly, a lower correlation coefficient of 0.70 was calculated (Fig. 3, *top*). In T2-weighted images, liver delineation was comparable with T2 images in the group without contrast application and the correlation coefficient calculated was 0.96 (Fig. 3, *bottom*). Assessment of individual liver lobe sizes in two different mouse strains revealed a significant interstrain variability (Table 1).

DISCUSSION

The approach of MR-based liver volumetry in mice provides accurate and precise volume determination of the regenerating liver in mice. Most importantly, T1-weighted images without contrast application demonstrated exceptional postoperative anatomy because of superior water-fat tissue contrast. Liver delineation in T1 images without paramagnetic contrast was highly accurate resulting in a correlation coefficient between the liver volume calculated and the liver weight measured of 0.98. On the other hand, the correlation coefficient calculated from T2-weighted images was slightly inferior because postoperative edematous changes around the regenerating

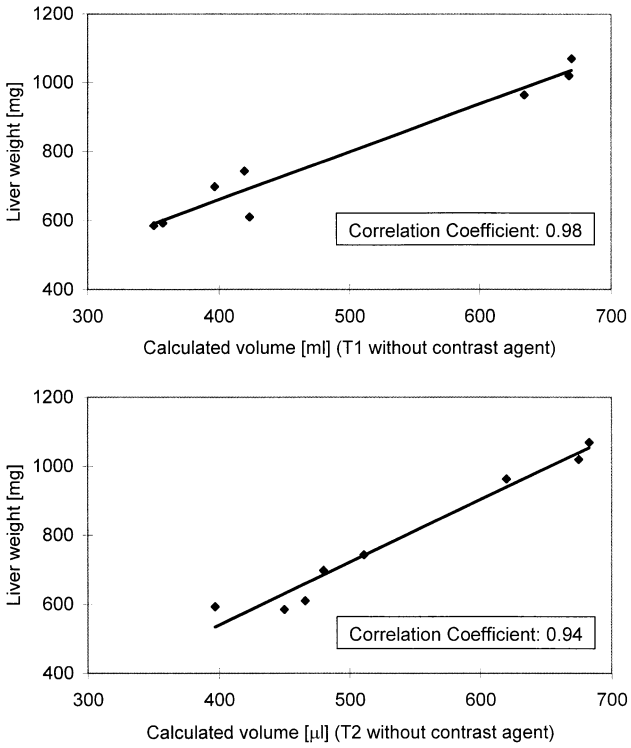

Fig. 2. Correlation between liver mass at autopsy and magnetic resonance (MR) volumetry in T1-weighted (*top*) and T2-weighted (*bottom*) images without intravenous paramagnetic contrast agent application.

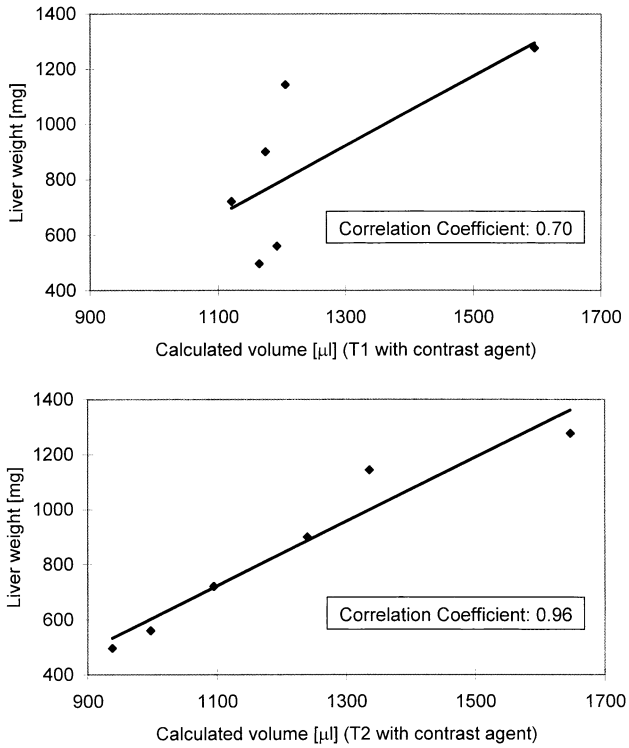

Fig. 3. Correlation between liver mass at autopsy and magnetic resonance (MR) volumetry in T1-weighted (*top*) and T2-weighted (*bottom*) images with intravenous paramagnetic contrast agent application.

liver with high signal intensity impeded precise liver delineation. The correlation coefficients obtained using the MR protocol applied in our series (VIBE 3D sequence) compare favorably with the correlation between final liver volume and postmortem liver weights reported in a similar experiment in rats.[11] The VIBE sequence generates a 3D volume image that allows continuous imaging in thin slices without gap. As the 3D space of interest is entirely covered by the VIBE sequence, optimal quality images with high resolution can be generated. Furthermore, this technique allows improved depiction of even very small lesions because of the ultrathin slices compared with other commercial sequences.

Contrast-enhanced T1-weighted images indicated high signal intensity areas that were caused by edematous changes, both in the regenerating liver and the adjacent omental and intestinal tissue. As depicted in Fig. 4, liver delineation on the left resection border was difficult because of nonexisting soft-tissue image contrast between hepatic tissue and adjacent structures. Consequently, the correlation coefficient was lowest in the series of T1-weighted images after intravenous contrast application. As expected, paramagnetic contrast application did not significantly interfere with liver delineation in T2-weighted images. It is known that T2 images are characterized by long relaxation times and therefore the effect of paramagnetic contrast agents is minimal and does

Table 1. Liver lobe size (relative to total liver weight) in Balb C and C57/B6 mice

	Mouse Strain		
Liver Lobes	**Balb C (n = 32) Adult Male**	**C57/B6 (n = 7) Adult Male**	***T* test**
Left lobe (%)	34.4 ± 2.0	30.8 ± 1.3	$P < 0.0001$
Median lobe (%)	26.2 ± 1.9	39.9 ± 8.0	$P < 0.0001$
Right Superior lobe (%)	16.6 ± 1.4	16.2 ± 1.7	P = NS
Right inferior lobe (%)	14.7 ± 1.4	12.3 ± 1.4	$P < 0.0001$
Caudate lobe (%)	8.1 ± 1.0	8.8 ± 1.4	P = NS

The relative weight of each liver lobe was determined in adult male Balb C and C57/B6 mice and indicated to be considerably different in the left, median, and right inferior lobes. No differences were detected in the right superior and caudate lobes.
NS = not significant.

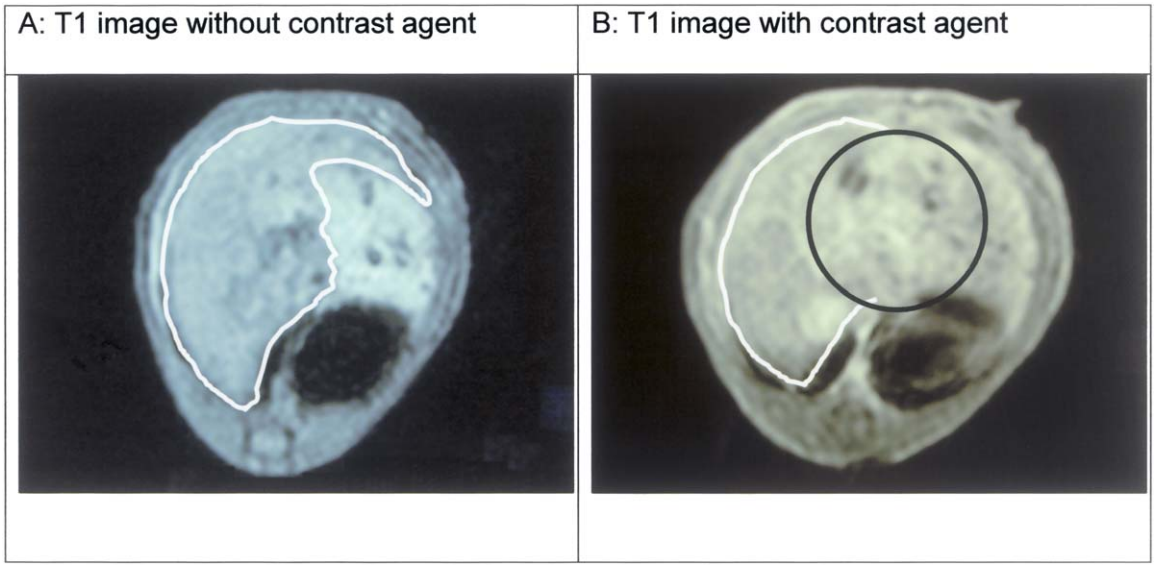

Fig. 4. Liver delineation in T1 images without paramagnetic contrast agent (**A**) using the VIBE sequence generates a three-dimensional volume image in thin slices without gap. Optimal quality images with high resolution allowed superior liver delineation and consequently highly accurate liver volume determination. Contrast-enhanced T1-weighted images (**B**) indicated high signal intensity attributable to edematous changes, both in the regenerating liver and the adjacent omental and intestinal tissue. Liver delineation on the left resection border was therefore difficult because of the disturbance of the normal soft-tissue image contrast between hepatic tissue and adjacent structures after partial hepatectomy. Consequently, the correlation coefficient between liver volume determined in magnetic resonance (MR) volumetry and liver weight was lowest in the series of T1-weighted images after intravenous contrast application.

not affect the soft-tissue contrast differences. Intravenous paramagnetic contrast injection in mice can be technically demanding and, based on the data acquired, seems unnecessary for a reliable MR-based volume determination with regard to regenerating mouse livers after partial hepatectomy.

The method developed offers a significant refinement[14] regarding animal experimentation in experimental hepatology as compared with other current options. The use of repetitive volume measurements rather than harvesting regenerating livers at specific time-points results in a significant reduction with regard to the amount of animals needed to generate a reliable hepatic regeneration curve. Furthermore, testing the same animal repeatedly over time reduces interanimal variability. Paired statistical data analysis allows for the reliable detection of notable differences between experimental groups and a smaller number of animals is required per group. The regenerative capacity after partial hepatectomy of different mouse strains with markedly different liver lobe sizes (Table 1) can be directly compared using the noninvasive volumetric method proposed. This is particularly important when using genetically modified transgenic or knock-out animals based on different genetic backgrounds with regard to surgical models of liver regeneration research.

CONCLUSION

The MR-based volumetric protocol developed allows accurate liver volume determination during hepatic regeneration in a mouse model. Repetitive determination of the hepatic volume during liver regeneration in the same animal offers the major advantage of reducing interindividual differences between animals. By measuring the time course of hepatic regeneration in mouse strains with different genetic backgrounds, the liver regenerative capacity of individual animal strains can easily be determined. MR-based liver volumetry without the need for additional paramagnetic contrast agents can therefore be a useful tool with regard to experimental hepatology.

Drs. Inderbitzin and Gass contributed equally to this work.

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Herpes Simplex Virus Amplicon Delivery of a Hypoxia-Inducible Angiogenic Inhibitor Blocks Capillary Formation in Hepatocellular Carcinoma

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Tumor hypoxia induces vascular endothelial growth factor (VEGF) expression, which stimulates tumor angiogenesis. The VEGF pathway is inhibited by soluble VEGF receptors (soluble fetal liver kinase-1 [sFlk-1]) that bind VEGF and block its interaction with endothelial cells. Herpes simplex virus (HSV)-derived amplicons are replication-incompetent viruses used for gene delivery. We attempt to attenuate angiogenesis and inhibit hepatoma growth through amplicon-mediated expression of sFlk-1 under hypoxic control. A multimerized hypoxia-responsive enhancer (10xHRE) was cloned upstream of the sFlk-1 gene (10xHRE/sFlk-1). An amplicon expressing 10xHRE/sFlk-1 was genetically engineered (HSV10xHRE/sFlk-1). SK-HEP-1 human hepatoma cells were transduced with HSV10xHRE/sFlk-1 and incubated in normoxia (21% O_2) or hypoxia (1% O_2). Human umbilical vein endothelial cell assay evaluated capillary inhibition. Western blot assessed sFlk-1 expression. SK-HEP-1 flank tumors (n = 24) in athymic mice were treated with HSV10xHRE/sFlk-1. Media from hypoxic SK-HEP-1 transduced with HSV10xHRE/sFlk-1 yielded an 80% reduction in capillary formation ($P < 0.005$), whereas normoxic SK-HEP-1 yielded a 25% reduction ($P < 0.05$). Western blot of SK-HEP-1 transduced with HSV10xHRE/sFlk-1 demonstrated greater sFlk-1 expression in hypoxia vs. normoxia. SK-HEP-1 tumors treated with HSV10xHRE/sFlk-1 yielded a 72% reduction in volume vs. the control group ($P < 0.000001$). HSV amplicon-mediated delivery of a hypoxia-inducible soluble VEGF receptor substantially reduces new vessel formation and tumor growth in hepatoma. (J GASTROINTEST SURG 2004;8:812–823)

KEY WORDS: Flk-1, herpes simplex virus, hypoxia-inducible factor 1, vascular endothelial growth factor, gene therapy

The selective delivery of therapeutic agents to tumor cells remains a primary challenge with regard to cancer gene therapy. However, lower tumor oxygen levels compared with surrounding normal tissues have recently been used to successfully target cancer.[1] Hypoxia is a prevalent feature of solid malignancies that develops as tumors proliferate and outgrow their vasculature. Tumor hypoxia is correlated with metastatic spread and resistance to conventional chemoradiation.[2–5] These hypoxic tumors may possess a more aggressive and malignant phenotype.[2] In primary hepatocellular carcinoma (HCC), hypoxia has been associated with the production of angiogenic stimulators and tumor growth.[6,7] In addition, widespread disease at the time of diagnosis and associated cirrhosis often preclude curative operative resection.[8] Therefore, the development of new therapeutic modalities directed toward hypoxic HCC is necessary. Lower hepatoma oxygen levels compared with surrounding normal liver parenchyma may be used to target antiangiogenic cancer therapy.

Tumor hypoxia stimulates the production of angiogenic factors, such as the vascular endothelial

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growth factor (VEGF), that promote tumor growth and spread.[9] Hypoxia increases VEGF expression by inducing the transcriptional regulator hypoxia-inducible factor 1 (HIF-1) that binds to the hypoxia-responsive element (HRE) in the VEGF promoter. HIF-1 is a heterodimer composed of the hypoxia-inducible α subunit (HIF-1α) and the constitutively expressed β subunit (HIF-1β).[10] The interaction between HIF-1 and HRE is the principal stimulus for VEGF expression in cancer cells.[11] VEGF is the main regulator of tumor angiogenesis and the most potent endothelial cell mitogen.[12,13] Binding of VEGF to its membrane-bound endothelial cell receptors stimulates the VEGF signaling pathway.

VEGF receptor-2 (Flk-1) is the primary VEGF receptor on endothelial cells.[14] After VEGF binds to Flk-1, the receptor dimerizes and initiates an intracellular signal through receptor tyrosine kinase activity.[15] This signal stimulates endothelial cell differentiation and survival, capillary tube formation, and vascular permeability.[16,17] Conversely, blocking the interaction between VEGF and Flk-1 has inhibited human tumor xenograft growth in mice.[18] Soluble forms of the VEGF receptor, lacking their membrane spanning domains, have recently been used to interrupt the VEGF signaling pathway.[19] Soluble receptors such as sFlk-1 bind VEGF and block its interaction with membrane-bound receptors, thus inhibiting angiogenesis.

Soluble VEGF receptors may be delivered to tumor cells by replication-incompetent herpes simplex viruses (HSV amplicons). Genetically engineered to lack viral sequences necessary for replication, HSV amplicons function as gene delivery vehicles and elicit minimal cytotoxicity.[20] They have been used successfully to deliver immunomodulatory cytokines for cancer gene therapy.[21,22] HSV amplicons transduce both dividing and quiescent cells and exhibit the capacity to transport large transgenes approaching 150 kb.[23]

This study explores the potential application of a hypoxia-inducible soluble VEGF receptor in HCC. Our intention was to attenuate angiogenesis and inhibit hepatoma growth through amplicon-mediated delivery of sFlk-1 under hypoxic control.

MATERIAL AND METHODS

Cell Culture and Hypoxic Treatment

SK-HEP-1 cells are a well-described human HCC cell line. SK-HEP-1 cells were obtained (American Type Culture Collection, Manassas, VA), maintained in a 5% CO_2 humidified incubator at 37 C, and subcultured twice a week. Cells were grown in minimum essential medium supplemented with 10% fetal calf serum (FCS).

Human umbilical vein endothelial cells (HUVEC) were obtained (Cambrex Corp., East Rutherford, NJ), maintained in a humidified incubator at 37 C, and subcultured twice a week. HUVEC cells were grown in endothelial cell growth medium-2 (EGM-2) (Cambrex Corp., East Rutherford, NJ). All HUVEC culture surfaces were treated with 0.2% gelatin.

Hypoxic conditions were created by the displacement of oxygen with nitrogen in a triple-gas incubator (NuAire, Inc., Plymouth, MN). Oxygen concentrations as low as 1% could be reached and maintained. After plating and transfection procedures, cells were routinely incubated for 12 hours in 21% O_2 to allow for attachment and stabilization before hypoxic exposure.

VEGF Expression

SK-HEP-1 cells were plated at 1×10^5 cells per well in six-well flat-bottom plates (Costar; Corning, Inc., Corning, NY) and incubated in either 21% O_2 or 1% O_2. After a 24-hour incubation, cell lysates were collected (Cell Signaling Technology, Inc., Beverly, MA) and protein concentrations of cell lysates were determined using the Bradford method (BioRad Laboratories, Inc., Hercules, CA). VEGF enzyme-linked immunosorbent assay (ELISA; R&D Systems, Inc., Minneapolis, MN) was performed in quadruplicate on 100 μg of protein from each sample.

HIF-1α Expression

SK-HEP-1 cells were plated at 5×10^6 cells/dish in 100 mm culture dishes (Costar; Corning, Inc., Corning, NY). Cells were incubated in either 21% O_2 or 1% O_2 for 1, 5, and 24 hours. HIF-1α is a nuclear protein and therefore nuclear extracts were collected from both normoxic and hypoxic cells at these time points (Active Motif, Inc., Carlsbad, CA). The protein content of nuclear extracts was determined according to the Bradford method (Protein Assay Reagent; BioRad Laboratories, Inc., Hercules, CA) by measuring absorbance at 595 nm (Beckman DU 640 spectrophotometer; Beckman Instruments, Inc., Fullerton, CA). HIF-1α ELISA (BD Biosciences Clontech, Palo Alto, CA) was performed in triplicate on 30 μg of nuclear protein from each time point. In addition, western blot analysis for HIF-1α was performed on SK-HEP-1 nuclear extracts collected at 5 and 24 hours after normoxic or hypoxic incubation. 50 μg of nuclear protein from each sample was assessed with a mouse monoclonal anti-HIF-1α antibody (1:1000 dilution; Novus Biologicals, Inc.,

Littleton, CO). Equal protein loading was confirmed using Ponceau S staining.

Construction of Hypoxia-Responsive Enhancer

Complementary 41 base pair oligonucleotides were constructed encoding the HIF-1 recognition sequence from the promoter of the human VEGF gene (Invitrogen Corp., Carlsbad, CA). These monomeric HRE were designed with *Xanthomonas holcicola* (Xho I) and *Streptomyces albus* (Sal I) compatible ends for multimerization and cloning. The complementary sequences were (5′-TCGAGCCACAGTGCATACGTGGGCTCCAACAGGTCCTCTTG-3′) and (5′- TCGACAAGAGGACCTGTTGGAGCCCACGTATGCACTGTGC-3′).[24] Paired oligomers were annealed and the 5′ ends of the double-stranded HRE product were phosphorylated with T4 polynucleotide kinase (New England Biolabs, Inc., Beverly, MA) for subsequent multimerization. Ten copies of the HRE fragment were tandemly ligated with Quick Ligase (New England Biolabs, Inc., Beverly, MA). The resulting 10xHRE enhancer was confirmed with sequence analysis and restriction digests.

Enhancer Function

We cloned the 10xHRE enhancer into the Xho I site of the plasmid gene light 3 (pGL3) promoter vector (Promega Corp., Madison, WI) upstream of the minimal simian virus 40 (SV40) promoter, forming the luciferase reporter plasmid 10xHRE/pGL3. SK-HEP-1 cells were plated at 1.5×10^6 cells per well in six-well flat-bottom plates (Costar; Corning, Inc., Corning, NY) and transfected with 1.6 μg of the 10xHRE/pGL3 plasmid using Lipofectamine 2000 (Invitrogen Corp., Carlsbad, CA). The original pGL3 promoter plasmid was used as a control in these experiments. Cells were incubated in either 21% O_2 or 1% O_2 for 18 hours. Cell lysates were collected and a luciferase assay (Luciferase Assay Kit; Promega Corp., Madison, WI) was performed using a single injection luminometer (MicroLumat Plus; EG&G Berthold, Oak Ridge, TN). Protein concentrations of cell lysates were determined using the Bradford method (BioRad Laboratories, Inc., Hercules, CA) to normalize luciferase activity between transfected groups.

Construction of Vector Expressing Hypoxia-Responsive sFlk-1

A plasmid containing the sFlk-1 gene was obtained (InvivoGen, San Diego, CA). The sFlk-1 gene was isolated by restriction digest with *Nocardia corallina* (Nco I) and *Neisseria mucosa heidelbergensis* (Nhe I) (New England Biolabs, Inc., Beverly, MA). The Nhe I site was blunted through exonuclease digestion with Klenow (New England Biolabs, Inc., Beverly, MA). 10xHRE/pGL3 was digested with Nco I and Xba I to remove the luciferase gene and the *Xanthomonas badrii* (Xba I) site was blunted (Klenow; New England Biolabs, Inc., Beverly, MA). The sFlk-1 gene was ligated between the former Nco I and Xba I sites of 10xHRE/pGL3 (Quick Ligase; New England Biolabs, Inc., Beverly, MA), forming the 10xHRE/sFlk-1 plasmid. The sequence was confirmed using polymerase chain reaction (PCR) and restriction digests.

Construction of Amplicon Expressing 10xHRE/sFlk-1

The vector pHSVminOriSmc (plasmid herpes simplex virus minimal origin of replications multiple cloning site), containing the HSV packaging sequences (pac) and lacking the genes required for viral replication, was used. The10xHRE/sFlk-1 sequence with its poly-A tail was PCR amplified from the 10xHRE/sFlk-1 plasmid using forward (5′-GATAAGGATCCGAGCTCTTACGCGTGCTAGC3′) and reverse (5′-TGACTGGGTTGAAGGCTCTCAAGGGCATCG-3′) primers (Invitrogen Corp., Carlsbad, CA). These primers maintained a *Brevibacterium albidum* (BamH I) restriction site downstream of the poly-A tail and introduced a BamH I site upstream of 10xHRE. The amplified PCR product containing 10xHRE/sFlk-1 was digested with BamH I (New England Biolabs, Inc., Beverly, MA) and ligated into pHSVminOriSmc at the BamH I site (Quick Ligase; New England Biolabs, Inc., Beverly, MA) creating the plasmid pHSV-10xHRE/sFlk-1. The sequence was confirmed using PCR and restriction digests. Using the vector pHSV-10xHRE/sFlk-1 as a template, the HSV amplicon expressing 10xHRE/sFlk-1 (HSV10xHRE/sFlk-1) was packaged, purified, and titered.[25,26] Viral pellets were resuspended in 100 μl of PBS and stored at −80 C until use. An amplicon expressing the lacZ reporter gene (HSVlacZ) was manufactured in the same fashion and used as a control in these experiments.

Capillary Inhibition by HSV10xHRE/sFlk-1

SK-HEP-1 cells were plated at 1×10^5 cells per well in six-well plates using Opti-MEM supplemented with 1% FCS and transduced with 5×10^4 transducing units (TU) of HSV10xHRE/sFlk-1 or HSVlacZ. Cells were incubated at either 21% O_2 or 1% O_2 for 3 days. After this incubation, conditioned media from the SK-HEP-1 cells was collected, supplemented with 10 ng/ml VEGF (Sigma-Aldrich Corp., St. Louis, MO), and used to plate HUVEC.

After a 14-hour incubation, two independent observers counted capillary tubes under light microscopy. Standard lacZ staining was performed on SK-HEP-1 cells transduced with HSVlacZ at the time of HUVEC plating. The number of SK-HEP-1 cells staining positively in hypoxia vs. normoxia was used to compare transduction efficiencies at these oxygen concentrations.

sFlk-1 Western Blot

SK-HEP-1 cells, which had been used to condition media for the HUVEC assay, were lysed with cell lysis buffer (Cell Signaling Technology, Inc., Beverly, MA). Protein concentrations of cell lysates were determined using the Bradford method (BioRad Laboratories, Inc., Hercules, CA) and 50 μg of protein from each sample was loaded into 5% sodium dodecyl sulfate (SDS) polyacrylamide gels (BioRad Laboratories, Inc., Hercules, CA). After gel electrophoresis in Tris-glycine-SDS (TGS pH 8.4; Fisher Scientific International, Inc., Hampton, NH), protein was transferred to polyvinylidene difluoride (PVDF) membranes for 1.5 hours in TGS containing 20% methanol at 250 mA and 4 C. Membranes were incubated for 16 hours in 4% milk containing either a 1:500 dilution of a mouse monoclonal anti-Flk-1 antibody (R&D Systems, Inc., Minneapolis, MN) or a 1:1000 dilution of a control goat polyclonal anti-β-actin antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After primary antibody incubation, the membranes were blocked in phosphate-buffered saline (PBS) containing 4% milk. Flk-1 and β-actin membranes were incubated in a secondary antibody (goat anti-mouse immunoglobulin G complexed to horseradish peroxidase [IgG-HRP] and donkey anti-goat IgG-HRP, respectively [Santa Cruz Biotechnology, Inc., Santa Cruz, CA]) diluted 1:1000 in PBS containing 4% milk. Membranes were washed five times in PBS. Protein bands were visualized with enhanced chemiluminescence (ECL Plus Western Blotting Detection System; Amersham Biosciences, Inc., Piscataway, NJ) and exposed on X-OMAT autoradiography (AR) film (Eastman Kodak Corp., Rochester, NY).

Establishment and Treatment of Flank Tumors

All animal procedures were performed with the approval of the Memorial Sloan-Kettering Institutional Animal Care and Use Committee. Six week-old athymic mice were obtained from Charles River Laboratories (Charles River Laboratories, Inc., Wilmington, MA). Mice were anesthetized with intraperitoneal injection of ketamine and xylazine (100 mg/kg ketamine, 10 mg/kg xylazine) for all procedures. Mice were housed allowing three per cage and allowed food and water ad libitum.

Twelve athymic mice underwent bilateral subcutaneous flank injections with 1×10^6 SK-HEP-1 cells. After tumor volumes reached approximately 20 mm^3, mice were randomized to receive 1×10^6 TU of HSV10xHRE/sFlk-1 (n = 8), 1×10^6 TU of HSV-lacZ (n = 8), or 50 μl of PBS (n = 8) through direct intratumoral injection. The flank tumors of each animal received the same treatment. Tumor volumes were assessed over 5 weeks.

Statistical Analysis

Data are expressed as the mean ± standard deviation (SD). Comparisons between groups were performed with a two-tailed Student's *t* test.

RESULTS

VEGF Expression

SK-HEP-1 cells incubated at either 1% O_2 or 21% O_2 were examined for VEGF expression using ELISA. VEGF levels were 189.6 ± 18.6 pg/ml in hypoxic SK-HEP-1 cell lysates and 35.0 ± 2.1 pg/ml in normoxic SK-HEP-1 cell lysates. These levels represent a 5.4-fold increase in VEGF expression after 24 hours of incubation in hypoxia compared with normoxia ($P < 0.001$, Fig. 1, *A*).

HIF-1α Expression

SK-HEP-1 cells incubated at either 1% O_2 or 21% O_2 were examined for HIF-1α expression using ELISA. SK-HEP-1 cells demonstrated a 1.8-fold increase in HIF-1α expression after 1 hour of hypoxic incubation compared with normoxic cells ($P < 0.01$)—a 3.0-fold increase after 5 hours ($P < 0.01$), and a 5.6-fold increase after 24 hours ($P < 0.005$, Fig. 1, *B*). Western blot analysis confirmed increased HIF-1α levels in hypoxia compared with normoxia at 5 and 24 hours (Fig. 1, *B*).

Luciferase Activity

10xHRE enhancer function was assessed using luciferase reporter assay. After incubation in either 1% O_2 or 21% O_2, SK-HEP-1 cell lysates underwent analysis for luciferase expression. In 1% O_2, SK-HEP-1 cells transfected with 10xHRE/pGL3 yielded a 45-fold increase in luciferase activity compared with cells transfected with pGL3 alone ($P < 0.01$, data not indicated). In 21% O_2, 10xHRE/pGL3 transfection resulted in a 2.5-fold increase in luciferase activity compared with pGL3 ($P < 0.05$, data not indicated).

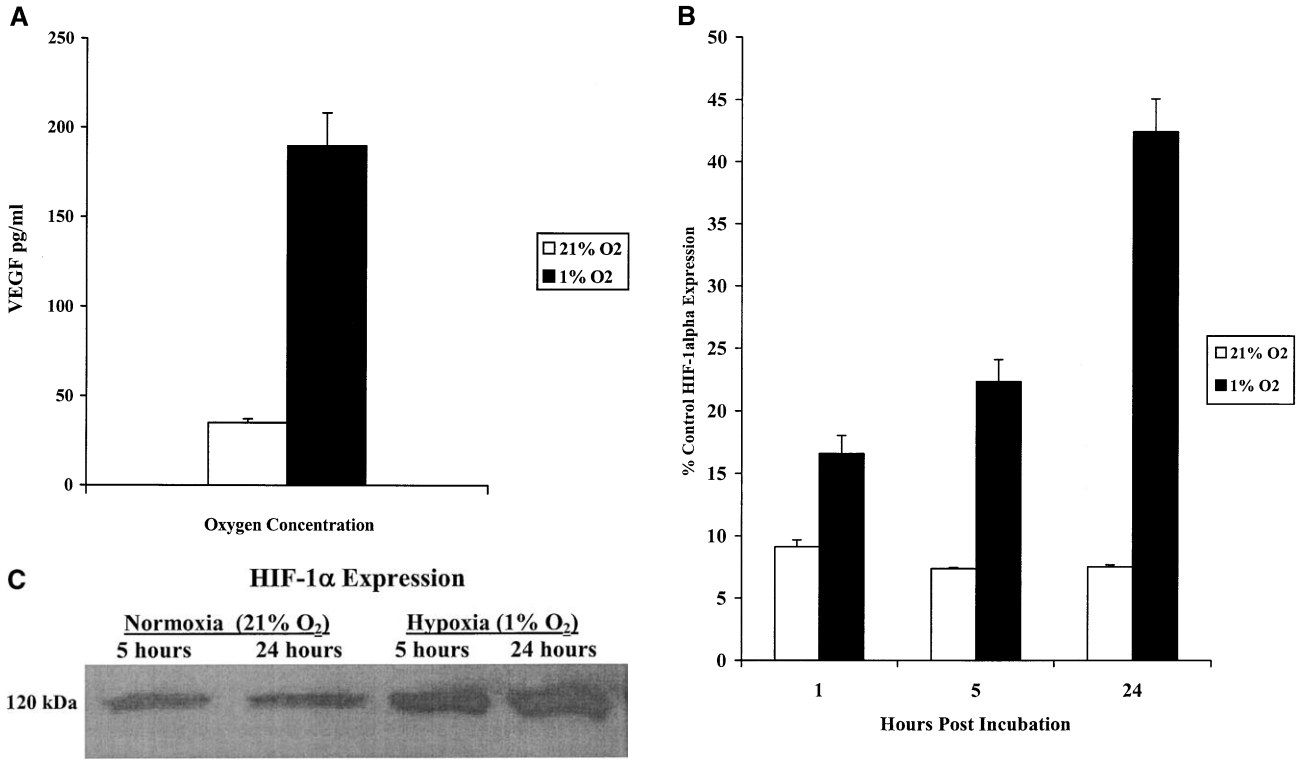

Fig. 1. Vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 (HIF-1α) levels in SK-HEP-1 cells after hypoxic (1% O_2) or normoxic (21% O_2) incubation. (**A**) SK-HEP-1 human hepatoma cells increased VEGF expression in hypoxia. After 24 hours of incubation in either 1% O_2 (*black bar*) or 21% O_2 (*white bar*), SK-HEP-1 cell lysates underwent VEGF enzyme-linked immunosorbent assay (ELISA, R&D Systems, Inc., Minneapolis, MN). VEGF levels increased 5.4-fold in hypoxia vs. normoxia ($P < 0.001$). (**B**) SK-HEP-1 cells upregulated hypoxia-inducible factor 1α (HIF-1α) expression in hypoxia. SK-HEP-1 cells were incubated in either 1% O_2 (*black bar*) or 21% O_2 (*white bar*) and nuclear extracts were collected at 1, 5, and 24 hours. HIF-1α ELISA was performed (BD Biosciences Clontech, Palo Alto, CA) on 30 μg of nuclear protein from each sample. At 24 hours, there was a 5.6-fold increase in HIF-1α expression in hypoxia compared with normoxia ($P < 0.005$). (*Bottom*) HIF-1α western blot confirmed increased HIF-1α expression in hypoxia after 5 and 24 hours of hypoxic (1% O_2) or normoxic (21% O_2) incubation.

These experiments demonstrate that the 10xHRE enhancer significantly improved paired gene expression in hypoxia with favorable specificity for an oxygen-deprived environment.

HUVEC Assay: HSV Amplicon-Mediated Delivery of Hypoxia-Regulated sFlk-1

Inhibition of capillary formation by HSV10xHRE/sFlk-1 transduction was assessed using HUVEC assay. Media conditioned using SK-HEP-1 cells cultured under normoxic conditions and transduced with HSVlacZ yielded 99 ± 15 capillary tubes per well, whereas media from cells transduced with HSV10xHRE/sFlk-1 produced 74 ± 16 tubes per well. This represented a 25% reduction in capillary formation in normoxia ($P < 0.05$, Fig. 2, *A*). Media conditioned using hypoxic SK-HEP-1 cells transduced with HSVlacZ indicated a mean of 127 ± 42 capillary tubes per well. Conditioned media from hypoxic cells transduced with HSV10xHRE/sFlk-1 resulted in a mean of 26 ± 14 tubes per well. This represented an 80% reduction in capillary formation under hypoxic conditions ($P < 0.005$, Figs. 2, *B* and 3). Using lacZ staining, no difference in HSV amplicon transduction was observed between normoxic and hypoxic SK-HEP-1 cells, with both groups exhibiting 90% transduction efficiency. These results demonstrate that HSV10xHRE/sFlk-1 significantly inhibits angiogenesis, particularly under low oxygen conditions.

10xHRE/sFlk-1 Expression

SK-HEP-1 cell lysates underwent western blot analysis for sFlk-1 expression after HSV10xHRE/sFlk-1 transduction. Equivalent transduction of HSV amplicon into SK-HEP-1 cells under normoxic and

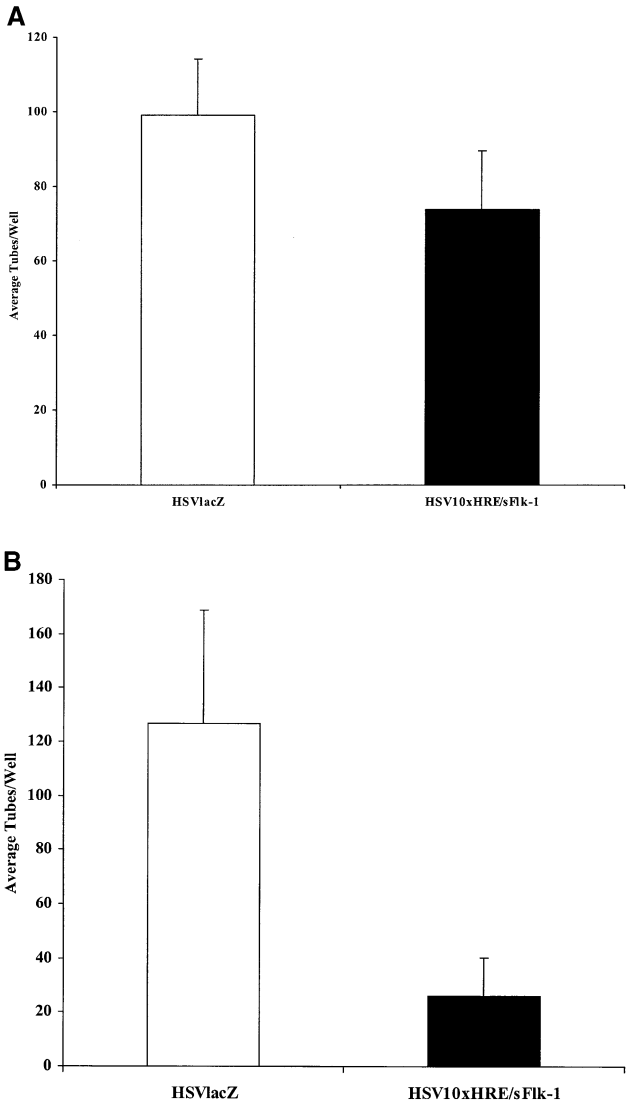

Fig. 2. Human umbilical vein endothelial cell (HUVEC) assay evaluated capillary inhibition after herpes simplex virus multimerized hypoxia responsive enhancer/ soluble fetal liver kinase-1 (HSV10xHRE/sFlk-1) transduction of SK-HEP-1 human hepatoma cells. Conditioned media was collected from SK-HEP-1 cells that had been transduced with HSV10xHRE/sFlk-1 or control HSVlacZ (lactose fermentation of b-galactosidase) and incubated at either 1% O_2 or 21% O_2. HUVEC cells were plated in the presence of this conditioned media. (***A***) Under normoxic conditions, transduction with HSV10xHRE/sFlk-1 reduced capillary formation by 25% vs. HSVlacZ ($P < 0.05$). (**B**) In hypoxia, HSV10xHRE/sFlk-1 transduction reduced capillary formation by 80% compared with HSVlacZ ($P < 0.005$).

hypoxic conditions was confirmed using lacZ staining. Nontransduced SK-HEP-1 cells did not express sFlk-1 under hypoxic or normoxic conditions. After transduction with HSV10xHRE/sFlk-1, greater sFlk-1 protein levels were detected in lysates prepared from transduced hypoxic SK-HEP-1 cells compared with their normoxic counterparts (Fig. 4). These results demonstrate that HSV10xHRE/sFlk-1 preferentially expresses sFlk-1 under conditions of reduced oxygen tension.

Inhibition of Tumor Growth

SK-HEP-1 cells were injected into the flanks of athymic mice to produce an in vivo tumor model. There was no significant difference in tumor volumes between HSVlacZ and PBS-treated tumors at any of the measured time points. As early as 1 week after HSV10xHRE/sFlk-1 delivery, there was a significant reduction in tumor volume. At this early time point, HSV10xHRE/sFlk-1 transduction decreased tumor volume by18% vs. control amplicon ($P < 0.01$) and 32% vs. PBS ($P < 0.001$, Fig. 5). This inhibition of tumor growth persisted throughout the entire observation period. At the conclusion of the 5-week study, tumors treated with HSV10xHRE/sFlk-1 indicated a mean volume of 78 ± 19 mm^3, whereas tumors treated with HSVlacZ indicated a mean volume of 280 ± 36 mm^3 and those treated with PBS exhibited a mean volume of 292 ± 38 mm^3. This represented a 72% reduction in tumor volume with HSV10xHRE/sFlk-1 transduction vs. control amplicon ($P < 0.000001$) and a 73% reduction vs. PBS ($P < 0.000001$, Fig. 5). These results demonstrate that HSV10xHRE/sFlk-1 can considerably inhibit tumor growth in vivo.

DISCUSSION

VEGF plays a critical role with regard to capillary tube formation and the survival of newly formed blood vessels.[13] Recent research has further elucidated the role of VEGF as a key regulator in cancer-related angiogenesis. VEGF has induced endothelial cell proliferation and tumor growth, potentially contributing to metastatic spread.[27–30] Studies have demonstrated that blocking VEGF at either the protein or mRNA level can decrease tumor vascularization and resulting tumor volume.[31,32] Moreover, elevated levels of VEGF have been correlated with increased invasiveness and a poor prognosis in various malignancies.[27–30] Hypoxia is the primary inducer of VEGF expression in tumors.[33] Targeting tumor hypoxia may therefore enhance cancer treatments that aim to suppress VEGF.

Tumor hypoxia promotes increased levels of the transcriptional regulator HIF-1. The direct relationship between elevated HIF-1 levels and the production of angiogenic proteins such as VEGF has been

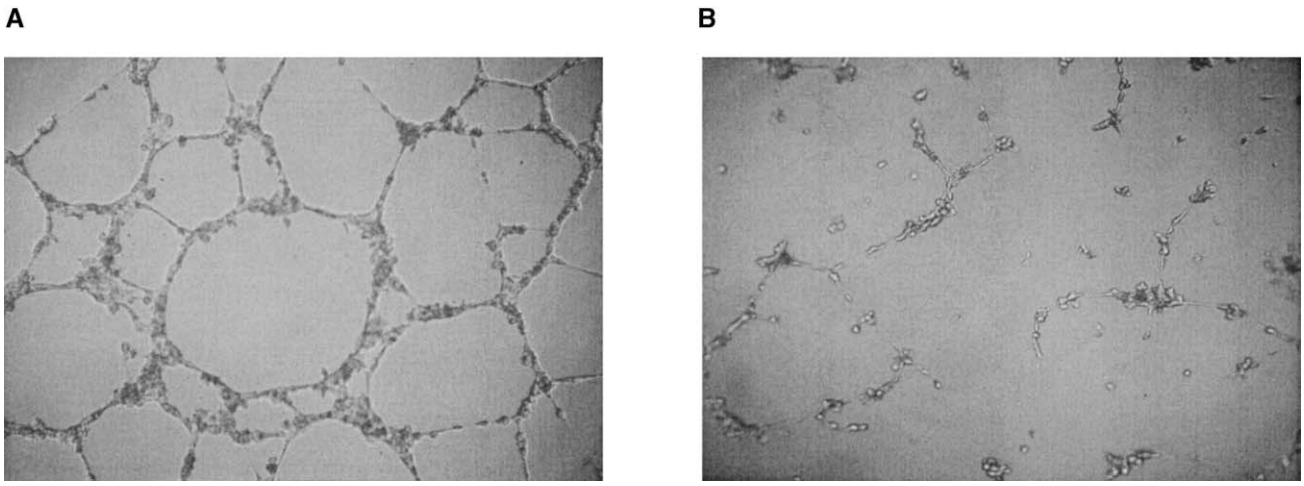

Fig. 3. Human umbilical vein endothelial cell (HUVEC) assay evaluated capillary inhibition. (**A**) HUVEC plated in media derived from hypoxic SK-HEP-1 cells transduced with control HSVlacZ (lactose fermentation of b-galactosidase). (**B**) HUVEC plated in media derived from hypoxic SK-HEP-1 cells transduced with herpes simplex virus multimerized hypoxia responsive enhancer/ soluble fetal liver kinase-1 (HSV10xHRE/sFlk-1). There was a significant reduction in capillary tube formation after transduction with HSV10xHRE/sFlk-1 compared with control HSVlacZ.

confirmed in HCC.[34] Enhanced VEGF expression has been demonstrated immunohistochemically in HCC and increased expression has been observed with poorly differentiated tumors.[35] Greater VEGF staining has been identified in HCC, which exhibit satellite lesions and vascular invasion, potentially contributing to distant metastases.[36] In addition to this enhanced tumor growth, low tumor oxygen levels are associated with resistance to conventional chemotherapy and radiation therapy.[4,5] In this study, we use hypoxia, a tumor trait that promotes VEGF expression and a more malignant phenotype, to selectively direct antiangiogenic gene therapy.

There are two principal VEGF receptors: VEGF receptor-1 (feline McDonough strain [fms]-like tyrosine kinase-1 [Flt-1]) and VEGF receptor-2 (fetal

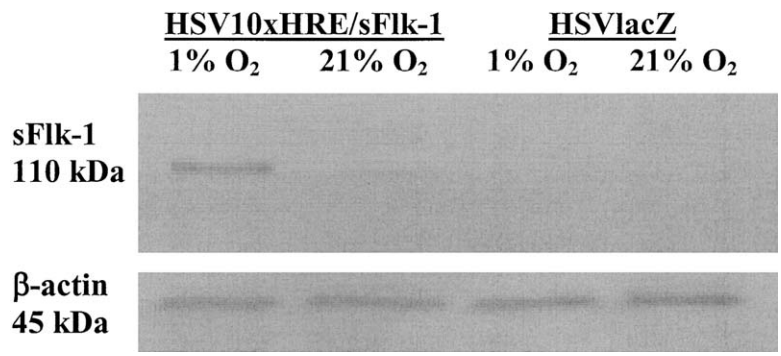

Fig. 4. Western blot assessment of soluble fetal liver kinase-1 (sFlk-1) expression after herpes simplex virus multimerized hypoxia responsive enhancer/ soluble fetal liver kinase-1 (HSV10xHRE/sFlk-1) transduction. After transduction with HSV10xHRE/sFlk-1 or control HSVlacZ (lactose fermentation of b-galactosidase), SK-HEP-1 human hepatoma cells were incubated in either 1% O_2 or 21% O_2. Cell lysates were collected and underwent western blot analysis for sFlk-1 protein. Hypoxic SK-HEP-1 cells transduced with HSV10xHRE/sFlk-1 exhibited greater sFlk-1 expression compared with similarly transduced normoxic cells. SK-HEP-1 cells did not express sFlk-1 after transduction with HSVlacZ.

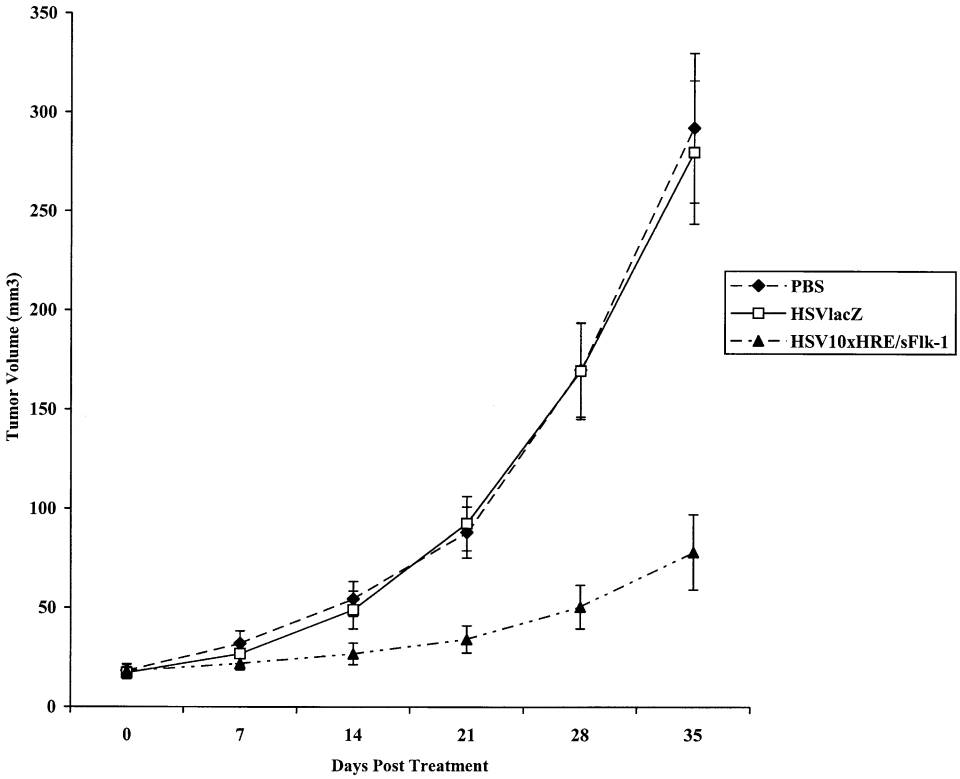

Fig. 5. Treatment of SK-HEP-1 flank tumors with herpes simplex virus multimerized hypoxia responsive enhancer/soluble fetal liver kinase-1 (HSV10xHRE/sFlk-1) in athymic mice (n = 24). SK-HEP-1 human hepatoma flank tumors were treated with phosphate-buffered saline (PBS) (n = 8) (*long dashed line with diamond*), HSVlacZ (lactose fermentation of b-galactosidase) (n = 8) (*solid line with square*), and HSV10xHRE/sFlk-1 (n = 8) (*long dash, short dash line with triangle*). There was no significant difference in tumor volumes between the PBS-treated and control HSVlacZ-transduced groups. Five weeks after treatment, HSV10xHRE/sFlk-1 yielded a 73% reduction in tumor volume vs. PBS-treated tumors ($P < 0.000001$) and a 72% reduction in volume vs. tumors treated with HSVlacZ ($P < 0.000001$).

liver kinase-1 [Flk-1]).[14,15] However, only Flk-1 is expressed exclusively on endothelial cells.[18] After VEGF binds to membrane-bound Flk-1, the receptor dimerizes and transduces an intracellular receptor tyrosine kinase signal that leads to endothelial cell proliferation and capillary formation.[37] Soluble forms of both Flk-1 and Flt-1 have inhibited endothelial cell activation by VEGF. Soluble Flk-1 delivered by an adenoviral vector has demonstrated decreased angiogenesis during wound healing in mice.[38] Regarding the treatment of cancer, soluble forms of Flt-1 have inhibited tumor xenograft growth in athymic mice. Tumors derived from a lung cancer cell line had reduced in vivo capillary formation and tumor volume after administration of adenovirus expressing sFlt-1.[39] Soluble forms of Flk-1 delivered using adenoviral vectors have effectively treated human pancreatic tumors in mice. There was approximately a 75% decrease in tumor volume compared with the control group 6 weeks after intravenous injection of adenovirus expressing sFlk-1.[40] In sarcoma and melanoma cell lines, inhibition of vessel formation and a reduction in tumor size have been observed after sFlk-1 transfection.[41] Soluble VEGF receptors have therefore demonstrated considerable usage with regard to blocking angiogenesis and solid tumor growth.

In this study, we targeted the production of sFlk-1 to tumor hypoxia, the primary stimulus for VEGF expression. We demonstrated that SK-HEP-1 human hepatoma cells upregulated HIF-1α and VEGF levels under hypoxic conditions. Our 10xHRE hypoxia-responsive enhancer significantly increased paired gene expression in hypoxic cells. The 10xHRE enhancer was paired with the sFlk-1 gene to form the 10xHRE/sFlk-1 plasmid. A HSV amplicon expressing 10xHRE/sFlk-1 (HSV10xHRE/sFlk-1) was genetically engineered and demonstrated increased sFlk-1 production at low oxygen concentrations. This

HSV amplicon was determined to preferentially inhibit HUVEC capillary formation in hypoxia and SK-HEP-1 flank tumor xenograft growth in athymic mice.

HSV amplicons are efficient gene delivery vehicles. These amplicons exhibit the identical viral envelope, tegument, and capsid as replication-competent herpes viruses.[42] They can therefore transduce a wide range of mitotically active and postmitotic cell types.[23] Recently, the ability to produce helper virus-free HSV amplicon stocks has nearly eliminated their contamination with cytotoxic recombinant viruses.[25,42,43] We used these helper virus-free HSV amplicon stocks in our current experiments as they have elicited minimal cytopathic effects in vitro.[20] Other advances in amplicon processing and purification have allowed for higher titers of amplicon stocks approaching 10^8 TU/ml.[23,42] Furthermore, the possibility of reversion to a wild-type replication-competent virus is extremely low, as amplicons carry less than 1% of the HSV genome.[42]

A potential limitation of amplicon-mediated gene transfer is the transient duration of gene expression. Decreasing levels of gene expression over time are related to the loss of vector DNA during cell division as well as DNA degradation within the cell.[23] Nevertheless, the ability of the HSV10xHRE/sFlk-1 amplicon to inhibit tumor growth over 5 weeks is not unexpected, as HSV amplicons have maintained stable gene transduction for more than 2 months.[44] The mitotic segregation of the episomal HSV amplicon, however, should lead to a progressively reduced fraction of transduced tumor cells. The extent of SK-HEP-1 tumor inhibition observed in vivo suggests that the secreted transgene product, sFlk-1, is stable and regionally localized to the tumor environment. Recently, integrated forms of the HSV amplicon platform have been developed that exhibit expression profiles of longer duration.[45] These more recent forms of the HSV amplicon could provide more stable maintenance of hypoxia-regulated sFlk-1 expression and thus enhance therapeutic benefit.

Reports reveal that the median oxygen concentration for head and neck, cervical, pancreatic, and breast cancers is 1%–4%, with most tumors exhibiting more hypoxic regions.[46–49] We therefore used 1% O_2 to mimic hepatoma hypoxia in our in vitro experiments. In addition, the atmospheric oxygen concentration of 21% was used as the normoxic standard, although the actual oxygen concentration of normal tissues may be lower than this.[49,50] Nonetheless, reports reveal that there is minimal difference in multimerized HRE enhancer function between 5% O_2 and 21% O_2.[1] Despite the lack of inducibility above 5% O_2, there remains a low base line level of gene expression under normoxic conditions.[1] This activity may have contributed to the less pronounced, but significant reduction in capillary formation demonstrated in normoxic HCC cells transduced with HSV10xHRE/sFlk-1. However, the side effects of inhibiting high VEGF levels in normal normoxic tissues are limited in adults, as elevated levels of VEGF are restricted to wound healing and the menstrual cycle.[51,52] Further studies are necessary to assess the systemic effects of anti-angiogenic gene therapy.

In summary, we have demonstrated that an HSV amplicon expressing sFlk-1 under hypoxic control can be used as definitive therapy for established HCC by reducing angiogenesis and tumor growth. Hypoxia is a common solid tumor condition and HSV amplicons are able to transduce a wide array of cell types. Therefore, this hypoxia-driven angiogenic inhibitor may indicate broad therapeutic applicability.

We thank Guo-jie Ye, Ph.D., for his advice with regard to cloning procedures and his knowledge of HSV vectors. We also thank Sam Yoon, M.D., for his invaluable assistance with the HUVEC assay and Wade Narrow for preparation of helper virus-free HSV amplicon stocks.

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Discussion

Dr. S. Vickers (Birmingham, AL): Dr. Pin, thank you for the chance to review your manuscript before your presentation. I have three questions. You have performed an elegantly designed study, but gene therapy is challenged by three major areas: vector targeting, vector selectivity in targeting, and vector infectivity and toxicity. In your study, you used a considerate way to address the issue of focusing on the HIF-inducible hypoxic factor and targeting toward that, but the two other areas remain challenging. My questions, which are fairly simple, are as follows.

First, because you would be relying on a solid tumor bystander effect, what data exists that Flk-1 or Flt-1 will, in fact, pass through desmosomes and would allow you to effect VEGF in a solid tumor?

Also, you have used a nonreplicative herpes virus. Many studies with herpes virus have actually used a conditional replicative herpes virus. You have given one injection and, as can be observed with your in vivo model, your tumors regrow at 35 days. Have you used more than one model and do you get an immunological response to that herpes virus when you do that? Does the herpes virus, like the adenovirus, have the same hepatotrophic effect so that you actually increase your toxicity to the liver?

Those are the two main questions. Hopefully, you will continue your work. It was very nicely done.

Dr. R. H. Pin: Thank you, Dr. Vickers, for your questions. In terms of exhibiting a bystander effect, the soluble Flk-1 receptor that we used is secreted by the cell and binds to surrounding extracellular VEGF. This interaction prevents VEGF from binding to membrane-associated VEGF receptors on nearby endothelial cells, thus interrupting the VEGF signaling pathway. In this sense, a bystander effect occurs and anti-angiogenic therapy can be directed to hypoxic tumor areas even if only a fraction of the cells are infected with our amplicon.

With regard to your second question, we have only used a single direct injection of amplicon to determine the duration of its efficacy. Using multiple injections may potentiate the antiangiogenic effect and the level of tumor growth inhibition. If further treatments had been given, we would not anticipate a significant immunologic reaction to the herpes viral amplicon. In these initial experiments, the amplicon was administered directly into the tumor, thus limiting the systemic exposure. More importantly, though, is that the amplicon that we constructed does not replicate and cannot lyse the host cell. It simply serves as a vector for soluble Flk-1 expression and elicits minimal cytopathic and immunologic responses. Unlike the adenovirus, the herpes virus does not exhibit a hepatotrophic effect. Genetically engineered herpes amplicons have demonstrated the ability to infect a wide array of tumor types. This lack of hepatotrophism makes our amplicon an attractive vector for targeting liver tumors while sparing normal surrounding liver parenchyma. Soluble Flk-1 production will be directed specifically to hypoxic tumor cells that can drive our hypoxia-responsive enhancer and transgene expression.

Dr. M. Callery (Boston, MA): That was a very polished presentation. Early on, I believe you indicated that an intact proteasome degradation process is required to eliminate HRE gene expression in normoxia. As you also know, proteasome inhibition is being considered in some malignancies as an effective antitumor approach. Can you drive HRE gene expression in normoxia by eliminating or inhibiting proteasome degradation?

Dr. R. H. Pin: HIF-1α is the oxygen-regulated transcription factor that interacts with the HRE to enhance gene expression. In normoxia, the HIF-1α polypeptide undergoes prolyl hydroxylation in its oxygen-dependent degradation domain. This signal targets HIF-1α for destruction through a ubiquitin-proteasome pathway mediated by the von Hippel–Lindau protein. Recent research has demonstrated that inhibition of prolyl hydroxylases or proteasome activity can prevent HIF-1α degradation, leading to higher normoxic levels. It is therefore possible to drive HRE-associated gene expression in normoxia through proteasome inhibition.

Nonobese Diabetic Mice Have Diminished Gallbladder Motility and Shortened Crystal Observation Time

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Diabetes and obesity are strongly associated and are risk factors for cholesterol gallstone disease. Leptin-deficient and leptin-resistant diabetic obese mice have enlarged, hypomotile gallbladders. In addition, bile from gallbladders of leptin-deficient mice has enhanced cholesterol crystal formation, whereas bile from gallbladders of leptin-resistant mice has delayed crystal observation time. To determine the effect of diabetes alone, we hypothesized that leptin-normal, nonobese diabetic (NOD) mice would have reduced biliary motility and rapid crystal formation. Twenty control and 9 prediabetic and 11 diabetic NOD, 12- to 26-week-old mice underwent glucose measurement and cholecystectomy for muscle bath stimulation with neurotransmitters. An additional group of 200 control and 78 NOD 12-week-old mice underwent microscopic bile examination for cholesterol crystal formation. Compared with control mice, prediabetic NOD mice had similar glucose levels and gallbladder volumes. Diabetic NOD mice had higher sugar levels and larger gallbladder volumes ($P < 0.001$) than control mice. Prediabetic NOD gallbladders had less contractility ($P < 0.01$) than control gallbladders, and contractility worsened ($P < 0.01$) in diabetic NOD mice. NOD mice formed cholesterol crystals earlier than did control mice ($P < 0.05$). Nonobese diabetic NOD mice have (1) decreased gallbladder contraction to neurotransmitters, which worsens with development of diabetes, and (2) rapid crystal formation. We conclude that diabetes alone alters gallbladder motility and cholesterol crystal formation. (J GASTROINTEST SURG 2004;8:824–830)

KEY WORDS: Diabetes mellitus, cholesterol, gallstones, gallbladder, motility

Diabetes is a major health care problem in the United States, with 18.2 million adults diagnosed with diabetes mellitus.[1] The prevalence of diabetes has increased 61% since 1990,[2] and diabetes has become the sixth leading cause of death of U.S. citizens.[1] Diabetes is a known risk factor for the development of cholesterol gallstone disease, and 30% of adults with diabetes develop cholelithiasis.[3] The pathogenesis of cholesterol gallstones is known to require three interrelated elements: cholesterol supersaturation of bile, decreased gallbladder motility, and cholesterol pronucleating agents. Diabetes is strongly associated with hypertriglyceridemia and elevated low-density lipoprotein (LDL) levels,[4] which may promote cholesterol supersaturated bile. In addition, gallbladders of diabetic patients have been shown to be enlarged with decreased emptying in response to meals.[3,5] However, the influence of diabetes on human cholesterol crystal formation has not been studied.

Insulin-resistant diabetes correlates strongly with obesity, and prior studies from our laboratory with obese leptin-deficient and leptin-resistant (Lep^{ob} and Lep^{db}, respectively) mice have demonstrated that these mice have severe insulin-resistant diabetes, hypertriglyceridemia, and hypercholesterolemia.[6–8] These mice also have dramatically reduced gallbladder contractility in response to neurotransmitter stimulation in an in vitro muscle bath,[6,7] which correlates with both hyperglycemia and hypertriglyceridemia.[8] In addition, bile from Lep^{ob} mice has faster cholesterol

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crystallization and growth compared with bile from control mice.[9–11] However, Lep^{db} mice have lower biliary cholesterol saturation and prolonged crystal observation time (COT).[12] Because the Lep^{ob} and Lep^{db} mice are obese and have abnormal leptin as well as diabetes, the etiology of these abnormalities remains unclear. Therefore, in this study the non-obese, insulin-deficient diabetic (NOD) mouse was studied to determine if diabetes alone plays a role in gallbladder dysmotility and/or cholesterol crystal formation.

MATERIAL AND METHODS

Animals and Diets

Experiment A. To study gallbladder contraction, 20 lean control C57BL/6J and 20 NOD 7-week-old female mice were obtained from the Jackson Laboratory (Bar Harbor, ME). The mice were housed in cages of five mice each in a light- (6:00 AM–6:00 PM) and temperature- (22° C) controlled environment for 6–16 weeks. All mice received a standard, low-cholesterol Chow diet (Ralston Purina, St. Louis, MO). NOD mice develop a beta cell insulinitis that leads to rapid and severe insulin-deficient diabetes at varying time intervals.[13] Twenty-five percent of female NOD mice develop diabetes by 12 weeks of age, and 80% develop diabetes by 24–30 weeks of age.

At 13 weeks of age, 10 NOD animals and 10 age-matched C57 control animals were studied. The remaining animals were housed until 18 weeks of age, when the NOD animals were checked weekly for the development of diabetes. NOD mice were lightly anesthetized with isoflurane and underwent tail stick for blood glucose assessment with a Freestyle glucometer (TheraSense, Inc., Alameda, CA). Once the NOD animals were determined to have blood sugars in the mid to high 200s, they were fasted overnight for study. For each diabetic NOD mouse, a C57 mouse was also fasted for study as an age-matched control. All mice were anesthetized with xylazine (15 mg/kg) and ketamine (50 mg/kg) and weighed, and they underwent cholecystectomy. Their gallbladders were placed in ice-cold, preoxygenated modified Krebs' solution (containing [in mmol/L]: NaCl, 116.6; $NaCO_3$, 21.9; KH_2PO_4, 1.2; glucose, 5.4; $MgCl_2$, 1.2; KCl, 3.4; and $CaCl_2$, 2.5). Whole blood was obtained by aspiration from the right heart, and livers were removed and weighed.

Experiment B. To determine cholesterol crystal formation in bile, 200 C57BL/6J and 78 NOD 8-week-old female mice were obtained from Jackson Laboratory. Animals were housed for 4 weeks and fed a standard Chow diet (Ralston Purina). At 12 weeks of age, all animals were fasted overnight and anesthetized with xylazine (15 mg/kg) and ketamine (50 mg/kg). Animals were weighed and underwent cholecystectomy. Whole blood was obtained from the right heart before liver excision and weighing.

Bile and Serum Glucose Analysis

Experiment A. Each gallbladder was removed intact, and bile was carefully aspirated from the fundus with a 30-gauge needle. Bile was then placed into a microtube, spun at 15,000 rpm for 5 minutes, and measured with a micropipette. Whole blood was also spun at 15,000 rpm for 5 minutes to separate serum. Serum was then warmed to 39° C, and glucose was measured with Freestyle glucose strips and glucometer.

Experiment B. Gallbladders were harvested intact and placed into microtubes. A small slit was cut into the body of each gallbladder for bile release. Microtubes were then spun at 15,000 rpm for 5 minutes, and bile was measured with a micropipette to determine volume. Bile was grouped into 160-µl aliquots (12 C57 pools and 6 NOD pools), microfiltered at 15,000 rpm for 5 minutes, kept sterile, and placed into a heated water bath at 39° C.

In Vitro Muscle Bath

Experiment A. Gallbladders were sutured at both ends with 7-0 polypropylene sutures and suspended longitudinally in 3-ml muscle bath chambers filled with modified Krebs' solution, warmed to 39° C, and oxygenated with 95% O_2/5% CO_2 carboxygen mix. Gallbladders were allowed to equilibrate at 0.025 *g* tension. Optimal length was then determined by stimulation with 10^{-5} mol/L acetycholine (ACh) (Sigma Chemical, St. Louis, MO) at 0.025-*g* increases until maximal gallbladder contraction was obtained. Gallbladders were maintained at their optimal lengths while neuropeptide Y (NPY) (Sigma Chemical) at 10^{-8}, 10^{-7}, and 10^{-6} mol/L doses and cholecystokinin (CCK) (Sigma Chemical) at 10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} mol/L doses were added. Responses were measured with Windaq/Ex (Dataq Instruments, Akron, OH) computer software. Gallbladders were rinsed with modified Krebs' solution every 15 minutes and after every neurotransmitter dosing. Gallbladder lengths and weights were measured and used to calculate the cross-sectional area. Gallbladder contractile responses were then expressed as Newtons per centimeter squared (N/cm^2).

Light Phase Microscopy

Experiment B. In the nucleation study, 3 µl of bile was placed on a microscope slide and examined under

polarized light microscopy every day for 18 days. Both birefringent liquid "maltese cross" and solid cholesterol monohydrate crystals were counted separately in 10 high-power fields.

Statistical Analysis

Experiment A. Data analyses were performed with SigmaStat Statistical Software (Jandel Corporation, San Rafael, CA). All data are expressed as mean ± SEM. To study the effects of diabetes, mice were divided into three subsets based on glucose levels. All C57 mice were nondiabetic, and the NOD mice were divided into those with fasting glucose levels less than 200 mg/dl (prediabetic) and those with levels greater than 200 mg/dl (diabetic). According to these criteria, seven of ten 13-week-old NOD animals were prediabetic and three were diabetic. In comparison, two of ten 23-week-old (range, 22–27 weeks) NOD animals were prediabetic and eight were diabetic. Mouse body weights, liver weights, serum glucose, gallbladder volume, and neurotransmitter responses were determined by two-way analysis of variance and Student's *t* test.

Experiment B. For the nucleation study, liquid and solid crystals were analyzed separately according to three parameters. COT was the first day on which crystals were observed. Crystal growth rate was the slope of the growth curve during the time of maximal crystal formation. Crystal mass was the total number of crystals observed within the 18 days of the experiment. The two groups were compared by analysis of variance and Student's *t* test with SigmaStat (SPSS, Inc., Chicago, IL) statistical software. A value of $P < 0.05$ was considered statistically significant in both studies.

RESULTS

Age, Body and Liver Weights, Serum Glucose, and Gallbladder Volume

Experiment A. Data for age, body and liver weights, serum glucose levels, and gallbladder volumes are shown in Table 1. Each control mouse was studied as an age-matched control with each NOD mouse. The average age of the control mice was 18.1 weeks. The mean age of the prediabetic NOD mice was 15.6 weeks, and the mean age of the diabetic NOD mice was 20.4 weeks. The body weights of the prediabetic NOD mice were larger than those of the control animals ($P < 0.001$) but not significantly different ($P = 0.11$) from the diabetic NOD animals. No statistical differences were present among any of the groups for liver weight. The serum glucose levels of the control and prediabetic NOD mice were the same (149 mg/dl) but markedly less than the glucose level of the diabetic NOD group (317 mg/dl, $P <$ 0.001 versus both groups). The mean gallbladder volume of the control mice was 16.5 µl, which were similar to the pre-diabetic NOD mice (20.7 µl) but smaller than the gallbladders of the diabetic NOD mice (36.6 µl, $P < 0.01$).

Muscle Bath

Gallbladder responses to ACh are shown in Figure 1. The contractile responses of both of the prediabetic and diabetic NOD groups were significantly less ($P <$ 0.01) than the responses of the control mice (0.09 and 0.04 versus 0.39 N/cm^2) but were not statistically different from each other ($P = 0.14$). Gallbladder responses to NPY at the 10^{-8}, 10^{-7}, and 10^{-6} mol/L concentrations are shown in Figure 2. Again, the control mice had the highest contractility, with the prediabetic NOD mice and the diabetic NOD mice showing a significantly reduced ($P < 0.01$) response. The gallbladder responses to CCK at the 10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} mol/L concentrations (Fig. 3) were also highest for the control mice, intermediate for the prediabetic NOD mice ($P < 0.01$ versus controls), and least in the diabetic NOD mice ($P < 0.01$ versus control mice and prediabetic NOD mice).

Nucleation Results

Experiment B. Liquid and solid crystal results for control and prediabetic NOD mice at 12 weeks of age appear in Table 2. COT, the first day of detection

Table 1. Age, body weight, liver weight, serum glucose, and gallbladder volume for control, pre-diabetic nonobese diabetic (NOD), and diabetic NOD mice

Strain	Age (wk)	Body weight (g)	Liver weight (g)	Glucose (mg/dl)	GB volume (µl)
Control	18.1 ± 1.2	17.5 ± 0.2	0.95 ± 0.03	149 ± 7	16.5 ± 2.2
Prediabetic NOD	15.6 ± 1.5	20.3 ± 0.5*	1.03 ± 0.03	149 ± 14	20.7 ± 3.8
Diabetic NOD	20.4 ± 1.4	17.9 ± 1.2	0.94 ± 0.06	316 ± 30†	36.6 ± 7.6*

Values are given as mean ± SEM.
*$P < 0.01$ versus control mice.
†$P < 0.001$ versus control and prediabetic NOD mice.

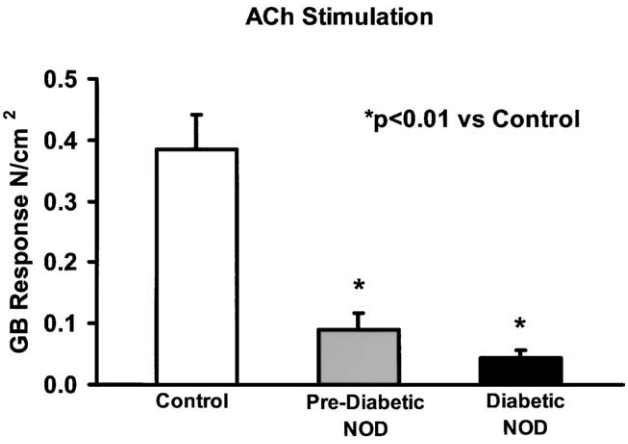

Fig. 1. Gallbladder (GB) stimulation with acetylcholine. Acetylcholine caused brisk contraction in the control animals but lesser contraction in both the prediabetic and diabetic NOD mice ($P < 0.01$).

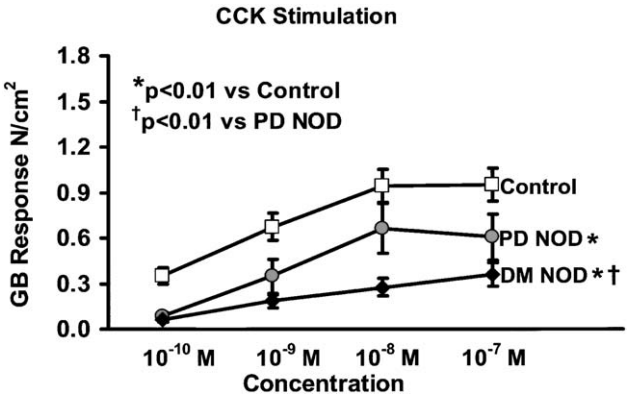

Fig. 3. Gallbladder (GB) stimulation with cholecystokinin (CCK). Again, control animals had the greatest contractile responses to CCK. Prediabetic (PD) NOD mice had reduced contraction ($P < 0.05$ versus control), and diabetic (DM) NOD mice had the lowest responses ($P < 0.05$ versus both groups).

of crystals, was significantly earlier for the prediabetic NOD mice for both liquid and solid crystals than for control mice ($P < 0.05$). No statistical differences were observed in the crystal growth rate or the overall crystal mass for either liquid or solid crystals between the prediabetic NOD and control animals.

DISCUSSION

In this study, NOD mice, which have a progressive development of insulin-deficient diabetes, demonstrate increasing serum glucose levels and enlarging gallbladders over a 10-week period. At an average age of 16 weeks, prediabetic NOD mice have decreased gallbladder reactivity to neurotransmitter stimulation in a muscle bath compared with nondiabetic C57 control mice. At an average age of 20 weeks, diabetic NOD mice have markedly enlarged gallbladders that respond very poorly in vitro to ACh, NPY, and CCK. Thus, NOD mice have an early abnormality in gallbladder contraction that worsens with the development of diabetes. In addition, prediabetic NOD mice have shortened cholesterol crystal observation without a change in crystal growth or mass.

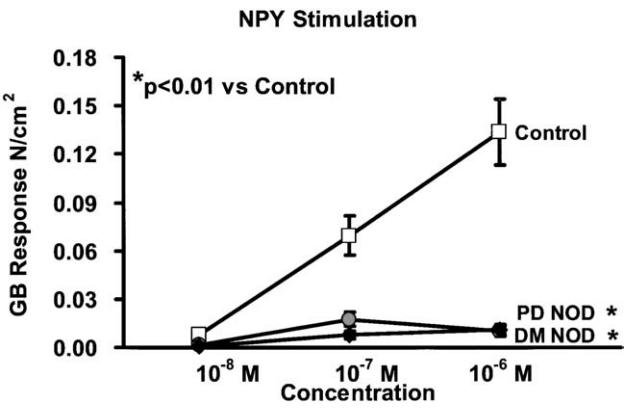

Fig. 2. Gallbladder (GB) stimulation with neuropeptide Y. Contraction to neuropeptide Y was greatest for the control animals and significantly reduced ($P < 0.01$) in the prediabetic (PD) and the diabetic (DM) NOD mice.

Our finding that the gallbladders of the diabetic NOD mice fed a standard Chow diet were enlarged (37 μl at an average age of 20 weeks) is consistent with the previous findings of Bouchard et al.[14] They found that after 8 weeks on a lithogenic diet, 18-week-old female NOD mice had gallbladder volumes greater than 50 μl. These murine observations are also consistent with many gallbladder imaging studies in diabetic patients that have reported that these patients have larger resting gallbladder volumes.[3,5] In addition, Nakeeb et al.[15] found that elevated fasting gallbladder volumes and poor response to a fatty meal correlated with elevated blood sugar levels in nondiabetic subjects. In the prediabetic NOD animals, the gallbladder volumes were slightly larger than controls (20.3 versus 16.5 μl), but this difference was not statistically significant.

The prediabetic NOD mice demonstrated decreased gallbladder motility compared with the control mice, and the diabetic NOD mice had an even more dramatic reduction in gallbladder contractility. This finding is consistent with the marked decreases in gallbladder contractility seen in the insulin-resistant diabetic models of the Lep^{ob} and Lep^{db} mice[6–8] and suggests that elevated serum glucose levels per se may have a detrimental effect on gallbladder emptying. In these leptin-related models, we have shown that

Table 2. Crystal observation time, crystal growth, and crystal mass of both liquid and solid crystals

	Liquid crystals			Solid crystals		
Strain	**COT (days)**	**Crystal growth***	**Crystal mass***	**COT (days)**	**Crystal growth***	**Crystal mass***
Control	12.3 ± 1.5	0 ± 0	3.2 ± 1.1	6.8 ± 0.8	1.7 ± 0.7	74 ± 15
Prediabetic nonobese diabetic	5.8 ± 3.1†	0 ± 0	2.8 ± 1.4	2.2 ± 0.7†	1.9 ± 1.3	124 ± 39

Values are given as mean ± SEM.
*Crystal growth is expressed as crystals per high-power field per day, and crystal mass is expressed as crystals per high power field.
†$P < 0.05$ versus control mice.

serum glucose and insulin are inversely correlated with gallbladder response to neurotransmitters.[8]

Gallbladder contraction is regulated by intrinsic cholinergic neurons as well as by direct binding of neurotransmitters on myocyte receptors.[16–18] The cholinergic ganglionic plexus lies between the serosa and muscle layers, intrinsic to the gallbladder.[18] These postganglionic nerves are present in the whole organ muscle bath, and prior studies have shown that CCK can readily access the subserosal gallbladder ganglia and activate the intrinsic cholinergic postganglionic nerves.[18,19] In addition, ACh is known to cause autoexitation by action on gallbladder nerves.[20] Because CCK and ACh potentiate a neuronal mechanism, diabetic neuropathy may be responsible, in part, for the observed decrease in gallbladder contraction of the NOD mice. Human studies of diabetic patients grouped into those with evidence of peripheral or autonomic neuropathy versus diabetic patients without neural complications found that gallbladder ejection fraction was reduced only in diabetic patients with neuropathy.[3] In addition, prior studies with NOD mice have demonstrated gastroparesis, a common sequelae of vagal neuropathy.[21]

Gallbladder myocytes are also known to have surface muscarinic[20] and CCK-A receptors[16] and, when directly stimulated by CCK, act via G proteins to utilize intracellular calcium to induce contraction.[16,17] We previously demonstrated that gallbladder myocytes from Lep^{ob} diabetic, obese mice are foreshortened, perhaps due to hyperglycemia.[22] In addition, the myocytes from Lep^{ob} and Lep^{db} mice had a reduced response to CCK compared with lean control myocytes. These observations are consistent with the diminished gallbladder response observed in the NOD mice in this study.

Diabetes may also affect alterations in the density or sensitivity of ACh or CCK receptors or prevent neurotransmitters from accessing their receptors. Sugars can react nonenzymatically with amino groups in proteins, lipids, and nucleic acids to form advanced glycation end-products (AGE).[23,24] These products are thought to have many effects, including covalent cross-linking of collagen and protein matrix.[23] The cross-linking of the matrix may lead to stiffening of the gallbladder wall itself, limiting its contraction, or may impair CCK egress through blood vessel basement membranes, preventing CCK interaction with neural or myocyte receptors. AGE are also known to initiate oxidative modification of LDL,[18] which is not recognized by the LDL receptor and may lead to the serum lipid disorders characteristic of diabetes. We have also shown in Lep^{ob} and Lep^{db} mice that gallbladder contraction correlates inversely with serum cholesterol and triglyceride levels.[8] Diabetic NOD mice also have serum lipid abnormalities[13] that may contribute to their gallbladder dysmotility.

Previous studies from our laboratory have shown that the Lep^{ob} mice have shortened COTs[9–11] and Lep^{db} mice have prolonged COTs[12] compared with controls. In this study, prediabetic NOD mice fed a nonlithogenic diet also demonstrate faster cholesterol crystal appearance. This observation suggests that pronucleators are present in the bile of NOD mice. Uchida et al.[25] studied NOD mice before and after the development of diabetes and found that plasma cholesterol increased from 57 mg/dl before diabetes to 124 mg/dl after diabetes. In addition, they found that biliary cholesterol and cholesterol saturation index also increased after the onset of diabetes.[25] Moreover, Bouchard et al.[14] demonstrated that NOD mice form gallstones rapidly on a high-cholesterol diet.

The present study with the NOD mice demonstrates that diabetes per se plays a role in gallbladder dysmotility and cholesterol crystal formation. Incomplete gallbladder emptying, combined with bile prone to rapid crystal formation, may lead to gallstone formation. Moreover, NOD mice have increased biliary cholesterol saturation.[14,25] Thus, diabetes itself, in the absence of obesity, may play a role in the pathogenesis of cholesterol gallstone formation.

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Discussion

Dr. Thomas Magnuson (Baltimore, MD): Shannon, that was an excellent talk and I want to congratulate you on that and thank you for the opportunity to review the paper. This study represents the latest in the evolution of papers from your lab on looking at obesity and diabetes and its impact on gallstone disease, which, as you showed in your first couple of slides, is particularly relevant to society today, as obesity and diabetes are increasing at exponential proportions.

A couple of quick questions. One question on the mechanism, if you could comment. What do you think is going on here? You demonstrated that in the NOD mice, even in the prediabetic NOD mice without the evidence of hyperglycemia, they also had a major impact on gallbladder contraction or motility, and you can't really ascribe that to hyperglycemia per se since their serum glucoses were similar to control. So do you think this is a myocyte effect or a neurotransmitter or a receptor effect?

Second question. Your lab has done a lot of work on bile composition in addition to motility, and have you done any work in these animal models looking at bile composition with respect to pronucelators or cholesterol saturation? There must be another factor involved in gallstone disease in this model.

And lastly, as the third question, have you looked at insulin? Can you reverse this effect or prevent the effect, the motility effect, by giving these animals insulin in their prediabetic state to counteract the effect of diabetes?

Thank you very much.

Dr. Graewin: Thank you for your comments. Your first question asked about the mechanism. First of all, we call these young NOD mice, prediabetic. Even at this early age, they have histologic evidence

of insulinitis. The glucose levels that we measured were after a 12- to 14-hour fast. These mice probably have elevated postprandial sugars that we have not measured.

The exact mechanism is not certain, but we think that the gallbladder dysmotility may be related to advanced glycation end products, or AGEPs. These end products are concentration-dependent. They are seen in normal, non-diabetic people and animal models, but they increase as glucose goes up. These products are known to occur early in the disease process, and they cause cross-linking in basement membranes. Because of this cross-linking in basement membrane, I am theorizing that cholecystokinin may not be able to traverse to the gallbladder. Alternately, this effect may be on the cholecystokinin receptors themselves or with the myocytes and connective tissue, resulting in a stiffened gallbladder. We also know from prior studies that these myocytes are foreshortened, which may result from the hyperglycemia.

Your second question was about bile composition. Others have shown that the NOD mice, if they are studied at a young age, have fairly normal biliary lipid profiles that worsen with their diabetes. Others have also shown that these AGEPs, the advanced glycation and products, affect the LDL receptors. Thus, the CSIs, their cholesterol saturation indices, do go up after they develop diabetes. We have also demonstrated shortened biliary cholesterol crystal observation time in NOD mice.

Your third question asked whether the gallbladder dysmotility could be restored in NOD mice with insulin. We have not done that study yet, but we have looked at treatment in the insulin-resistant diabetic Lep^{ob} and Lep^{db} mice. In Lep^{ob} mice we have used pioglitazone, which is a PPAR gamma agonist known to increase insulin sensitivity. Preliminary findings with pioglitazone have not restored gallbladder motility, which was reversed in another study with leptin. However, ciliary neurotrophic factor, or CNTF, did restore gallbladder motility in diabetic leptin-resistant Lep^{db} mice. They also had dramatic reductions in their fasting serum glucose levels.

Dr. Frank Moody (Houston, TX): It is a lovely presentation and it shows proof of concept, but the obese patient, many of them will have gallstones but don't have diabetes; I am sure you are well aware of that. The majority of them have insulin-resistance before they get fat. There are a lot of other factors, but a common denominator is the deposition of triglycerides in skeletal muscle, possibly in gut smooth muscle. Have you measured your triglyceride levels in your gallbladder walls?

Dr. Graewin: No, we have not yet measured triglyceride levels in the gallbladder walls. We do know that human diabetics and diabetic mice have hypertriglyceridemia that is a risk factor for gallstones in humans and correlates with decreased gallbladder motility in obese mice.

Nitrergic Mechanisms Mediating Inhibitory Control of Longitudinal Smooth Muscle Contraction in Mouse Small Intestine

Tatsuya Ueno, M.D., Judith A. Duenes, B.A., Abdalla E. Zarroug, M.D., Michael G. Sarr, M.D.

Studies using genetic manipulation to investigate mechanisms of control of physiologic function often necessitate mouse models. However, baseline functional analysis of murine small intestinal motility has not been well defined. Our aim was to define nitrergic mechanisms regulating mouse small intestinal longitudinal muscle. Endogenous nitric oxide (NO) is an important neuroregulatory substance mediating inhibition of contractile activity in murine small bowel. Full-thickness muscle strips of jejunum and ileum from C57BL/6 mice (n ⩾6 mice) cut in the direction of longitudinal muscle were studied. Numerous conditions of electrical field stimulation (EFS) and effects of exogenous NO and NO donors were studied in the absence or presence of inhibitors of nitric oxide synthase (NOS) and 1H-[1,2,4]-oxadiazaolo-[4,3-**a**]-quinoxalin-1-one (ODQ), a downstream inhibitor of guanylyl cyclase. EFS induced a frequency-dependent inhibition of contractile activity in both jejunum and ileum ($P < 0.05$). As the voltage of EFS was increased, inhibition turned to excitation in the jejunum; in contrast, the ileum demonstrated a voltage-dependent increasing inhibition ($P < 0.05$ each). EFS-induced inhibition was blocked by NOS inhibitors and ODQ. NO donors inhibited spontaneous contractile activity abolished by ODQ. NO appears to be an endogenous inhibitory neurotransmitter in murine longitudinal small bowel muscle. Nitrergic mechanisms mediate inhibitory control of murine longitudinal small intestinal muscle. Differences exist in neuroregulatory control between jejunum and ileum that may be related to their known difference in motor patterns. (J GASTROINTEST SURG 2004;8:831–841)

KEY WORDS: Smooth muscle, longitudinal smooth muscle, contractility, mouse, electrical field stimulation, nitric oxide, nitric oxide synthase, guanylyl cyclase

Increasingly, the mouse is being used to study many aspects of gastrointestinal physiology because of our ability to understand and alter its genotypic expression.[1,2] Study of the control of gastrointestinal motility classically has used rodent models in the rat and guinea pig and mammalian models in the dog, pig, and human.[3] Systemic and locoregional control of gastrointestinal motility in the mouse model is less well defined. As our laboratory pursues these questions using genomically altered mice, the characterization of nitrergic mechanisms in the normal mouse model is both important and essential before the study of genetically altered models. The aims of our experiments were to extend previous work[4] and to explore and define neural mechanisms controlling longitudinal smooth muscle contractile activity in the jejunum and ileum of the normal mouse. Our study focused on nitrergic inhibitory mechanisms. We designed our experiments to evaluate many different parameters of electrical field stimulation, a neurally mediated event, to uncover different subsets of nonadrenergic, noncholinergic (NANC) nerves. In our previous work,[4] we

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examined the more classic cholinergic and adrenergic mechanisms.

METHODS

Tissue Preparation and Recording of Contractile Activity

Procedures and animal care were performed according to the guidelines of the Animal Care and Use Committee of the Mayo Foundation in accordance with the guidelines of the National Institutes of Health and the U.S. Public Health Service *Policy on the Human Use and Care of Laboratory Animals*.

We studied male C57BL/6 mice 12–20 weeks of age that weighed 20–30 g (Harlan, Indianapolis, IN). The mice were allowed free access to water and food and maintained in a controlled environment with altering 12-hour periods of light and darkness. Tissue was obtained after anesthesia was achieved by initial inhalation of isoflurane (Abbott Laboratories, North Chicago, IL) followed by 50 mg/kg intraperitoneal sodium pentobarbital (Ampro Pharmacy, Arcadia, CA). The entire jejunoileum was removed, divided into three portions, each of which had a length of about 10 cm, and kept in chilled, modified Krebs-Ringer bicarbonate solution (concentration in mM: 118.3 NaCl, 4.7 KCl, 2.5 $CaCl^2$, 1.2 $MgSO^4$, 25.0 $NaHCO^3$, 1.2 KH^2PO^4, 0.26 calcium disodium edetate, and 11.1 glucose) throughout the process of preparation of muscle strips. The first 10 cm was considered jejunum and the last 10 cm as ileum. Each segment was opened along the mesenteric border, and under an operating microscope, eight transmural tissue strips, 2 × 6 mm (width by length) were cut in the direction of longitudinal muscle. We specifically studied transmural muscle strips, and we did not strip off the mucosa, submucosa, or circular muscle, because we wanted to study the intact neuromuscular unit with the gut wall. These muscle strips were tied at both ends with 5-0 silk and suspended vertically in a 10-ml tissue bath filled with modified Krebs-Ringer bicarbonate solution, maintained at 37.5° C, and bubbled with 95% oxygen and 5% carbon dioxide (Puritan-Bennet Corporation, Lenexa, KS). The silk tie from one end of the strip was connected to a fixed hook, while the other silk tie was attached to a stationary metal hook connected to a noncompliant force transducer (Kulite Semiconductor Products, Inc., Leonia, NJ) to measure isometric contractile force. Sixteen muscle strips from each mouse were prepared and mounted in these tissue chambers within 45 minutes of tissue harvest. Contractile activity was monitored in real time by two eight-channel recorders (Grass 7D Polygraph; Grass Instrument Co., MA) and converted to digital signals by a computerized data acquisition system. Digital signals, displayed and stored on a microcomputer (Reason 486; Reason Technology, Inc., Minneapolis, MN), were evaluated for online analysis or later analysis using specialized software (see Data Analysis). At the end of experiments, each muscle strip was blotted superficially twice, and wet weight was recorded for standardization.

Experimental Design

After a 60-minute equilibration period with washout of the modified Krebs-Ringer bicarbonate bath solution every 15 minutes, each strip was stretched incrementally at 5- to 10-minute intervals until its optimal length (L_O) was achieved, defined previously[4] as the length at which further stretching did not further increase the amplitude or frequency of spontaneous contractions. All subsequent experiments were performed at L_O, and bath solutions were changed at least once every 60 minutes. After L_O was infachieved, equilibration periods of 3 hours in jejunum and 1 hour in ileum were allowed to achieve stable and consistent stabilize spontaneous contractile activity based on our previous study.[4] At least four muscle strips were used for each experiment, except for the NO donor experiments, in which two muscle strips were used. Each muscle strip was used only for a single titled experiment, and all experiments were finished within 6 hours after achieving L_O.

Effect of Partial and Total Neural Blockade on Spontaneous Contractile Activity. After recording 1-hour baseline activity, NANC conditions (partial neural blockade) were established by adding phentolamine, propranolol, and atropine (phentolamine, propranolol, and atropine; all $5{\cdot}10^{-6}$ M in jejunum and $1{\cdot}10^{-6}$ M in ileum) to the bath solution based on previous work.[4] Baseline activity under these NANC conditions was measured for 1 hour. After that, tetrodotoxin (TTX; $5{\cdot}10^{-6}$ M), a sodium channel blocker that inhibits almost all neural transmission, was added to four of the eight chambers (total enteric neural blockade), and contractile activity was measured thereafter for 30 minutes.

Electrical Field Stimulation (EFS). After establishing NANC conditions or conditions of total neural blockade with TTX as described earlier, EFS experiments were performed using a model SD7 Grass stimulator (Grass Instruments, Quincy, MA). This stimulator delivers square waves across the muscle strip via platinum electrodes positioned 10 mm apart in the tissue bath. After each EFS, muscle strips were not subjected to the next EFS until spontaneous contractile activity had returned to pre-EFS activity; spontaneous activity usually reestablished within

5–10 minutes. The different parameters (see later) of EFS were delivered in random order within each experimental condition.

Frequency, Voltage, and Pulse Width-Effect Curve. According to multiple preliminary experiments that established optimal inhibitory parameters of EFS that gave a stable, reliable response, the following conditions were chosen to establish frequency-inhibition, voltage-inhibition, and pulse width-inhibition relationships. For the frequency experiment, frequency of stimulation was evaluated at 1, 2, 5, 10, 20, 50, and 100 Hz, keeping voltage, pulse width, and duration of stimulation constant at 10 V, 0.5 millisecond, and 10 seconds, respectively, in jejunum and 20 V, 0.1 millisecond, and 10 seconds, respectively in ileum. For the voltage experiment, each strip was exposed to voltages of 10, 20, 50, and 100 V, keeping the pulse width constant at 0.5 millisecond in jejunum and 0.1 millisecond in ileum and the frequency and duration of stimulation constant at 20 Hz and 10 seconds, respectively. In different muscle strips, we also determined the effect of these same voltages at frequencies of 1, 2, 5, 10, 50, and 100 Hz for both jejunum and ileum. For the pulse width experiment, the pulse width was varied from values of 0.1, 0.5, and 1 millisecond in jejunum and 0.05, 0.1, and 0.5 millisecond in ileum while keeping the voltage, frequency, and duration of stimulation constant at 20 V, 20 Hz, and 10 seconds, respectively. In separate muscle strips, we also determined the effect of these same pulse widths at frequencies of 1, 2, 5, 10, 50, and 100 Hz in both jejunum and ileum.

We chose the primary parameters based on multiple preliminary pilot studies to record primarily EFS-induced inhibition of contractile activity. On any experimental day, each muscle strip received a maximum of 14 stimulations in an attempt to avoid EFS-induced rundown of neurotransmitter release; a total of more than six mice per experiment was therefore required to have an n of six or more mice per condition.

Stimulation for Longer Periods. To determine the effect of different durations of stimulation, experiments using different muscle strips were designed as follows. Experimental conditions of EFS were chosen that gave good, reproducible inhibition of spontaneous contractile activity. Stimulation at frequencies of 5, 20, and 100 Hz were evaluated for 10-, 30-, and 60-second stimulation. In the jejunum, these differing durations of stimulation were evaluated at both 10 and 100 V, keeping the pulse width constant at 0.5 millisecond; for the ileum, the differing durations of stimulation were evaluated at both 20 and 100 V, keeping the pulse width at 0.1 millisecond. Each muscle strip was studied to a maximum of 18 simulations.

Effect of NOS and Guanylyl Cyclase Inhibition on Response to EFS. To investigate nitrergic mechanisms, the NO synthase inhibitors, N^G-amino-L-arginine (L-NNA; $1{\cdot}10^{-4}$ M), N^G-nitro-L-arginine methyl ester (L-NAME; $1{\cdot}10^{-4}$ M), and N^G-monomethyl-L-arginine methyl (L-NMMA; $1{\cdot}10^{-3}$ M), were administered into the bath after the first series of EFS experiments. Doses of these inhibitors were chosen based on previous work in the literature.[5,6] After a 30-minute equilibration period with the NOS inhibitors in the bath solution, the following EFS conditions were evaluated, varying only the frequency of stimulation at 5, 20, 50, and 100 Hz. The voltage, pulse width, and duration of stimulation were kept constant at 20 V, 0.5 millisecond, and 10 seconds, respectively, in the jejunum and at 20 V, 0.1 millisecond, and 10 seconds, respectively, in the ileum. The specific inhibitor[7] of soluble guanylyl cyclase 1H-[1,2,4]-oxadiazaolo-[4,3-**a**]-quinoxalin-1-one (ODQ: 1·10–^{5} M) was studied in separate muscle strips under these same conditions.

Effect of Exogenous NO and NO Donors. NO dissolved in distilled water ($1{\cdot}10^{-5}$ M and $3{\cdot}10^{-5}$ M) was prepared as described previously[8,9] and was given directly into the bath solution in eight tissue chambers; 10 minutes after completing this experiment, the NO donors sodium nitroprusside (SNP: $1{\cdot}10^{-7}$ M to $1{\cdot}10^{-4}$ M) and S-nitroso-N-acetylpenicillamine (SNAP: $1{\cdot}10^{-7}$ M to $1{\cdot}10^{-4}$ M) were studied in two muscle strips for each NO donor. SNP was given in a noncumulative manner with a 30-minute equilibration period, whereas SNAP was given in cumulative manner at 5-minute intervals. After completing this experimental series, ODQ was administered into the bath solution, and only the greatest doses of SNP ($1{\cdot}10^{-4}$ M) and SNAP ($1{\cdot}10^{-4}$ M) were administered again into the bath.

Drugs

All drugs (atropine, phentolamine, propranolol, TTX, SNP, SNAP, ODQ, L-NNA, L-NAME, L-NMMA) were purchased from Sigma Chemical Co. (St. Louis, MO) and diluted with distilled water, except for ODQ and SNAP, which were dissolved in dimethyl sulfoxide. NO gas was obtained from Praxair (Rochester, MN).

Measurement of Contractile Activity

Total contractile activity was quantified by measuring the integral of contractile force as the area under the curve (AUC) with data acquisition software system (Acknowledge 3.2; Biopac Systems, Inc.,

Goleta, CA). All values were corrected for wet weight of each muscle strip.

Spontaneous and Postneural Blockade. Measurements of baseline spontaneous contractile activity were obtained for two separate 10-minute durations beginning 15 minutes and 45 minutes before the addition of phentolamine, propranolol, or atropine (to establish partial neural blockade, i.e., NANC conditions) or TTX (to establish total neural blockade); these two values were meaned for each muscle strip to obtain baseline spontaneous activity. With phentolamine, propranolol, and atropine, contractile activity was measured for two separate 10-minute intervals beginning 15 minutes and 45 minutes after the addition of phentolamine, propranolol, and atropine, and these two values were then meaned. For TTX, contractile activity was measured only once for 10 minutes beginning 15 minutes after the addition of TTX.

EFS Experiments. Contractile activity during EFS was quantified and presented as the percentage of the spontaneous contractile activity; spontaneous contractile activity for all EFS was measured for the 2 minutes just before each separate EFS. Because multiple pilot and preliminary observations with a 10-second duration of EFS showed a biphasic response (initial inhibition followed by return of contraction during the 10 seconds of EFS), we analyzed separately the first 4 seconds and the last 6 seconds (Fig. 1). For the experiment involving a 60-second duration of stimulation, the first 4 seconds, the next 6 seconds, and each 10-second duration thereafter were quantified separately.

NO and NO Donors. Contractile activity was measured for 20 seconds after administration of exogenous NO, for 1 minute after administration of SNP, and for 2 minutes after administration of SNAP, and compared with spontaneous activity measured for the 2 minutes immediately before administration of these agents.

Data Analysis

Mean values of contractile activity were calculated separately for each mouse, and mean values across all mice were then calculated. Comparisons before and after differential conditions (neural blockade, EFS, and nitrergic inhibitors or agonists) were made using paired Student's *t* test and when appropriate using paired, one-way analysis of variance (ANOVA). Data are expressed as mean ± SEM.

RESULTS

Effect of Partial and Total Neural Blockade on Baseline Contractile Activity

Spontaneous contractile activity showed regular contractions in jejunum and ileum. Partial neural blockade established by the combination of phentolamine, propranolol, and atropine and total neural blockade by TTX had no effect on spontaneous contractile activity in jejunum and ileum as measured by pattern, frequency, amplitude, or total contractile activity (data not shown).

Electrical Field Stimulation

In general, EFS induced monophasic inhibitory or biphasic responses (inhibition followed by stimulation) during the electrical stimulation, but the response was highly dependent both on the condition

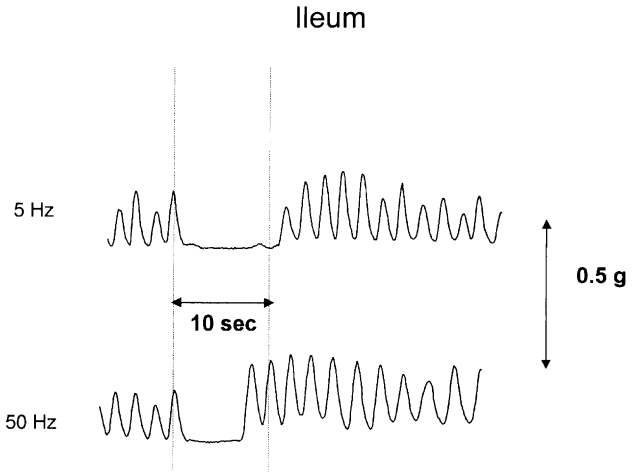

Fig. 1. Typical tracing of electrical field stimulation experiment in mouse ileal longitudinal muscle at 5 Hz (*top*) and 50 Hz (*bottom*) (50 V, 0.1-millisecond pulse duration for 10-second stimulation).

of EFS (i.e., frequency, voltage, pulse width, and duration of stimulation) (Fig. 1) and on anatomic site (jejunum versus ileum). All EFS-induced inhibition was blocked by TTX in both jejunum and ileum under all experimental conditions (data not shown), confirming the neurally mediated response to EFS.

Frequency Experiment (1–100 Hz). Under the conditions selected of constant voltage and pulse width, EFS induced an overall inhibition of contractile activity for the 10 seconds of stimulation in both jejunum and ileum ($P < 0.01$ each) that was greater at the higher frequencies of EFS (Fig. 2). In the jejunum, inhibition was first noted at frequencies of 2 Hz or higher (Fig. 2*A*); inhibition increased up to frequencies of 20 Hz, after which higher frequencies did not further increase the percentage of inhibition of baseline. The extent of inhibition was similar whether measured during the initial 4 seconds or during the last 6 seconds of EFS (Fig. 2*A*). In the ileum, although the inhibition was again prominent, the pattern varied somewhat from the jejunum. Inhibition became prominent at frequencies of 20 Hz or higher and became progressively greater as frequency increased to 100 Hz ($P < 0.01$) (Fig. 2*B*). As in jejunum, the effect of EFS was inhibitory throughout the 10 seconds of EFS.

Voltage Experiments (10–100 V). When the voltage was increased, keeping pulse width and duration of stimulation constant, the frequency-dependent EFS-induced inhibition of contractile activity was

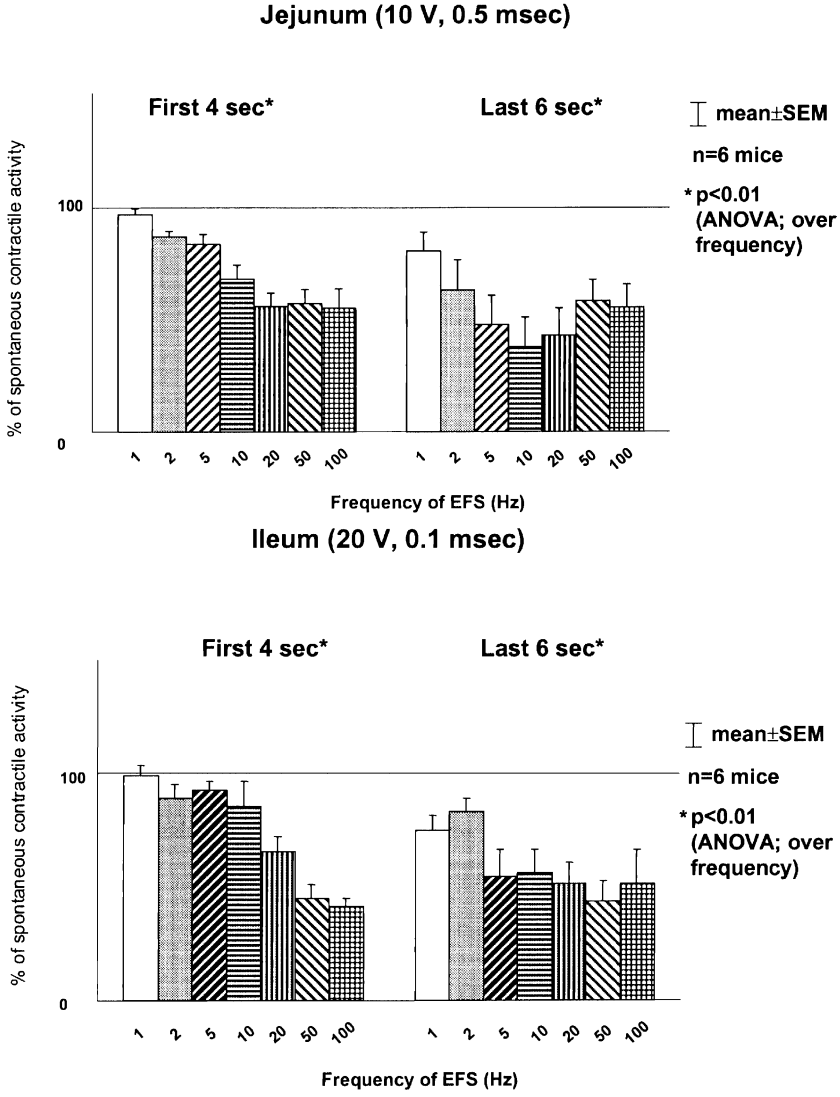

Fig. 2. Frequency-dependent inhibition of mouse jejunal (**Upper**) and ileal (**Lower**) muscle strips as analyzed separately for the first 4 seconds and the last 6 seconds of the 10-second electrical field stimulation.

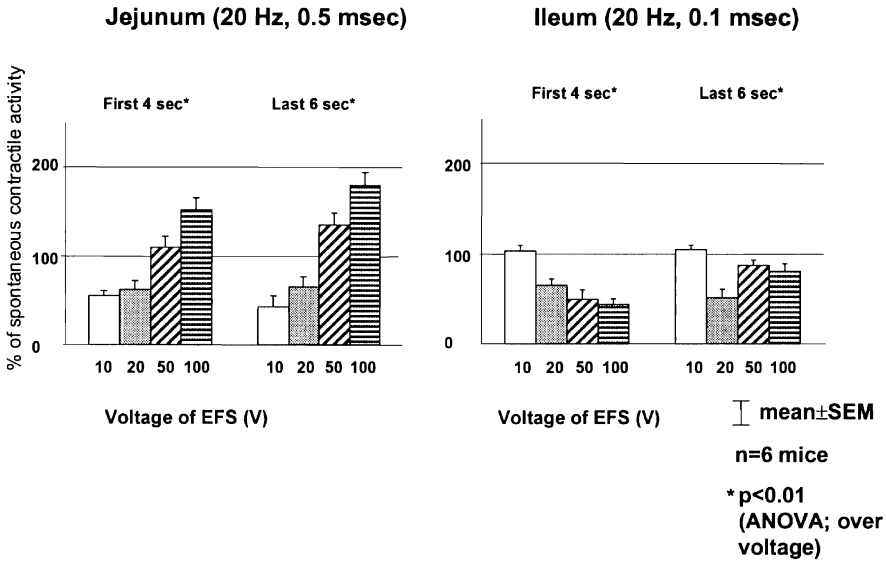

Fig. 3. Voltage-dependent response of mouse jejunal and ileal muscle strips to electrical field stimulation as analyzed separately in first 4 seconds and last 6 seconds of electrical field stimulation.

abolished in the jejunum at 50 and 100 V, and a net augmentation of contractile activity in both the first 4 seconds and the last 6 seconds of stimulation was observed ($P < 0.01$) (see Fig. 3 for data at 20 Hz). In contrast, in the ileum, frequency-dependent EFS-induced inhibition was enhanced progressively in the first 4 seconds of stimulation as voltage was increased to 100 V ($P < 0.01$); inhibition was maintained even during the last 6 seconds ($P < 0.01$). These patterns in the jejunum and in the ileum tended to persist when the various voltages were evaluated at the other frequencies of stimulation (1, 2, 5, 10, 50, and 100 Hz; data not shown).

Pulse Width Experiments (0.05–1 millisecond). When the pulse width of EFS was increased from 0.1 to 1 millisecond in jejunum and from 0.05 to 0.5 millisecond in the ileum, the net effect in the first 4 seconds was inhibition in both jejunum and ileum ($P < 0.01$) (Fig. 4). The percentage of inhibition did not change markedly at any specific pulse width. In

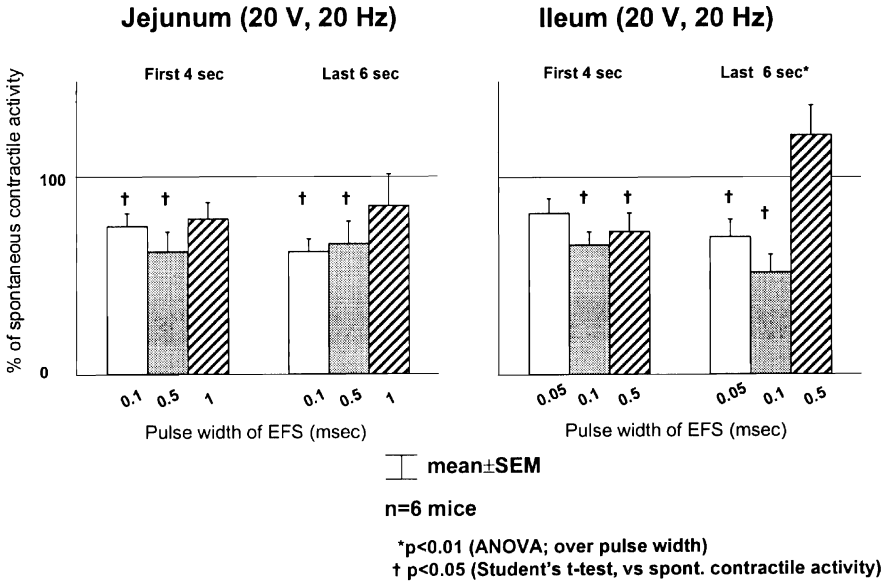

Fig. 4. Effect of the different pulse width on the inhibition induced by electrical field stimulation. Except for the last 6 seconds in ileum, pulse width–dependent response was not observed.

contrast, when the effect of increasing pulse width was analyzed over the last 6 seconds of EFS, the longest pulse width (1 millisecond) had little change in effect in jejunum but caused a net increase in contractile activity in ileum ($P < 0.01$).

Duration of Stimulation Experiments (10–60 seconds). When the duration of stimulation was increased, the initial inhibition of contractile activity seen in jejunum and ileum in both the first 4 seconds and next 6 seconds was not maintained thereafter at frequencies of EFS of 5, 20, and 100 Hz (Fig. 5). In the jejunum, the net inhibition of contractile activity noted in the first 4 seconds ($P < 0.01$) was no longer present after the first 10 seconds of EFS, and contractile activity thereafter was not different from the pre-EFS baseline activity. In the ileum, a somewhat different pattern was seen. Again, net inhibition was evident in the first 10 seconds of EFS ($P < 0.01$), but contractile activity increased thereafter to levels greater than pre-EFS baseline activity beginning at about 20 seconds after the start of EFS for the higher frequencies of stimulation (20 Hz and 100 Hz); EFS at 5 Hz had a pattern similar to the jejunum.

Effect of Nitrergic Inhibition on EFS. There were no apparent differences in spontaneous contractile activity when quantified as total contractile activity, frequency of contraction, or amplitude of contractions or when analyzed by overall subjective pattern of contractions when phentolamine, propranolol, and atropine (NANC conditions) or phentolamine, propranolol, and atropine and either L-NNA, LNAME, L-NMMA, or ODQ was added to the bath (data not shown). When EFS was then initiated after pretreatment of the muscle strips with these nitrergic blockers, a markedly different response to EFS was seen (Fig. 6*A*). Overall, the nitrergic blockers tended to reverse the EFS-induced inhibition. This blockade of EFS-induced inhibition was most evident during the last 6 seconds of the 10 seconds of EFS duration in both jejunum (Fig. 6*B*) and ileum (Fig. 6*C*). The blockade of nitrergic-dependent EFS-induced inhibition was most pronounced with ODQ, L-NNA, and L-NAME.

Effect of Exogenous NO and NO Donors. When exogenous NO was administered in doses ($3 \cdot 10^{-5}$ and 10^{-5} M) that inhibit rat and dog jejunal longitudinal muscle contractile activity,[8,9] no inhibition of spontaneous contractile activity was seen in jejunum (Fig. 7*A*) or in ileum (data not shown). Separate experiments with rat tissue known to be responsive to NO using these NO solutions confirmed the biologic activity of the NO solutions. In contrast, exogenous administration of the NO donors SNP and SNAP did partially inhibit contractile activity in a dose-dependent manner in jejunum and ileum (Fig. 7*B*); this inhibition was abolished completely by ODQ ($P < 0.05$) in jejunum and partially in ileum.

DISCUSSION

Despite the tremendous progress made in using genetically altered mouse models to study various mechanisms of gut cellular biology, basic knowledge

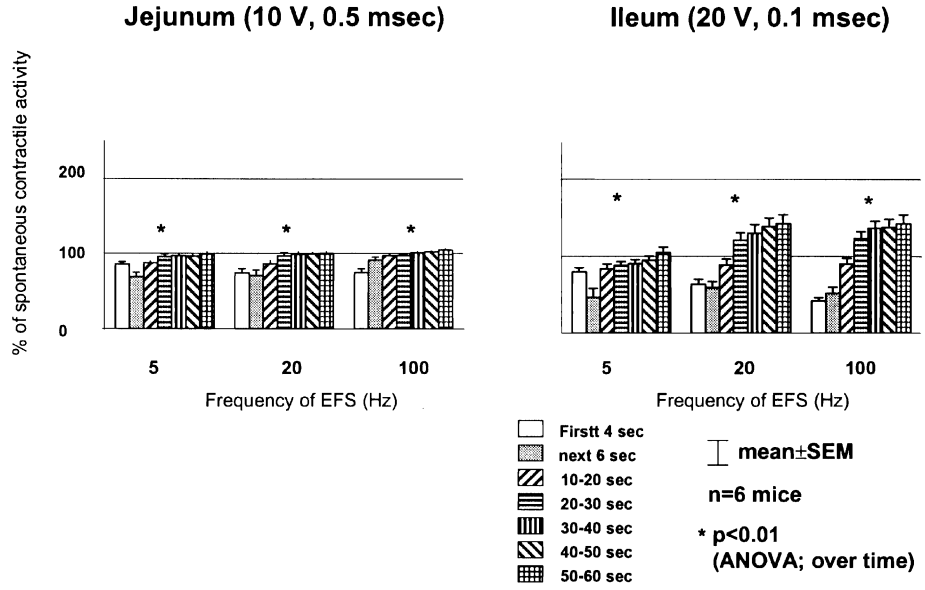

Fig. 5. Response to electrical field stimulation applied for 60 seconds. The inhibition seen at the first 10 seconds of jejunal and ileal muscle strips did not last longer than 20 seconds.

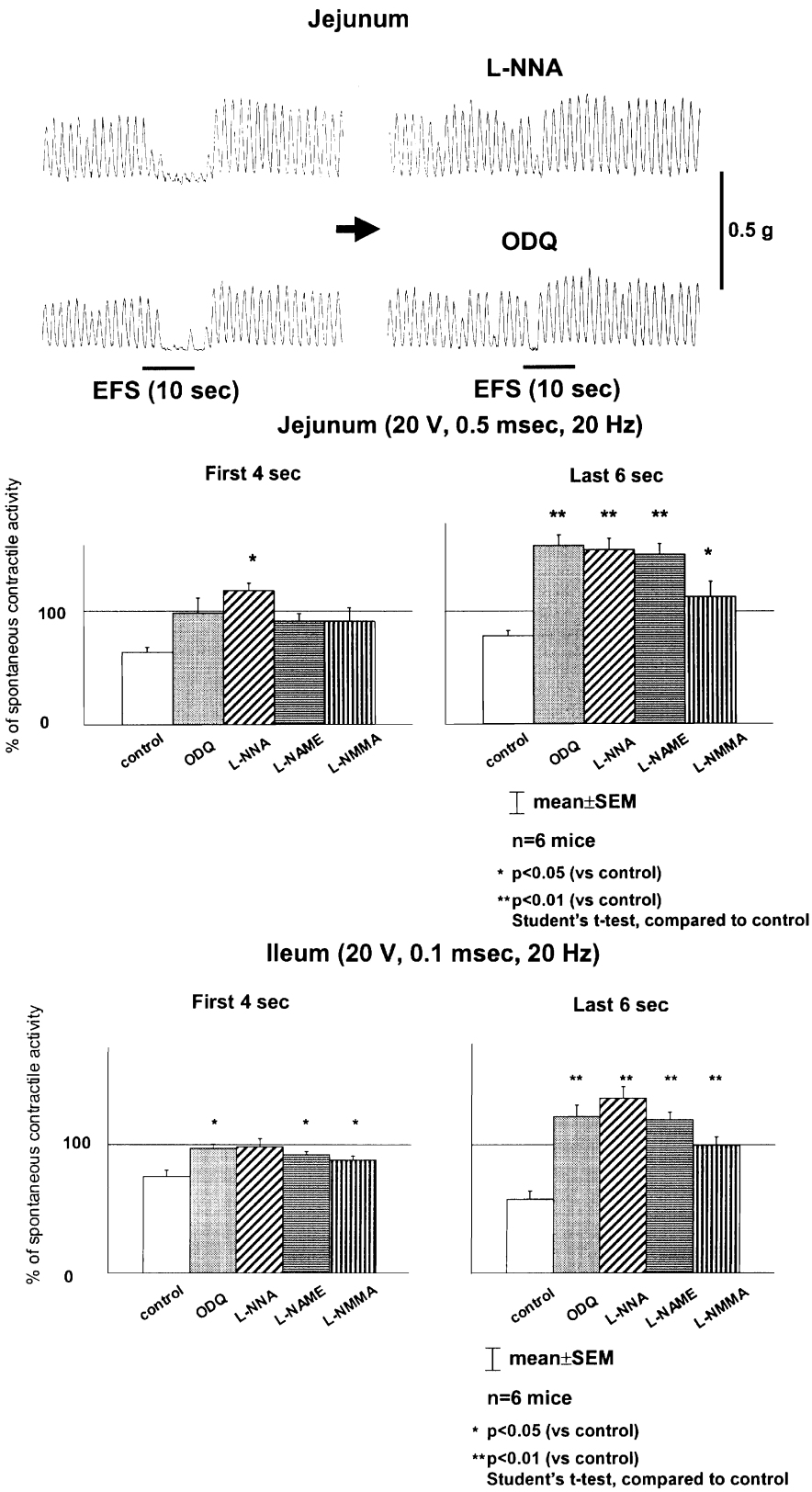

Fig. 6. (**Upper panels**) Typical recording of jejunal muscle strips measuring the effect of the nitrergic blockers N^G-amino-L-arginine and 1H-[1,2,4]-oxadiazaolo-[4,3-**a**]-quinoxalin-1-one on the response to electrical field stimulation (EFS). (**Lower four panels**) Measuring the effect of each NO antagonist on the inhibitory response by EFS. EFS (frequency, voltage, pulse width, and duration of the stimulation response): jejunum; 20 Hz, 20 V, 0.5 millisecond, 10 seconds; and ileum; 20 Hz, 20 V, 0.1 millisecond, 10 seconds.

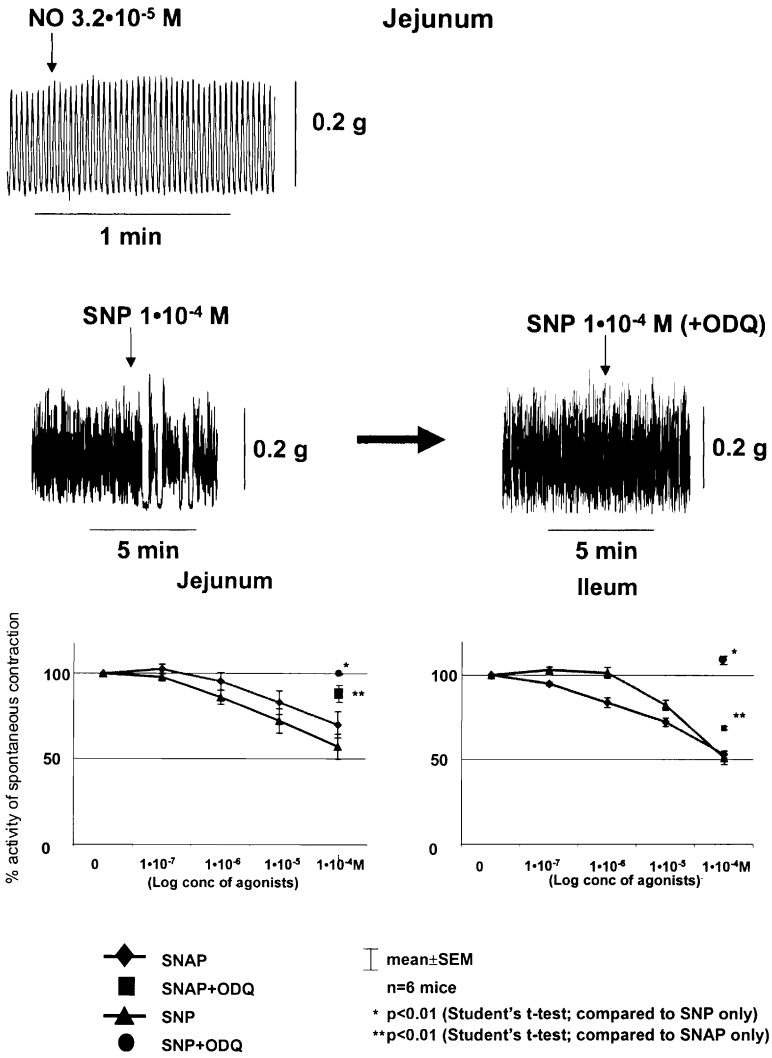

Fig. 7. (**Upper panels**) Typical tracing of the inhibitory response to nitric oxide (NO) donors and *S*-nitroso-*N*-acetylpenicillamine (SNAP) and the effect of specific cGMP blocker 1H-[1,2,4]-oxadiazaolo-[4,3-**a**]-quinoxalin-1-one (ODQ) on the inhibition. (**Lower two panels**) Dose-response curve of sodium nitroprusside and SNAP and the effect of ODQ on the inhibitory response to NO donors.

of contractile properties of mouse smooth muscle of the gut is limited. Our study was designed to explore nitrergic mechanisms mediating neural inhibition of small intestinal contractile activity in longitudinal smooth muscle of mouse small intestine. The key findings of our study were threefold: first, tonic baseline input from the enteric nervous system to mouse small intestinal longitudinal muscle is minimal; second, smooth muscle responses to different conditions of EFS (changes in frequency, voltage, pulse width, and duration of stimulation) exhibit anatomic variability (jejunum versus ileum); and third, neurally mediated NANC inhibitory input to mouse longitudinal muscle is mediated in large part by nitrergic mechanisms.

Our first interest was whether the enteric nervous system maintained a tonic excitatory or inhibitory input to mouse longitudinal smooth muscle. By selectively inhibiting either cholinergic and adrenergic nerves through receptor blockade, nitrergic nerves by inhibiting NO production or guanylyl cyclase, or all neural transmission with sodium channel blockade, we could uncover no evidence of tonic enteric neural regulation of longitudinal muscle. While this finding is consistent with rat ileal (and jejunal) longitudinal and circular muscle,[9,10–12] there are species and regional differences. For instance, we have shown previously that canine ileal (and jejunal) longitudinal muscle has a dominant tonic excitatory input from the enteric nervous system,[13] whereas the circular

muscle from these same regions is suppressed by a tonic neural inhibition.[14] These differences reflect the complexity of control and regulation of gut smooth muscle contractile activity.

NO has been shown to be an important neurotransmitter in many regions of the gut in multiple species, including human,[15] dog,[15] rat,[9] and many others. In the mouse specifically, others have implicated NO as playing a primary role as an inhibitory neurotransmitter.[7,16,17] Our current work not only confirmed this preliminary work but extends these observations and those of Satoh and colleagues[18] to explore anatomic differences in NANC neurotransmitters.

We used EFS and the differing responses to varying the electrical parameters of EFS in an attempt to implicate both the role of NO in inhibitory modulation of contractile activity and the presence of other important NANC neurotransmitters. The role of neurally mediated release of NO was confirmed by blocking the inhibitory effects of EFS by TTX (pan neural inhibition of neurotransmission) and by several inhibitors of NO synthase (the source of NO) and of guanylyl cyclase (downstream mediator of NO effects). In addition, the exogenous application of NO donors reproduced a net inhibition of contractile activity that was blocked by downstream inhibition of guanylyl cyclase activity. These experiments provide compelling evidence to implicate a role for endogenous neural release of NO from the mouse muscle strips mediating an inhibitory effect.

Our inability to measure an inhibitory effect of exogenous NO dissolved in distilled water remains unexplained. Our previous work in human,[15] rat,[9] and canine[15] small intestine using NO dissolved in water in a similar experimental set-up gave reproducible inhibition of contractile activity. Indeed, we tested our NO solution used in these mouse experiments in a rat preparation and showed inhibition. In contrast, the NO donors (SNP and SNAP) induced reproducible, dose-dependent inhibitory responses. These findings suggest that NO cannot adequately diffuse from the bath solution across the mouse serosa to affect the longitudinal smooth muscle.

Possibly of more interest were our observations of differing responses in jejunum and ileum. While both anatomic regions responded with increasing inhibition as the frequency of EFS was increased from 1 Hz to 20 Hz, the response was less prominent in the jejunum at higher frequencies (20–100 Hz). Whether this represents a greater release of NO or the release of another inhibitory neurotransmitter in the ileum is unknown. Satoh et al.[18] suggested that vasoactive intestinal peptide and pituitary adenyl cyclase activating peptide are additional, region-specific inhibitory neurotransmitters in mouse small intestinal longitudinal muscle; interestingly, their work suggested a role for these other neurotransmitters in jejunum and colon, respectively, and not in ileum. We, of course, cannot exclude the presence of another inhibitory neurotransmitter(s) such as the purine neurotransmitter adenosine triphosphate.[19]

As the voltage was increased, the inhibitory effect increased in the ileum, while in the jejunum, net inhibition changed to net excitation at voltages of 50 V or greater, again suggesting the neural release of a different profile of endogenous neurotransmitters with a net excitatory response in the jejunum. Both the differing effects of varying frequency and voltage serve to reinforce the concept of the complexity and the anatomic diversity of regulating mechanisms controlling contractile activity throughout the gut as well as between species. The motor patterns of the jejunum and ileum differ in vivo and may in part be related to these different mechanisms of inhibitory control by the enteric nervous system. The different response in jejunum versus ileum appears to allow a differential anatomic control that might prove important under various physiologic conditions, stimuli, or times of stress. Our future work will attempt to dissect the different neurotransmitters involved in these effects through the use of genetically engineered knockout mice and the use of specific receptor antagonists.

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Inhibition of the Vanilloid Receptor Subtype-1 Attenuates TNBS-Colitis

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Primary sensory neurons are important in regard to the initiation and propagation of intestinal inflammation. The vanilloid receptor subtype-1 (VR-1) is a cation channel located on the sensory nerves that, when stimulated, release proinflammatory peptides. Previous reports have indicated that inhibition of VR-1 with capsazepine (CPZ), a VR-1 antagonist, attenuates dextran sodium sulfate (DSS) colitis in rats. DSS-induced colitis resembles ulcerative colitis with regard to its pathologic features. In this study, we examined the effect of CPZ on trinitrobenzene sulfonic acid (TNBS)-induced colitis, an experimental model of intestinal inflammation that most closely resembles the histologic and microscopic features of Crohn's disease. Colitis was induced by administering a single enema of 100 mg/kg TNBS in 50% ethanol via catheter to lightly anesthetized rats. Subsets of rats were treated with either 1 μmol/kg/ml of CPZ or CPZ-vehicle via enema for 6 days. Seven days after TNBS administration, rats were sacrificed and inflammation was assessed using a validated macroscopic damage score (MDS) and by measuring myeloperoxidase (MPO) activity. In addition, histologic examination was performed. TNBS administration resulted in reproducible chronic erosive lesions extending into the muscularis propria and extensive recruitment of neutrophils in the distal colon. MDS and MPO scores were considerably elevated in the TNBS colons when compared with the TNBS vehicle animals. TNBS rats treated with CPZ enemas exhibited a substantial reduction in MDS and MPO scores and demonstrated dramatically improved pathologic findings. Topical CPZ resulted in considerable attenuation of TNBS-induced colitis. These results support the role of VR-1 and sensory neurons with regard to intestinal inflammation. (J GASTROINTEST SURG 2004;8:842–848)

KEY WORDS: Colitis, neurogenic inflammation, sensory neurons, TNBS-colitis, vanilloid receptor subtype-1

Neurogenic inflammation refers to the stimulation of sensory neurons that signal bimodally to the central nervous system where pain perception is received as well as peripherally where local inflammatory and immune responses occur. Bayliss proposed the idea of an "axon reflex" in 1901 after stimulation of the dorsal root ganglion resulted in peripheral vasodilatation. Early studies examined the effects of physical stimulants, such as heat and pressure, as well as chemical irritants such as acid, with regard to initiating neurogenic inflammation. More recent work has investigated specific neurotransmitters and the receptors responsible for completing the reflex arc. Specifically, small neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) present in sensory neurons are known to regulate pain and inflammation.[1,2] For example, SP released by sensory neurons in response to peripheral tissue damage or noxious stimuli may signal pain to the spinal cord while also causing inflammation in the periphery. This peripheral inflammatory response involves mast cell release of histamine, cytokine release, and vasodilatation with resultant edema. The acute inflammation may be adaptive in that it represents

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the initial step in wound healing, however, in susceptible individuals, the inflammation may become chronic.

Neurogenic inflammation has been examined in diverse human chronic inflammatory states as well as in animal models. For example, SP receptors exhibited dramatic increases in radioligand binding in the colons of both Crohn's and ulcerative colitis patients and this increase in receptor binding was restricted to tissues involved in mediating the inflammatory response such as arterioles, venules, and lymphoid aggregates.[3] In rats, both *Clostridium difficile* toxin A enterocolitis and dextran sulphate sodium (DSS)-induced colitis were prevented by either chemical or surgical ablation of sensory neurons or pretreatment of a SP receptor antagonist.[4–7] However, what is unclear regarding these studies is the stimulus that causes the sensory nerve to release the proinflammatory peptides.

Recently, a nonselective cation channel termed the vanilloid receptor subtype-1 (VR-1) was sequenced, cloned, and found to be expressed on primary sensory neurons.[8] Known agonists to VR-1 include heat, acid, or capsaicin (the active ingredient in hot chili peppers). Stimulation of VR-1 results in the release of SP and CGRP from sensory nerve terminals. Additionally, capsazepine (CPZ), a selective VR-1 antagonist, has been developed which inhibits the in vitro effects of both capsaicin and heat on VR-1.[9] We have recently determined in rats that either sensory nerve denervation or pretreatment with CPZ prevents DSS-induced colitis.[10] Pathologically, DSS colitis resembles human ulcerative colitis with crypt shortening, epithelial ulceration, and neutrophil infiltration suggesting that VR-1 may be important with regard to the pathogenesis of inflammatory bowel disease (IBD).

In the present study, we examined the effect of VR-1 inhibition in trinitrobenzene sulfonic acid (TNBS)-induced colitis in the rat. TNBS-colitis results in erosions, ulcerations, and massive transmural infiltration of inflammatory cells that most closely resembles Crohn's disease. Additionally, in deference to the previous experiments, we administered CPZ (the VR-1 antagonist) after the initial induction of TNBS-colitis to examine the potential effects of VR-1 inhibition once inflammation occurred.

MATERIAL AND METHODS

TNBS-Induced Colitis

A dosage of 100 mg/kg of TNBS (Sigma, St. Louis, MO) diluted in 50% ethanol was administered rectally via a polyethylene catheter (outer diameter [OD] 2 mm) 4 cm from the anus to 200 g Sprague–Dawley rats (Charles Rivers Laboratories, Raleigh, NC) lightly anesthetized with ketamine (40 mg/kg) and xylazine (10 mg/kg). For 7 days, the rats were monitored for colitis and then sacrificed. A cohort of rats (n = 6) received only TNBS-vehicle. A cohort of rats (n = 6) received a single enema of CPZ 1 hour after initial TNBS administration. A dosage of 0.5 ml of CPZ (Sigma, St. Louis, MO) made with 100 μg CPZ dissolved in 10% Tween/10% ethanol was administered using a flexible catheter (OD 4 mm) inserted exactly 4 cm from the anus. Rats were maintained in a head-down position for 30 minutes to prevent solution leakage. A final group of control rats (n = 6) were treated with enema infusion of CPZ vehicle. All groups of rats maintained their weights and did not demonstrate any toxic symptoms. The experiments were approved by the institution's animal care and use committee.

MPO Activity

Segments of proximal and distal colon were removed after euthanasia and stored frozen at −80°C. The specimens were weighed, place in a plastic tube on ice, and 0.5% of hexadecyltrimethyllammonium bromide (HTAB) in 50 mM KH_2PO_4 (pH 6) was added to each sample. Samples were homogenized on ice using Polytron tissue homogenizer (Kinematica, Littaw, Switzerland) for 15 seconds and subsequently underwent three cycles of freeze/thawing. All samples were fortified with additional HTAB buffer to equal 1 ml HTAB/50 mg wet weight. The samples were then vortexed and 500 μl of each was transferred to microfuge tubes. The tubes were centrifuged in a microfuge at 4°C for 2 minutes and the absorbance of each supernatant was read at 460 nm for 0, 30, and 60 seconds after addition of 2.9 ml of O-dianisidine dihydrochloride to 0.1 ml supernatant. One unit of myeloperoxidase (MPO) activity was defined as the degradation of 1 mol of peroxide per minute at 25; the results are expressed in units per gram of protein.

Macroscopic Damage Score

After rapid removal of the colon, the specimen was flushed with saline, cut open, and photographed. The photographs of the colonic specimens were then scored by a blinded observer who was unaware of the treatment. Scores were assessed using a previously established tissue damage scoring system[11] (Table 1).

Histology

After 7 days of TNBS administration, the rats were euthanized and sections of cecum, transverse colon,

Table 1. Macroscopic damage score (gross specimens were photographed and damage was assessed by a blind observer)

Score	Tissue Damage
0	no inflammation
1	swelling or redness
2	swelling and redness
3	1 or 2 ulcers
4	more than 2 ulcers or 1 large ulcer
5	mild necrosis
6	severe necrosis

and descending colon were removed and placed in paraformaldehyde. The specimens were then paraffin-embedded and subsequently cut into 5-μm sections. The sections were stained with hematoxylin and eosin (H&E) and examined with light microscopy. Little to no damage was observed in the ascending or transverse colon, therefore MPO, macroscopic damage scores (MDS), and histology were all obtained from the descending colon. This is consistent with previous reports using the TNBS model, where the most intense inflammation is observed only on the left side of the colon.

Statistical Analysis

Results are expressed as the mean ± the standard error of the mean (SEM). Differences among groups were examined using one-way analysis of variance (ANOVA) with the Dunnett test when comparing treatment vs. control groups and the Tukey–Kramer test was used when comparing all groups. Statistical analysis was done using GraphPad Instat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA). Significance was assumed to occur at P less than 0.05.

RESULTS

Histology

Light microscopic examination of the distal colon demonstrated severe inflammation in all rats treated with TNBS. Erosive lesions were present in the mucosa and extended into the muscularis propria. Extensive recruitment of neutrophils were also demonstrated throughout the submucosa (Fig. 1, *B*). No damage was observed in rats given only TNBS-vehicle (not indicated for clarity), CPZ-vehicle (not indicated), or TNBS-vehicle plus CPZ-vehicle (Fig. 1, *A*). Rats administered TNBS and then CPZ revealed little to no pathologic effects (Fig. 1, *C*). Specifically, no mucosal erosions were present and minimal to no neutrophils could be determined in the submucosa.

MPO Activity

MPO activity is a useful method for evaluating granulocyte infiltration in colonic tissues after the induction of colitis. A significant increase in MPO activity was observed in rats given TNBS and CPZ-vehicle compared with rats given only TNBS-vehicle and CPZ-vehicle (2.31 ± 0.50 vs. 0.82 ± 0.41; $P < 0.05$). Rats administered TNBS and then CPZ 1 hour later demonstrated a significant reduction of MPO compared with the control group (TNBS-vehicle + CPZ-vehicle) levels (0.65 ± 0.49 vs. 2.31 ± 0.50; $P < 0.05$) (Fig. 2).

Macroscopic Damage Score

Rats administered CPZ-vehicle and TNBS-vehicle demonstrated little histological damage whereas rats administered TNBS and CPZ-vehicle exhibited a significant elevation in pathologic injury (0.65 ± 0.45 vs. 3.00 ± 0.55; $P < 0.05$). Rats administered TNBS and then CPZ 1 hour later demonstrated a significant reduction in macroscopic damage score when compared with rats given TNBS (1.33 ± 0.23 vs. 3.00 ± 0.55; $P < 0.05$). No statistical difference was observed between control (TNBS-vehicle + CPZ-vehicle) rats and rats administered TNBS and CPZ (0.65 ± 0.45 vs. 0.33 ± 0.23) (Fig. 3).

DISCUSSION

In this study, we demonstrated that antagonism of VR-1 results in the prevention of pathological and histological effects of intracolonic administration of TNBS. TNBS-induced colitis results in a highly reproducible model of severe and often transmural inflammation that resembles Crohn's disease. After induction of TNBS-colitis, CPZ, a specific antagonist of VR-1, significantly reduced MPO activity, MDS, and gross histological findings. These results suggest that VR-1 may play a pivotal role with regard to the initiation and propagation of experimental colitis.

Neurogenic inflammation refers to the stimulation of primary sensory neurons that results in both central signals being conveyed to the spinal cord and brain, as well as peripheral signals being transmitted to small blood vessels, immune complexes, and the epithelium itself. Central signals result in the sensation of pain, whereas peripheral signals result in local inflammation. Neurogenic inflammation has been investigated in diverse organ systems including the lung[12], skin[13],

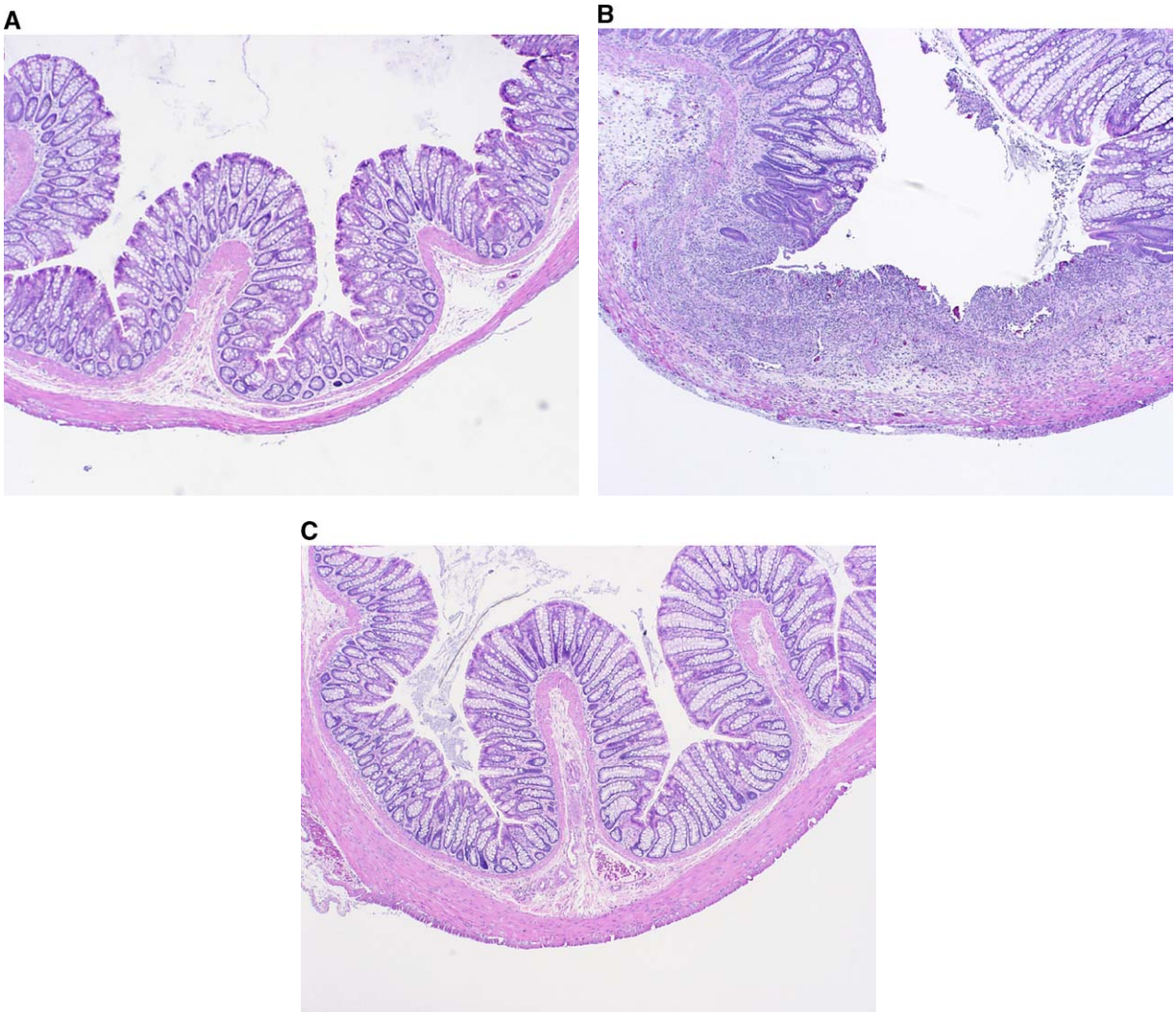

Fig. 1. Pathological effects of trinitrobenzene sulfuric acid (TNBS) colitis with or without capsazepine (CPZ) (10×). No damage was observed in animals treated with TNBS-vehicle and CPZ-vehicle (**A**). Severe damage with mucosal and submucosal ulcerations with edema and neutrophil infiltration was observed in animals administered TNBS and CPZ-vehicle (**B**). Near complete histological protection was determined in animals treated with TNBS and CPZ (**C**).

bladder,[14] pancreas,[15] and the intestine[16] and has indicated clinical correlations relative to asthma, bullous pemphigoid, cystitis, chronic pancreatitis, and IBD, respectively. In many of these studies, sensory neurons have been determined to be critical with regard to initiating inflammation and antagonism of the receptor to the neuropeptide released by the sensory nerve results in a reduction of the inflammatory response. However, the signal that results in the release of the proinflammatory peptides has remained elusive.

VR-1 has been recently sequenced and cloned and represents a class of nonselective cation channels located on primary sensory neurons[8]. Stimulation of VR-1 results in the release of proinflammatory peptides such as SP and CGRP that result in nociception and inflammation. CPZ, a synthetic antagonist to VR-1, inhibits these effects in vitro and in vivo. Pretreatment with CPZ in both an acute model of enterocolitis[17] and a more chronic model of DSS-induced colitis[10] prevented the pathological damage normally observed in these model systems. However, in both of these intestinal inflammation models, administration of CPZ after induction of inflammation did not prevent colitis.

TNBS-induced colitis is a commonly used model of gut inflammation. TNBS-colitis results in extensive erosions and ulcerations, submucosal edema, and

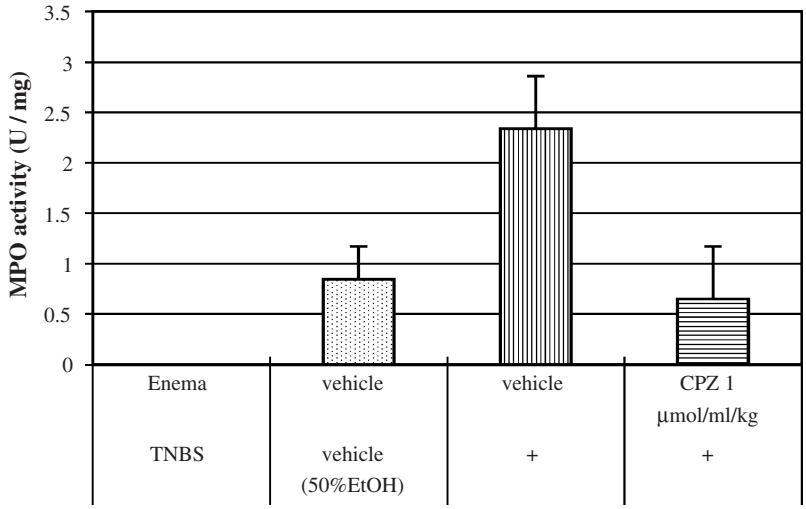

Fig. 2. Myleoperoxidase (MPO) activity in trinitrobenzene sulfuric acid (TNBS)-colitis with or without capsazepine (CPZ) treatment. A significant reduction in MPO was noted between TNBS-colitis animals and animals treated with CPZ (*$P < 0.05$). No difference was observed between TNBS-vehicle + CPZ vehicle and TNBS + CPZ animals. EtOH = ethanol.

infiltration of inflammatory cells that are most acutely apparent after 7 days. TNBS-colitis is attenuated with antitumor necrosis factor-α antibody,[18,19] inhibition of the interlenkin (IL)-12 receptor[20], and other Thelper-1 (Th-1) cytokine antagonists. As such, this model closely resembles Crohn's disease. In the present study, we administered CPZ 1 hour after initiation of TNBS-colitis indicating that antagonism of VR-1 after the induction of colitis prevents the initiation of the inflammatory cascade.

This study, in conjunction with our previous work,[10] indicates that sensory neurons seem to be critical with regard to modulating inflammation in the intestine. Sensory neurons containing VR-1 seem to be stimulated by a yet unknown antagonist that results in proinflammatory peptide release. Although several candidate antagonists for VR-1 have been suggested, their precise role with regard to in vivo models of inflammation is unclear. Further investigation into the measurement of tissue levels of these potential

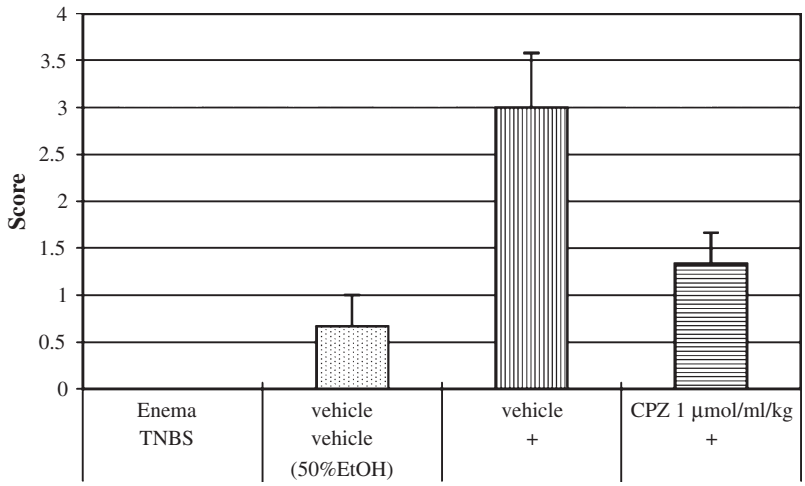

Fig. 3. Macroscopic damage score (MDS) trinitrobenzene sulfuric acid (TNBS)-colitis with or without capsazepine (CPZ) treatment. A significant reduction in MDS was noted between TNBS-colitis animals and animals treated with CPZ (*$P < 0.05$). No difference was observed between TNBS-vehicle + CPZ vehicle and TNBS + CPZ animals. EtOH = ethanol.

VR-1 antagonists in inflammatory states is ongoing. Additionally, these compounds could be readily tested in human inflammatory states such as IBD. Finally, VR-1 antagonists may represent a therapeutic mechanism to inhibit the inflammatory cascade. Our provocative results indicating that CPZ is effective after the induction of TNBS-colitis suggests that inhibition of neurogenic inflammation may be beneficial for chronic-relapsing diseases such as Crohn's disease.

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Discussion

Dr. J. Becker (Boston, MA): I would like to congratulate this group for this work. I think it is important for all of us to begin to consider novel strategies for the treatment of IBD. This study certainly supports the work that we have done with substance P antagonists, both in a chemically induced colitis model and in a spontaneous pouchitis model. I wonder if your group has been given the opportunity to observe any of the other newer IBD models in animals such as the knockout models or some of the other genetic models now available?

Dr. Mantyh: Thank you for those kind comments. We are actually pursuing those exact studies, Dr. Becker. We are going to be observing the IL-10

knockout mouse and we have actually started to raise a colony of those. I believe your group has published information concerning the pouchitis model in the rat and that is an excellent model in itself. Perhaps we might be borrowing your techniques, also.

Dr. S. Mulvihill (Salt Lake City, UT): Chris, this was a very nice piece of work and thank you for bringing it to this meeting. We have learned a considerable amount in recent years regarding efferent mechanisms in the enteric nervous system that control inflammation, blood flow, motility and the like, but the afferent mechanisms are still relatively mysterious. I wonder if you can give us any insight from this work in terms of what the linkage might be between your insulting agent and the stimulation of that vanilloid receptor? In other words, what is the mechanism of that afferent stimulation that then sets off the efferent arc resulting in inflammation?

Dr. Mantyh: That is the black box that we are trying to crack open. We are not sure what that is, however, there are several proposed antagonists concerning the VR-1 receptor on sensory neurons. Some of them appear to be cell wall products such as arachidonic acid, prostaglandins, and this very unusual compound known as anandamide, which is actually a derivative of THC—the compound in marijuana.

Dr. F. Moody (Houston, TX): Very nice presentation. I was curious regarding other possible mechanisms. I take it you did not actually measure the release of serotonin, histamine, or anything else in the tissue. Did you do any experiments where you simply put in lidocaine pretreatment to see if you could prevent the release of substance P? Many inflammatory pathways could be involved here. Could it be that you just happened to look at this pathway and this is what you determined?

Dr. Mantyh: Have we tried lidocaine? We have not done that, but that is a good experiment to do. The Swedish group, Dr. Ove Lundgren, has actually published information regarding that in *Science* a couple of years ago with very provocative results. That would be a fairly easy experiment to do and we should potentially pursue it.

Dr. J. Matthews (Cincinnati, OH): Can you comment on whether VR-1 inhibition lessens the injury or accelerates the repair?

Dr. Mantyh: Good question. VR-1 stimulation seems to increase the damage in the colon. There is some evidence from the UCLA group that the VR-1 receptor may be protective in the stomach and the reason for that is because it seems to release CGRP that produces vasodilatory effects and the increased blood flow then causes the ulcer to heal faster. The way I perceive inflammation is as a spectrum that may eventually result in healing and we may just be observing the inflammatory effects. At some point in the future, perhaps several weeks or months later, healing will actually occur. So, in my opinion, it is just a matter of semantics.

Effect of Body Mass Index on Nonalcoholic Fatty Liver Disease in Patients Undergoing Minimally Invasive Bariatric Surgery

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The risk factors for nonalcoholic fatty liver disease in patients undergoing bariatric surgery are under study. We wanted to determine the correlation between nonalcoholic fatty liver disease and patient factors such as obesity and liver function tests. A retrospective analysis was performed on 177 nonalcoholic morbidly obese patients who underwent laparoscopic Roux-en-Y gastric bypass with liver biopsy, to identify risk factors for nonalcoholic fatty liver disease. The histologic grade of liver disease was compared with preoperative body mass index, age, and liver function tests. Simple steatosis and steatohepatitis were present in 90% and 42% of patients, respectively. Elevated transaminaselevels were an independent risk for liver disease. Body mass index and liver disease were not correlated with univariate analysis. Regression analysis performed on age, body mass index, and liver disease demonstrated that the risk for liver disease increased with body mass index in the younger (<35 years old) age group and decreased with body mass index in the older (>45 years old) age group. There was a high incidence of steatosis and steatohepatitis in these nonalcoholic bariatric patients, and elevated transaminase level was indicative of disease. Body mass index was a positive risk factor for liver disease in younger patients but a negative risk factor in the older patients. (J GASTROINTEST SURG 2004;8:849–855)

KEY WORDS: Nonalcoholic fatty liver disease, morbid obesity, laparoscopic bariatric surgery

Nonalcoholic fatty liver disease (NAFLD) is defined as the accumulation of excessive fat (5%–10% of organ weight) in the liver of individuals who consume no more than two alcoholic beverages per day.[1] The spectrum of NAFLD extends from fatty replacement (simple steatosis) to nonalcoholic steatohepatitis (NASH).[2] In adults, the typical histopathologic manifestations of NASH include steatosis (especially in zone 3, near the hepatic venules), ballooned hepatocytes, lobular inflammation, and zone 3 perisinusoidal fibrosis.[1] An autopsy study found simple steatosis and NASH in 70% and 18.5% of obese patients, respectively; these figures were 35% and 2.7% in nonobese patients, respectively.[3] Up to 11% of nonalcoholic obese patients with abnormal liver function tests but no clinical liver disease have histopathologic evidence of cirrhosis[4]; on a similar note, 10%–30% of patients with NAFLD develop cirrhosis after 10 years,[5–7] although only a minority have liver failure. The 5-year survival of patients diagnosed with NASH is 67%; death often is from comorbid disease.[8]

The pathophysiology of NASH has been described to follow a "two-hits hypothesis."[9] The first hit, or insult, to liver homeostasis involves accumulation of

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triglycerides in the liver (i.e., steatosis) secondary to overeating. This situation raises the level of intracellular oxidant stress, which in turn makes the hepatocyte vulnerable to a second insult. The second insult may involve an acute oxidant stress load, an upregulation of tumor necrosis factor α, or another environmental and/or genetic factor that can induce necrotic cell death in susceptible hepatocytes; this in turn will produce inflammation.[10] Considering the etiologic factors involved, it is not surprising that NASH is associated with diagnoses such as obesity, insulin-resistant diabetes, hyperlipidemia, hypertension, and the metabolic syndrome.[11]

The impact of NASH on operative mortality and long-term outcome in bariatric surgery is unclear. In one retrospective study of 126 surgeons who performed 86,500 bariatric procedures (via the open approach) in individuals not suspected to have liver disease, a grossly cirrhotic liver was found in 0.14% of patients.[12] The operative mortality rate in these cirrhotic patients was approximately five times that of bariatric patients without cirrhosis.[12] Whether the effect of unexpected cirrhosis on minimally invasive bariatric operative mortality will be similar to the effect observed on open operative mortality remains to be seen. Currently, there are no clinical or biochemical tests that accurately detect NASH[1,13]; a definitive diagnosis relies on liver biopsy.

In 2002, approximately 75,000 bariatric procedures were performed in the United States; in comparison, the annual rate was 10,000–15,000 in the mid 1990s.[14] One of the main factors responsible for this increase is the widespread application of minimally invasive bariatric procedures.[14] Such a large increase in the number of bariatric procedures (which, by most indicators, will continue to increase) combined with the evolving knowledge about NASH[1,10] led us to characterize the incidence of NASH in our bariatric population and to determine whether there were preoperative risk factors for NASH. We confirmed previously reported risk factors but also found an unexpected protective effect of body mass index (BMI) on NASH in older patients.

PATIENTS AND METHODS

Permission to review medical records for this study was obtained from the institutional review board. Between November 2001 and April 2003, data were collected on 177 nonalcoholic morbidly obese patients who underwent minimally invasive (laparoscopic) gastric bypass with liver biopsy, supervised by one surgeon (C.T.F.). Routine preoperative evaluation included history and physical examination (with BMI calculation), cardiopulmonary testing, and serologies, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), alkaline phosphatase (AP), total protein (TP), albumin (Alb), hepatitis panel, and human immunodeficiency virus testing.

The primary indication for bariatric surgery was BMI of greater than 40 (weight [kg]/height [m^2]); if a patient had a BMI of 35–40 and significant comorbidity attributable to obesity, the patient also was considered for a bypass.[15] The criterion for a "nonalcoholic" was two or fewer drinks per day (one drink = 12 oz. beer, 5 oz. wine, or 1.5 oz. liquor). All patients underwent a laparoscopic Roux-en-Y gastric bypass with intraoperative liver biopsy. There were no intraoperative or postoperative complications related to the liver biopsy. The diagnosis of NASH was based on the presence of steatosis and portal inflammation (with or without fibrosis, cirrhosis, or Mallory bodies) with the exclusion of other liver diseases such as alcoholic hepatitis, viral hepatitis, drug-induced hepatitis, hemochromatosis, and Wilson's disease.[1] The grading scales for steatosis and steatohepatitis are shown in Table 1.

The Kruskal-Wallis test (KaleidaGraph; Synergy Software, Reading, PA) was used to evaluate univariate relationships. The level of significance was defined as $p < 0.05$. Cumulative logit regression analysis using the SAS System for Windows (Cary, NC), Version 8.2 was performed to detect multivariate relationships. A backwards elimination procedure was used to determine important factors associated with steatohepatitis and steatosis. Variables included in the modeling process were age, BMI, sex, AST, ALT, TB, AP, and Alb along with two-level interactions for BMI and sex and BMI and age. Variables were retained in the model if they maintained a significance level of 0.05.

Table 1. Grading scales for steatosis and steatohepatitis

Steatosis		Steatohepatitis	
Grade	**Description**	**Grade**	**Description**
0	None	0	None
1	Mild	1	Mild pericellular fibrosis confined to zone 3
2	Moderate	2	Moderate pericellular fibrosis confined to zone 3 with no or minimal fibrosis
3	Severe	3	Severe septal fibrosis
		4	Cirrhosis

RESULTS

A consecutive group of 186 nonalcoholic obese patients (mean age, 40 years; median age, 40.0 years; age range, 18–68 years; 155 women [83%]) who underwent laparoscopic gastric bypass with liver biopsy was reviewed. The mean BMI was 48.9 kg/m^2 (median, 46.9 kg/m^2; range, 35–86 kg/m^2). Of this group, nine liver biopsies either were not obtained or were not readable secondary to insufficient specimen quantity, which left 177 biopsy samples for analysis. Of note, the planned operation was not altered if any of the 186 patients secondary to an intraoperative finding. The distribution of the various grades of steatosis and steatohepatitis is shown in Table 2. Only 10% of patients did not have any steatosis, and none of these patients had steatohepatitis. On the other hand, 58% of the patients did not have any steatohepatitis; in this group, the number of patients with grade 0, 1, 2, and 3 steatosis was 18, 63, 19, and 2, respectively.

Determination of a univariate relationship between either liver disease and other factors (age, BMI, AST, ALT, TB, AP, or Alb) is shown in Table 3 (steatosis) and Table 4 (steatohepatitis). For example, the average age of patients with grade 0, 1, 2, or 3 steatosis is shown in the first line of Table 3, along with the results of the Kruskal-Wallis test (the nonparametric equivalent of analysis of variance testing). In summary, Table 3 indicates that there was no simple correlation of steatosis versus age, BMI, TB, AP, or Alb; however, nonparametric testing of either AST or ALT against steatosis revealed that elevation of one or both of these transaminases was correlated with the presence of steatosis. Similarly, elevation of AST and/or ALT was correlated with the presence of steatohepatitis (Table 4), whereas none of the other factors correlated. Because there only was one patient with grade 3 (severe) steatohepatitis, this individual was grouped with the grade 2 patients for the analysis. Plots of the raw data for some of the variables examined in Tables 3 and 4 are shown in Fig. 1; these scattergrams reveal that at least some of the data have a nonnormal distribution (especially in Fig. 1, *A* and *B*, which show the AST data).

Consideration of three-variable relationships revealed an interaction among BMI, age, and presence of liver disease. To illustrate this relationship, some of the raw data are replotted (Fig. 2). The patients were segregated into five age ranges, and each age range was further segregated according to grade of steatosis (Fig. 2, *A*). The mean BMI of these age–grade "isobars" then was calculated and plotted. The plot suggests that younger patients with a higher BMI had greater risk of steatosis and that older patients with a higher BMI had less risk of steatosis (Fig. 2, *A*). Analogous findings are shown in Fig. 2, *B*; that is, the risk of steatohepatitis appears to be elevated in younger patients with a higher BMI, but the risk of steatohepatitis decreases with increasing BMI in the older age groups.

The putative relationship among BMI, age, and grade of disease was confirmed with cumulative regression analysis. For both steatosis and steatohepatitis, the interaction between age and BMI was significant ($P = 0.0106$ and 0.0010, respectively). At lower ages, as BMI increased, the probability of no disease increased. For the middle ages, there was little effect of BMI on the probability of no disease. At the higher ages, as BMI increased, the probability of no disease increased. In other words, the effect of BMI on the probability of disease in younger subjects was opposite the probability of disease in older subjects. For both steatosis and steatohepatitis, the regression analysis implied a BMI or an age at which the risk of disease is constant regardless of the other value; these BMI or age values (along with their confidence intervals) are given in Table 5. For example, there was no effect of BMI on the risk for steatohepatitis in a 40-year-old patient. Another interpretation of the model is that the odds of steatohepatitis were significantly increased with a 1-unit increase in BMI for ages 32 and younger and that the odds were significantly decreased with a 1-unit increase in BMI for ages 50 and older.

Table 2. Distribution of the various grades of steatosis and steatohepatitis

	No. of patients			
Liver disease	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**
Steatosis	18	71	65	23
Steatohepatitis	102	59	15	1

DISCUSSION

The clinical importance of cryptogenic hepatitis, of which NASH is commonly the underlying cause, is controversial.[13] The controversy appears to be related to the rate at which NAFLD progresses to clinical disease with complications of portal hypertension, hepatic insufficiency, and so on. For example, unsuspected cirrhosis was found in about 1:1000 bariatric patients in a worldwide questionnaire survey[12]; this statistic seems counter to the estimate that

Table 3. Correlation of various factors with the grade of steatosis

Factor	Grade of steatosis 0	1	2	3	*P* value
Age (yr)	34.7 ± 12.3 (18)	40.8 ± 11.0 (71)	42.2 ± 10.9 (65)	38.5 ± 11.1 (23)	0.0661
BMI (kg/m^2)	48.6 ± 10.3 (18)	48.7 ± 7.9 (70)	49.1 ± 9.0 (65)	50.4 ± 7.9 (23)	0.6049
AST (U/L)	19 ± 6 (18)	21 ± 8 (66)	31 ± 33 (63)	43 ± 46 (21)	<0.001
ALT (U/L)	22 ± 10 (18)	25 ± 14 (66)	40 ± 44 (63)	61 ± 69 (21)	<0.001
TB (mg/dL)	0.4 ± 0.2 (18)	0.4 ± 0.2 (66)	0.5 ± 0.2 (63)	0.5 ± 0.2	0.7825
AP (U/L)	78 ± 22 (18)	82 ± 22 (66)	77 ± 24 (63)	85 ± 37 (21)	0.4098
Alb (g/dL)	3.8 ± 0.3 (18)	3.7 ± 0.4 (66)	3.9 ± 0.4 (63)	3.7 ± 0.6 (21)	0.6879

Data are reported as mean ± SD, with the number of observations in parentheses. The *P* values were generated with the Kruskal-Wallis test.

640,000 individuals in the United States may have cirrhosis secondary to NAFLD.[13] Furthermore, only 1% of patients on transplant waiting lists have NAFLD as their underlying diagnosis.[13] The disparity between the estimates of disease progression and the actual observations may be secondary to the relatively long time that NAFLD requires for progression to cirrhosis.[1] Most patients undergo bariatric surgery at a relatively young age (age, 30–50 years),[16] at which time laboratory or pathologic evidence of NAFLD may be found. The clinical manifestations of NAFLD often do not develop, however, for another decade.[1,13] Given the current state of knowledge, the clinical relevance of NAFLD will continue to evolve.

Other groups have documented steatosis and steatohepatitis in 65%–80% and 2–33%, respectively, of patients undergoing a bariatric procedure with concomitant liver biopsy.[17–19] No cases of cirrhosis were discovered in these reports (<100 patients each). Our rates of steatosis and steatohepatitis (90% and 42%, respectively) were somewhat higher than these rates; the reason for this is unclear. We did not uncover any unsuspected cirrhosis in our 177 patients, which is consistent with the 0.1% incidence described earlier. In general, the vast majority of our patients had some degree of steatosis, but fewer than half had steatohepatitis, and the majority of these cases were mild (i.e., grade 1; see Table 2).

Univariate analysis of factors associated with NAFLD in our patients revealed, not surprisingly, that elevation of AST and/or ALT was associated with both steatosis and steatohepatitis. The degree of transaminase elevation in the typical patient was not dramatic, however; the average transaminase elevation (from a non-normal distribution; see Fig. 1) in a patient with NAFLD was only about twice that of a patient without disease. No other independent variables for disease were found. The lack of a simple relationship between BMI and NASH has been documented by others.[19,20] Our multivariate analysis, however, found an unexpected interaction among age,

Table 4. Correlation of various factors with the grade of steatohepatitis

Variable	Grade of steatohepatitis 0	1	2	*P* value
Age (yr)	40.0 ± 11.4 (102)	41.8 ± 10.5 (59)	37.5 ± 12.3 (16)	0.2968
BMI (kg/m^2)	49.0 ± 8.1 (101)	48.8 ± 9.4 (60)	50.6 ± 8.6 (15)	0.5071
AST (U/L)	20 ± 8 (96)	35 ± 37 (58)	41 ± 46 (14)	<0.001
ALT (U/L)	25 ± 14 (96)	46 ± 55 (58)	54 ± 55 (14)	0.0001
TB (mg/dL)	0.5 ± 0.2 (96)	0.4 ± 0.2 (58)	0.5 ± 0.2 (14)	0.2349
AP (U/L)	78 ± 22 (96)	85 ± 30 (58)	69 ± 16 (14)	0.0806
Alb (g/dL)	3.7 ± 0.4 (96)	3.9 ± 0.4 (58)	3.8 ± 0.6 (14)	0.1627

Data are reported as mean ± SD, with the number of observations in parentheses. The *P* values were generated with the Kruskal-Wallis test. Note that for these analyses, the one patient with grade 3 steatohepatitis was grouped with the grade 2 patients.

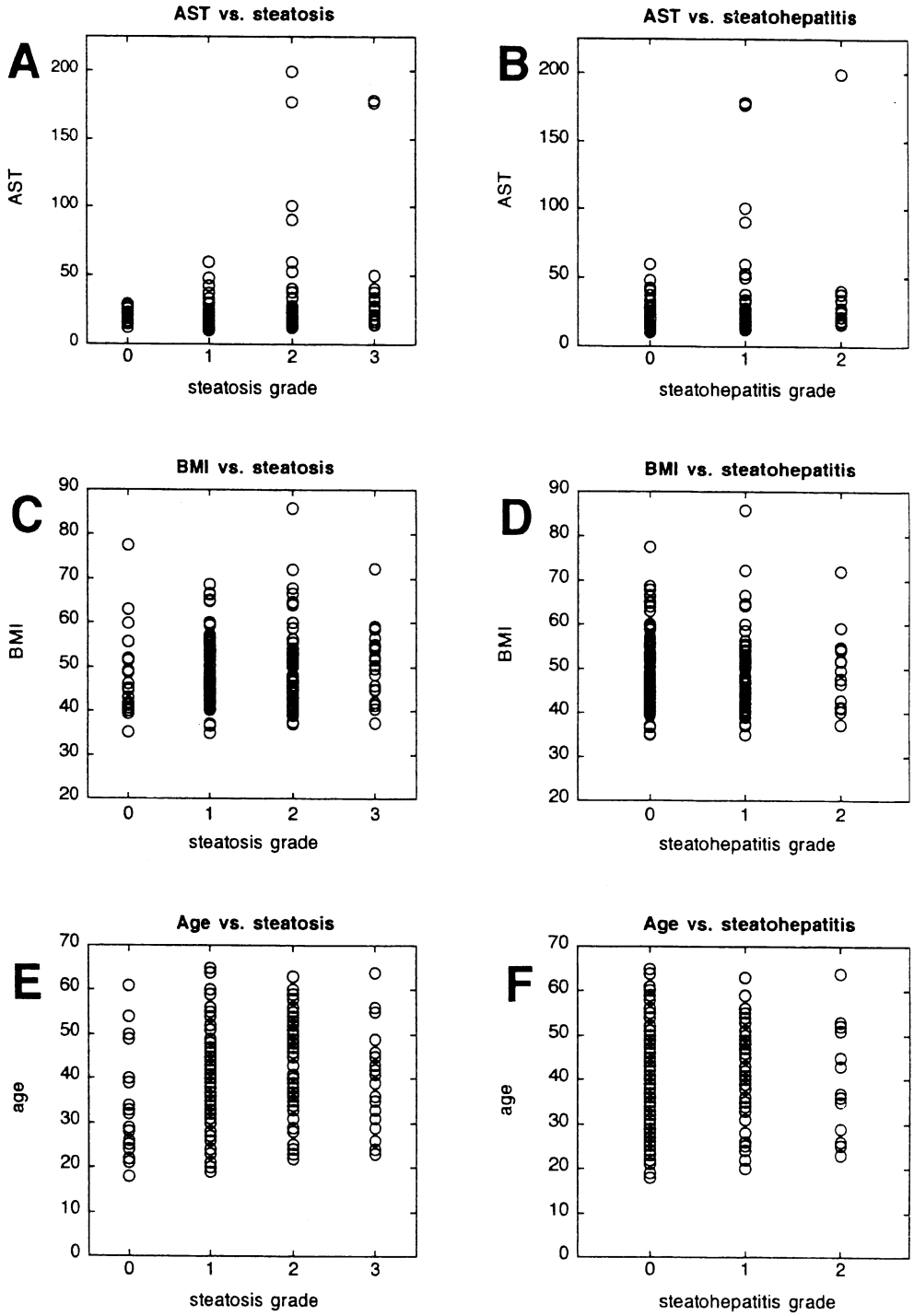

Fig. 1. Raw data plots of single selected variables vs. grade of liver disease. (**A**) Aspartate aminotransferase (AST) vs. grade of steatosis. (**B**) AST vs. grade of steatohepatitis. (**C**) Body mass index (BMI) vs. grade of steatosis. (**D**) BMI vs. grade of steatohepatitis. (**E**) Age vs. grade of steatosis. (**F**) Age vs. grade of steatohepatitis.

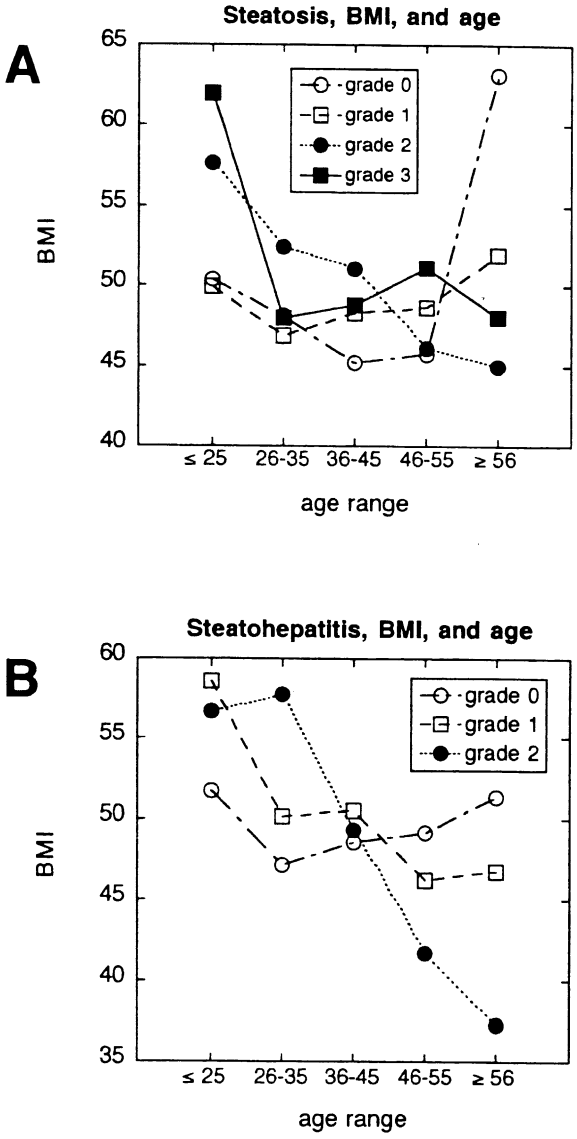

Fig. 2. (**A**) Plot of steatosis grade, body mass index (BMI) and age. For each age range, the mean BMI at each disease grade was plotted; each line represents the relationship between age and BMI at a constant disease grade. (**B**) Similar plot using steatohepatitis grade, BMI, and age.

BMI, and liver disease grade: at younger ages, increasing BMI was associated with both steatosis and steatohepatitis, whereas at older ages, increasing BMI was associated with less disease. In other words, BMI was a positive risk factor for NAFLD in the younger group but a negative risk factor in the older group. This finding seems somewhat counterintuitive, given what is known about the pathophysiology of NAFLD. Furthermore, the clinical implications of this paradoxical effect of BMI are unclear. This finding will need to undergo further examination. The reason that increased BMI may have a protective effect against NASH in older patients is at this point only speculative.

Table 5. Value and 95% confidence intervals for age and body mass index values at which there is a change in the direction of the effect of the other variable

Variable	Steatosis	Steatohepatitis
Age (yr)	45.7 (36.2–55.3)	40.3 (33.8–46.9)
Body mass index (kg/m^2)	54.6 (47.3–62.1)	50.4 (45.1–55.7)

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The Practice of Bariatric Surgery at Academic Medical Centers

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The growing demand for laparoscopic bariatric surgery has led to an increase in the development of new bariatric surgical practices. Proper hospital facilities and an experienced bariatric surgical team are necessary to ensure optimal patient results. We surveyed academic centers participating in the University HealthSystem Consortium to examine the current practice of bariatric surgery. The survey questioned (1) availability of resources and equipment designed for the morbidly obese, (2) accidents, equipment problems, and workers' compensation relating to the care of bariatric surgical patients, (3) credentialing of bariatric surgeons, and (4) suggestions for improvements in the bariatric surgery program. Twenty-five institutions that perform bariatric surgery responded. Although the majority of institutions noted that they had basic bariatric equipment, some organizations did not have facility resources such as high-weight operating room tables and computed tomography scanners or transfer devices. Twenty-eight percent of institutions reported having accidents or equipment problems and 40% of institutions had workers' compensation claims relating to the care of bariatric patients. With regard to credentialing, 60% of institutions required the surgeons to have performed a minimum number of procedures prior to granting privileges. Suggested improvements included the need for more specialized bariatric equipment, enhancement of the education of all members of the bariatric surgical team, and designation of a bariatric physician who would coordinate care. This survey of bariatric surgery practices at academic medical centers demonstrates that the practice of bariatric surgery could be improved with regard to availability of bariatric equipment and resources and credentialing of surgeons. (J GASTROINTEST SURG 2004;8:856–861)

KEY WORDS: Gastric bypass, bariatric surgery, workers' compensation, credentialing

A recent surge in the number of bariatric operations being performed in the United States has resulted in a corresponding increase in the number of centers developing a bariatric surgical practice. For example, the number of centers performing bariatric surgery within the University HealthSystem Consortium (UHC) database has increased from 64 in 1999 to 84 in 2002.[1] Bariatric surgery, like other complex surgical procedures, should be performed by surgeons with experience and demonstrated competency. The clinical setting of a comprehensive bariatric program must have adequate support from a multidisciplinary health care team and appropriate institutional resources and unique equipment for the care of morbidly obese patients.[2] The outcome of bariatric surgery is often a reflection of appropriate structure and processes of care specifically tailored for the morbidly obese.[1] Important structural components of a bariatric surgery program include experienced surgeons and health care professionals who implement standardized selection criteria as well as operative and postoperative care and the availability of specialized equipment (e.g., large wheelchairs, beds, gurneys, operating room tables), diagnostic technology, critical care staffing, and rehabilitation facilities outfitted for the safe care of bariatric patients. Important processes of care include clinical pathways for bariatric surgery, organized support groups, and a system for long-term follow-up and outcome reporting. Because bariatric

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surgery is becoming a frequently performed operation, a clinical pathway can reduce medication and treatment errors by standardizing care, reduce cost by omitting unnecessary tests, and reduce the surgeons' work load. These structural components and processes of care have been stressed as integral components for a successful practice of bariatric surgery.

The growing number of bariatric surgery centers being developed across the United States raises a potential concern about the quality of care being delivered for bariatric surgical patients at institutions not fully equipped to care for the morbidly obese. The American College of Surgeons published a guideline recommending appropriate necessary staffing and facilities at institutions that perform bariatric surgery[3] (Table 1). We recognize that appropriate facilities for morbidly obese patients are necessary to complement an experienced bariatric surgical team (surgeons, anesthesiologists, nurses, coordinators, dieticians). We surveyed the practice of bariatric surgery at academic institutions with regard to availability of appropriate facilities and resources for management of the morbidly obese and credentialing of surgeons performing bariatric surgery. We hypothesized that bariatric surgery performed at institutions with inappropriate facilities and resources for management of the morbidly obese can lead to higher accidental injury and workers' compensation claims.

MATERIAL AND METHODS

The UHC is an alliance of academic health centers that provides resources aimed at improving performance levels in clinical, operational, and financial areas. A survey was sent via e-mail to bariatric physicians and/or risk managers of participating institutions of the UHC inquiring about the practice of bariatric surgery.

The survey was divided into five areas of questioning: (1) bariatric surgical practice demographics (presence of a bariatric surgery program, number of surgeons performing bariatric surgery, number of bariatric surgical cases performed yearly, presence of a formalized multidisciplinary bariatric surgery program); (2) availability of bariatric equipment capable of supporting the super-super obese (computed tomography [CT] scanner, appropriate patient clothing, large blood pressure cuffs, high-weight scales, high-weight hospital beds, large hospital chairs, lift devices, high-weight operating room tables, large wheelchairs, special surgical equipment for the morbidly obese, large walkers, and high-weight gurneys); (3) accidents, equipment problems, and workers' compensation related to the care of bariatric surgical patients; (4) credentialing process of bariatric surgeons (if bariatric surgery is considered as a core [part of general surgery] or noncore [not part of general surgery and requiring additional certification/training]); and (5) suggested improvements in the bariatric surgery program. Association between institutions with accidents/equipment problems and institutions with workers' compensation was determined using Fisher's exact tests. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL). A value of $P < 0.05$ was considered statistically significant.

Table 1. Recommendations by the American College of Surgeons for facilities performing bariatirc surgery

- An experienced bariatric surgery team, including surgeons, anesthesiologists, nurses, nutritionists, and recovery room staff
- Availability of specialists in cardiology, pulmonology, rehabilitation, and psychiatry
- Special operating room tables and surgical equipment to accommodate morbidly obese patients
- Available intensive care unit and recovery room capable of providing critical care to obese patients
- Hospital beds, commodes, chairs, and wheelchairs to accommodate the morbidly obese
- Radiology and other diagnostic equipment capable of handling the morbidly obese
- Long-term follow-up care facilities, including rehabilitation facilities, psychiatric care, nutritional counseling, and support groups

Adapted from "Recommendations for facilities performing bariatric surgery."[3]

RESULTS

Twenty-five of the 29 institutions responding to the survey performed bariatric surgery. The median number of bariatric surgeons was two, and the median number of bariatric procedures performed annually was 150. Twenty-four of 25 institutions were considered to have a formal bariatric surgery program. The majority of institutions had available large gowns (87%), large blood pressure cuffs (96%), high-weight scales (91%), high-weight hospital beds (87%), high-weight bedside commodes (87%), and large wheelchairs (91%). However, more than a quarter of institutions did not have available high-weight CT scanners (20%), floor-based commodes (28%), lift or transfer devices (68%), bariatric-specific surgical instruments (68%), large hospital chairs (60%), large walkers (60%), large gurneys (36%), or high-weight operating room tables (68%).

Seven of 25 (28%) institutions reported accidents or equipment problems relating to the care of bariatric patients. The reasons for the accidents or equipment problems were related to the use of equipment

inadequate to accommodate the morbidly obese. This survey, however, did not question whether patient injury occurred as a result of these accidents/equipment problems. Ten of 25 (40%) institutions had workers' compensation claims relating to the care of bariatric patients. We believe that the majority of accidental injury occurred because of inadequate equipment or lack of appropriate equipment for management of the morbidly obese, which can lead to claims for personal injury. Inappropriate equipment/resources for management of the morbidly obese can also lead to higher occupational injury and hence higher number of workers' compensation claims. There was a trend for institutions reporting accidents to also have workers' compensation claims. Four of seven institutions who reported accidents/equipment problems relating to the care of bariatric surgical patients also had workers' compensation claims; in contrast, only 3 of 18 institutions without accidental injuries had workers' compensation claims (57% versus 17%, $P = 0.06$). We were not able to elucidate the reasons for workers' compensation claims.

Bariatric surgery was categorized as a noncore privilege in 40% of institutions. Only 60% of institutions required a minimum number of procedures to be performed by the surgeon before granting bariatric surgery privileges. Fifty-two percent of institutions used qualitative bariatric surgical outcome measures to evaluate the surgeon's competence for reappointment. The three most common suggestions for improvement of their respective bariatric surgery programs were (1) availability and access to bariatric equipment for the care of morbidly obese patients, (2) an increase in educational programs and training for all staff members caring for bariatric surgery patients, and (3) designation of a bariatric physician who would coordinate the care and implement a formalized program in bariatric surgery.

DISCUSSION

Bariatric surgery is best performed in the context of a unique program that combines appropriate facilities and equipment and an experienced bariatric surgical team to ensure safe practice. In this survey, we found that the majority of institutions performing bariatric surgery have adequate basic bariatric equipment; however, there could be improvement in hospital resources for CT scanners, operating room tables, surgical instruments, and transfer devices designed to accommodate the morbidly obese. The use of equipment not specifically made to accommodate the morbidly obese can lead to patient injury or equipment malfunction. In this survey, more than a quarter of institutions performing bariatric surgery had reported accidents or equipment problems related to the care of bariatric patients. In addition, there was a trend in association between institutions reporting accidents and institutions reporting workers' compensation ($P = 0.06$). The results from this survey suggest that implementation of appropriate facilities and resources specifically tailored to accommodate the morbidly obese could potentially minimize patient and health care workers' injuries and potentially reduce workers' compensation claims.

Appropriate bariatric equipment improves patient and health care workers' safety, provides accuracy of information, increases efficiency in patient care, and improves satisfaction and patient comfort. Consideration should be given to analyzing special patient needs, starting with the clinic visit, the operating room, the recovery room, the ward, and the radiology suite. The clinic should have appropriate scales, office furniture, and examining tables. Standard examining tables have a height of 33 inches from the floor and a width of 26 inches. Bariatric examination tables are often powered with adjustable height and width that increases to 27.5 inches. Diagnostic equipment such as large blood pressure cuffs should be available to ensure accurate readings. The hospital should have appropriate hospital beds, operating room tables, commodes, and shower chairs. The weight limit of a standard hospital bed is normally 500 lb, whereas a bariatric bed can hold up to 1,000 lb with special features such as side rail adjustment, larger width, and hand controls. Similarly, a standard wheelchair has a width of 18–22 inches, whereas a bariatric wheelchair can hold up to 750 lb and has a width of 24–30 inches. Transfer of patients is an important safety issue to both the patients and health care workers. Traditional lateral transfer devices include plastic bags, rollers, and slides. New transfer technology includes the air transport mats, which could allow the transfer of bariatric patients by a single person. Optimal facilities for care of bariatric surgical patients may be developed by a bariatric task force, which should consist of surgeons, nurses, transportation personnel, radiology, and clinic personnel. One of the goals of the bariatric task force is to investigate the facilities' resources and the need for additional bariatric equipment and thereby identify limitations of the facility. For example, many bariatric surgical centers can accommodate a 400-lb patient, but few centers have the capability to accommodate a patient weighing 700 lb. Personnel at each individual institution should know the limitations of equipment and resources of their facility and the surgeons should

select candidates for bariatric surgery based on their facility limitations.

Accidents or equipment problems relating to the care of bariatric surgical patients are not uncommon. In this survey, more than a quarter of institutions performing bariatric surgery reported accidents or equipment problems relating to the care of bariatric patients. The use of standard equipment in the management of morbidly obese patients can result in equipment malfunction, inaccurate information, and/or patient injury. We also found that institutions reporting accidents tended to report workers' compensation claims. Inadequate facility resources for the care of bariatric surgical patients put health care providers at risk for occupational injury. The transfer process of the patient from the operating room to the recovery room, to the ward, and to the radiology suite can be physically challenging for health care workers, particularly in patients with a body mass index greater than 60 kg/m^2 or who are bedridden. Specialized lifting devices and training of staff members in the transfer process are essential to minimize occupational injury. Forty percent of the responding institutions reported workers' compensation claims relating to the care of bariatric patients. Although this survey did not elucidate the causes of injury to the health care workers, it is known that patient transfer and manual handling tasks are common reasons for musculoskeletal injuries in the workplace. Retsas et al.[4] reported that approximately one third of all occupational injuries were associated with lifting patients, which comprised one half of all causes of injuries from direct patient care activities. The transfer devices offer a potential solution to reducing the harmful physical exposures to health care workers. Evanoff et al.[5] reported that implementation of patient lifts can be effective in reducing occupational musculoskeletal injuries to nursing personnel. Only 68% of institutions in this survey had available transfer devices for the care of bariatric surgical patients.

Credentialing of bariatric surgeons should be standardized to ensure optimal quality of surgical care for bariatric surgical patients. Like other subspecialties, bariatric surgeons need to demonstrate a baseline technical experience in this uniquely challenging patient population to be eligible for hospital privileges. The ASBS has published guidelines for granting privileges in bariatric surgery (Table 2). The surgeon's experience in bariatric surgery is one of the most important factors predictive of outcomes. The learning curve of laparoscopic gastric bypass has been estimated at 75−100 cases.[6,7] In a study of laparoscopic gastric bypass, early operative experience of the surgeon (first 75 cases) was associated with a longer hospital stay and more major complications.[7] Casey et al.[8] found that litigation associated with bariatric surgery was caused by patient pain and suffering or death related to complications of bariatric surgery. Often the surgeon believed that the primary reason for complications was error in technique.[8] Therefore, it is important for individual institutions to grant bariatric surgery privileges based on education, training, and experience. A review of the surgeon's outcome data within 6 months of initiation of a new program should be performed. Although we did not specifically examine the credentialing process at each individual institution, we did find that 40% of institutions considered bariatric surgery as a noncore privilege and only 60% of institutions required a minimum number of procedures to be performed by the surgeon before granting privileges. In addition, only 52% of institutions use qualitative outcome measures to evaluate the surgeons' competence for reappointment. The results from this survey suggest that institutions performing bariatric surgery should adopt the guideline put forth by the American Society for Bariatric Surgery in credentialing surgeons for bariatric surgery with an understanding that an experienced surgical team is an important component in maintaining

Table 2. Guidelines by the American Society for Bariatric Surgery for granting privileges in bariatric surgery

Global Credentialing Requirements
- Have credentials at an accredited facility to perform gastrointestinal and biliary surgery.
- Perform bariatric surgery within a multidisciplinary integrated program.
- Document a program in place to prevent, monitor, and manage complications.
- Document a system in place to provide follow-up.

Provisional Bariatric Surgery Privileges
- Complete a bariatric training course of at least 2 days.
- Document three proctored cases in which the assistant is a fully trained bariatric surgeon.
- Complete an approved preceptorship program.

Open or Laparoscopic Bariatric Surgery Privileges
- For open bariatric surgery, the surgeon must document three proctored cases in which the assistant is a fully trained bariatric surgeon, and document acceptable perioperative complication rates for 10 open bariatric surgical cases.
- For laparoscopic bariatric surgery, the surgeon must have privileges to perform open bariatric surgery and advanced laparoscopic surgery, document three proctored cases, and document acceptable perioperative complication rates in 15 laparoscopic bariatric surgical cases.

Adapted from "Recommendations for facilities performing bariatric surgery."[3]

the quality of surgical care for bariatric surgical patients.

The limitation to this survey is that we examined the practice of bariatric surgery only at academic hospitals that self-reported by responding to this survey. The survey was sent only to academic hospitals, and the results may not accurately reflect bariatric surgery practices at community hospitals. Further, acquisition of equipment is an ongoing process at most hospitals, and our survey represents past activity not accounting for continual improvement. Also, we did not quantify the number and severity of workers' compensation claims or the total institutional cost of such. The questions on credentials that asked whether a minimum number of operations was required did not stipulate the minimum requirement of the American Society for Bariatric Surgery guideline. Finally, we used the term "accidents" loosely, not necessarily implying patient consequences but rather including equipment malfunction and other correctable events.

CONCLUSIONS

The increasing number of bariatric surgical operations performed in the United States today requires that every effort be made to improve and ensure quality of care. The morbidly obese are a challenging population from an intraoperative technical standpoint as well as regarding the complexities of preoperative and postoperative management. Perioperative success depends on the presence of appropriate staffing, operating room and hospital facilities, and an experienced bariatric surgical team. This survey stressed the need for appropriate equipment and facilities capable of handling the morbidly obese and credentialing of bariatric surgeons. Morbidly obese patients have special facility requirements to ensure the safety of both the patients and the health care provider. Institutions performing bariatric surgery or institutions initiating a new bariatric surgery program should have the required operating room and hospital facility resources, trained staff, available specialists, and methods for long-term follow-up. By having these prerequisite resources, the likelihood of patient and health care worker injury and associated workers' compensation claims may be reduced. Credentialing of surgeons is an equally important task to maintain the quality of surgical care for bariatric surgical patients.

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Discussion

Dr. B. Schirmer (Charlottesville, VA): Congratulations on a very nice paper. Do you have any data that reflected outcomes in relationship to deficiencies? In other words, did you have any data that showed if an institution was deficient in these areas of equipment or credentialing, then it translated into lower outcomes? And my second question for you is, did you ask the question if any surgeons did have their privileges rescinded because of poor outcomes?

Dr. Nguyen: In this survey we did not inquire about outcome of bariatric surgery, but we did find that lack of appropriate bariatric equipment can result in accidental injury and noted an association between reports of accidents and workers' compensation claims. We have no information on restriction privileges.

For your second question, what about outcomes related to surgeon's credentialing? We did not ask that in this survey.

Dr. M. Murr (Tampa, FL): Ninh, I enjoyed your presentation. Is there a way to track patient safety data and correlate it to the absence of a core bariatric program or specialized equipment?

Dr. Nguyen: Each individual institution should have a mechanism to track accidental injury and patient safety data, particularly for bariatric patients.

Dr. J. Kral (Brooklyn, NY): This is extremely important now, with a cottage industry setting up bariatric surgical services. As you pointed out, the willingness of institutions to invest is of key importance, but I want to emphasize that the investment must be up front. The current business model makes hospital owners unwilling to provide enough resources to do a safe or adequate job. For you who are entering into this field: Don't get "snookered" into the mentality of "pay-as-you-go, show your results, do enough cases and we will let you grow with your program"! Your survey very nicely shows that there is often a failure to provide all of the necessary services.

I would like to make a plea for one particular service. There has to be a socioeconomic back-up team to see that the patients have resources, partly intellectual and cultural to understand the necessity of follow-up and partly financial. Patients have to afford to be able to keep doctors' appointments, they have to afford the blood tests, or there has to be means to remunerate this. Unfortunately, some of the disasters we are seeing with long-term follow-up are because the resources weren't there. It is crucial to look at the totality of the investment.

Let me take issue with one point—a real dilemma of this whole field! If you are operating on 150 patients a year, in 3 years you will have 450 patients. If you fail to recognize that every one of those 450 patients is going to need individualized care over a long period of time, you are making a serious mistake. This work cannot be *outsourced*, as is currently the case.

The old model, typified by cardiothoracic surgeons who are the technicians but have teams that do everything preop and everything postop, doesn't work as well with bariatric surgery patients. They require more of a "hands-on" approach from the surgeon. Only the surgeon can gather all of the relevant information—it is difficult to delegate this to nurses or bariatric physicians. Several programs have failed because of a self-defeating mechanism: do "enough" volume for a long enough period of time and you will have to stop operating just to take care of your patients. This behavioral surgery requires life-long maintenance.

Dr. Nguyen: I agree that the hospital should invest in appropriate bariatric equipment if they want to have a bariatric program. Our study demonstrated that appropriate equipment is not only essential but it is important to ensure the safety of the patient and health care worker. I will give an example. When I began at UC-Davis we already had an established program in bariatric surgery. However, when I moved down to UC-Irvine, I had to start up a bariatric practice from scratch. An integral part of starting a new bariatric program is to form a "bariatric task force." This task force will be responsible for identifying deficient areas in the management of bariatric patients and essential equipment for the care of morbidly obese patients. You should evaluate all areas of the hospital, including the waiting room, the clinic, the operating room, the recovery room, the hospital ward, and even the radiology suite. As a bariatric surgeon, it is your responsibility to ensure that the hospital facility has appropriate equipment and a staff well trained in the care of bariatric patients.

Dr. G. Telford (Milwaukee, WI): This is a continuation of the last discussant's comments. I think some surgeons who are considering performing this operation don't know the magnitude of the problem. We have gotten to the point in Milwaukee where people advertise that they have a nicer waiting room with larger chairs, and the next thing you know, you have got to have new furniture.

Have you put together a list from your experience of what you recommend to surgeons who are contemplating performing gastric bypass procedures?

Dr. Nguyen: That is a good point. The American College of Surgeons has published guidelines with recommendations for facilities performing bariatric surgery. Their guidelines, however, contain only broad categories of recommendations with little specifics. I think we need something more concrete and specific. The American Society for Bariatric Surgery is coming out with the guidelines for Centers of Excellence and may contain a more specific guideline in terms of facility resources and equipment.

Dr. L. Way (San Francisco, CA): Your survey showed some deficiencies that you recommended be improved, but I just wonder, it seems to me that the hospitals you surveyed form a unique group that is likely to be the best there is.

The questions I have are, what proportion of bariatric surgery is being done in hospitals like this, and do you have any information about the other group, which could even be the majority, for all I know, and the status of their programs in the same terms that you analyzed these?

Dr. Nguyen: You are correct. These are academic health centers, and of the 120 or so academic centers associated with the University Health System Consortium, 84 were performing bariatric surgery in 2002. That number increased from 64 in 1999. We have no data available for community hospitals and private clinics performing bariatric surgery.

G Protein Polymorphisms Do Not Predict Weight Loss and Improvement of Hypertension in Severely Obese Patients

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Both the gene encoding the α subunit of G stimulatory proteins (*GNAS1*) and the β3 subunit gene (*GNB3*) of G proteins are associated with obesity and/or hypertension. Moreover, the TT/TC825 polymorphism of *GNB3* predicts greater weight loss than the CC825 polymorphism in obese patients (mean body mass index, 35 kg/m^2) undergoing a structured nonpharmacologic weight loss program. Gastric banding enforces a low-calorie diet by diminishing the need for volitional adherence. It is unknown whether these polymorphisms predict the variable weight loss in patients after bariatric surgery. Three hundred and four severely obese patients (mean ± SEM age, 42 ± 1 years; 245 women and 59 men; mean ± SEM body mass index, 43.9 ± 0.3 kg/m^2) followed prospectively for at least 3 years after surgery were genotyped for the *GNB3* C825T, G814A, and *GNAS1* T393 polymorphisms. All analyses were performed blinded to the phenotypic characteristics of the study group. Frequencies of polymorphisms were comparable to those previously published. No polymorphism studied predicted 3-year weight loss or was associated with high blood pressure in severely obese patients after gastric banding. Multivariate analysis of potentially confounding factors such as reoperation rate or use of sibutramine or orlistat revealed similar results ($P > 0.1$). Regardless of the mechanism(s) involved for these discordant findings, *GNB3* C825T, G814A, and *GNAS1* T393C polymorphisms do not seem to be reliable predictors of long-term weight loss. (J GASTROINTEST SURG 2004;8:862–868)

KEY WORDS: G protein, *GNB3* C825T, *GNB3* G814A, *GNAS1* T393C, severe obesity, treatment outcome

Obesity is a multifactorial disease caused by the interaction of genetic factors and the environment.[1] Sedentary lifestyle, high-fat energy-dense diet, and a genetic predisposition to obesity all play a part in the epidemic[2] affecting the health of populations worldwide.[3] Effective prevention and treatment to achieve weight loss and reduce comorbidities, such as the very costly components of the metabolic syndrome,[4] are mandatory. The most common primary methods of treatment of obesity require life-long volitional control of the quantity and/or composition of ingested nutrients and are disappointingly ineffective.[2,5] Operations restricting the capacity and outflow from the stomach, thus imposing a low-calorie diet with less need for volitional control,[6] are relatively effective for treating severe obesity.[7] However, just as for conventional dietary treatment, reliable predictors of poor weight loss and complications are lacking.

Recently, the C825T polymorphism[8] of the β subunit (*GNB3*) and the T393C polymorphism[9] of the α subunit (*GNAS1*) of G proteins were demonstrated to be highly predictive for the identification of obese individuals who would benefit from weight-lowering drugs such as sibutramine in addition to a structured weight loss program. Other studies indicate the value of the *GNB3* C825T polymorphism as a pharmacogenetic marker to predict responses to antihypertensive[10,11] and antidepressant drugs.[10,12]

Physiologic and cell biology investigations support the notion that the *GNB3* 825T allele status affects various body functions, including control of systemic,[13] renal,[14] and coronary blood flow[11]; atrial

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potassium currents[15]; lipolysis[16,17]; and immune responses.[18] Moreover, the *GNB3* C825T polymorphism has been the subject of many clinical and pharmacologic investigations. For example, the 825T allele has been shown to be associated with essential hypertension,[11] stroke,[19] left ventricular hypertrophy,[20] alterations in body weight regulation,[21–23] and transplant survival.[24] However, negative results from association studies[25–28] have also been published that may relate in part to ethnic effects.[21]

Another polymorphism (G814A) results in the replacement of glycine by serine at position 272; thus a highly conserved amino acid motif (Ile-Ile-Cys-Gly-Ile-Thr-Ser-Val) is affected.[29] This polymorphism is not associated with either obesity or hypertension, but haplotype analysis in the German sample indicates a significant association of the 814A allele with the 825C allele ($P = 0.0041$).[29]

The purpose of the present study was to evaluate whether the *GNB3* C825T and G814A polymorphisms and the *GNAS1* T393C polymorphism predict rate of hypertension and/or outcome (weight loss, reoperation rate) 3 years after implantation of an adjustable gastric band.

PATIENTS AND METHODS

Patients

Sample size estimation was performed based on the effects on weight loss using polymorphism 825-TT[8] frequency (13.1%) in obese subjects. A sample size of nine patients in each group would be sufficient to detect a more than 4-kg difference after 1 year at 95% power, both with an average of 0.001.[30] Therefore, minimal sample size was calculated to be 67. Taking into account the variability of reported frequencies (whites, 5%[27]–13.1%[8]), we decided to include at least 300 patients in the present study. The first 304 patients undergoing laparoscopic adjustable banding over a 5-year period, among 469 consecutive severely obese patients described earlier,[31] were entered into the study.

Patient characteristics were age of 42 ± 1 years (mean ± SEM), 81% female, height of 167 ± 1 cm (mean ± SEM), and body mass index (BMI) of 43.9 ± 0.3 kg/m^2 (mean ± SEM). Exclusion criteria were BMI of less than 35 kg/m^2, age younger than 18 or older than 70 years, and alcoholism or drug abuse determined at interview, or through information from family or referring physician.

A multidisciplinary team consisting of a physician specializing in obesity, a bariatric surgeon, a dietician, and a psychologist assessed each patient before laparoscopic gastric banding. Data were entered prospectively into our computerized database (Obesity Base 2000, Praxis, Zürich, Switzerland). Patients were fully informed about all procedures and gave written consent. The study was approved by the local ethics committee and complied with the Declaration of Helsinki.

Hypertension

Hypertension was diagnosed in patients taking hypotensive drugs or having elevated systolic and/or diastolic blood pressure (≥130 or 85 mm Hg, respectively).[4]

Gastric Banding

Laparoscopic adjustable gastric banding was performed as described earlier.[32,33] Briefly, the inflatable band encircled the cardia of the stomach and was sutured in place to form a small pouch (volume < 20 mL; Fig. 1). The injectable subcutaneous port was sutured external to the lower third of the sternum and attached via tubing to the inflatable gastric band. Four to 6 weeks after surgery, the band was inflated for the first time using contrast (Iopamiro 200, iopamidol; Bracco, Milan, Italy). Indications for inflation were less than 1-kg weight loss per month or absence of fullness reported during a semistructured interview after a standard meal (half the size of a typical preoperative meal). Deflation was indicated for obstruction, nightly aspiration, or vomiting more than twice per week.

Complications resulting in reoperation, blood pressure, and weight were recorded at each office visit. One patient died of myocardial infarction during her third postoperative year, and data up to her death were included in the analysis.

Insufficient weight loss, defined as less than 50% excessive weight loss after primary operation, or failure to lose weight continuously for the previous 3 months, or initial excessive weight loss of more than 50% but subsequent weight regain greater than 10% of the lowest weight achieved that reduces excessive weight loss to less than 50%, was treated medically by the addition of either sibutramine (serotonin and norepinephrine reuptake inhibitor, Reductil; Abbott, Baar, Switzerland)[34] or orlistat (gastrointestinal lipase inhibitor, Xenical; Roche, Basel, Switzerland)[35] or by reoperation with the addition of distal gastric bypass (biliopancreatic diversion with or without duodenal switch position)[36,37] depending on the clinical outcome before the diagnosis of insufficient weight loss.

DNA Genotyping

At least 18 months postoperation, 20 mL of EDTA venous blood was collected. DNA preparation from

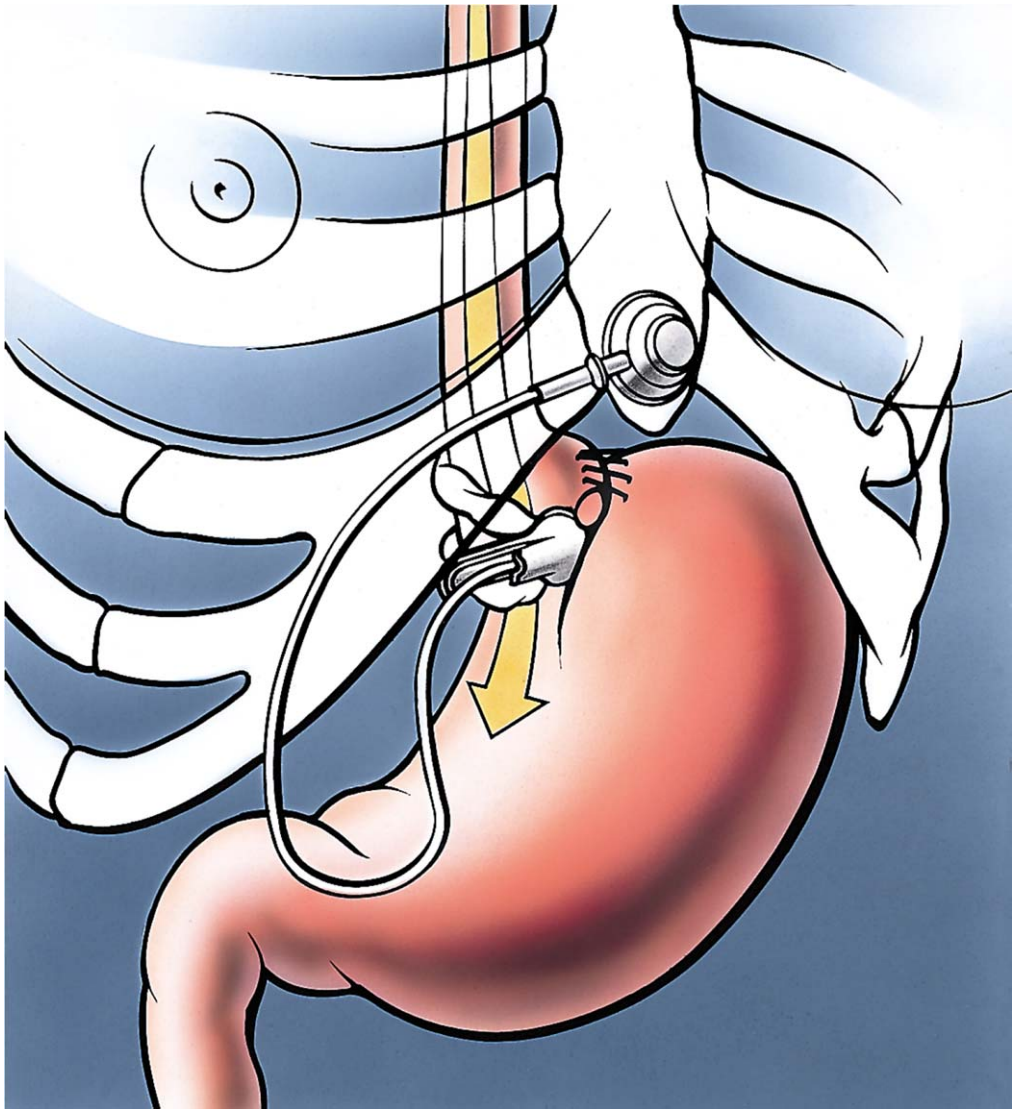

Fig. 1. Gastric band with port and tube system. The adjustable gastric band encircles the cardia of the stomach forming a small pouch. The tube leads from the gastric band to the port, which is sutured subcutaneously external to the lower third of the sternum allowing transcutaneous adjustment of the band.

EDTA-blood was performed on the GenoM-48 (GenoVision, Vienna, Austria) robotic workstation using the GenoPrep DNA isolation kit and the "Higher Yield" protocol according to the manufacturer's recommendations. Purified DNA was quantified with a GENios Plus spectrophotometer (Tecan Instruments, Männedorf, Switzerland) and PicoGreen (Molecular Probes, Eugene, OR) as fluorescent dye.

Genotypes of the *GNB3* G814→A and C825→T polymorphisms and of the *GNAS1* T393→C polymorphisms were determined by polymerase chain reaction (PCR) and subsequent sequence analysis.

For the *GNB3* G814→A and C825→T polymorphisms, the oligonucleotide primers 5′-TGACCCACTTGCCACCCGTGC-3′ (forward) and 5′-GCAGCAGCCAGGGCTGGC-3′ (reverse) were used. PCR was performed essentially as described by Rosskopf et al.[29]

The resulting PCR fragments (268 base-pairs) were sequenced in both directions on a ABI 3100 capillary sequencer with the forward and reverse PCR primers as sequencing primers and the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany).

For the *GNAS1* T393→C polymorphism, the biotinylated forward primer 5′-CTCCTAACTGACATGGTGCAA-3′ and the reverse primer 5′-TAAGGCCACACAAGTCGGGGT-3′ were used.

Table 1. Frequency of polymorphisms and outcome parameters 3 years after implantation of an adjustable gastric band

				P
Patients genotyped for the C825T polymorphism of *GNB3*	CC	TC	TT	
No. of patients (%)	143 (47.0)	122 (40.1)	39 (12.8)	
Weight loss (kg)	32.4 ± 2.4	30.8 ± 1.1	31.2 ± 0.8	0.888
Change in systolic blood pressure (mm Hg)	7 ± 1	5 ± 1	6 ± 3	0.953
Change in diastolic blood pressure (mm Hg)	7 ± 1	6 ± 1	7 ± 2	0.650
Change in frequency of hypertension (%)	15.3	13.1	15.3	0.299*
No. of reoperations (%)	34 (23.8)	30 (24.6)	10 (25.6)	0.968*
No. treated with sibutramine (%)	21 (14.7)	15 (12.3)	1 (2.6)	0.122*
No. treated with orlistat (%)	31 (21.7)	34 (27.9)	7 (17.9)	0.331*
Patients genotyped for the G814A polymorphism of *GNB3*	GG	GA	AA	
No. of patients (%)	266 (87.5)	37 (12.2)	1 (0.3)†	
Weight loss (kg)	31.1 ± 0.9	31.6 ± 2.4	28.4	0.422
Change in systolic blood pressure (mm Hg)	6 ± 1	1 ± 3	5	0.713
Change in diastolic blood pressure (mm Hg)	7 ± 1	7 ± 2	15	0.737
Change in frequency of hypertension (%)	15.8	5.5	0	0.557*
No. of reoperations (%)	63 (23.7)	11 (29.7)	0 (0)	0.616*
No. treated with sibutramine (%)	33 (12.4)	4 (10.8)	0 (0)	0.970*
No. treated with orlistat (%)	60 (22.6)	12 (32.4)	0 (0)	0.356*
Patients genotyped for the T393C polymorphism of *GNAS1*	TT	TC	CC	
No. of patients (%)	82 (27.0)	150 (49.3)	72 (23.7)	
Weight loss (kg)	31.2 ± 1.5	30.8 ± 1.2	31.8 ± 1.5	0.760
Change in systolic blood pressure (mm Hg)	3 ± 2	6 ± 1	6 ± 2	0.232
Change in diastolic blood pressure (mm Hg)	6 ± 1	7 ± 1	7 ± 1	0.156
Change in frequency of hypertension (%)	11.4	15.3	16.7	0.652*
No. of reoperations (%)	19 (23.2)	38 (25.3)	17 (23.6)	0.922*
No. treated with sibutramine (%)	8 (9.8)	20 (13.3)	9 (12.5)	0.725*
No. treated with orlistat (%)	21 (25.6)	34 (22.7)	17 (23.6)	0.881*

P values are calculated using MANOVA at 3 years.
**P* values analyzed using χ^2.
†This patient has been included in the GA group for statistical analysis.

The exact details of the PCR conditions are available on request. PCR products were analyzed by pyrosequencing with the reverse primer 5′-GGCACGTTCATCACACTCAG-3′ on the PSQ96 MA system (Biotage AB) according to the manufacturer's instructions. The sequencing results were analyzed with the PSQ96 SNP software.

Statistical Analysis

Analysis of variance for repeated measures or χ^2 was used where appropriate and corrected for multiple comparisons (SPSS version 11.0 for Windows, Chicago, IL). Weight loss and hypertension were analyzed by multivariate analysis taking into account potentially confounding parameters such as reoperation rate and the use of additional sibutramine or orlistat. Age, gender, and preoperative BMI were defined as covariance. All results are presented as mean ± SEM in the text, Table 1, and Fig. 1. Reported *P* values were two-sided.

RESULTS

Frequency of Polymorphisms and Selected Demographic Patient Data

Frequencies of *GNB3* C825T, G814A, and *GNAS1* T393C were comparable to those previously reported in obese white subjects[8,9,21] (Table 1) and were in the Hardy-Weinberg equilibrium (*GNB3* 825 CC: 0.7, TT: 0.4 and 814 GG: 0.9, AA: 0.1; *GNAS1* TT: 0.5, CC: 0.5). For all three polymorphisms, age ([in years]: *GNB3* 825: CC: 42 ± 1, TC: 41 ± 2, TT: 41 ± 2; $P = 0.574$; *GNB3* 814: GG: 42 ± 1, GA: 40 ± 2, AA: 54, $P = 0.339$; *GNAS1* 393: TT: 43 ± 1, TC: 41 ± 1, CC: 41 ± 1, $P = 0.535$), distribution of gender (females [in %]: *GNB3* 825: CC: 76.9, TC: 83.6, TT: 84.6; $P = 0.310$; *GNB3* 814: GG: 81.2, GA: 75.7, AA: 0, $P = 0.087$; *GNAS1* 393: TT: 82.9, TC: 82.7, CC: 73.6, $P = 0.230$), and preoperative BMI ([in kg/m^2]: *GNB3* 825: CC: 43.5 ± 0.4, TC: 44.4 ± 0.5, TT: 44.1 ± 0.9, $P = 0.416$; *GNB3* 814: GG: 44.1 ± 0.3,

GA: 43.2 ± 0.9, AA: 43.7, $P = 0.645$; *GNAS1* 393: TT: 43.9 ± 0.6, TC: 43.7 ± 0.4, CC: 44.5 ± 0.6, $P = 0.591$) were similar between patients homozygous for the wild-type alleles and heterozygous or homozygous for the variant alleles, respectively, as were systolic blood pressure ([in mm Hg]: *GNB3* 825: CC: 133 ± 2, TC: 130 ± 2, TT: 131 ± 3, $P = 0.611$; *GNB3* 814: GG: 131 ± 1, GA: 127 ± 3, AA: 130, $P = 0.429$; *GNAS1* 393: TT: 131 ± 2, TC: 130 ± 1, CC: 131 ± 2, $P = 0.939$), diastolic blood pressure ([in mm Hg]: *GNB3* 825: CC: 85 ± 1, TC: 85 ± 1, TT: 85 ± 2, $P = 0.928$; *GNB3* 814: GG: 85 ± 1, GA: 85 ± 2, AA: 100, $P = 0.315$; *GNAS1* 393: TT: 85 ± 1, TC: 84 ± 1, CC: 87 ± 1, $P = 0.190$), and patients with hypertension ([in %]: *GNB3* 825: CC: 60.1, TC: 67.2, TT: 61.5, $P = 0.480$; *GNB3* 814: GG: 64.7, GA: 51.4, AA: 100, $P = 0.217$; *GNAS1* 393: TT: 62.2, TC: 61.3, CC: 68.1, $P = 0.610$). There was only one patient homozygous for the A allele of the *GNB3* G814A polymorphism. This patient was included into the GA group for further statistical analysis.

Complications and Reoperations

Reoperation occurred cumulatively over 3 years in a total of 74 patients (8.1%/year) due to complications of the band or port/tube system (more than one complication occurred in eight patients [2.6%]). Port/tube complications consisted of infection (n = 5), port-site discomfort (n = 14), port dislocation (n = 2), and tube leakage (n = 4). Band complications were band slippage (n = 22), leak (n = 10), band intolerance (n = 19), and migration (n = 1). Complication and reoperation rates reported include all complications and reoperations occurring over the study period after both primary operation and reoperations. Additional intake of sibutramine was indicated in 12.2% of the patients (n = 37) for a mean duration time of 8.3 ± 1.0 months. Orlistat was prescribed in 23.7% of the patients (n = 72) with a mean time of 14.9 ± 1.9 months.

Genotype-Dependent Weight Loss

Three years after gastric banding, mean weight loss of the study group was 25% ± 0.6% of preoperative weight (31.2 ± 0.8 kg, $P < 0.001$). For each polymorphism analyzed, carriers of the homozygous wild-type, homozygous, or heterozygous variant genotype resulted in similar weight loss (Table 1). Defining age, gender, and preoperative BMI as covariance, in multivariate analysis, neither reoperation rate (C825T: $P = 0.374$; G814A: $P = 0.935$ and T393C: $P = 0.738$) nor additional use of weight-reducing drugs ($P > 0.2$) showed any significant impact of these potentially confounding factors on weight loss as related to the respective genotype.

Genotype-Dependent Outcome of Hypertension

After the patients lost 31.2 ± 0.8 kg of weight, systolic blood pressure (diastolic blood pressure) decreased by 6.1 ± 1.4 mm Hg (6.3 ± 0.8 mm Hg), resulting in blood pressure 3 years after gastric banding of 125 ± 0.9 mm Hg (78 ± 0.6 mm Hg) (Table 1). Rate of hypertension was reduced by 22.9% (preoperative, n = 192; after 3 years, n = 148, $P < 0.001$) during the study period. The effect of weight loss on hypertension 3 years after gastric banding was not modulated by any of three polymorphisms investigated, a finding that persisted after multivariate analysis (see Patients and Methods for details).

DISCUSSION

Obesity is considered to be "on track as No. 1 killer"[38] in humans! Therefore, efficient long-term strategies to reduce body weight, as achieved by bariatric surgery, are mandatory. Restrictive procedures, such as adjustable gastric banding, are very effective to induce long-term sustained weight loss in well-selected patients.[39] However, outcome, with respect to weight loss, varies considerably between patients. As we recently demonstrated, melanocortin-4 receptor gene variants determine the outcome of bariatric treatment of severe obesity.[40,41] The present study demonstrates that the three polymorphisms (*GNB3* C825T, G814A, and *GNAS1* T393C) of G proteins predict neither weight loss nor reduction in the rate of hypertension. Our findings are in contrast to earlier studies demonstrating that G protein polymorphisms might predict outcome of pharmacologic (sibutramine) and nonpharmacologic weight loss programs after 1 year.[8,9]

Operations restricting the capacity and outflow from the stomach, thus imposing a low-calorie diet with less need for volitional control,[6] are relatively effective in treating severe obesity.[7] One would expect that patients undergoing implantation of an adjustable gastric band who carry a polymorphism of *GNB3* or *GNAS1* would lose less weight than would patients who do not carry a variant investigated, in an analogous manner as observed in the patients undergoing nonpharmacologic weight loss programs that include caloric reduction. Therefore, the question arises of whether restriction achieved by an adjustable gastric band in the present study overrides the subtle polymorphism-dependent differences in

weight loss observed using a structured nonpharmacologic weight loss program.[8,9] Regardless of the mechanisms involved for these discordant findings, further studies are needed to elucidate potential metabolic pathways explaining the lack of polymorphism-related effect on weight loss in patients with gastric banding but a positive effect on weight loss in patients using lifestyle modifications.

The complications of bariatric surgery that result in costly reoperations represent a major drawback. We recently demonstrated that melanocortin-4 receptor gene variants are predictors of a five times higher complication rate in patients undergoing gastric banding.[40,41] Whether G proteins that are not obviously involved in appetite regulation, such as melanocortin-4 receptor variants, would also influence rate of complications after gastric banding is unknown. As was demonstrated in the present study, the complications of gastric banding that result in reoperations were not modulated by any of the three polymorphisms investigated. Therefore, one might hypothesize that G proteins are known modulators of intracellular lipolytic activity[16,17] but seem not to be involved in a feedback loop of appetite regulation.

The association between obesity and hypertension is well documented, although the exact nature of this relation remains unclear.[42] In contrast to earlier studies,[43] including patients with BMI above 30 kg/m^2, frequencies of patients carrying the different polymorphisms were similar in hypertensive and nonhypertensive patients in our study population (mean BMI, 43.9 $\pm$ 0.3 kg/m^2). As expected, average systolic and diastolic blood pressures decreased by 6.1 $\pm$ 1.4 and 6.3 $\pm$ 0.8 mm Hg ($P < 0.001$), respectively, after a weight loss of 25% $\pm$ 0.6% of preoperative weight (31.2 $\pm$ 0.8 kg, $P < 0.001$) at 3 years. In accordance with earlier studies,[44] the rate of hypertension decreased from 63.2% to 48.7% at 3 years ($P < 0.001$) as a consequence of considerable weight loss. Although earlier studies demonstrated an association of hypertension,[11] stroke,[19] and left ventricular hypertrophy[20] with some polymorphisms of G proteins investigated in the present study and despite the known functional significance of the *GNB3* TC825 polymorphism,[43] the rate of hypertension was not modulated before or 3 years after bariatric surgery by either *GNB3* TC825 polymorphism or any other polymorphisms investigated. Whether these discordant findings are due to the higher BMI in our patients compared with the population of Hauner et al.[8] or are due to ethnic differences[21,27] awaits further study. Thus, G proteins seem to predict hypertension in never weight–reduced obese subjects in a BMI-dependent manner but do not modulate the weight loss–induced change of blood pressure.

The described polymorphisms of G proteins are neither reliable predictors of intermediate-term weight loss nor predictors of resolution of hypertension due to considerable weight loss following adjustable gastric banding. The mechanisms underlying these discordant findings, with respect to weight loss and hypertension, between patients on a structured weight loss program and patients undergoing adjustable gastric banding remain to be elucidated.

We are indebted to our hard-working data entry team, particularly Andreas Juchli, Diana Strassmann, Beatrice Arn, and Andreas Kinzel; to Monica Scheumann for organizing data collection; to Markus Sprenger, Jürg Mühlemann, and the IT Team for managing ObesityBase; to Dr. Horber (Praxis) for the ongoing care of our patients; and to the team of Bioscientia for performing genotyping.

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The Benefits of a Dedicated Minimally Invasive Surgery Program to Academic General Surgery Practice

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In 2001, a dedicated minimally invasive surgery (MIS) program was established at a large university hospital. Changes included improvement and standardization of equipment and instruments, patient care protocols, standardized orders, and staff education. The aim of this study was to evaluate the impact of this program on an academic surgery practice. From January 1999 through October 2003, hospital and departmental databases were reviewed for all records pertaining to general surgery cases. Data trends were analyzed by regression analysis and are expressed as mean ± SEM. In 1999, 15.0 ± 0.1% of all general surgery cases were MIS cases compared with 30.2 ± 0.1% in 2003 ($P < 0.0001$). During this period, the number of patients requiring conversion from a laparoscopic to an open approach decreased from 14.4% to 4.0% ($P = 0.0007$). In 1999, 30% of appendectomies were laparoscopic, compared with 92% in 2003 ($P < 0.0001$). This increase in the rate of laparoscopic appendectomy resulted in a decrease in average length of hospital stay for all patients with acute appendicitis, from 5.5 ± 1.0 days in 1999 to 2.7 ± 0.2 days in 2003 ($P < 0.0001$), and a decrease in total hospital cost per case, from $6569 ± 400 in 1999 to $4819 ± 175 in 2002 ($P < 0.001$). Total operating room time per case for cholecystectomy decreased from 131 ± 3.7 to 108 ± 3.2 minutes ($P < 0.0001$), and actual surgery time decreased from 95 ± 4.1 to 74 ± 4.0 minutes ($P = 0.0006$). Implementation of a dedicated MIS program resulted in a significant increase in the number of MIS cases and percentage of general surgery cases performed by MIS. This increase in the utilization of MIS resulted in reduced length of stay and cost and has been accompanied by improvements in operating room efficiency. Changes in practice associated with development of an MIS program have had measurable institutional benefits. (J GASTROINTEST SURG 2004;8:869–873)

KEY WORDS: Laparoscopy, program development, cost, volume

The introduction of minimally invasive surgical (MIS) techniques to general surgical practice has revolutionized patient care. Although widely accepted as the standard of care in the management of many gastrointestinal disorders, such as gallstone and reflux disease, laparoscopic surgery is often viewed as inefficient and costly compared with open surgery. This perception is based on the acquisition cost of minimally invasive equipment, longer duration of surgery, and increased operating room expenditures. In addition, the introduction of MIS to a hospital requires training of hospital staff and personnel. In an era of limited resources and cost containment, these issues dampen hospital enthusiasm for introducing new laparoscopic technology and procedures.

Some evidence suggests that a dedicated MIS program provides improved operating room efficiency and surgical volumes compared with MIS performed outside the context of a dedicated program. For example, when comparing laparoscopic cholecystectomy performed by a dedicated MIS team compared with that performed without a trained team, decreased operative time, an improvement in patient care, and decreased costs to the patient have been observed.[1,2] In the academic environment, the introduction of a full-time director of MIS resulted in a 100%

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increase in laparoscopic surgery volume and an increase in MIS research and training.[3]

Recognizing the importance of the current and future role of MIA in academic general surgical practice and resident training and the potential benefit to an academic institution in the form of improvements in patient care, a dedicated MIS program was established at a large university hospital in 2001. The aim of this study was to evaluate the impact of this program on an academic surgery practice, including surgical volumes and approaches, operating room efficiency, and cost.

MATERIAL AND METHODS

Under the direction of a fellowship trained faculty member, changes were implemented to standardize and improve the MIS practice of a busy academic general surgery practice at a university hospital. Changes included improvement and standardization of equipment and instruments, patient care protocols, postoperative orders, and staff education. Instrument standardization included acquisition of new, reusable instruments and elimination of disposable instruments. In addition, imaging equipment was updated and made more available by increasing the number of towers. Changes in the surgical management of patients with acute appendicitis and symptomatic gallstones were studied to ascertain the impact of a dedicated MIS program on the practice of common general surgical operations. In addition, trends in the number of advanced laparoscopic gastrointestinal procedures were studied to ascertain the impact of this program on the referral practice within the institution. These index procedures included laparoscopic small bowel, colon, esophageal, stomach, hepatic, pancreatic, adrenal, and spleen surgery. Hospital and departmental databases were reviewed for all records pertaining to general surgery cases performed from January 1999 through October 2003. Data trends were analyzed by regression analysis.

RESULTS

After the introduction of a dedicated MIS program in 2001, a dramatic increase in the number of minimally invasive operations was observed. The average monthly number of minimally invasive general surgery cases increased from 25 in 1999 and 2000 to 61 in 2003 (Fig. 1, *A*). In addition, the percentage of all general surgery cases performed via a minimally invasive approach increased from 15% in 1999 and 2000 to nearly 30% in 2003 (Fig. 1, *B*). These trends were statistically significant ($P < 0.001$ by regression analysis).

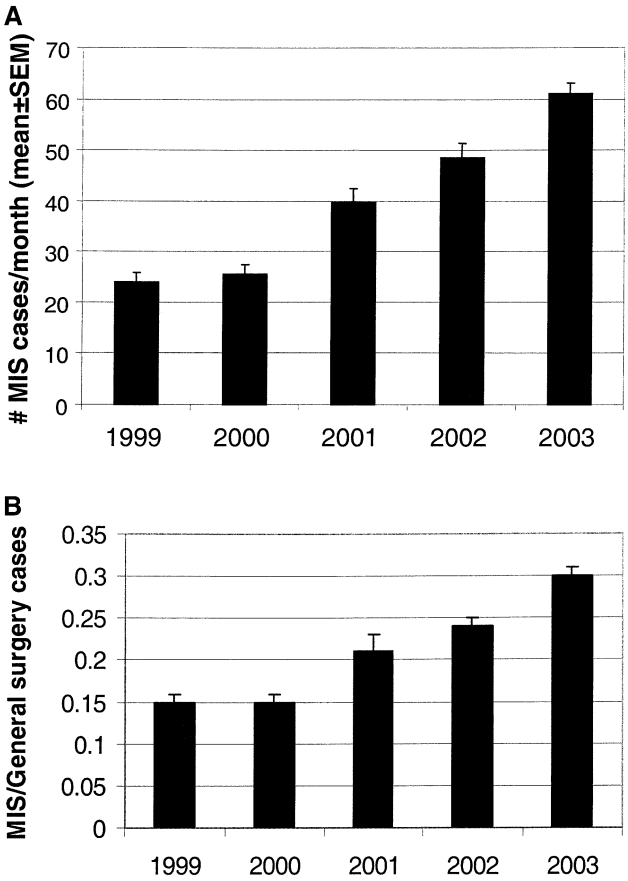

Fig. 1. (**A**) Average number of minimally invasive surgery (MIS) cases per month by year. (**B**) Percentage of all general surgery cases done via a minimally invasive approach. Data are given as monthly mean ± SEM; $P < 0.0001$ by regression analysis.

The impact of the dedicated MIS program on choice of operative approach and conversion rates were analyzed. In the case of appendectomy, a significant increase in the use of the laparoscopic approach was seen after introduction of the program. Thirty-one percent of appendectomies were laparoscopic in 1999. By 2003, 92% were laparoscopic (Fig. 2). This trend was highly significant ($P < 0.0001$). A significant increase in the number of index cases was also observed. In 1999, 37 advanced minimally invasive cases were performed. By 2003, the yearly number of the index cases had significantly increased to 145 per year (Table 1). This increase in the number of advanced laparoscopic cases included increases in the number of commonly performed operations and

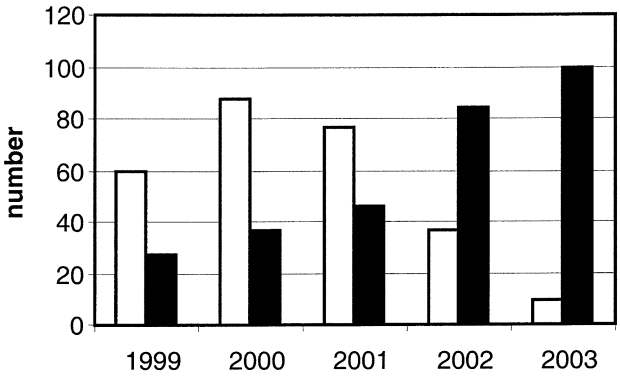

Fig. 2. Number of open (*open*) and laparoscopic (*shaded*) appendectomies by year. $P < 0.0001$ by regression analysis.

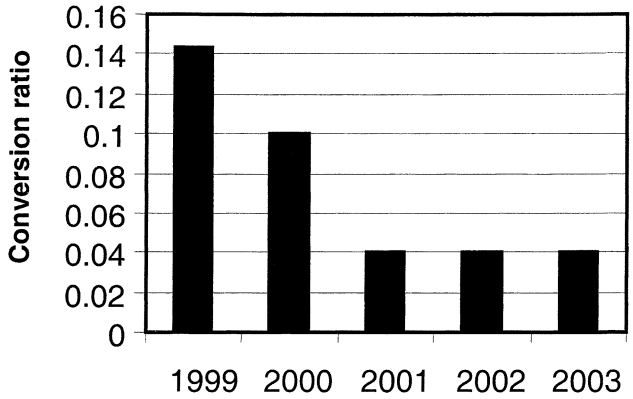

Fig. 3. Ratio of conversion of laparoscopic to open cholecystectomy by year. $P = 0.007$ by regression analysis.

the introduction of operations previously not performed at the institution. For example, the number of laparoscopic antireflux procedures increased more than two-fold from 34 in 1999 to 74 in 2003. Before 2001, all colectomies were open. With the introduction of the MIS program, the number of laparoscopic colectomies increased from 10 in 2002 to 20 in 2003. Similarly, 31 laparoscopic esophageal, hepatic, gastric, and pancreatic operations were performed between fall of 2001 to fall of 2003. During this same period, a significant reduction was observed in the rate of conversion of laparoscopic to open cholecystectomies, from 14.4% in 1999 to 4.0% in 2003 (Fig. 3).

Laparoscopic appendectomy was associated with a shorter length of hospital stay in any given year of the study (Fig. 4). Yearly average length of hospital stay ranged from 1.4 to 2.0 days for laparoscopic appendectomy and 3.2 to 5.5 days for open appendectomy. As the percentage of appendectomies performed by a laparoscopic approach increased between 1999 and 2002, the average length of hospital stay for all patients with acute appendicitis decreased from 5.5 days to 2.7 days ($P < 0.0001$). Similarly, a significant reduction in average total hospital costs for patients with acute appendicitis was observed. The average total cost was $6569 in 1999 compared with $4819 in 2002 (Table 2). Cost data were not available for the calendar year 2003.

Changes implemented with the MIS surgery program included standardization and improvement of instruments and imaging equipment and training of a dedicated nursing and operating room staff. For laparoscopic cholecystectomy, average disposable instrument costs decreased from $526 to $119 per case. These changes also resulted in significant improvements in operating room efficiency. A significant reduction in overall operating room time and surgery times was observed. The mean ± SEM operating room times for patients undergoing laparoscopic cholecystectomy decreased from 131 ± 3.7 minutes in 1999 to 108 ± 3.2 minutes in 2003 (Fig. 4). The mean ± SEM surgical times decreased from 95 ± 4.1 minutes in 1999 to 74 ± 4.0 minutes in

Table 1. Advanced minimally invasive cases per year

Year	No. of cases
1999	37
2000	37
2001	60
2002	96
2003	145

Advanced minimally invasive surgical cases include esophageal, gastric, colon, small bowel, liver, pancreas, spleen, adrenal.

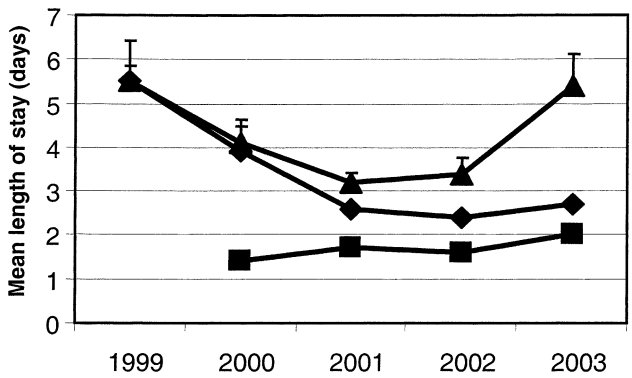

Fig. 4. Average length of hospital stay by year for patients undergoing appendectomy by approach: all patients undergoing appendectomy (diamond line), patients undergoing laparoscopic appendectomy (square line), and patients undergoing open appendectomy (triangle line). Data are given as yearly mean ± SEM; $P < 0.001$ by regression analysis for all patients.

Table 2. Appendectomy costs by approach

	Mean total hospital costs ($)		
	All patients*	Laparoscopic	Open
1999	6569	Not Available	6569
2000	5662	3318	5846
2001	4646	4086	4982
2002	4819	4224	6432

Data are yearly mean cost in U.S. dollars.
*$P < 0.001$.

2003. These trends were highly significant ($P < 0.0001$ and $P = 0.006$, respectively) (Fig. 5).

DISCUSSION

In the fall of 2001, a dedicated MIS program was established at a large academic, referral center. Changes instituted under the direction of a fellowship-trained program director included improvement and standardization of equipment and instruments, patient care protocols, standardized postoperative orders, staff education and establishment of a dedicated MIS operating room team, and limited marketing on the part of the hospital and health plan. The implementation of this program has resulted in significant changes to the general surgery practice.

Increases in the number of MIS cases and the percentage of general surgery cases performed via a minimally invasive approach were observed. This was accompanied by an increase in the number of advanced laparoscopic surgery cases not previously performed at the institution. These increases in case volume have previously been reported elsewhere.[3] In addition, a significant change in the operative approach to common diseases was observed. At the current time, more than 90% of appendectomies are performed via a laparoscopic approach, whereas less than one third were performed laparoscopically before development of the program. This represents an evolution in surgeon preference, likely in response to improvement in feasibility of laparoscopic appendectomy stemming from improvements in imaging, equipment, and staff training.[4]

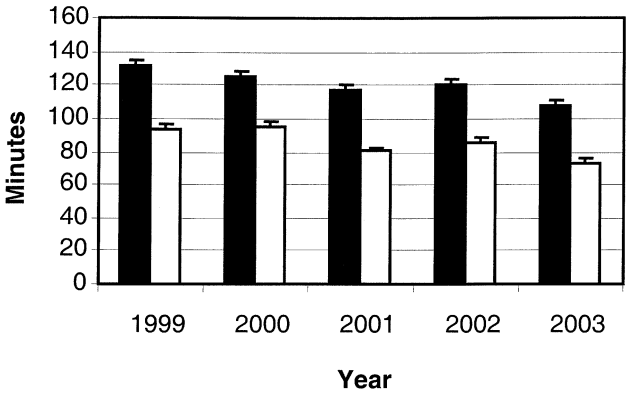

Fig. 5. Mean ± SEM operating room time (*filled bar*) and surgery time (*open bar*) for laparoscopic cholecystectomy by year. Both trends are significantly shorter, $P < 0.001$ by regression analysis.

The increase in the use of a laparoscopic approach to common diseases such as appendicitis and more complicated advanced minimally invasive operations has also resulted in significant reductions in length of hospital stay and hospital cost. In this study, length of hospital stay was significantly shorter for patients with acute appendicitis treated laparoscopically compared with those treated with an open approach. As the use of laparoscopic appendectomy increased, the overall length of hospital stay for patients with acute appendicitis decreased by 2.8 days, from 5.5 days in 1999, when the majority of appendectomies were open, to 2.7 days in 2003, when most appendectomies were laparoscopic. At our current volume of 140 appendectomies per year, this is a reduction of 392 patient-days per year, creating an opportunity for additional hospital admissions for other conditions. Similarly, a dramatic reduction in the total hospital costs associated with treating patients with acute appendicitis has occurred. Average cost associated with the laparoscopic approach was significantly lower than cost for the open approach. As the percentage of patients treated via a laparoscopic approach increased, overall total hospital costs associated with the treatment of all patients with acute appendicitis decreased an average of $1750 per patient. This translates into a savings of $245,000 per year for the institution. Further savings were generated through the use of reusable instruments, the standardization of surgeon preference cards, and the use of patient care protocols.[5,6]

In addition to savings resulting from decreased length of hospital stay and standardization of instruments, improvements in operating room efficiency and surgery times were observed. Others have reported similar improvements in operating room efficiency.[1,7] At our institution, a 23-minute reduction in overall operating room time was observed between 1999 and 2003. Most of this time savings resulted from a 21-minute reduction in average surgery times. At a current volume of approximately 350 cholecystectomies per year and an operating room cost of $17 per minute, this translates into a potential savings of $136,850 per year. As seen in other studies, we observed an added benefit of a dedicated minimally invasive team in a lower rate of open conversion.[7,8]

These data may be criticized based on the retrospective and unmatched nature of data collection and comparisons between open and laparoscopic approaches. The purpose of this study was not to compare surgical approaches but rather to provide an analysis of the impact of program development on the overall practice of general surgery at our institution. The increased use of a laparoscopic approach to common diseases like appendicitis and gallstones has resulted in significant reductions in hospital stay and cost to the institution. Similar dramatic savings have been previously reported.[6,7] In addition, improvements in patient outcomes with lower conversion rates and increased exposure of the surgical trainees to advanced laparoscopic procedures have occurred. At our institution, a dedicated MIS program is an asset and worthwhile investment for the academic surgery department and hospital.

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Visuospatial Skills and Computer Game Experience Influence the Performance of Virtual Endoscopy

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Advanced medical simulators have been introduced to facilitate surgical and endoscopic training and thereby improve patient safety. Residents trained in the Procedicus Minimally Invasive Surgical Trainer-Virtual Reality (MIST-VR) laparoscopic simulator perform laparoscopic cholecystectomy safer and faster than a control group. Little has been reported regarding whether factors like gender, computer experience, and visuospatial tests can predict the performance with a medical simulator. Our aim was to investigate whether such factors influence the performance of simulated gastroscopy. Seventeen medical students were asked about computer gaming experiences. Before virtual endoscopy, they performed the visuospatial test PicCOr, which discriminates the ability of the tested person to create a three-dimensional image from a two-dimensional presentation. Each student performed one gastroscopy (level 1, case 1) in the GI Mentor II, Simbionix, and several variables related to performance were registered. Percentage of time spent with a clear view in the endoscope correlated well with the performance on the PicSOr test ($r = 0.56$, $P < 0.001$). Efficiency of screening also correlated with PicSOr ($r = 0.23$, $P < 0.05$). In students with computer gaming experience, the efficiency of screening increased (33.6% ± 3.1% versus 22.6% ± 2.8%, $P < 0.05$) and the duration of the examination decreased by 1.5 minutes ($P < 0.05$). A similar trend was seen in men compared with women. The visuospatial test PicSOr predicts the results with the endoscopic simulator GI Mentor II. Two-dimensional image experience, as in computer games, also seems to affect the outcome. (J GASTROINTEST SURG 2004;8:874–880)

KEY WORDS: Medical simulators, endoscopy, endoscopic training, validation studies, visuospatial tests

The development of modern technology in natural orifice surgery, which makes more therapeutic options possible, has increased the demands on the examiner. In endoscopic retrograde cholangiopancreatography, for example, there is a shift from a majority of previously diagnostic examinations to new therapeutic endoscopic techniques, with noninvasive magnetic resonance cholangiopancreatography replacing most of the invasive diagnostic endoscopic retrograde cholangiopancreatography procedures.[1,2] The increasing therapeutic skills necessary to be an expert examiner together with the large number of procedures necessary to gain competence pose a need for improved education and training.[3] Flexible endoscopy is the second most common procedure performed by surgeons in the United States,[4] but it is not incorporated to a sufficient extent, in neither Sweden nor the United States, in the curriculum of gastroenterologists or surgeons. Virtual endoscopic training could be a way to increase the technical skills, because natural orifice surgery requires that the examiner is familiar with both the pathophysiologic findings and the handling of the instrument. The introduction of advanced medical simulators has added a new dimension in the training of technical skills without causing additional trauma or complications to the patients.

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Seymour et al.[5] demonstrated, in a prospective randomized, double-blinded study, that residents who reached expert criterion levels in the Procedicus Minimally Invasive Surgical Trainer-Virtual Reality (MIST-VR) (Mentice AB, Gothenburg, Sweden) laparoscopic simulator performed laparoscopic cholecystectomy 29% faster and were five times less likely to make mistakes than a control group without simulator training. Sedlack and Kolars[6] demonstrated that introducing a 6-hour curriculum in computer-based colonoscopy simulation provided a significant advantage to gastrointestinal fellows compared with traditionally trained ones.

The virtual endoscopy simulator GI Mentor II (Simbionix, Cleveland, OH) can identify differences between beginners and experts.[3,4] Little, however, has been reported as to whether factors like gender, computer gaming experience, and visuospatial skills have any effect on the performance of virtual endoscopy. Furthermore, the importance of flow as a factor influencing the outcome in natural orifice surgery has not been described. Flow has been described as an extremely rewarding experience that occurs when a person is fully involved in an activity. To experience flow while being engaged in any activity, individuals must perceive a balance between their skills and the challenges posed by the object with which they interact, and both their skills and challenges must be above a critical threshold.[7–9]

The aim of this study was to investigate whether these factors predict the outcome of virtual gastroscopy performed by medical students.

MATERIAL AND METHODS

Seventeen medical students at Karolinska University Hospital—Campus Huddinge participated in the study. None of them had previous experience with the simulators. They were all attending the surgical semester. All of them performed the test program on an individual basis. They received standard oral instructions by one test leader (L.E.). Only the test subjects who were supervised by the main test leader (L.E.) were included in this study to avoid discrepancies in the oral instructions that were given. The test program consisted of three parts. The first part was a visuospatial test, the Pictorial Surface Orientation test (PicSOr). The PicSOr test was made available to us courtesy of Dr. Anthony Gallagher, Emory Endosurgery Unit, Emory University School of Medicine, Atlanta, GA. The PicSOr test was validated by Gallagher et al.[10] and is described in Fig. 1.

The students then performed a gastroscopy (case 1, module 1) in the GI Mentor II (Fig. 2).

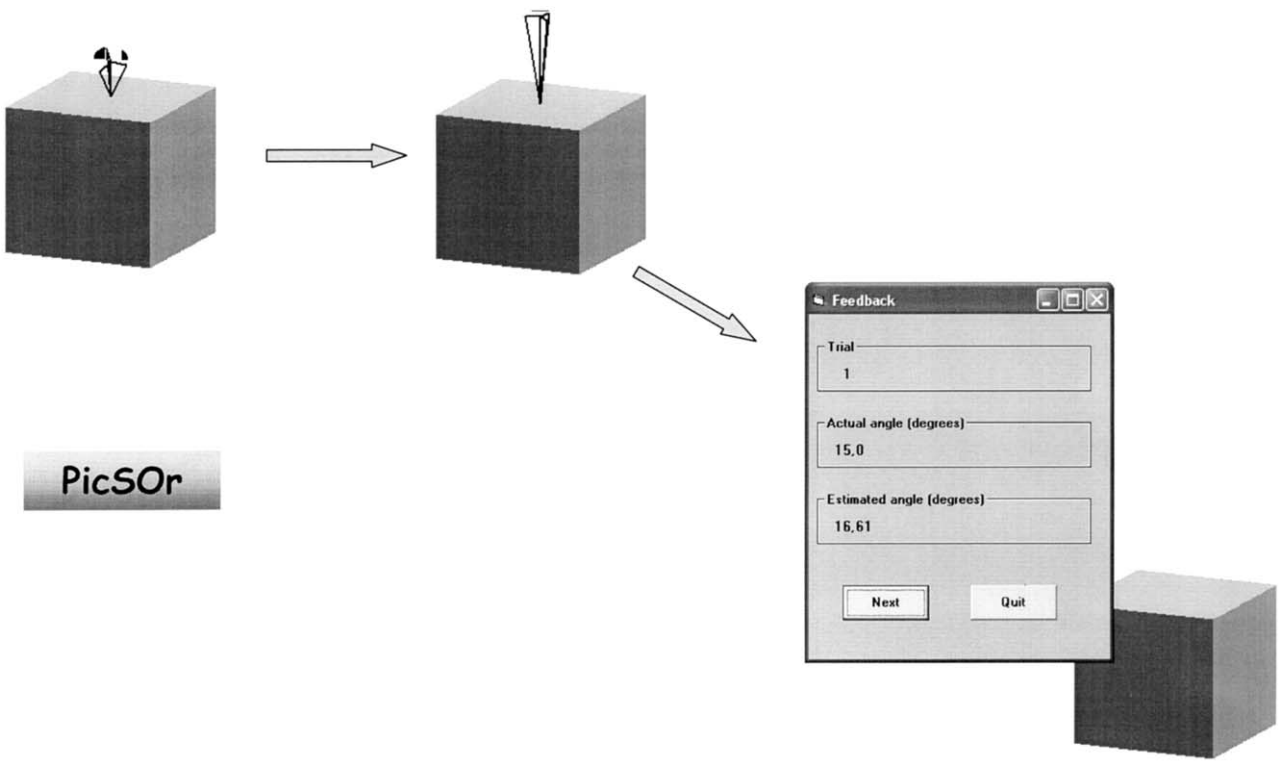

Fig. 1. Pictorial Surface Orientation (PicSOr) test. Adapted from Gallagher et al.[10]

All students were asked to perform the gastroscopy in accordance with the following instructions.

1. Go down to the second duodenum as quickly as you can with the instrument.
2. On your way up, inspect as much of the mucosa as you can.
3. Perform retroversion of the instrument in the stomach and inspect the cardia from below.
4. After inspection of the cardia, straighten out the instrument and inspect parts of the mucosa not yet inspected.
5. On withdrawal of the instrument from the esophagus, deflate the stomach.

The test subjects were also asked to fill out a questionnaire, providing some background factors like age and experience with computer games and with medical simulators.

Computer game experience was defined as one of two options: occasionally or daily. Not having computer game experience was defined as never playing computer games.

Finally, they were asked to fill out a questionnaire to measure the factors associated with flow like enjoyment, concentration, feeling of control, exploratory use, and challenge that they thought the virtual endoscopy posed. The flowsheet was based on the questionnaire by Ghani and Deshpande[11] (Fig. 3). Participation was voluntary, and the study was approved by the local ethics committee.

Statistical Analysis

Data were analyzed using the statistical software JMP 5.1 for Windows (SAS Institute Inc., Cary, NC) and presented as mean ± SEM values. Student's unpaired *t* test was used for comparisons of two mean values in groups with a normal distribution. In groups not normally distributed, the nonparametric Wilcoxon/Kruskal-Wallis test (rank sums) were used. For comparison between the PicSOr scores and the metrics of the GI Mentor II, linear regression analysis was used.

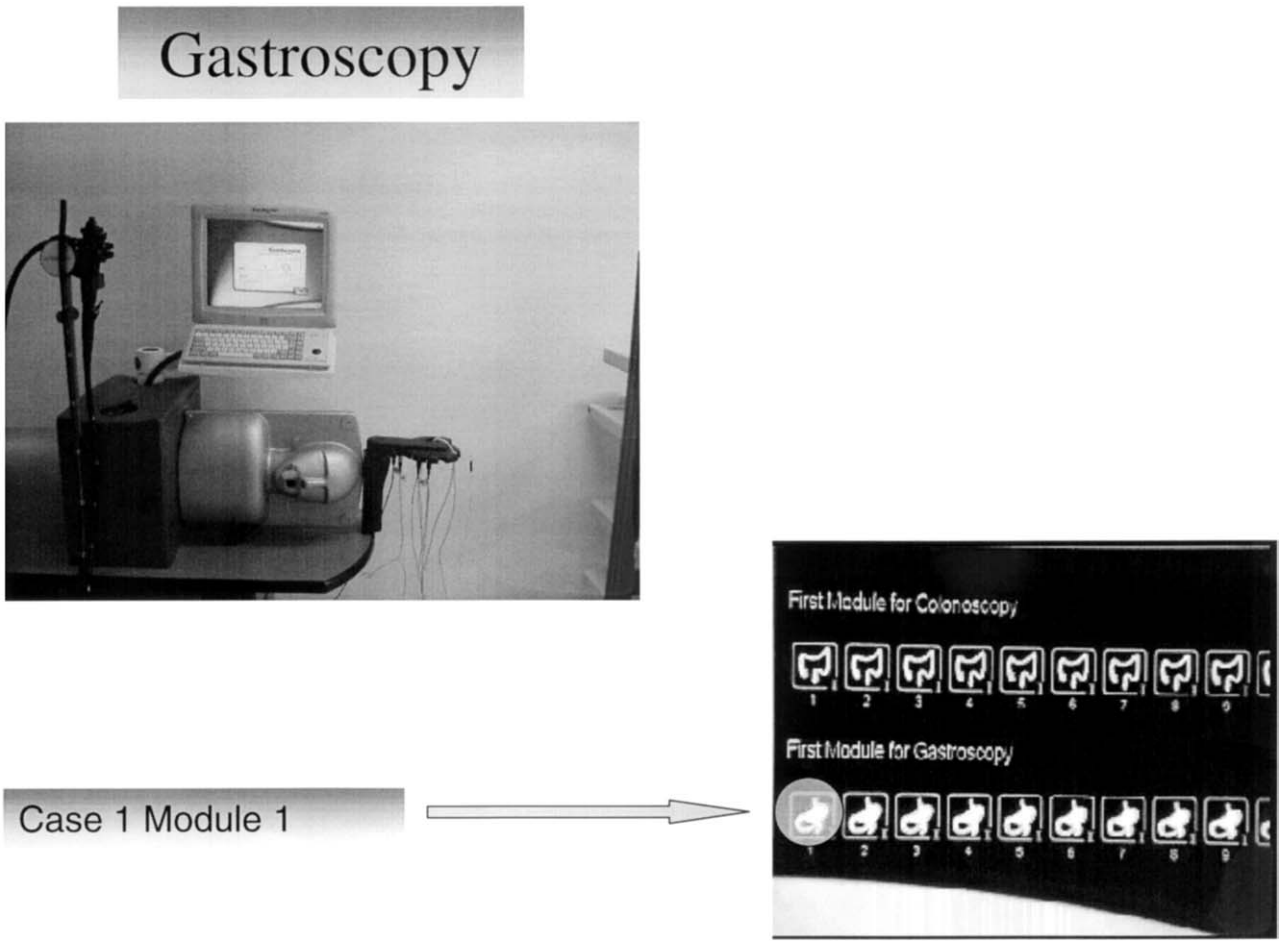

Fig. 2. GI Mentor II.

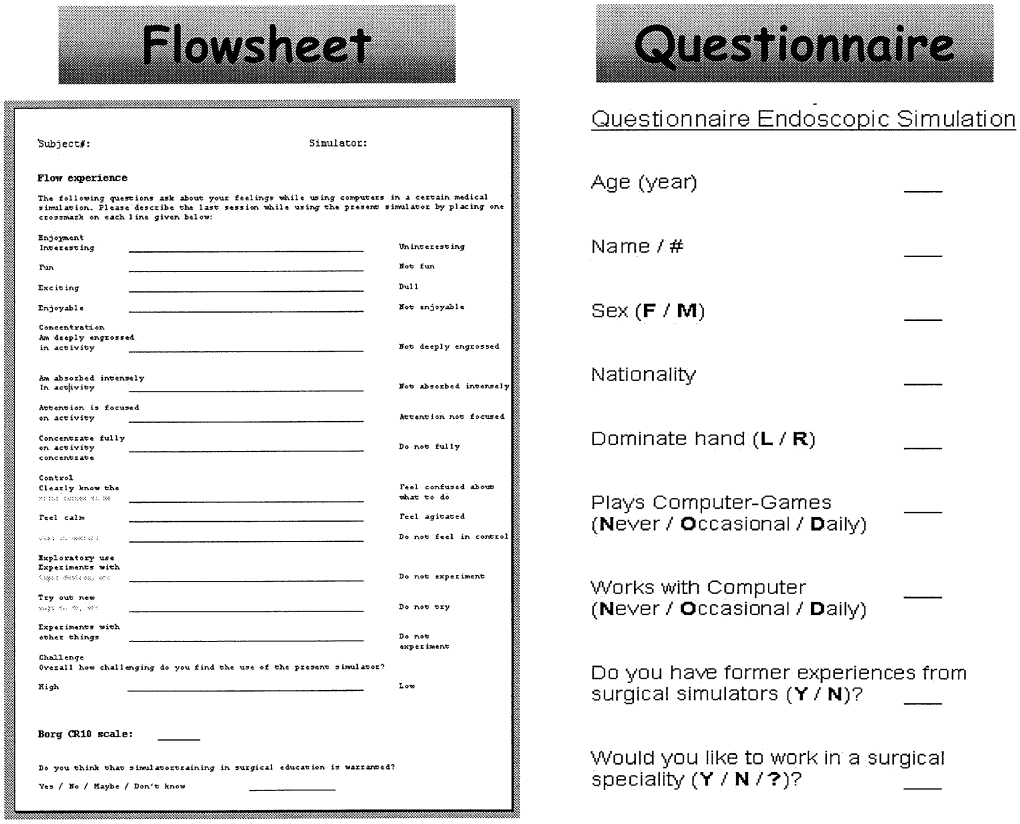

Fig. 3. Flowsheet was based on the questionnaire by Ghani and Deshpande.[11]

RESULTS

The results of the PicSOr test correlate well with the metrics of the GI Mentor and are summarized in Table 1. The correlations between PicSOr and two of the GI Mentor parameters—"Percent of time spent with a clear view" and "Efficiency of screening"—are significant, although the R^2 values are not very prominent. The correlations are gender specific, demonstrating a high correlation in men, whereas the correlations between PicSOr and the GI Mentor metrics are not significant in women. The correlation between some of the individual parameters of the flowsheet and metrics of the GI Mentor also seem to be gender specific. The parameter "Feeling in control" in the flowsheet correlated much better with "Efficiency of screening," "Percent of mucosal surface examined," and "Total endoscopy time" in men than in women, whereas the parameter "Exciting" demonstrated a different pattern with a stronger correlation with "Efficiency of screening" in women. The parameter "Exciting" did not correlate significantly with "Percent of mucosal surface examined" of the GI Mentor in women but demonstrated a P value of 0.08 compared with 0.52 in men. The results are summarized in Table 2.

When both the PicSOr test and the parameter "Control" of the flowsheet were correlated to the performance in the GI Mentor, there were gender-specific differences. In men, there was a good correlation with "Efficiency of screening" ($P = 0.05$) and "Percent of time spent with clear view" ($P < 0.05$), whereas there was no correlation in women. The parameter control is the mean value of the three individual parameters "Clearly know the right things to do," "Feel calm," and "Feel in control." In women, there was, on the other hand, a correlation between PicSOr and "Enjoyment" and the parameter "Percent of the mucosal surface examined" ($P < 0.05$) in GI Mentor. In men, "Enjoyment" and PicSOr correlated with "Percent of the mucosal surface examined" ($P < 0.05$) and "Efficiency of screening" ($P < 0.05$). The parameter "Enjoyment" is the mean value of the four individual parameters: "Interesting," "Fun," "Exciting," and "Enjoyable." There were no gender-specific differences between the five subgroups of the flowsheet: "Enjoyment," "Concentration," "Control," "Exploratory use," and "Challenge."

Table 1. Summary table of the correlation between the performance of the PicSOr test and metrics of the GI Mentor

Performance	R^2	*P*	F ratio
Total population (n = 17)			
Total endoscopy time (secs)	0.13	0.15	2.24
Percent of time spent with clear view	0.56	<0.001	18.78
Efficiency of screening	0.23	0.05	4.54
Percent of mucosal surface examined	0.18	0.09	3.27
Male population (n = 8)			
Total endoscopy time (secs)	0.39	0.10	3.80
Percent of time spent with clear view	0.66	<0.05	11.85
Efficiency of screening	0.47	0.06	5.23
Percent of mucosal surface examined	0.39	0.10	3.85
Female population (n = 9)			
Total endoscopy time (secs)	0.02	0.69	0.17
Percent of time spent with clear view	0.05	0.56	0.38
Efficiency of screening	0.15	0.31	1.18
Percent of mucosal surface examined	0.32	0.11	3.28

Although the two groups "PC gamers" (n = 5) and "Nongamers" (n = 12) were different in size and the gaming group was small, there was a significant difference in "Efficiency of screening." The students who played computer games were 11% more efficient than those who did not play computer games. The students who played computer games were also 1 minute 24 seconds faster in performing the virtual endoscopy task. There was a trend toward PC gamers inspecting more of the mucosal surface ($P = 0.07$) and spending less time with a clear view ($P = 0.07$) (Table 3).

DISCUSSION

Our aim was to determine whether background factors and visuospatial skills of the test subjects were correlated to their performance in the virtual endoscopy simulator GI Mentor II. The PicSOr test was highly predictive of the outcome in one of the gastroscopy metrics ("Percent of time spent with a clear view") and demonstrated a significant but not as strong correlation with "Efficiency of screening" (Table 1). The finding is in accordance with that of Gallagher et al.,[10] who found a correlation between PicSOr and the performance in a laparoscopic cutting task. In our study, there were gender-specific differences in the correlation between PicSOr and the metrics of the GI Mentor, with a stronger correlation found in men. In the study by Gallagher et al., there was a predominance of males (14 men and 4 women in one of the series). Gender-specific differences in the performance of laparoscopic simulators like the Minimally Invasive Surgical Trainer-Virtual Reality (MIST-VR) have been previously demonstrated.[12] In our study, we did not, however, find any significant gender-specific differences in the performance of the simulator, although there was a trend toward men

Table 2. Summary table of the correlation between individual parameters of the flowsheet and metrics of the GI Mentor

	EXCITING			FEEL IN CONTROL		
Performance	R^2	*P*	F ratio	R^2	*P*	F ratio
Total population (n = 17)						
Total endoscopy time (secs)	0.11	0.20	1.83	0.02	0.59	0.30
Percent of time spent with clear view	0.04	0.46	0.59	0.14	0.14	2.44
Efficiency of screening	0.05	0.37	0.84	0.03	0.51	0.46
Percent of mucosal surface examined	0.03	0.42	0.53	0.07	0.31	1.09
Male population (n = 8)						
Total endoscopy time (secs)	0.01	0.91	0.01	0.59	<0.05	8.70
Percent of time spent with clear view	0.28	0.18	2.30	0.13	0.38	0.90
Efficiency of screening	0.04	0.63	0.26	0.66	<0.05	11.56
Percent of mucosal surface examined	0.05	0.58	0.33	0.62	<0.05	9.96
Female population (n = 9)						
Total endoscopy time (secs)	0.25	0.17	2.33	0.20	0.23	1.75
Percent of time spent with clear view	0.18	0.26	1.50	0.38	0.08	4.32
Efficiency of screening	0.04	**<0.05**	6.34	0.13	0.33	1.08
Percent of mucosal surface examined	0.37	0.08	4.09	0.06	0.52	0.45

Table 3. Influence of computer gaming experience on the performance in the GI Mentor II

Performance	PC gamers (n = 5)	*P*	Nongamers (n = 12)
Total endoscopy time (seconds)	385 ± 22	<0.05	469 ± 23
Percent of time spent with clear view	94.2 ± 2.6	0.07	97.0 ± 0.5
Efficiency of screening	33.6 ± 3.1	<0.05	22.6 ± 2.8
Percent of mucosal surface examined	50.8 ± 2.3	0.07	44.9 ± 1.7

performing better in two of the metrics ("Time of endoscopy," $P = 0.07$, and "Efficiency of screening," $P = 0.08$). The flowsheets presented to the students after the simulator tests also indicate gender-specific differences in the response to simulator training. In men, the individual parameter "Feel in control" correlated well with the performance in GI Mentor, whereas "Excited" correlated better in women. The gender differences in how men and women react to and perform in simulator training in our study should be interpreted cautiously and further studies are advocated. The possible gender differences in regard to simulator training, however, represent an important issue to be considered when designing future educational programs to ensure that we do not just make new "boys' toys." This fact is further emphasized when looking at PC gamers and nongamers. The two groups differed regarding both "Efficiency of screening" and "Endoscopy time," with the PC gamers performing better in our study. There was also a trend toward PC gamers performing better regarding the other parameters of the GI Mentor, although the differences were not significant ($P = 0.07$). In diagnostic gastroscopy, one of the key issues is to inspect the mucosa thoroughly to find early cancers and flat adenomas that have a high potential for malignant transformation.[13] The majority of the students in the PC gamer group were men (four of five). Grantcharov et al.[12] also found that PC gamers made fewer errors in a medical simulator (MIST-VR) than did those without gaming experience. This might indicate that those who have experience with interpreting two-dimensional images and constructing them into a three-dimensional environment, like PC gamers, have an obvious advantage in image-guided surgery and endoscopy. Sedlack and Kolars[6] found that gastroenterology fellows who completed a 6-hour curriculum with computer-based colonoscopy simulation performed colonoscopy better for the first 30 colonoscopies than did a control group without simulator training. Our results confirm that those without this experience might have a disadvantage and thus probably need more time with simulator training to reach criterion levels. It is important that this obvious discrepancy regarding background factors and visuospatial abilities in students not exclude them from a career in natural orifice surgery; it should mainly indicate the importance of including simulator training early in undergraduate medical education for students with less visuospatial capabilities to gain experience through extended training in the simulators. It is more important to reach expert criterion levels in the simulator than to perform an examination a sufficient number of times.[5,14]

CONCLUSION

Our study results demonstrate that the visuospatial test PicSOr can predict performance in the GI Mentor II virtual endoscopy simulator. Correlation between the PicSOr test and performance with the GI Mentor is in favor of those with experience in creating a three-dimensional image from a two-dimensional presentation, like in computer gaming. The results of our study support the introduction of simulators early in natural orifice surgery training and that reaching expert criterion levels should be the goal rather than performing a certain number of virtual endoscopies. It also indicates that three-dimensional perception experience, as in computer gaming, improves performance in endoscopic simulation.

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Discussion

Dr. D. Scott (New Orleans, LA): I think you should be applauded for a very eloquent study, and I think the most interesting question to us is, perhaps, what is the utility of this information? Say we test our trainees initially on the PicSOr. Does it help you in any way with your logistics of training, or ultimately do you see this as perhaps even a screening tool to test whether or not individuals seeking a surgical career have this ability?

Dr. Enochsson: No, I think it should not be used as a screening test as to whether people are going to be good endoscopists or not, but what this study demonstrates is rather that there seems to be a gap between the 2-D image experience in people; some are very used to using the computer and analyzing the 2-D image, whereas others are not. Our study indicates that those who don't have the experience should train with a medical simulator rather early on.

Dr. N. Soper (Chicago, IL): I, too, enjoyed the presentation very much. However, you have only reported on the initial experience. Have you gone to the next level and looked at potential curricula or other means that would then lead to improvements in task performance on the virtual endoscopy or on the PicSOr?

Dr. Enochsson: No, we haven't. We are doing that. But we know also from the article by Sedlack and Kolars (*American Journal of Gastroenterology* 99:33–37,2004) that those who trained in the simulator had a clinical effect by doing better colonoscopies. This effect lasted for approximately 30 colonoscopies compared to those who didn't train in the simulator. We are looking at that also.

Dr. D. McClusky (Atlanta, GA): This is good work. We also use these tests at Emory, and I know that Dr. Anthony Gallagher has worked closely with you on this. Have you identified any other tests that highlight differences in endoscopic proficiency? I know you have used the PicSOr, but have you looked at other visio-spatial or maybe perceptual tests?

Dr. Enochsson: Yes, we have. There are some mental rotation tests which we received, courtesy of Dr. Gallagher and also Dr. Hedman. We have found some interesting correlations between those tests and the performance in the simulator. We have extracted these data, but they are not analyzed fully yet.

Evaluation of Vagus Nerve Function Before and After Antireflux Surgery

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We sought to evaluate vagus nerve integrity before and after antireflux surgery and to compare it with symptomatic outcome. Antireflux surgery patients were recruited. Patients with disorders associated with vagus dysfunction or who took medications with anticholinergic effects were excluded. Each patient underwent a sham-feeding–stimulated pancreatic polypeptide (PP) test before and after surgery. A symptom survey was also administered. Twenty patients completed preoperative testing; their mean age was 57 years, and postoperative testing results were available for 16 of them. Of the 20, 14 (70%) had an appropriate increase in PP level with sham-meal preoperatively. All 4 patients with an abnormal preoperative test remained abnormal, and 5 of 12 (42%) with a normal preoperative test had an abnormal postoperative result; thus 9 of 16 (56%) had an abnormal postoperative PP test. In 15 patients, assessments of bowel function were obtained before and after surgery. Six of 15 (40%) patients developed new or worse symptoms (diarrhea in 4, flatus in 2). The symptoms did not correlate with PP results. This suggests that some patients referred for antireflux surgery have evidence of abnormal vagus function that persists after surgery. Many patients (42%) with normal testing before surgery develop an abnormal test after surgery. There was no correlation between PP tests and the development or worsening of bowel symptoms. (J GASTROINTEST SURG 2004;8:881–887)

KEY WORDS: Reflux, surgery, fundoplication, vagus

The approach to gastroesophageal reflux disease (GERD) has been in a state of flux for the past decade. The availability and marketing of proton pump inhibitors have resulted in relief of symptoms and control of mucosal disease in the majority of GERD patients. Because GERD is often a chronic condition, most patients will require long-term, often life-long, treatment. Proton pump inhibitors appear to provide adequate maintenance in the majority of patients. The advent of an effective laparoscopic approach to antireflux surgery has increased the number of patients who elect to undergo surgery for GERD.

From the available data, it appears that antireflux surgery provides control of symptoms and mucosal disease at least as well as does medication, perhaps slightly better in some studies.[1,2] Surgery also allows many patients to have their symptoms controlled without taking medications. Antireflux surgery, however, is not without side effects. Symptoms commonly seen after antireflux surgery include dysphagia, inability to belch, bloating, and diarrhea.[3] The cause of dysphagia can often be identified and corrected with esophageal dilation and occasionally repeated surgery.[4] Inability to belch is an expected outcome after fundoplication and most patients learn to compensate for this symptom. The development of bloating and diarrhea is more difficult to explain and also may occur in many patients after fundoplication.[3] Mechanical changes in the cardia (lack of accommodation to liquids) may be related to some of these symptoms, but vagus injury during the fundoplication has been proposed as an etiologic factor.

Presented at the Forty-Fifth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, Louisiana, May 15–19, 2004 (oral presentation).

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Our ability to assess vagus nerve function in vivo is limited and usually based on the physiologic consequences noted after truncal vagotomy. For example, tests of gastric acid secretion using Congo red staining have been used to determine the completeness of a surgical vagotomy.[5] The response of acid secretion to insulin-induced hypoglycemia is a good test of vagus innervation, but it is not without some risk when used clinically.[6] Small bowel manometry can be used to assess the normal conversion from a fasting to a fed pattern after a meal, which is also dependent on intact innervation.[7] Unfortunately, the absence of this conversion is probably neither specific nor sensitive. Finally, pancreatic secretion is at least partially dependent on an intact vagus nerve, and substances secreted from the pancreas can be assayed.[8] We have considerable experience with several of these techniques but have elected to use a sham-meal–stimulated pancreatic polypeptide (PP) assay for this study. The aims of this study were to test vagus nerve function, using the sham-meal–stimulated PP assay, before and after antireflux surgery and to compare the results of those tests with symptomatic outcome.

METHODS

Consecutive patients referred to our center for antireflux surgery were considered for the study. Exclusion criteria included prior esophageal, stomach, or chest surgery; use of medications with anticholinergic effects; chronic disease such as diabetes or renal failure that had the potential to interact with the testing; and unwillingness to return for the postoperative testing. Informed consent was obtained from patients who agreed to the study and met inclusion and exclusion criteria. Approval for the study was granted by the Mayo Clinic Institutional Review Board in August 1998.

Before their scheduled surgery, patients presented for the meal-stimulated PP test after an overnight fast. A small intravenous catheter was inserted into a vein in the patient's arm. Two baseline blood samples (5 ml) were obtained. The study assistant then prepared a bacon and cheese sandwich in the room with the patient. The meal was given to the patient, and they were asked to chew the meal but to not swallow it. Each bite was chewed and then discarded into a container. Samples for PP determination were then obtained every 5 minutes for a total of 30 minutes. Hence there were a total eight samples obtained during each test (two baseline and six after sham-feeding). The samples were processed, and PP levels (pg/ml) were assayed with rabbit anti-human PP and radiolabeled PP as previously described.[9] The average of the two baseline samples was used as the baseline PP level. The highest level obtained with any one of the subsequent six determinations after the sham-meal was considered the maximum. The maximum level was subtracted from the average of the baseline samples to give the increase in PP seen after sham-feeding. In our laboratory, an increase in PP of greater than or equal to 25 pg/ml with sham-feeding is considered to be normal.[10] The patients then underwent a laparoscopic Nissen fundoplication performed according to our previously described technique.[11] The posterior vagus nerve was identified in all cases and was included within the fundoplication. The anterior vagus nerve was usually not identified and was left adherent to the anterior esophagus. The patients returned at least 1 week after surgery and had a follow-up PP test performed.

All patients were contacted in follow-up at least 12 months after their operation and asked to answer a structured questionnaire regarding their bowel habits before and after the surgery (Table 1). Specific symptoms sought included diarrhea, constipation, abdominal pain, bloating, and distention.

Table 1. Bowel habit questionnaire

Answer the first three questions taking into account how you were BEFORE surgery.

1. Before your surgery for acid reflux, did you have any troubles with your bowels? (yes or no)

2. If you answered yes, what kind of problem did you have?
   Diarrhea
   Constipation
   Abdominal pain
   Bloating or distention
   Other (please explain)
3. Before surgery, how many bowel movements did you have each day?

Answer the next four questions taking into account how you are now (AFTER surgery).

1. Since your surgery for acid reflux, have you had any troubles with your bowels? (yes or no)

2. If you answered yes, what kind of problem do you have?
   Diarrhea
   Constipation
   Abdominal pain
   Bloating or distention
   Other (please explain)
3. Since surgery, how many bowel movements do you have each day?

4. Compared with before surgery, are your bowel symptoms now (same, worse, better)?

RESULTS

A total of 20 patients were enrolled in the study and completed preoperative testing. The mean age was 57 years (age range, 36–80 years). Follow-up testing was obtained in 16 patients and was performed a mean of 45 days (range, 6–134 days) after surgery. The other 4 patients were lost to follow-up or refused to return for the second set of tests. Baseline PP levels varied widely but the mean before surgery, 103 pg/ml (range, 54–179 pg/ml), did not change after surgery, 106 pg/ml (range, 40–242 pg/ml). The PP increase in response to sham-feeding varied greatly between individual patients (Fig. 1), but on average, it was less after surgery (41.4 pg/ml; range, 11–257 pg/ml) than before surgery (73.3 pg/ml; range, 5–334 pg/ml) ($P < 0.05$).

Using our lower limit of normal cutoff of a 25-pg/ml increase after sham-feeding, 14 of 20 (70%) of these patients with severe reflux had an expected increase in PP level with sham-feeding in the preoperative period. In the postoperative period, 5 of 12 (42%) who had a normal preoperative test developed an abnormal result. All four of the patients with an abnormal preoperative test remained abnormal after surgery. Thus, in the 16 patients studied after surgery, 9 of 16 (56%) had an abnormal PP test (Fig. 2). We examined the clinical characteristics of these two groups and found that their gender ratio and number of days between surgery and postoperative testing did not differ. The nine patients with an abnormal postoperative test were on average more than a decade older (59 years; range, 36–72 years) than those with a normal test (47 years; range, 38–66 years) ($P < 0.05$).

In 15 patients, detailed symptom assessments of bowel function were obtained before and after surgery, with one patient lost to symptomatic follow-up. New or worse symptoms developed in 6 of 15 (40%) patients after surgery (diarrhea in 4, flatus in 2). In the patients with worsening symptoms, 5 of 6 (83%) had a normal preoperative PP test and 3 of 6 (50%) had a normal test after surgery. Similarly, 7 of 9 (78%) of those with either no change or an improvement in their bowel symptoms had a normal preoperative test and 4 of 9 (44%) had a normal postoperative test (Fig. 3). Hence, there was no symptomatic correlation with the results of PP testing.

DISCUSSION

GERD is very common and usually well controlled with medical therapy.[12] Some patients may have symptoms that are not completely controlled with medication or may desire an alternative treatment that does not require daily medication use. The advent of a laparoscopic approach has resulted in a

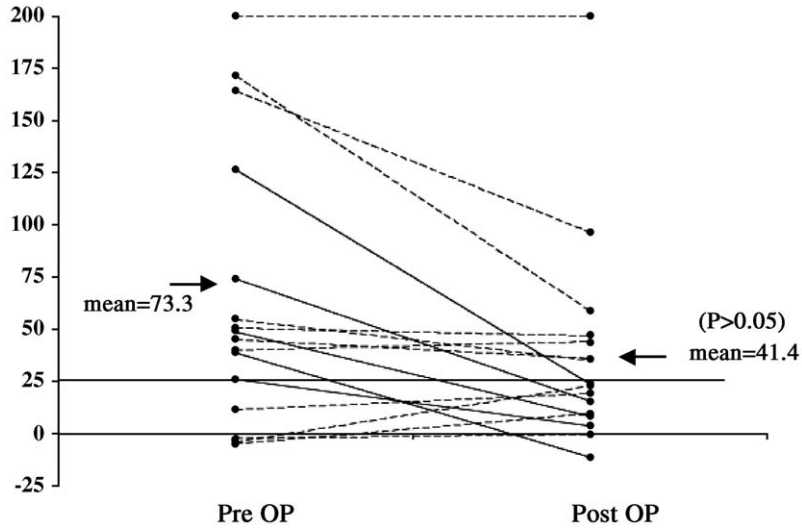

Fig. 1. Increase in pancreatic polypeptide after sham-feeding (pg/ml) before and after antireflux surgery. Seven patients had normal tests (>25 pg/ml increase) in the preoperative and postoperative periods, whereas four patients had abnormal tests in both periods (dotted lines). Five patients had normal tests before surgery, which became abnormal after surgery (solid lines). The mean increase fell from 73.3 to 41.1 pg/ml after surgery ($P < 0.05$). Note that the patient at the top with an indicated increase of 200 pg/ml actually had an increase of 334 pg/ml before surgery and 257 but are compressed to better illustrate the remainder of the data.

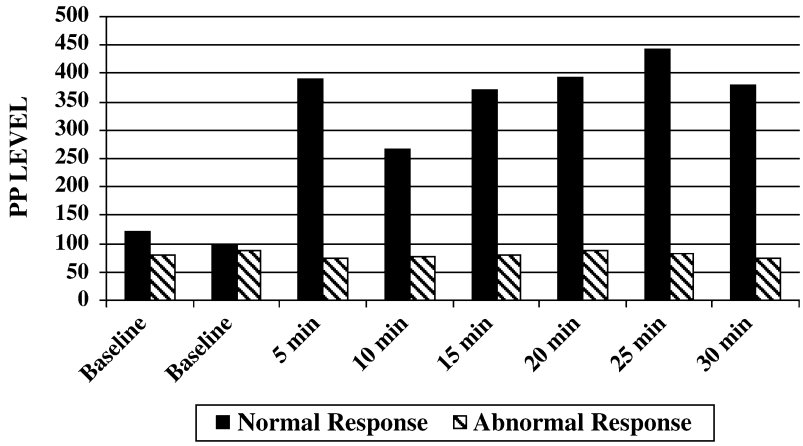

Fig. 2. Pancreatic polypeptide (PP) levels from two patients obtained after sham-meal stimulation. The patient illustrated with the solid bars had normal PP levels before stimulation with a normal increase after the sham-meal. The second patient (illustrated with the hatched bars) had similar basal levels but no increase with the sham-meal.

marked increase in the number of patients electing to undergo antireflux surgery. Patients can expect to have their symptoms of heartburn and regurgitation controlled in more than 90% of well-selected cases.[12] The response of atypical symptoms is less complete, which is also the case with medical therapy.[13] The clinical response to antireflux surgery is considered relatively durable, but recent reports have challenged this durability by showing a large number of surgical patients back on medication after surgery.[14] This therapy is often given, perhaps inappropriately, in patients without recurrence of classic reflux symptoms or objective evidence of reflux.[15,16] Nonetheless, many centers continue to effectively treat a large number of GERD patients with surgery.

Several symptoms may develop or worsen after antireflux surgery, with the most common of these being dysphagia. The incidence of postoperative dysphagia is difficult to quantify, but it appears to be significant in approximately 10% of patients.[17] Most cases resolve spontaneously or with esophageal dilation, but some persist and on occasion require a revision of the fundoplication.[4] Difficulty with gas, both upper abdominal (belching) and flatulence, is also common after surgery. In a large study of patients randomized to antireflux surgery or omeprazole therapy, difficulty belching and increased flatulence were

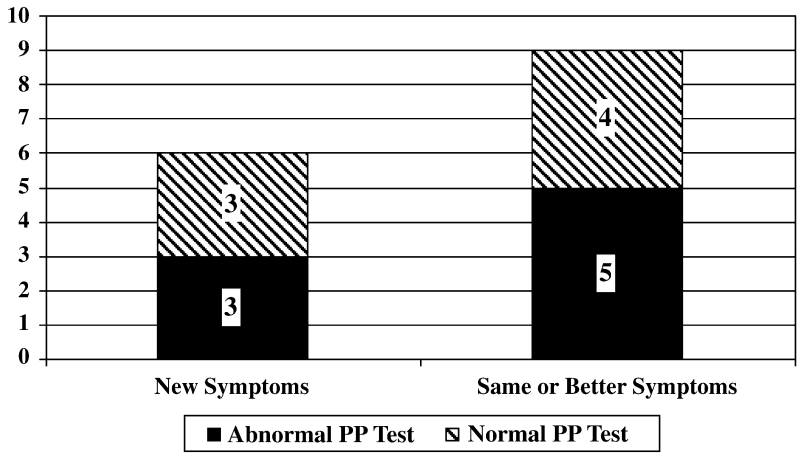

Fig. 3. Relationship of pancreatic polypeptide (PP) test of vagus function to the development of new postoperative symptoms. Six patients developed new symptoms after surgery, but only three of six had an abnormal postoperative PP test. Nine patients had no new symptoms or improvement of symptoms that were present before surgery; similarly, five of nine had an abnormal postoperative PP test.

more common in the surgery group.[2] The cause of these symptoms is unknown; one explanation is that the fundoplication exerts a mechanical effect on the lower esophageal sphincter, which both strengthens it and decreases transient lower esophageal sphincter relaxations. Because we know that both normal individuals and those with reflux swallow large volumes of air routinely,[18] the presence of a fundoplication may prevent venting of gas from the proximal stomach and cause increased abdominal distention and, subsequently, flatus.

Diarrhea is a more recently recognized complication of antireflux surgery, occurring in up to 18% of patients.[3] The potential cause for this symptom is unclear, although overlap may exist between GERD and irritable bowel syndrome in some patients, particularly those with nonerosive GERD.[19] Some investigators have suggested that underlying irritable bowel syndrome may be exacerbated by surgery or may become the dominant set of symptoms once the GERD is controlled.[20] However, in a previous study, we found preoperative bowel dysfunction to be relatively rare.[3] Another possible explanation for diarrhea is that patients develop rapid gastric emptying after antireflux surgery, which behaves like the dumping syndrome.[21] More rapid gastric emptying after the creation of a fundoplication is attributed to loss of accommodation in the stomach, thereby preventing the fundus from expanding to contain the liquid portion of the meal.[22] Finally, it is possible that some patients develop gastrointestinal dysmotility due to damage to the vagus nerve during surgery. Dysmotility may cause diarrhea by impairing the clearance of bacteria from the upper small intestine, which can result in bacterial overgrowth and malabsorption.[23,24] In this study, we expected that some patients would develop evidence of vagus dysfunction after surgery and that they would be predisposed to diarrhea. We confirmed that some develop evidence of vagus dysfunction after antireflux surgery and that the mean increase in PP level with meal stimulation is less after surgery, but this dysfunction and change in levels did not seem to correlate with symptoms. In the only similar study, Vu et al.[25] evaluated vagus nerve integrity after antireflux surgery using the insulin-induced hypoglycemia method of vagus stimulation. PP responded normally in 11 of their 12 patients. These two studies are difficult to compare because both were fairly small and used different methods and normal ranges.

It was also interesting that the patients with an abnormal PP test after surgery were, on average, a decade older than those with a normal test. This could be indicative of generalized loss of neural function with aging, which has been suggested in several studies.[25,26] There has not been a comprehensive look at postoperative symptoms in older patients, but several small series have indicated that antireflux surgery can be successfully performed in older patients.[27–29] Postoperative symptoms did not seem to be related to either age or gender in our study.

In summary, the results of this study confirmed that evidence of vagus dysfunction is present in some patients before antireflux surgery and that additional patients develop evidence of vagus dysfunction after surgery. But, most important, the dysfunction did not correlate with worsening or development of new symptoms in this small sample of patients. We therefore suggest that an abnormal indirect test of vagus function such as the meal-stimulated PP test not be used to implicate an inadvertent vagotomy as the etiology of an individual patient's postoperative symptoms.

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Discussion

Dr. P. Crookes (Los Angeles, CA): I was asked to discuss this paper and I thank the society for letting me make a few comments. I think we all agree this is a really timely and interesting paper, and it has interesting implications even for legal redress when a patient is dissatisfied with the result of a Nissen fundoplication. I also think that it was a very remarkable admission by the presenting author on the first slide that "anti-reflux surgery can produce good and durable treatment of reflux disease"!

However, because the implications are so strong, we do have to be rigorous about the methodology, and I think the one thing that I would perhaps ask the authors, if they have really been fair, is, do you think that you studied these patients too early? I had a chance to look at the paper, and it seemed that you basically invited them back 1 week afterward, and there were a couple of outliers, but the mean was only about 6 weeks; one was 134 days afterward. So the abnormalities you observed may only represent some very transient vagal nerve dysfunction.

If you were to accept your data on its face value, it means that one of the most highly experienced teams in the country "bags" the vagus nerve 50% of the time but that it doesn't matter. However, I think it is too early to assess the relationship of the symptoms and it is probably too early to do the test.

The last thing I would like to ask, and maybe this is not fair to ask a gastroenterologist, but have you identified what aspect of the surgery might cause the injury? Is it just nonspecific cautery, or traction with the sling around the esophagus and that sort of thing, or the creation of the wrap and putting a stitch through the vagus? And do you think you have modified the technique in light of these results?

Dr. DeVault: Thank you very much for those comments. First, in regard to the timing of the study, the majority of the patients actually were studied between 4 and 8 weeks. There were a couple of outliers. We found it quite challenging to get patients to come back for the testing, which actually surprised us somewhat. Four to 8 weeks was our goal, although we didn't achieve that in all of our patients.

In regard to the cause of vagus injury, I don't know—it is my assumption that if there is vagus nerve injury, the trauma comes from traction on the nerve, not transection. The nerve may be traumatized but not transected and could improve over time. The symptom data were obtained at least a year after the surgery. We actually added this part of the study after we had completed the physiologic evaluation, so there was a bit of disconnect between when the tests were done and when the final symptom data

were collected. I will agree with that as a limitation of the study.

Dr. J. Kral (Brooklyn, NY): In the rationale for this study, you indicated that the vagotomy might cause diarrhea through bacterial overgrowth. Is there any evidence that this is the mechanism for diarrhea associated with vagotomy?

Dr. DeVault: No, there are not a lot of data, but there certainly are patients who have a vagus nerve injury, diabetics, for instance, who we as gastroenterologists believe develop diarrhea because of motility changes followed by bacterial overgrowth. This diarrhea often responds to antibiotics. At times we can document this by culturing the proximal duodenum.

Omeprazole Does Not Reduce Gastroesophageal Reflux: New Insights Using Multichannel Intraluminal Impedance Technology

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Proton pump inhibitors are the mainstay of medical management in gastroesophageal reflux disease. Although they provide relief from most symptoms, reflux may persist. We hypothesize that omeprazole does not reduce the total amount of gastroesophageal reflux but simply alters its pH characteristics. Six asymptomatic volunteers had combined 24-hour impedance pH monitoring before and after 7 days of omeprazole (20 mg BID). Multichannel intraluminal impedance was used to identify reflux episodes, which were classified as acid (pH $<$ 4), weak acid (pH $>$ 4 but decrease $>$ 1 pH unit) and nonacid (pH $>$ 4 and decrease $<$ 1 pH unit) by pH measurements 5 cm above the lower esophageal sphincter (LES). A gastric pH sensor located 10 cm below the LES was used to verify the action of omeprazole. Impedance detected a total of 116 reflux episodes before and 96 episodes after omeprazole treatment. The median number of reflux episodes (18 versus 16, $P = 0.4$), median duration of reflux episodes (4.7 versus 3.6 minutes, $P = 0.5$), and total duration of reflux episodes (27.2 versus 42.4 minutes, $P = 0.5$) per subject were similar before and after omeprazole. Acid reflux episodes were reduced from 63% before to 2.1% after omeprazole ($P < 0.0001$), whereas nonacid reflux episodes increased (15% to 76%, $P < 0.0001$). Weak acid reflux episodes did not change (22.4% to 21.8%, $P = 1.0$). The proportion of reflux episodes greater than pH 4 increased from 37% to 98% ($P < 0.0001$). In normal subjects, omeprazole treatment does not affect the number of reflux episodes or their duration; rather it converts acid reflux to less acid reflux, thus exposing esophagus to altered gastric juice. These observations may explain the persistence of symptoms and emergence of mucosal injury white on proton pump inhibitor therapy. (J GASTROINTEST SURG 2004;8:888–896)

KEY WORDS: Gastroesophageal reflux, nonacid reflux, omeprazole, intraluminal impedance

With the introduction of histamine-2 blockers in the 1970s and proton pump inhibitors (PPIs) in the 1980s, antisecretory agents have been the primary therapy of gastroesophageal reflux disease (GERD) for nearly three decades. A large body of literature documents the fact that PPI therapy is effective in relieving the symptom of heartburn and healing erosive esophagitis in 80–90% of patients with GERD. It has become increasingly clear, however, that despite adequate PPI treatment, typical GERD symptoms may remain, atypical symptoms such as cough and hoarseness may be unchanged, [1]and Barrett's esophagus may emerge.[2] These observations emphasize that PPI treatment, while altering the pH of the gastric juice, does not correct the defects in the gastroesophageal barrier and consequently does not eliminate the presence, frequency, or volume of refluxed gastric content. Until recently, there was no way to investigate this hypothesis.

The presence of gastroesophageal reflux is typically quantified by esophageal luminal pH 5 cm above the upper border of the lower esophageal sphincter (LES) over a 24-hour period. In fact, many authors have used increased esophageal acid exposure to define the presence of GERD.[3,4] This definition is clinically useful and relevant in most patients. Measurement of nonacid (pH $>$ 4) reflux has always been difficult and generally restricted to experimental

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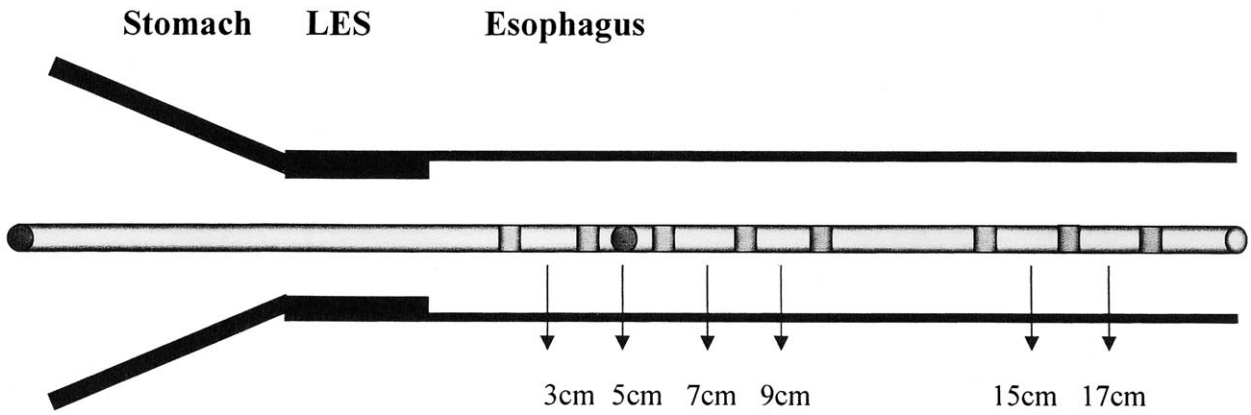

Fig. 1. Schematic representation of the multichannel intraluminal impedance pH catheter in situ. pH sensors are located in the stomach (distal tip) and at 5 cm above the upper border of the lower esophageal sphincter (LES). Impedance electrodes are placed in such a way that intraluminal impedance is recorded at 3, 5, 7, 9, 15, and 17 cm above the upper border of the LES.

studies. Classic methods of measuring nonacid reflux such as esophageal aspiration and scintigraphy are tedious and have limitations that prevent their routine clinical use. Multichannel intraluminal impedance (MII) technology has been introduced as a method of measuring esophageal bolus presence and transit and detecting reflux independent of its pH. Normative data for bolus transit and gastroesophageal reflux quantification by MII have been established, allowing its confident clinical use to measure these entities.[5–9] MII allows the detection of intraesophageal fluid bolus independent of its pH. This permits not only the identification of liquid, gaseous, or mixed (liquid plus gaseous) intraesophageal material but also the direction in which it travels (down or up the esophagus). Further, the use of MII in conjunction with a pH sensor allows discrimination of acid from nonacid reflux. In this study, we assess the effect of omeprazole on the quantity and pH of gastroesophageal reflux over a 24-hour period in an effort to improve our understanding of the treatment of GERD.

PATIENTS AND METHODS

Six asymptomatic volunteers (three men and three women; age range, 29–50 years) were recruited to

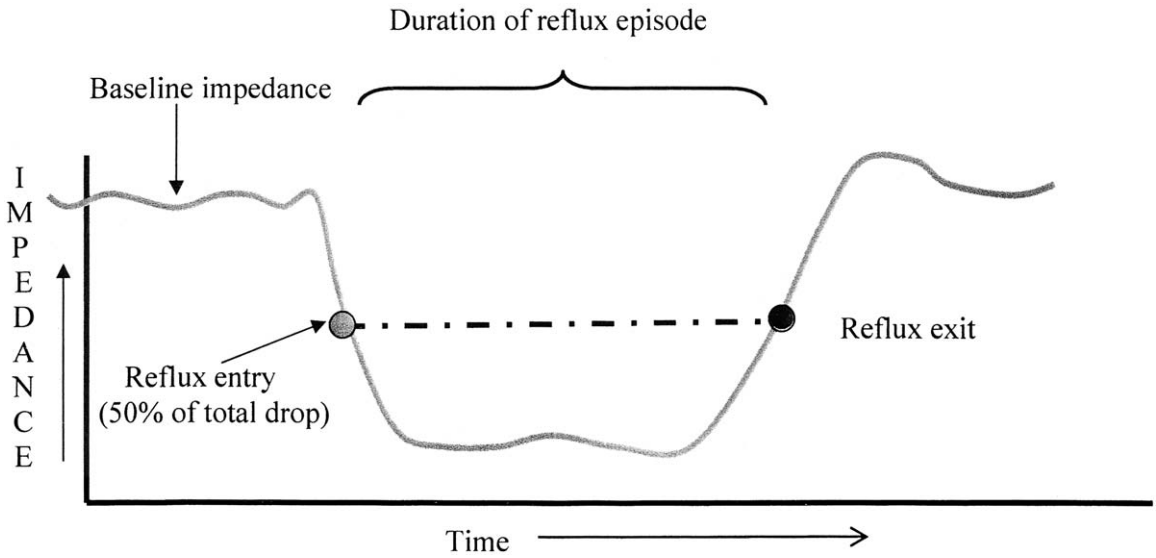

Fig. 2. Schematic showing impedance changes when a reflux bolus passes through two impedance electrodes (one impedance sensor). Reflux duration is measured as the time taken from reflux bolus entry to exit, as shown.

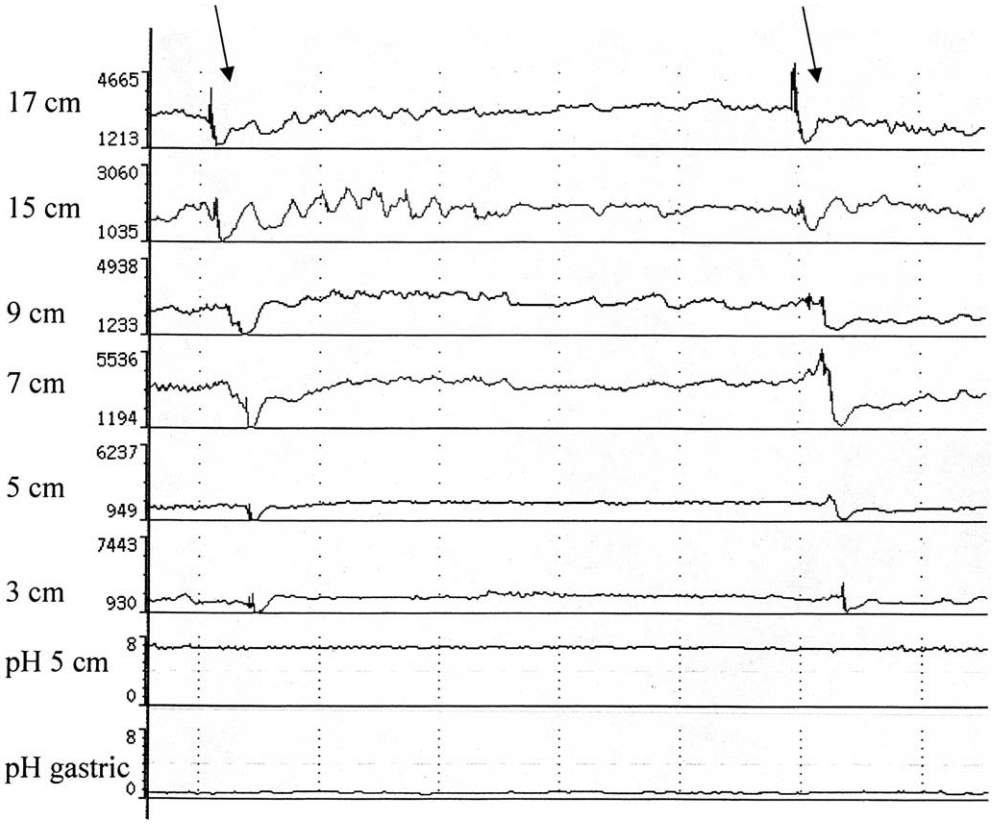

Fig. 3. Tracing from the multiluminal intraluminal impedance pH catheter showing normal swallows in a preomeprazole therapy study. Note the progressive and sequential impedance drops in sensors (*arrows*) showing downward peristaltic movement of the swallowed bolus. There was no change in the esophageal pH at 5 cm or the acidic intragastric pH tracing.

participate in this study. The protocol was approved by the local institutional review board. All study subjects underwent esophageal manometry, 24-hour pH monitoring, and barium esophagography to confirm normal anatomy and function of the esophagus. Each volunteer had a 24-hour impedance pH monitoring with the catheter positioned as described later. During the monitoring period, volunteers ate three standard meals, and all of their activity was recorded in a diary. After the 24-hour control study, volunteers were administered a 1-week regiven of 20 mg omeprazole BID (30 minutes before breakfast and dinner). After 7 days, while on omeprazole therapy, each subject underwent a second 24-hour monitored period with identical standard meals and similar activity as their control monitored period.

A combined impedance and pH catheter was used for the study (Sandhill Scientific Inc., Denver, CO). The catheter is 2.1 mm in diameter and is fitted with six impedance and two pH sensors (Fig. 1). The catheter was passed transnasally, and the six impedance sensors (two ring electrodes measure impedance at their midpoint and constitute one sensor) were positioned 3, 5, 7, 9, 15, and 17 cm from the upper border of the LES. Of the two incorporated pH sensors, the proximal sensor was located 5 cm above the upper border of LES and the distal was located 15 cm lower, in the gastric fundus. The distal gastric pH sensor was used to confirm the effect of omeprazole therapy.

MII detects intraesophageal contents (saliva, food, gastric juice) by identifying the change in the intraesophageal impedance (electrical resistance) between the paired ring electrodes. An empty esophagus is characterized by high electrical impedance, which decreases when the paired electrodes are bathed by a liquid bolus. The drop in impedance lasts as long as the bolus remains between the paired electrodes, thus measuring the duration of bolus presence at that esophageal level. The nature of impedance variation helps identify the physical nature of the bolus (liquid, gaseous, mixed). Sequential changes in impedance along the length of the catheter allow determination of the direction of bolus movement—cephalad to caudad, as with swallowing, or caudad to cephalad,

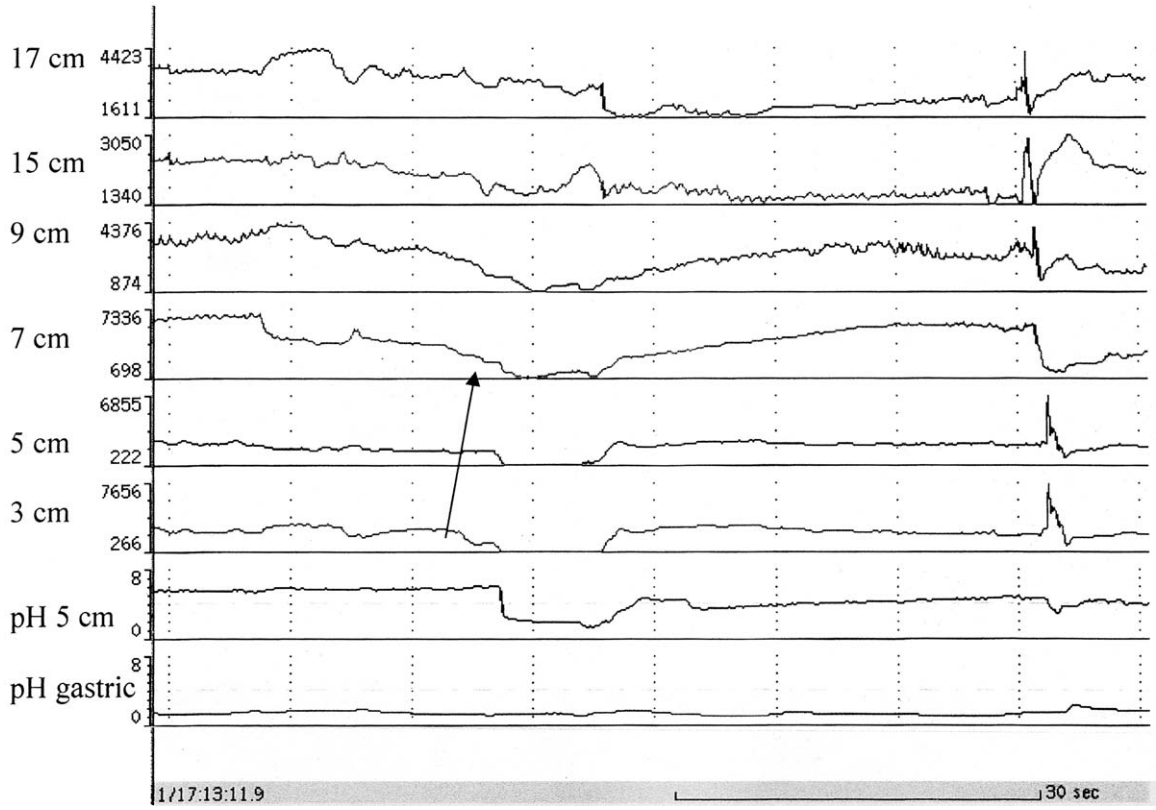

Fig. 4. Acid reflux episode. Note the retrograde reflux bolus movement seen clearly in the sensors at 3, 5, and 7 cm (*arrow*). There is a concurrent fall in esophageal pH at 5 cm above the lower esophageal sphincter. The gastric pH records normal acidic pH.

as with gastroesophageal reflux. Concurrent intraesophageal pH measurement allows characterization of the reflux episode as acid (pH < 4), weak acid (pH > 4 but decrease >1 pH unit), and nonacid (pH > 4 and drop <1 pH unit). The duration of reflux was measured as the time the impedance at 5 cm above the upper border of LES remained altered due to the presence of refluxate. Patterns of impedance pH changes are illustrated in Figures 2 through 5.

Each recording was analyzed by two authors independently (Bioview Analysis version 4.7.1.4; Sandhill Scientific Inc., Denver, CO). Discordant readings were reviewed until agreement was reached. Reflux episodes consisting of only gaseous reflux, without evidence of liquid reflux, were excluded from the study. The total number of reflux episodes; the number of acid, weak acid, and nonacid episodes; and the total duration of reflux time were recorded for each study. Gastric pH sensor data were reviewed to confirm that gastric pH baseline was raised above pH 4 while on omeprazole.

We hypothesized that after PPI therapy all acid episodes would be converted to nonacid or weak acid episodes. With $\alpha = 5\%$ and $\beta = 10\%$, and a person-to-person variability (SD) of 9, the estimated sample size is 14, or 7 volunteers in each limb (before and after PPI). Data were analyzed using the Wilcoxon matched pairs test and Fisher exact test.

RESULTS

All volunteers had a 24-hour impedance pH monitoring off and on omeprazole therapy (median, 24.45 hours versus 24.75 hours before and after omeprazole, respectively; $P = 0.8$). Overall impedance detected a total of 116 reflux episodes before and 96 after omeprazole treatment ($P > 0.05$). The median number of reflux episodes per subject, however, was similar before and after omeprazole therapy (18 versus 16, $P = 0.4$) (Fig. 6). The total duration of reflux episodes was also similar (27.2 minutes before versus 42.4 minutes after omeprazole, $P = 0.5$), as was the median duration of reflux episodes per subject (4.7 minutes versus 3.6 minutes, $P = 0.5$) (Fig. 7).

PPI treatment had a profound effect on the pH of the reflux episodes. Omeprazole reduced the proportion of acid reflux episodes from 63% to 2.1% ($P <$

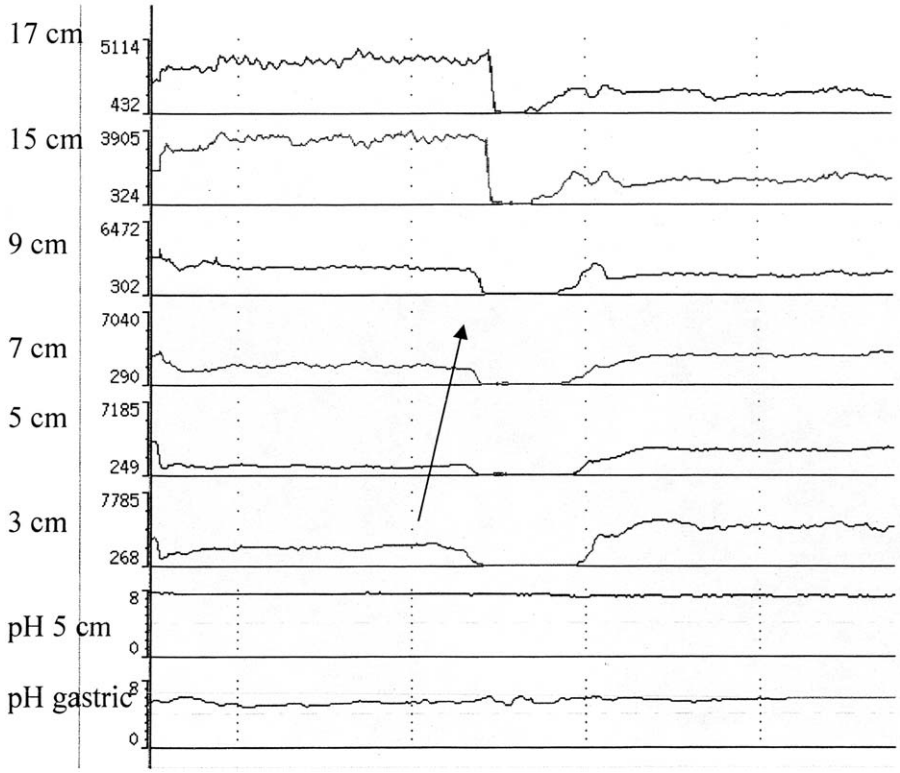

Fig. 5. Nonacid reflux episode. Retrograde reflux movement is clearly evident in most impedance sensors (*arrow*) without any change in the esophageal pH sensor located 5 cm above the lower esophageal sphincter. Note that the intragastric pH remains well above 4 due to omeprazole therapy.

0.0001). Correspondingly, the proportion of nonacid reflux episodes increased from 14.6% to 76% ($P < 0.0001$). The proportion of weak acid reflux episodes did not change (22.4% to 21.8%, $P = 1.0$). Together, nonacid and weak acid reflux episodes increased from 37.1% to 97.9% ($P < 0.0001$) (Figs. 8 and 9).

DISCUSSION

The development of intraluminal impedance technology has the potential to provide significant new insight into the pathophysiology and treatment of esophageal diseases. For the first time, we have a readily reproducible and quantifiable means to measure esophageal bolus transit and the capacity to detect both acid and nonacid reflux episodes over a 24-hour period in an ambulatory setting. We have used this new technology to study the effects of PPI treatment on gastroesophageal reflux. PPIs are currently the mainstay of GERD therapy. It is well established that they relieve the symptoms of "acid peptic" reflux disease, namely heartburn, and heal erosive "acid peptic" esophagitis. It is also becoming clear that nonacid reflux persists under PPI therapy and can result in significant symptoms and mucosal injury. For example, respiratory symptoms commonly associated with nonacid reflux often fail to respond to PPI therapy. [1,7,10,11] Pulmonary disease secondary to GERD occurs from neural reflexes provoked by the physical presence of refluxed fluid in the esophagus or by direct aspiration, both mechanisms unlikely to be affected by PPI therapy. Further, there is evidence suggesting that less acid reflux (pH 3–5) may encourage the development of Barrett's esophagus. [2,12–15] These clinical observations have raised concerns that empirically treating all GERD patients with long-term PPI therapy may be inappropriate.

We have shown that PPI therapy alters the H^+ ion concentration in the refluxed fluid but not the amount of gastroesophageal reflux. In a 24-hour ambulatory setting, omeprazole had little to no effect on either the number of reflux episodes or the duration of reflux episodes. Vela and colleagues[16] reported nearly identical findings in reflux patients monitored over a short-term 2-hour postprandial period in a reflexogenic (right lateral decubitus) position. This study included 12 GERD patients monitored over a 2-hour postprandial period in a reflexogenic position (right lateral). The number of reflux episodes actually increased from 217 to 261 ($P > 0.05$, NS) after PPI

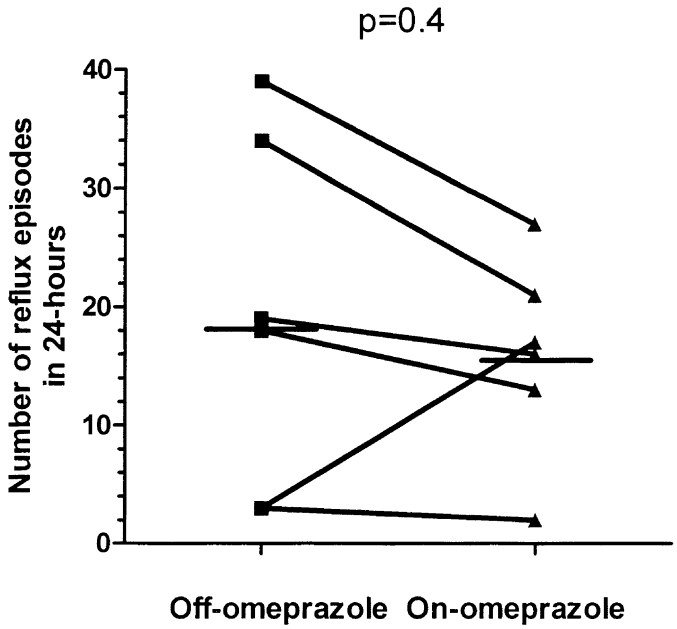

Fig. 6. Total number of reflux episodes off and on omeprazole therapy are similar (n = 6). Bold horizontal lines mark the median (18 versus 16, $P = 0.4$).

therapy. Our results, differing from the Vela et al. study in that we studied ambulatory normal subjects over a 24-hour period, show a net decrease in the number of episodes. It should be noted that PPI medications are antisecretory agents as well as antacids. A decrease in volume of gastric secretions may account for the decrease we observed on therapy, particularly given that the subjects were normal subjects. This may not be evident in patients with GERD in the presence of an altered gastroesophageal barrier. Numerous variables affect the frequency of reflux episodes, including the gastroesophageal barrier function, the quantity and type of food consumed, and intermittent gastric distention. Thus, a reduction in the volume of gastric juice may not necessarily have a direct impact on the number of reflux episodes.

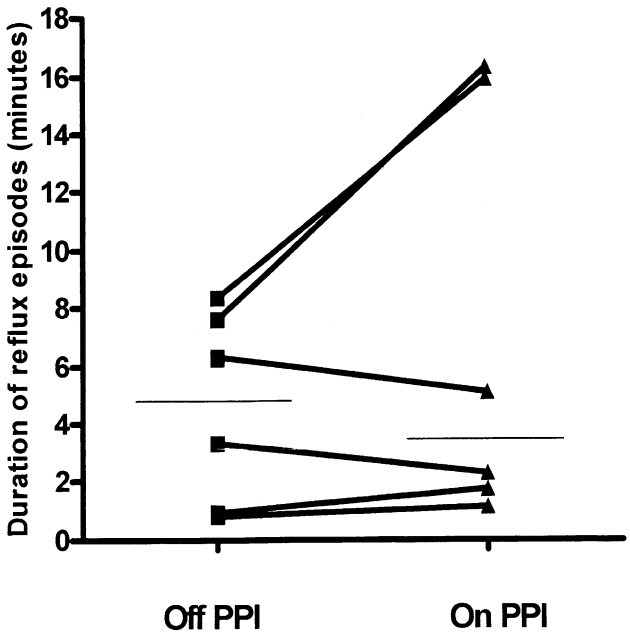

Fig. 7. Total durations of reflux episodes before and after omeprazole therapy are similar in each subject. Horizontal lines mark the median (4.7 versus 3.6 minutes, $P = 0.5$). PPI = proton pump inhibitor.

We elected to first study normal subjects with a normal gastroesophageal barrier to test our hypothesis. Although it is relevant to study patients with GERD, the presence of an incompetent gastroesophageal barrier could cause bias in the results in favor of persistent reflux, because previous studies have shown that omeprazole does not affect the gastroesophageal motor function or LES pressure.[17] If our hypothesis was confirmed in normal subjects with a normal gastroesophageal barrier, it is likely to be true in patients with a hiatal hernia or a structurally defective LES.

We used a 2.1-mm catheter that extended into the stomach to measure gastric pH to confirm the effectiveness of acid reduction by omeprazole. Although the catheter was placed through the LES, it is unlikely that its presence influenced the results. Previous studies have shown that the presence of a

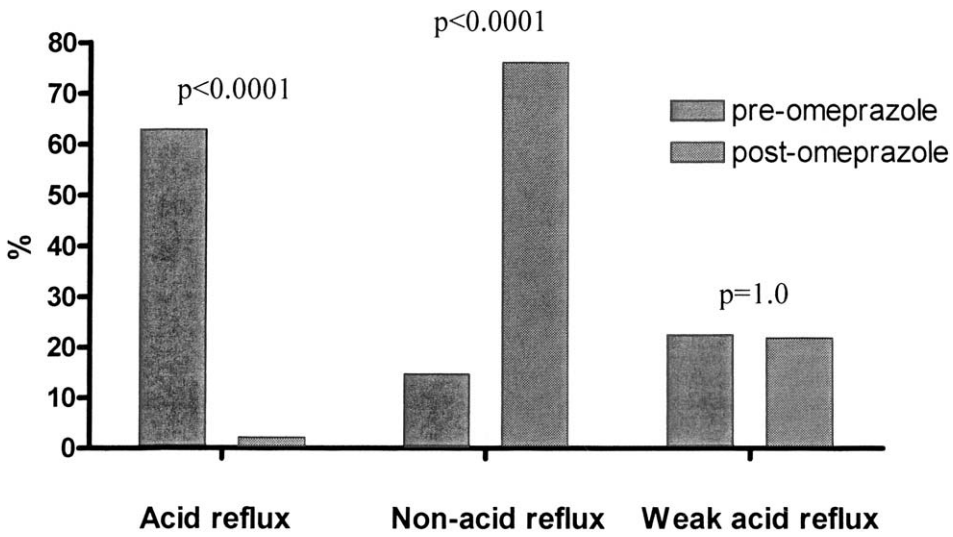

Fig. 8. Cumulative proportions of acid, weak acid, and nonacid reflux episodes before and after omeprazole therapy.

trans-sphincter catheter does not change the amount of gastroesophageal reflux.[18]

This is the first study of its kind to analyze the effect of omeprazole on the amount of reflux over a 24-hour period. Although a small number of subjects were studied, we believe that a type II error is unlikely due to the much larger number of reflux episodes analyzed (116) and the dramatic effect of omeprazole on the pH of the refluxed material.

Impedance measurement requires an extremely low voltage of 0.00025 μW to be transmitted across the electrodes at a frequency of 1–2 kHz with an electrical current limited to 8 μA. This is below the stimulation threshold for nerves and esophageal or cardiac muscle. It is unlikely that the technology used in this study could effectively alter the function of the LES or esophagus. Consequently, we have confidence in our findings, using combined 24-hour impedance and pH monitoring, that PPI therapy does not affect the number or duration of reflux episodes. PPIs convert the pH of the refluxed gastric juice from acidic to less acidic. These findings may explain the persistence of symptoms and the emergence of mucosal injury while on PPI therapy, profoundly affecting our understanding of the treatment of GERD.

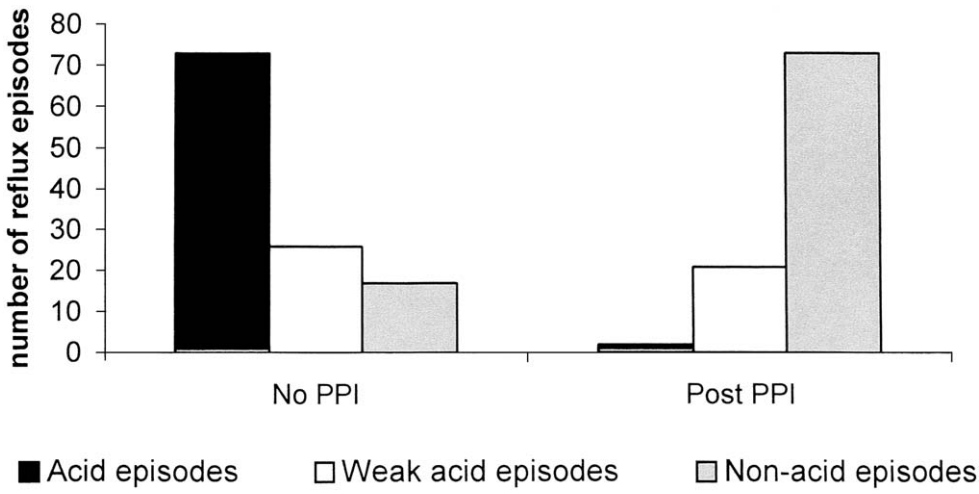

Fig. 9. Number of acid, weak acid, and nonacid reflux episodes before and after omeprazole therapy. PPI = proton pump inhibitor.

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Discussion

Dr. L. Khaitan (Atlanta, GA): First, I would like to thank the authors for providing me with the manuscript in advance. MII [multichannel intraluminal impedance] technology is a new technology that we have begun to use and apply in the diagnosis of our patients, and I think it is very exciting. I have really enjoyed your presentation and your paper. This group has asked a simple question: Does PPI [proton pump inhibitor] therapy decrease reflux or simply nonacidify the reflux that is present? I believe that you have answered this with a nice matched-paired analysis by studying six asymptomatic patients on and off 20 mg of omeprazole BID. You have demonstrated that reflux persists but is no longer acid. I do have a few questions.

We know that PPIs decrease symptoms and heal esophagitis, so what is the clinical significance of this persistent nonacid reflux? Also, should we start using this technology to study all of our patients with suspected GERD, or should we use this technology selectively? Interestingly, although you did not see a significant decrease in the number of episodes per patient, five of these six patients did show a decrease in the number of reflux episodes on the PPI therapy, and the overall number was statistically significant if you averaged out the group. I would be interested to know what your thoughts are.

Also, was there any change in the proximal extent of the reflux on and off the PPI therapy, as now we can quantify this by having the MII sensors at different levels within the esophagus? Your group has extensive experience with the Bilitec, and how does this technology compare with that? Is the nonacid reflux that we know prior to the patients being on the PPI therapy all bile, or is there some other component to that refluxate?

I really enjoyed your presentation. I think this is very important work, and I believe it will change how we approach GERD in the future.

Dr. A. P. Tamhankar: Thank you for your comments. I might ask you to repeat your questions, because there were so many. I will try to answer them as I remember them.

Dr. Khaitan: The first question was, you know that PPIs treat patient symptoms and heal esophagitis, so what is the significance of the nonacid reflux?

Dr. Tamhankar: Most literature will say that PPIs will treat symptoms and heal esophagitis in about 80–90% of cases. So there are a number of patients who will not benefit from PPIs. A lot of physicians will say that the PPIs have not been given in adequate doses, and there are nighttime breakthroughs of acid reflux. At the same time, there are data with the advent of impedance technology that have started to pinpoint nonacid reflux with the symptoms and show quite significantly that some nonacid episodes will cause symptoms.

Again, there is good evidence that bile reflux at higher pH is going to damage the esophageal mucosa. We have been seeing over the last so many years with the advent of PPIs that the classic peptic complications of strictures and esophagitis are reducing in numbers but that Barrett's and adenocarcinoma are increasing. So regarding the whole picture, I think there is some evidence that nonacid reflux is the incriminator in these pathologies, and as we study and have more clinical data accumulated with more impedance technology, this is going to be the way to go.

Dr. Khaitan: Was there a change in the number of reflux episodes on and off therapy?

Dr. Tamhankar: Some volunteers did reduce their reflux episodes. We had only six volunteers, so studying more would obviously get us the right picture. There is one other small study on 10 GERD patients by Villa et al., and they have exactly similar results. However, their study was a smaller study, for 2 hours postprandial, and the reflux is an imposition. It is difficult to comment on what would happen if we had more volunteers, but I think statistically this is similar, so we should take the results as they are right now.

A few volunteers had their duration of reflux episodes increased. So cumulatively, the time of reflux presence versus the number probably matches the total amount of reflux in the esophagus.

About your question regarding the height, we didn't specifically look at the height. We were focusing our data analysis at 5 cm above the LES, which is the standard position. So I can't really answer that. But clearly, the duration was higher in a few volunteers and lower in a few volunteers, so we just have to see as we do more studies.

Dr. Khaitan: How does this compare with the Bilitec?

Dr. Tamhankar: There are very few studies about omeprazole or PPIs having an effect on the bile secretion, and again, even if the bile secretion is reduced or increased, or whatever happens to it, we don't know if affects the duodenal gastric reflux. But just by intuitive guess, I would suggest that if reflux doesn't change, then the bile reflux will not change as well, which means that part of the reflux will be just nonacid, or less acid in gastric juice. Whatever bile was coming before will still come, perhaps damaging more and resulting in a relatively higher pH.

Dr. L. Stewart (San Francisco, CA): I take it you have not looked at this in patients?

Dr. Tamhankar: No.

Dr. L. Way (San Francisco, CA): Based upon previous work, we know that the PPIs don't change the misbehavior of the sphincter or the body of the esophagus. It would be hard to imagine, therefore, that the reflux would stop with the PPIs. One profound effect, however, that might influence the phenomenon would be the substantial decrease in gastric acid secretion, the volume of secretion in the stomach. So just on reasoning alone you might suspect that the reflux would continue but it would be, in magnitude, somewhat less. I think that you need to consider this other factor before you are through analyzing the work you are doing.

Dr. Tamhankar: We thought about that. There are a few studies, especially by the anesthesiologists, who look at the gastric volumes after anesthetic induction when they do intravenous PPI therapy, and there are good data there showing a decrease in volume over the first few hours afterward, but those studies are purely on intravenous PPIs. There are no substantial data on oral PPIs.

Second, reducing the volume of gastric secretion alone may not necessarily have the exact proportional effect on the reflux, because there are multiple factors. There could be reflux depending on how much people have been eating, how much distention the food has caused, and so on. Although it will have some impact, I am not sure it is purely decreasing the volume, and how much it decreases with oral therapy is also not clear.

Dr. Way: Of course, gastric analysis shows there is a profound effect on gastric secretion with these drugs. So I don't think that there would be a question about the volume decreasing.

Multiorgan Resection for Gastric Cancer: Intraoperative and Computed Tomography Assessment of Locally Advanced Disease Is Inaccurate

Kari L. Colen, M.D., Stuart G. Marcus, M.D., Elliot Newman, M.D., Russell S. Berman, M.D., Herman Yee, M.D., Spiros P. Hiotis, M.D., Ph.D.

Multiorgan resection of locally advanced gastric cancer has previously been associated with increased morbidity. This study was performed to determine the actual prevalence of pathologic T4 disease in multiorgan gastric resection specimens excised for presumed clinical T4 gastric cancer. A prospective oncology database was queried to identify gastric cancer patients who underwent en bloc multiorgan resection for clinical T4 lesions. Four hundred eighteen patients with gastric cancer underwent gastrectomy between 1990 and 2002. Multiorgan resection was performed in 21 of 418 (5%) patients. Multiorgan resection was not associated with a significant increase in morbidity or mortality. Pathologically confirmed T4 disease was present in only 8 of 21 (38%) patients; the pathologic T stage in all remaining patients was T3 (13 [62%]). Fifteen patients were evaluated by preoperative computed tomography scan. Preoperative computed tomography was inaccurate in assessing T4 lesions, with a positive predictive value of only 50%. Multiorgan resection was safely performed in patients with locally advanced gastric cancer. Pathologic T4 disease was present in only one third of multiorgan resections performed for en bloc excision of locally advanced gastric cancer. Improved methods for intraoperative assessment of disease extension to adjacent viscera should be investigated. (J GASTROINTEST SURG 2004;8:897–900)

KEY WORDS: Gastric cancer, gastrectomy, resection, T4, locally advanced

Definitive surgical management of gastric cancer may often pose significant technical challenges to the experienced surgeon. R0 resection, with negative microscopic and macroscopic margins, has clearly been associated with improved long-term survival for patients with gastric cancer in prior studies.[1] Complete R0 resection for patients who present with bulky tumors sometimes necessitates the removal of adjacent organs.[2,3] Although this can usually be accomplished safely, the resection of adjacent organs in addition to gastrectomy has been associated with an increase in postoperative morbidity.[4–6] Ideally, multiorgan resection in conjunction with gastrectomy for gastric cancer would be reserved for patients with true histologic invasion of adjacent organs (T4 disease).[7]

The proper identification of patients with T4 gastric cancer can be difficult. Diagnostic modalities such as computed tomography (CT) scanning, endoscopic ultrasound, or laparoscopic ultrasound are useful in providing a clinical T stage in patients with locally advanced gastric cancer.[8–10] High-quality CT scans are widely available at most, if not all, major medical centers in the United States and often are used for preoperative assessment of the patient with gastric cancer. Despite a wealth of medical literature on CT in patients with abdominal neoplasms, little is

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known about the true accuracy of CT in patients with T4 gastric cancer.[11,12]

In addition to imaging modalities, intraoperative clinical assessment by an experienced surgeon is critical for determining the necessity of multiorgan resection for patients with gastric cancer. When performed for the purpose of en bloc resection of bulky disease, multiorgan resection is ideally performed in patients with true invasion of adjacent organs by a primary gastric cancer. This determination is usually made by the apparent adherence of a gastric neoplasm to adjacent structures and is reserved to the best judgment of an experienced surgeon. Little data exist to evaluate the accuracy of this intraoperative assessment of apparent T4 gastric cancer.

The purpose of this study was to determine the accuracy of intraoperative assessment of T4 gastric cancer, and to similarly determine the accuracy of preoperative CT in patients with presumed T4 disease. Data in this study were obtained from a prospective gastrointestinal oncology database and were retrospectively reviewed.

MATERIAL AND METHODS

A retrospective analysis of a prospective gastrointestinal oncology database was performed. Patients included in this study were treated between 1990 and 2002. Medical records of identified patients were retrospectively reviewed to supplement information maintained in the database. Patients with a diagnosis of primary gastric cancer who underwent removal of multiple organs for the purpose of en bloc resection of a locally advanced tumor were included. Patients were excluded if organs other than stomach were removed for reasons other than en bloc resection of locally advanced disease. For example, all patients who underwent splenectomy for management of intraoperative splenic trauma were excluded from this study. Intraoperative decisions to proceed with removal of additional organs for the purpose of achieving an R0 resection was based on the judgment of the operating attending surgeon. Fifteen patients had CT scans taken before surgery; this information was reviewed to determine whether invasion into adjacent organs was predicted by preoperative imaging.

RESULTS

Among a total of 418 patients that underwent gastrectomy for gastric cancer between 1990 and 2002, 21 patients underwent en bloc multiorgan resection for presumed T4 disease (14 men and 7 women; median age, 67 years). Subtotal gastrectomy was performed in 7 patients and total gastrectomy was performed in 14 patients. Organs resected in addition to stomach included the spleen (n = 13), distal pancreas (n = 12), colon (n = 5), small bowel (n = 3), and liver (n = 2). Seven patients had one additional organ resected, 11 patients had two additional organs resected, and 3 patents had three additional organs resected (Table 1).

Postoperative median length of stay was 11 days. Complications following resection included wound infection (n = 5), pneumonia (n = 4), pleural effusion (n = 3), urinary tract infection (n = 2), sepsis (n = 2), arrhythmia (n = 1), pancreatic fistula (n = 1), intra-abdominal abscess (n = 1), and a dislodged arterial angiocatheter (n = 1).

The cumulative postoperative morbidity was 39%. Nine patients had no complications. Two patients died without leaving the hospital, for an in-hospital postoperative mortality rate of 10%. No significant difference in morbidity or mortality was observed in patients undergoing multiorgan resection for gastric cancer compared with a control group of patients with gastric cancer who underwent gastrectomy alone. Morbidity following multiorgan resection was 39%, compared with 36% in control patients ($P > 0.05$, χ^2 analysis). Mortality was 10% after multiorgan resection, compared with 3% in control patients ($P > 0.05$, χ^2 analysis; Table 2). Survival following resection was determined by Kaplan-Meier analysis (Fig. 1).

Histopathology revealed a diagnosis of adenocarcinoma of the stomach in all 21 patients. Among the 21 total patients who underwent multiorgan resection, only 8 had a pathologic T4 tumor identified on histopathology. The remainder of patients (13 of 21) had T3 disease. Thus, the accuracy of intraoperative clinical assessment for a presumed T4 gastric cancer was only 38%.

Table 1. Patient demographics and additional organs resected

	Pathologic T3 disease	Pathologic T4 disease
Median age (yr)	63.5	68.5
Gender (m/f)	10:3	4:4
Total gastrectomy (n)	10	4
Subtotal gastrectomy (n)	3	4
Additional organs resected (n)		
Spleen only	1	1
Pancreas only	0	1
Pancreas, spleen	7	1
Pancreas, spleen, colon	3	0
Colon	2	1
Small bowel, colon	0	2
Small bowel, liver	0	1
Liver	0	1

Table 2. Postoperative and in-hospital morbidity data

	30-Day and in-hospital morbidity	Postoperative mortality
Multiorgan resection	7/18 (39%)	2/21 (10%)
Control group (gastrectomy only)	28/78 (36%)	2/78 (3%)

$P > 0.05$ (χ^2 analysis, NS).

Fifteen patients were assessed by preoperative CT. Among all preoperative CT scans interpreted as positive for invasion into an adjacent organ by a locally advanced gastric cancer, only half were correct in this assessment; the positive predictive value of preoperative CT in assessing T4 gastric cancer was only 50% (Table 3).

DISCUSSION

Only 5% of patients with gastric cancer at our institution underwent en bloc resection of additional organs at the time of gastrectomy. In all of these patients, multiorgan resection was specifically performed for the purpose of removing all evaluable disease, to achieve an R0 resection. Multiorgan resection in this small group of patients was performed without a significant increase in morbidity or mortality compared with gastrectomy alone. A much larger series on multiorgan resection for gastric cancer from the Memorial Sloan-Kettering Cancer Center has associated multiorgan resection with a significant increase in morbidity.[13,14] Unfortunately, that series did not exclude patients who underwent removal of additional organs for purposes other than en bloc resection of bulky disease (such as splenectomy for management of intraoperative splenic trauma). Thus, the increased morbidity in that series may be the result of intraoperative complications, rather than a direct association with the removal of adjacent, adherent organs.

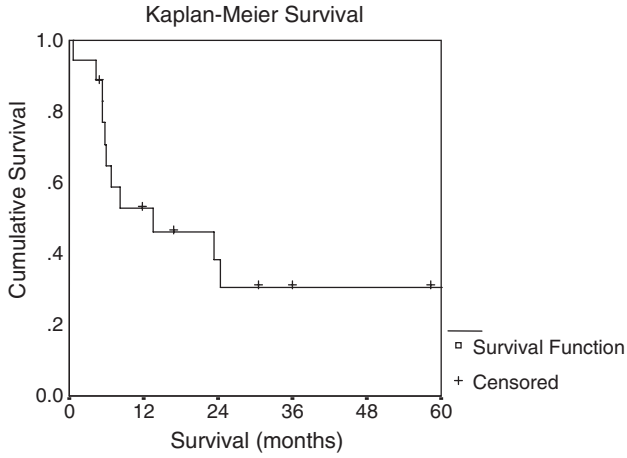

Fig. 1. Kaplan-Meier analysis for survival after multiorgan resection for locally advanced gastric cancer.

Table 3. Accuracy of clinical T staging by preoperative computed tomography (CT)

Pathologic T stage	Clinical T stage (based on CT)		Total
	T3	T4	
T3	6	2	8
T4	5	2	7
Total	11	4	15

Positive predictive value = 50%; negative predictive value = 55%; sensitivity = 29%; specificity = 75%.

Preoperative CT did not accurately predict the presence of T4 disease in the majority of patients undergoing multiorgan resection for gastric cancer. A positive predictive value of only 50% for this modality indicates that, at our institution, CT overstaged patients who were suspected of having invasion into adjacent organs half of the time. Given the inclusion of patients in this study from 1990 on (when the availability and experience with endoscopic ultrasound were limited), most patients had not been evaluated with preoperative endoscopic or laparoscopic ultrasound for T and N staging. These more sensitive modalities are currently used at our institution for all patients with gastric cancer. The likelihood exists that preoperative staging for locally advanced gastric cancer will become increasingly accurate as endoscopic and laparoscopic ultrasound becomes more available and experience with these modalities improves.[15,16]

Intraoperative assessment of presumed T4 disease was incorrect in 13 of 21 cases. Only eight patients who underwent multiorgan resection actually had invasion of an additional organ by the primary gastric cancer on histopathology. Many patients who had a T3 lesion on histopathology were noted to have close encroachment by the tumor upon an adjacent organ, without actual invasion (Fig. 2). This was often accompanied by a densely adherent layer of fibrous tissue between the cancer and adjacent uninvolved organ. Despite the inaccuracy of intraoperative assessment, the removal of adjacent organs, when it can be done safely, is still advocated if the suspicion exists that T4 disease is present. The alternative of "peeling" an adherent tumor off of an adjacent structure carries an unacceptable risk of leaving behind a microscopic positive margin.

Multiorgan resection, when performed in patients with locally advanced gastric cancer for the purpose of achieving an R0 resection, is not associated with an increase in morbidity or mortality. Clinical staging of patients by intraoperative assessment or preoperative CT is inaccurate. Histopathology reveals T4

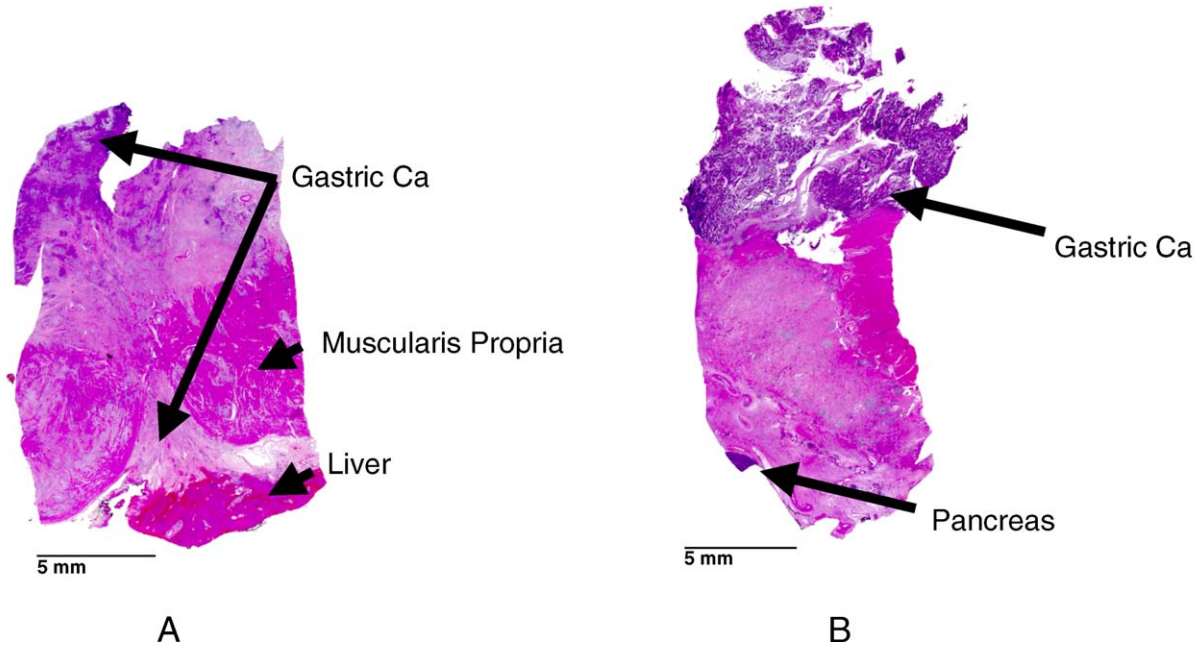

Fig. 2. Invasion of the liver by a T4 gastric adenocarcinoma (Ca) is demonstrated by (**A**) histopathology. Many patients who undergo resection of adjacent organs for presumed invasion by locally advanced gastric cancer do not actually have T4 gastric cancer on histopathology. Close encroachment by the tumor on an adjacent organ, without actual invasion, is often accompanied by a densely adherent layer of fibrous tissue between the cancer and an adjacent uninvolved organ (**B**).

disease in only 38% of patients who undergo multiorgan resection for presumed T4 gastric cancers. Despite the inaccuracy of clinical staging by intraoperative assessment or CT, multiorgan resection is still advocated when it can be safely offered to the gastric cancer patient for the purpose of completely removing all disease. The alternative of leaving a positive margin behind as a result of avoiding multiorgan resection may subject the patient to an increased risk for a poor outcome in longterm survival.

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Percutaneous Endoscopic Gastrostomy

Jeffrey L. Ponsky, M.D.

It has been more than 20 years since the first description of an endoscopic alternative to surgical gastrostomy.[1] The original technique for the performance of percuatneous endoscopic gastrostomy has been termed the "pull" technique, and it is the most commonly employed today.[2] A variation of the method, the "push" technique, is very similar and employs the same principles to provide safe, percutaneous access to the stomach.[3] Little has changed since the original descriptions of the method, but refinements have added for patient safety and ease of performance.

Indications for percuatneous endoscopic gastrostomy include the need for enteral nutrition in patients unable to swallow, the provision of supplemental feedings or bile replacement, and for gastric decompression in cases of chronic intestinal obstruction secondary to benign or malignant disease.

METHOD

Feedings are withheld for 8 hours prior to the procedure to ensure an empty stomach. A preoperative antibiotic, usually one with gram-positive coverage, is given just before the procedure is begun in order to provide prophylaxis against peritubal infection. The patient is generally positioned supine and conscious sedation administered. Posterior pharyngeal anesthesia is often provided with topical spray.

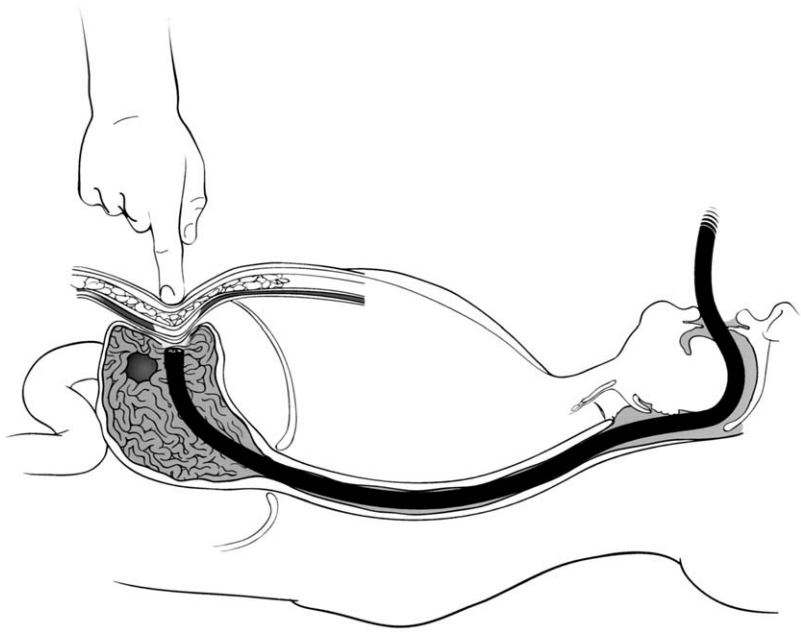

Fig. 1. Gentle finger pressure at the proper site should produce a clear mound of indentation of the gastric wall seen endoscopically.

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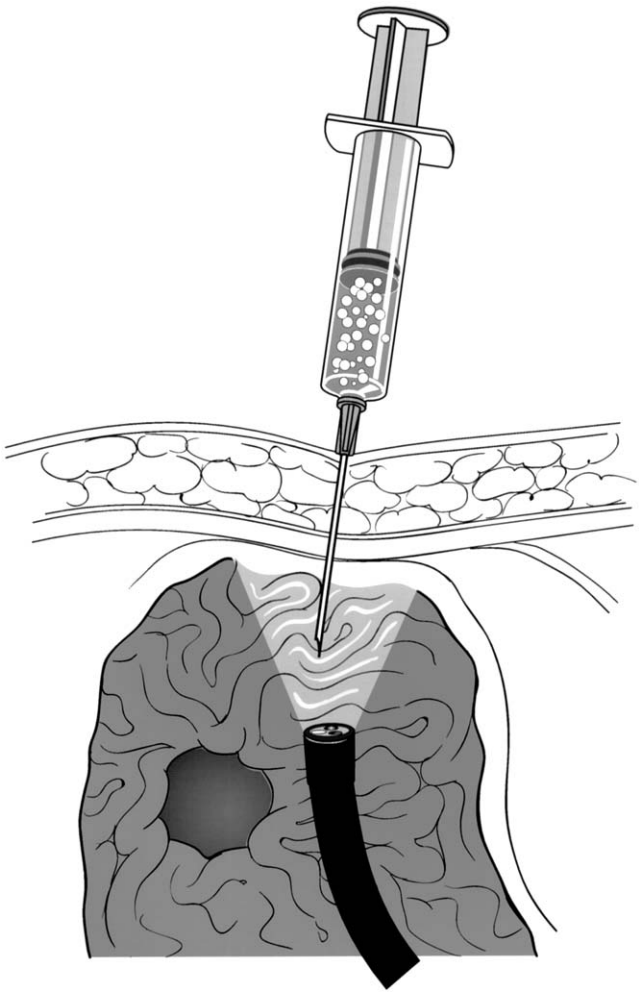

Fig. 2. Using the "safe tract" technique, the needle of the syringe with fluid in the barrel is advanced with traction on the plunger until air bubbles are seen in the chamber. This should occur at the same moment that the needle is seen entering into the gastric lumen. Should air be noted in the syringe prior to needle entry into the gastric lumen, another site should be selected, because bowel is likely to be present between the abdominal wall and the stomach.

The abdomen is prepared and draped in a sterile fashion. The gastroscope is introduced under direct vision into the esophagus and advanced into the stomach. The gastric contents are aspirated and the stomach is inspected. The duodenal bulb may also be surveyed because additional pathology may be noted. The scope is withdrawn proximal to the incisura, and the assistant performing the abdominal portion of the procedure uses gentle finger pressure in various areas to ascertain the point at which the stomach and abdominal wall are in closest contact. It is important to take extra time at this juncture to correctly identify the point of optimal puncture. Although transillumination of the abdominal wall was originally described in order to best localize the correct site of puncture, today we have abandoned this in favor of finger pressure (Fig. 1) in concert with performance of the "safe tract" technique. This maneuver, originally described by Foutch et al.,[4] has greatly enhanced the safety and accuracy of PEG placement and has served as a cornerstone for the safe performance or more difficult procedures such as direct percutaneous jejunostomy.

When a site of clear gastric indentation is noted with external finger pressure, the safety of that site is tested using the safe tract method. A syringe containing several milliliters of local anesthetic is introduced just through the skin at the selected point. With constant observation by the endoscopist, the needle is advanced while suction is applied to the barrel of the syringe. If the correct site has been chosen, the endoscopist will see the needle tip enter the gastric lumen at the same moment that the assistant identifies air bubbling into the barrel of the syringe (Fig. 2). Should air appear in the syringe prior to the needle's appearance in the stomach, the needle has entered another air-containing viscus, colon, or small bowel on its way to the stomach. In such a case, the needle is withdrawn and another site for puncture is selected. When the appropriate site is agreed on, local anesthetic is infiltrated into the skin and a 0.5 cm incision is made in the skin. Inside the stomach, a snare is placed and opened over the site of proposed entry. A large-bore needle (14 gauge) is thrust through the apposed gastric and abdominal walls into the waiting snare loop. The loop is then closed around the needle. A long looped suture is then introduced through the puncturing needle into the gastric lumen (Fig. 3). When several inches of suture have entered the

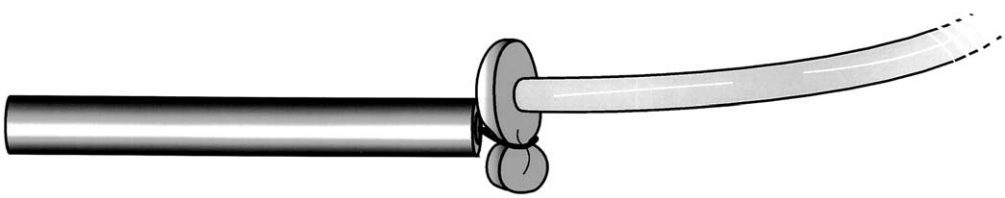

Fig. 3. To aid in the second passage of the scope, half the head of the gastrostomy tube is tightly snared through the scope and used to guide the scope into the esophagus.

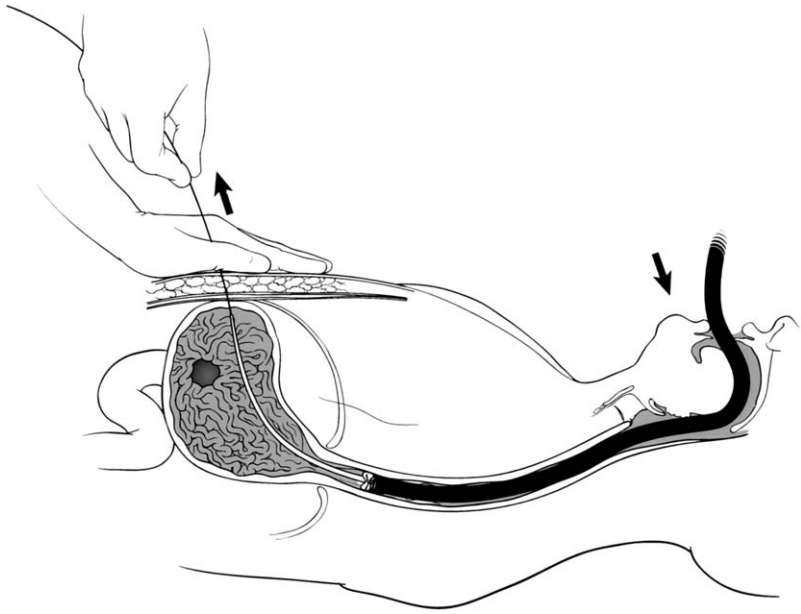

Fig. 4. The scope-tube combination is pushed and pulled into position, and approximately halfway down the esophagus the snare is loosened and the head of the tube is released.

stomach, the snare is loosened and retightened around the suture itself; the needle is withdrawn slightly to facilitate this. The snare with the contained suture is then removed, along with the gastroscope, from the patient's mouth.

The suture is next affixed to the tapered end of the gastrostomy tube, and the tube is well lubricated. The abdominal assistant will next pull on the abdominal end of the suture to "pull" the tube down through the esophagus and into the gastric lumen. To ensure proper tube placement, it is desirable to reinsert the gastroscope to follow the tube down into the stomach and document its correct placement. Reinsertion of the endoscope may be difficult and time consuming, and may be facilitated by grasping one side of the tube's mushroom head with a snare passed

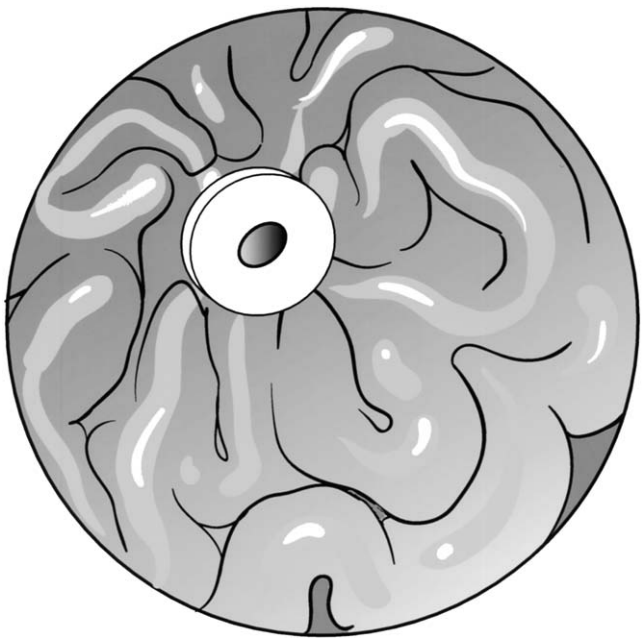

Fig. 5. The head of the tube should come to rest in loose contact with the gastric mucosa.

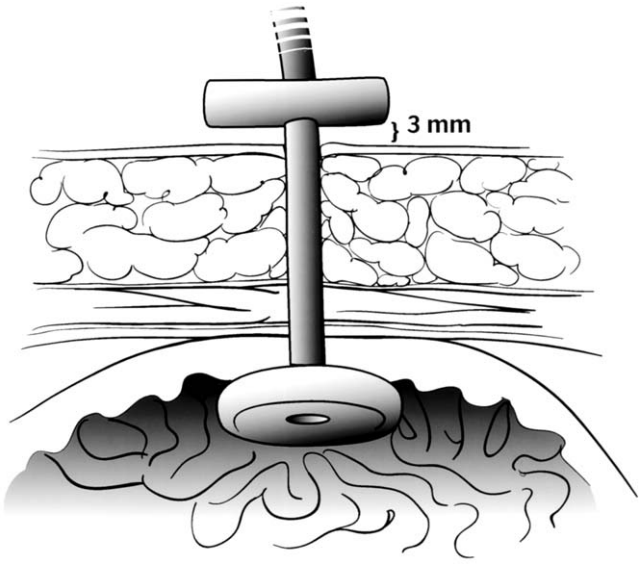

Fig. 6. With the tube pulled gently up, the external crossbar should be placed approximately 3 mm from the abdominal skin to avoid excessive tension and ischemia of the intervening tissue, between the head of the tube and the crossbar.

through the endoscope (Fig. 4) and following the tube as it is being pulled through the esophagus. Once the tube is in the midesophagus, approximately 30 cm., pull is halted and the snare is loosened and removed from the head of the tube. Then pulling on the tube is resumed, and the scope is gently pushed after it to document its final position lying gently against the gastric mucosa (Fig. 5). The gastroscope is then removed.

An outer crossbar or disc is applied to the tube in order to hold the gastric and abdominal walls in contact and to prevent inward migration of the catheter. In the past there has been a compulsion to apply this crossbar tightly in order to ensure adherence of the gastric and abdominal walls. This is not only unnecessary but extremely dangerous. Excess tension here produces ischemia in the interposed tissue of the abdominal wall and leads to necrosis, infection, peritubal leakage, and often tube extrusion.[5] It is preferable to allow several millimeters of space between the outer crossbar and the skin (Fig. 6). Experience and experimental work has demonstrated that that tight apposition of the gastric and abdominal walls is not necessary.

Feeding adapters are applied to the tube and simple tape is used to hold it against the abdominal wall. Although many choose to use an occlusive dressing, this may promote a moist environment and maceration of the skin. The tube may be used for feedings immediately following placement.

Percutaneous endoscopic gastrostomy is now a well-established method, and is the preferred method for placement of a gastrostomy without laparotomy. It represents one of the first forays into the area of minimally invasive surgery.

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Review Article

Current Management of Gastric Cancer

Ulf H. Haglund, M.D., Ph.D., Bengt Wallner, M.D., Ph.D.

Gastric cancer is a topic of great interest for surgeons as well as for epidemiologists. However, in many countries it is not of major interest for oncologists because of limited benefit from radiotherapy and cytostatics.[1] Epidemiologic studies have revealed great differences in gastric cancer incidence among countries and a significantly decreasing incidence of gastric cancer around the world during the past 50 years. Surgeons today have learned to perform total gastrectomy, with a low postoperative mortality and morbidity. In specialty centers, very low frequencies of postoperative complications are reported. However, the surgical strategy is still a matter of intense debate. This review presents the modern management of gastric cancer, the evidence supporting it, as well as the issues of controversy regarding the best surgical treatment.

INCIDENCE AND RISKS

The incidence of gastric cancer has been declining during recent decades but is still the second most common cause of cancer death in the world. There are very significant differences in the incidence of gastric cancer among different countries. Japan, China, and Russia are countries with a high incidence of gastric cancer, whereas in the United States, the white population is in the lower incidence range. Men have roughly twice as high an incidence of gastric cancer as women. The incidence among Japanese men is almost 80 cases per 100,000 inhabitants a year, whereas it is only slightly above 10 cases per 100,000 inhabitants a year among white U.S. men.[1] The decline in incidence is not seen for all types of gastric cancer. Although the total incidence of gastric cancer is decreasing, the incidence of cancer located in the gastric cardia is increasing.[2,3]

It is generally believed that environmental factors early in life are important factors in the differences in gastric cancer incidence found in the different parts of the world. Supporting this are reports indicating that the gastric cancer incidence found among second- and third-generation offspring from immigrants originating from high-incidence countries, but moving to low-incidence areas, approaches the low incidence of the native populations.[4]

Gastric cancers constitute more than 90% of all gastric tumors. From a pathologic-anatomic point of view, gastric cancer most often can be subdivided into an intestinal type and a diffuse type according to the Finnish pathologist Lauren.[5] This classification system has been widely adopted all over the world. The incidence of the diffuse type of gastric cancer seems to be fairly constant over time and among countries. This form is, from a prognostic point of view, the less favorable one. It is the intestinal type that is common in the high-incidence countries, and it is this type of gastric cancer that has had a declining incidence over the years.[1]

Gastric cancer is most often staged according to the TNM classification proposed by the International Union Against Cancer (UICC)[6] (Table 1). The classification according to the American Joint Committee of Cancer[7] is similar but differs slightly from the UICC version according to definitions of N factors and stage grouping. There is an international agreement to use the UICC staging system. In addition, the Japanese Research Society for the study of Gastric Cancer (JRSGC) has published guidelines for the standardization of surgical treatment and pathologic evaluation of gastric cancer.[8] According to the TNM classification, T1 tumors are located within the mucosa (T1a) or reach the submucosa (T1b). This stage of tumor is often referred to as early gastric cancer (EGC). Such tumors may have nodal metastasis (N1). EGC is quite common in Japan (25–50% of all gastric cancer) but constitutes less than 10% of gastric cancer in the western part of the world.[9,10] This form of gastric cancer has a very good prognosis, with reported 5-year survival of more than 90%. Therefore, screening programs are carried out in Japan to identify patients with EGC. Gastric cancer that is not EGC is called advanced gastric cancer, regardless of the spread within the gastric wall, nodal involvement, or distant metastasis.

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Table 1. International unified TNM classification of gastric cancer

T factor			
Tx	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	Carcinoma in situ: Intraepithelial tumor without invasion of the lamina propria		
T1	Tumor invades lamina propria or submucosa		
T2	Tumor invades muscularis propria or subserosa		
T3	Tumor penetrates serosa (visceral peritoneum) without invasion of adjacent structures		
T4	Tumor invades adjacent structures		
N factor			
Nx	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Metastasis in 1–6 regional lymph nodes		
N2	Metastasis in 7–15 regional lymph nodes		
N3	Metastasis in more than 15 regional lymph nodes		
M factor			
Mx	Distant metastasis cannot be assessed		
M0	No distant metastasis		
M1	Distant metastasis		
Stage grouping			
Stage 0	Tis	N0	M0
Stage Ia	T1	N0	M0
Stage Ib	T1	N1	M0
	T2	N0	M0
Stage II	T1	N2	M0
	T2	N1	M0
	T3	N0	M0
Stage IIIa	T2	N2	M0
	T3	N1	M0
	T4	N0	M0
Stage IIIb	T3	N2	M0
Stage IV	T4	N1, N2, N3	M0
	T1, T2, T3	N3	M0
	Any T	Any N	M1

Adapted from UICC-TNM Classification of Malignant Tumours. 5th ed. Berlin: Springer Verlag; 1997.

The JRSGC guidelines have in a systematic way identified the perigastric lymph nodes that may be involved with gastric cancer cells from a primary gastric cancer[8] (Fig. 1). The type of lymph node resection (D1, D2, and D3; see later) is defined according to this system. Recently, evidence supporting the concept that nodal metastases in gastric cancer may first involve one to five sentinel nodes, at least in EGC, has been presented.[11]

Cancer of the gastric cardia represents a special problem in the respect that a clear definition and distinction from lower esophageal cancer are missing.[12] For the purpose of this review, cancers arising from the esophagogastric junction or below but infiltrating the esophagogastric junction are considered as cancer of the cardia. This type of adenocarcinoma is increasing in incidence. Although gastric cancer has an association with *Helicobacter pylori* infection,[1] this seems not to be true for cardia cancer.[13]

DIAGNOSIS AND PREOPERATIVE STAGING

Screening programs for gastric cancer are used in Japan but generally not in the western world. It has been discussed that patients undergoing a gastric resection for ulcer more than 20 years ago should be considered for screening because they have an increased risk for cancer in the remainder of the stomach.[14] However, with the current very low rate of gastric resection for ulcer disease and the advanced age of most of these patients, this has not been considered to be cost-effective. Therefore, in the western world patients are investigated for gastric cancer only when they have symptoms. Abdominal pain, dyspepsia, and weight loss are frequent symptoms, as are anorexia and dysphagia (Table 2).[15] Anemia and fatigue are also frequently found in gastric cancer patients. Today, fiberoptic esophagogastroduodenal endoscopy is the most frequent form of diagnostic investigation (Table 3). Combined with biopsies, this modality may be conclusive in the vast majority of cases at the first attempt (>98%).[1,16] If a definitive cancer diagnosis cannot be reached but suspicions were aroused during the examination, repeat examinations without delay are required. An upper gastrointestinal series using the double-contrast technique may be an equally good diagnostic procedure but does not allow biopsies; it therefore has to be followed by an endoscopy. Gastric cancer can be subdivided according to the endoscopic appearance and classified as described by the Japanese Endoscopic Society,[17] but this is not done routinely by most endoscopists in the western part of the world.

Computed tomography (CT) scans can be used to evaluate the extent of the primary tumor and to reveal nodal and distant metastases. However, compared with the findings at laparotomy, CT may frequently underestimate tumor involvement. In general, nodes have to be 10 mm to be detected routinely on CT.[18] Endoscopic ultrasonography (EUS) is an investigation used increasingly in the preoperative workup of patients with esophageal and gastric cancers. EUS does not, however, reliably define T2 from T3 and T3 from T4 tumors. Lymph nodes may be detectable when they are greater than 3 mm.[18] According

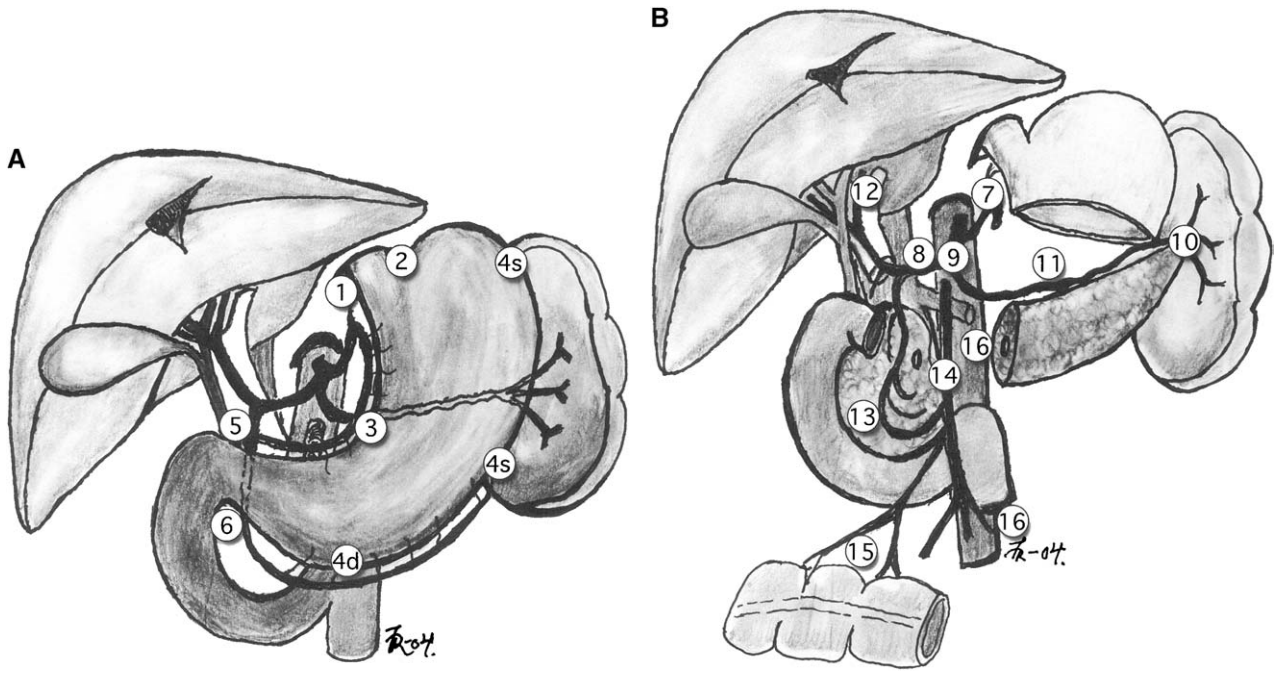

Fig. 1. (**A** and **B**) Lymph node characterization, adapted from Japanese Research Society for Gastric Cancer.[8] Depending on the location of the cancer in the stomach, removal of lymph nodes 1–6 constitutes a D1 resection; additional removal at lymph nodes 7–12 constitutes a D2 resection. A D3 resection also includes lymph nodes 13 and 14.

to Kuntz and Herfarth,[18] the T category and node involvement can be staged with 60–90% and 50–80% accuracy, respectively, with the use of EUS.[18] Modern magnetic resonance imaging (MRI) probably has the potential to be at least as accurate as EUS, but comparative studies are lacking at the moment. Also, laparoscopy has been used for staging of patients with gastric cancer. Distant metastasis has been detected in patients in whom CT did not reveal signs of unresectability.[19] Laparoscopy can be combined with laparoscopic ultrasound examination, but data from comparative studies are still lacking.

SURGICAL TREATMENT OF GASTRIC CANCER

Surgery With Intent to Cure

Most modern series from specialist centers report postoperative mortality rates of less than 1–2% and limited postoperative morbidity after total gastrectomy, even in elderly patients. This is a significant improvement compared to series reported 30 years ago. However, population-based studies have demonstrated less satisfying results, indicating that much could be gained from referring patients with gastric cancer to units with specialized surgeons and a high volume of patients.[15,20] The patient prognosis following surgery for gastric cancer is dependent on whether there is serosal extension and on whether there is lymph node involvement. Patients with distant metastasis from gastric cancer cannot be cured.

Table 2. Symptoms frequently associated with gastric cancer

Abdominal pain
Weight loss
Dyspepsia
Dysphagia
Anorexia

SURGERY FOR CANCER OF THE GASTRIC CARDIA

For cancers located in the gastric cardia, several surgical possibilities are available. There are, however, no randomized controlled studies with the end point of long-term survival available to guide the surgeon. A total gastrectomy with a Roux-en-Y esophagojejunostomy via a thoracoabdominal incision is one option. The blood circulation in the long Roux limb may be compromised, and this procedure has a reputation for increased risks of anastomotic insufficiency. A resection of the upper part of the stomach and the distal part of the esophagus may be an alternative, according to Ivor Lewis, as could a transhiatal

Table 3. Investigative procedures for gastric cancer

Procedure	Comment
Gastroscopy	Key procedure with biopsies for histopathological examination
Upper gastrointestinal series	Has to be followed by gastroscopy for biopsies
Endoscopic ultrasound (EUS)	Effective for preoperative staging (T and N factor)
Computed tomography (CT)	Reveals metastatic disease and, to a lesser exstent, lymph node involvement (N and M factors)
Magnetic resonance imaging (MRI)	May be more sensitive than CT; may have the potential to replace both EUS and CT in the future (T, N, and M factors)
Diagnostic laparoscopy	More effective if combined with laparoscopic ultrasound

esophagectomy without thoracotomy and cervical esophagogastrostomy. Surgeons anxious to perform an extensive lymph node dissection prefer to perform a thoracotomy and a three-field node dissection. There is, however, no good evidence supporting the benefit of extended lymph node dissection for cancer of the gastric cardia. In procedures involving a thoracotomy, the esophagogastric anastomosis can be located in the thorax or in the neck. The latter location is preferred by some because an anastomotic leak in the neck has less effect on the patient.[12,22]

A total gastrectomy and a Roux-en-Y esophagojejunostomy with or without a pouch is most often used when operating on cancers located in the upper half of the stomach. A proximal resection with an esophagoantral anastomosis could theoretically be an alternative, but this procedure is not frequently used due to the severe bile reflux esophagitis frequently seen after this procedure.[22]

TOTAL OR PARTIAL GASTRECTOMY?

For a long time, the surgical treatment of distal gastric cancers was controversial. Some surgeons have considered a subtotal gastrectomy to be sufficient, provided an upper margin of at least 5–6 cm could be achieved, depending on the depth of tumor penetration of the gastric wall.[23,24] Others have advocated total gastrectomy *en principe*. Two randomized controlled trials have addressed this issue—a French[25] and an Italian[26] multicenter trial. The French study included 201 patients; 169 of them completed the trial and were evaluated (the total gastrectomy group included 76 patients and the subtotal gastrectomy group included 93 patients). There were no significant differences between the two groups in basic characteristics such as age, sex, size of tumor, serosal invasion, and nodal extension. There were four postoperative deaths in this study, one in the total gastrectomy group and three in the subtotal gastrectomy group. Nonlethal complications were found in 33% of the patients in the total gastrectomy group compared with 34% in the subtotal gastrectomy group. Regardless of the type of surgery, survival was closely related to lymph node involvement and serosal extension. The 5-year survival rate without lymph node invasions was 69% compared with 18% with node involvement ($P < 0.001$). The 5-year survival rate without serosal extension was 64% compared with a rate of 16% with serosal extension ($P < 0.001$). The extent of gastric resection did not influence survival of the matched groups with equal nodal involvement or serosal extension.[25]

The Italian study included 622 patients from 28 centers: 319 randomized to subtotal gastrectomy and 303 to total gastrectomy. Both groups of patients were operated on using the technique of D2 gastrectomy (see section on Extent of Nodal Dissection below for more details) as described by Nakajima and Kaiitani[27]. The characteristics of the patients in the two groups (age, sex, site and size of tumor, wall invasion, and nodal status) were well matched. The total 5-year survival rate calculated for 301 patients at risk was approximately 65%. Survival was heavily influenced by TNM stages—T3-T4 tumors indicated significantly worse survival than T2. Also, nodal involvement significantly influenced survival. However, surgical treatment did not influence the survival rate, which was 65% in the subtotal gastrectomy group and 62% in the total gastrectomy group.[26]

In a small study, Robertson et al.[28] compared an R1 subtotal gastrectomy (a subtotal gastrectomy with a D1 lymph node dissection) with an R3 total gastrectomy (with a D3 node dissection). Included in the two groups were 25 and 29 patients, respectively. The groups were comparable in age and stage of disease. One postoperative death occurred in the R3 group. Of the 29 patients operated on with total gastrectomy and D3 lymph node dissection, 14 had a left subphrenic abscess in the postoperative period. Overall survival was significantly better in the R1 subtotal gastrectomy group.

The conclusion from these studies is that a subtotal gastrectomy is an adequate surgical procedure for

patients with gastric cancer located in the distal half of the stomach, allowing a 6-cm proximal resection margin. This margin has to be verified by frozen section. These studies also reemphasize that nodal involvement and extent of wall invasion are the two important factors that determine long-term survival after surgery for gastric cancer.

EXTENT OF NODAL DISSECTION

The rate of long-term survival after surgery for gastric cancer is higher in Japan than in the western world.[8] As discussed, EGC, the stage of gastric cancer with the very best prognosis, is much more common in Japan. This fact explains part of the difference. The more precise histopathologic examinations in Japan and, hence, the more adequate cancer staging also means better survival for patients with cancers correctly staged at lower stages. In addition, it has been proposed that the much more extensive lymph node dissection generally performed by the Japanese surgeons when operating for gastric cancer could be an important part of this difference in gastric cancer survival.[8] Nonrandomized studies have further supported the concept that an adaptation of the Japanese surgical technique could improve the results of gastric cancer surgery in the west.[29,30] Two European randomized trials that tested the hypothesis that more extensive lymph node dissection was associated with higher long-term survival have been presented recently—a British study[31,32] and a Dutch study.[33]

In the British study, a D1 resection (involving lymph stations 1–6 as indicated in Fig. 1) was compared with a D2 dissection (lymph nodes 1–12 in Fig. 1, depending on the tumor location). A total of 737 patients with histologically proven adenocarcinoma were registered from 32 participating surgeons over 7 years. Three hundred thirty-seven patients were found to be ineligible at staging laparotomy. The study, therefore, was performed on 400 patients divided into two equal groups. The patients in the groups were comparable regarding age, tumor location, disease stage, and gastric resection. It was found that the D2 group had higher postoperative hospital mortality (13% versus 6.5%) and higher overall postoperative morbidity (46% versus 28%). The postoperative hospital stay was longer for the D2 patients. The excess postoperative morbidity and mortality in the D2 group were reported mainly to be due to the distal pancreaticosplenectomy and splenectomy, respectively, as parts of the D2 procedure with certain cancer locations.[31] In a later publication, 5-year survival was reported to be 35% for D1 resection and 33% for D2 resection.[32] Death from gastric cancer was similar in the two groups, as was recurrence-free survival. It was concluded that D2 resection offered no survival benefit for patients with gastric cancer.[32]

In the Dutch study, 996 patients were entered to obtain 711 randomized study patients. D1 resections were compared with D2 resections. Also in this study, patients in the D2 group had a significantly higher rate of complications postoperatively (43% versus 25%), more postoperative deaths (10% versus 4%), and a longer hospital stay. Five-year survival rates were similar in the two groups (45% for the D1 group and 47% for the D2 group). Patients who had R0 resections (no indication of remaining cancer at surgery) had a 43% cumulative risk for relapse at 5 years after a D1 resection compared with 37% with a D2 dissection. These authors concluded that the routine use of a D2 lymph node dissection in patients with gastric cancer is not evidence supported.[33]

Wu and co-workers from Taiwan[34] reported a randomized study comparing 110 patients operated on with a D1 procedure with 111 having a D3 operation (also involving lymph nodes 13 and 14; see Fig. 1). The two groups of patients were well matched. Total gastrectomy was performed in 27.3% and 20.7% (not significant) of the two groups, respectively. Operating time was significantly longer and blood loss was significantly greater in the D3 group of patients. Postoperative morbidity was significantly greater in the D3 group (17.1% versus 7.3%, respectively). The difference was found to be largely due to a greater incidence of abdominal abscesses.[34] There were no fatalities in either group. Long-term results are not yet available.

A recent Cochrane collaboration study, based on the two randomized studies,[31–33] two nonrandomized studies, and 11 cohort studies has been published.[35] The authors suggest a D2 procedure could be considered the preferred treatment for fit patients with intermediate stage (II–III) gastric cancer, but the hard evidence to support this remains to be presented. To minimize postoperative morbidity and mortality, the Cochrane study recommends that only surgeons who have extensive experience and specialist training should perform gastric resection for cancer.

RECONSTRUCTION AFTER TOTAL GASTRECTOMY

The reconstruction after gastric resection has been a matter of controversy. The postoperative symptomatic outcome after resection for gastric cancer was reported by Buhl et al.[36] to be poor after proximal gastric resection and esophagoantral reconstruction; it was equally poor following a distal partial gastric

Table 4. Oncologic treatment for gastric cancer

Strategy	Evidence
Neoadjuvant chemotherapy	Not sufficient evidence for routine use Large phase III studies ongoing
Adjuvant chemotherapy	Survival benefit suggested in inconclusive studies Not sufficient evidence for routine use
Adjuvant chemoradiation	Survival benefit suggested in inconclusive studies Not sufficient evidence for routine use
Palliative treatment	Justified in selected cases Not sufficient evidence for routine use
Intraperitoneal chemotherapy	Survival benefit suggested in inconclusive studies Not sufficient evidence for routine use

resection with gastrojejunostomy end-to-side and total gastrectomy with a jejunal pouch reconstruction modified from Hunt[37] and Lawrence et al.[38] However, in a similar and also nonrandomized study, significantly more postoperative symptoms such as dyspepsia and dysphagia were demonstrated after total gastrectomy with esophagojejunostomy compared with proximal gastric resection with esophagoantral reconstruction and partial gastrectomy followed by gastrojejunal loop reconstruction.[39] The majority of patients with total gastrectomy had a jejunal pouch in this study. These two reports support the concept that a partial gastric resection and gastrojejunostomy should be performed provided it does not compromise the cancer procedure.

Recently, Fukuhara et al.[40] proposed that after partial gastric resection for cancer, a Roux-en-Y reconstruction is superior to a Billroth I or II reconstruction in regard to bile reflux and reflux symptoms. However, it is well known from previous ulcer treatment studies that a Roux reconstruction is frequently complicated by late stomal ulcers if the procedure is not combined with a vagotomy[41]; in addition, the Roux loop frequently causes stasis.[42] Therefore, non–Roux loop gastrojejunostomy is considered the gold standard.

Svedlund et al.[43] demonstrated in a randomized controlled study that patients reconstructed with a jejunal S-shaped pouch after total gastrectomy for cancer of the stomach had fewer symptoms than patients undergoing total gastrectomy without pouch reconstruction. Again, patients with a subtotal gastrectomy and gastrojejunostomy did significantly better than patients with total gastrectomy. This was especially true for the symptoms of indigestion and diarrhea. Patients with a pouch reconstruction improved postoperatively with time to achieve very good symptomatic results similar or even superior to those following partial resection.[43] In another study, the same group reported that reconstruction with an S-shaped pouch was associated with continuous weight gain many years after total gastrectomy for cancer.[44] It is generally suggested that in patients with a favorable tumor stage, pouch reconstruction after total gastrectomy should be considered.[22] Other procedures such as jejunal interposition[45] and ileocecal interposition[46] between the esophagus and the duodenum

Table 5. Management of gastric cancer—summary

Location of cancer	Surgical management	Evidence
Distal cancer	Partial resection with gastrojejunostomy	Solid supportive evidence
Proximal cancer	Total gastrectomy with esophageal jejunual anastomosis	Solid supportive evidence
	For fit patients with stage II-III tumors: could be considered D2 dissection and pouch reconstruction	Weak supportive evidence
Cancer of the cardia	Either a total gastrectomy with high anastomosis, esophageal-gastric resection with thoracotomy, or esophagectomy without thoracotomy	No supportive evidence in choice of procedure

have been described but have not been generally accepted.

CYTOSTATIC TREATMENT

In a review of the literature, Janunger et al.[47] determined the 5-year survival rate for the total population of patients with gastric cancer to be 15–25%. For details of the many individual studies of treatment with chemotherapy, the reader is referred to this review. Janunger and co-workers included in a meta-analysis studies of systemic and intraperitoneal chemotherapy administered before, during, or after surgery for advanced disease. A special analysis was performed that included 21 randomized studies of the use of adjuvant systemic chemotherapy for gastric cancer. Preoperative or neoadjuvant chemotherapy did not demonstrate any significant benefit. Intraperitoneal therapy also showed no detectable survival benefit. Postoperative cytostatic treatment showed a significant benefit. However, when western and Asian studies were analyzed separately, Janunger et al.[47] found no survival benefit for the treated patients in the western populations; consequently, the survival benefit was found only in Asian patients. In patients with advanced disease, the meta-analysis demonstrated prolonged survival. The survival benefit was in the range of 3–9 months. It was concluded that there is insufficient evidence to recommend adjuvant chemotherapy after surgery for gastric cancer as a routine treatment. It was furthermore concluded that some patients with advanced disease will have a clinically important benefit from palliative chemotherapy, and this treatment is recommended for patients who are otherwise in good health.[47,48] In a review by Sugarbaker et al.,[49] a benefit of intraperitoneal therapy was found in patients with a high likelihood of microscopic recurrent disease, as with T3 and T4 tumors and one or two positive nodes. Intraperitoneal therapy was also advocated for patients with advanced disease that allows only palliative resections[49] (Table 4).

In conclusion, gastric cancer is declining in incidence but remains the number 2 cause of cancer death in the world. Environmental factors in early life are important in the incidence of intestinal type of gastric cancer. Esophagogastroscopy with biopsies for microscopy is the cornerstone in the diagnosis of gastric cancer. CT and, in the future, probably MRI are helpful for preoperative staging. Surgery is the only treatment modality with the potential to cure. A partial gastric resection should be performed if a tumor-free resection margin of 6 cm is possible (Table 5). If not, a total gastrectomy should be considered. There is no support from randomized studies for routine D2 dissection, although it is suggested on the basis mainly on cohort studies that in stage II and III patients, a more extensive lymph node dissection might be associated with prolonged cancer free survival. A D2 resection is therefore proposed for fit patients provided the surgeon is well experienced with this procedure. If a total gastrectomy is performed, a reconstruction that includes a jejunal pouch should be considered in patients with favorable tumor stages. For cancer of the gastric cardia, a total gastrectomy with high anastomosis, a thoracoabdominal gastroesophageal resection, or an esophagectomy without thoracotomy could be used. There is insufficient evidence to support the routine use of adjuvant chemotherapy in patients operated on for gastric cancer. In some patients, chemotherapy could be of significant palliative value.

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A Rare Diagnosis for a Pancreatic Mass: Splenosis

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Splenosis, the autotransplantation of splenic tissue, has been designed to preserve organ functions after splenectomy. We present the first case of laparoscopic resection of a pancreatic splenosis, in a patient who had undergone a splenectomy 31 years before, complaining of abdominal pain and diarrhea. Abdominal computed tomography (CT) scan showed an enhancing hypervascular 3-cm solid mass in the body of the pancreas, mimicking a pancreatic cancer or a neuroendocrine tumor. A diagnostic laparoscopy was planned, and a 3-cm peripancreatic nodule with a long pedicle was visualized, with many nodules close to the tail of the pancreas and in the greater omentum. They were all resected, and the specimens obtained were immediately sent for frozen-section examination, which confirmed the diagnosis of heterotopic splenic tissue. Splenosis should be included in the differential diagnosis of the pancreatic masses in patients with previous splenic surgery. A hypervascular mass on CT scan should be regarded as an adenocarcinoma of the pancreas until proven otherwise. The possibility of a neuroendocrine tumor mandates an octreotide scan and gastrointestinal hormones dosage. In the unlikely event that all tests may produce equivocal results, a diagnostic laparoscopy is mandatory, in order to obtain an accurate histopathologic diagnosis. (J GASTROINTEST SURG 2004;8:913–914)

KEY WORDS: Autotransplantation, spleen, splenosis, pancreas, accessory spleen

INTRODUCTION

Having observed severe and frequently fatal manifestations of septicemia in patients after splenectomy,[1] gastrointestinal surgeons designed *splenosis*, the autotransplantation of splenic tissue that usually follows traumatic rupture of the spleen, to preserve organ function.[2–4] The splenic tissue implants are generally numerous, and the preferred location is the greater omentum,[2,3] because the vascularization of the greater omentum provides ideal conditions for regeneration and neovascularization.[3]

CASE REPORT

A 52-year-old man presented with an 18-month history of abdominal right upper quadrant pain and diarrhea. Thirty-one years before, he had undergone a splenectomy for a severe blunt splenic injury, with heterotopic autotransplantation of splenic tissue into the greater omentum. Abdominal computed tomography (CT) scan, performed with the use of intravenous contrast material and abdominal magnetic resonance imaging (MRI) showed an enhancing hypervascular 3-cm solid mass in the body of the pancreas (Fig. 1). The indium 111-octreotide scan suggested the presence of a neuroendocrine tumor of the pancreas: however, determination of gastrointestinal hormonal serum levels revealed no abnormal values. A diagnostic pancreatic laparoscopy was planned. After the induction of pneumoperitoneum, a 30° high-quality, digital three-chip videolaparoscope was inserted through the umbilicus using the "open technique." The stomach was grasped and lifted with the forceps,

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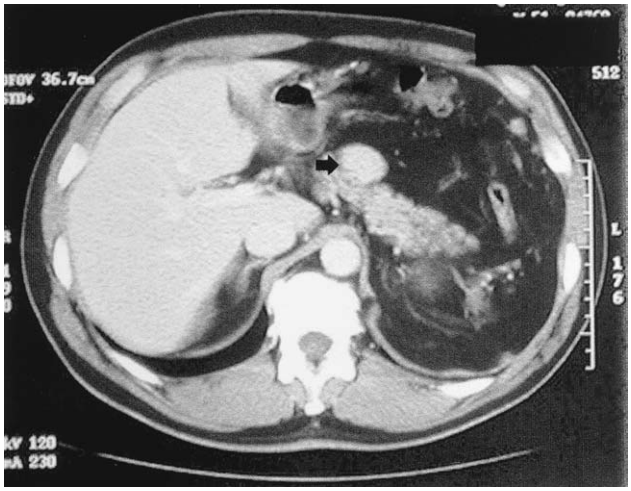

Fig. 1. A hypervascular, solid mass is present in the body of the pancreas on CT scan (*arrow*).

and the transparent window close to the greater curvature was divided by scissors, so that an excellent visualization of the body and the tail of the pancreas was obtained. A 3-cm peripancreatic nodule with a long pedicle was visualized (Fig. 2). In addition, many other nodules were present close to the tail of the pancreas and in the greater omentum. They were all resected, and the specimens obtained were immediately sent for frozen-section examination, which confirmed the diagnosis of heterotopic splenic tissue. A diagnosis of splenosis was made. The postoperative course was uneventful, and the patient was discharged on the fourth postoperative day.

DISCUSSION

Pancreatic splenosis can be very difficult to diagnose preoperatively, and it should be always considered in the differential diagnosis for islet cell tumors, pancreatic carcinoma, and even metastases. Islet cell tumors of the pancreas occur in up to 1.6% of the population,[5] and patients with functioning tumors typically present with the symptoms from hormonal overproduction. Insulinomas are homogeneous, hypervascular masses, while gastrinomas are often moderately vascular.[6] Pancreatic adenocarcinoma has a peak incidence in the seventh decade of life.[5] The masses are usually hypovascular, as shown on CT images.[5] Laparoscopic examination, with ultrasonography, provides an accurate differential diagnosis of the pancreatic masses, with assessment of the size and the extent of the local dissemination.[7] In our case, the octeotride scan suggested the presence of an endocrine tumor of the pancreas, despite the fact that the gastrointestinal hormones, serum values were within normal ranges. In this unclear situation, we performed a diagnostic laparoscopy, with a careful exploration and histopathologic examination of the surgical specimens, which enabled accurate diagnosis. Despite the benign nature of the lesion, surgical exploration was necessary due to the symptomatic nature of the mass, probably related to the torsion of the pedicle. In fact, the patient's symptoms resolved postoperatively, which suggests that the cause may have been related to this lesion.

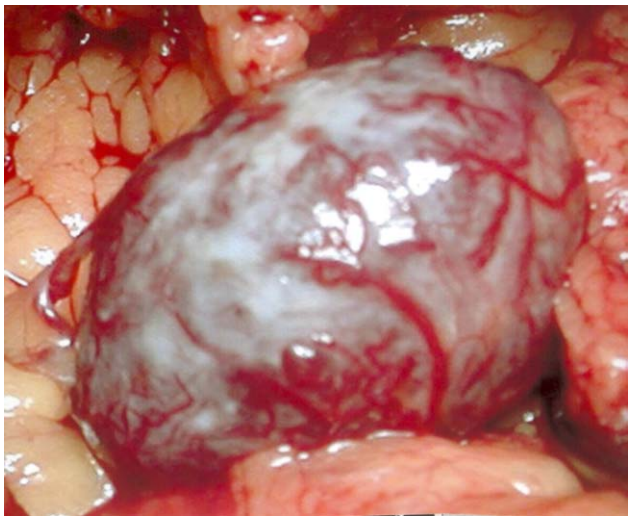

Fig. 2. A 3-cm peripancreatic mass is seen at laparoscopy.

In conclusion, splenosis should be included in the differential diagnosis of the pancreatic masses in patients with previous splenic surgery. A hypervascular mass on CT scan should be regarded as an adenocarcinoma of the pancreas until proven otherwise. The possibility of a neuroendocrine tumor mandates an octreotide scan and measurement of gastrointestinal hormonal levels.

In the unlikely event of all tests producing equivocal results, a diagnostic laparoscopy is mandatory, in order to obtain an accurate histopathologic diagnosis.

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