The First Year

John L. Cameron, M.D., Keith A. Kelly, M.D. Co-Editors

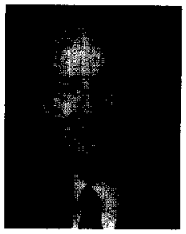
Just one year ago the first issue of the JOURNAL OF GASTROINTESTINAL SURGERY was published after a great deal of planning, and with much enthusiasm and optimism. The driving force was the concept that a journal devoted solely to gastrointestinal surgery was needed, and would appeal to readers and authors alike. Moreover, this new journal would be a voice for our Society, The Society for Surgery of the Alimentary Tract (SSAT). It would lend legitimacy to our specialty, gastrointestinal surgery, and it would attract others to the field. No other American journal had these aims.

One year later these initial goals are well on the way to being achieved. The JOURNAL, through the strong efforts of our Editorial Board and our Publisher, and with the enthusiastic support of the SSAT, is off to a strong start. The six issues of the first volume have struck a balance between innovative clinical papers and exciting basic research. These issues have also included comments from SSAT members, major addresses from SSAT officers, symposia, a consensus conference, letters to the editors, and a memorial. The content has been well received, as evidenced by the rapid growth of our circulation in the first year. All members of the SSAT receive the JOURNAL. Moreover, all senior general surgical residents in the United States and Canada receive the JOURNAL through the generous sponsorship of United States Surgical Corporation. In addition, many other readers have subscribed on their own. Even in these days of electronic communication, a printed journal that is attractively produced, containing high-quality scientific material entirely focused on the field of gastrointestinal surgery, is a sought-after item. Nonetheless, in this rapidly changing era of electronic communication, your Editors along with our capable Publisher are well aware of these trends and will move with them as they continue to develop.

Your Editors send thanks to the SSAT for the opportunity to serve the JOURNAL, to the authors who have sent us such superb material, and to the Editorial Board and reviewers for their fine work. We are especially grateful to our readers who, through their support and subscriptions, have made the JOURNAL possible. Please let us know what you like and don't like about your JOURNAL, and most importantly, we ask for your suggestions as to how it can be improved.

We look forward to the future.

Change, Relationships, and Accountability: Marks of a Vibrant Society



Tom R. DeMeester, M.D.

A society is a group of persons who come together for the purpose of advancing a shared interest. The key components are the persons and the interest. The interest of The Society for Surgery of the Alimentary Tract is implied in its name. We are a group of persons whose feelings, concerns, and attention are focused on surgery of the alimentary tract to the point that we accept accountability for its growth and development. This interest is pursued through individuals acting together to bring about change. The study of this activity and the individuals involved identifies characteristics that distinguish our Society—that give it a personality. The honor of serving as your president has allowed me an opportunity to know in detail our Society. I am convinced that we have a Society with a vibrant personality—one that is pulsating with life, vigor, and activity. The stories of how our Society has changed over the years are fascinating. Knowing them provides insight into what we are.

Around 1957 Dr. Robert Turell had a dream (Fig. 1)—a dream, in his words, "of launching a new surgical organization oriented to the problems of the alimentary tract and of creating a research or educational foundation."[1] The surgical climate at the inception of our Society was, according to Dr. Turell, one of "exaltation of the then budding exotic field of cardiovascular surgery and the debasement of other fields, especially the prosaic, if not the outright pedestrian, field of gastrointestinal surgery."[1] At the time there was no dearth of surgical societies, but their memberships, as he stated, "were shut tight to new, younger progressive surgeons. The only hope of gaining membership was through either death or resignation of members and worse yet, politics at times played an ugly but deciding role."[1] Dr. Turell discussed the possibility of a new society with scores of prominent surgeons and most considered the idea a "speculation if not a gamble."[1]

Because he was an optimist by nature and realized that progress without change is a myth, Dr. Turell proceeded to pursue his dream. He contacted Dr. Warren Cole, who exerted a "soothing and reassuring effect" on him[1] (Fig. 2). He agreed to help under the condition that Dr. John Waugh would join the venture (Fig. 3). The "make-or-break" conference with Dr. Waugh took place in Rochester, Minnesota. After learning about the details of the plan and the meeting with Dr. Cole, Dr. Waugh pledged his full cooperation. He also confessed that he had been entertaining similar thoughts for almost identical reasons.

The founding membership consisted of authors who had contributed papers to six issues of the *Surgical Clinics of North America* edited by Dr. Turell and the authors of the chapters in his textbook *Diseases of the Colon and Anorectum.* All but one of the individuals accepted. The sole person who declined waged an active campaign against the birth of the Society. In the end, he provided the wind that made the kite go higher.

The Society was incorporated on March 30, 1960, as the Association for Colon Surgery and by 1962 the winds of change were blowing. In the beginning it was thought more prudent to limit the focus of the

From the Department of Surgery, University of Southern California School of Medicine, Los Angeles, Calif.
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Reprints requests: Tom R. DeMeester, M.D., Professor and Chairman, Department of Surgery, University of Southern California School of Medicine, 1510 San Pablo St., Suite 514, Los Angeles, CA 90033-4612.

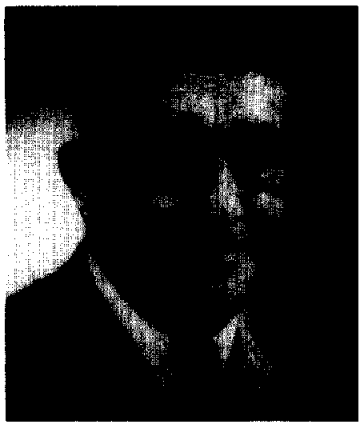

Fig. 1. Dr. Robert Turell, who dreamed of launching a new surgical organization oriented to the problems of the alimentary tract.

Fig. 2. Dr. Warren Cole, who exerted a soothing and reassuring effect on Dr. Turell and agreed to help form a society if Dr. John Waugh would join the venture.

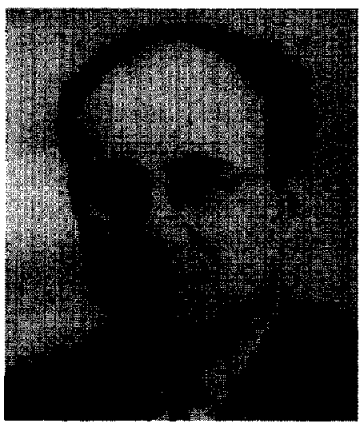

Fig. 3. Dr. John Waugh, who pledged his full support and cooperation to Dr. Turell in developing a new surgical society.

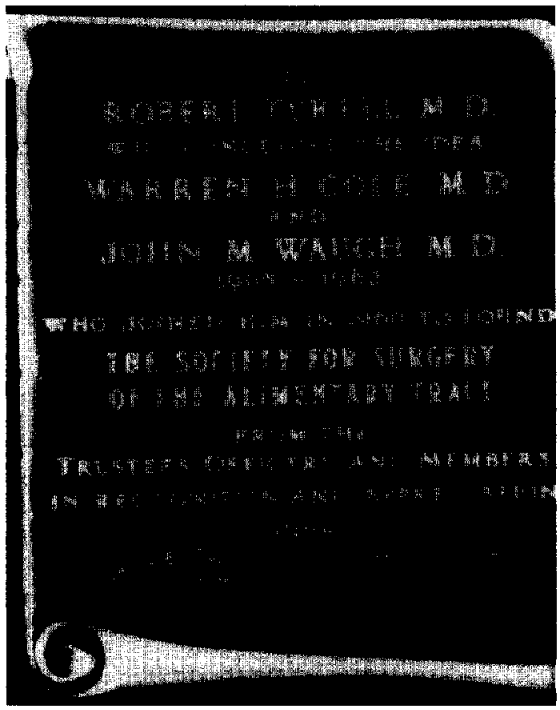

Fig. 4. A plaque honoring the three founding members.

Society to the colon only. Dr. Cole, after much discussion, proposed to the Board of Trustees that the name be changed to The Society for Surgery of the Alimentary Tract to reflect an original and wide interest in the entire alimentary tract. Dr. Turell, on accepting the nomination as the Society's seventh president, summed up our beginning succinctly by stating "we started with anal pruritus and we ended up with generalized dermatitis."[2] In 1968, eight years after it was formed, the Society honored the three founders with a plaque, which is on permanent display in the Department of Surgery of Mount Sinai Medical Center (Fig. 4).

Dr. Robert Zollinger,[3] the Society's third president, gave a convincing address entitled "Justifying our Existence." The address later became known as our "Declaration of Independence." He emphasized that by expanding the scope of the Society from the colon to the entire alimentary tract, all doubts as to the need for another surgical society were dispelled. In his mind, "the dramatic advances in cardiovascular surgery had in no way dampened the enthusiasm of those challenged by the equally dramatic problems involving the alimentary tract."[3] He noted that papers related to the alimentary tract made up less than half of the programs of other societies, including the American College of Surgeons Clinical Congress, and that our Society was the only one focused on surgical

problems of the entire alimentary tract. As far as I am aware, this situation still exists today.

Dr. Zollinger counted at least seven different groups of surgeons with an interest in problems of the alimentary tract, depending on the patient's age and the location of the disease. As examples he cited general surgeons, thoracic surgeons, pediatric surgeons, and coloproctologists. He stressed that to justify our existence these various fields must be represented in our Society. My presence before you is evidence that his advice was heeded.

The requirements for membership in the Society have been in constant debate since its inception. The story is a study of how change can be resisted. In 1965, five years after the founding of the Society, the Board of Trustees directed that the membership should be enlarged rather than restricted to a small group. The first constitution of the Society was approved that same year. The requirements for membership were (1) fellowship in the American College of Surgeons or its equivalent and (2) demonstrated interest in the function and disease of the alimentary tract as evidenced by unpublished fundamental research or publication of significant papers. Two years after the Board's mandate, Dr. Jonathan Rhoads, Chairman of the Membership Committee, reported that "there were no hard and fast rules about eligibility"[4] for membership except that the candidate be certified in his or her specialty. According to Dr. Rhoads, the committee seemed "to have smiled upon people who have had eight to ten or more published papers."[4] The fact that the committee did not attempt to limit membership by the number of publications was in his mind "a very favorable factor and the Society should look forward to a very healthy growth."[4] Despite this initial open policy, by 1981, sixteen years later, the requirement for at least 10 publications became the law of the membership committees.[5] As expected, growth flattened.

Three years later, in 1984, the Board of Trustees became concerned over the lack of growth and again decided that the publication requirement should be liberalized. Dr. James Thompson, Chairman of the Board, reported to the membership that this decision was the most important action taken at the board meeting. He stated that "the ascendancy of our collegial organization, the American Gastroenterological Association (AGA), to a position of great importance, many believe, dates from its adoption of the recommendation of Dr. Mort Grossman that it be an egalitarian and not an elitist organization. I have to assure you," he said, "it was a difficult decision for a bunch of pure elitists to make, but we did it."[6] Then he made a passionate speech to the membership. "The message is to go out and look at the people in your community who are practicing surgery and find those who fit the criteria, generous as they are, and propose them for membership."[6] At that time, the only membership criteria were certification by the American Board of Surgery or its equivalent, and an interest in gastrointestinal surgery. Despite this pronouncement, the membership committee continued to require a minimum of two publications.[7]

In 1993 President-Elect Dr. Bernard Langer suggested that there were three exceedingly important issues facing the Society. "First, the promulgation of advanced training programs in gastrointestinal surgery throughout North America; second, the need to increase substantially the membership of the Society to include virtually all surgeons in North America who practice alimentary tract surgery; and third, further discussion regarding starting our own journal of gastrointestinal surgery."[8] During his presidency, Dr. Langer convened a task force that recommended to the Board a campaign to aggressively recruit members, a change in the membership process to one of direct application, and the creation of a trainee membership. The proposed criteria for membership were (1) a degree from a medical school acceptable to the Board of Trustees, (2) a license to practice medicine in the applicant's state, providence, or country, (3) certification by a board that is a member of the American Board of Medical Specialties, the Royal College of Physicians and Surgeons in Canada, or an equivalent body, and (4) an interest in surgical aspects of digestive disease. Membership is also available to career scientists with an M.D., Ph.D., or a equivalent degree from an institution acceptable to the Board of Trustees.

The most important part of Dr. Langer's proposal was that applications for membership could be initiated by the applicant. All that was necessary was to fill out a form and return it signed and accompanied by a short letter from an active or senior member. At our Executive Session on Wednesday morning, the membership will be asked to approve the new constitution and bylaws that incorporate these requirements since the requirements for membership were never specifically spelled out in the constitution. Since the requirements were very general in our original constitution, these changes have already been activated by the Membership Committee. The appointment of Dr. Robert Beart as chairman has turned the committee into a recruiting force and their efforts, along with the changes mentioned, have been effective (Fig. 5).

The story of the Society journal is one where change engendered competition. The main objective of the founders was the establishment of an effective new organization that would live in harmony with the already established surgical organizations. This auto-

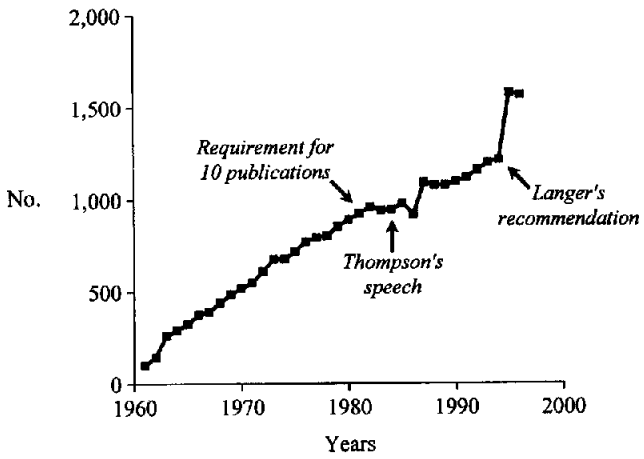

Fig. 5. SSAT growth in membership showing the effects of a requirement for 10 publications, Dr. Thompson's speech, and Dr. Langer's recommendations.

matically eliminated consideration of an independent journal. Despite this, the question arose about the publication of the papers presented at the first annual meeting. Drs. Cole and Turell announced that "Dr. Zollinger, editor of the *American Journal of Surgery*, expressed an interest in the papers and that the Society made a commitment to that journal for one year."[9] Dr. Louis Buie, editor of *Diseases of the Colon and Rectum*, also had an interest in the papers but the group questioned the wisdom of publishing in a specialty journal when their aim was to appeal to a broader readership. Dr. Walter Maddock, one of the editors of *Archives of Surgery*, expressed his interest in the papers and stated that his journal would be beneficial since it had a "captive circulation of about 40,000."[9] Despite the competition, Dr. Zollinger managed to have the *American Journal of Surgery* publish the papers presented at the annual meeting of the Society for the next 10 years. Only once during that period, in 1965, did the Society review the possibility of publishing its own journal, but thought it not desirable at the time.[10] In 1970, through the persuasive efforts of Dr. Zollinger, the *American Journal of Surgery* became the official journal of the Society with the caveat that all members would subscribe to the journal.

In 1984 the issue of a Society journal was again raised. Dr. Frank Moody presented to the Board the possibility of having *Surgical Gastroenterology* become the official journal of the Society with the Society having a major voice in the running of the journal and establishing its policies. The discussion was described as long, agonizing, and painful. In the end, Drs. James Thompson, Paul Jordon, and Scott Jones were charged to discuss the issue delicately with Dr. Zollinger.[11] As expected, the group recommended that the Society remain with the *American Journal of Surgery* and so it did.

The issue resurfaced once more in 1993 as one of Dr. Langer's three important decisions facing the Society. The issue became part of the agenda of the special task force convened during his presidency. In response to the report of the task force, the Board appointed a Publication Committee, chaired by Dr. Keith Kelly, to study the issue. At its October 1995 meeting, the Board accepted the recommendation of the Publication Committee to proceed with establishing an SSAT journal.[12] The name selected was the JOURNAL OF GASTROINTESTINAL SURGERY. Most important, the journal was to be owned and copyrighted by the Society. The Board made the decision to have two co-editors and appointed Drs. Keith A. Kelly and John L. Cameron to those positions. These two distinguished surgeons from two distinguished institutions, serving as joint editors, have enhanced and dignified our new journal immensely.

The story of our Society becoming part of Digestive Disease Week is one where change came about through the threat of being excluded. It also involved taking some risk, an unnatural thing for a society to do. Our Society from the beginning has shown an interest in integrating with other professional organizations. This factor, more than any other, has changed our character and identity. It all started when Dr. Helger Jenkins in 1964, four years after our beginning, urged that a committee be appointed to work out a joint membership with gastroenterologists. He thought that "a little missionary work would be helpful to them as well as to us."[13] Subsequently he was proven to be a prophet. Apparently in response to his request, a Liaison Committee to the AGA was appointed by the Board around 1966. Dr. Lloyd Nyhus chaired the committee. Their charge was to explore possible ways of bringing the two societies interested in gastrointestinal diseases into closer relationship.[14] The purpose was for cross-fertilization of new ideas and the formation of a united front in governmental relationships. The committee found it impossible to schedule a joint meeting with the AGA and the whole issue would have been dropped if it was not for the death of a prominent person in Minneapolis from ulcerative colitis.

The family of the deceased individual established the Digestive Disease Foundation of Minneapolis for the purpose of funding research in the broad scope of digestive diseases. In February 1967, Dr. Nyhus, who was still attempting to make contact with the AGA, attended a conference on Digestive Disease as a National Problem. This conference was sponsored by the Digestive Disease Foundation of Minneapolis, the

National Institute of Arthritis and Metabolic Diseases, and the AGA. The purpose of the conference was to stress to the federal government the overall importance of digestive diseases as they affect the American public. The total monies lost per year to individuals, to industry, and to the nation were documented in an impressive manner. Details regarding the prominence of the problem, the need for continued research, the need for manpower, and a plan to provide for these needs in the future were presented. It was implied that undue attention was given to cardiovascular problems and none or very little to alimentary tract problems. As a direct result of the conference, the National Institute of Arthritis and Metabolic Disease identified the problem of gastrointestinal disease for an in-depth study. It was of interest to Dr. Nyhus that the gallbladder was singled out as an area where greater research efforts were urgently needed. As expected, this got the attention of the surgeons.[15]

The following year Dr. Nyhus reported to the Society that the AGA had taken an interest in our Society because of the desire to have surgeons involved in discussions about digestive disease with governmental agencies. This provided an opportunity for the two committees to discuss a variety of issues including the possibility of a joint annual meeting. At that time the annual meeting of the SSAT was held in conjunction with the American Medical Association meeting, and it was suggested that we change our meeting dates to coincide with those of the AGA. Our interest was piqued partly because the AGA was after research dollars and partly because the gallbladder was singled out as a common problem for which research was needed.

Dr. Robert Zeppa at the time was chairman of a splinter organization of the AGA called the Gastroenterology Research Group. He had gained considerable background information concerning individuals and subject matter that might be helpful in further discussions regarding the integration of the two societies. Dr. Nyhus recommended that Dr. Zeppa be placed on the Liaison Committee, that our Society make no major move to alter its meeting dates but monitor the situation carefully by having members of our Society infiltrate the AGA committee, that all members of the Liaison Committee also be members of the AGA, and that the composition of our committee be known to the AGA so that our members could be members of their committee as well, or at least be invited to sit in on the work of their committee.[16]

The AGA, in moving toward its goal of obtaining research dollars, formed both a Federation of Disease Societies and a Digestive Disease Foundation. Dr. Morton Grossman spent 45 minutes with the Board of Trustees of the SSAT at its 1970 meeting,[17] explaining that the goal of the Federation and the Foundation was to develop a National Digestive Disease Institute similar to the National Cancer Institute. The purpose of the institute would be to support research and education of the lay public, unify public relations, and initiate legislation regarding digestive diseases. He expressed the hope that our Society would join both organizations. There was considerable discussion of Dr. Grossman's presentation. Dr. Turell, our founder, was against the idea, stating he was afraid our Society would be swallowed up and could do better by itself. Dr. Nyhus did not agree. He believed that if the Federation should get a National Digestive Disease Institute with our Society not involved, surgeons would not be represented. Dr. James Hardy concurred. The decision was made to join both the Federation and the Foundation.

When the action of the Board was reported at the Society's annual business meeting, Dr. Ward Griffen took the issue of integration with the AGA one step further and recommended that the membership be polled regarding moving the meeting of our Society to coincide with the AGA meeting.[18] He stated that by doing so many of the members who also belong to the AGA would benefit by going to both meetings and it might help to improve our program. Dr. Dunphy stated "that this was a very reasonable proposal and that the Board should resurface the issue at the next annual meeting with something that tended in this direction." At the meeting a year later, and after a lively discussion during which Dr. Moody dispelled the myth likening the AGA to an octopus, it was decided to poll the membership. Dr. William Silen, out of frustration, sent a strong letter to the Chairman of the Board, Dr. William Scott.[19] He encouraged a clear enunciation of the Society's aims, broadening the membership policies, improving the scientific caliber of the papers presented, and supporting the move to meet with the AGA. The latter he thought would salvage the Society, enhance immeasurably its educational goals, and improve the gastroenterologic research done by surgeons. In response a poll of the membership was taken.

At the 1972 meeting Dr. Zeppa, Chairman of the Liaison Committee, reported that the Digestive Disease Foundation had become a moot issue in that the AGA had achieved its goal by having a bill signed into law by the President of the United States that did not set up a separate institute but changed the name of the National Institute for Arthritis and Metabolic Diseases to the National Institute for Arthritis, Metabolic and Digestive Disease.[20,21] This indicates that a senior individual in that institute will be concerned with the outlay of money to support digestive disease

research. It was expected that research dollars would begin to flow. With respect to the Federation of Digestive Disease Societies, Dr. Zeppa reported that it was still active but entangled in discussion over organizational issues.

At the same meeting Dr. Nyhus, Secretary of the Society, reported that the poll of the membership showed that 80% were strongly in favor of changing the date and location of the meeting to coincide with the AGA in a so-called Digestive Disease Week (DDW), and that arrangements for a combined meeting in New York were set for May 1973.

The combined meeting went exceedingly well and most members enthusiastically supported the motion to continue the arrangement. The Liaison Committee to the AGA, headed by Dr. Zeppa, had done its job well and was retired. In October 1974, six months after the annual meeting, Dr. Zeppa and Dr. Moody were authorized to attend the newly formed Digestive Disease Week Council as representatives of our Society. So it was that DDW came into being.

Four years later in his presidential address entitled "Cooperation to Meet the Challenges." Dr. Zeppa[22] reviewed the Society's decision to join the Digestive Disease Week Council. He noted that as a consequence of this decision the following occurred: first, financial benefit and stability came to each of the four societies, namely, the AGA, the American Association for the Study of Liver Diseases (AASLD), the American Society for Gastrointestinal Endoscopy (ASGE), and our Society; second, attendance at our meeting increased, particularly the special lectures; third, the quality of our program improved, mainly because of the competition for "ears"; fourth, the educational benefits for our members were expanded by the diversity of programs available; and fifth, there was increased opportunity for dialogue, formal and informal, with our medical colleagues. Dr. Zeppa concluded that the membership was to be congratulated for its wise decision. The growth in DDW attendance and income has confirmed the wisdom of the decision and it all started with Dr. Jenkin's suggestion that we do a little "missionary" work (Figs. 6 to 9)!

Our integration into DDW was a sign that other

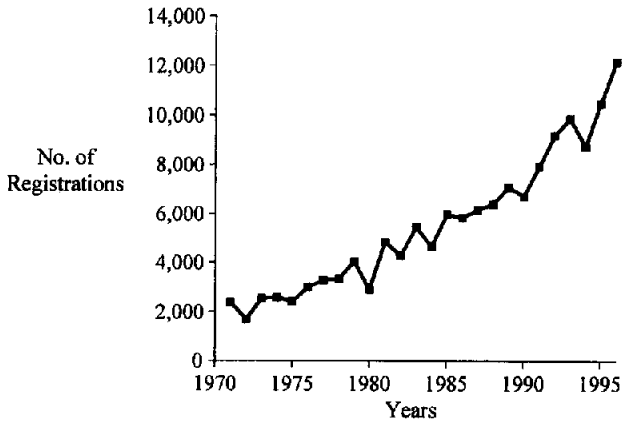

Fig. 6. Digestive Disease Week attendance since its inception in the early 1970s.

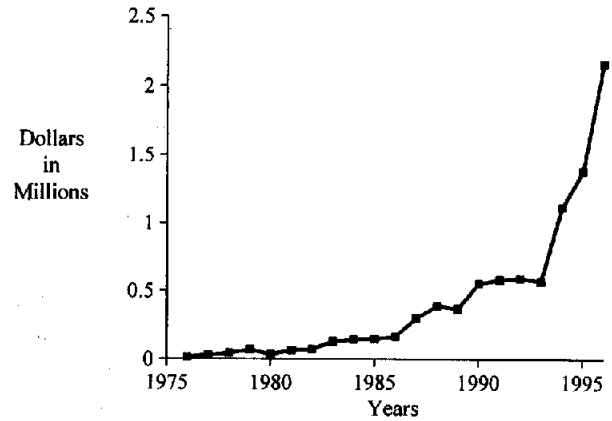

Fig. 7. Net profit of Digestive Disease Week since its inception.

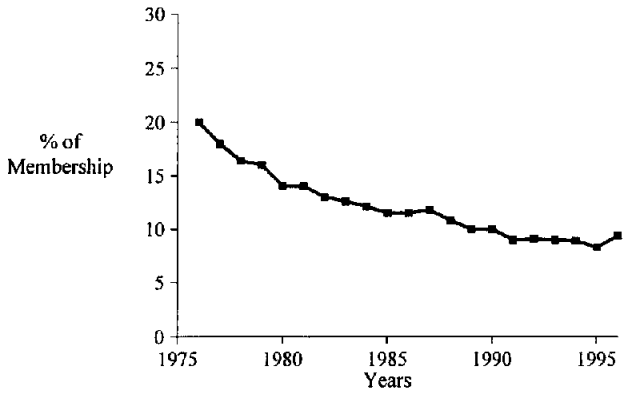

Fig. 8. The percentage of the total membership of the four societies that are members of the SSAT. The percentage is used in the formula to distribute the profits of Digestive Disease Week to the four societies.

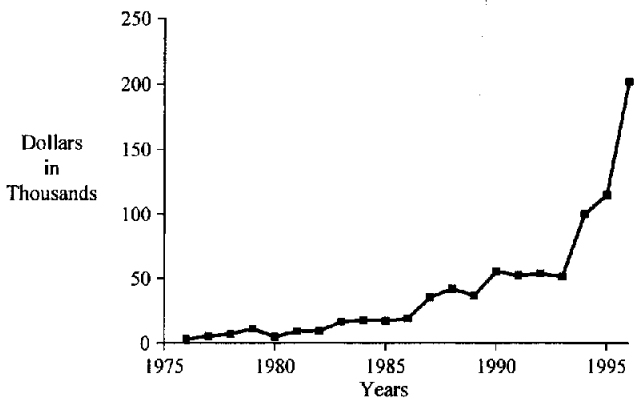

Fig. 9. Growth in the SSAT portion of the profit from Digestive Disease Week.

interactions would follow. In 1991 Dr. William Silen, Chairman of the Board of Trustees, appointed an ad hoc committee to examine the possibility of formalizing a relationship between our Society and the United States Section of Collegium Internationale Chirurgiae Digestivae (CICD). It seemed logical that there should be some appropriate linkage between the societies representing the national and international surgeons interested in the alimentary tract. Dr. Robert Condon, then Secretary-Treasurer of CICD, was appointed chair of the committee. A formal proposal submitted by the CICD in 1992 provided the funding, including honorarium and travel expenses, for the "State-of-the-Art" address at the annual SSAT meeting.[23] Our Society, while retaining the prerogative of choosing the speakers, would acknowledge this financial support in its annual program book and would also list the annual CICD Breakfast Forum. We agreed to remind the newly elected members of our Society of the opportunity to join the CICD. The proposal was accepted unanimously.

More recently we have furthered our relationship with the component societies of DDW by contributing to combined clinical symposiums, organizing a yearly consensus conference, and integrating appropriate oral and poster presentations of our papers into AGA focused research sections and the president's plenary poster session. This year's joint symposium on minimally invasive surgery represents an effort to integrate with the Society of American Gastrointestinal Endoscopic Surgeons (SAGES). Further, the Society is exploring the possibilities of joining the Federated Societies of Gastroenterology and Hepatology. This group has been formed to maximize coordination, cooperation, and efficiency among its members in global programs regarding professional, scientific, educational, and public policy. Our voice on global issues, for example, the delivery of medical care, would almost certainly be more effective in this group.

Accountability for the teaching and science of gastrointestinal surgery is a characteristic that our Society accepted from its inception. Our founder, Dr. Turell, in his presidential address, told of his dream of creating a research and educational foundation. He believed that this was of vital importance and would solidify our existence. In his address to the Society as its seventh president, he judged the society of his dreams to be a "robust reality," whereas the foundation was still a "paper tiger."[1] In practical terms, creating a research and educational foundation required

Table I. SSAT Career Development Award recipients

Year	Name	University affiliation at time of award	Current university affiliation	Current academic rank	Societies*	Funded
1987	Barbara L. Bass	George Washington	Maryland	Professor	SSAT, AGA, SUS	NIH (1yr)
1988†	Steven E. Raper	Michigan	Pennsylvania	Associate Professor	SSAT, SUS	NIH (2 yr)
1989	Stanley W. Ashley	Washington	Harvard	Associate Professor	SSAT, AGA, SUS	NIH (1 yr)
1991	Tien C. Ko	Texas Medical Branch	Texas Medical Branch	Assistant Professor	AGA, SUS	NIH (1 yr)
1991	Jeffrey B. Matthews	Harvard	Harvard	Associate Professor	SSAT, AGA, SUS	NIH (2 yr)
1992	Neil E. Seymore	Texas Southwestern	Yale	Assistant Professor		None
1993	John J. Ferrara	Tulane	Tulane	Professor	SSAT, ASA, SUS	None
1994	Richard A. Hodin	Harvard	Harvard	Associate Professor	SSAT, AGA, SUS	NIH (2 yr)
1995	J. Augusto Bastidas	Stanford	Stanford	Assistant Professor	SSAT	None
1996	Stephen Summers	Maryland	Maryland	Assistant Professor	SSAT	None

Note: The 1990 recipient declined the award.
*SSAT = The Society for Surgery of the Alimentary Tract; AGA = American Gastroenterological Association; SUS = Society of University Surgeons; ASA = American Surgical Association.
†Expanded to two-year support.

the development of an enduring source of enrichment money. The first move in realizing this dream occurred at the Board of Trustees meeting in October 1985. Dr. Bernard Jaffe, Chairman of the Ad Hoc Committee on Research and Education, recommended that the Board issue a policy statement supporting the development of a two-year program for postresidency experience in research and clinical surgery of the digestive tract for the purpose of providing leadership for the discipline in the future.[24] The committee further recommended that the Society sponsor a Career Development Award to support individuals in this advanced experience. There was an avalanche of discussion about the proposal, largely over the need to increase the dues in order to fund the award and the possible interference it would have with the training of general surgery residents. There was more enthusiasm for the research part and less for the clinical part. Dr. Jaffe was asked to redraw the proposal.

The next year Dr. Jaffe recommended a proposal that focused only on supporting research, which the Board happily approved. However, the treasurer, Dr. Larry Cheung, indicated that the Society had insufficient funds to implement the proposal. A variety of solutions without substance were proposed and as a consequence a final decision was not reached. By the fall Board meeting, the Society had received a check for $18,864.00 as its share of the DDW profits and Dr. Cheung announced that sufficient funds were now available to fund the fellowship. The Board responded by approving the establishment of a two-year Career Development Award and authorized the funding of the first year at $15,000. Drs. David Nahrwold and Jaffe were to work out the process of application and selection with the understanding that the first award would be given in 1987. The award was subsequently increased stepwise to its current level of $40,000 per year. To date, 10 awards have been given, and 8 of the 10 recipients of these awards currently hold university appointments and six are receiving ongoing funding from the National Institutes of Health (Table I). Indeed the program has been a success.

To secure an enduring source of enrichment money to support our educational and scientific mandate, The Society is investigating the possibility of joining the American Digestive Health Foundation initially formed by the AGA and ASGE, and subsequently joined by the AASLD. The mission of the Foundation is to support research and education regarding the cause, prevention, treatment, and cure of digestive disease. The partnership currently supports four initiatives: basic research, clinical outcomes research, technology research, and applying the discoveries made in these areas to restructuring of the health care market. It seems as though history is about to repeat itself, but this time the government is not listening. Consequently a serious effort must be made to encourage industry to support research. I strongly believe we should be part of this effort.

The Society still has looming before it the challenge of being accountable for the quality of training

in the performance of complex gastrointestinal surgery. The presidential addresses of both Dr. John L. Cameron[25] ("Is Fellowship Training in Alimentary Tract Surgery Necessary?") and Dr. Keith A. Kelly[26] ("New Directions in Gastrointestinal Surgery") addressed this issue. In response Dr. Bernard Langer, during his year as president of the Society, proposed disbanding the Ad Hoc Research and Education Committee and appointing a new Education Committee to evaluate the need for advanced training programs beyond the general surgical residency. Dr. Carlos Pellegrini was appointed chairman. The committee met with the executives of the American Board of Surgery to exchange views about advanced training. The Society was urged that such fellowships should meet the Accreditation Council for Graduate Medical Education (ACGME) guidelines and scrupulously avoid interfering with the general surgery residency training at the institutions where these fellowships are awarded. After discussing various facets of the issue, the Board directed Dr. Pellegrini to develop a written proposal for an SSAT fellowship. The proposal should contain elements that ensure a substantial experience in difficult gastrointestinal operations, protection of the local general surgical residency program, and an oversight and accrediting mechanism by our Society. A fully developed comprehensive proposal was presented to the Board of Trustees in May 1996.[27] After a polarizing discussion, it was moved to table the proposal. The issue still looms before us. I dream that change in this area will also occur.

Like an individual, a society grows and develops by the process of change. Change is usually brought about through the interaction of its members and is driven by a sense of accountability. People who really make an impact model the quality of accountability. It gives them the energy to interact with others to bring about change. The process is catalyzed by a shared feeling of anxiety and apprehension among the membership about an issue, and by the perception that consequences will occur if no action is taken. Both provide the desire and willingness to organize which, according to Peter Drucker, is the most powerful human tool to effect change. A basic ingredient is the development of relationships marked by grace, reasonableness, and truthfulness. Through relationships our purposes are sharpened, clarified, expanded, and take on common ownership. The ability of our Society to develop relationships within the milieu of DDW is our strength, and will lead to changes beyond our boundaries that will affect the whole of digestive disease for the better. The American College of Surgeons focuses on the practice of surgery. The Society of University Surgeons and the American Surgical Association focus on the science of surgery. The Society for Surgery of the Alimentary Tract needs to focus on the integration of the practice and science of surgery into other disciplines of medicine. Herein lies our uniqueness, and our greatness.

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Should Hepatic Resections Be Performed at High-Volume Referral Centers?

Michael A. Choti, M.D., Helen M. Bowman, M.S., Henry A. Pitt, M.D., Julie Ann Sosa, M.D., James V. Sitzmann, M.D., John L. Cameron, M.D., Toby A. Gordon, Sc.D.

Recent studies have demonstrated the relationship between clinical outcomes of complex surgical procedures and provider volume. Hepatic resection is one such high-risk surgical procedure. The aim of this analysis was to determine whether mortality and cost of performing hepatic resection are related to surgical volume while also examining outcomes by extent of resection and diagnosis, variables seen with this procedure. Maryland discharge data were used to study surgical volume, length of stay, charges, and mortality for 606 liver resections performed at all acute-care hospitals between January 1990 and June 1996. One high-volume provider accounted for 43.6% of discharges, averaging 40.6 cases per year. In comparison, the remainder of resections were performed at 35 other hospitals, averaging 1.5 cases per year. Data were stratified into these high- and low-volume groups, and adjusted outcomes were compared. The mortality rate for all procedures in the low-volume group was 7.9% compared to 1.5% for the high-volume provider ($P < 0.01$, relative risk = 5.2). No overall differences were observed between low- and high-volume providers in total hospital charges. When analyzing by procedure type and diagnosis, lower mortality was seen in the high-volume center for both minor and major resections, as well as resections for metastatic disease. It was concluded that hepatic resection can be performed more safely and at comparable cost at high-volume referral centers. (J GASTROINTEST SURG 1998;2:11-20.)

In recent years, studies have examined the relationship between clinical outcomes and hospital case volume for a variety of medical and surgical procedures.[1-13] The procedures analyzed have ranged from cardiac operations to complex gastrointestinal surgical procedures. These studies have demonstrated that high-volume regional providers can deliver complex care with improved short-term outcomes at lower costs than community hospitals. However, no study has examined the relationship between outcomes and provider volume for hepatic resections, a commonly performed procedure in the United States.

Improved overall long-term survival in patients with liver malignancies has resulted in an increased number of liver resections being performed with an increasingly aggressive surgical approach.[14-17] Liver resection, unlike other high-risk surgical procedures, is an operation with variable complexity and extent of surgery. In addition, such operations are performed for a variety of malignant and benign conditions with variable comorbid diseases. These factors all may have an impact on outcome. Thus this analysis was performed to determine whether mortality and cost of performing hepatic resection are related to surgical volume.

METHODS

Data Source

This study examined the relationships between provider volume and outcomes for all patients who underwent hepatic resection in Maryland from January 1, 1990, through June 30, 1996. Information pertaining to the hospital discharges used in this study was obtained from the Maryland Health Services Cost Review Commission. This database includes records for every discharge from all 52 nonfederal acute-care hospitals in Maryland. Each discharge record contains information on demographic characteristics, primary and secondary diagnoses, procedures performed during that hospital stay, length of stay, hospital charges, and discharge status. Case selection for this study was

From the Department of Surgery, Department of Health Policy and Management School of Hygiene and Public Health, The Johns Hopkins Medical Institutions, Baltimore, Md.

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Reprint requests: Michael A. Choti, M.D., The Johns Hopkins Hospital, 600 N. Wolfe St., Halsted 614, Baltimore, MD 21287. E-mail: mchoti@welchlink.welch.jhu.edu.

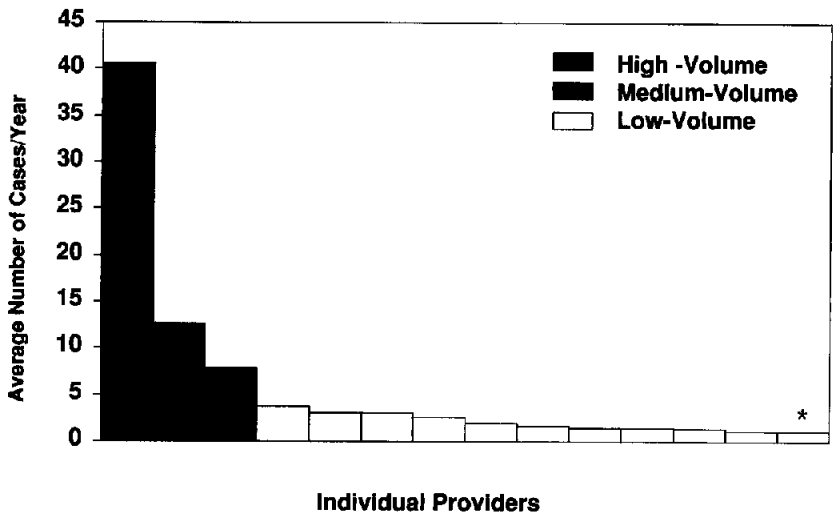

Fig. 1. Average volume of cases per year by provider (n = 14). High volume, >15 resections per year; medium volume, 7 to 15 per year; low volume, <7 per year. *Twenty-two additional low-volume providers not shown who performed <1.0 resections per year.

based on primary ICD-9 procedure codes 50.22, partial hepatectomy, and 50.3, hepatic lobectomy.[18]

The distribution of discharges by hospital revealed that one hospital accounted for 43.6% of discharges and averaged 40.6 resections per year (Fig. 1). The remaining resections were performed at 35 other hospitals and averaged 1.5 resections per year. Therefore discharges were stratified into high- and low-volume provider groups (high volume, more than 15 per year; low volume, less than or equal to 15 per year). An alternative stratification was performed for high-, medium-, and low-volume providers (high volume, more than 15 per year; medium volume, between 7 and 15 per year; and low volume, less than 7 per year) for relative risk analysis of mortality.

Outcomes

Primary outcomes studied were average length of stay, average total hospital charges, and in-hospital mortality. Analysis was performed for all discharges in the data set (606 procedures) and findings were stratified separately by procedure and primary diagnosis. Procedures were grouped into minor (ICD-9 procedure code 50.22, partial hepatectomy) and major (50.3, hepatic lobectomy) liver resections. Primary diagnoses were grouped into primary liver cancer (ICD-9 codes 155.0 and 155.2), metastatic cancer (ICD-9 codes 153.0, 153.9, 154.0, 157.0, 157.4, 197.0, and 197.7), and all other diagnoses, which includes liver resection for trauma, benign neoplasms, and infectious processes. The specific type of primary liver malignancy (i.e., hepatocellular cancer) was not identifiable from this data set, nor was the site of primary cancer in those who underwent resection for metastatic disease. Cryosurgery and other nonresectional therapy were excluded from the analysis.

Patient Characteristics

Patient characteristics for those treated by the high-volume provider compared to the group treated by the low-volume providers are shown in Table I. The age and sex distributions for the two groups were similar. The mean ages at the high- and low-volume providers were 54.3 and 55.4 years, respectively; males made up 50.4% of the high-volume patients and 54.4% of patients at other hospitals. The racial characteristics of the groups differed, however; patients treated by the high-volume provider were more likely to be white (82.2% vs. 67.3%, $P < 0.01$).

The number of comorbidities was analyzed using the Dartmouth-Manitoba adaptation of the Charlson comorbidity index.[19,20] This methodology is a validated index based on secondary diagnoses listed in the discharge abstracts. Instead of calculating a weighted comorbidity score, the number of comorbidities, 0, 1, 2, or more, was calculated. No overall differences in the number of comorbidities were observed between the high- and low-volume providers. When comparing the number of comorbidities by procedure and diagnosis, no differences were observed by provider group in patients undergoing either minor or major liver resections (Table II). In patients undergoing resection for primary liver cancer, a higher number of comorbidities was seen in the low-volume provider

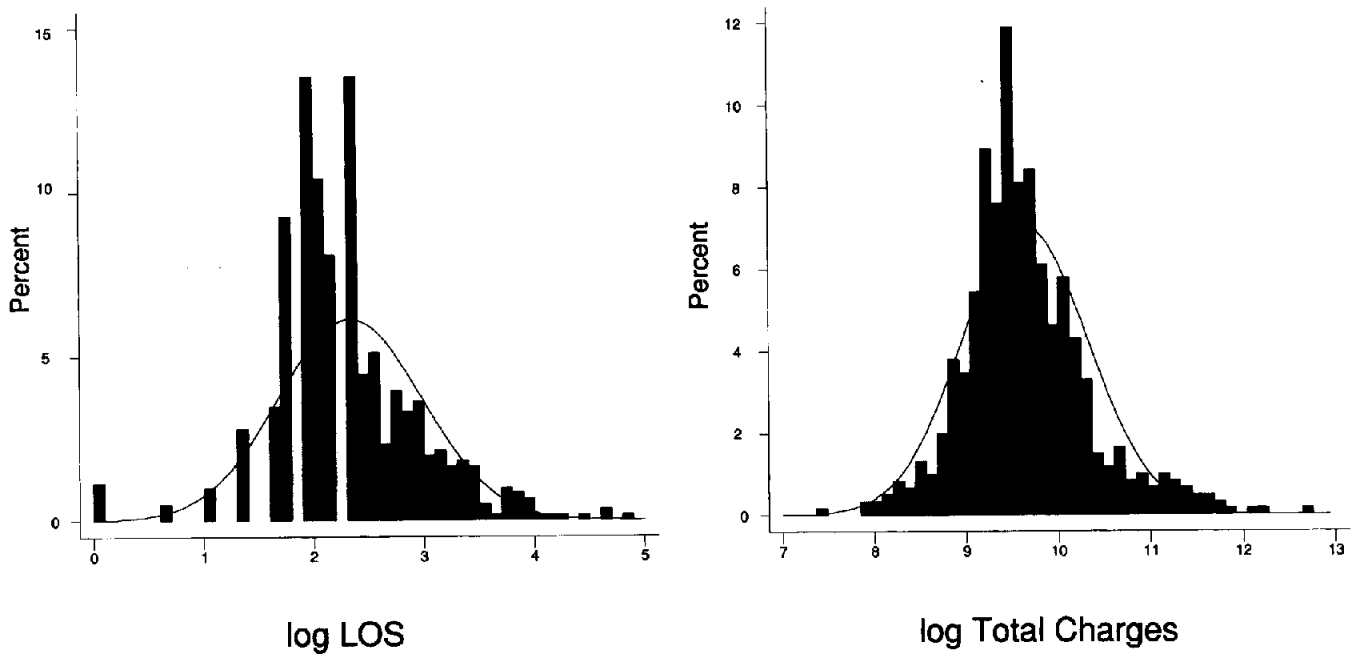

Fig. 2. Distribution of length of stay *(LOS)* and total charges after natural log transformation. Transformed variables were used for statistical analyses.

Table I. Patient characteristics by provider group for all liver resections (n = 606)

Demographic variables	High-volume provider (n = 264)	Low-volume providers (n = 342)	*P* value
Mean age (yr)	54.3	55.4	0.45
Sex			
Male	50.4%	54.4%	0.33
Female	49.6%	45.6%	
	100.0%	100.0%	
Race			
White	82.2%	67.3%	<0.01
African-American	12.1%	27.5%	
Other	5.7%	5.3%	
	100.0%	100.1%	
No. of comorbidities			
0	72.0%	69.6%	0.81
1	23.9%	25.7%	
≥2	4.2%	4.7%	
	100.1%	100.0%	

group ($P < 0.05$). No difference was observed between groups in those patients undergoing liver resection for metastatic cancer or other diagnoses.

Statistical Analysis

The statistical analysis of dichotomous and categorical variables such as sex, race, number of comorbidities and mortality was conducted using the chi-square statistic. Continuous variables were compared using Student's *t* test. One assumption of linear regression models is that continuous variables must be normally distributed. Since length of stay and total charges were not normally distributed, a natural log transformation was performed (Fig. 2). All subsequent statistical analyses were performed on the transformed data. Multiple linear regression models were performed to assess the relationship between hospital

Table II. Number of comorbidities by procedure and diagnosis

Stratification	Provider group		*P* value
	High-volume provider	Low-volume providers	
All discharges, by procedure			
All procedures (N = 606)	n = 264	n = 342	
No. of comorbidities			
0	72%	69%	0.81
1	24%	26%	
≥2	4%	5%	
	100%	100%	
Minor (partial hepatectomy) (N = 374)	n = 176	n = 198	
No. of comorbidities			
0	70%	71%	0.91
1	26%	24%	
≥2	4%	5%	
	100%	100%	
Major (≥hepatic lobectomy) (N = 232)	n = 88	n = 144	
No. of comorbidities			
0	76%	67%	0.36
1	21%	29%	
≥2	3%	4%	
	100%	100%	
All discharges, by diagnosis			
Primary liver cancer (N = 109)	n = 61	n = 48	
No. of comorbidities			
0	64%	35%	0.01
1	26%	54%	
≥2	10%	11%	
	100%	100%	
Metastatic cancer (N = 285)	n = 125	n = 160	
No. of comorbidities			
0	70%	65%	0.42
1	27%	29%	
≥2	3%	6%	
	100%	100%	
All other diagnoses (N = 212)	n = 78	n = 134	
No. of comorbidities			
0	82%	88%	0.48
1	17%	11%	
≥2	1%	1%	
	100%	100%	

volume and average length of stay and average total charges, adjusted for age, race, sex, and the number of comorbidities. Poisson regression was used to examine the relationship between hospital volume and mortality, adjusted for age, race, sex, and the number of comorbidities. Stata 5.0 software (Stata Corporation, College Station, Tex.) was used for statistical analysis.

RESULTS

Overall Outcomes

For the 606 total discharges, the overall length of stay was 13.0 days, the average total charges were $20,498, and the in-hospital mortality rate was 5.1%. A number of differences in outcomes were noted between the group of patients treated at the high-volume provider compared to outcomes in the low-volume group. The unadjusted average length of stay (12.7 vs. 13.2 days) and hospital charges ($17,923 vs. $22,485) did not differ between provider groups (Table III). The unadjusted hospital mortality rate was 1.5% in the high-volume group compared to 7.9% in the low-volume group ($P < 0.01$). Adjusted outcomes are shown in Table IV. The adjusted average length of stay was less at the low-volume providers (11.1 vs. 9.8, $P < 0.05$) with no difference in total charges. Relative risk of mortality was 5.2 times higher at the low-volume providers compared to the high-volume provider ($P < 0.01$).

Outcomes by Procedure

Of the liver resections, 374 (62%) were minor and 232 (38%) were major liver resections. The average overall length of stay, total charges, and in-hospital mortality differed according to the extent of the liver resection. The average length of stay for minor liver resections was 11.2 days compared to 16.0 days for major resections ($P < 0.001$). Total charges were $17,085 and $25,999 for minor and major resections ($P < 0.001$), respectively. The in-hospital mortality rate was 3.7% for minor resections compared to 7.3% for major liver resections (P = not significant [NS]).

When comparing unadjusted outcomes by procedure, the average length of stay was shorter at the low-volume providers for minor resections (11.7 vs. 10.8 days, $P < 0.01$) but was no different for major resections (see Table III), and average total charges were higher at the low-volume providers for major resections ($21,090 vs. $30,000, $P < 0.05$) but no different for minor resections. Unadjusted in-hospital mortality was higher at the low-volume providers compared to the high-volume provider for both minor and major resections (minor: 1.1% vs. 6.1%, $P < 0.05$; major: 2.3% vs. 10.4%, $P < 0.05$). After adjusting for age, sex, race, and comorbidities (see Table IV), both the average length of stay and the total charges for minor resections only were less at the low-volume providers compared to the high-volume provider. The adjusted relative risk (RR) of mortality remained higher at the low-volume providers for both minor and major resections (minor: RR = 5.3, $P < 0.05$; major: RR = 4.4, $P = 0.05$).

Outcomes by Diagnosis

One hundred nine patients (18%) underwent resection for primary liver cancer. Resection was performed for metastatic cancer in 285 patients (47%), and 202 (33%) resections were performed for other diagnoses including trauma, benign tumors, and infection. Hepatic resection for primary liver cancer was associated overall with a significantly longer length of stay ($P < 0.01$), higher average total charges ($P < 0.05$), and greater in-hospital mortality ($P < 0.01$) compared to resection for metastatic cancer. The average length of stay for primary liver cancer was 15.1 days compared to 11.5 days for patients undergoing resection for metastatic disease; the average total charges for primary liver malignancy were $23,191 compared to $17,459 for metastatic disease; and the overall in-hospital mortality rate was 11.0% compared to 2.8% for those undergoing resection for metastatic disease.

Both unadjusted and adjusted average length of stay and hospital charges did not differ between low- and high-volume providers when examining by primary diagnosis (Fig. 3). The unadjusted mortality rate, however, did differ between groups (see Table III and Fig. 4). The mortality rate for primary liver cancer was 18.8% in the low-volume provider group compared to 4.9% in the high-volume group ($P \leq 0.05$). No deaths occurred in patients undergoing liver resection for metastatic disease at the high-volume provider and eight deaths occurred in the low-volume provider group (0% vs. 5.0%, P = NS). The adjusted relative risk of mortality again did not reach statistical significance when comparing low- to high-volume providers for those with metastatic disease (RR >5.0, P = NS) but was borderline significant between provider groups for resections for primary liver cancer. For liver resections performed for nonmalignant diagnoses, no differences were seen between low- and high-volume providers for all measured outcomes.

Table III. Unadjusted averages for outcomes of interest by procedure type, diagnosis, and provider group

Stratification	Average length of stay			Average total charge			Mortality (%)		Relative risk of mortality	
	High-volume	Low-volume	*P* value*	High-volume	Low-volume	*P* value*	High-volume	Low-volume	Low- vs. high-volume	*P* value*
By procedure										
All procedures	12.7	13.2	0.15	17,923.49	22,485.24	0.49	1.5%	7.9%	5.21	<0.01
Minor (partial hepatectomy)	11.7	10.8	<0.01	16,340.23	17,747.56	0.08	1.1%	6.1%	5.33	0.03
Major (≥hepatic lobectomy)	14.9	16.6	0.98	21,090.02	28,999.56	0.02	2.3%	10.4%	4.58	0.04
By diagnosis										
Primary liver cancer	14.8	15.4	0.09	20,312.83	26,848.67	0.70	4.9%	18.8%	3.81	0.05
Metastatic cancer	11.6	11.4	0.88	17,273.92	17,603.39	0.68	0.0%	5.0%	>5.00	NS†
All other diagnoses	13.0	14.6	0.63	17,095.89	26,751.30	0.42	1.3%	7.5%	5.82	0.09

*All *P* values are from univariate regression models; linear regression for average length of stay and average total charges, and Poisson regression for mortality.
†NS = not significant; $P = 0.04$ by chi-square analysis.

Table IV. Adjusted* averages for outcomes of interest by procedure type, diagnosis, and provider group

Stratification	Average length of stay			Average total charge			Relative risk of mortality	
	High-volume	Low-volume	*P* value†	High-volume	Low-volume	*P* value†	Low- vs. high-volume	*P* value†
By procedure								
All procedures	11.1	9.8	0.02	15,434.80	15,326.42	0.90	5.20	<0.01
Minor (partial hepatectomy)	10.4	8.4	<0.01	14,730.56	12,425.31	<0.01	5.25	0.03
Major (≥hepatic lobectomy)	12.6	11.9	0.56	17,127.41	20,318.97	0.08	4.37	0.05
By diagnosis								
Primary liver cancer	13.3	10.9	0.15	18,184.93	18,505.02	0.90	2.88	0.14
Metastatic cancer	10.1	9.5	0.31	14,445.41	14,137.53	0.76	>5.00	NS‡
All other diagnoses	10.8	9.8	0.39	14,854.96	15,925.39	0.53	5.92	0.09

*Regression models are adjusted for age, sex, race, and number of comorbidities. Multiple linear regression models for average length of stay and average total charge, Poisson regression models for mortality.
†All *P* values are from regression models.
‡NS = not significant; $P = 0.04$ by chi-square analysis.

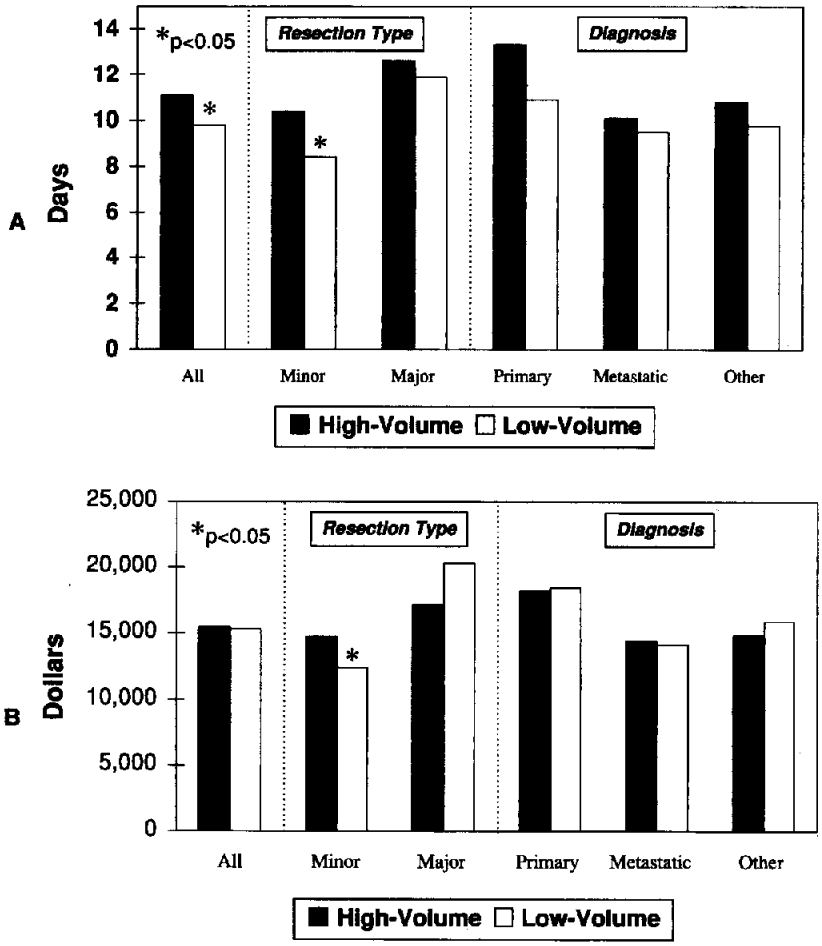

Fig. 3. Adjusted average length of stay **(A)** and total charges **(B)** by high- and low-volume provider group, adjusted for age, sex, and number of comorbidities. *P <0.05 vs. high-volume provider.

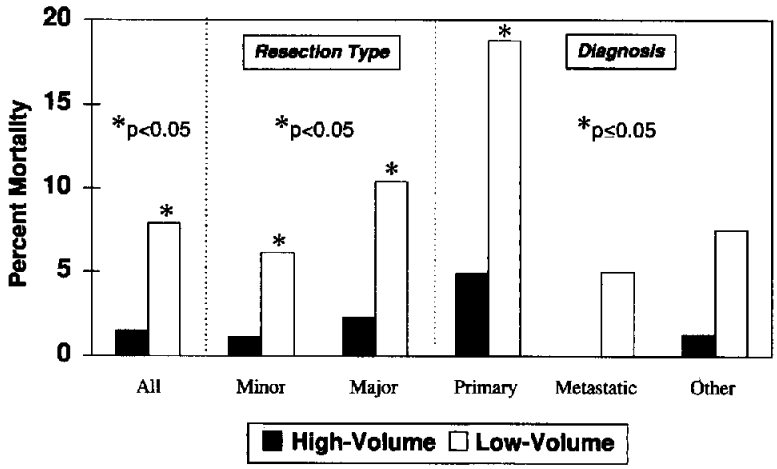

Fig. 4. In-hospital mortality by high- and low-volume provider (unadjusted). *$P \leq 0.05$ vs. high-volume provider.

Table V. Association of hospital surgical volume and in-hospital mortality for patients undergoing liver resection

Hospital volume	No. of hospitals	Total cases	Deaths	Mortality rate (%)	Adjusted* relative risk	*P* value
≤7	33	209	20	9.6	6.4	<0.01
7-15	2	133	7	5.3	3.4	0.05
>15	1	264	4	1.5	1.0	NA

NA = not applicable.
*Poisson regression adjusted for age, sex, race, and number of comorbidities.

Relative Risk by Hospital Volume

A more detailed comparison of mortality and relative risk by hospital volume was performed using a three-way volume stratification of high-, medium-, and low-volume hospitals. In-hospital mortality rates using this grouping were 1.5%, 5.3%, and 9.3%, respectively (Table V). Patients treated at hospitals performing between 7 and 15 resections per year had a relative risk of in-hospital mortality that was 3.4 times that at the high-volume center; patients treated at hospitals performing fewer than 7 resections per year had a 6.4 times greater risk of death.

DISCUSSION

This study suggests that hepatic resections performed at a high-volume center are associated with improved safety and similar total charges compared to low-volume hospitals. The same trend was seen regardless of extent of liver resection or primary diagnosis. The reason for the improved results at the high-volume center is likely multifactorial and may reflect the increased expertise that is associated with larger numbers of procedures performed by experienced surgeons, anesthesia staff, nurses, and other support staff. With early recognition of problems and familiarity with complex patients, adverse outcomes may be averted. With hepatic surgery in particular, improved outcomes may be associated with the use of newer surgical techniques and equipment, which may only be available where a greater number of procedures are being performed. These differences might include the routine use of intraoperative ultrasonography, argon beam coagulation, ultrasonic dissection, and anatomic resectional techniques.

This study demonstrated that major liver resections were associated with overall increases in length of stay, hospital charges, and in-hospital mortality compared to minor resections. These results parallel findings in some series in which higher mortality rates were associated with more extensive liver resections,[21,22] whereas others report no differences in mortality by extent of resection.[23] One might expect a greater difference in outcomes between high- and low-volume providers with major liver resections than with minor liver resections. This study, however, found a significantly improved mortality rate at the high-volume provider regardless of the extent of resection. Furthermore, major hepatic resections carried a lower mortality risk at the high-volume provider (2.3%) than minor resections at the low-volume providers (6.1%).

Liver resection in patients with primary hepatic malignancy was associated with overall worse outcomes than in those undergoing resection for metastatic disease. This finding is not unexpected given the often associated cirrhosis and other comorbid conditions in patients with hepatocellular carcinoma.[14-16] Indeed, a higher overall comorbidity index was observed in those patients undergoing liver resection for primary liver cancer. Although difficult to ascertain from this data set, one can expect that in the majority of patients undergoing liver resection for metastatic disease, the metastasis was from primary colorectal cancer. Other series have reported similar differences in hospital mortality between liver resections for metastatic colorectal cancer and primary liver cancer.[17,24] Although the present study demonstrated improved crude in-hospital mortality at the high-volume center for patients undergoing resection for both primary and metastatic cancer, when adjusted, the mortality did not differ significantly between provider groups.

This study has several limitations. Because only one hospital was included in the high-volume group, observed differences may not necessarily be related to volume alone but may be associated with variables not analyzed. This single high-volume center is a large academic institution with house staff, facilities, and referral patterns not seen in many community hospitals. Specific experienced surgeons or unique operative techniques associated with such a center may have an impact on outcomes that is independent of case volume. Provider groups were divided in such a way,

however, because the greatest difference in volume was seen at this breakpoint. To attempt to correlate them specifically with case volume, clinical outcomes were also compared using three volume categories: high, medium, and low. Medium-volume centers performing between 7 and 15 resections per year included two hospitals—one community hospital and one academic center. Such a sensitivity analysis demonstrated a "dose-response" effect between mortality and provider volume, suggesting a real association.

A second limitation is that only in-hospital clinical outcomes were measured. Clearly, postdischarge deaths, readmissions, and quality of life could not be examined using this administrative data set. Similarly, total hospital charges were those accrued during the one in-patient stay and may have included a variable number of preoperative diagnostic tests including visceral arteriography and CT portography. Similarly, limited data may have failed to clearly account for the extent of the surgical procedure. In addition, simple wedge resection of the liver may be associated with concomitant resection of other organs (e.g., colon). Coding problems with such an administrative data set may also oversimplify the complexity of the hepatic resection. Stratification by procedure type (major vs. minor) attempted to account for extent of liver resection, but other unknown features of the surgical procedure may have a significant impact on outcome variables.

In summary, these data lend support for the referral of patients for liver resection to high-volume centers. As with other complex surgical procedures, these operations are costly and carry a relatively high risk of in-hospital death. Studies such as this help to identify those procedures best suited for triage of patients to high-volume centers.

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Discussion

Dr. W.C. Meyers (Worchester, Mass.). You have shown that the risk seems to be greater in the low-volume institutions, but what about the technique? Do you think with inexperience that there will be fewer lesions detected? You have one high-volume center, which I presume is your own, and so your more important statistical data may be from your median-volume group and that probably should be emphasized in your report. If the length of stay was equal in the two sets, how did you adjust for length of stay in the patients who died intraoperatively?

Dr. M.A. Choti. The first question related to whether there were differences in surgical technique that may have influenced these results. Certainly this is a possibility. With analysis of these short-term outcomes, surgical technique, as well as other associated factors related both to the individual surgeon and the institution overall, may indeed have affected these results. With this data set it is difficult to know the precise reason. Your second question relates to the fact that a single hospital was in the high-volume provider group. Ideally it would be preferable to stratify with a different breakpoint with more providers in the high-volume group. This distribution was somewhat unusual in that one center performed 44% of the operations and was a distinct outlier. Any other breakpoint would have been suboptimal. One option may have been to exclude the one outlier and analyze only the lower-volume providers. This, however, would have reduced the number in the sample size and reduced the power of the study. Another study, which is yet to be published, examined outcomes of liver resection in California. In that report there was a larger sample size and multiple hospitals in the group of high-volume providers. That study found similar results. Your last question was related to average length of stay and whether intraoperative mortality may have had an impact on the shorter length of stay in the low-volume provider group. We did not look at that specifically. It is certainly possible that mortality may have had an impact on length of stay.

Dr. M. Didolkar (Baltimore, Md.). What was your definition of mortality? Was it in-hospital or 30-day mortality? Did you collect data from hospital records or from the state registry?

Dr. Choti. We examined only in-hospital mortality. The data used were from the same data set for all providers—the Maryland State discharge data.

Surgical Management of Hepatocellular Carcinoma: Resection or Transplantation?

Benjamin Philosophe, M.D., Paul D. Greig, M.D., Alan W. Hemming, M.D., Mark S. Cattral, M.D., Ian Wanless, M.D., Imran Rasul, M.D., Nancy Baxter, M.D., Bryce R. Taylor, M.D., Bernard Langer, M.D.

Liver resection or transplantation offers the best opportunity for cure of hepatocellular carcinoma (HCC). To determine the relative roles for resection and transplantation and to evaluate the patient and tumor characteristics that might predict survival, the records of 125 patients treated for nonfibrolamellar HCC at The Toronto Hospital between 1981 and 1996 were reviewed. No adjuvant chemotherapy or antiviral protocols were used. Resection was the first operation in 67 patients; one underwent re-resection. Sixty patients underwent transplantation including two who had previously had a resection; 40 had known or suspected HCC and 20 had incidental tumors identified in the explanted liver. The incidence of cirrhosis was 49% for resection and 88% for transplantation. The incidence of hepatitis B virus (HBV) was 58% and 33%, respectively. The operative mortality rate for resection was 4.4% (9.4% in cirrhotic and 0 in noncirrhotic patients) and 13.3% for transplantation. The 5-year cumulative recurrence rate was 55% following resection and 20% following transplantation ($P < 0.001$). The 5-year Kaplan-Meier survival rates were 38% for resection and 45% for transplantation—60% for transplanted HBV-negative and 17% for HBV-positive patients ($P < 0.001$). After resection, recurrent HCC accounted for 86% of deaths, whereas recurrent HBV was responsible for 42% of deaths after transplantation. By univariate analysis, following resection, vascular invasion, advanced stage, multiple tumors, and lack of a capsule were predictive of survival; cirrhosis, HBV, age, tumor size, number, and grade were not. By multivariate analysis, only vascular invasion was predictive for resection and HBV for transplantation. Resection and transplantation are complementary methods of treating HCC. With the current organ shortage, resection should be considered first-line treatment. HBV-positive patients with HCC should only undergo transplantation in combination with effective antiviral therapy. (J GASTROINTEST SURG 1998;2:21-27.)

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world and is responsible for more than 1 million deaths annually. The most important risk factors for development of HCC are previous infection with hepatitis B virus (HBV), hepatitis C virus, or alcoholic cirrhosis.[1] The geographic variation in incidence relates to the prevalence of underlying liver diseases that contribute to its development. The opportunities for surgical management are limited by the fact that patients often present with either advanced malignancy or severe underlying liver disease.

Resection has been the traditional curative approach to the treatment of HCC. The operative risk of resection in patients with cirrhosis has limited its application, but with improvements in surgical technique and supportive care, as well as better patient selection, a progressive decrease in operative mortality and improvement in 5-year survival have been observed.[2,3] The majority of treatment failures result from recurrent HCC in the liver.[4,5]

Transplantation has also been used as treatment for HCC and has the theoretical advantages of not only removing the known primary lesion but also curing the underlying liver disease in which the tumor developed and reducing the likelihood of new primary tumors. The mortality and survival rates for liver transplantation for HCC have also been improving over the past two decades,[2] and some authors have suggested that transplantation should be the pre-

From the Hepatobiliary/Pancreatic and Liver Transplantation Services, Department of Surgery, University of Toronto, and The Toronto Hospital, Toronto, Ontario, Canada.

Presented at the Thirty-Eighth Annual Meeting of The Society for Surgery of the Alimentary Tract, Washington, D.C., May 11-14, 1997.

Reprint requests: Bernard Langer, M.D., The Toronto Hospital, en 9-242, 200 Elizabeth St., Toronto, Ontario M5G 2C4, Canada.

ferred treatment even in some resectable cirrhotic patients.[6]

The purpose of this study was to assess the relative roles of resection and transplantation for HCC in a single center that offers both modalities of treatment and to evaluate patient and tumor characteristics that might be predictive of long-term survival in order to improve patient selection.

PATIENTS AND METHODS

A retrospective chart review and careful follow-up were carried out in 125 patients with nonfibrolamellar HCC who were managed surgically at The Toronto Hospital between 1981 and 1996.

All patients with known or suspected HCC were initially evaluated as candidates for liver resection. Patients had to be able to tolerate a major procedure and have minimum impairment of liver function (Child-Pugh class A or early B) and no complications of cirrhosis (i.e., ascites, bleeding, or encephalopathy). The tumors had to be confined to the liver and technically resectable with a 1 cm margin. Liver transplantation was reserved for patients who had unresectable tumors confined to the liver, with minimal or no symptoms. Patients with extensive involvement of the liver by tumor or significant tumor-related symptoms were not offered transplantation. In addition, one third of the transplanted patients with HCC had unsuspected or "incidental" tumors discovered in the explanted liver by the pathologist following transplantation for end-stage liver disease.

Preoperative evaluation initially included abdominal ultrasonography, computed tomographic (CT) scanning, and chest x-ray examination. More recently, magnetic resonance imaging has been added to the preoperative assessment and intraoperative ultrasonography has been the final diagnostic procedure. Angiography was commonly used in the first decade of the study but it has been used infrequently since then. Preoperative assessment of liver function included standard liver profile, coagulation studies, and occasionally liver biopsy of the nontumor liver to assess the potential for regeneration. The majority of cirrhotic patients were Child class A.

Surgical resection was the first operation performed in 67 patients and transplantation in 58. Nearly two thirds of the resections were major lobectomies (Table I) because of the size or location of the tumor. This ratio was the same in patients with and without cirrhosis. Three of the resected patients developed recurrent disease and had a second operation; one required re-resection and the other two underwent transplantation. In the analysis of data, the recurrence rate was therefore based on 68 resection operations and 60 transplant operations. The survival rate was based on 65 patients who had resections and 60 patients who received transplants. Follow-up was complete in 98% of patients and the mean follow-up was 28 months. Adjuvant chemotherapy was not used in any patient, and there was no standardized protocol of adjuvant antiviral therapy in use during this period of time, although one patient was given Lamivudine before and after transplantation in 1996.

There were significant differences between the two treatment groups as a result of the different selection criteria (Table II). Approximately half of the resection patients had cirrhosis based on gross appearance and microscopic confirmation, whereas the vast majority (88%) of the transplanted patients had cirrhosis. On the other hand, 58% of the resection patients were HBV positive, based on HBsAg determination, compared to 33% of the transplant patients. Tumor size was determined from pathologic examination or imaging study reports and tended to be larger in the resection patients compared to the transplant patients. Tumor numbers were derived from pathology reports. There were many more patients with multiple tumors in the transplantation group than in the resection group; however, this is biased by the availabil-

Table I. Extent of liver resection

	No.
Right lobe	27
Extended right lobe	7
Left lobe	11
Left lateral segment	8
Other segment/subsegment	15
TOTAL	68

Table II. Differences between patient groups

	Resection	Transplantation
Patient characteristics		
Cirrhotic	32	53
Noncirrhotic	33	7
HBV positive	38	20
HBV negative	27	40
Median age (yr)	58	56
Tumor characteristics		
Size		
0-2 cm	5	23
2-5 cm	32	26
>5 cm	28	8
No. of tumors		
Single	53	33
Multiple	12	27

ity of the whole organ to the pathologist in transplant patients in which occult tumors could be found.

Operative mortality was defined as death during the same hospital admission or within 30 days of the operation for discharged patients. Kaplan-Meier curves were plotted for overall survival of the resection and transplant groups. Comparisons were done using the log-rank test. Cumulative recurrence rates were calculated using life-table analysis. The prognostic variables were analyzed for their possible influence on overall survival using the Cox proportional hazards model for the two groups separately. Each variable was first subjected to a univariate analysis. Multivariate analyses were then performed with factors entered in a forward stepwise manner; models were compared with the log-likelihood ratio criterion. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

The overall operative mortality rate for liver resection was 4.4% (9.4% in cirrhotic and 0 in noncirrhotic patients). The operative mortality for transplantation was 13.3%.

Table III. Causes of late death following resection and transplantation

	Resection (No.)	Transplantation HBV+ (No.)	Transplantation HBV− (No.)
Recurrent HCC	19	1	3
HBV/hepatitis	0	8	—
Other	3	2	5
TOTAL	22	11	8

The 5-year cumulative recurrence rate for HCC was 55% following resection and 20% following transplantation ($P < 0.001$) (Fig. 1). Most of the recurrences in the resected patients were in the liver at a distance from the resection margin, and were likely new primary tumors. Five transplanted patients developed recurrent tumor, each with widespread metastatic HCC. All five had known or suspected cancer at the time of their transplant.

Survival curves for both groups of patients are shown in Fig. 2 and are similar for transplantation (45% at 5 years) and resection (38% at 5 years). The influence of cirrhosis on survival in resected patients is shown in Fig. 3. Although the operative mortality rate in cirrhotic patients was slightly higher than that in noncirrhotic patients, the 5-year survival rates were similar in cirrhotic (38%) and noncirrhotic (40%) patients. Fig. 4 depicts survival in resected patients according to HBV status and does not show any significant difference in survival between HBV-positive (49%) and HBV-negative (17%) patients ($P = 0.38$). In the patients treated by transplantation, however, there is a marked difference in 5-year survival between patients who are HBV positive (17%) and those who are HBV negative (60%, $P = 0.001$), as shown in Fig. 5. The major cause of late death in HBV-positive transplanted patients was recurrence of hepatitis B in the transplanted liver. The frequency of deaths from other causes was not significantly different between HBV-positive and HBV-negative patients (Table III). In transplanted patients, the survival of those with known or suspected tumors was similar to the survival of patients with incidental tumors (Fig. 6).

Table IV shows all of the factors that were examined as potential predictors of mortality following either resection or transplantation. Stage was defined according to American Joint Committee on Cancer criteria,[7] and tumor grading was based on Edmondson criteria.[8] For resection, univariate analysis sug-

Table IV. Factors examined as potential predictors of mortality following resection and transplantation

Variables	Resection Univariate	Resection Multivariate	Transplantation Univariate	Transplantation Multivariate
Vascular invasion	0.0001*	0.0003*	0.228	
Advanced stage	0.0001*		0.527	
Multiple tumors	0.0002*		0.066	
Lack of capsulation	0.039*		0.454	
Size (larger)	0.453		0.645	
Grade (higher)	0.539		0.754	
Cirrhosis	0.502		0.338	
Age (younger)	0.608		0.029*	
HBV	0.387		0.003*	0.03*

*Significant; $P < 0.05$.

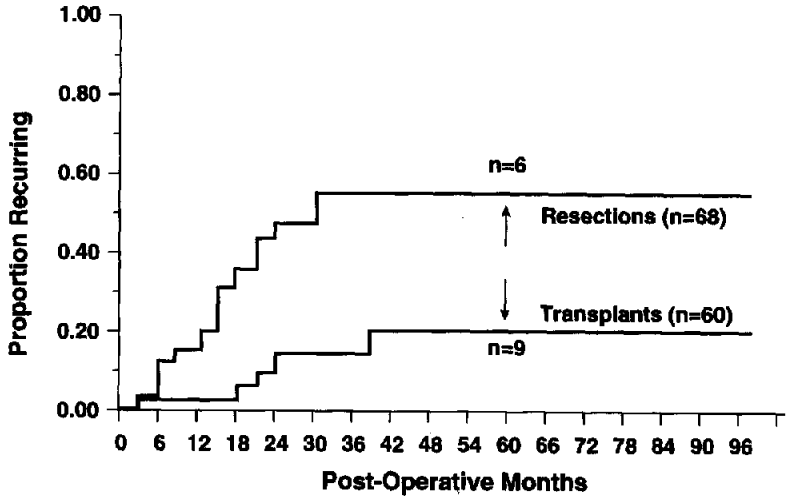

Fig. 1. Cumulative recurrence rate following 68 liver resections and 60 liver transplants for HCC ($P < 0.001$).

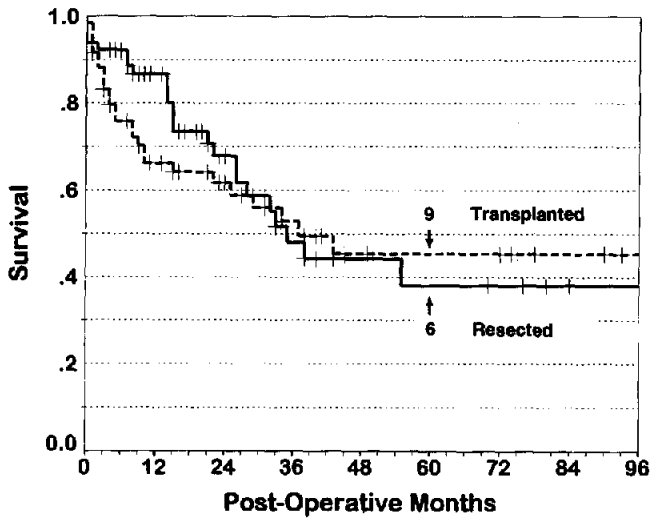

Fig. 2. Survival curves for 65 patients following resection and 60 patients following transplantation for HCC.

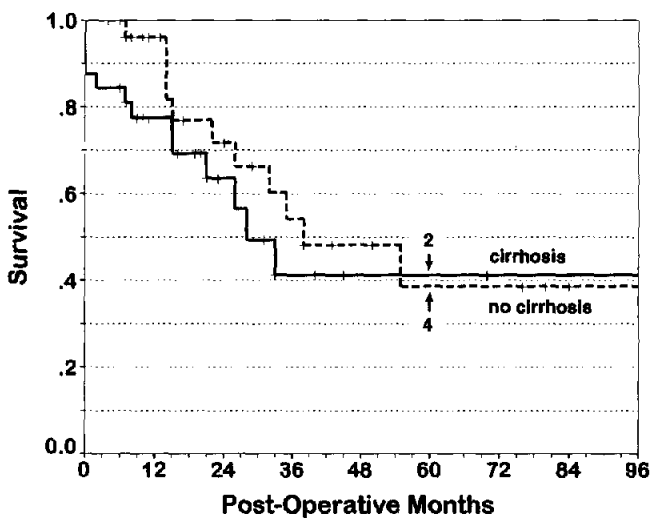

Fig. 3. Survival following resection for HCC in 32 patients with cirrhosis and 33 without cirrhosis.

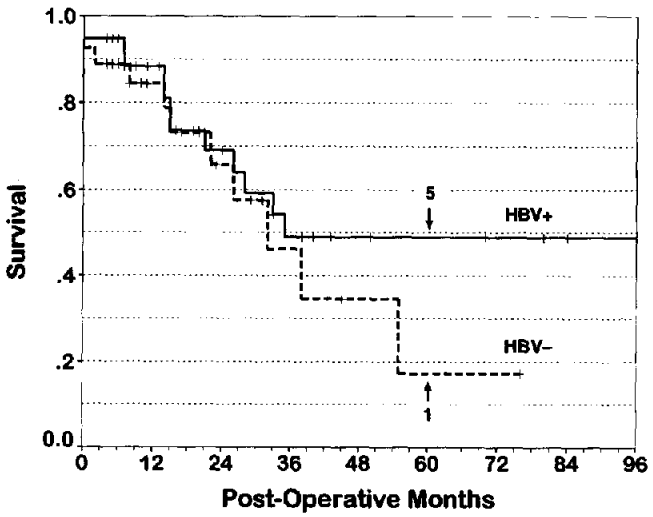

Fig. 4. Survival following resection for HCC in 38 HBV-positive patients and 27 HBV-negative patients ($P = 0.38$).

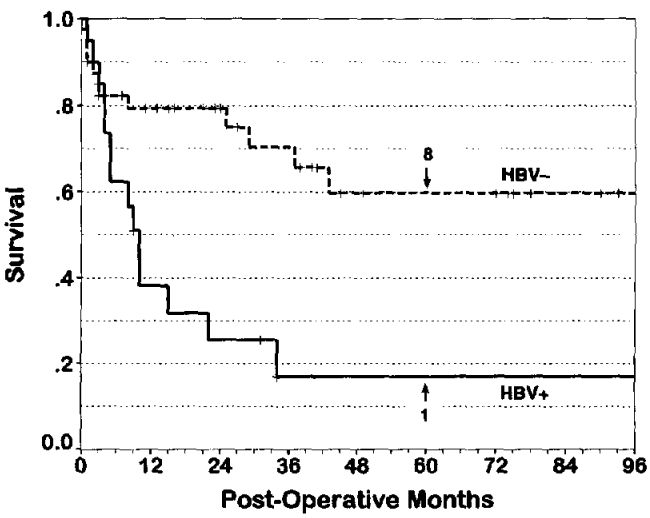

Fig. 5. Survival following transplantation for HCC in 40 HBV-negative patients and 20 HBV-positive patients ($P = 0.001$).

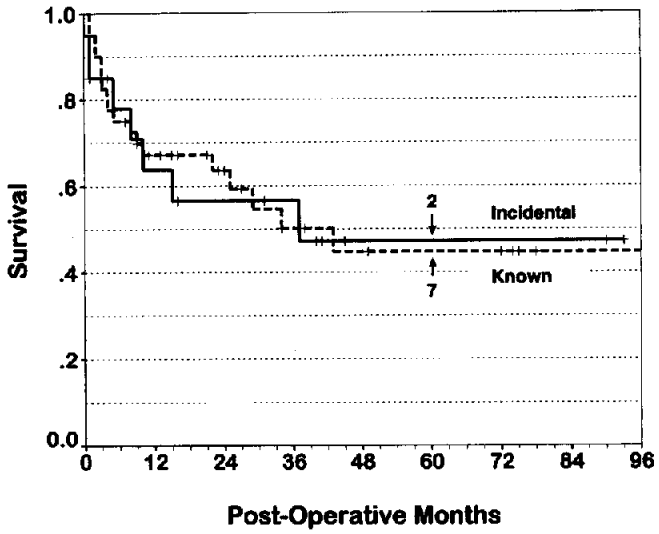

Fig. 6. Survival following transplantation in 40 patients with known or suspected HCC and 20 patients with incidental HCC.

gested that the tumor factors of vascular invasion, advanced stage, multiple tumors, and lack of a capsule appear to be significant. In the multivariate analysis, however, only vascular invasion was significant. In the transplanted patients, age and hepatitis B status appeared to be significant predictors on univariate analysis, but only hepatitis B status was a predictor on multivariate analysis.

DISCUSSION

In 1977 Foster and Berman,[9] in their classic liver tumor survey, reported an operative mortality following resection for HCC of 58% in cirrhotic and 12% in noncirrhotic patients and 5-year survival of 0 in cirrhotic and 27% in noncirrhotic patients. With improvement in the techniques of liver resection, anesthesia, and perioperative care, and improved methods of selecting patients for operation, operative mortality has progressively improved and is now in the range of 5% to 10% for cirrhotic patients and less than 5% for noncirrhotic patients. Five-year survival following resection is currently reported to be between 25% and 40% in both cirrhotic and noncirrhotic patients.[10-13] This change is likely the result of earlier diagnosis from screening programs and improved imaging, as well as better selection of patients, taking into account those factors that have been considered as predictive of survival. These have included single tumors,[13,14] presence of a capsule,[15] absence of vascular invasion,[14,16] and small size.[15,16] Comparison of results between centers is difficult because of variation in selection criteria and lack of standardization of staging and reporting. Our finding of vascular invasion as the only predictor of survival after resection using multivariate analysis is in keeping with other recent reports.[14,16]

The early published reports of transplantation for HCC noted a high early recurrence rate of tumor and poor survival. In 1988 Pichlmayr[17] questioned whether transplantation had any role to play in the treatment of hepatic malignancies. It was apparent, however, since patients who were found to have incidental tumors had the same survival rates as those without HCC,[18] that some early HCC can be cured with transplantation. More recent reports have indicated that with careful selection and low operative mortality, lower recurrence rates and improved survival can be achieved.[2] The tumor-related factors currently considered to be important as predictors of both recurrence and survival have included the following: no tumor-associated symptoms,[19] early stage,[20-22] small size (<2 to 5 cm),[6,19-22] single tumors or fewer than three,[6,19,21,22] low α-fetoprotein,[22] encapsulation of the tumor,[21,22] and absence of vascular invasion.[20-22] To further improve the results of transplantation for HCC, preoperative chemotherapy or chemoembolization[19,23] and/or postoperative adjuvant chemotherapy[23,24] are being investigated; the role of this strategy remains to be defined.

The data from our center confirm those of others in showing that careful patient selection allows one to carry out resective surgery with low operative risk even in patients with cirrhosis. Our recurrent rate of 55% is comparable to that of other authors and constitutes the major problem limiting long-term survival.[5,25] In a small number of patients re-resection is possible, and one of our patients is alive and disease free 8 years following the first of his two liver resections. Use of chemotherapy, either systemically, by intra-arterial infusion, or by chemoembolization, has not been shown to improve survival.[25,26] Methods of adjuvant therapy to reduce the occurrence of new tumors in the susceptible liver may be more appropriate, and the report of the use of synthetic retinoids to decrease the recurrence rate of HCC after curative treatment shows promise as a potential adjuvant to surgical resection.[27]

In the case of transplantation, our data indicate that the incidence of tumor recurrence in patients selected with early and minimally symptomatic disease is not as high as previously reported. The survival of the HBV-negative patients is similar to that achieved in other groups of transplant patients, indicating an important role for transplantation in carefully selected patients with known or suspected HCC. Moreover, these results question the role of adjuvant chemotherapy for all patients, since our survival of HBV-negative patients is comparable to what has been reported when adjuvant chemotherapy is used.[19,23,28]

Our study failed to identify any tumor-associated factors predictive of survival following transplantation. However, this may have been the result of the small sample size, selection bias, or other factors. There is a need to better define the predictive value, if any, of tumor size, multiplicity, presence of a capsule, and gross vascular invasion. This can only be achieved by studying larger numbers of patients using standardized staging and multivariate analysis of data.

The data reported in this study reinforce our previously reported opinion[29] and that of others[30] that patients who are HBV positive should not undergo transplantation without effective antiviral therapy to prevent the recurrence of hepatitis B in the graft, which under the influence of immunosuppression has a rapidly progressive course. The best protocol has not yet been developed. The limited availability of hepatitis B immune globulin restricts its widespread use. Lamivudine or other similar antiviral drugs hold promise; however, the emergence of mutant strains of HBV in response to these agents is now well recognized.

Theoretically, liver transplantation should provide the best long-term survival for patients with HCC, and it has been suggested that it be the treatment of choice in patients with cirrhosis, even those with early potentially resectable disease.[6] In our patient population of HBV-negative patients, a 5-year survival of 60% was achieved with a low incidence of recurrence. The practical problem, however, is that there are insufficient livers available in most centers to offer transplantation to patients with resectable HCC.

CONCLUSION

At the present time we consider both resection and transplantation to be appropriate and complementary methods of treating HCC. Given the limitation in organ availability and the absence of prospective randomized trials, resection should be considered first-line treatment for HCC in low risk patients who have good preservation of hepatic function and whose tumors are technically resectable with an adequate margin. Effective adjuvant therapy directed at reducing the rate of recurrence of tumors in the liver is needed to improve survival.

Liver transplantation is effective treatment for selected patients with HCC who are HBV negative. The importance of currently suggested selection criteria, including size, multiplicity, capsulation, and vascular invasion, which might be predictive of outcome remains to be determined. Patients with HCC who are HBV positive should only undergo transplantation as part of a prospective study using antiviral protocols.

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Discussion

Dr. C. Baker (Chapel Hill, N.C.). Did you analyze the 20 patients in whom incidental carcinoma was seen at the time of transplantation? It is a small group but I would like to know what happened to them.

Dr. P.D. Greig. Yes, we did. Further analysis of the transplant patients has been performed. The recurrence and survival rates for this incidental group of 20 patients are equivalent to rates for the 40 patients with no suspected tumor. We also found no difference when stratifying for those with and without hepatitis B.

Dr. J. Roslyn (Philadelphia, Pa.). I wonder if you could comment on what type of intraoperative assessment is used to determine whether the lesion is resectable? Do you use intraoperative ultrasound?

Dr. Greig. Yes, we do use intraoperative ultrasound. It is useful in identifying other tumors that would preclude resection and is helpful in making the final decision as to the extent of resection. Intraoperative ultrasound has become an essential part of our intraoperative assessment of these patients.

Biliary CA 19-9 Values Correlate With the Risk of Hepatic Metastases in Patients With Adenocarcinoma of the Pancreas

Richard C. Montgomery, M.D., John P. Hoffman, M.D., Eric A. Ross, Ph.D., Lee B. Riley, M.D., Ph.D., John A. Ridge, M.D., Ph.D., Burton L. Eisenberg, M.D.

Serum values of the tumor-associated antigen CA 19-9 are useful as an independent predictor of survival in patients with adenocarcinoma of the pancreas. However, the utility of biliary CA 19-9 values is unknown. This study was undertaken to determine whether biliary CA 19-9 levels are predictive of hepatic metastases. Between 1991 and 1996, thirty-eight patients treated for adenocarcinoma of the pancreas were evaluated using a biliary CA 19-9 assay. Bile was obtained from percutaneous stents placed during the perioperative period. Five of the 38 patients had low serum levels of CA 19-9 (<2 U/ml) and were excluded from the study. Twenty-seven (80%) of the 33 patients developed distant metastases: five pulmonary, five peritoneal, and 17 hepatic. Liver metastases were discovered initially in 10 and after resection of the primary tumor in seven (median interval 10 months). Biliary CA 19-9 values were significantly higher in patients with hepatic metastases (median 267,400 U/ml; range 34,379 to 5,000,000 U/ml) compared to patients without metastatic disease (median 34,103 U/ml; range 6,620 to 239,880 U/ml; $P < 0.006$). Patients with hepatic, peritoneal, and pulmonary metastases had median survivals of 8, 14, and 35 months, respectively ($P < 0.0041$). All patients without metastatic disease are alive (median follow-up 13 months). Biliary CA 19-9 values are associated with a stepwise increase in the risk of developing metastatic disease. Patients with biliary CA 19-9 levels greater than 149,490 U/ml have an increased risk of developing recurrent disease in the liver and may warrant further hepatic evaluation or therapy. (J GASTROINTEST SURG 1998;2:28-35.)

Since its initial description by Koprowski et al.,[1] the tumor-associated antigen CA 19-9 has become the predominant tumor marker for the diagnosis of adenocarcinoma of the pancreas.[2] Recent evidence has shown that serum CA 19-9 is useful as an independent predictor of recurrence and survival after resection.[3-6] Although serum CA 19-9 values correlate with tumor burden and metastatic disease,[7] accurate predictions regarding the pattern of recurrence using this tumor marker obtained from serum cannot be made. In addition, few studies have shown a use for CA 19-9 values obtained from sources other than the serum, and no study to date has examined the potential use of biliary CA 19-9 as a predictor of metastatic disease.

One of the most common reasons for an aborted pancreatic resection is the intraoperative detection of unsuspected hepatic metastases. Studies on patterns of recurrence show that the liver is a common site of failure after pancreatic resection.[8-10] This knowledge has led many investigators to search for preoperative or intraoperative predictors of hepatic metastases in an effort to identify these high-risk patients for proper staging and treatment.

Since serum CA 19-9 is a known tumor marker for patients with adenocarcinoma of the pancreas, an interesting question is whether biliary CA 19-9 values can accurately identify patients at risk for developing or having hepatic metastases after resection for adenocarcinoma of the pancreas.

In patients with benign and malignant disease of the pancreas and biliary tree, bile contains identifiable amounts of the CA 19-9 antigen. In addition, small pancreatic tumors produce detectable levels of the CA 19-9 antigen,[11] and work using mathematic modeling

From the Departments of Surgical Oncology and Biostatistics, Fox Chase Cancer Center, Philadelphia, Pa.
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Reprint requests: John P. Hoffman, M.D., Department of Surgical Oncology, Fox Chase Cancer Center, 7701 Burlholme Ave., Philadelphia, PA 19111.

suggests that the CA 19-9 antigen could be detected prior to clinical identification of the primary tumor.[7] Therefore it is theoretically possible for a clinically undiscovered hepatic metastases to produce sufficient CA 19-9 antigen to allow detection. If a correlation between the value of the biliary CA 19-9 and the presence or development of hepatic metastases could be documented, it could prove useful in the preoperative workup and postoperative treatment of these patients. The purpose of this study was threefold: (1) to determine if biliary CA 19-9 values are correlated with serum CA 19-9 values; (2) to determine if biliary CA 19-9 alone can predict survival in patients with adenocarcinoma of the pancreas; and primarily (3) to determine if biliary CA 19-9 values are associated with the development of hepatic metastases.

MATERIAL AND METHODS

The Tumor Registry at the Fox Chase Cancer Center was reviewed for the period between 1991 and 1996. Thirty-eight patients were identified who were diagnosed with adenocarcinoma of the pancreas and had perioperative CA 19-9 assays performed on bile samples. Hospital, clinic, and personal physician records were obtained and reviewed for data pertaining to each patient's clinical course. Data collected included age, sex, presenting symptoms, diagnostic tests, method of initial biliary decompression, biliary CA 19-9 value, serum CA 19-9 value, stage of disease, operative procedure, intraoperative findings, postoperative course, adjuvant therapy, morbidity, interval to failure, site of failure, survival status, and date of last evaluation. When appropriate, patients or their personal physicians were contacted to establish current survival status.

Biliary samples were obtained from percutaneous stents placed during the preoperative period in 20 patients (including all patients eventually undergoing bypass), and either preoperatively or immediately postoperatively in all patients undergoing resection. Bile samples obtained closest to the operative period were chosen for analysis when multiple data points were encountered. Serum samples were drawn before treatment and then every 3 months for 1 year (after 1 year, samples were drawn every 3 to 6 months). Biliary and serum CA 19-9 levels were determined using a CA 19-9 radioimmunoassay kit manufactured by Abbott Laboratories, Chicago, Illinois. The recommended normal value of 37 U/ml was used for the serum assays. However, a normal value for biliary samples has not been determined.

Five patients with preoperative levels of serum CA 19-9 less than 2 U/ml were considered nonsecretors and were excluded, leaving 33 potential subjects for analysis. When possible, serum alkaline phosphatase and serum bilirubin values were matched to each serum and biliary CA 19-9 value, and patient records were evaluated in an effort to exclude nonmalignant causes of CA 19-9 elevation.

Thirty-three patients were included in this study, 16 males and 17 females with an average age of 64 years (range 41 to 80 years). Twenty-two had tumors confined to the pancreatic head, 10 had tumors involving the pancreatic body, and one had a tumor confined to the pancreatic tail.

Thirty-one patients underwent surgical exploration. There were 22 Whipple procedures, one total pancreatectomy, and eight palliative bypasses. Two patients were found to have metastatic disease during the preoperative workup and did not undergo exploration.

Using the American Joint Committee on Cancer Staging System,[12] patients were classified as follows: six patients were stage I, two were stage II, 14 were stage III, and 11 were stage IV. Tumor stages included two T1b lesions, 20 T2 lesions, and 11 T3 lesions as determined by CT scan or pathologic examination. Tumor differentiation was verified at the Fox Chase Cancer Center; tumors were well differentiated in three patients, moderately differentiated in 20, and poorly differentiated in nine. Of the 23 patients who underwent resection, 16 were resected with negative margins, two with positive margins, and five with close (<2 mm) margins.

Seventeen patients were enrolled in two separate phase II trials of neoadjuvant therapy consisting of 5-fluorouracil and mitomycin C combined with external beam radiotherapy. Eight of these patients were eventually deemed unresectable because of metastatic disease. Thirteen patients received postoperative adjuvant therapy using a similar regimen, and one patient did not receive any adjuvant therapy. Two patients who had metastatic disease on presentation were treated with primary chemotherapy and radiation therapy.

Categorical and continuous variables were evaluated using the log-rank and Cox-Mantel tests with respect to disease-free and overall survival. For further statistical analysis, serum CA 19-9 data were divided into preoperative values and values obtained between 1 and 3 months postoperatively, whereas the biliary CA 19-9 value obtained closest to the initiation of surgical treatment was used. The results of the proportional hazards regression model were visualized using Kaplan-Meier survival curves. Empiric sensitivity and specificity curves for the development of hepatic metastases were created by computing the sensitivity and specificity of a variety of different cut points defined by each observed biliary CA 19-9

value. A plot of the estimated probability of hepatic metastases as a function of the bile CA 19-9 was constructed using predicted values derived from a linear logistic regression fit to the presence or absence of hepatic metastases. The covariant for this regression model was the natural log of the biliary CA 19-9 values. A significance level of 5% was used for all calculations.

RESULTS

Categorical and continuous data were analyzed using a univariate regression analysis, and their association with respect to disease-free and overall survival is shown in Table I. Neither age, sex, nor adjuvant treatment was associated with improved survival. However, lower stage of disease, well-differentiated tumors, and negative margin status were associated with improved survival. In addition, preoperative and postoperative CA 19-9 values were significantly associated with overall survival as were the biliary CA 19-9 values.

Statistical analysis using Spearman's correlation coefficient revealed that the postoperative serum CA 19-9 values were significantly correlated with the biliary CA 19-9 values ($P < 0.02$; Fig. 1). Increasing serum and bile CA 19-9 values were correlated with decreased disease-free ($P < 0.001$) and overall ($P < 0.02$) survival.

Preoperative serum CA 19-9 values ranged from 5 to 34,379 U/ml with a median value of 472 U/ml; CA 19-9 values obtained between 1 and 3 months postoperatively ranged from 12 to 89,500 U/ml with a median value of 156 U/ml. The patients with preoperative serum CA 19-9 values less than the median value of 472 U/ml had significantly longer overall survival (21 vs. 11 months; $P < 0.029$) compared with patients who had preoperative serum values greater than the median value (Table II). In addition, serum values obtained between 1 and 3 months postoperatively that were less than the median value of 156 U/ml were predictive of improved disease-free survival (14 vs. 5 months; $P < 0.028$) and overall survival (35 vs. 12 months; $P < 0.017$; Table II).

Biliary CA 19-9 values ranged from 6620 to 5,000,000 U/ml with a median value of 149,490 U/ml. Patients with biliary CA 19-9 values below the median value had significantly longer disease-free (13 vs. 1 month; $P < 0.04$) and overall (21 vs. 11 months; $P < 0.013$) survival compared to patients with biliary values above the median (Fig. 2).

Table I. Effects of variables on survival

Variable	*P* value
Age	<0.79
Sex	<0.78
Neoadjuvant treatment	<0.18
Stage	<0.0001
Differentiation	<0.02
Negative margins	<0.0002
Preoperative serum CA 19-9	<0.01
Postoperative serum CA 19-9	<0.006
Biliary CA 19-9	<0.0002

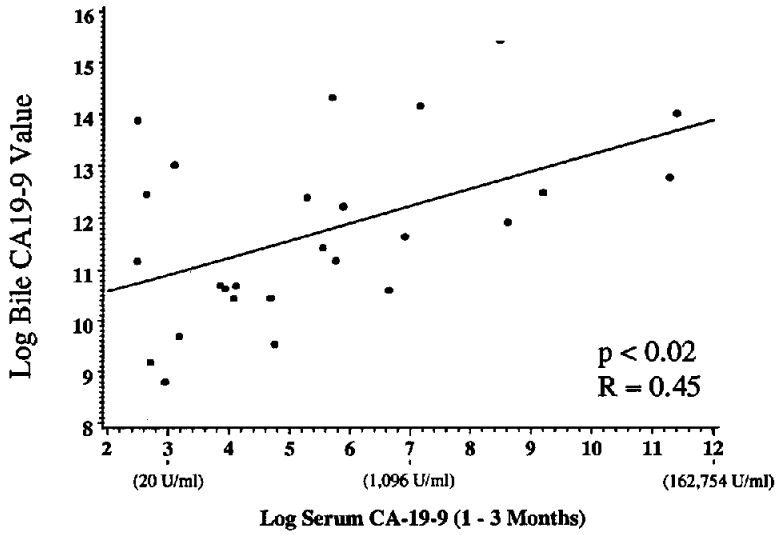

Fig. 1. Correlation between biliary CA 19-9 and postoperative serum CA 19-9 values. Line represents best fit to observed data.

Twenty-seven (80%) of the 33 patients developed metastatic disease. Sites of recurrence included pulmonary (n = 5), peritoneal (n = 5), and hepatic (n = 17) metastases. Nine of the 10 patients with pulmonary and peritoneal metastases had a recurrence after pancreatic resection, with a median interval to failure of 17 and 10 months, respectively. Of the 17 patients with hepatic metastases, 10 were found at the initial exploration; seven of these patients underwent bypass, one was maintained with a percutaneous stent, and two who had small peripheral hepatic lesions underwent resection. Seven patients were diagnosed with hepatic metastases during postoperative follow-up or during maintenance chemotherapy with a median interval to detection of 10 months.

Overall survival for the entire study group was 13 months (all five patients without evidence of disease are alive with a median follow-up of 13 months). The median survival of those patients who developed hepatic, peritoneal, and pulmonary metastases was 8, 14, and 35 months, respectively ($P < 0.0041$).

Table II. Serum CA 19-9 as a predictor of survival using the median value as a threshold

CA 19-9 values	Overall survival (mo)
Preoperative serum CA 19-9	
>472 U/ml (median)	11
<472 U/ml	21
Postoperative CA 19-9 (1-3 mo)	
>156 U/ml (median)	12
<156 U/ml	35

The values for biliary CA 19-9 correlated with a stepwise increase in the risk of developing metastatic disease with the lowest values associated with no metastases, intermediate values with pulmonary and peritoneal metastases, and the highest values with hepatic metastases (Fig. 3; $P < 0.01$). Biliary CA 19-9 values were significantly higher in patients with metastatic disease in the liver (median 267,400 U/ml; range 34,379 to 5,000,000 U/ml) compared to patients without metastatic disease (median 34,103 U/ml; range 6620 to 239,880 U/ml; $P < 0.006$). Despite the correlation between increasing biliary CA 19-9 values and the development of metastatic disease, the ranges of the biliary CA 19-9 values for patients with different metastatic sites overlapped (see Fig. 3). In fact, biliary CA 19-9 values for patients with hepatic metastases were not significantly different from those values in patients who developed peritoneal metastases (median 91,890 U/ml; range 44,198 to 1,059,200 U/ml), although the median value for the patients with hepatic metastases was higher than that for patients with peritoneal metastases. This may be related to undetected hepatic metastases in the peritoneal group because the patient with the highest value from the peritoneal group (3 times the next patient's value) was found to have undetected hepatic metastases at autopsy. Although the biliary and serum CA 19-9 values were correlated, serum values were not predictive for the site of metastatic disease.

Sensitivity and specificity curves as a function of the risk of developing hepatic metastases were com-

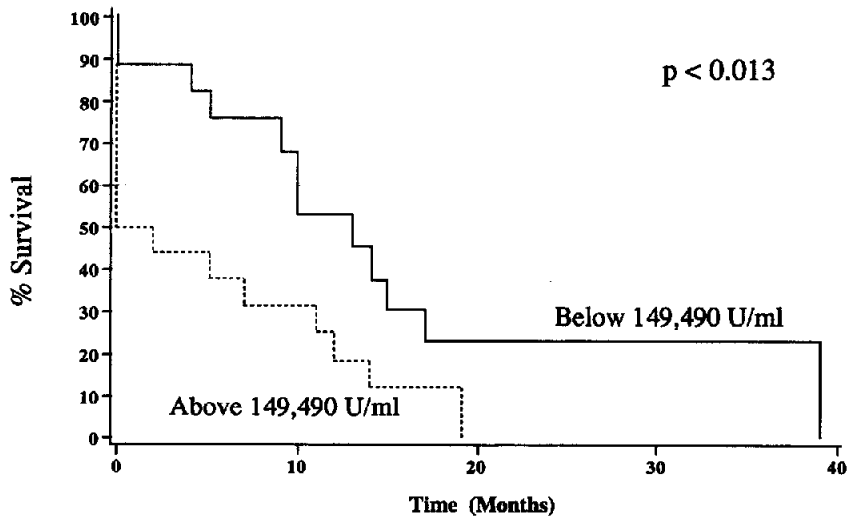

Fig. 2. Overall survival of patients with biliary CA 19-9 values below the median (149,490 U/ml) compared to patients with biliary CA 19-9 values above the median.

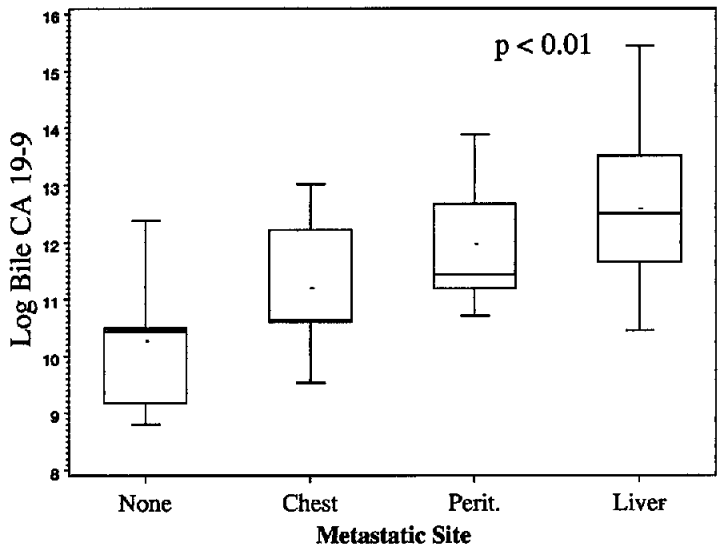

Fig. 3. Box and whisker plot of biliary CA 19-9 values grouped by site of metastatic recurrence. Box represents data point between the twenty-fifth and seventy-fifth percentiles. Line in the box represents the median value and the dash represents the mean value. Whiskers represent range of data from lowest to highest value.

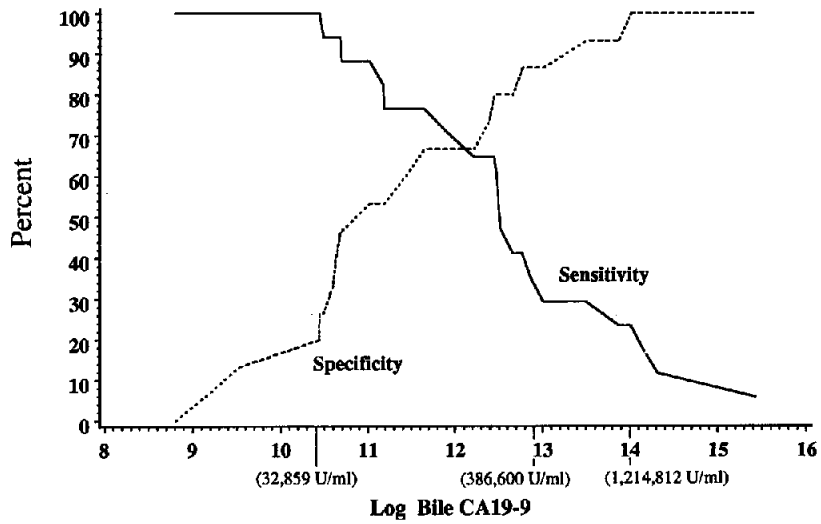

Fig. 4. Empiric sensitivity and specificity curves generated from individual biliary CA 19-9 values calibrated to the presence or absence of hepatic metastases.

puted using each observed biliary CA 19-9 value (Fig. 4). Sensitivity is defined as the proportion of patients with disease who test positive, whereas specificity is defined as the proportion of patients without disease who test negative. As the threshold for biliary CA 19-9 values increased, a higher specificity with regard to identifying hepatic metastases was obtained. However, as these values increased, there was a decrease in the sensitivity in identifying all patients at risk for developing hepatic metastases.

A plot of the estimated probability of hepatic metastases as a function of the biliary CA 19-9 value is shown in Fig. 5. The predicted values are derived from a linear logistic regression analysis calibrated to the presence or absence of hepatic metastases. As biliary CA 19-9 values increased, an increase in the

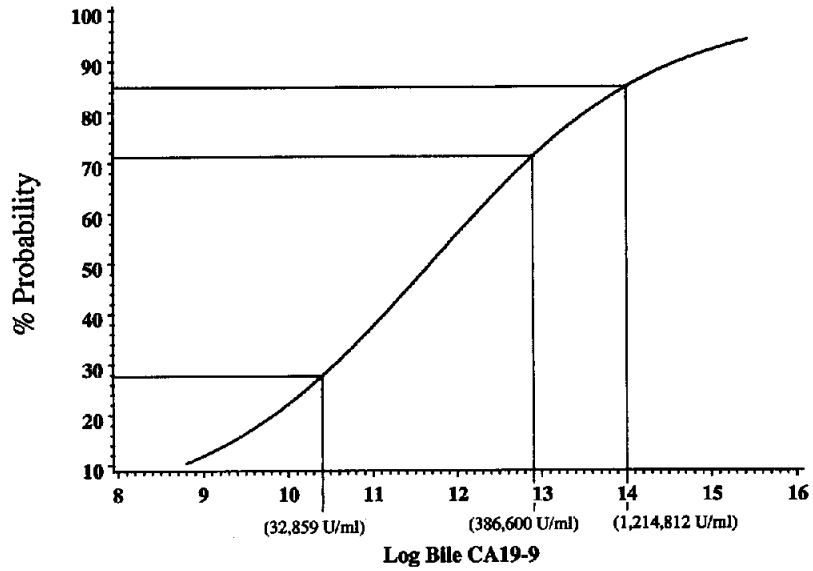

Fig. 5. Estimated probability of developing hepatic metastases as a function of individual biliary CA 19-9 values.

probability of developing hepatic metastases was clearly observed.

DISCUSSION

The propensity to develop metastatic disease continues to make adenocarcinoma of the pancreas one of the deadliest malignancies treated in the United States today, accounting for approximately 27,800 deaths in 1996 alone.[13] Lymphatic spread can occur early in tumor development, and some studies have shown that as many as 75% of patients with T1 tumors confined to the pancreas will have microscopic lymphatic metastases at the time of resection.[14,15] Despite the widespread use of computed tomography and angiography in the preoperative evaluation of these patients, one of the most common reasons for an aborted pancreatic resection remains the discovery of unsuspected hepatic metastases. Since the only hope for long-term survival is surgical resection, significant time and expense are expended to identify distant spread of disease as well as high-risk patients who may benefit from neoadjuvant or adjuvant therapy.

The tumor-associated antigen CA 19-9 is the sialylated Le^{ab} antigen and is present in salivary mucus, serum, and physiologic exocrine pancreatic secretions.[2] Immunohistochemical techniques demonstrate the CA 19-9 antigen in several different gastrointestinal malignancies as well as in nonneoplastic epithelia from the pancreas, stomach, liver, and biliary tract.[16] Thus benign conditions such as cholelithiasis, cholangitis, and biliary obstruction can result in an elevated serum CA 19-9 level, which can cause confusion when this assay is used as a diagnostic test.

Although decompression of the biliary tree results in a rapid fall in the serum CA 19-9 levels, these values do not return to normal in patients with malignant disease as opposed to patients with nonmalignant biliary disease.[17,18] CA 19-9 values have been shown to parallel documented tumor burden and decrease in response to treatment.[19] In addition, serum CA 19-9 marker concentrations have been mathematically shown to closely follow tumor burden and the development of hepatic metastases in a patient with adenocarcinoma of the pancreas.[7] If metastatic disease is present in the liver, then theoretically the CA 19-9 antigen shed by metastatic tumor would correlate with tumor burden and be detectable in biliary samples.

In this study, biliary CA 19-9 values were directly correlated with serum CA 19-9 values in patients with adenocarcinoma of the pancreas (see Fig. 1), and biliary CA 19-9 values alone were independent predictors of survival. When we arbitrarily assigned the median value as our threshold, a clear difference in disease-free and overall survival between the patients with biliary values greater than the median of 149,490 U/ml could be seen in comparison to patients with biliary values less than the median value (see Fig. 2). Despite the correlation between biliary and serum CA 19-9, serum values did not correlate with the site of metastatic disease.

We attempted to minimize the effect biliary obstruction had on the CA 19-9 value by reviewing con-

current liver function tests and by using bile samples obtained after a period of decompression. Although the retrospective nature of this study prevented complete resolution of this problem, CA 19-9 was produced by the tumor in sufficient amounts to correlate with the different sites of metastatic disease. Biliary CA 19-9 values are associated with the risk of developing metastatic disease at any site. However, complete independent discrimination between separate metastatic sites using the biliary value is not possible because of the overlapping ranges of the biliary CA 19-9 values (see Fig. 3). Regardless, higher values are significantly associated with the greatest risk of developing hepatic metastases. In fact, the biliary CA 19-9 value may be more sensitive than we can demonstrate because a patient with peritoneal metastases by imaging studies (and included in that group for analysis), and the highest biliary CA 19-9 value for that group, was found to have hepatic metastases only after autopsy.

Since elevated biliary CA 19-9 values are associated with hepatic metastases, we turned our attention to identifying individual biliary CA 19-9 values that could be used as threshold values to stratify patients into separate groups with opposing risks of hepatic metastases. Sensitivity and specificity curves constructed from our data reveal that as biliary CA 19-9 values increase, the specificity also increases. Unfortunately the converse is true with regard to sensitivity, which decreases as biliary CA 19-9 values increase. Thus any threshold value chosen will require a tradeoff between sensitivity and specificity. Therefore choosing a threshold value will require a knowledge of the consequences of false negative and false positive results.

A threshold with a high specificity will reduce the number of false positive results. This situation would be more desirable when attempting to identify patients who may benefit from a more thorough, and presumably expensive, diagnostic workup. Higher specificity is more desirable when attempting to identify those patients with a high enough risk to justify treatment with a more intensive systemic or hepatic-directed therapy.

However, some patients who are at risk of developing hepatic metastases will not be identified using any of the higher biliary CA 19-9 values because of the lower sensitivity associated with these values. Depending on the threshold value chosen, patients with values above the threshold will have a risk that is sufficiently high to justify a more intensive workup or additional treatment.

Keeping these limitations in mind, a patient's probability of developing hepatic metastases can be identified using the biliary CA 19-9 value (see Fig. 5). For example, based on our results, patients with a biliary CA 19-9 value greater than 1,214,812 U/ml have an 85% probability (sensitivity 24%; specificity 100%) of developing hepatic metastases. Patients with values greater than 386,600 U/ml have a 70% probability (sensitivity 35%, specificity 86%) of developing hepatic metastases. Conversely, patients with a biliary value of 32,859 U/ml have a 27% probability (sensitivity 100%, specificity 20%) of developing hepatic metastases. Clearly the biliary CA 19-9 value can be useful in identifying those patients at a high risk of developing hepatic metastases despite the fact that some patients at risk will not be identified.

Some caution must be exercised when attempting to use any of our threshold values for generalized purposes, however, since statistically significant threshold values will vary depending on the time interval used and the population size.[20] Although our data are strongly suggestive, enough variability exists within this small series of patients to prevent the application of our threshold values to the general population. A prospective study with a large group of patients is needed to identify statistically sound threshold values that can be considered suitable for routine use.

At present, the absence of effective adjuvant therapy for metastatic pancreatic cancer is the most important obstacle preventing the potential use of the biliary CA 19-9 assay to identify patients at risk for hepatic metastases. Although biliary CA 19-9 may provide information indicating the presence of hepatic metastases, systemic adjuvant treatment should not be based on this value alone in the absence of identifiable disease. Once prospective studies clarify the parameters surrounding the use of the biliary CA 19-9 assay, trials can be designed using this assay to identify patients with a high risk of developing hepatic metastases and examine the potential effects of hepatic-directed adjuvant therapy such as continuous postoperative infusion chemotherapy.[21] Currently, the use of biliary CA 19-9 assays for anything other than an indication for a more intensive diagnostic search for metastatic disease is not recommended.

In summary, biliary CA 19-9 is correlated with serum CA 19-9 and increasing values are correlated with decreased survival. Biliary CA 19-9 values alone are predictors of survival and are associated with the development of hepatic metastases with the higher values carrying the greatest risk. In our study, patients with biliary CA 19-9 values greater than the median value of 149,490 U/ml were at greater risk of developing hepatic metastases compared to patients with lower values. Patients with biliary CA 19-9 values greater than 386,600 U/ml have a 70% probability of developing hepatic metastases. Because of the inaccu-

racy of current imaging techniques in the detection of small hepatic metastases, the biliary CA 19-9 value may be more specific than we are able to demonstrate. Prospective studies on larger populations of patients with adenocarcinoma of the pancreas are needed before highly accurate threshold values can be established for clinical use.

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A Novel Strategy for Inhibiting Growth of Human Pancreatic Cancer Cells by Blocking Cyclin-Dependent Kinase Activity

Hideaki Iseki, M.D., Tien C. Ko, M.D., Xiang Ying Xue, M.D., Annie Seapan, B.S., Courtney M. Townsend, Jr., M.D.

Pancreatic cancers frequently carry mutations in the K-*ras*, p53, and p16 genes, which regulate cell proliferation. Transition from G1 to S phase of the cell cycle requires activation of cyclin-dependent kinase 2 (Cdk2), which is inhibited by olomoucine and roscovitine. The purpose of this study was to determine whether olomoucine and roscovitine can block Cdk2 kinase activity and inhibit proliferation of four human pancreatic cancer cell lines with various genetic alterations. Human pancreatic carcinoma cell lines BxPC-3, PANC-1, Capan-2, and CAV were treated with olomoucine or roscovitine. Cdk2 kinase activity was determined using histone H1 as the substrate. Cell cycle distribution was analyzed by DNA flow cytometry. Cell numbers were quantitated by Coulter counter. Olomoucine and roscovitine blocked Cdk2 activity in all four pancreatic cancer cell lines. Both compounds also inhibited cell proliferation in a dose-dependent fashion. Roscovitine was at least threefold more potent than olomoucine for both Cdk2 activity and cell proliferation. We have shown that Cdk inhibitors, olomoucine and roscovitine, block proliferation of human pancreatic cancer cells regardless of their mutations in K-*ras*, p53, or p16 genes. These compounds represent a novel therapeutic strategy with potential therapeutic benefits for pancreatic cancers. (JGASTROINTEST SURG 1998;2:36-43.)

Pancreatic cancer is the second leading cause of gastrointestinal cancer–related deaths and the fifth most common cause of all cancer deaths in the United States.[1] It is estimated that there will be 27,600 new cases in 1997 with an overall 5-year survival rate of less than 5%.[1] This poor prognosis attests to the very aggressive nature of pancreatic cancer and its propensity to spread early into regional lymph nodes and distant organs. Surgery remains the treatment of choice for localized disease, which is present in only 10% to 15% of affected individuals.[2] Even after curative resection, the 5-year survival rate is approximately 12%.[1,2] Conventional radiation therapy and systemic chemotherapy have been ineffective in improving the prognosis for advanced disease.[3] Clearly, new strategies are needed if significant improvement in survival is to be achieved in pancreatic cancer. The development of novel therapeutic approaches requires an understanding of the molecular alterations that occur during pancreatic tumorigenesis.

During the past 10 years, advances in molecular techniques have identified three frequently altered genes in pancreatic cancer: K-*ras*, p53, and p16. K-*ras* is a member of the ras oncogene family and encodes a 21 kDa membrane-bound protein involved in signal transduction. Point mutation at codon 12 of the K-*ras* oncogene is found in at least 75% of pancreatic adenocarcinomas.[4,5] This leads to inappropriate activation of the ras pathway resulting in uncontrolled cell proliferation. p53 is a transcription factor and activates genes involved in cell cycle control and apoptosis. This tumor-suppressor gene is mutated in 50%

From the Department of Surgery, The University of Texas Medical Branch, Galveston, Tex.
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Reprint requests: Tien C. Ko, M.D., Department of Surgery, The University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0542.

to 70% of pancreatic carcinomas.[5-7] p16 (also known as MTS1/INK4A) is a tumor-suppressor gene that is deleted or mutated in nearly 80% of pancreatic carcinomas.[8] Both p53 and p16 send inhibitory signals to the cell division cycle.

Progression through the cell cycle requires sequential activation of a family of protein kinases, the cyclin-dependent kinases (Cdks).[9,10] Cdk is activated when it is bound to a specific cyclin protein,[11] and the cyclin/Cdk complex can be inhibited by Cdk inhibitory proteins (CKIs).[12,13] The cell cycle is regulated at two key checkpoints: the G1/S transition and the G2/M transition.[14] Cdk2 activity is required for G1/S transition,[15,16] and Cdk1 (also known as cdc2) is essential for G2/M transition.[9,10] Cdk4 and Cdk6 are activated during early to mid-G1 transition and are specifically inhibited by the 16 kDa product of the p16/MTS1 gene.[17] p53 blocks cell cycle progression by transcriptionally activating the $p21^{Cip1}$ gene,[18-20] which can inhibit all known Cdks. Thus both p53 and p16 mutations can lead to increased Cdk activity and uncontrolled proliferation.

Recently two compounds, olomoucine and roscovitine, have been shown to inhibit Cdk activity.[21,22] These compounds are purine analogues with structural similarity to adenosine 5′-triphosphate (ATP) and can compete for ATP binding to Cdks. The specificity of these compounds for Cdks is dictated by the side chain residues of the purine ring. Iso-olomoucine and olomoucine differ only in the placement of the methyl group, yet iso-olomoucine is relatively ineffective in blocking Cdk activity.[23] Olomoucine and roscovitine have been shown to inhibit proliferation of 60 human tumor cell lines comprising nine tumor types including central nervous system cancer, non–small cell lung cancer, breast cancer, colon cancer, renal cancer, ovarian cancer, prostate cancer, melanoma, and leukemia.[24,25] The average IC_{50} for olomoucine and roscovitine is 60.3 μM and 16 μM, respectively. The sensitivity of cancer cells to these Cdk inhibitors does not require the presence of wild-type p53. Previously we have shown that both olomoucine and roscovitine can inhibit proliferation of human gastric cancer cell lines.[23] In the present study we evaluated the effects of these compounds on Cdk2 kinase activity and cell proliferation in four human pancreatic cancer cell lines that have various genetic alterations in the K-*ras*, p53, and p16 genes.

MATERIAL AND METHODS

Olomoucine, 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine, and iso-olomoucine, 6-benzylamino-2-(2-hydroxyethylamino)-7-methylpurine, were obtained from Calbiochem (La Jolla, Calif.) and dissolved in dimethylsulfoxide (DMSO) to a concentration of 100 mmol/L. Roscovitine, 2-(1-D,L-hydroxymethyl-propylamino)-6-benzylamino-9-isopropylpurine, was a generous gift of Dr. L. Meijer (Centre National de la Recherche Scientifique, Roscoff, France) and was dissolved in DMSO to a concentration of 10 mmol/L. Culture media were obtained from Mediatech Inc. (Herndon, Va.) and fetal bovine serum (FBS) was obtained from Hyclone Laboratories (Logan, Utah). FBS was heat inactivated by incubation at 60° C for 30 minutes. RNase A was purchased from Boehringer Mannheim (Indianapolis, Ind.). For kinase assays the rabbit polyclonal anti-Cdk2 antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.). Histone H1 protein was obtained from Life Technologies. [γ-^{32}P]ATP was obtained from DuPont New England Nuclear (Boston, Mass.). All other reagents were purchased from Sigma Chemical (St. Louis, Mo.).

Cell Culture and Proliferation Assay

The human pancreatic adenocarcinoma cell line CAV was established in our laboratory[26,27] and was maintained as monolayer cultures in DMEM:RPMI 1640 (1:1) medium supplemented with 10% (vol/vol) FBS. Three other human pancreatic adenocarcinoma cell lines, BxPC-3, PANC-1, and Capan-2, used in this study were obtained from the American Type Culture Collection (ATCC, Rockland, Md.). BxPC-3 cells were cultured in RPMI 1640 medium with 5% FBS; PANC-1 cells were grown in DMEM medium with 5% FBS; and CAPAN-2 cells were incubated in McCoy's 5A medium with 10% FBS. All cells were maintained at 37° C in a humidified atmosphere containing 5% carbon dioxide.

For proliferation assays, cells were seeded into 12-well plates (1.5 to 5.0 × 10^4 cells per well) and incubated in 5% to 10% FBS for 24 hours. To study the effects of FBS on cell proliferation, cultures were washed twice with Dulbecco's phosphate-buffered saline (PBS) and incubated for an additional 48 hours in various concentrations of FBS (0% to 10%). To examine the effects of Cdk inhibitors on cell proliferation, cells were treated with 0.3% DMSO (vehicle), olomoucine (10 to 300 μM), roscovitine (1 to 30 μM), or iso-olomoucine (300 μM) for various times before harvesting. Cells were dispersed with trypsin and counted using a model Zf Coulter counter (Coulter Electronics, Hialeah, Fla.).

DNA Flow Cytometry

BxPC-3 cells (0.5 × 10^6 cells per 100 mm plate) were incubated in 5% FBS for 24 hours, then washed twice with PBS. Culture medium was changed to 0% to 10% FBS. After 48 hours, cells were harvested by trypsin and collected by centrifugation. Cell pellets were resuspended in 0.3 ml low-salt stain (4 mmol/L sodium citrate, 0.1% Triton X-100, 30 mg/ml polyethylene glycol [molecular weight 8000; PEG 8000], and 50 μg/ml propidium iodide) plus 10 μl of 2 mg/ml RNase A and incubated at 37° C for 20 minutes. Cells were lysed by adding 0.3 ml high salt stain (400 mmol/L NaCl, 0.1% Triton X-100, 30 mg/ml PEG 8000, and 50 μg/ml propidium iodide) and incubating at 4° C overnight. Stained nuclei (10,000 per sample) were analyzed by FACScan (Becton Dickson, San Jose, Calif.), and the percentage of the S-phase population of cells was determined by Modfit LT program (Verity, Maine).

Cdk2 Kinase Assay

Cells (0.5 to 1.5 × 10^6 cells per 100 mm plate) were incubated in 5% to 10% FBS for 24 hours, then washed twice with PBS. Culture medium was changed to 0% to 10% FBS. After 48 hours, cells were harvested by scraping and collected by centrifugation. Protein lysates were prepared in NP40 buffer as described previously.[28] The lysates were clarified by centrifugation and protein concentrations were quantitated by the Bradford assay (Bio-Rad Laboratories, Hercules, Calif.). Cdk2 activity was assayed essentially as described by Dulic et al.[15] Samples (50 to 100 μg of protein) were incubated with anti-Cdk2 (0.5 μg) antibody or preimmune serum (1 μl) for 3 hours at 4° C, followed by a 2-hour incubation with protein A-Sepharose. Immunocomplexes were collected by centrifugation and washed three times in NP40 buffer and three times in reaction buffer (20 mmol/L Tris at pH 7.5 and 4 mmol/L $MgCl_2$). The kinase assay was performed for 30 minutes at 37° C in 10 μl of reaction buffer containing 2.4 μg histone H1, 40 μM [γ-^{32}P]ATP (10 Ci/mmol) plus either 0.3% DMSO, olomoucine (10 to 300 μM), roscovitine (1 to 30 μM), or iso-olomoucine (300 μM). The reaction was stopped by adding 12 μl 2× Laemmli buffer (125 mmol/L Tris at pH 6.8, 4% sodium dodecyl sulfate [SDS], 20% glycerol, 10% β-mercaptoethanol, and 0.5 mg/ml bromophenol blue). Samples were boiled for 5 minutes, then resolved by 10% SDS-polyacrylamide gel electrophoresis. The gels were dried, and phosphorylated histone H1 proteins were visualized by autoradiography and quantitated by a Lynx densitometer (Applied Imaging, Pittsburg, Pa.).

RESULTS

Cdk2 Kinase Activity Correlates With the S-Phase Population of Human Pancreatic Cancer Cells

In nontransformed cells, Cdk2 is activated during late G1 and remains active throughout the S-phase.[15,16] If this is true for pancreatic cancer cells, then cultures with an increasing S-phase population of cells should exhibit augmented Cdk2 enzyme activity. We tested this hypothesis by determining Cdk2 kinase activity and the S-phase population in human pancreatic adenocarcinoma cells, BxPC-3, that were treated with various concentrations of FBS (0% to 10%) for 48 hours. Protein lysates were prepared and assayed for Cdk2 kinase activity as described in Material and Methods. To determine nonspecific enzyme activity, protein lysates from 10% FBS–treated cultures were also immunoprecipitated with a preimmune serum and analyzed for kinase activity. Cells from parallel cultures were stained with propidium iodide and analyzed by a flow cytometer. The results are shown in Fig. 1. Increased Cdk2 activity was noted in

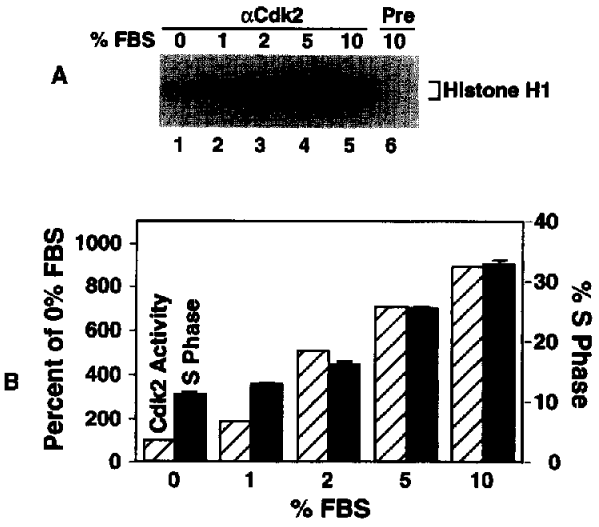

Fig. 1. Effects of fetal bovine serum *(FBS)* on Cdk2 kinase activity and the S-phase population in BxPC-3 cells. Cells were grown in 5% FBS for 24 hours, then incubated for an additional 48 hours in 0% to 10% FBS. Protein lysates were immunoprecipitated with anti-Cdk2 antibody *(αCdk2)* or preimmune serum *(Pre)* and assayed for kinase activity using histone H1 as the substrate. **A,** Autoradiogram of the kinase assay showing phosphorylated histone H1. **B,** Densitometric analysis of the autoradiogram was performed and results were expressed as percentage of 0% FBS (hatched bars). Parallel cultures were processed for cell cycle analysis. Cell nuclei were stained with propidium iodide and analyzed for percentage of S-phase population by flow cytometry (solid bars). Results were expressed as mean ± standard error of the mean.

cells maintained in higher concentrations of FBS (see also Fig. 2, *A*). When cells were incubated in 10% FBS, they had eightfold greater Cdk2 activity than cells maintained in 0% FBS. Similar increases were noted when cells were analyzed for S-phase population. Cells in 10% FBS had threefold greater S-phase fractions than cells maintained in 0% FBS. At each concentration of FBS tested, the increase in Cdk2 functional activity correlated with increased S-phase population of cells (see Fig. 1). Similar alterations in Cdk2 enzyme activity (Fig. 3, lanes 1 and 2) and S-phase fraction (results not shown) were observed in three additional pancreatic cancer cell lines (PANC-1, Capan-2, and CAV) that were cultured in the absence or presence of 5% to 10% FBS. These results suggest that Cdk2 activity is associated with G1/S transition in human pancreatic carcinoma cells and may be crucial for cell proliferation.

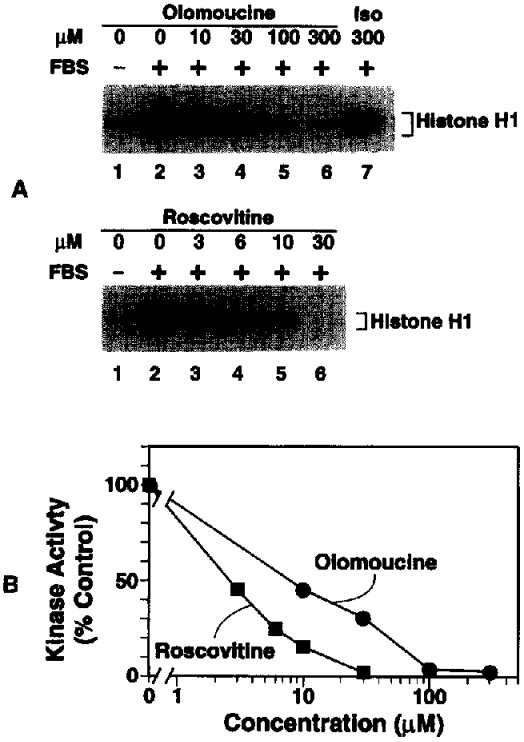

Fig. 2. Effects of olomoucine and roscovitine on Cdk2 kinase activity in BxPC-3 cells. Cells were incubated in 0% (−) or 5% (+) fetal bovine serum *(FBS)* for 48 hours. Cell lysates were immunoprecipitated with anti-Cdk2 antibody and assayed for kinase activity in the presence of olomoucine (0 to 300 μM), roscovitine (0 to 30 μM), or iso-olomoucine (300 μM) using histone H1 as the substrate. **A,** Autoradiogram of the kinase assay showing phosphorylated histone H1. **B,** Densitometric analysis of the autoradiogram. Results were expressed as percentages of control values (5% FBS, lane 2) for olomoucine (closed circles) or roscovitine (closed squares).

Olomoucine and Roscovitine Inhibit Cdk2 Kinase Activity in Human Pancreatic Cancer Cells

Olomoucine and roscovitine are potent inhibitors of Cdk2 kinase activity in human gastric cancer cells.[23] We determined whether olomoucine and roscovitine inhibited Cdk2 kinase activity in four human pancreatic cancer cell lines that have different genetic alterations. First, we examined the effects of different doses of these compounds on Cdk2 enzyme activity in BxPC-3 pancreatic cancer cells. Protein lysates were prepared from cells cultured in 0% or 5% FBS for 48 hours and immunoprecipitated with anti-Cdk2 antibody. The immunocomplexes were assayed for kinase activity in the presence of increasing concentrations of olomoucine, roscovitine, or iso-olomoucine. As shown in Fig. 2, *A* and *B*, olomoucine and roscovitine inhibited Cdk2 activity in a dose-dependent fashion, with the IC_{50} value of 10 μM for olomoucine and 3 μM for roscovitine. At the maximum concentrations tested, both compounds reduced Cdk2 activity to levels below those from cells cultured in 0% FBS (see Fig. 2, *A*, lanes 1 and 6). Furthermore, olomoucine, at 300 μM, inhibited Cdk2 activity by 97% (lane 6), whereas iso-olomoucine inhibited Cdk2 activity by 43% (lane 7). These results indicate that olomoucine and roscovitine are potent inhibitors of

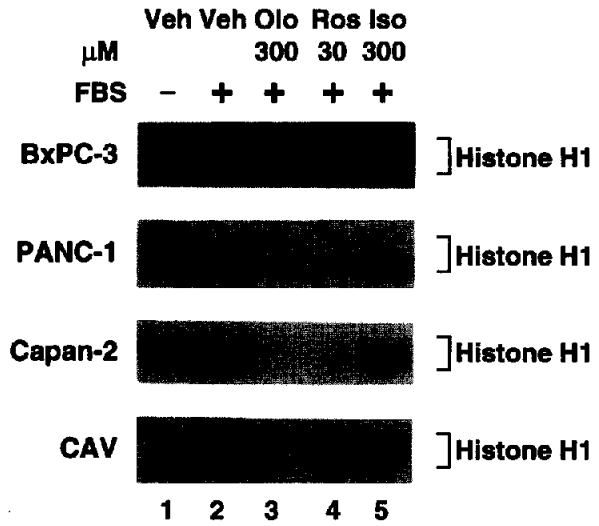

Fig. 3. Effects of olomoucine and roscovitine on Cdk2 kinase activity in four human pancreatic cancer cell lines. Cells were incubated in absence (−) or presence (+) of 5% or 10% fetal bovine serum *(FBS)* for 48 hours. Protein lysates were immunoprecipitated with anti-Cdk2 antibody and assayed for kinase activity in the presence of 0.3% DMSO *(Veh)*, 300 μM olomoucine *(Olo)*, 30 μM roscovitine *(Ros)*, or 300 μM iso-olomoucine *(Iso)* using histone H1 as the substrate.

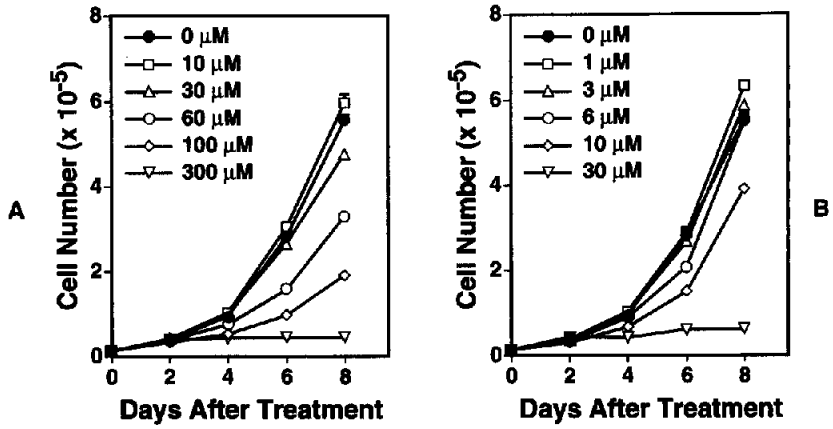

Fig. 4. Time course of the effects of olomoucine and roscovitine on proliferation of BxPC-3 cells. Cells were treated with various doses of olomoucine or roscovitine for 8 days. Cells were counted every other day from triplicate wells and expressed as mean ± standard error of the mean. **A,** Growth curve after treatment with olomoucine (0 to 300 μM). **B,** Growth curve after treatment with roscovitine (0 to 30 μM).

Cdk2 kinase activity in BxPC-3 cells. Roscovitine was threefold more potent than olomoucine, whereas iso-olomoucine was significantly less effective in blocking Cdk2 activity.

Next we examined the effects of a single dose of olomoucine (300 μM), roscovitine (30 μM), or iso-olomoucine (300 μM) on Cdk2 enzyme activity in three additional pancreatic carcinoma cell lines—PANC-1, Capan-2, and CAV. As shown in Fig. 3, both olomoucine and roscovitine blocked activation of Cdk2, whereas iso-olomoucine had a small effect on Cdk2 activity. These results demonstrate that both compounds were able to inhibit Cdk2 activity in all four human pancreatic cancer cells used for this study.

Olomoucine and Roscovitine Inhibit Proliferation of Human Pancreatic Cancer Cells

Since olomoucine and roscovitine inhibited Cdk2 activity, which is required for cell cycle progression,[15,16] we determined their effects on proliferation of human pancreatic cancer cells. First, we performed a time-course experiment examining the effects of various doses of olomoucine, roscovitine, or iso-olomoucine on proliferation of BxPC-3 cells. As shown in Fig. 4, *A*, olomoucine inhibited cell proliferation in a dose-dependent fashion beginning 4 days after treatment. By day 8, cultures treated with 300 μM of olomoucine had greater than a 90% reduction in the number of cells compared to control cells (0 μM). As expected, iso-olomoucine, which had little Cdk2 inhibitory activity (see Fig. 2), also had a significantly less antiproliferative effect on BxPC-3 cells than olomoucine over the 8-day treatment course (results not shown). These results suggest that the antiproliferative effect of olomoucine is mediated through its inhibition of Cdk2 activity. Similarly, roscovitine also inhibited BxPC-3 cell growth in a dose-dependent fashion beginning 4 days after treatment (Fig. 4, *B*). To better define the potency of these two compounds, proliferation assays were performed on four human pancreatic carcinoma cell lines to determine the IC_{50} values for growth suppression after 6 days of treatment. Both olomoucine and roscovitine inhibited proliferation of all four cell lines in a dose-dependent fashion (Fig. 5). The IC_{50} values of olomoucine on BxPC-3, PANC-1, Capan-2, and CAV cells were 80, 90, 50, and 90 μM, respectively. The IC_{50} values of roscovitine on BxPC-3, PANC-1, Capan-2, and CAV cells were 10, 13, 18, and 13 μM, respectively. These results demonstrate that roscovitine was three- to eightfold more potent than olomoucine in suppressing the proliferation of human pancreatic cancer cells. Iso-olomoucine, at equal molar concentrations, had significantly less growth-inhibitory activity on each cell line tested compared to olomoucine (results not shown). Despite various alterations in K-*ras*, p16, and p53 genes, all four human pancreatic cancer cell lines were similarly inhibited by both olomoucine and roscovitine.

DISCUSSION

Pancreatic cancer remains a major health challenge in the United States as well as worldwide. It is a biologically aggressive tumor and early diagnosis is hampered by its location within the abdomen. Most pa-

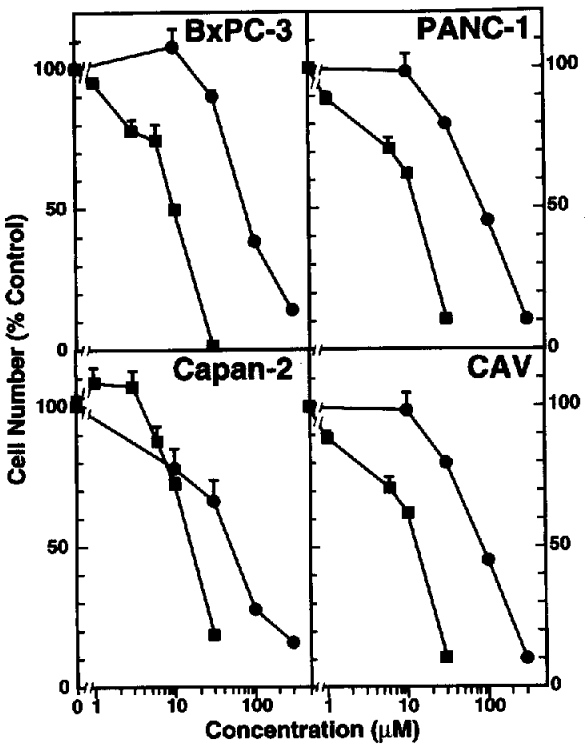

Fig. 5. Dose curves of the effects of olomoucine and roscovitine on proliferation of four human pancreatic cancer cell lines. Cells were incubated in fetal bovine serum (5% or 10%) with 0.3% DMSO (control), olomoucine (10 to 300 μM; closed circles), or roscovitine (1 to 30 μM; closed squares) for 6 days. Cell were counted from triplicate wells and results were expressed as percentages of control values, mean ± standard error of the mean.

tients do not develop symptoms until the tumor has spread to regional lymph nodes or distant organs. The exact pathogenesis of pancreatic adenocarcinoma is not known, but like other epithelial tumors it requires multiple somatic mutations to develop the malignant phenotype. The most common genetic alterations observed in pancreatic cancer cells are found in the K-*ras*, p53, and p16 genes.[4-8] The protein products of these genes are involved in the control of signal transduction pathways, cell cycle machinery, and apoptosis. K-*ras* is an oncogene, whereas p53 and p16 act as tumor suppressors. When a cancer cell acquires several of these defects, it can give rise to a clone of cells with a selective growth advantage over the surrounding cells. Current radiation and chemotherapy regimens offer a marginal survival advantage for advanced pancreatic cancer.[3]

Although cells have multiple kinase pathways that regulate cell proliferation, we chose to block the functional activity of Cdk2 because it regulates a key transition point in the cell cycle.[15,16] Moreover, Cdk2 acts downstream of the three cell cycle regulators, K-*ras*, p53, and p16, which are frequently mutated in pancreatic cancer cells. Others have attempted to control pancreatic cancer growth by selectively targeting K-*ras* or p53.[29,30] This strategy has the advantage of specifically affecting only cells that carry the mutated genes. However, cancer cells without the targeted genetic alteration would not be inhibited by this treatment.[29] We selected several pancreatic cancer cell lines for our investigation because their genetic alterations have been well characterized. The K-*ras* gene is mutated in PANC-1 and Capan-2 cell lines but is unaltered in BxPC-3 cells.[31] The p53 protein is abnormally expressed in BxPC-3 and PANC-1 cells, but not in Capan-2 cells.[32] The p16 tumor suppressor gene is deleted in both BxPC-3 and PANC-1 cells and is normally expressed in Capan-2.[33] CAV is a moderately to well-differentiated pancreatic ductal cancer cell line established in our laboratory and is maintained both as a cell line and as xenografts in nude mice.[26,27] Its genetic alterations have not been characterized and served as an unknown control. We have shown that both olomoucine and roscovitine inhibited proliferation of all four cell lines. These results suggest that Cdk inhibitors can block cell proliferation without the presence of wild-type p53 or p16 tumor suppressor genes. Moreover, both compounds blocked growth of cells with activated K-*ras* protein. These results indicate that compounds which inactivate Cdk activity may be effective in pancreatic cancers regardless of their molecular phenotype.

The activity of Cdk is regulated by positive and negative phosphorylation of several of its amino acid residues.[34,35] Cdk activation also requires the binding of a regulatory protein (cyclin)[11] and is inhibited by inhibitory proteins (CKIs).[12,13] Although we have only examined compounds that directly inhibit Cdk activity, it is obvious that molecules which suppress cyclin expression, augment CKI activity, or interfere with Cdk phosphorylation/dephosphorylation may also block cell proliferation. These agents are potential candidates for treatment of pancreatic adenocarcinomas. One possible problem with a therapeutic strategy that targets cell cycle regulators is one of specificity and toxicity. Olomoucine and roscovitine also inhibit cell proliferation in nontransformed cells (unpublished observations, T.C.K.), and their toxicity in animals has not been tested.

We have shown that both olomoucine and roscovitine inhibited Cdk2 activity in pancreatic cancer cells. Both compounds also should block cdc2 activity, which has been shown in human gastric cancer cells.[23] Olomoucine was originally synthesized to inhibit enzymes involved in metabolism of certain plant growth hormones.[36] Later, olomoucine was found to be a po-

tent inhibitor of cyclin B/cdc2 kinase during an extensive survey of purine analogues as potential inhibitors of Cdks.[21] The specificity of olomoucine has been examined using 35 highly purified protein kinases reconstituted from baculovirus-infected Sf9 insect cells or from starfish oocytes.[21] Under in vitro conditions, only Cdk2, cdc2, Cdk5, and to a lesser extent, erk1 are substantially inhibited by olomoucine. The IC_{50} values for Cdk2, cdc2, and erk1 are 7, 7, and 30 μm, respectively.[21] Roscovitine was developed to be a more potent and selective inhibitor of Cdks.[22] The IC_{50} values of roscovitine for Cdk2, cdc2, and erk1 are 0.7, 0.65, and 30 μM, respectively. We have found similar IC_{50} values for these compounds on Cdk2 kinase activity in pancreatic cancer cells. However, the IC_{50} values for olomoucine and roscovitine on cell proliferation were several-fold higher than those for Cdk2 enzyme activity. These differences may be due to a decrease in the uptake, an increase in intracellular degradation, or compartmentalization of these compounds, resulting in decreased intracellular activity.

In summary, this study demonstrates that the Cdk inhibitors olomoucine and roscovitine are novel antineoplastic compounds with potential therapeutic benefits for pancreatic carcinomas regardless of their genetic alterations. Moreover, inhibiting cell cycle regulators such as Cdks appears to be a promising novel strategy in the development of effective chemotherapeutic agents.

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Enterocyte Apoptosis Is Increased Following Small Bowel Resection

Michael A. Helmrath, M.D., Christopher R. Erwin, Ph.D., Cathy E. Shin, M.D., Brad W. Warner, M.D.

The intestinal mucosa is in a steady state of turnover as the rate of cellular proliferation is balanced by the rate of cell death. Although it is accepted that adaptation after small bowel resection (SBR) results in increased proliferation, its effect on apoptosis is not known. The purpose of this study was to determine the effect of adaptation following SBR on rates of enterocyte apoptosis. Male ICR mice underwent either 50% proximal SBR or sham operation (bowel transection/reanastomosis). After 12 and 24 hours, and 3 and 7 days, rates of proliferation were measured in the ileum as the percentage of crypt cells incorporating bromodeoxyuridine. Apoptosis was quantitated by end labeling of DNA strand breaks and propidium iodide staining of the number of apoptotic bodies per crypt and villus. Significant increases in enterocyte proliferation (30% to 40%) as well as apoptosis (57% to 87%) occurred at all time points following SBR when compared with sham-operated mice. Adaptation following SBR increases both the rate of enterocyte proliferation and the rate of apoptosis. Understanding the pathophysiology of intestinal adaptation and therapeutic interventions designed to augment this important response will require complete characterization of their effects on both proliferation and apoptosis. (J GASTROINTEST SURG 1998;2:44-49.)

The mucosa of the gastrointestinal tract is in a tightly regulated steady state of turnover that balances the rate of enterocyte proliferation with the rate of enterocyte loss. The exact mechanism(s) for enterocyte loss is not well understood. It has long been held that cells which are produced in the crypts migrate and differentiate up to the villus tip whereby they are simply exfoliated into the intestinal lumen.[1,2] Thus during conditions of increased enterocyte proliferation, there is a direct relationship between cellular production and exfoliation for purposes of maintaining homeostasis.[1,3] On the other hand, there is mounting evidence to suggest that apoptosis, and not simple exfoliation of enterocytes from the villus tips, may account for most of the cell loss into the lumen of the gut.[4-6]

Apoptosis, or programmed cell death, is an energy-dependent, highly ordered process that can be prevented by inhibitors of RNA and protein synthesis.[7] The importance of this process is underscored in p53 knockout mice in whom apoptosis is inhibited. In these animals the symmetry of cellular proliferation and death is perturbed, and neoplastic transformation is observed at multiple sites.[8]

Another hyperplastic condition is the adaptive response of the intestine to massive small bowel loss. This important compensatory response results in taller villi, deeper crypts, and enhanced mucosal surface area. These morphologic changes may be the result of an alteration of the balance between enterocyte proliferation and death. Although it is accepted that intestinal adaptation following small bowel resection (SBR) enhances enterocyte proliferation, its effect on rates of apoptosis have not been previously characterized. The purpose of this study, therefore, was to determine the effect of adaptation following intestinal resection on rates of enterocyte apoptosis.

From the Division of Pediatric Surgery, Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio.

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Reprint requests: Brad W. Warner, M.D., Division of Pediatric Surgery, Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229-3039. E-mail: Brad.Warner@chmcc.org.

MATERIAL AND METHODS

Animals

A protocol for this study was approved by the Institutional Animal Care and Use Committee of the Children's Hospital Research Foundation (Children's Hospital Medical Center, Cincinnati, Ohio). Male ICR mice (The Jackson Laboratory, Bar Harbor, Maine) were housed in groups of four at 21° C on 12-hour day/night cycles (6 AM to 6 PM). At approximately 8 to 10 weeks of life, the mice were randomized to undergo either a 50% proximal SBR with reanastomosis or a sham operation (transection of the bowel with reanastomosis only).

Small Bowel Resection Procedure

Details of this model have been previously reported.[9] Briefly, the mice were anesthetized with inhaled 2% isoflorane. The abdomen was clipped, prepared with povidone iodine solution, and draped sterilely. Operations were performed with the aid of an operating microscope. Through a midline incision the ileocecal junction was identified. Sham operations consisted of division and reanastomosis of the bowel approximately 15 cm proximal to the ileocecal junction. In mice undergoing SBR, the bowel was divided approximately 15 cm proximal to the ileocecal junction and again 2 to 3 cm distal to the ligament of Treitz. The intervening 15 cm of small intestine was removed (50% resection). The mesentery of the resected intestine was ligated and intestinal continuity was restored by an end-to-end, single-layered anastomosis with interrupted 9-0 monofilament suture (Ethicon, Inc., Somerville, N.J.). The abdomen was closed and the mice were resuscitated with an intraperitoneal injection of 0.9% saline solution (2 ml) and allowed to recover in a warmed incubator (33° C). After 2 hours, they were returned to their regular facilities and provided with water, ad libitum, for the first postoperative night. Thereafter a liquid rodent diet (Micro-Stabilized Rodent Liquid Diet LD 101/101A, Purina Mills Inc., St. Louis, Mo.) was provided ad libitum.

Tissue Harvest

Mice from both groups were killed by an overdose of inhaled methoxyfluorane after 12 and 24 hours, and 3 and 7 days. The ileum 2 cm distal to the anastomosis was immediately excised and luminal feces gently removed with cotton swabs. The bowel was suspended with a 3.1 gm weight, its length recorded, and the distal 7 cm immersed in liquid nitrogen for subsequent measurements of DNA and protein. The remaining ileum was used for histologic examination to determine a proliferative index and apoptotic index as described below.

DNA and Protein Measurement

Individual ileum samples were thawed and homogenized (PowerGen, Fisher Scientific Co., Pittsburgh, Pa.). DNA content was determined using the Burton modification of the diphenylamine procedure,[10] with calf thymus DNA as standard. Total protein was quantitated by a modified Lowry assay,[11] with bovine serum as the standard. Both DNA and protein contents are expressed per centimeter of ileum.

Proliferative Index

One hour before the mice were killed, they were given an intraperitoneal injection of 5-bromodeoxyuridine (BrdU) provided as "labeling reagent" (1 ml/100 gm body weight; Zymed Laboratories Inc., San Francisco, Calif.). At the time the animals were killed, the ileum was removed and fixed in 10% neutral buffered formalin for 24 hours and then stored in 80% ethanol until embedded in paraffin. Once embedded, 5 μm slices were cut, mounted on poly-l-lysine–coated slides, and deparaffinized with xylene. Sections were then rehydrated with ethanol (100%, 95%, and 75%) and endogenous tissue peroxidase was inactivated with 30% hydrogen peroxide in methanol (1:9). BrdU incorporation into proliferating crypt cells (S-phase) was detected using a biotinylated monoclonal anti-BrdU antibody system with streptavidin-peroxidase as a signal generator. The staining reagents and methods were provided in kit form (Zymed Laboratories Inc.). An index of crypt cell proliferation rate was derived by the percentage of crypt cells incorporating BrdU. Ten representative crypts were counted (ability to visualize the crypt-villus junction on both sides of the crypt) from each animal. The investigator was blinded as to the origin of the tissue section during the scoring procedure.

Apoptotic Index

Apoptosis is quantitated by immunohistochemical labeling of DNA strand breaks in enterocytes and confirmed by morphometric analysis of propidium iodide–stained tissue sections. Labeling of DNA strand breaks was accomplished using the ApopTag kit (Oncor, Gaithersburg, Md.) following the instructions of the manufacturer. In short, formalin-fixed, paraffin-embedded ileal specimens were deparaffinized with xylene and then rehydrated with a series of ethanol washes (100%, 95%, and 70%). Protein was digested with proteinase-K and endogenous per-

oxidase activity was quenched with 2% hydrogen peroxide. The slides were then incubated with a reaction mixture containing terminal deoxynucleotidyl transferase and its substrate digoxigenin–11-deoxyuridine triphosphate at 37° C for 1 hour. The reaction was terminated and an antidigoxigenin-peroxidase antibody was applied for 30 minutes at room temperature. Hydrogen peroxide was added as a chromogenic substrate. The slides were then counterstained with methyl green, washed in 100% butanol, dehydrated with xylene, and mounted. Different sections from embedded ileum were then stained with propidium iodide for morphologic examination to confirm the findings of the labeling procedure as above. A quantitative index of apoptosis was derived by counting the number of apoptotic bodies per crypt and villus that were both nick-end labeled and moving abnormal morphology (pyknotic nuclei, condensed chromatin, and nuclear fragmentation). Blinded scoring of 50 crypts and villi per mouse was performed in triplicate.

Statistical Analysis

Results are presented as mean values ± standard error of the mean (SEM). Student's *t* test was used for comparisons of group means. A *P* value less than 0.05 was considered significant.

RESULTS

The overall survival was 20 of 28 (71%) for mice that underwent sham-operation and 23 of 28 (82%) for mice that underwent SBR. The greater mortality in the sham-operated group occurred early in the experiments and was due to bleeding at the mesenteric margin of the divided bowel. As expected, adaptation occurred in the ileum of the SBR mice as demonstrated by greater DNA content after 24 hours as well as in protein content at the 3- and 7-day intervals (Table I).

Proliferation was substantially increased in the

Table I. Ileal DNA and protein content

Group (n)	DNA (mg/cm)	Protein (mg/cm)
12 hr Sham (5)	0.04 ± 0.004	2.22 ± 0.15
12 hr SBR (5)	0.05 ± 0.005	2.30 ± 0.13
P value	0.24	0.7
24 hr Sham (5)	0.036 ± 0.005	1.71 ± 0.22
24 hr SBR (7)	0.051 ± 0.004	2.19 ± 0.07
P value	0.036	0.12
3-day Sham (5)	0.029 ± 0.003	1.75 ± 0.14
3-day SBR (6)	0.07 ± 0.005	2.79 ± 0.11
P value	<0.001	<0.001
7-day Sham (5)	0.039 ± 0.01	1.77 ± 0.19
7-day SBR (5)	0.09 ± 0.019	3.75 ± 0.21
P value	<0.001	<0.001

Ileum was harvested from mice at various time points following either 50% proximal small bowel resection (SBR) or sham operation (transection of the bowel with reanastomosis only).
Values are presented as mean ± SEM, and statistical comparisons were made between the two groups at each time point using Student's *t* test.

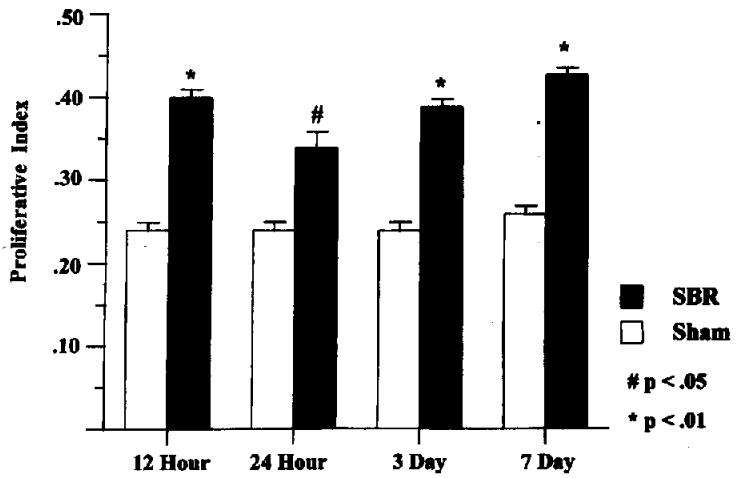

Fig. 1. Ileal crypt cell proliferation rates from mice at various times following either 50% proximal small bowel resection *(SBR)* or sham operation (bowel transection with reanastomosis only). A proliferative index is calculated by the number of crypt cells incorporating 5-bromodeoxyuridine divided by the total number of cells within the crypt. Ten crypts from each animal were counted per mouse; n = 5 from each group at each time point.

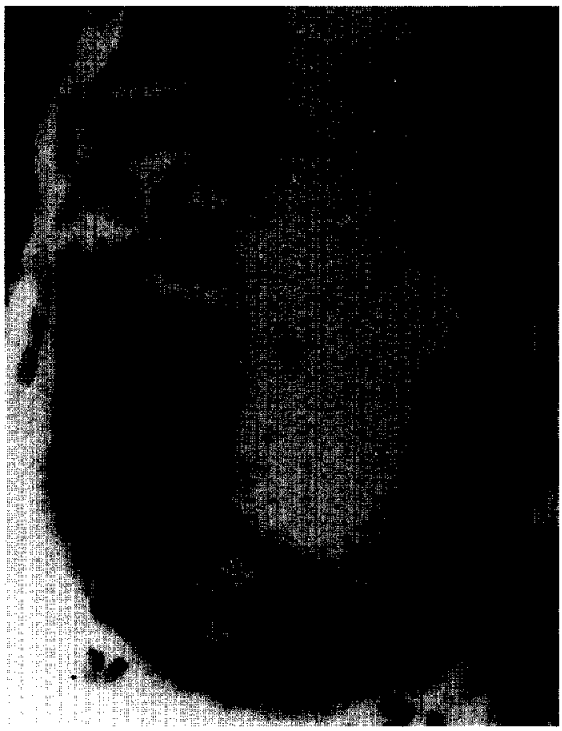

Fig. 2. Propidium iodide–stained ileal crypt from a mouse 3 days following 50% proximal SBR. Apoptotic bodies are identified *(arrows)* by nuclear condensation and fragmentation. (Original magnification ×50.)

ileum of mice that underwent SBR and was greater than in sham-operated mice at every time point (Fig. 1). The increase in proliferation following SBR was roughly 30% to 40% greater when compared with sham-operated mice at each time point. The values obtained for the proliferative index at these time points following SBR or sham operation are consistent with our previous observations.[9]

The appearance of apoptotic bodies was more frequently observed in the ileum after SBR at all time points in both crypts and villi (Fig. 2). These observations were quantitated by the apoptotic index and confirmed to be greater following SBR in the crypt (Fig. 3) and villus units at all time points studied (Fig. 4). When compared with sham-operated mice, SBR resulted in apoptotic indices that were 57% to 86% greater in the crypts and 63% to 87% greater in the villi for each time point.

DISCUSSION

In the present study we have shown for the first time that apoptosis, as documented by scoring the presence of apoptotic bodies, is increased in the ileum following small bowel resection. This increase in apoptosis occurred in the context of enhanced enterocyte proliferation. These findings suggest that

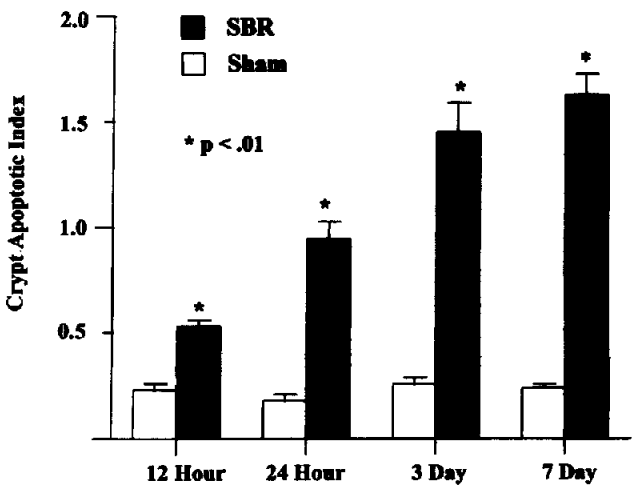

Fig. 3. Rates of apoptosis in ileal crypts at various times following either 50% proximal small bowel resection *(SBR)* or sham operation (bowel transection with reanastomosis only). An apoptotic index was derived by counting the number of apoptotic bodies per crypt that were both in situ end labeled for DNA strand breaks and demonstrated abnormal morphology (pyknotic nuclei, condensed chromatin, and nuclear fragmentation). Blinded scoring of 50 crypts and villi per mouse was performed in triplicate.

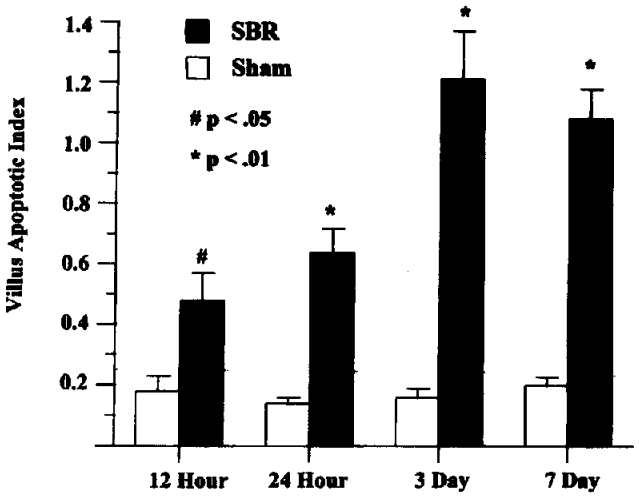

Fig. 4. Rates of apoptosis in ileal villi at various times following either 50% proximal small bowel resection *(SBR)* or sham operation (bowel transection with reanastomosis only). An apoptotic index was derived by counting the number of apoptotic bodies per villus that were both in situ end labeled for DNA strand breaks and demonstrated abnormal morphology (pyknotic nuclei, condensed chromatin, and nuclear fragmentation). Blinded scoring of 50 villi per mouse was performed in triplicate.

apoptosis is an integral component of adaptive response of the intestine to massive small bowel loss.

Our murine model of SBR is a reproducible and efficient system for the study of intestinal adaptation. The values that we measured with regard to DNA and protein content in the ileum after SBR in this study closely approximate those that we have previously reported.[9] We did not measure changes in villus height and crypt depth in this study since we have already monitored substantial changes in these parameters using this model.[9] The ileum, and not the jejunum, was used for analysis since this is the site of greatest adaptive change following intestinal resection.[12] Further, most of the jejunum is removed during a proximal intestinal resection, thus leaving little tissue for subsequent analysis.

The limitations of visualization and scoring apoptotic bodies in the gastrointestinal tract as a means to estimate the rate of apoptosis have been elegantly addressed by Potten[13] and include an unknown duration of time for the apoptotic process, as well as uncertainty as to the route of elimination of the apoptotic bodies (e.g., epithelial cell or macrophage phagocytosis, luminal extrusion). Also, since apoptotic bodies represent fragmented remnants of a cell, the number of apoptotic bodies identified in a tissue section could originate from a single or multiple cells. The apoptotic index, therefore, should probably not be equated with a cell death score. This phenomenon probably accounts for the fact that we observed a greater magnitude of increase in apoptosis relative to the increase in proliferation.

Another technique for the detection of apoptosis that we used to corroborate our morphologic observations takes advantage of the presence of fragmented DNA in the cells undergoing apoptosis. Free 3′-OH ends are end labeled in situ with exogenous dUTP or dATP by a terminal deoxynucleotidyl transferase enzyme.[4,5,14] This method is also imprecise and is influenced by such factors as delayed fixation[5] and nonspecific background staining.[14] In addition, DNA strand breaks induced by other factors such as injury or frank necrosis cannot be distinguished from apoptosis.

It is presently not clear where the predominant site of apoptosis is within the crypt/villus axis. Apoptotic cell death has been observed to occur only at the tip of the villus in one report[4] and was identified in both the crypt and the villus in another.[5] Other investigators, however, have identified apoptosis occurring only in the crypts at approximately the fourth cell position, which coincides with the site of stem cells.[14,15] In our study we identified increased numbers of apoptotic bodies in both villi and crypts following SBR. In the crypts we also observed the greatest frequency of apoptosis occurring at or near the fourth cell position.

The number of apoptotic bodies that we recorded in the ileal crypts of sham-operated mice was very similar to the frequency that has been previously reported.[15] Our measurements of the number of apoptotic bodies along the villus, however, tended to be less than those previously reported.[5] We believe that this was due to the fact that we were fairly stringent in what we were willing to call an apoptotic body. Further, an apoptotic body was not counted unless the morphology was validated by in situ end-labeling.

In addition to scoring morphologic changes and in situ end-labeling, other techniques that might allow for a more precise and objective quantitation of apoptosis include measuring the expression of various factors known to correlate with apoptotic cell death. Although extremely complex, a few factors are worthy of consideration. Expression of deoxyribonuclease I has been linked to apoptosis in the intestine.[16,17] Radiation-induced apoptosis in the gastrointestinal tract has been shown to coincide with the expression of p53[18] and is ablated in mice that are p53 deficient.[19] The product of the protooncogene *bcl*-2 is known to play a role in cell survival and act as an inhibitor of apoptosis.[20] Negligible levels of *bcl*-2 in the small intestine (hence, increased apoptosis) and greater content in the colon have been proposed to explain the greater frequency of carcinoma and adenomas that are known to develop in the colon.[14] Homologues of *bcl*-2 including *bax* and *mcl*-1 have all been identified in intestinal epithelial cells and probably play integral, interdependent roles during apoptosis.[21-24]

Therapy designed to enhance adaptation following massive intestinal loss may prove useful to reduce parenteral nutrition requirements and to improve patient survival. Although most approaches have focused on the augmentation of enterocyte proliferation through the administration of various trophic agents or growth factors, an alternative strategy directed toward retarding apoptosis may be advantageous.[25] Furthermore, the beneficial effects of these agents may result from a mechanism of impeding apoptosis in addition to enhancing proliferation. One example is interleukin-11, which was shown to be trophic to the intestine in rats following a 90% SBR.[26] This same growth factor was found to retard apoptosis and accelerate proliferation in the intestine following combined chemotherapy and radiation.[27]

The significance of increased apoptosis following massive intestinal resection is presently unknown. However, we speculate that as enterocyte proliferation is increased, the rate of enterocyte apoptosis must also increase to maintain homeostasis. The establishment of a new set point in the balance of proliferation to apoptosis is probably vital toward the development

of taller villi and deeper crypts that characterize the adaptive response. Comprehension of both proliferation and rate of programmed cell death is an important step toward elucidating the mechanism for intestinal adaptation. Since apoptotic cell death is an active process requiring transcription and translation, therapy directed toward inhibiting apoptosis may provide a more effective, novel strategy in the management of patients with the short gut syndrome or other pathologic gastrointestinal conditions.[25]

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The Utility of Intracorporeal Ultrasonography for Screening of the Bile Duct During Laparoscopic Cholecystectomy

Justin S. Wu, M.D., Deannia L. Dunnegan, R.N., Nathaniel J. Soper, M.D., F.A.C.S.

Different strategies and imaging modalities have been used to detect common bile duct (CBD) stones during laparoscopic cholecystectomy. We prospectively compared fluoroscopic intraoperative cholangiography (FIOC) and laparoscopic intracorporeal ultrasonography (LICU) in patients undergoing laparoscopic cholecystectomy for this purpose. In a consecutive series of 607 laparoscopic cholecystectomies, FIOC was used in the first 407 patients, whereas LICU was preferentially applied to the subsequent 200 patients. When LICU documented CBD stones, the duct was flushed with saline solution after intravenous administration of glucagon, and stone persistence or absence was confirmed by FIOC and/or repeat LICU. In the FIOC group, 10 patients were converted to open cholecystectomy and 16 patients did not undergo FIOC. Among the remaining 381 patients, FIOC was successful in 370 (97%). In the LICU group, two patients were converted and LICU was not performed in 26 patients. In the remaining 172 patients, the cystic duct (CBD) junction and the CBD were visualized in all cases ($P < 0.05$ vs. FIOC). The mean (± SEM) times required to complete FIOC and LICU were 15.1 ± 0.4 minutes and 5.3 ± 0.2 minutes, respectively ($P < 0.0001$). Choledocholithiasis was detected in 25 patients (7%) undergoing FIOC and in 22 patients (13%) undergoing LICU ($P < 0.05$). In the LICU group, the mean sizes of the stones cleared by ampullary dilatation and flushing (17 of 22, 77%) and those requiring more invasive methods (5 of 22, 23%) were 1.6 ± 0.2 mm and 2.7 ± 0.3 mm, respectively ($P < 0.01$). Sludge was seen in the CBD by LICU in 10 patients (6%), which disappeared with flushing in all cases. LICU is accurate, safe, and permits more rapid evaluation of bile duct stones than FIOC during laparoscopic cholecystectomy. LICU may be overly sensitive in detecting small stones and sludge, which are of questionable significance. Stones 2 mm or less can usually be cleared by flushing, whereas larger ones often require invasive techniques for removal. (J GASTROINTEST SURG 1998;2:50-60.)

Different strategies and imaging modalities have been used to detect common bile duct (CBD) stones during laparoscopic cholecystectomy. Fluoroscopic intraoperative cholangiography (FIOC) has largely replaced static cholangiography as the standard technique to screen for choledocholithiasis and to clarify ductal anatomy in order to reduce bile duct injuries during laparoscopic cholecystectomy. The incidence of concomitant choledocholithiasis during laparoscopic cholecystectomy is 5% to 10%.[1-3] This incidence is higher when patients have preoperative or intraoperative signs of CBD stones (ultrasonographic findings of dilated CBD >6 mm, history of jaundice or pancreatitis, elevated serum liver enzymes, cystic duct stones, or dilated cystic duct >4 mm) as opposed to the 1% to 5% incidence in patients without these findings.[4,5] However, FIOC itself is not without risk and costs. Many surgeons argue against routine FIOC because of the small risk of iatrogenic ductal injury as well as the increased length and cost of the procedure.[6-10] In addition, FIOC carries a finite rate of failure to cannulate the cystic duct of 1% to 10% as well as false positive and negative studies.[6,11-14]

Recently, laparoscopic intracorporeal ultrasonography (LICU) has been suggested to be as accurate but more rapid than FIOC in screening for choledocholithiasis during laparoscopic cholecystectomy.[11,14-22] The accuracy of defining ductal anatomy

From the Department of Surgery, Washington University School of Medicine, and the Washington University Institute for Minimally Invasive Surgery, St. Louis, Mo.
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Reprint requests: Nathaniel J. Soper, M.D., Washington University School of Medicine, Department of Surgery, Box 8109, Ste. 6108, One Barnes Hospital Plaza, St. Louis, MO 63110.

by LICU, however, has not been as good as that of FIOC.[14,23] This role may not be as important during laparoscopic cholecystectomy since meticulous dissection, and not cholangiography, may be the best method of preventing iatrogenic biliary injuries.[4,20] Also, LICU allows the laparoscopic surgeon to "see" beyond the visible surfaces, which may be very valuable in difficult dissections and for other purposes.[24] We therefore changed to a policy of routine LICU to assess the CBD for stones during laparoscopic cholecystectomy and prospectively evaluated this method with respect to the former policy of routine FIOC.

MATERIAL AND METHODS

Consecutive patients (N = 607) undergoing laparoscopic cholecystectomy by the senior author (N.J.S.) were entered prospectively in a database. The demographics of the patients are presented in Table I. The laparoscopic operation was performed using four access ports with surgical trainees performing most operations as described previously.[25] After the gallbladder neck and the proximal cystic duct were dissected, an initial assessment of the distal cystic duct and CBD was made using FIOC or LICU (see below). FIOC was used in the first 407 patients, whereas LICU became available for use in the subsequent 200 patients. Patients who were converted to open operations before screening of the CBD and patients who were not screened by their respective imaging modalities were excluded from the study. When the imaging study documented CBD stones, the duct was flushed with saline solution after intravenous administration of glucagon, and stone persistence or absence was confirmed by FIOC or LICU.

More invasive means of treating duct stones were employed when indicated and included laparoscopic transcystic choledochoscopy or choledochotomy, conversion to laparotomy with CBD exploration, or postoperative endoscopic retrograde cholangiography with sphincterotomy. Laparoscopic cholecystectomy was then completed in all patients, who were subsequently admitted to the hospital overnight and discharged when they were able to tolerate oral feedings and experienced minimal abdominal discomfort. Patients were examined in the outpatient office at 1 month postoperatively and thereafter as clinically indicated. Data that were recorded included duration of the operation (from skin incision to skin closure), time to obtain the cholangiogram or LICU, pertinent findings, intraoperative and postoperative complications, duration of postoperative hospitalization, and postoperative evidence of retained CBD stones.

Table I. Demographics of study groups

	FIOC group	LICU group
Consecutive number	407	200
Age (yr)*	49 ± 1	49 ± 1
Weight (kg)*	78 ± 1	85 ± 2
Female sex (%)	303 (74%)	149 (74%)
ASA class*	1.9 ± 0.1	2.0 ± 0.1

FIOC = fluoroscopic intraoperative cholangiography; LICU = laparoscopic intracorporeal ultrasonography; ASA = American Society of Anesthesia.
*Mean ± standard error of the mean.

Fluoroscopic Intraoperative Cholangiographic Technique

Cholangiograms were obtained after catheterization of the cystic duct. A clip was first placed across the junction of the cystic duct with the gallbladder, and a small cystic ductotomy was made just distal to the clip. Atraumatic dissecting forceps were used to palpate and squeeze the cystic duct gently, beginning near its junction with the CBD and "milking" the duct toward the incision to discover and remove cystic duct stones.[25] The cystic duct was then intubated with a 4 F ureteral catheter and stabilized with a cholangioclamp (Karl Storz Endoscopy-America, Inc., Culver City, Calif.) that was placed through a subcostal port. Fluorocholangiography was performed using a standard C-arm connected to a digital image processor (OEC Diasonics, Inc., Salt Lake City, Utah). Iodinated contrast medium (Conray-60 [dothalamate sodium; Mallinckrodt Medical, St. Louis, Mo.] diluted 1:1 with sterile saline solution) was injected slowly and stopped if the pancreatic duct started to fill. When filling defects thought to be air bubbles were seen, the bile and air bubbles were aspirated and/or flushed into the duodenum under fluoroscopic imaging. Duct calculi were identified as discrete filling defects that did not change shape with aspiration or irrigation. When small stones were seen or contrast medium did not enter the duodenum, x-ray imaging was repeated after intravenous administration of 1 mg of glucagon. Successful FIOC examination consisted of complete visualization of the extrahepatic bile ducts. Time to perform the FIOC was calculated from placement of the cystic duct clip to the time of catheter removal.

Laparoscopic Intracorporeal Ultrasound Technique

Intracorporeal ultrasonography of the extrahepatic bile ducts was performed after dissecting the hepatocystic triangle and placing a clip on the proximal cys-

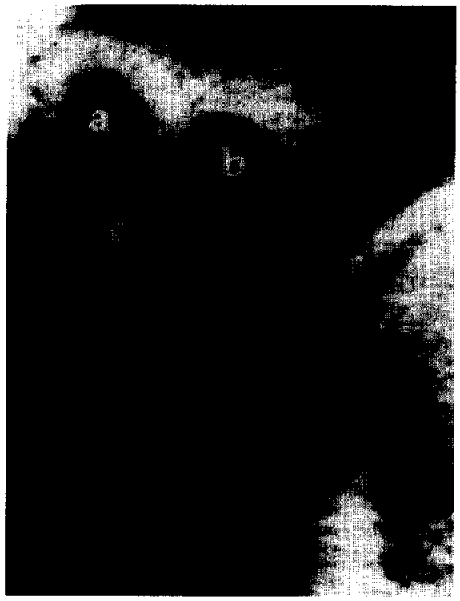

Fig. 1. Transverse view of the hepatoduodenal ligament by laparoscopic intracorporeal ultrasonography ("Mickey Mouse head" image). a = Common bile duct; b = hepatic artery, c = portal vein.

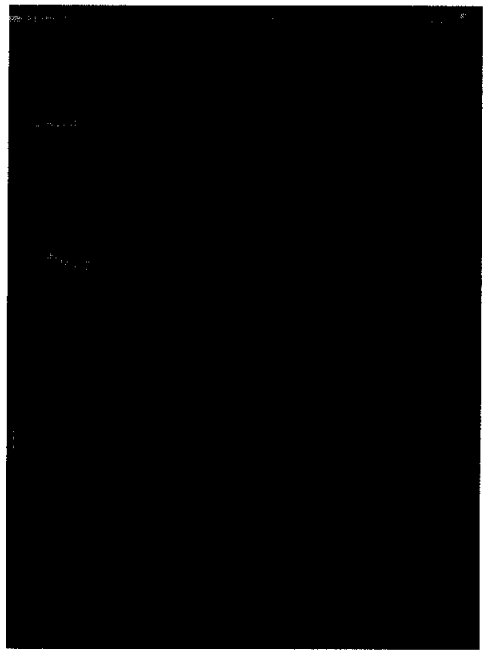

Fig. 2. Laparoscopic intracorporeal ultrasonography revealing a common bile duct *(CBD)* stone *(straight arrow)*. Note the echogenic object within the CBD casting a discrete acoustic shadow *(curved arrow)*. PV = portal vein; IVC = inferior vena cava.

tic duct. Examinations were done via the epigastric port using a rigid 7.5 MHz linear-array 10 mm diameter transducer equipped with a Doppler flow-detection system (Tetrad Corporation, Englewood, Colo.). The transducer was placed directly on the porta hepatis and transverse scanning was performed in real time from the cystic duct-CBD junction to the terminal end of the CBD. The probe was positioned to obtain the cross-sectional image configuration of the three tubular structures in the hepatoduodenal ligament: CBD, hepatic artery, and portal vein (Fig. 1). The image of these structures usually resembled the silhouette of a "Mickey Mouse head."[17] Visualization of the distal CBD and the pancreatic duct as they join at the papilla was achieved by clockwise rotation of the probe. In some patients, saline solution was instilled into the right upper quadrant to enhance acoustic coupling and facilitate the examination.[22] Notation was made of the diameter of the mid and distal common bile ducts, the presence, location, and size of stones or sludge, and the completeness of the ultrasound examination. Successful LICU examination was defined as complete visualization of the biliary tree from the cystic duct-CBD junction to the ampulla of Vater.

After the sonographic examination, the cystic duct was incised and "milked" for stones, and the distal duct was double clipped. Time to perform the ultrasound examination was calculated from the placement of the first clip until placement of the second clip. Duct calculi were identified ultrasonographically as echogenic objects within the CBD, which cast discrete acoustic shadows (Fig. 2). Sludge was defined as small ($\leq$1 mm diameter) areas of mobile echogenic material without discrete acoustic shadowing (Fig. 3, *A*). When calculi or sludge was identified, 1 mg of glucagon was administered intravenously, and the CBD was flushed with saline solution injected through a cystic duct catheter in an attempt to clear the duct by irrigation. FIOC or repeat LICU (Fig. 3, *B*) was then performed to confirm the persistence or absence of the stones.

Data Analyses

The InStat Biostatistics computer program (GraphPad Software, Inc., San Diego, Calif.) was used to perform Fisher's exact test to compare discrete variables and the Mann-Whitney U test to compare continuous data. Statistical significance was designated at the $P < 0.05$ level. Summary data in the text

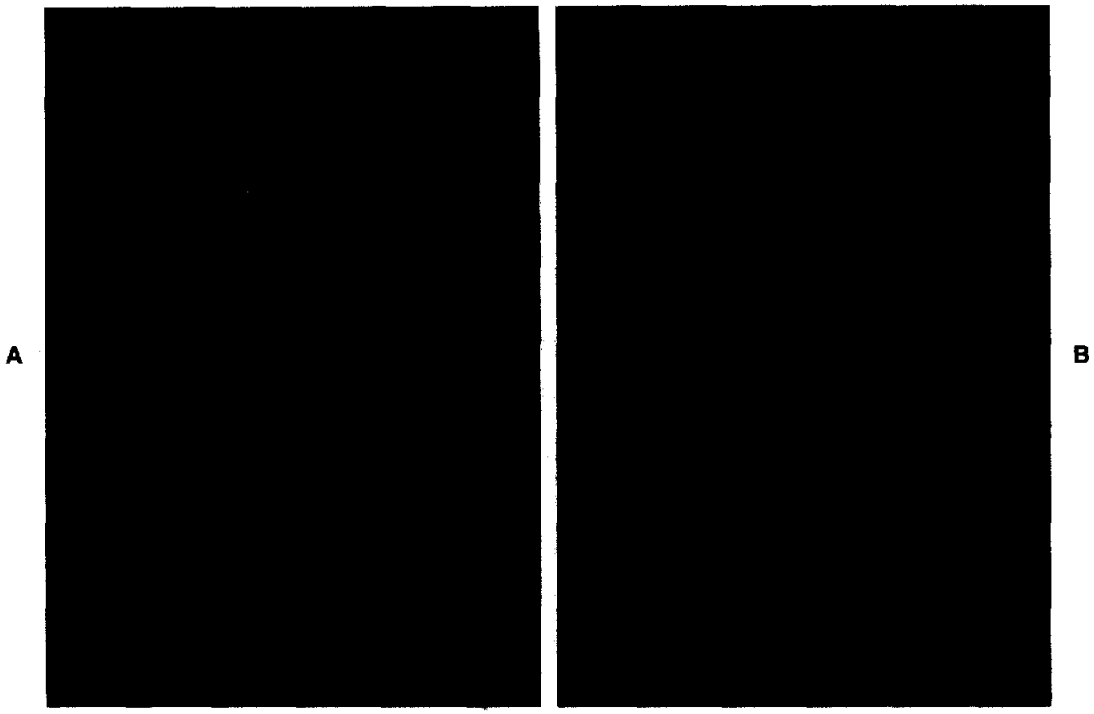

Fig. 3. Laparoscopic intracorporeal ultrasonography revealing common bile duct sludge. **A,** Note the small area of echogenic material *(arrow)* without discrete acoustic shadowing in contrast to Fig. 2. **B,** Successful clearance of the common bile duct after saline irrigation through a catheter *(arrow)* via the cystic duct.

are expressed as mean ± standard error of the mean (SEM).

RESULTS

The indications for laparoscopic cholecystectomy were similar for both the FIOC and LICU groups. The most common preoperative indication was symptomatic cholelithiasis (81% and 87%, respectively), followed by acalculous cholecystitis (8% and 6%), acute cholecystitis (5% and 4%), and gallstone pancreatitis (6% and 2%). In addition, laparoscopic cholecystectomy was performed in the LICU group for gallbladder polyps (1%) and porcelain gallbladder (1%). In the FIOC group, 10 patients (2%) were converted to open cholecystectomy because of adhesions or severe inflammation (Table II). FIOC was not performed in 16 patients (4%) because of lack of equipment, technical problems, critically ill status, pregnancy, or training for LICU (Table III). In the remaining 381 cases, the CBD was successfully imaged by means of FIOC in 370 patients (97%).

In the LICU group, two patients (1%) were converted to an open operation because of adhesions (n = 1) or severe inflammation (n = 1). Twenty-six patients did not undergo LICU for various reasons (Table III). Early in our experience with LICU, 12 patients with preoperative suspicion of choledocholithiasis or the diagnosis of chronic acalculous cholecystitis underwent FIOC directly without LICU. All of these exceptions occurred in the first 55 cases, and we now routinely image the CBD by LICU in these patients. In addition, five patients intraoperatively suspected of having ductal anomalies or the potential for injury also underwent FIOC directly without LICU; an aberrant right hepatic duct and a cystic duct entering the right hepatic duct were found in two patients each, whereas one patient had a torn cystic duct caused by dissection. Lack of available equipment or technical problems precluded the use of LICU in four patients, and five patients required expedient operations because of a concomitant procedure or critically ill status, so imaging was avoided. In all of the remaining 172 patients, the cystic duct-CBD junction and the terminal CBD were clearly visualized ($P < 0.05$ vs. FIOC group; Table II). The mean times required to complete FIOC and LICU were 15.1 ± 0.4 minutes and 5.3 ± 0.2 minutes, respectively ($P < 0.0001$).

Table II. Results of common bile duct screening

	FIOC group	LICU group
No. of patients	407	200
No. of conversions to open operation*	10 (2%)	2 (1%)
CBD screening not attempted by		
FIOC	16 (4%)	—
LICU	—	26 (13%)
Attempted screening	381	172
Visualization of CBD		
Unsuccessful	11 (3%)†	0 (0%)
Successful	370 (97%)	172 (100%)‡
Time to perform study (min; mean ± SEM)	15.1 ± 0.4	5.3 ± 0.2§

FIOC = fluoroscopic intraoperative cholangiography; LICU = laparoscopic intracorporeal ultrasonography; CBD = common bile duct.
*Before screening for CBD stones.
†Inability to cannulate (n = 8), avulsed cystic duct (n = 3).
‡$P < 0.05$.
§$P < 0.0001$.

Table III. Reasons for not attempting to screen the common bile duct

	FIOC group	LICU group
Preoperative suspicion of CBD stones*	—	7†
Acalculous cholecystitis*	—	5†
Suspect ductal anomalies	—	5†‡
Equipment problems/unavailability	9	4
Concurrent operations	—	4
Learning LICU	4	—
Critically ill status	2	1
Pregnancy	1	—
TOTAL	16	26

FIOC = fluoroscopic intraoperative cholangiography; LICU = laparoscopic intracorporeal ultrasonography; CBD = common bile duct.
*All within the first 55 patients in the LICU group; no longer used as exclusion for LICU.
†FIOC without LICU.
‡Cystic duct off right hepatic duct (n = 2); aberrant right hepatic duct (n = 2); torn cystic duct (n = 1).

Table IV. Intraoperative detection and management of common bile duct stones/sludge

	FIOC group	LICU group
CBD stones detected	25 (7%)	22 (13%)*
Successful treatment of stones		
Glucagon and saline flush	10/25 (4%)	17/22 (77%)*
Extraction techniques†	15/15 (100%)	5/5 (100%)
CBD sludge detected	0 (0%)	10 (6%)‡
Successful treatment of sludge		
Glucagon and saline flush	—	10/10 (100%)§

FIOC = fluoroscopic intraoperative cholangiography; LICU = laparoscopic intracorporeal ultrasonography; CBD = common bile duct.
*$P < 0.05$.
†Laparoscopic transcystic extraction, laparoscopic or open CBD exploration with T-tube placement, and/or postoperative endoscopic retrograde cholangiography.
‡$P < 0.0001$.
§One patient had FIOC showing only air bubbles but was readmitted postoperatively after clinically passing a CBD stone.

Table V. Size of common bile duct by laparoscopic intracorporeal ultrasonography

	Patients with stones/sludge	Patients without stones/sludge
No. of patients	32	150
Maximum CBD diameter (mm)*	5.5 ± 0.4	4.8 ± 0.1
Range (mm)	2.0-10.0	2.0-10.0
Location of maximium CBD diameter		
Mid CBD	18 (56%)†	121 (81%)
Distal CBD	14 (44%)†	29 (19%)

CBD = common bile duct.
*Mean ± standard error of the mean.
†$P < 0.01$ vs. patients without stones/sludge.

Table VI. Mean size of common bile duct stones detected by laparoscopic intracorpeal ultrasonography

	Clearance by glucagon and saline flush	Removal by invasive techniques
No. of patients	17/22 (77%)	5/22 (23%)
Mean diameter of stone (mm)	1.6 ± 0.2	2.7 ± 0.3*
Range (mm)	1.0-2.0	2.0-3.0

*$P < 0.01$.

Choledocholithiasis was detected in 25 patients (7%) in the FIOC group and in 22 patients (13%) undergoing LICU ($P < 0.05$; Table IV). In the FIOC group, CBD stones were cleared by flushing with saline solution after intravenous glucagon in 10 patients (40%), whereas more invasive techniques were required in 15 (60%). In contrast, most patients with CBD stones in the LICU group had the stones successfully cleared with saline irrigation after intravenous glucagon (77%, $P < 0.05$ vs. FIOC), whereas five patients (23%) required extraction techniques. CBD sludge was detected in 10 patients (6%) in the LICU group, which was apparently cleared by saline flushes after intravenous glucagon administration, as evidenced by repeat LICU and/or FIOC. CBD sludge was never visualized by FIOC.

In the LICU group, maximal bile duct diameter ranged from 2 to 10 mm and the mean was similar in those with (5.5 ± 0.4 mm) and without (4.8 ± 0.1 mm) duct stones/sludge. The location of the maximal bile duct diameter was more commonly found in the mid (supraduodenal) rather than the distal CBD (Table V). However, the distal duct was more likely to be the largest part of the CBD in patients with ductal stones/sludge than in patients without these findings ($P < 0.01$). The mean size of the stones cleared by ampullary dilatation with glucagon and saline irrigation was 1.6 ± 0.2 mm, whereas more invasive methods were required for larger stones with a mean diameter of 2.7 ± 0.3 mm ($P < 0.01$; Table VI).

There was no morbidity or mortality associated with either FIOC or LICU. Median duration of hospitalization was 1 day for patients in both groups. All patients were seen in follow-up within 1 month postoperatively. Three patients in the FIOC group (0.8%) were readmitted to the hospital because of ductal problems. One patient had a retained CBD stone (false negative rate of 0.3%), despite a normal fluoroscopic intraoperative cholangiogram, which was successfully treated by endoscopic retrograde cholangiography. A second patient who did not undergo screening because of a concomitant procedure was readmitted for presumed retained stone(s), which resolved spontaneously during readmission, and a third patient developed a cystic duct stump leak. One patient in the LICU group was readmitted for abdominal discomfort, elevated liver function values, and a presumed retained CBD stone, which resolved spontaneously. In that particular case, screening of the CBD by LICU showed sludge but no stones, FIOC revealed air bubbles, and a repeat LICU showed clearance of the sludge after irrigation. The presumed false negative rate of LICU was therefore 0.6%.

Cost data were available for operating room time and disposable equipment. Table VII shows that screening with FIOC costs approximately $145 more

Table VII. Cost analysis*

	FIOC group	LICU group
Operating room time†	$10.53/min	$10.53/min
Mean time of use	15 min	5 min
Average cost	$158	$53
FIOC supplies (contrast, catheter)	$ 40	—
Total cost	$198	$53

FIOC = fluoroscopic intraoperative cholangiography; LICU = laparoscopic intracorporeal ultrasonography.
*Does not include professional fees or charges for capital depreciation.
†Based on "second half-hour" operating room cost.

than screening with LICU. These data do not include professional fees or charges for capital depreciation of equipment.

DISCUSSION

Although the patients were not randomized into the FIOC and the LICU groups, the current study analyzed the evolution of our techniques of CBD screening during laparoscopic cholecystectomy and provided insight regarding the advantages and disadvantages of the two imaging modalities. The senior author (N.J.S.) initially performed static cholangiography during laparoscopic cholecystectomy, demonstrating in a prospective randomized trial that selective application of intraoperative cholangiography was equally safe and less costly than routine static cholangiography.[6] Subsequently, fluoroscopic techniques for intraoperative cholangiography were applied routinely, after this procedure was revealed to be accurate, safe, and more rapid than static intraoperative cholangiography for screening the CBD; mean cholangiography times decreased significantly from 24 minutes for static images to 14 minutes for FIOC.[4] Recently many studies have suggested that laparoscopic sonography is as accurate as cholangiography in detecting CBD stones.[11,14-22] Because of these preliminary reports, as well as our initial successful experience with LICU,[11] we initiated the routine performance of LICU to evaluate the CBD for stones during laparoscopic cholecystectomy. However, since ductal anatomy is better appreciated by FIOC,[11] cholangiography was performed when ductal anomalies or injuries were suspected. Currently our aims for using routine LICU are to detect ductal stones and teach surgical trainees how to perform LICU. The ongoing prospective database has been continued during this transition, allowing the authors to compare FIOC and LICU in consecutive patients undergoing laparoscopic cholecystectomy.

One disadvantage of FIOC is its generation of potentially hazardous ionizing radiation, precluding its use in one pregnant patient in this series, before LICU was available. Another disadvantage of FIOC is the necessity to cannulate the cystic duct and the potential danger of perforating/avulsing the cystic duct with the cholangiocatheter. The former occurred in eight patients (2%) and the latter in three (1%), precluding performance of FIOC. Fortunately none of these 11 patients had postoperative evidence of choledocholithiasis or ductal complications. Other series have reported a similar failure rate in performing FIOC of 2% to 10%.[1,2,12-14] In contrast, the cystic duct-CBD junction and the distal CBD were successfully visualized, without iatrogenic ductal injury caused by sonographic scanning, in all patients in the LICU group. The sonographic images obtained by LICU scanning using a transducer placed through the epigastric trocar were transverse cuts through the hepatoduodenal ligament and oblique images through the head of the pancreas. This technique, as opposed to longitudinal scans of the ductal system obtained by placing the transducer through the umbilical port as suggested by others,[22,26] allowed examination of the papillary region without interposition of the duodenum, which may account for some failures in visualizing the terminal CBD.[26] Also, the transducer is easily and rapidly placed via the epigastric port without changing the laparoscopic imaging arrangement. A disadvantage of transverse scanning using this technique is that the bile ducts proximal to the common duct are poorly visualized. Fortunately most ductal stones are located in the distal CBD, and only one patient was presumed to have retained CBD stones following negative LICU. Thus the advantages of LICU are clearly seen in that it does not rely on a specific cystic duct size for cannulation, there is less risk of perforating or avulsing the cystic duct, and ionizing radiation is eliminated.

Advocates of routine cholangiography point out that abnormalities of the ductal system detected radiographically can alter the conduct of cholecystectomy

and prevent ductal injury. LICU was not used in this study to identify ductal anatomy, although with increased experience the ductal anatomy can be demonstrated well. Additional anatomic information, such as arterial and venous structures, can also be obtained by means of LICU and not by FIOC.[17,18] The most critical technical maneuver during laparoscopic cholecystectomy is to dissect the infundibulum of the gallbladder from both the ventral and dorsal aspects of the hepatocystic triangle and thereby demonstrate the cystic duct arising in continuity from the gallbladder neck,[6] hence exposing the "critical view" of safety[27] rather than relying on a cholangiogram for preventing iatrogenic ductal injuries. If ductal injuries were suspected, FIOC was indeed useful for ductal evaluation. In this current series with 170 patients undergoing LICU, there were no major ductal injuries without cholangiography.

Another advantage of LICU is the significantly reduced time required for its performance compared to that of FIOC. The evolution of our strategy to screen the CBD has shown tangible differences in the operating time required, beginning with a mean of 24 minutes for static cholangiography,[6] to 15 minutes for FIOC,[4] to only 5 minutes for LICU. Furthermore, disposable cholangiocatheters and related equipment are unnecessary, and specialized fluoroscopic tables are not required. These advantages translate to a lower cost, averaging $145 per patient at our institution, which is especially important in the current cost-containment environment.

The incidence of CBD stones detected by LICU was significantly greater than that detected by FIOC. Although both imaging techniques were not performed in all patients to determine the false negative and positive rates as well as their precise sensitivity and specificity, it is interesting that the incidence was almost twofold greater in the LICU group. One possible explanation is that LICU is more sensitive than FIOC in detecting small stones, as has been previously suggested.[11,14] Unfortunately data for the diameter of CBD stones detected by FIOC were not collected prospectively. The data showed that the majority of the stones detected by LICU (77%) were easily cleared from the CBD by saline irrigation. In contrast, only 40% of the stones detected by FIOC were cleared by the saline flush technique, whereas more invasive extraction techniques were required to remove the remainder of stones. Furthermore, within the LICU group, stones less than 2.0 mm (mean diameter 1.6 ± 0.2 mm) were always successfully cleared by saline irrigation and intravenous glucagon administration, whereas stones greater than 2.0 mm (mean diameter 2.7 ± 0.3 mm) could not be cleared by saline irrigation and required invasive extraction techniques. Thus not only does LICU seem to be more sensitive than FIOC in detecting CBD stones, but LICU may be overly sensitive in identifying small stones that may be clinically irrelevant even if they had not been cleared by saline irrigation.

Additional information from LICU undetectable by FIOC was the presence of sludge in the CBD.[15] Although it may be difficult to differentiate between bile duct sludge and artifacts, adherence to well-known sonographic criteria allows this distinction.[28] In this series, sludge was demonstrated in 6% of patients and was easily cleared by saline irrigation techniques. The significance of sludge is not well established, and many clinicians believe it to be clinically insignificant. However, recent studies suggest that biliary sludge may not be innocuous, potentially causing acute cholangitis[29] and acute pancreatitis.[30] Thus the apparent greater sensitivity of the LICU to detect sludge may be useful, for instance, in patients whose indication for cholecystectomy is gallstone pancreatitis.[14]

The maximum CBD diameter detected by LICU did not predict the presence or absence of CBD stones/sludge. However, the location of the maximum CBD diameter was found significantly more often in the distal CBD in patients with ductal stones/sludge than in patients without CBD stones/sludge. Nevertheless, definitive diagnosis of CBD stones by LICU relied on actual visualization of an echogenic mass with acoustic shadowing.

As a result of these findings, our current policy is to treat small (<2.0 mm) CBD stones with transcystic saline irrigation and intravenous glucagon administration; unsuccessful clearance of these stones detected by repeat LICU, as well as the presence of sludge, may not require further intervention because they will probably be insignificant clinically (Fig. 4). We also attempt to flush stones 2.0 mm or larger, realizing that this technique will often be unsuccessful, necessitating invasive extraction techniques. Fortunately these stones can usually be successfully treated using laparoscopic or endoscopic techniques, thus preserving the goals of minimally invasive therapy and minimizing postoperative hospitalization and convalescence.

CONCLUSION

LICU offers the following advantages over FIOC in screening the CBD for stones during laparoscopic cholecystectomy:

1. No ionizing radiation
2. No iodinated contrast material
3. Less invasive
   Can be performed before any tissue dissection

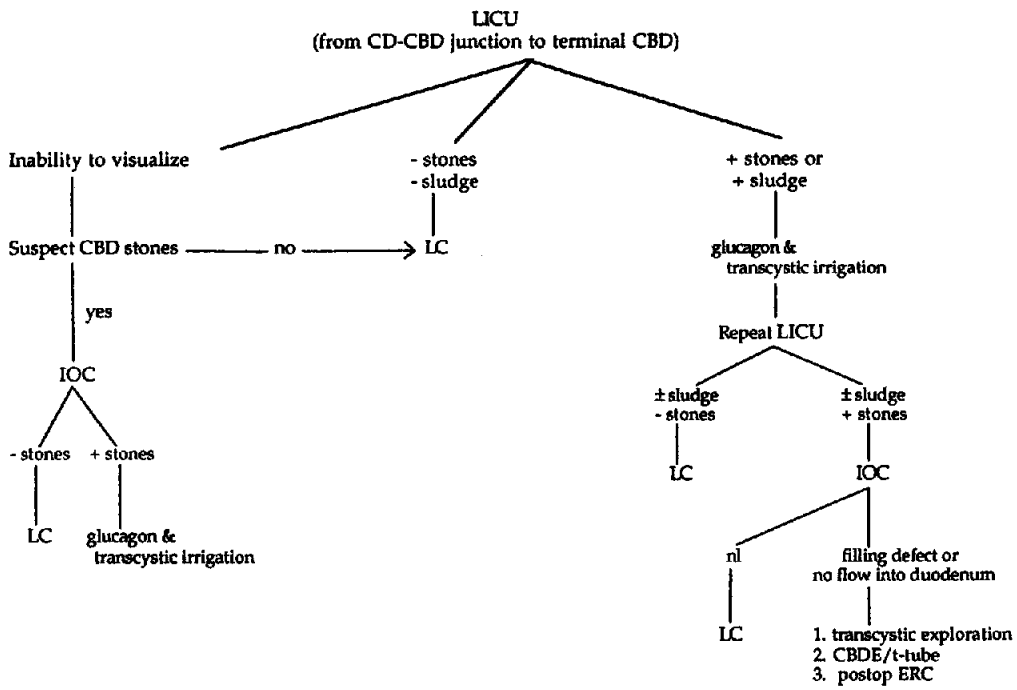

Fig. 4. Proposed new algorithm for screening and management of choledocholithiasis during laparoscopic cholecystectomy. LICU = laparoscopic intracorporeal ultrasonography; CD = cystic duct; CBD = common bile duct; LC = laparoscopic cholecystectomy; IOC = intraoperative cholangiography; ERC = endoscopic retrograde cholangiography; CBDE = common bile duct exploration.

Does not depend on cystic duct cannulation
Less risk of injury to cystic or common ducts

4. Higher success rate in visualizing distal CBD
5. Detects sludge
6. More rapid
7. More convenient and less cumbersome
   No fluoroscopic table, C-arm, or x-ray technician required
   Can be repeated easily at any time
8. Less costly

LICU is accurate, safe, and permits more rapid evaluation of bile duct stones. We routinely screen the CBD with LICU during laparoscopic cholecystectomy to allow trainees to master this relatively new technique, which may be very helpful for visualizing structures deep to the surface during other laparoscopic procedures.[24] Selective use of LICU to screen for choledocholithiasis on the basis of preoperative investigations or intraoperative findings is certainly an alternative. LICU may be overly sensitive in detecting small CBD stones and sludge, which are of questionable significance. Stones 2 mm or less can usually be cleared by flushing, whereas larger ones will most likely require duct exploration or endoscopic retrograde cholangiography for removal. The convenience of LICU also allows it to be easily repeated at any stage of the operation. Selective use of FIOC can be complementary if the LICU examination is incomplete or ductal injuries/anomalies are suspected.[14] LICU has the potential to replace FIOC for screening the CBD in most patients undergoing laparoscopic cholecystectomy.

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Discussion

Dr. K. Lillemoe (Baltimore, Md.). I have no doubt that you have answered the question that this technique is very valuable in the detection of common duct stones. Of course, the other role of cholangiography is identification of anatomy and avoidance of bile duct injury. The classic injury with laparoscopic cholecystectomy, as described by Dr. Meyers and the group at Duke University, has involved the mistaken identity of the cystic duct and the common duct and actually clipping and dividing of the common duct. In that situation, an intraoperative cholangiogram will be obviously abnormal, showing no filling of the proximal biliary tree. What safeguards does this sonographic, technique have to avoid such an injury in the patients in whom that same classic injury may occur. Furthermore, can you give us any information about the role of this technique in the identification of bile duct anatomy and the prevention of injury.

Dr. J. Wu. Looking at this series, we felt that the iatrogenic injuries were really due to lack of careful dissection, and we do not believe that screening for ductal anomalies or ductal anatomy really helps to prevent these injuries. During the dissection, however, if we are concerned that there could be a ductal anomaly or a question of iatrogenic injury, we would proceed with cholangiography in addition to ultrasonography.

Dr. W. Richards (Nashville, Tenn.). How many procedures are required to train a surgeon to use this technology? You were able to perform the ultrasound screening in 5½ minutes. I doubt many of us would be able to do it in that amount of time.

Dr. Wu. There were 16 patients in the cholangiography group who were excluded for various reasons. Four of those patients were used to learn the technique of ultrasonogra-

phy. After becoming comfortable with the use of ultrasonography in just those four patients, we proceeded with the 200 consecutive laparoscopic ultrasound studies. As I stated, within the first 55 ultrasound patients, we felt comfortable with cholangiography so when there was high index of suspicion, we proceeded with the cholangiography. After the first 55 patients, then we screened all patients with ultrasonography. When we studied the first 50 patients with ultrasonography, we found, much to our surprise, that the mean time to perform ultrasonography was 5.6 minutes. For the latest 50 cases, it was 5.1 minutes.

Dr. S. Grundfest (Cleveland, Ohio). You stated that intraoperative ultrasonography was more sensitive for detecting smaller stones, but how do you know that the small stones were not just flushed out when you performed the operative cholangiography.

Dr. Wu. We do not know that. We believe that this may have occurred in several patients during the cholangiography.

Dr. J. Becker (Boston, Mass.). You suggested that it may be an overly sensitive technique. Do you have any data to suggest what would be the fate of the sludge and small stones if you had left them in place?

Dr. Wu. We would also like to know what the significance of sludge is. We feel that it does pose a potential problem to the patients. If we do flush the sludge and it does not clear, we would not attempt any further extraction and we would not send the patient for postoperative endoscopic retrograde cholangiography. We would just follow them clinically. On the other hand, there are two papers that have reported gallstone pancreatitis due to sludge, and another report showed acute cholangitis due to sludge, but we feel that the incidence of this is very low.

Dr. L. Way (San Francisco, Calif.). When you state that the sensitivity is very high, you imply that there is the possibility of an increasing number of false positive results. Do you have independent validation of what you were interpreting on these ultrasound images as sludge and small stones. How do you know for sure that is what they really were.

Dr. Wu. Prior to this, we conducted a multicenter trial screening the common bile duct using both ultrasonography and cholangiography. In all 100 patients, we confirmed the ultrasound findings with the cholangiogram. As a result we felt comfortable interpreting the ultrasound images ourselves.

Dr. M. Dayton (Salt Lake City, Utah). One of the advantages of cholangiography is the ability to simultaneously visualize the entire biliary tree. How effective is this technique going to be in finding intrahepatic stones?

Dr. Wu. We only went as far as the cystic duct common bile duct junction and the distal common bile duct. With the transducer through the epigastric port, we could not see the proximal biliary duct, but we feel that most of the stones are either in the mid or distal duct. To date, only 1 out of the 200 patients had a retained stone. There could have been a stone in the proximal duct, but if one really wants to image the proximal duct, the transducer can be moved to the umbilicus and the camera can be moved to another port.

The Consequences of a Major Bile Duct Injury During Laparoscopic Cholecystectomy

Todd W. Bauer, M.D., Jon B. Morris, M.D., Adam Lowenstein, M.D., Charles Wolferth, M.D., Francis E. Rosato, M.D., Ernest F. Rosato, M.D.

Bile duct injury is perhaps the most feared complication of laparoscopic cholecystectomy. The focus of this study was on the immediate and short-term outcome of patients who have undergone repair of major bile duct injuries with respect to hospital stay, perioperative interventions, and reoperations. The records of patients who underwent surgery at three academic hospitals in Philadelphia (Hospital of the University of Pennsylvania, Thomas Jefferson University Hospital, and Graduate Hospital) from 1990 to 1995 for repair of a major biliary injury following laparoscopic cholecystectomy were reviewed. A major biliary injury was defined as any disruption (including ligation, avulsion, or resection) of the extrahepatic biliary system. Small biliary leaks not requiring surgery were excluded. Thirty-two patients sustained major bile duct injuries. The injury was recognized immediately in 10 patients. The remaining 22 patients had pain (59%), jaundice (50%), and/or fever (32%) as the symptom heralding the injury. Bismuth classification was as follows: 13% of patients were class I, 63% were class II, 7% were class III, 7% were class IV, and 10% were class V. Biliary reconstruction included a Roux-en-Y hepaticojejunostomy in 30 patients and two were primary repairs. There was one postoperative death from multiorgan system failure. The mean length of hospital stay after repair was 17 ± 8 days. Over a mean follow-up period of 11.5 ± 10.5 months, 11 patients (38%) required 19 emergency readmissions, most commonly for cholangitis. Five patients (17%) required postoperative balloon dilatation for biliary stricture. At follow-up 18 patients (62.0%) remain asymptomatic with normal liver function values, eight (28%) are experiencing episodic cholangitis, and three (10%) are asymptomatic with persistently elevated liver function values. The consequences of a major biliary tract injury following laparoscopic cholecystectomy include a complex operative repair resulting in a lengthy postoperative stay with an increased risk of death, an excessive number of perioperative diagnostic and therapeutic studies, frequent readmissions (often as emergencies), and a lifelong risk of restricture. The "cost" to these patients remains enormous. (J GASTROINTEST SURG 1998;2:61-66.)

In the past several years laparoscopic cholecystectomy has proved to be the preferred technique for removal of the gallbladder.[1] Laparoscopic as compared to open cholecystectomy has yielded several benefits including decreased length of hospital stay, less postoperative discomfort, and a shorter convalescent period for patients.[1] However, major bile duct injury, one of the most feared complications of cholecystectomy, has been shown to occur 2.5 to 4 times more frequently during laparoscopic as compared to open cholecystectomy.[2-4] The operative management of bile duct injuries has been reviewed by several authors.[2,5-8] Many of these excellent studies have focused on operative strategy and technique. This study was conducted to assess the immediate and short-term consequences among patients who undergo repair of major biliary tract injuries sustained during laparoscopic cholecystectomy.

METHODS

The patients included in this series were managed at three academic centers in Philadelphia including the Departments of Surgery at the Hospital of the

From the Departments of Surgery, Hospital of the University of Pennsylvania (T.W.B., J.B.M., and E.F.R.), Thomas Jefferson University Hospital (A.L. and F.E.R.), and Graduate Hospital (C.W.), Philadelphia, Pa.

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Reprint requests: Jon B. Morris, M.D., Gastrointestinal Surgery, Department of Surgery, 4 Silverstein, Hospital of the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104.

University of Pennsylvania, Thomas Jefferson University Hospital, and Graduate Hospital. The 32 patients included in this review sustained a major biliary tract injury during laparoscopic cholecystectomy over a period of 5 years. Major biliary tract injuries were defined as disruption of the extrahepatic biliary system including ligation, avulsion, and resection. Cystic duct leaks, bilomas, and small duct lacerations were excluded. The hospital records of these patients were reviewed including detailed operative reports, inpatient records, and outpatient follow-up records.

RESULTS

Laparoscopic cholecystectomy was performed at a referring institution in 29 (91%) of the 32 patients, and three were performed at one of our institutions. The incidence of bile duct injury for each of the surgeons is not known. The mean age of the patients was 47 years (range 21 to 74 years), and 75% were female. The indication for laparoscopic cholecystectomy was symptomatic cholelithiasis in 20 patients (62%) and acute cholecystitis in 12 (38%). The operation was converted to an open procedure in 15 cases, and a biliary injury was known or suspected before conversion to an open procedure in eight patients. Injury was known or suspected after conversion to an open procedure in two patients but was never recognized in five. Overall the biliary injury was recognized at the time of the original cholecystectomy in 10 patients (31%).

Seven patients underwent intraoperative cholangiography during their laparoscopic cholecystectomy. Five patients did not undergo intraoperative cholangiography, and in 20 of the 32 patients it was not known whether or not intraoperative cholangiography was performed. Among the seven patients who underwent cholangiography, the injury was immediately recognized in five cases. Among the five patients who did not undergo cholangiography, the injury was immediately recognized in only one. Among the remaining 20 patients, there was immediate recognition of the injury in five.

Biliary injury was immediately recognized in 10 (31%) of the 32 patients. Seven patients (22%) showed signs or symptoms of injury during their initial hospital stay by postoperative day 3.6 ± 2.0. The remaining 15 patients (47%) presented following discharge on postoperative day 8.6 ± 3.8. Overall the most common symptoms were pain (59%), jaundice (50%), and fever (32%). Four patients underwent unsuccessful repair prior to referral to our centers. Primary bile duct repair was attempted in two of these patients, loop hepaticojejunostomy in one, and Roux-en-Y hepaticojejunostomy in one.

Preoperative Management

Of the 10 patients whose injuries were immediately recognized, six underwent immediate repair. Three of these patients had incurred their injuries at one of our institutions. The other three patients underwent immediate repair and were then referred to our institution for revision of a failed reconstruction. Three patients were referred to us immediately following their injury prior to attempt at repair. Two of these patients had biliary catheters in place in the proximal and distal bile ducts; one patient underwent repair on the day of referral, the other patient 2 days later. The third patient had no biliary catheters in place and underwent endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC) followed by operative repair 3 days after referral. One patient underwent biliary T-tube placement and was referred 4 months later with a stricture.

The median length of time from injury to referral for definitive repair was 14 days (range 0 days to 2.5 years) and from injury to repair, 16.5 days. Operative repair was performed on the day of referral in four patients. The remaining patients typically underwent repair within 4 days of referral.

After referral, most patients underwent studies to define their anatomy and to provide biliary drainage. Twenty (69%) of the 29 referred patients underwent ERCP, 17 (59%) underwent PTC, and in one patient cholangiography was performed through an indwelling catheter. Of the 12 patients who did not undergo PTC for drainage, five had indwelling catheters providing biliary drainage. Only four patients did not undergo either ERCP or PTC. Three of these four patients had indwelling catheters present from the original cholecystectomy (Table I).

Operative Repair

Bismuth classification of the injury was possible in 30 patients. Bismuth classification was as follows: 13% of patients were class I, 63% were class II, 7% were class III, 7% were class IV, and 10% were class V. Two (7%) had concomitant injury to the right hepatic artery. One patient had erosion of a biliary stent through the common bile duct into the duodenum 30 months following stent placement for a common bile duct injury. Four of the reconstructions were revisions of previous repairs.

Two patients who had a resection of the common bile duct underwent primary bile duct anastomosis over a stent. The remaining 30 (94%) underwent Roux-en-Y hepaticojejunostomy, 29 of which were over a stent. One patient underwent a two-stage repair with placement of a T-tube followed by choledochojejunostomy 41 days later. Twenty-one anastomoses (70%) were to a single duct, and three (10%) were to two ducts. Six (20%) involved an anastomosis to a "neoconfluence" (common septum). In no case was it necessary to perform an anastomosis to the left hepatic duct after dropping the hilar plate. Thirty-one (97%) of 32 patients had stents placed at the time of repair. Most of these stents remained in place for 30 to 90 days, and many were still in place at the time of follow-up.

Outcome

One patient with a history of angina, congestive heart failure, and chronic obstructive pulmonary disease died of multiorgan system failure following Roux-en-Y choledochojejunostomy for a common bile duct resection. The patient developed jaundice on postoperative day 4 following laparoscopic cholecystectomy and was taken for exploratory laparotomy, which revealed biliary ascites and a transected common bile duct. After referral, he underwent CT-guided drainage of a large volume of bilious ascites. Thirteen days after transfer, he underwent choledochojejunostomy. Postoperatively he could not be weaned from the ventilator; he developed sepsis and died of multiorgan system failure on postoperative day 19.

A variety of other postoperative complications occurred and are listed in Table II. The mean length of hospital stay for the biliary reconstruction was 17 ± 8 days (range 5 to 24 days). Follow-up data were available for 29 patients at 11.5 ± 10.5 months. Thirteen patients (45%) required 22 readmissions. There were three elective readmissions in three patients and 19 emergency readmissions, most commonly for cholangitis and stricture, in 11 patients (Table III). The average length of stay for the readmissions was 7 ± 5 days. Five patients (17%) developed postoperative strictures, which have been managed by percutaneous transhepatic balloon dilatation, and one patient required operative revision of the anastomosis.

During the 1-year follow-up period a large number of diagnostic and therapeutic studies were performed (Table IV). The outcome of the biliary reconstruction was as follows: 18 (62%) of the 29 patients are asymptomatic with normal liver function values,

Table I. Preoperative studies or interventions in 29 referred patients with major bile duct injuries

	No.	(%)
ERCP	20	69
PTC	17	59
CT	10	34
Ultrasonography	9	31
Biliary stent placement	9	31
Nuclear biliary scan	3	10
Hepatic arteriography	1	3

ERCP = endoscopic retrograde cholangiopancreatography; PTC = percutaneous transhepatic cholangiography; CT = computed tomography.

Table II. Early complications after repair of major bile duct injury

	No. of patients
Wound infection	2
Pleural effusion	2
Hepatic abscess	1
Pancreatitis	1
Stress gastritis, upper gastrointestinal bleeding	1
Abdominal wall hematoma	1
Pulmonary failure	1
Right subclavian vein thrombosis and reactive thrombocytopenia	1
Death from multiorgan system failure	1

Table III. Readmissions following complex biliary reconstruction

Reason for readmission	No. of readmissions
Elective readmissions	
Excision of wound granuloma	1
Incisional hernia repair	2
TOTAL	3
Emergency readmissions	
Cholangitis without stricture	7
Cholangitis with stricture	5
Hepatic abscess	4
Partial small bowel obstruction	1
Infected intra-abdominal hematoma	1
Revision of strictured anastomosis	1
TOTAL	19

eight patients (28%) suffer from symptoms related to intermittent cholangitis or stricture, and three patients (10%) remain asymptomatic with persistently elevated liver function values.

A Bismuth class II injury was the most common injury, occurring in 63% of patients. Of the patients with Bismuth class II injuries, 63% were asymptomatic at follow-up, closely approximating the overall outcome (Table V). Three (75%) of the four patients with Bismuth class I injuries were asymptomatic at follow-up. All of the patients with Bismuth class III and IV injuries (total of 4 patients) were symptomatic at follow-up—also included in this group was the one postoperative death. Two of the three patients with Bismuth class V injuries were asymptomatic at follow-up.

Table IV. Evaluations following repair of major bile duct injury*

	Total No. of studies
Office visits	159
Serum liver function tests	153
Cholangiography	115
Abdominal x-rays	81
CT	35
Ultrasonography	23
Endoscopic retrograde cholangiopancreatography	12
Nuclear biliary scans	6
PTC	6
Upper gastrointestinal barium studies	4
MRI	1

MRI = magnetic resonance imaging; other abbreviations as in Table I.
*Data obtained from 29 patients.

DISCUSSION

Major bile duct injury is perhaps the most serious complication of laparoscopic cholecystectomy and occurs at 2.5 to 4 times the incidence when compared to open cholecystectomy.[2-4] Repair of these injuries, usually with a Roux-en-Y biliary-enteric anastomosis, can be a technically demanding operation. This results in prolonged hospitalization and greater discomfort for the patient. However, the greatest concern is the long-term morbidity imposed by such an injury. Our short-term and early (1 year) follow-up was notable for the significant number of emergency readmissions, interventions, and reoperations. After approximately 1 year of follow-up, we noted an operative mortality rate of 3% and a reoperation rate of 3%. Initially 12 (41%) of the 29 patients were symptomatic. After further intervention (one reoperation and multiple balloon dilatations), only eight (28%) remain symptomatic, 18 (62%) are asymptomatic, and an additional three (10%) are asymptomatic with elevated liver function values. The long-term significance of this last group is unknown (Fig. 1).

Analysis of our data shows an association between obtaining an intraoperative cholangiogram and immediate detection of an injury. This association, although not statistically significant, may be due to a selection bias in those patients undergoing intraoperative cholangiography; the surgeon's suspicion of an injury may prompt him or her to obtain an intraoperative cholangiogram, which confirms the injury.

In our series Bismuth classification of injury served as a predictor of outcome, although this relationship is not statistically significant because of the small number of patients with class I, III, IV, and V injuries (see Table V). Patients with class I and II injuries had a better outcome than those with class III and IV injuries. The patients with class V injuries, however, also had a good outcome. The correlation between

Table V. Bismuth classification of injury and outcome of patients

			Outcome			
Bismuth class	No.	(%)	Asymptomatic (%)	Abnormal liver function tests (%)	Symptomatic (%)	Other
I	4	13	75	0	25	
II	19	63	63	16	21	
III	2	7	0	0	100	1 death
IV	2	7	0	0	100	
V	3	10	67	0	33	
Overall	30	100	63	10	28	

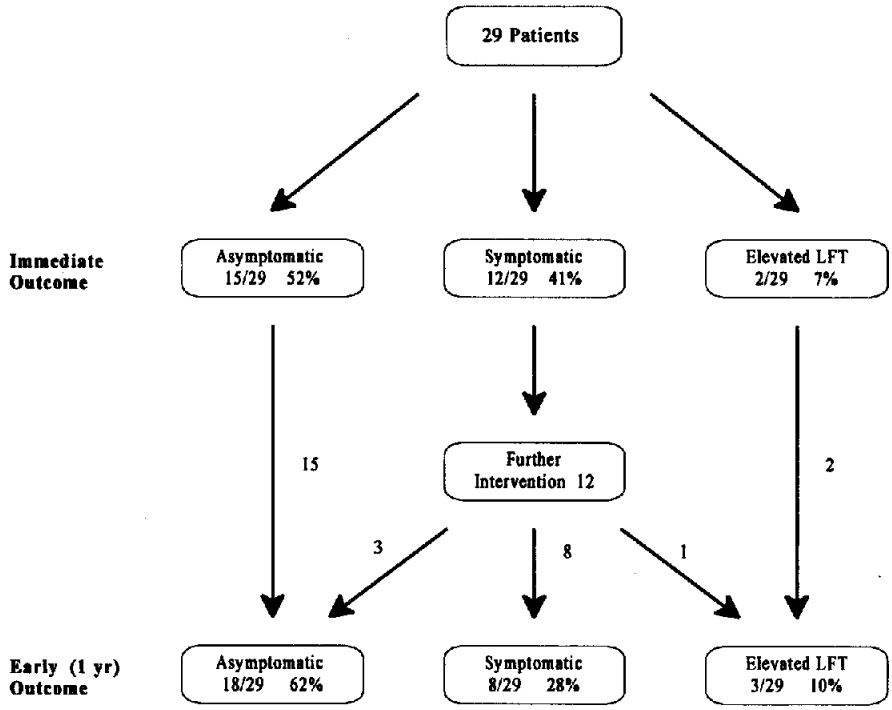

Fig. 1. Immediate and early outcome after repair of major bile duct injury. LFT = liver function tests.

higher Bismuth class and poor outcome has been demonstrated by other series including the recent review by Lillemoe et al.[9] from the Johns Hopkins Hospital. They found Bismuth class III or higher to be a predictor of poor outcome in their series.

We were not able to establish a relationship between the type of repair and outcome of patients, since only two patients underwent primary repair. One of these patients was lost to follow-up and the other is asymptomatic with elevated liver function values.

Early results after repair of major bile duct injuries vary greatly from one series to another. Lillemoe et al.[9] recently reported a 92% success rate following surgical reconstruction with a follow-up of 33.4 months. Our lower success rate may, in part, be attributable to our shorter follow-up interval. Three (25%) of the 12 patients who initially were symptomatic following repair improved with further intervention and are currently asymptomatic. Perhaps additional patients who were still symptomatic at follow-up may be symptom free after a longer follow-up period.

The long-term morbidity of these injuries is less well known because of the relatively recent introduction of laparoscopic cholecystectomy. However, long-term follow-up of biliary injuries after open cholecystectomy has demonstrated results similar to ours.[10-12] Frattaroli et al.[10] reported on 194 patients with biliary injuries (74 resulting from open cholecystectomy) with a mean follow-up of 9.3 years with an operative mortality rate of 2.6%, a reoperation rate of 9.6%, and a "good" (absence of cholestasis and infection) result rate of 79.6%. Chapman et al.[11] reported on 130 biliary injuries after open cholecystectomy with a mean follow-up of 7.2 years with an operative mortality rate of 1.8%, a reoperation rate of 9.8%, and a "good" (no biliary symptoms and no need for intervention) result rate of 76%.

It is evident that the short-term and lifelong "cost" to these patients is enormous. Patients often undergo numerous studies (ERCP, PTC, CT, and liver function tests) to detect their injuries. The operative repair for major biliary injuries, the long hospital stay, and the numerous interventional radiology procedures represent a major medical expense. Savader et al.[13] calculated the mean hospital and interventional radiology charges as $51,411 per patient for the patients who underwent treatment of laparoscopic-related bile duct injuries at the Johns Hopkins Hospital from 1990 to 1995. Patients are unexpectedly forced out of work for the duration of this time in-

cluding the convalescent period at home. This time lost from work often results in a financial loss to the patient. A significant but unmeasured cost is the quality-of-life change in these patients. Litigation resulting from biliary injuries represents a substantial financial outlay. Kern[14] has reported a 20-fold increase in litigation claims resulting from injuries following cholecystectomy since the widespread adoption of laparoscopic cholecystectomy in 1990. Kern estimates that approximately 200 cases of litigation involving laparoscopic cholecystectomies are filed annually in the United States.

A biliary injury following laparoscopic cholecystectomy is a tragedy for both the patient and the surgeon. Both have expectations of a simple operation and a short recovery period. However, they are faced with a major operative repair, prolonged hospitalization, numerous postoperative complications, and possibly litigation. These consequences have a lasting impact on both the patient and the surgeon.

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Cumulative Incidence of Colorectal and Extracolonic Cancers in MLH1 and MSH2 Mutation Carriers of Hereditary Nonpolyposis Colorectal Cancer

Kevin M. Lin, M.D., M. Shashidharan, M.D., Alan G. Thorson, M.D., Charles A. Ternent, M.D., Garnet J. Blatchford, M.D., Mark A. Christensen, M.D., Patrice Watson, Ph.D., Stephen J. Lemon, M.D., M.P.H., Barbara Franklin, B.S.N., Beth Karr, B.S.N., Jane Lynch, B.S.N., Henry T. Lynch, M.D.

The extracolonic tumor spectrum of hereditary nonpolyposis colorectal cancer (HNPCC) includes cancer of the endometrium, ovaries, stomach, biliary tract, and urinary tract. This study was designed to determine the penetrance of colorectal and extracolonic tumors in HNPCC mutation carriers. Forty-nine patients (22 females and 27 males) were identified with an MSH2 germline mutation, and 56 patients (28 females and 28 males) were identified with an MLH1 mutation. Cumulative incidence by age 60 (lifetime risk) and mean age of cancer diagnosis were compared. The lifetime risk of extracolonic cancers in MSH2 and MLH1 carriers was 48% and 11% respectively ($P = 0.016$). Extracolonic cancer risk in MSH2 females and males was 69% and 34%, respectively ($P = 0.042$). Mean age of extracolonic cancer diagnosis was significantly older for MSH2 males than females (55.4 vs. 39.0, $P = 0.013$). No difference was observed in colorectal cancer risk between MLH1 and MSH2 carriers (84% vs. 71%). Colorectal cancer risk was 96% in MSH2 males compared to 39% in MSH2 females ($P = 0.034$). No differences in colorectal and extracolonic cancer risks between MLH1 females and males were identified. The risk of extracolonic cancer by age 60 was greater in MSH2 mutation carriers than in MLH1 carriers. Gender differences in colorectal and extracolonic cancer risk were observed for MSH2 carriers only. These phenotypic features of HNPCC genotypes may have clinical significance in the design of genotype-specific screening, surveillance, and follow-up for affected individuals. (J GASTROINTEST SURG 1998;2:67-71.)

Fifteen to 20 percent of the total colorectal cancer burden is associated with a positive family history (cancer in one or more parents, siblings, or children).[1-4] Hereditary nonpolyposis colorectal cancer (HNPCC) accounts for 4% to 7% of all cases of colorectal cancer. Familial adenomatous polyposis represents about 1%. Inflammatory bowel disease, Peutz-Jeghers, and familial juvenile polyposis all contribute approximately 1%. The natural history of HNPCC, an autosomal dominant inherited disorder, has been studied in detail.[5,6] A family history of colorectal cancers consistent with the Amsterdam criteria is the usual means of identifying HNPCC families.[7-9] Recent identification of the genes responsible for HNPCC have enabled patients to be conclusively identified as having HNPCC. This is accomplished by analyzing the DNA of peripheral blood lymphocytes for these genetic defects.

The majority of HNPCC kindreds have a germline mutation in one of four mismatch repair genes (hMLH1, hMSH2, hPMS1, or hPMS2).[10-19] The repair of DNA mismatches during cellular replication requires the normal function of four protein subunits. MSH2 and guanidine-thymidine binding protein first combine to form a heterodimer that recognizes and binds to base-pair mismatches on DNA. MLH1 and

From the Section of Colon and Rectal Surgery, Department of Surgery, Creighton University School of Medicine (K.M.L., M.S., A.G.T., C.A.T., G.J.B., and M.A.C.), and the Department of Preventive Medicine and Public Health and the Hereditary Cancer Institute at Creighton University (P.W., S.J.L., B.F., B.K., J.L., and H.T.L.), Omaha, Neb.

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Reprint requests: Alan G. Thorson, M.D., Creighton University Medical Center, Section of Colon and Rectal Surgery, 601 N. 30th St., Ste. 3520, Omaha, NE 68131-2197.

PMS2 protein subunits then attach to the heterodimer to form a complex that excises the DNA defect and allows for polymerization.[20] Germline mutations of MSH2 and MLH1 consist of missense (frameshifts) or nonsense (deletions) mutations in different exons of the chromosome. This genetic alteration results in a premature stop codon that produces a shortened malfunctioning protein subunit of DNA mismatch repair.[13,20-24]

The majority of HNPCC tumors show genomic instability from defective DNA mismatch repair.[4,5] Kindred members who carry the HNPCC genetic mutation will have a 78% lifetime risk for colorectal cancers and 43% for endometrial cancers. Increased risk of extracolonic cancers such as those in the stomach (19%), biliary tract (18%), urinary tract (10%), ovaries (9%), brain (1%), and small bowel (1%) has also been observed.[25] Other extracolonic cancers such as those in the breast, pancreas, liver, larynx, bronchus, lung, esophagus, sarcoma, leukemia, and central nervous system have been noted in some HNPCC families but they may be incidental.[26-29]

We hypothesized that there is phenotypic variation in the frequency of cancer expression within HNPCC based on MSH2 or MLH1 genotype and gender. The purpose of this study was to determine the cumulative incidence of colorectal and extracolonic cancers within HNPCC mutation carriers. The mean age of cancer diagnosis was also examined.

METHODS AND STATISTICAL ANALYSIS

The study group was composed of 105 patients. Seventy-eight underwent genetic testing and 27 were determined to be obligate gene carriers under the assumption that the genetic mutations in the members of a single kindred were identical by descent. Twenty-two females and 27 males from two kindreds were identified with MSH2 germline mutations. Twenty-eight females and 28 males from two kindreds were identified with MLH1 germline mutations. Cumulative incidence by age 60 and mean age of extracolonic and colorectal cancers were calculated for each genotype and compared. Cancer cumulative incidence was calculated with Kaplan-Meier curves and analyzed with the log-rank test for trend. Mean age of cancer diagnosis was analyzed using the unpaired Student's *t* test.

RESULTS

The cumulative incidence of extracolonic cancers to age 60 was 48% in MSH2 carriers compared to 11% in MLH1 carriers ($P = 0.016$) (Fig. 1). With gender-specific comparisons, the cumulative incidence of extracolonic cancer was 69% in MSH2 females compared to 19% in MLH1 females ($P = 0.024$) and 34% in MSH2 males compared to 5% in MLH1 males ($P = 0.046$) (Fig. 2).

The cumulative incidence of extracolonic cancers was 69% in MSH2 females compared to 34% in MSH2 males ($P = 0.042$) (Fig. 2) and 19% in MLH1 females compared to 5% in MLH1 males (P = not significant [NS]). Endometrial cancers were the most common extracolonic cancer in both genotypic females with a cumulative incidence of 36% in MSH2 compared to 19% in MLH1 (Table I). The specific extracolonic cancers in males are enumerated in Table II.

Mean age of extracolonic cancer diagnosis was 55.4 years for MSH2 males compared to 39.0 years for MSH2 females ($P = 0.013$). This age difference for extracolonic cancers was not observed between MLH1 males and females (41.0 vs. 43.0 years). Mean age of endometrial cancer was 40.5 years for MSH2 and 43.0 years for MLH1 (P = NS). Mean age of colorectal cancers was 47 years for MSH2 males compared to 52 years for MSH2 females (P = NS) and 45 years for MLH1 males compared to 45.4 years for MLH1 females (P = NS).

The cumulative incidence of colorectal cancer was 84% in MLH1 and 71% in MSH2 carriers (P = NS) (Fig. 3). The cumulative incidence of colorectal cancer was 96% in MSH2 males compared to 39% in MSH2 females ($P = 0.034$) (Fig. 4) and 94% in

Table I. Extracolonic cancers in females

Cancer	Genotype MSH2 (n = 22)	Genotype MLH1 (n = 28)
Endometrial	4	3
Ovarian	1	1
Cervical	2	
Esophageal	1	
Leukemia	1	

Table II. Extracolonic cancers in males

Cancer	Genotypes MSH2 (n = 27)	Genotypes MLH1 (n = 28)
Kidney	2	
Bladder	1	
Ureter	2	
Stomach	1	1
Thyroid	1	
Brain	1	

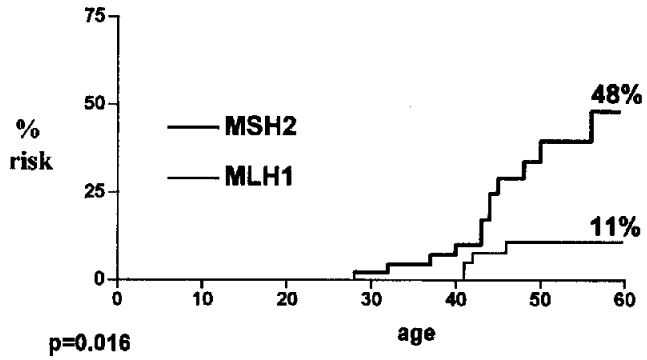

Fig. 1. Lifetime extracolonic cancer risk in MSH2 mutation carriers vs. MLH1 mutation carriers.

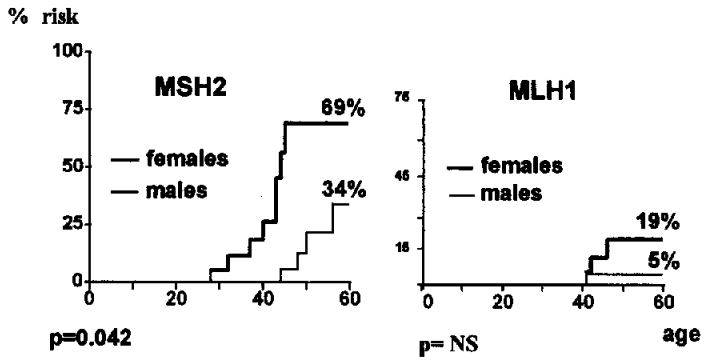

Fig. 2. Lifetime extracolonic cancer risk for females and males with the MSH2 genotype vs. the MLH1 genotype.

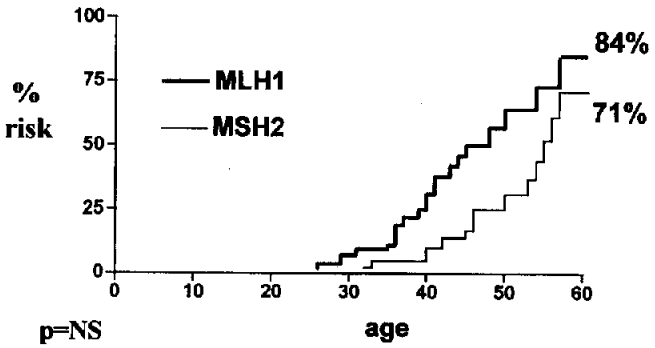

Fig. 3. Lifetime colorectal cancer risk in MLH1 mutation carriers vs. MSH2 mutation carriers.

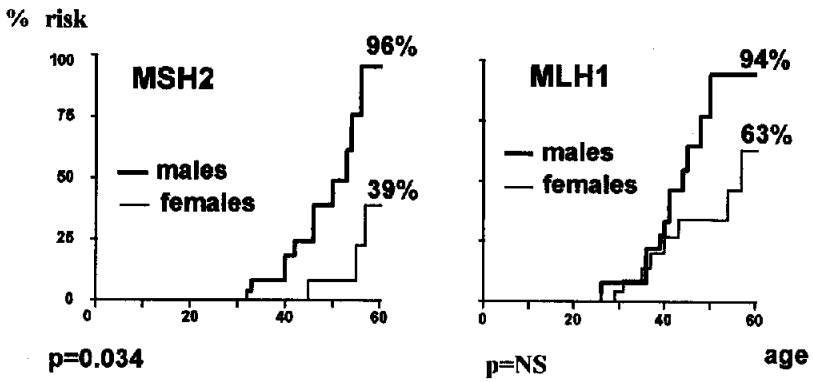

Fig. 4. Lifetime colorectal cancer risk for males and females with the MSH2 genotype vs. the MLH1 genotype.

MLH1 males compared to 63% in MLH1 females (P = NS). Thus gender differences in colorectal and extracolonic cancer risks were observed in MSH2 but not in MLH1 carriers.

DISCUSSION

Phenotypic differences between MSH2 and MLH1 genotypes have been suggested in the literature.[30] The overall lifetime risk of colorectal cancer is estimated at 80% in both genotypes. Although the risk of endometrial cancer appears greater in MSH2 (61%) than in MLH1 (42%) carriers, this difference has not been shown to be statistically significant. MSH2 carriers have been reported to have a significantly increased relative risk (RR) of cancer of the urinary tract (RR = 75.3), stomach (RR = 19.3), and ovaries (RR = 8.0) compared to the general population. Both genotypes have shown a higher relative risk of small bowel cancer (RR >100) compared to the general population. This study did not calculate the overall extracolonic cancer risk between the two genotypes. Our study found an increased overall risk of extracolonic cancers in MSH2 compared to MLH1 carriers (48% vs. 11%, P = 0.016). This observation held true for gender-specific comparisons. The marked excess of extracolonic cancers among MSH2 mutation carriers is an interesting phenomenon and is the only evidence to date that mutations in different mismatch repair genes have different effects.

Gender appears to play a role in phenotypic expression. A study involving 67 HNPCC gene carriers showed a lifetime risk to age 70 for all cancers of 91% in males and 69% in females.[31] The lifetime risk of colorectal cancers was reported to be significantly greater for males than females (74% vs. 30%, P = 0.006). This study did not calculate colorectal cancer risks based on specific genotypes. Our study demonstrated a gender difference in colorectal cancer risk only in MSH2 carriers with increased risk in males, which is more specific but remains consistent with the preceding study.

The risk of uterine cancer (42%) exceeded that for colorectal cancer (30%) in females in that study.[31] Our study demonstrated that females expressed a significant excess of extracolonic cancers over their male counterparts in MSH2 mutation carriers. This finding is expected because of the contribution from endometrial and ovarian cancers in females.

The clinical implications of our findings of genotype and gender differences in cancer risk apply most obviously to female members of HNPCC families. Gynecologic cancers are the most common manifestation of HNPCC after colorectal cancers. Thus gynecologic cancer screening and prophylactic surgery are the only extracolonic cancer–preventing measures widely recommended in HNPCC. Specific organ cancer risk estimates for each genetic HNPCC subgroup are needed to appropriately plan cancer prevention strategies.

CONCLUSION

Significant heterogeneity in phenotypic expression of extracolonic cancer between MLH1 and MSH2 carriers has been demonstrated. Gender differences in colorectal and extracolonic cancer expression within the MSH2 genotype were also noted. Based on our findings, closer surveillance for extracolonic cancers is recommended in MSH2-positive members, especially MSH2 females. These phenotypic features of HNPCC genotypes may have clinical significance in the design of genotype specific screening, surveillance, and follow-up recommendations for affected individuals.

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Discussion

Dr. J.E. Fischer (Cincinnati, Ohio). It seems to me that perhaps the outcome of the study is really determined by the groupings of the cancers. The cancers that are higher risk are mostly gynecologic with some stomach and urologic carcinoma. One might argue that the presence of one case of cancer of the stomach in each group was highly significant and equivalent because this is a very rare cancer. Is there another way of comparing these two genetic defects that are really gender neutral if you fail to consider the contribution of the gynecologic cancer.

Dr. K.M. Lin. It is true that the endometrial cancers are the most common extracolonic cancers in these patients. We looked at the overall extracolonic cancer risks and found that the risks were significantly different between the two genotypes. If you eliminated the endometrial cancers, I believe there would still be an excess of extracolonic cancers in the female MSH2 population in this study. We have performed similar studies in a larger population, which demonstrated that the MSH2 genotypes expressed a marked excess of extracolonic cancers regardless of endometrial cancers.

Dr. Fischer. To what do you ascribe this seeming difference in virulence?

Dr. Lin. It is not certain why the HNPCC genotypes have different phenotypic expression. As I have mentioned, there are four protein subunits that are involved in DNA mismatch repair in HNPCC. The proper function of these subunits is crucial for DNA repair. Which subunit is more important than the rest in repairing DNA has yet to be determined. We may find that there are different degrees of malfunction in DNA repair. A specific HNPCC genotype may prove to have only partial malfunction of DNA repair and thus express fewer cancers than other genotypes.

Outcome Analysis of Long-Term Survivors Following Pancreaticoduodenectomy

W. Scott Melvin, M.D., Karl S. Buekers, B.S., Peter Muscarella, M.D., Jerome A. Johnson, Ph.D., William J. Schirmer, M.D., E. Christopher Ellison, M.D.

The long-term sequelae of pancreaticoduodenectomy are not completely understood. In the present study nutritional status, pancreatic function, and subjective quality-of-life parameters were evaluated in 45 patients who had previously undergone either pylorus-preserving pancreaticoduodenectomy (PPPD) or standard pancreaticoduodenectomy (SPD). Quality-of-life parameters, as measured by the Short Form-36 health survey, demonstrated no significant differences between the subgroups and normal control subjects in six of the eight domains for physical and mental health. Patients who had undergone SPD were noted to have significantly lower scores for general health and vitality than either age-matched control subjects or those who had undergone PPPD. No differences in nutritional parameters or indicators of pancreatic exocrine function between the two groups were identified. An elevated hemoglobin A_{1C} value was seen in only one patient who was not diabetic preoperatively. Our data indicate that long-term survivors of pancreaticoduodenectomy generally feel as good as their normal counterparts, although SPD may result in some health satisfaction deficits. Nutritional status and pancreatic exocrine function are not improved in patients undergoing a pylorus-preserving procedure, and postoperative pancreatic endocrine dysfunction is unusual in both groups. (J GASTROINTEST SURG 1998;2:72-78.)

Pancreaticoduodenectomy is indicated for the potential cure or surgical palliation of pancreatic and periampullary neoplasms and some benign diseases.[1] Initially associated with significant perioperative mortality, few long-term survivors were reported. Recent advances in anesthesia, critical care, and surgical technique have resulted in an operative mortality rate of less than 5% and have allowed a greater number of patients with a wide variety of diagnoses to be treated. In 1978 Traverso and Longmire[2] published their support for pylorus-preserving pancreaticoduodenectomy, which suggested improved nutritional status. Many reports followed that validated their results, but these demonstrated only small improvements in survival or hospital course, and few included long-term data. In the 1900s approximately 5000 pancreaticoduodenectomies per year are being performed for a variety of indications,[3] and the number of long-term survivors is increasing. It was, therefore, the object of this study to accurately quantify the effects of pylorus-preserving pancreaticoduodenectomy (PPPD) and standard pancreaticoduodenectomy (SPD) on quality of life, nutrition, metabolism, and pancreatic function in long-term survivors.

METHODS

Patients

Patients undergoing pancreaticoduodenectomy have been entered into a computerized database since 1985 at our institution. We used this database to identify patients who were alive a minimum of 12 months following pancreaticoduodenectomy for inclusion in this study. Patients were contacted and asked to participate in our survey and to submit to further laboratory investigations and physical examination.

Evaluations

Quality of life was assessed by the Short Form-36 health survey (SF-36, United States version 1.0), as provided by the Medical Outcomes Trust. A stan-

From the Division of General Surgery, College of Medicine, The Ohio State University, Columbus, Ohio.
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Reprint requests: W. Scott Melvin, M.D., Assistant Professor, Division of General Surgery, Ohio State University Hospitals, 410 W. 10th Ave.-N711 Doan Hall, Columbus, OH 43210-1228.

dardized question-and-answer format was used over the telephone and scores were entered into a database. These scores were grouped according to the eight health domains and converted to transformed scores that then could then be compared to normal standards of age-matched control subjects from the United States population as supplied by the Medical Outcomes Trust from responses of more than 1000 respondents. The control group differed depending on the average age of the subgroup examined.

Nutritional parameters were identified through a variety of methods. Physical examination and anthropomorphic measurements were obtained. Ideal body weight (IBW) was calculated and compared to normal values using 1983 Metropolitan Life height and weight tables. Body mass index (BMI) was calculated by dividing the patient's weight in kilograms by the height in meters squared (kg/m^2). Body impedance analysis was performed using standard tetra-polar technique and the body impedance analysis machine (model 101A, R.J.L. Systems, Mt. Clemens, Mich.) to estimate the percentage of lean body mass. Visceral protein mass was determined by measuring the serum concentrations of albumin and transferrin. Immune competence was indirectly assessed by measuring total lymphocyte counts. Other laboratory tests included hemoglobin, white blood cell count, platelet count, serum electrolytes, liver enzymes, fasting glucose, and serum calcium concentrations. Pancreatic exocrine function was indirectly assessed by determining the necessity of pancreatic enzyme replacement and the frequency of bowel movements. Pancreatic endocrine function was evaluated in all patients by determining serum levels of hemoglobin A_{1C} (HbA_{1C}).

Surgery

All patients were operated on by a single group of surgeons using similar techniques. The decision to perform an antrectomy or a PPPD was made intraoperatively at the discretion of the operating surgeon and was based on the clinical scenario. Surgical technique for a PPPD included preservation of the proximal 3 cm of duodenum with reconstruction utilizing an end-to-side duodenojejunostomy in two layers. Reconstruction following antrectomy was done with an antecolic loop gastrojejunostomy with a similar anastomotic technique. Pancreatic reconstruction was performed by means of an end-to-side pancreaticojejunostomy with a mucosa-to-mucosa ductal anastomosis using interrupted absorbable sutures. A single-layer biliary-enteric anastomosis was then created using absorbable sutures. No stents were used for either anastomosis.

Statistical Analysis

Patients were divided into subgroups based on type of operation and indication for surgery. Results were expressed as mean ± standard deviation. Comparisons of proportions were performed using Fisher's exact test. The Student's t test for unpaired samples and the Mann-Whitney U test were used to compare mean values of data between groups. P values <0.05 were considered significant.

RESULTS

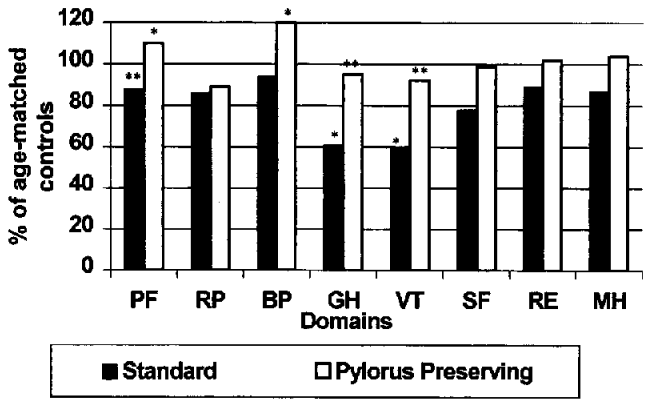
A total of 156 eligible patients were entered in the database during the study period. Of the 61 patients considered to be survivors, eight were lost to further follow-up, four refused to participate, and four had active disease. These patients were excluded from the study. Complete quality-of-life data were obtained from 45 patients, and laboratory and physical examination data were obtained from 25 patients. Of the 45 patients, there were 24 females and 21 males; 24 patients underwent PPPD and 21 patients underwent SPD. The indication for surgery was malignancy in 27 patients and benign disease in 18. The average overall age was 57.3 years (52.1 years for the PPPD group vs. 61.9 years for the SPD group). The mean period of postoperative observation time was 5.5 years with a range of 17 to 143 months. Mean postoperative observation time for the PPPD group (4.8 years) was not considered to be statistically different from that of the SPD group (6.1 years). Demographic data are presented in Table I.

Complete quality-of-life data were obtained in 45 patients. The eight domains of the SF-36 were individually compared to evaluate differences between both subgroups and age-matched control subjects (Fig. 1). For the three best measures of mental health (social functioning, role emotional, and mental health), there were no significant differences between either the total group or each subgroup when compared to normal controls. The three scales that have the best validity in assessing measures of physical health are termed physical function, role physical, and bodily pain. Each of these scales addresses a different aspect of physical health. Postoperative patients did not demonstrate any significant decrease in these measures. In fact, in the scales for physical functioning and bodily pain, patients who had undergone PPPD demonstrated improved scores when compared to normal control subjects but not when compared to the group that underwent SPD. General health and vitality are scales that demonstrate validity in assessing both physical and mental health. In both domains, significantly lower scores were noted in the subgroup of patients who had undergone SPD com-

Table I. Preoperative patient demographics

	PPPD (n = 21)	SPD (n = 24)	*P* value
Age (mean ± SD)	52.1 ± 14.5 yr	61.9 ± 9.6 yr	0.013*
Sex (M/F)	14/7	12/12	0.366†
Follow-up time (mean ± SD)	57.3 ± 40.4 mo	73.0 ± 36.7 mo	0.182*
Malignancy	57.1%	50.0%	0.767†

PPPD = pylorus-preserving pancreaticoduodenectomy; SPD = standard pancreaticoduodenectomy; SD = standard deviation.
*By two-sample Student's *t* test.
†By two-tailed Fisher's exact test for comparison of frequencies. *P* values <0.05 are considered significant.



* p<0.05 vs age-matched controls
** p<0.05 standard vs pylorus preserving

Fig. 1. Short Form-36 health survey results for patients undergoing standard vs. pylorus-preserving pancreaticoduodenectomy. Mean group values are reported as percentages of values in age-matched control subjects. PF = physical function; RP = role physical; BP = bodily pain; GH = general health; VT = vitality; SF = social functioning; RE = role emotional; MH = mental health.

pared to those who had undergone PPPD. This difference could not be explained by the younger age in the PPPD group alone. We compared the scores computed as a percentage of normal in age-matched controls for the two groups and there was still a significant difference. There was no difference seen between responses in patients who had undergone resection for malignancy when compared to those who had a benign process.

Nutritionally, the mean values of the various measurements obtained were all within normal limits. No significant deviations from normal populations were seen in any of the parameters we assessed (Table II). Twenty-five patients underwent complete anthropomorphic measurements, physical examination, and laboratory testing, with 12 patients having undergone SPD and 13 patients PPPD. None of the patients had a severe weight deficit, identified as an IBW less than 70%. Only 5 of the 25 had any weight deficit noted. Following PPPD, two patients had a moderate weight deficit (70% to 79% of IBW). Following SPD, two patients had a mild deficit (80% to 89% IBW) and one patient had a moderate deficit. BMI was considered less than normal if below 18 kg/m^2. Below-normal values were obtained in two patients following PPPD and in only one after SPD. Mild obesity, a BMI of between 25 and 30 kg/m^2, was observed in six patients following PPPD, and severe obesity was seen in one patient (BMI 31.1 kg/m^2). Average lean body mass was 66.2 ± 11.2 kg for all patients. No differences in lean body mass were observed between patients undergoing PPPD or SPD (63.3 ± 11.1 kg vs. 69.4 ± 10.5 kg). When compared to normal values of 75% to 90% for men and 70% to 82% for women, no patients had less than normal body fat. For all patients the mean total lymphocyte count was within normal limits (1.72 K/μl). Three patients following PPPD and two patients following SPD were below

Table II. Postoperative nutritional parameters at time of follow-up

	PPPD (n = 12)	SPD (n = 13)	*P* value
Body weight (pounds)	142.3 ± 30.3	147.2 ± 29.9	0.605*
Body mass index	23.6 ± 3.8	25.2 ± 4.9	0.253*
% Ideal body weight	104.0 ± 16.2	109.5 ± 20.9	0.355*
% Lean body mass	69.4 ± 11.0	63.3 ± 11.5	0.142*
Albumin (g/dl)	3.65 ± 0.52	3.92 ± 0.42	0.112*
Transferrin (mg/dl)	297.7 ± 80.8	273.6 ± 46.2	0.807*
Total lymphocyte count (K/μl)	1.80 ± 0.70	1.79 ± 0.63	0.727*

Abbreviations as in Table I; values are listed as mean ± standard deviation.
*By two-sample Mann-Whitney U test. *P* values <0.05 are considered significant.

Table III. Postoperative indicators of pancreatic exocrine function at the time of follow-up

	PPPD (n = 21)	SPD (n = 24)	*P* value
Patients with more than 3 stools per day	47.6	70.8	0.138*
Patients requiring oral pancreatic enzyme replacement	19.0	20.8	1.000*

Abbreviations as in Table I; values are listed as percentages.
*By two-tailed Fisher's exact test for comparison of frequencies. *P* values <0.05 are considered significant.

the lower limit of normal. Albumin levels were below normal in four patients, following SPD in two and following PPPD in two.

Twenty-four of the 25 patients tested had normal HbA_{1C} values with an average frequency of 6.1% (normal range 4.4% to 8.0%). A value of 8.8% was obtained in a single patient who was not previously identified as diabetic. Four other patients, previously identified as having insulin-dependent diabetes, had normal HbA_{1C} values. Nineteen of the 45 patients surveyed regularly were taking pancreatic exocrine supplements for significant steatorrhea. Twelve of the 21 PPPD patients and seven of the 24 SPD patients were taking enzymes. Only eight patients reported a stool frequency averaging more than three per day, and only four of these patients had ever taken pancreatic enzyme supplements (Table III).

DISCUSSION

Pancreaticoduodenectomy has historically been associated with significant perioperative and long-term postoperative morbidity and mortality. Recent reports have clearly documented the decreasing perioperative mortality of pancreaticoduodenectomy and better long-term prognosis.[4-6] Thus more long-term survivors of pancreaticoduodenectomy are being followed in surgical practices. Therefore this study was performed to evaluate and document the physiologic and nutritional sequelae that may be seen in these long-term survivors.

Pancreaticoduodenectomy was popularized and evaluated as a surgical procedure that included a 30% gastrectomy rate.[1] The outcome of patients surviving the postoperative period was generally favorable; however, many patients had gastrointestinal disturbances. In 1969 Fish et al.[7] evaluated six patients with a follow-up of 20 to 84 months. All patients had a significantly decreased weight, between 10 and 40 pounds, as well as decreased serum albumin concentrations. Much of the postoperative symptomatology was attributed to the gastrectomy.[8-10] Thus in 1978 Traverso and Longmire[2] readvocated preservation of the distal stomach and pylorus when pancreaticoduodenectomy was performed. In 1980 they evaluated eight patients with a follow-up of 2 to 12 months.[11] All of these patients had abnormal pancreatic exocrine function, although most were generally healthy. In 1988 Fink et al.[12] compared six long-term survivors of SRD with six patients who had undergone PPPD. Their postgastrectomy-type symptoms were identical; however, a delay in liquid-phase gastric emptying was seen in patients with an antrectomy compared to PPPD.

Quality-of-life measurements have recently come to the forefront of medical analysis as an overall as-

sessment of patients' well-being during illness and treatment. A variety of instruments have been developed to assess the impact of illness and treatment on patients' physical and mental health, as well as their personal feelings of well-being. The SF-36 has been developed and modified over several years as an instrument to quantify the effect of acute illness on an individual's well-being. It has been extensively utilized and validated in a variety of clinical settings; these include assessing treatment for medical conditions such as chronic obstructive pulmonary disease, diabetes mellitus, and angina, as well as surgical interventions including coronary artery bypass grafting and joint replacement. The SF-36 does not address specific disease processes, and it does not include questions concerning specific symptoms. We chose the SF-36 as an assessment tool because, to our knowledge, it has not been previously applied to patients following pancreaticoduodenectomy and because it is a valid and reliable instrument for assessing overall quality of life.

Quality of life as an outcome measure has been reported for patients with other gastrointestinal diseases including gastroesophageal reflux disease and postoperative pancreaticoduodenectomy. In 1995 McLeod et al.[13] presented their findings after evaluating 25 long-term survivors of pancreaticoduodenectomy. They measured outcomes in quality of life using six different methodologies including the disease-specific Gastrointestinal Quality of Life Index and Visick scales. No differences were seen between their group of patients and age-matched control subjects who had undergone cholecystectomy alone. No difference was seen between the types of operations performed. Only one other study has assessed quality of life following pancreaticoduodenectomy. Patel et al.[14] used the Memorial-Slone Kettering Pain Assessment Card and questioned 23 patients (21 after SPD and 2 after PPPD) an average of 32 months following surgery. There were no differences between the groups and both groups scored high, demonstrating little deficit in function following surgery.

We were able to discern very little difference in quality of life as measured by the SF-36 in our patients. The eight domains of the SF-36 evaluated different aspects of an individual's health status. The most significant differences were seen in the domains of vitality and general health. Both of these domains are valid in assessing overall physical and mental health in combination. General health is specifically assessed by asking patients to rank their own health in general as excellent, very good, good, fair, or poor. The second part asks them to compare their health to that of others and quantify as true or false whether their health is excellent, if they expect their health to get worse, and whether they are healthier or sicker than other people they know. In the domain of vitality, participants are asked to grade their feelings of pep, nervousness, cheerfulness, calmness, and happiness. Additionally, they are questioned as to whether they consider themselves to have a great deal of energy and whether they feel tired or worn out. Patients undergoing a SPD scored significantly worse than those undergoing PPPD and control subjects with regard to both questions. The reasons for these discrepancies are not clear. The difference in the raw score can perhaps be attributed, at least in part, to the difference in age, but when the scores from these patients were compared as a percentage of their "normal" counterparts of similar age, there was still a significant difference.

Overall nutritional status is difficult to quantify. IBW is a calculated estimate that can be used as a screening tool for malnutrition. Eighty percent of our patients fell within normal population limits for IBW. The remainder had a mild-to-moderate weight deficit, and two of these patients reported that their current weight was unchanged from their baseline weight. BMI is another measurement of a patient's nutritional state that has been utilized extensively but is more often used in patients with morbid obesity and, in fact, may not apply to patients with malnutrition.[15] The BMI was below the normal range in only three patients, and each of these patients was identified as having a moderate weight deficit by IBW. Body impedance analysis is a valid technique that is used to measure body composition and thus estimate the amount of body fat. The percentage of body fat was higher in the study patients than in normal subjects, and this may reflect losses of lean body mass during acute illness. Additionally, we compared our patient population with sex-matched normal individuals but not age-matched control subjects and found that older patients may have a higher percentage of body fat. Most important, however, is that few patients had low levels of body fat.

Protein malnutrition can occur more subtly than calorie malnutrition but can be accurately determined in euvolemic patients by measurement of serum proteins. Serum albumin was below normal in four patients and these patients also had below-normal values for body weight and all had low percentages of body fat. Three of the four patients with low albumin reported regular symptoms of steatorrhea despite pancreatic enzyme replacement. All patients had normal transferrin concentration. Our results were similar to those of Royall et al.[16] who reported the long-term nutritional consequences of 24 patients undergoing pancreaticoduodenectomy. The mean follow-up was 4.6 years. When compared with control subjects, patients had a lower body weight, but when values were

converted to BMI no difference was seen. Additionally, serum concentrations of albumin and transferrin were not different from control values. When patients were subgrouped into those undergoing SPD (n = 14) vs. PPPD (n = 10), body weight was equal and no difference was seen in biochemical parameters and body composition as determined by body impedance analysis. This is in contrast to the report of Zerbi et al.,[17] who demonstrated significantly better nutritional parameters in patients undergoing PPPD as compared to SPD. That group evaluated nutrition by body weight expressed as a percentage of preoperative weight and serum albumin. This was a large group of patients, 47 following SPD and 75 following PPPD, who were evaluated at 6 months postoperatively. Most of the patients evaluated required pancreatic exocrine supplementation because many of the patients had undergone pancreatic duct injection rather than pancreaticoenteric anastomosis at the time of their surgery.

In our study anemia was a common problem. Seven patients had serum hemoglobin concentrations below normal limits. This anemia was relatively mild in all but one of the patients, with a range of 10.8 to 11.9 g/dl. One patient with a hemoglobin concentration of 6.8 gm/dl was found to have a marginal ulcer 18 months after a SPD. These findings suggest that patients should be screened for anemia.

Based on our accumulated data and other published reports, we conclude that few early or late differences are seen between SPD and PPPD patients. The postoperative quality of life is generally good and nutritional parameters remain normal in most patients regardless of which procedure is performed. In addition, postoperative measurements of exocrine and endocrine pancreatic function are similar in both groups. Because both procedures have similar outcomes, it is reasonable for the surgeon to decide which operation to perform based on the individual clinical scenario.

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Discussion

Dr. B. Langer (Toronto, Ontario). It is very important to be looking at outcomes, in addition to operative mortality, tumor recurrence, and survival, in these types of operations. I am pleased to see that what you report corresponds almost exactly to what we reported at this meeting two years ago—that is, that the quality of life and nutritional status of patients following pancreaticoduodenectomy was pretty well indistinguishable from what was observed in a matched group of patients following cholecystectomy. You indicted that the parameter of "vitality" was the main area of difference in the two groups studied. I note that the two groups differed by approximately 10 years in mean age. Was that an age-corrected measure of vitality, and if it was not, would you think it would be a good idea to compare groups that are age matched, particularly when you are evaluating parameters that might be expected to change with advancing age?

Dr. W. S. Melvin. When we look at vitality in "normal populations," there is a trend toward decreased scores with increasing age; that is why I expressed these as percentages of normal. We actually used different control population age ranges for the PPPD group who were younger than the pa-

tients who had undergone antrectomy, so an attempt was made to control for age as there was some difference. We still observed a statistical significance despite the matching for age.

Dr. G. Aranha (Maywood, Ill.). Some surgeons have used a pancreaticogastrostomy reconstruction after pancreaticoduodenectomy. Did you have any such patients and does that make a difference in the nutritional evaluations of these patients.

Dr. Melvin. None of the patients who underwent pancreaticogastrostomy were included in this analysis.

Dr. D. Anderson (New Haven, Conn.). I wonder if you could comment on the time it took for exocrine or endocrine abnormalities to begin to appear in your survivors.

Dr. Melvin. Most of the patients who had problems really had them early in the postoperative period and we started to quantify that a bit when we were performing our PABA excretion test. I was unable to identify patients who developed pancreatitis late in their course. Overall, 20% of the patients were taking enzymes. They had been taking them intermittently during their entire postoperative course, not just recently.

Dr. L. W. Traverso (Seattle, Wash.). My question involves the use of disease-specific quality-of-life measurements vs. general health SF-36. Did you discern the multiple variables that might be involved with the higher vitality scores that are seen in patients undergoing a pylorus-preserving procedure?

Dr. Melvin. We did not perform multivariant analysis. That might have been able to help us identify some aberrancies. Two years ago Dr. Langer's group certainly gave us good information using several different disease-specific questionnaires and tools for determining quality of life. I would concur with your assumption and I too was puzzled as to why patients should feel better after a major operation. I think that it is a realization—that is, many of them have faced a serious illness and done well and so their perception of their general health is perhaps better. I think that a combination of generic tests such as this for overall outcome and quality of life is reasonable. That, probably combined with disease-specific tools, is probably the correct way to completely evaluate these patients.

Adenocarcinoma of the Duodenum: Factors Influencing Long-Term Survival

Taylor A. Sohn, M.D., Keith D. Lillemoe, M.D., John L. Cameron, M.D., Henry A. Pitt, M.D., Howard S. Kaufman, M.D., Ralph H. Hruban, M.D., Charles J. Yeo, M.D.

This single-institution retrospective analysis reviews the management and outcome of patients with surgically treated adenocarcinoma of the duodenum. Between February 1984 and August 1996, fifty-five patients with adenocarcinoma of the duodenum underwent surgery at The Johns Hopkins Hospital. Univariate analysis was performed to identify possible prognostic indicators. Curative resection was performed in 48 patients (87%): 35 of these patients (73%) underwent a pancreaticoduodenectomy (PD), whereas 27% (n = 13) underwent a pancreas-sparing duodenectomy (PSD). Patients undergoing PD were comparable to those undergoing PSD with respect to demographic factors, presenting symptoms, and tumor pathology. The remaining 13% of patients (n = 7) were deemed unresectable at the time of surgery and underwent biopsy and/or palliative bypass. PD was associated with an increase in postoperative complications when compared to PSD (57% vs. 30%), but this difference was not statistically significant. One perioperative death occurred following PD (mortality 2.9%). The overall 5-year survival rate for the 48 patients undergoing potentially curative resection was 53%. Negative resection margins ($P < 0.001$), PD ($P < 0.005$), and tumors in the first and second portions of the duodenum ($P < 0.05$) were favorable predictors of long-term survival by univariate analysis. Nodal status, tumor diameter, degree of differentiation, and the use of adjuvant chemoradiation therapy did not influence survival. These data support an aggressive role for resection in patients with adenocarcinoma of the duodenum (J GASTROINTEST SURG 1998;2:79-87.)

Adenocarcinoma of the duodenum is an uncommon neoplasm that represents less than 0.5% of all gastrointestinal malignancies.[1,2] Despite accounting for less than 10% of the total length of the small intestine, the duodenum is the site of 25% to 45% of all small bowel cancers.[3,4] Cancer can develop anywhere along the length of the duodenum; however, most tumors arise in the periampullary region. Therefore these neoplasms are often grouped with carcinoma of the pancreas, ampulla of Vater, and distal bile duct and are considered as the clinical entity of periampullary carcinoma. Of the periampullary neoplasms, duodenal cancer is the least common.[5]

The reported overall 5-year survival rate for resected adenocarcinoma of the duodenum ranges from 20% to 61%.[1-3,5-11] In recent decades, authors have attempted to define those factors that influence survival in patients with duodenal carcinoma. All studies to date agree that resection offers the only hope for long-term survival. However, the extent of resection, pancreaticoduodenectomy (PD) vs. pancreas-sparing duodenectomy (PSD), remains controversial. In addition, the prognostic significance of various pathologic factors has been examined in several studies. Lymph node status, surgical margins, and tumor differentiation have all been shown to have prognostic significance; however, the results of many studies are inconsistent, with the discrepancies likely due to small sample sizes.[1-3,5-11]

This single-institution retrospective review was undertaken to determine the natural history of patients with operatively managed adenocarcinoma of the duodenum. This analysis also attempts to identify demographic, operative, pathologic, and postoperative prognostic factors in the most uncommon of the periampullary cancers.

From the Departments of Surgery and Pathology, The Johns Hopkins Medical Institutions, Baltimore, Md.
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Reprint requests: Keith D. Lillemoe, M.D., Professor of Surgery, The Johns Hopkins Hospital, 600 North Wolfe St., Blalock 603, Baltimore, MD 21287-4603. E-mail: klillemo@gwgate 1.jhmi.jhu.edu.

PATIENTS AND METHODS

Between February 1984 and August 1996, fifty-five patients with adenocarcinoma of the duodenum underwent surgery at The Johns Hopkins Hospital. Patients with biopsy-proved adenocarcinoma of the duodenum who were deemed unresectable and did not undergo surgery were not included in this review. Demographic, perioperative, pathologic, and long-term survival data were collected retrospectively.

All pathologic specimens were reviewed by a single pathologist (R.H.H.) to confirm a duodenal origin of the adenocarcinoma. Based on criteria previously described, tumors arising from the head of the pancreas, distal bile duct, and ampulla of Vater were excluded from the analysis.[12] The tumor diameter, lymph node and margin status, and degree of differentiation were determined for each specimen.

Forty-eight (87%) of the 55 patients underwent potentially curative resection, whereas the remaining seven patients (13%) underwent palliative operative procedures. Resection was performed by either PD or PSD,[13] with the choice of procedure generally based on the location of the tumor. The majority of patients with proximal tumors (first and second portion of the duodenum) underwent PD, whereas patients with more distal lesions underwent PSD. Extended retroperitoneal lymph node dissection was not performed. Pylorus-preserving PD was routinely performed with hemigastrectomy reserved for cases in which the tumor involved the distal stomach or first portion of the duodenum. No patient required total pancreatectomy to achieve a margin-negative resection.

The overall incidence of postoperative complications was evaluated. The incidences of delayed gastric emptying, pancreatic fistula, bile leak, wound infection, pneumonia, and cholangitis were based on specific definitions. Delayed gastric emptying was defined as either (1) nasogastric tube left in place for 10 or more days plus one of the following: (a) emesis after removal of the nasogastric tube, (b) postoperative use of prokinetic agents after postoperative day 10, (c) reinsertion of the nasogastric tube, or (d) failure to progress with diet; or (2) nasogastric tube in place for less than 10 days plus two or me of the previously mentioned criteria (a through d).[14] A pancreatic fistula or bile leak was defined as more than 50 ml of amylase-rich or bilirubin-rich fluid draining from the region of the pancreaticojejunostomy or the hepaticojejunostomy, respectively, on or after postoperative day 10.[15] Wound infection was defined as a positive wound culture and the presence of pus in the wound necessitating opening the wound. Pneumonia was defined as positive sputum cultures with radiographic evidence of an infiltrate requiring antibiotics. Positive bile cultures associated with abnormal liver function tests and fever requiring antibiotics defined cholangitis. The need for reoperation in the immediate postoperative period was recorded. Perioperative mortality was defined as death during the initial hospitalization or within 30 days of surgery.

Adjuvant postoperative chemoradiation therapy was selectively offered to patients based on the preference of the patient and treating physician. The protocol for most treated patients consisted of external beam radiation with intravenous 5-fluorouracil as previously described.[5,12,16]

Follow-up information was obtained via direct patient contact, through review of physician/hospital records, and by contacting the United States Social Security Administration. Information regarding long-term survival was available for 54 (98%) of the 55 patients in the series. One patient who was lost to follow-up was without evidence of disease at 2 months after PD. The median follow-up for the 31 patients remaining alive was 36 months and follow-up was complete through August 1996.

Data are presented as mean ± standard error of the mean. Differences between the resection groups were evaluated by chi-square analysis, analysis of variance, and Fisher's exact test as appropriate. Survival analysis was performed using the method of Kaplan and Meier.[17] The log-rank test was used to evaluate differences in survival among different subgroups. The overall sample size did not allow for performance of a multivariate analysis. A P value of ≤ 0.05 was considered significant.

RESULTS

The mean age for the 55 patients was 61.4 ± 1.7 years (range 33 to 89 years), with a median age of 63 years. Thirty-two patients (58%) were male and 23 (42%) were female. Forty-six (84%) were white and nine (16%) were black. The most common presenting signs and symptoms were abdominal pain (46%), iron-deficiency anemia (35%), weight loss (30%), nausea/vomiting (22%), and obstructive jaundice (18%). Two patients were identified as having Gardner's syndrome, whereas there were no patients with familial polyposis.

Forty-eight patients (87%) were resected for cure and seven (13%) underwent palliative bypass surgery or open biopsy (Table I). Of the 48 patients undergoing curative resections, 35 (73%) underwent PD, whereas 13 (27%) underwent PSD. Of the 35 patients undergoing PD, 27 underwent pylorus-preserving procedures (77%), whereas eight patients underwent classic PD including distal gastrectomy. The mean operative time for PD was 6.8 hours, which was sig-

nificantly longer than that for PSD (5 hours, $P < 0.05$; Table II). The estimated blood loss and mean number of blood transfusions were similar. The median number of blood transfusions was zero for both procedures. Of the seven patients undergoing palliative operations, one was deemed unresectable because of invasion of the superior mesenteric vessels by a tumor in the third portion of the duodenum, whereas the remaining six patients had evidence of liver and/or peritoneal metastases.

Two deaths occurred in the series for an overall in-hospital or 30-day mortality rate of 3.6%. One death occurred in a patient undergoing PD (mortality 2.9%), whereas the second death occurred in a patient who had a palliative bypass. No deaths occurred in the PSD group. Forty-four percent of patients experienced one or more postoperative complications. The overall incidence of postoperative complications was greater following PD (57% vs. 30%); however, this difference did not reach statistical significance. The incidence of delayed gastric emptying was 26% following PD vs. 0% following PSD ($P < 0.05$). Twenty percent of patients undergoing PD developed a pancreatic anastomotic leak, whereas one patient developed a pancreatic fistula following PSD (not significant). There were no differences in the overall incidence of cholangitis (8%) and wound infection (6%) between the two groups of patients undergoing resection. One patient required reoperation and completion pancreatectomy following a PD in the immediate postoperative period secondary to breakdown of the pancreaticojejunostomy. Only one patient (14%) undergoing a palliative procedure developed a complication. The mean postoperative length of stay was longer following PD than PSD, with the difference approaching statistical significance ($P = 0.06$). There was one death observed during the first 90 days after hospital discharge, which occurred in a patient who had a palliative operation.

The average tumor diameter in the 48 resected specimens was 4.6 ± 0.4 cm. Fourteen resected tumors (29%) were located in the third or fourth portion of the duodenum. Lymph nodes were positive in 27 (56%) of 48 of the resected patients. Four (8%) of 48 resected patients had positive margins. Seventy percent of patients had tumors that were well to moderately differentiated, with the remainder (30%) being poorly differentiated.

The overall 1-, 2-, and 5-year survival rates for the entire cohort of 55 patients were 81%, 64%, and 47%, respectively. Resectable patients had not yet reached median survival at last follow-up, and their actuarial 5-year survival was 53%. This outcome is in contrast to that of unresectable patients in whom the median survival was 4 months, with no 5-year survivors (Fig. 1; $P < 0.0001$).

Demographic factors including age, sex, and race had no prognostic significance. Of the pathologic factors evaluated, only resection margin status achieved statistical significance. Patients with negative resection margins (n = 44) had a 5-year survival of 58% compared to 0% in those with positive resection margins (n = 4, $P < 0.001$; Fig. 2). Tumor diameter, lymph node status, and degree of tumor differentiation did not influence long-term survival. A trend toward improved survival was observed in patients with negative lymph nodes, but this difference did not achieve statistical significance. The 5-year survival in patients with node-negative resections was 66% com-

Table I. Operative management of 55 patients with adenocarcinoma of the duodenum

	No.	(%)
Current resection	48	87
Pancreaticoduodenectomy	35	73
Pylorus-preserving	27	77
Classic	8	23
Pancreas-sparing duodenectomy	13	27
Palliative procedures	7	13

Table II. Perioperative factors in 48 patients undergoing resection of adenocarcinoma of the duodenum

	Pancreaticoduodenectomy (n = 35)	Pancreas-sparing duodenectomy (n = 13)
Mean operative time (hr)	6.8 ± 0.2	5.0 ± 0.6*
Mean estimated blood loss (ml)	757 ± 99	656 ± 220
Mean blood transfusions (units)	1.5 ± 0.1	1.7 ± 0.2
Postoperative mortality (%)	2.9	0.0
Any complication (%)	57	30
Reoperation (%)	3	0
Postoperative length of stay (days)	20.4 ± 2.2	12.5 ± 2.4†

*$P < 0.05$.
†$P = 0.06$.

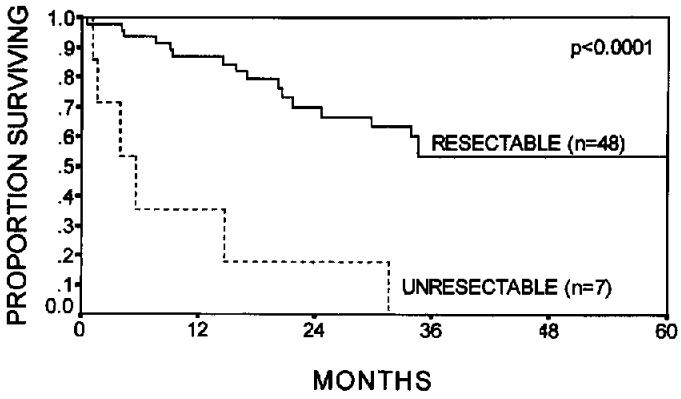

Fig. 1. Kaplan-Meier survival curves comparing patients with resectable duodenal cancers (n = 48) to those with unresectable tumors (n = 7; $P < 0.0001$).

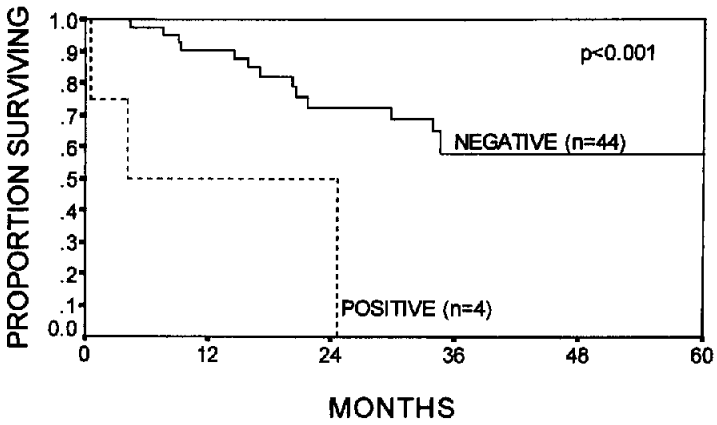

Fig. 2. Kaplan-Meier survival curves comparing resectable patients with negative resection margins (n = 44) to those with positive resection margins (n = 4; $P < 0.001$).

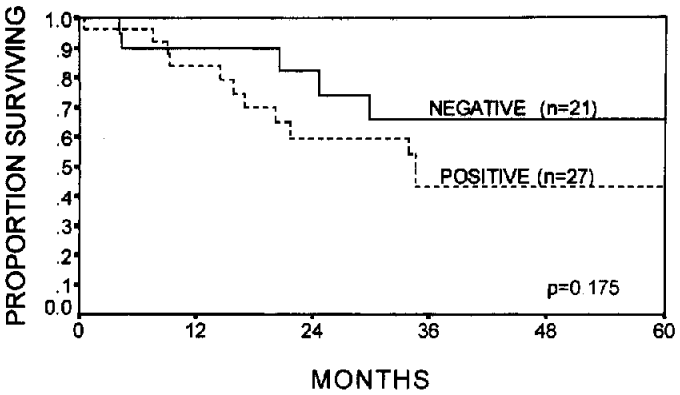

Fig. 3. Kaplan-Meier survival curves comparing resectable patients with node-negative tumors (n = 21) to those with node-positive tumors (n = 27; $P = 0.175$).

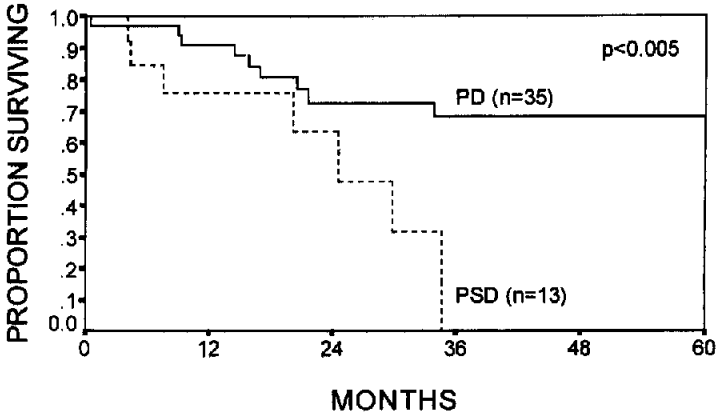

Fig. 4. Kaplan-Meier survival curves comparing patients undergoing pancreaticoduodenectomy *(PD)* (n = 35) to those undergoing pancreas-sparing duodenectomy *(PSD)* (n = 13; $P < 0.005$).

pared to 43% in those with node-positive resections ($P = 0.175$; Fig. 3).

The 35 patients undergoing PD had 1-, 2-, and 5-year survival rates of 91%, 73%, and 69% compared to 76%, 63%, and 0%, respectively, for the 13 patients undergoing PSD ($P < 0.005$; Fig. 4 and Table III). Similarly, the 34 patients with tumors of the first and second portion of the duodenum fared better than those (n = 14) with tumors in the third and fourth portion, with 1-, 2-, and 5-year survival rates of 91%, 72%, and 67% vs. 78%, 67%, and 13% ($P < 0.05$; Fig. 5 and Table III).

Eleven patients received adjuvant therapy after curative resection with 1-, 2-, and 5-year survival rates of 82%, 58%, and 39% (Fig. 6 and Table III), whereas 37 patients elected not to undergo adjuvant therapy with their 1-, 2-, and 5-year survival rates being 81%, 66%, and 48%, respectively ($P = 0.73$; Fig. 6 and Table III). It should be noted that the decision to receive adjuvant therapy was determined by patient and physician selection and was not randomized.

The PD and PSD groups were compared with regard to demographic factors, operative factors, and tumor pathology. The two groups were similar with respect to age, sex, and race. As noted previously (see Table II), estimated blood loss and transfusion were also not different between the two groups. The tumors in the two groups were similar in size and nodal status, but patients undergoing PSD had a higher incidence of both positive resection margins (23% vs. 3%; $P < 0.05$) and poorly differentiated tumors (50% vs. 24%; P = NS) (Table IV). Because margin status

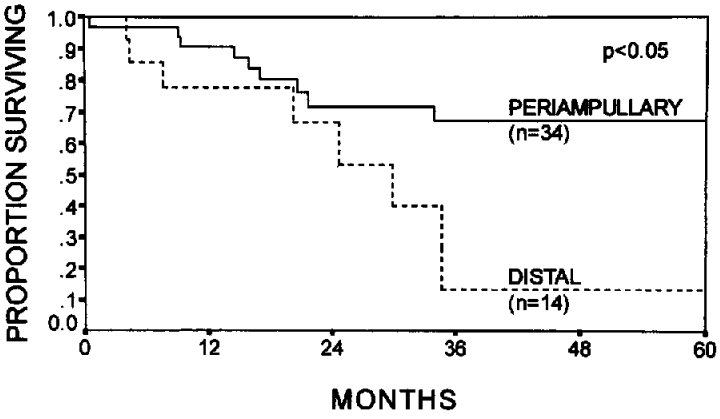

Fig. 5. Kaplan-Meier survival curves comparing resectable patients with periampullary tumors (n = 34) to those with distal tumors (n = 14; $P < 0.05$).

Table III. Factors influencing survival for adenocarcinoma of the duodenum

	Median (mo)	1 year (%)	2 year (%)	5 year (%)	*P* value
All patients					
Overall (n = 55)	35	81	64	47	—
Resectable (n = 48)	Not yet reached	88	70	53	<0.0001
Unresectable (n = 7)	4	36	18	0	
Resectable patients (n = 48)					
Negative margins (n = 44)	Not yet reached	90	72	58	<0.001
Positive margins (n = 4)	4	50	0	0	
Negative lymph nodes (n = 21)	Not yet reached	90	83	66	0.175
Positive lymph nodes (n = 28)	35	84	60	43	
Pancreaticoduodenectomy (n = 35)	Not yet reached	91	73	69	<0.005
Pancreas-sparing duodenectomy (n = 13)	24	76	63	0	
Periampullary (n = 34)	Not yet reached	91	72	67	<0.05
Distal (n = 14)	24	78	67	13	
Adjuvant therapy (n = 11)	27	82	58	39	0.73
No adjuvant therapy (n = 37)	35	81	66	48	

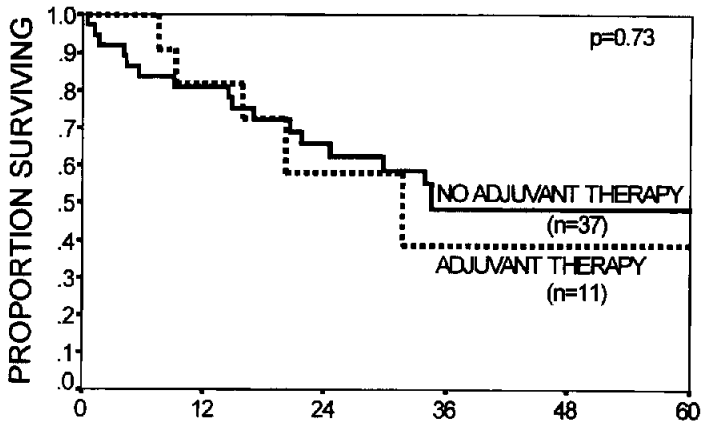

Fig. 6. Kaplan-Meier survival curves comparing resectable patients undergoing adjuvant therapy (n = 11) to those not undergoing adjuvant therapy (n = 37; $P = 0.73$).

Table IV. Pathologic characteristics of resected tumors

	Pancreaticoduodenectomy (n = 35)	Pancreas-sparing duodenectomy (n = 13)	*P* value
Tumor diameter (cm)			
Average	4.7 ± 2.6	4.5 ± 2.5	NS
Median	4.5	4	
Margin status			
Negative	97%	77%	<0.05
Positive	3%	23%	
Lymph node status			
Negative	40%	54%	NS
Positive	60%	46%	
Differentiation			
Good to moderate	76%	50%	NS
Poor	24%	50%	

NS = not significant.

was significant in the univariate model, survival in margin-negative patients undergoing PD and PSD was compared. The 1-, 2-, and 5-year survival rates for the PD group with negative margins were 94%, 75%, and 71% compared to 79%, 63%, and 0% for the PSD group ($P < 0.01$).

DISCUSSION

Adenocarcinoma of the duodenum is the least common of the periampullary tumors. In a recent review of patients undergoing PD at The Johns Hopkins Hospital in the 1990s, only 6% of tumors arose from the periampullary duodenum.[5] A number of reports have demonstrated a more favorable prognosis in tumors with a duodenal origin.[18-21] The recent experience at Johns Hopkins also reflects this finding, with the projected 3-year survival for duodenal cancer being 72% compared to 53% for ampullary cancer, 30% for pancreatic carcinoma, and 16% for distal bile duct cancer.

As with other periampullary malignancies, resection offers the only hope for long-term survival in patients with duodenal adenocarcinoma. Unresected the disease is uniformly fatal. Other factors influencing survival in patients with duodenal adenocarcinoma have not been well defined because of the relative infrequency of this tumor and the resulting small size of reported series of patients resected for cure.

In this series the 5-year survival for the entire co-

hort of patients was 47%. In those undergoing curative resection, however, 5-year survival was significantly improved in comparison to unresected patients (53% vs. 0%). In addition, resection with negative surgical margins, periampullary location, and PD all significantly favored survival in a univariate model. Demographic factors, intraoperative factors, adjuvant therapy and other pathologic factors did not influence survival. Results of multiple previous studies are consistent with regard to the favorable prognostic significance of tumor resection and negative margin status.[1-3,5-11,18-28] Likewise, most of these studies have failed to demonstrate prognostic significance for demographic factors or tumor grade.

The two largest areas of controversy with regard to prognosis for patients with adenocarcinoma of the duodenum are lymph node status and location of the primary tumor. The optimal type of resection to perform is also controversial, although this factor is closely related to the location of the primary tumor. Several authors have demonstrated poor prognosis associated with positive nodal status.[3,7,22,25] Conversely, multiple studies have shown no statistical difference, although the trend in most is toward improved survival in node-negative patients.[8-11,23,24] The current study also demonstrates a trend toward improved survival, but this difference does not achieve significance with 5-year survival being 66% vs. 43% ($P = 0.175$). Although patients with negative lymph nodes may fare better, this study demonstrates that long-term survival can still be achieved with node-positive tumors supporting an aggressive approach regarding resection of these tumors.

Several series have demonstrated no difference in survival between periampullary lesions and more distal lesions.[7,9,25] Conversely, two studies have demonstrated improved survival with more distal tumors.[2,6] The first study by Alwmark et al.[2] analyzed survival in 66 patients of whom only 19 were resected for cure. The average survival for patients with suprapapillar cancers was 23.1 months compared to 27.1 months for peripapillar cancers and 31.1 months for infrapapillar cancers. These figures included both resectable and unresectable patients. Of note, despite the better survival with more distal lesions, longer survivals were observed in those patients undergoing pancreaticoduodenal resection when compared to those undergoing pancreas-sparing procedures (mean 5-year survival 53 months vs. 41 months, respectively). These data are difficult to interpret because of the small number of resected patients and the inclusion of unresectable patients in the evaluation of tumor location as a prognostic indicator. In the other series, Lowell et al.[6] analyzed survival in 17 patients with resected adenocarcinoma of the duodenum. Seven patients had tumors of the distal duodenum, whereas 10 patients had proximal tumors. The median survival for patients with tumors in the first and second portion of the duodenum was 30 months. Those with more distal tumors had a median survival of 46 months ($P < 0.01$).

This study demonstrates that combined modality adjuvant therapy is not associated with a significant survival benefit after resection. The results should be interpreted with caution because of the retrospective, nonrandomized nature of the study as well as the small sample size. It is not unreasonable to postulate a survival benefit for adjuvant therapy following resection of duodenal adenocarcinoma, as adjuvant chemoradiation therapy has been reported to benefit patients with resected adenocarcinoma of the pancreas.[16,29,30]

The current analysis suggests that PD is the preferred operation for most patients with duodenal carcinoma. Several recent reports demonstrate that PD can be safely performed with operative mortality rates of less than 5%.[12,31-34] Although it is recognized that duodenal pathology and the association with soft pancreatic texture are associated with an increased likelihood of pancreatic anastomotic leak,[15] this operation should not be denied to those patients with periampullary duodenal cancers. The operative mortality rate in the current series for PD was 2.9% with the overall incidence of postoperative complications 57%, and the mean postoperative length of stay 20 days. These results, however, are not markedly different from the recent report concerning 650 pancreaticoduodenectomies performed at this hospital for both benign and malignant disease in the 1990s.[5] Furthermore, in the current analysis PD was not associated with a higher rate of complications than PSD. We conclude that tumor location and the ability to resect the tumor with adequate margins, with no concern for excessive morbidity and mortality, should determine the extent of resection.

CONCLUSION

The results of this study demonstrate, as with other periampullary cancers,[12,35,36] that aggressive resection is indicated in patients with adenocarcinoma of the duodenum. Large tumor size and the presence of positive nodes should not preclude resection if it can be performed with negative resection margins. Although patients with distal tumors may fare worse, resection still offers the only opportunity for long-term survival.

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Discussion

Dr. J. Kilkenny (Jacksonville, Fla.). I have a question regarding your 20% fistula rate. Is that different from your overall experience? Have you addressed that with your technique or the use of perioperative somatostatin?

Dr. T.A. Sohn. We do not routinely use somatostatin after a Whipple resection to decrease the pancreatic fistula rate. Our rate of fistulization in this series was 20%, which is consistent with our overall rate. In a recent series of 650 Whipple procedures performed at The Johns Hopkins Hospital, we looked at pancreatic fistula rates and found a slightly higher incidence of fistulization in patients with duodenal cancers and ampullary cancers likely as a result of a softer, normal pancreas. These rates in this study are consistent with our overall fistula rates.

Dr. L. Stewart (San Francisco, Calif.). Did patients who had positive margins all have pancreas-sparing procedures?

Dr. Sohn. Of the patients who had positive margins, three of them had PSD. The fourth patient underwent a PD.

Dr. Stewart. Did you compare the distal duodenal carcinomas to the periampullary tumor stage? Was there also a difference in survival based on stage?

Dr. Sohn. The curve I showed you included all patients. There was a higher percentage of positive margins in the PSD group. We therefore analyzed the data excluding the margin-positive patients and just compared margin-negative patients. In margin-negative patients undergoing PD, the 5-year survival rate was 71% as compared to 0% in the PSD group. This difference was also significant at the $P < 0.05$ level. We did not compare American Joint Committee on Cancer stage I, II, and III duodenal cancers. We did, however, compare node-negative to node-positive patients (stages I and II vs. stage III). The difference in survival was seen in both periampullary and distal tumors.

Dr. D. Schirmer (Charlottesville, Va.). I was particularly interested to see that you had no gastric emptying complications in the patients who underwent PSD. How exactly was the gastrointestinal tract reconstructed in those patients? What percentage of gastrectomy was done, if any? Was a vagotomy done? Do you have any insight as to why you had no gastric emptying delays?

Dr. Sohn. None of the patients undergoing PSD underwent gastrectomy or vagotomy. We think that the difference in the incidence of delayed gastric emptying is multifactorial. The first and second portions of the duodenum are retained maintaining the "peacemaker" function of this portion of the duodenum. In addition, with most of the duodenum still in place, motilin is still produced. I think that the third factor is that we are just performing a resection of bowel and primary anastomosis, and mostly end to end, without the additive factors of removing the head of the pancreas and the associated nerve and blood supply.

Dr. J. Hunter (Atlanta, Ga.). Given the poorer outcome of those who had the pancreas-sparing operation for their duodenal tumors, is this a function of the worst natural history of these tumors or is the operation you are performing less adequate? Should you be performing a more radical operation for those tumors in the third and fourth portions of the duodenum in order to try to achieve negative margins and try to improve the survival rates?

Dr. Sohn. I think that the difference in survival we are seeing is based on tumor location. Tumors in the third and fourth portions of the duodenum behave differently in that their pattern of spread both by direct invasion and lymph node involvement is directly into the root of the mesentery. For that same reason, we do not believe that patients with distal tumors would benefit from a PD because there would be no increase in the resection margin. It is not possible to obtain a wide clearance at the root of the mesentery.

Dr. A. Warshaw. (Boston, Mass.). To be fair, though, there are at least some reports that suggest the opposite—that is, that distal tumors have a better natural history and prognosis. Perhaps a wider operation might be helpful.

Dr. Sohn. Yes. We do acknowledge this possibility.

Thoracoscopic Splanchnicectomy for "Small Duct" Chronic Pancreatitis: Case Selection by Differential Epidural Analgesia

Edward L. Bradley III, M.D., Jonathan A. Reynhout, M.D., Gerald L. Peer, M.D.

Management of patients with intractable pain from "small duct" chronic pancreatitis has been difficult, often resulting in narcotic addiction and/or malnutrition from major pancreatic resection. Recently, denervation of sympathetic pain afferents from the pancreas by surgical splanchnicectomy has shown promise in relieving pain while preserving residual pancreatic function. However, results from surgical splanchnicectomy have been mixed in large part because of patient selection. Differentiating actual pancreatic pain from "pancreatic" pain caused by drug-seeking behavior, psychogenic diseases, or various somatically innervated conditions is clinically challenging at best. Between 1992 and 1996, twenty-two patients with 20 prior pancreatic operations, "small duct" chronic pancreatitis, and "pancreatic" pain requiring narcotics were evaluated. Each underwent differential epidural analgesia (DEA) using the following standard techniques: placebo, low-dose (sympathetic), and high-dose (somatic) blocks. Pain perceptions were recorded before and after DEA using a visual analogue scale (VAS). Six demonstrated a greater than 50% decrease in VAS pain after placebo injection and were eliminated from further study. In the remaining 16 patients, pain relief only occurred with sympathetic or somatic blockade. Greater and lesser splanchnicectomy (surgical splanchnicectomy) was performed 27 times in these 16 patients (11 bilateral, 6 synchronous) (5 unilateral; 2 right and 3 left) using thoracoscopic techniques in 14 patients and open thoracotomy in two. No significant surgical or anesthetic complications were encountered. Surgical splanchnicectomy resulted in an overall significant reduction in preoperative VAS scores (8.25 to 4.18; $P < 0.05$). Ten of 13 patients with DEA-predicted sympathetic pain experienced a greater than 50% decrease in VAS after surgical splanchnicectomy, but only two had complete relief. None of the three patients with DEA-predicted somatic pain were benefited by splanchnicectomy. During an average follow-up of 23.3 months, initial good results from surgical splanchnicectomy were maintained in 8 of 10 patients. The following conclusions were reached: (1) surgical splanchnicectomy is a safe, often effective technique for amelioration of intractable pain from "small duct" chronic pancreatitis and (2) DEA is a promising approach for identifying patients most likely to respond to surgical splanchnicectomy. (J GASTROINTEST SURG 1998;2:88-94.)

Eighty-five percent of patients with chronic pancreatitis will develop pain at some time during the course of their disease. Although surgical approaches to "large duct" chronic pancreatitis have been quite successful,[1] patients with persistent pain from "small duct" chronic pancreatitis have historically been a difficult group to manage. Medical therapy in this latter group has often been either inconclusive or ineffectual, and narcotic addiction is common. Surgical approaches have fared little better, with extensive ablations of pancreatic parenchyma either failing to relieve pain or leading to brittle diabetes, marginal ulceration, and serious malnutrition. As an alternative to pancreatic resection, efforts to eliminate pain and preserve residual organ function by pancreatic denervation have met with variable success, ranging from greater than 90% success in some instances[2] to being almost useless in others.[3] Since it is possible that this wide disparity in results is traceable to the widely recognized clinical problem of separating true pancreatic pain from other causes of abdominal pain, the current study was specifically designed to provide a coherent

From the Departments of Surgery (E.L.B. and J.A.R.) and Anesthesiology (G.L.P.), State University of New York (Buffalo), and Pain Management Services, Buffalo, N.Y.
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Reprint requests: Edward L. Bradley III, M.D., Chief of Surgery, Buffalo General Hospital, 100 High St., Buffalo, NY 14203.

group of patients with pain originating in the pancreas as determined by physiologic preoperative testing, in whom denervation of the pancreatic pain afferents by thoracoscopic splanchnicectomy could be tested.

PATIENTS AND METHODS

Between 1992 and 1996, twenty-two patients (14 males, 8 females; mean age 42.9 years) with unequivocal "small duct" chronic pancreatitis and persistent pain requiring narcotics agreed to participate in an institutional review board–approved protocol evaluating surgical splanchnicectomy. The initial patients were seen at Emory University; all subsequent procedures were performed at the State University of New York at Buffalo. Patient demographics are presented in Table I.

The diagnosis of chronic pancreatitis was made from the clinical history and the appearance of the main pancreatic duct on endoscopic pancreatography in 20 cases, and supported by findings from computed tomography in 16 patients. In addition, 15 of these patients had undergone 20 historical operations for chronic pancreatitis (cyst-enteric drainage in 12, pancreatic resections in 4, longitudinal pancreatojejunostomy in 2, and unknown in 2). Only patients with a main pancreatic duct diameter of less than 5 mm were entered into this study. The maximum diameter of the main pancreatic duct was determined from endoscopic retrograde cholangiopancreatography, after correcting for magnification error.

Subjective evaluation of the degree of pain was measured before and after both the differential epidural test and surgical splanchnicectomy. Patient responses to perceived pain were graded on a visual analogue scale (VAS) that ranged from 1 (least) to 10 (worst).

Differential epidural analgesia (DEA) was performed in the classic manner.[4] Following placement of the epidural catheter in the midthoracic epidural space, a placebo injection was followed by a low dose of lidocaine designed to block only unmyelinated sympathetic fibers (sympatholytic), and subsequently a high dose of the local anesthetic agent was administered to produce somatic nerve blockage (somatolytic). Sympathetic block was considered present when skin temperature increased one degree or when marked analgesia occurred in the presence of continued motor function. Somatic block was established when sensory examination to pinprick revealed analgesia in a dermatomal band on the abdomen. For evaluation purposes, all subjects were then advanced to surgical block in which anesthesia was produced in a dermatomal band pattern and marked weakness of hip flexors occurred. When significant improvement in VAS pain occurred after placebo injection alone, patients were considered placebo responders. Patients experiencing a marked reduction in VAS pain after documented sympatholytic block were considered examples of pancreatic pain, whereas those who obtained significant relief only after high-dose block were considered examples of nonpancreatic somatic pain.

Thoracoscopic splanchnicectomy was performed with a 10 mm camera port located under the tip of the scapula in the sixth intercostal space, and two 5 mm ports separated by 4 to 6 inches in the posterior axillary line. All procedures were done with a cuffed endotracheal tube capable of single-lung ventilation. Proper placement of the tube was verified by fiberoptic bronchoscopy.

Following deflation of the ipsilateral lung, the endoscope was introduced and the course of the greater and lesser splanchnic nerves was identified. In an equal number of cases, the nerves were either easily viewed through the pleura or required stripping of the overlying opaque pleura for identification. Once identified, the greater and lesser splanchnic nerves were elevated and segments excised between metallic clips. Fulguration of smaller nerve branches was also performed. At the completion of the procedure, a No. 20 chest tube was placed in the thoracic cavity and removed in the recovery room if a postoperative chest x-ray film failed to demonstrate a pneumothorax.

Early in our experience, unilateral splanchnicectomy was performed when pain was principally localized to one side or the other; contralateral splanchnicectomy was reserved for those experiencing pain on the opposite side after unilateral neurectomy. More recently, thoracoscopic procedures have been bilateral and synchronous, with the patient in a prone position on a spine table. Two of the 16 patients undergoing surgical splanchnicectomy required open thoracotomy, in both cases because of extensive pleural adhesions. Thoracoscopic techniques were used in all others.

Initially patients were seen biweekly for 1 month, then at 3-month intervals. At the time of each evaluation, VAS pain score were elicited.

RESULTS

Of the 22 original patients with "small duct" chronic pancreatitis, only six responded to placebo injection during DEA with a greater than 50% decrease in the VAS pain score. These placebo responders were returned to their primary care physicians with recommendations for formal pain management programs. Each of the remaining 16 patients underwent

Table I. Patient profiles and results of surgical splanchnicectomy

Patient	Sex	Age (yr)	Type of chronic pancreatitis	DEA-predicted origin of pain	Thoracoscopic splanchnicectomy		VAS pain			Length of follow-up (mo)
					Bilateral	Unilateral	Preop	Postop 3 mo	Current	
1	F	36	Alcoholic	SY		L	10	3	2	51
2	M	46	Alcoholic	SO	M		9	8	8	25
3	M	38	Alcoholic	SY	S		8	8	L 8; R 4	30
4	M	41	Alcoholic	SO	M		7	7	9	9
5	M	49	Alcoholic	SY	M		9	4	4	8
6	F	46	Idiopathic	SY		L	8	4	3	22
7	M	40	Alcoholic	SY		L	8	2	1	44
8	F	44	Pancreas divisum	SY		R	7	0	0	39
9	M	43	Idiopathic	SY	M		8	2	2	37
10	M	52	Alcoholic	SY	S		7	1	1	36
11	F	36	Alcoholic	SY	S		9	6	L 7; R 1	15
12	M	39	Alcoholic	SY	S		8	3	8	15
13	M	38	Idiopathic	SO	S		9	9	8	13
14	M	44	Alcoholic	SY	M*		8	2	6	9
15	M	56	Idiopathic	SY	S		8	8	8	11
16	F	38	Alcoholic	SY		R*	9	0	0	8

DEA = differential epidural anesthesia; VAS = visual analogue scale; SY = sympathetic pain; SO = somatic pain; S = synchronous; M = metachronous.
*Open thoracotomy

surgical splanchnicectomy, including three cases in which a significant reduction in VAS pain did not occur with sympathetic block, but only with full somatic block. Splanchnicectomy was performed 27 times in these 16 patients. The operation was unilateral in five cases (2 right and 3 left) and bilateral in 11 (6 synchronous and 5 staged).

Surgical splanchnicectomy resulted in a significant reduction in preoperative VAS scores (8.25 to 4.18; $P < 0.05$). Ten (76.9%) of 13 patients with DEA-predicted sympathetic pancreatic pain experienced a greater than 50% decrease in VAS pain after surgical splanchnicectomy. In contrast, none of the three patients with DEA-predicted somatic pain were benefited by splanchnicectomy (see Table I). VAS data were supported by narcotic use patterns. It is important to note that despite a significant reduction in overall pain perception and narcotic usage in patients with "small duct" chronic pancreatitis after surgical splanchnicectomy, only 2 of the 10 patients undergoing splanchnicectomy experienced complete pain relief. Furthermore, during follow-up, which now averages 23.3 months, the initially good results were maintained in 8 of the 10 patients.

Six patients experienced minimal atelectasis, which cleared quickly. No patient had diarrhea. One patient had intercostal neuritis, which responded to intercostal nerve injection with a long-acting analgesic. One other patient experienced an unexplained 3 cm area of hyperesthesia in the skin of the anterior chest wall between the sixth and seventh ribs, just below the areola. This resolved over 2 months' time.

DISCUSSION

A variety of approaches to pancreatic denervation have been used in the past. Mallet-Guy was apparently the first person to attempt any form of denervation of the pancreas in patients with chronic pancreatitis. Using a retroperitoneal approach, he performed unilateral left splanchnicectomy in 1942 with successful relief of pain. By 1950, Mallet-Guy and deBeaujeu[5] were able to report successful results in 84% of 37 patients followed for 1 year or more.

Confirmation of the value of pancreatic denervation in patients with chronic pancreatitis first appeared in the United States with the report of Reinhoff and Baker,[6] who described incidental pain relief in a patient with calcific pancreatitis following bilateral splanchnicectomy, sympathectomy, and vagectomy, which were being used to treat intractable hypertension. These observations were subsequently confirmed in other patients with chronic pancreatitis undergoing transthoracic sympathectomy for hypertension.[7-10] In 1983 Mallet-Guy[11] reported on his expanded series of 215 patients with chronic pancreatitis who had undergone left splanchnicectomy and celiac ganglionectomy. In 127 patients who had been followed for 5 years or more, relief of pain was observed in 88%. Despite these sporadic successes with surgical splanchnicectomy, in 1983 White[3] reported only a 20% success rate in relieving chronic pancreatic pain with left splanchnicectomy and celiac ganglionectomy using the technique devised by Mallet-Guy.[3] Thereafter interest in pancreatic denervation by splanchnicectomy waned.

A different approach to pancreatic denervation was suggested by Yoshioka and Wakabayashi[12] in 1958. After studying the neuroanatomy of postganglionic nerve fibers traveling from the celiac ganglion to the pancreatic parenchyma in 100 cadavers, they recommended a selective bilateral neurectomy of the fibers entering the pancreas from the right and left celiac ganglia. They reported that 35 to 36 patients with chronic pancreatitis experienced "excellent" results from this challenging technique. Little or no additional information concerning these patients was provided. In a modification of this selective parenchymal sympathectomy, Hiraoka et al.[13] extended the Yoshioka procedure to include more extensive denervation of the body and tail by completely freeing the pancreas from its retroperitoneal attachments. Successful relief of pain was achieved in both patients undergoing this modified procedure.[13] These excellent results, however, could not be reproduced in Denmark. Hoffman and Jensen[14] reported that of six patients subjected to selective parenchymal sympathectomy, two patients were unchanged and four experienced only temporary relief.

It was not until 1990, when Stone and Chauvin[2] reported that 14 of 15 patients with intractable pain from chronic pancreatitis experienced complete relief from transthoracic splanchnicectomy and concomitant bilateral truncal vagectomy, that interest was rekindled in splanchnicectomy. Miraculously, 14 of the 15 patients were not only cured of pain but were able to return to work, experienced a 29% mean increase in body weight, and were cured of preoperative drug addiction in 10 of 11 cases. Although the necessity for the concomitant vagectomy was not validated, the authors concluded that the delayed gastric emptying noted in 11 of 14 patients seemed a small price to pay for pain relief.

A thoracoscopic approach to the splanchnic nerves in patients with intractable pain from chronic pancreatitis was first reported by Cuschieri et al.[15] in 1994. Pain relief was observed in three of five patients observed for 8 months. Using the same posterior bilateral thoracoscopic approach to the splanchnic nerves employed by the Dundee group, Andren-Sandberg et

al.[16] demonstrated a significant reduction in pain in 13 of 14 patients with chronic pancreatitis over a median follow-up of 13 months. A decrease in VAS pain from 7 ± 12 to 3 ± 3 was noted 1 week after surgery and was maintained at this level for the duration of follow-up. However, no patient experienced complete relief, and each of the 14 patients required the postoperative use of nonnarcotic analgesics on a regular basis.

Maher et al.[17] performed ipsilateral thoracoscopic splanchnicectomy in 15 patients with chronic pancreatitis and pain localizing to one side. Pain recurred in seven cases and these patients then underwent contralateral splanchnicectomy. Over a median follow-up of 18 months, VAS pain significantly decreased from 6.5 before the operation to 2.0 postoperatively ($P < 0.01$). Seven of the 15 no longer require narcotics, five had a reduction in narcotic needs, and three received no relief.[17]

The foregoing experiences with surgical splanchnicectomy seem to suggest that splanchnicectomy can reduce pain in some but not all patients with chronic "pancreatic" pain. The results of the current study also support this contention. Surgical splanchnicectomy in our patients resulted in an overall significant reduction in VAS scores in keeping with other recent reports.[16,17] Moreover, the technique carries a negligible mortality risk and minimal morbidity. Aside from a few collected instances of postoperative hemorrhage,[16] chylothorax, intercostal neuritis, and time-limited postural hypotension,[17] difficulties are few and hospitalization is short.

Since the splanchnic nerves are thought to carry most if not all of the sympathetic pain afferents from the pancreatic parenchyma,[18] it is not surprising that splanchnicectomy relieves pain in patients with chronic pancreatitis. Rather, it is the failure of splanchnicectomy to *completely* relieve pain that is remarkable. Only 2 of our 16 patients experienced total relief of their pain. Reductions in pain level rather than complete loss of pain were also the norm in the experience of others.[16,17] It is not clear why this should be.

One possible explanation for incomplete pain relief after splanchnicectomy, could be technical. Despite anatomic drawings that have demonstrated little variation in the anatomy of the splanchnic nerves, we have found considerable variation in the location and number of branches of these nerves. In one of our early patients experiencing failure of pain control by splanchnicectomy (No. 3), review of the surgical video tape reveals that the left greater splanchnic nerve was never resected.

Another plausible explanation for suboptimal pain response from splanchnicectomy might be that some elements of pain in chronic pancreatitis are mediated by somatic nerve pathways. As all surgeons dealing with chronic pancreatitis know, the disease process often extends beyond the boundaries of the gland to involve the posterior abdominal parietes. Pain arising from chronic inflammation of the parietal tissues is true somatic exteroceptive pain, whose pathway is through the spinal nerves to the dorsal root ganglion, and then to the lateral spinothalamic tract. In support of this theory of mixed sympathetic and somatic involvement in some patients with chronic pancreatitis are our observations during DEA testing, in which we noted a decrease in VAS pain in some of our patients after sympathetic blockade but total anesthesia required a full somatic block. Moreover, the well-recognized failure of even total pancreatectomy to provide pain relief to some patients might be explained by somatic nerve involvement superimposed on sympathetic pain. If combined sympathetic and somatic nerve involvement is common in patients with chronic pancreatitis, an operation could be designed to add appropriate somatic denervation to the splanchnicectomy.

A final explanation for the frequent absence of total analgesia after splanchnicectomy might be that pancreatic pain afferents are present in mixed nerves, thereby bypassing the celiac ganglia and the splanchnic nerves. Although it is known that such fibers exist,[4] their relative importance in the neurophysiology of pancreatic pain is unknown.

A question has also arisen concerning the duration of analgesia provided by splanchnicectomy. Although Andren-Sandberg et al.[16] noted no attrition in postoperative analgesia during a 12-month follow-up, Maher et al.[17] described pain recurring over time in one patient after bilateral splanchnicectomy, and expressed concern that two more of their patients with initially good results might be experiencing worsening pain. Two of our patients with initially good results (Nos. 11 and 14) experienced worsening of pain at 12 and 8 months, respectively, although the pain did not return to preoperative levels. Long-term follow-up of patients undergoing splanchnicectomy will be necessary to address this issue.

Methods of patient selection for splanchnicectomy have varied from one investigator to another. Cuschieri et al.[15] observed that their worst results occurred in those patients with the most severe disease and proposed that splanchnicectomy would be most useful in "minimal" chronic pancreatitis. Maher et al.[17] noted that all three failures of splanchnicectomy developed in patients with more extensive disease, as

reflected by the need for previous pancreatic surgery. However, programmatic exclusion of patients with advanced disease and previous pancreatic surgery may not be appropriate deselection criteria, as demonstrated by the current study in which good results were obtained in a group of patients with severe chronic pancreatitis and multiple previous pancreatic procedures. Similar findings were reported by Andren-Sandberg et al.[16] in that good results were obtained in 12 of 14 patients who were disabled and seven of whom had undergone previous pancreatic surgery.

Because splanchnicectomy can be reasonably expected to only affect sympathetic pain afferents, the ability to determine which patients are actually experiencing sympathetically mediated pain should represent the optimal selection process. Maher et al.[17] attempted to screen their patients for sympathetic mediated pain by the response to celiac ganglion block; only those demonstrating a reduction in VAS pain with ganglionic blockade were accepted for surgery. However, celiac blockade is notoriously difficult to perform and interpret in chronic pancreatitis, and is occasionally followed by severe complications.[19]

A consistent and reliable method for identifying candidates for splanchnicectomy is critical, as it is clinically difficult to separate those patients with true pancreatic pain from those with drug-seeking behavior, psychogenic pain, or somatically innervated conditions such as somatic nerve entrapment, which can successfully masquerade as abdominal pain. DEA may represent a better approach for identifying placebo responders who could reasonably be expected to benefit from a nonsurgical approach such as a pain management program. Identification of placebo responders would also remove the placebo effect as an issue confounding evaluation of putative surgical approaches. Finally, separation of sympathetic responses from somatic responses could prevent unnecessary surgery in nonpancreatic conditions. Although this approach is logically appealing, differential epidural tests are not as precise as sometimes represented. Because of an overlap in the sensitivity of sympathetic and somatic fibers, the diffusion characteristics of the local anesthetic used, and the morphologic distribution of mixed spinal nerves, exact separation of patients with chronic pancreatitis into placebo, sympathetic, and somatic categories is not to be expected.[4]

This study has perhaps raised more questions than it has answered. Is there attrition with splanchnicectomy-induced analgesia over time? Should splanchnicectomy be combined with somatic nerve neurectomy? Do we completely understand the neuroanatomy of pancreatic pain afferents? Answers to these and other intriguing questions must be sought if only because neurectomy embodies two highly desirable characteristics for management of patients with chronic pancreatitis: preservation of residual pancreatic function and low surgical risk.

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Discussion

Dr. L. Stewart (San Francisco, Calif.). Did the decrease in the pain score correlate with the decreased need for analgesics?

Dr. E. L. Bradley. Yes. In general, they were parallel.

Dr. H. Reber (Los Angeles, Calif.). I am sure you agree that your numbers are small, and I think your follow-up is short too.

Dr. Bradley. Follow-up was 23 months on average.

Dr. Reber. In the patients who had some relief, was it permanent? If not, what did you do next?

Dr. Bradley. In this group, 10 out of the 13 had a good response, and eight continued to have a good response over a period of follow-up. There were two relapses at 12 months and 8 months, respectively, although the pain never returned to its original level. I would consider these two patients to be failures of the technique. Others who have used this technique for chronic pancreatitis have had similar experiences, that is, there is some failure with time, but it is not common.

Dr. K. Sharp (Nashville, Tenn.). Did you operate on both sides at the initial operation? Did you perform a bilateral thoracoscopic splanchnicectomy or did you use a unilateral or a staged procedure? Will these patients eventually develop problems such as pseudocyst or bile duct obstruction among others?

Dr. Bradley. We are using prone bilateral simultaneous synchronous procedures. We found that some patients, even if they had pain on the left side, would return with pain on the right side. So now we think the procedure should be bilateral. We do not believe the operation will have any effect on the natural history of chronic pancreatitis. Thus far we have not had any patient return with complications. Two of our patients have had a recurrence of clinical pancreatitis. When these denervation procedures are performed, the ability to detect peritoneal findings does not change.

Dr. M.G. Sarr (Rochester, Minn.). Is this really a good long-term successful operation?

Dr. Bradley. Professor Mallet-Guy actually reported 85% long-term good results in a group of 215 patients who underwent left celiac ganglionectomy. He did not remove the right side.

Dr. K. Lillemoe (Baltimore, Md.). Have you tried to use percutaneous celiac block as a predictor of results. I know it is a bit more invasive than an epidural, but could that give you more information with regard to predicting success? Finally, some people who talk about this technique have added a vagotomy. Have you considered adding a thoracoscopic vagotomy in hopes of helping with that percentage of pain that may be coming through other channels?

Dr. Bradley. Dr. Mayer has a similar group of patients undergoing thoracoscopic splanchnicectomy and he used celiac blockade as his method of patient selection. I think it is a good method. It is more difficult to perform than thoracic epidural and it also has some rather serious complications on occasion. So we have elected to use a procedure that can be performed without a fluoroscope and which has few complications associated with it. I think that the principle of using differential analgesia is an interesting one and it might be one that we can apply in the future. It is very difficult, when dealing with pain, to decide whether it is true sympathetic pain or some somatic condition that is masquerading as pancreatic pain.

Dr. Lillemoe. What about vagotomy?

Dr. Bradley. Vagotomy has been championed by Stone and his group. They have had remarkable success using an open splanchnicectomy, open thoracotomy, and a bilateral vagotomy and they found gastric stasis, which occurred in 11 of their 14 patients, was a small price to pay for the pain relief. Fourteen or 15 of their patients were not only cured of all of their pain but they were able to return to work and 10 out of 11 were no longer addicted to narcotics. Nevertheless, the question of whether a vagectomy carries any afferent pain fibers from the pancreas remains unanswered.

Cationic Liposome-Mediated Gene Transfer During Acute Pancreatitis: Tissue Specificity, Duration, and Effects of Acute Inflammation

Woody Denham, M.D., Jun Yang, M.D., Sally MacKay, Ph.D., Cynthia Tannahill, M.S., Gay Carter, B.A., Amer Abouhamze, B.S., Lyle L. Moldawer, Ph.D., James Norman, M.D.

Production of inflammatory cytokines in the pancreas, lung, and liver is believed to play a major role in the development of severe pancreatitis. This tissue-specific production could lend itself to directed anticytokine gene therapy if an appropriate delivery system could be developed. This study was undertaken to examine a novel approach for the delivery of protein-based therapies to the tissues involved during acute pancreatitis. Healthy mice received an intraperitoneal injection of cationic liposomes and a DNA plasmid containing the chloramphenicol acetyltransferase (CAT) reporter gene. Animals were killed at 12 hours and 1, 2, 3, 7, and 14 days with serum, pancreas, lung, and liver harvested. Acute pancreatitis was induced (cerulein, 50 μg/kg/hr intraperitoneally ×4) in additional mice before or after CAT transfection. The presence of pancreatitis was established in all animals by histologic scoring of pancreata and by serum amylase and lipase levels. CAT transfection efficiency was determined by quantitative CAT enzyme activity within tissue homogenates. Animals that received the liposome were successfully transfected with the CAT gene into the pancreas, lungs, and liver. Maximal transfection in each tissue occurred at 12 hours with decreasing CAT activity over the ensuing 14 days. No healthy animals receiving the CAT gene developed elevations in amylase, lipase, or any histologic parameter of pancreatitis. Transfection efficiency in the pancreas was markedly increased by preexisting or delayed induction of pancreatitis, whereas transfection of the lung and liver was increased to a lesser extent. Gene transfection into the pancreas, liver, and lungs is possible using a cationic liposome delivery system that does not induce pancreatitis or pancreatic inflammation. Pancreatic expression of the gene product is equal to or greater than that of the organs of the reticuloendothelial system and continues at very high efficiency rates during acute pancreatitis. (J GASTROINTEST SURG 1998;2:95-101.)

Acute pancreatitis is a severe noninfectious inflammation of the pancreas for which there is no specific therapy, mainly because of a poor understanding of its pathophysiology. Recent investigations implicate the proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) as major mediators responsible for the progression of acute pancreatitis. These cytokines are produced in high concentrations in the pancreas and subsequently in the lungs and liver of animals with experimental pancreatitis.[1-4] The importance of IL-1β and TNF-α during acute pancreatitis has been confirmed in a number of studies utilizing pharmacologic blockade that demonstrate attenuated severity and improved survival when either IL-1β or TNF-α is inhibited.[5-9]

The production of inflammatory cytokines in the pancreas, lungs, and liver during acute pancreatitis could lend itself to directed anticytokine gene therapy if an appropriate delivery system could be developed. Gene therapy offers a novel approach for the delivery of protein-based therapies to individual tissues involved in acute injury or inflammation and may

From the Departments of Surgery, University of South Florida, Tampa, Fla. (W.D., J.Y., G.C., and J.N.), and the University of Florida, Gainesville, Fla. (S.M., C.T., A.A., and L.L.M.).
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Reprint requests: James Norman, M.D., Department of Surgery, University of South Florida, Box 1289, Tampa General Hospital, Tampa, FL 33606.

be more effective than systemic administration of cytokine inhibitors, which is frequently an inefficient means of targeting excessive production in individual tissue compartments.[10,11] Historically the delivery of desired genes to target cells has been achieved by using naked DNA alone, viral delivery systems, or a combination of mammalian expression plasmids complexed with cationic liposomes.[10]

Previous studies of pancreatic transfection have centered on the use of adenoviral vectors. Direct pancreatic injection of an adenoviral vector demonstrated gene transfer into the pancreas, but acute pancreatic inflammation was induced (infiltration of leukocytes, enzyme release, and pancreatic edema) and the transfection was limited to those cells immediately adjacent to the site of injection.[12] Adenoviral mediated transfection by direct injection of the pancreatic duct will also achieve effective transfection but inflammatory cell infiltration is uniformly induced.[13] Pancreatic ductal injection of cationic liposomes has recently been studied, but this method resulted in only sporadic gene transfer to ductal cells and resulted in the induction of interstitial edema.[14]

The lack of an effective method to transfect foreign DNA into the pancreas has prompted these studies. In the hopes of finding a method by which we could regulate the pancreatitis-associated production of IL-1β and TNF-α in the pancreas, lung, and liver, we examined the transfection of these tissues using a recently described vector and cationic liposomes. Ideally, effective transfection of the pancreas would not induce pancreatic inflammation. Another ideal characteristic would include the ability to effectively transfect the pancreas, lungs, and liver during acute pancreatitis.

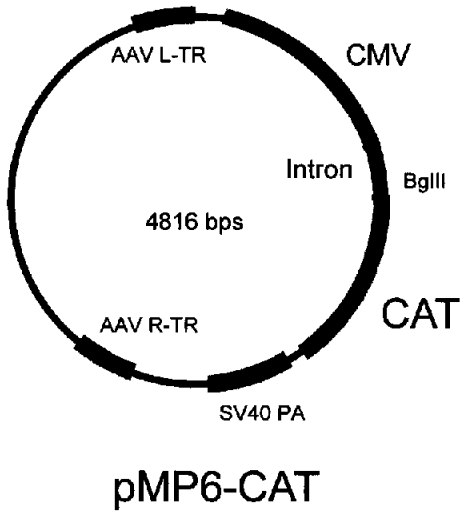

Fig. 1. Schematic map of plasmid pMP6-CAT detailing relevant structural components. PMP6-CAT is based on a pBR322 backbone with a cytomegalovirus *(CMV)* immediate-early promoter-enhancer, a 5′ rat preproinsulin heterologous intron, the CAT reporter gene, and an SV40 polyadenylation site. The promoter sequence and polyadenylation sites are flanked by adeno-associated virus terminal repeats.

MATERIAL AND METHODS

Animal studies were performed at an American Association for Accreditation of Laboratory Animal Care accredited facility in accordance with the Department of Animal Medicine at the University of South Florida. All animals were housed in 12-hour light/dark rooms with free access to standard laboratory chow and water.

Preparation of pMP6-CAT Plasmid

The bacterial reporter gene chloramphenicol acetyltransferase (CAT) was cloned into the pMP6 plasmid (Fig. 1), which was obtained from Mohan Philip (RPR-GenCell, Santa Clara, Calif.).[15] The plasmid expression cassette contains the cytomegalovirus immediate early promoter, which has the advantage of directing high levels of expression in a variety of tissues including lung, kidney, and vascular endothelium.[16] The combination of the cytomegalovirus early promoter, hybrid intron from the IgG variable region, and SV40 polyadenylation sequence is designed to increase the stability of the transgene messenger RNA (mRNA) and to direct the mRNA out of the nucleus and into the cytoplasm. Left and right adeno-associated virus terminal repeats flank the expression cassette and are intended to enhance transgene expression in primary and slowly dividing cells and to serve as viral origins of replication.

Alkaline lysis and potassium acetate precipitation (Plasmid Giga Kit, Qiagen, Inc., Chatsworth, Calif.) were used to isolate the plasmids. A commercial purification kit was used to remove endotoxin from the bacterial plasmid (LPS Extraction Kit, Qiagen, Inc.) with levels (1.1 to 4.4 endotoxin units [EU]/mg DNA) determined using a commercial limulus lysate assay (Etoxate test, Sigma Chemical, St. Louis, Mo.).

Cationic Liposome/DNA Preparation

Sterile, purified liposomes comprised of equimolar quantities of the cationic lipid dimethyldioctadecylammonium bromide (DDAB) and the neutral lipid dioleoylphosphatidylethanolamine (DOPE) in 1

mmol/L final concentration were obtained from RPR GenCell (Santa Clara, Calif.). Two hundred nanomoles of the cationic liposomes was added to 1 ml of lactated ringer's solution in a sterile conical tube. One hundred micrograms of pMP6-CAT DNA was added to the cationic liposomes, mixed gently, and allowed to stand at room temperature for 30 to 45 minutes prior to administration to the mice.

Animal Experiments

Transfection of Healthy Animals. Healthy adult male NIH Swiss mice (n = 30), weighing 20 to 25 g, were given a single intraperitoneal injection of liposomes containing 100 μg of the pMP6-CAT plasmid. All injections were performed with a sterile 25-gauge needle in the left lower quadrant of the abdomen. Mice were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and killed at 12 hours and 1, 2, 3, 7, and 14 days. Serum was collected for determination of amylase and lipase levels. Pancreata, lungs, and liver were harvested, immediately frozen in liquid nitrogen, and stored at −70° C until CAT activity was determined. Prior to placement in liquid nitrogen, the pancreas was divided and a portion fixed in 10% buffered formalin for histologic examination.

Transfection During Acute Pancreatic Inflammation. Additional adult male NIH Swiss mice (n = 8) underwent intraperitoneal injection of cationic liposomes and the pMP6-CAT plasmid. Twenty-four hours *later*, pancreatitis was induced by four hourly intraperitoneal injections of the cholecystokinin analogue cerulein (50 μg/kg). Animals were killed 8 hours later (maximal pancreatitis) and the pancreas, liver, and lungs were harvested as previously described.

To determine the effect of *preexisting* pancreatic inflammation on gene transfection, pancreatitis was induced in adult male mice (n = 8) using cerulein (50 μg/kg/hr intraperitoneally ×4). Cationic liposomes and the pMP6-CAT plasmid were administered intraperitoneally 8 hours after the induction of pancreatitis. The pancreas, lungs, and liver were harvested 48 hours after gene transfection to determine transfection efficiency.

Confirmation of Transgene Expression

Confirmation of transgene expression was through quantitative measures (butylation of [^{14}C]chloramphenicol) of bacterial CAT enzyme activity within the murine tissues. Briefly, frozen organs were homogenized in five weight:volumes of a mixture of 250 mmol/L Tris-HCl (pH 7.5), 5 mmol/L EDTA, and the protease inhibitors aprotinin (10 μg/ml), paraoxone (diethyl P-nitrophenyl phosphate, 1 mmol/L), and E-64 (trans-epoxysuccinyl-L-leucylamido-[4-guanido] butane, 0.183 μl/ml) (all from Sigma Chemical). After homogenization, tissue was subjected to two freeze-thaw cycles followed by centrifugation at 16,000 ×*g* for 5 minutes. One hundred microliters of the organ extract was added to 200 nmol of n-butyryl coenzyme A and 0.05 μCi of [^{14}C]chloramphenicol with overnight incubation of the mixture at 37° C. The butylated chloramphenicol species were then extracted into mixed xylenes and analyzed by liquid scintillation spectrometry. Data are presented as milliunits of CAT activity (mEU) per milligram of tissue protein ± standard error of the mean (SEM). The specific activity of recombinant CAT is approximately 8×10^8 mEU/mg protein. Total protein concentration of the cytosolic extract was determined with Bradford reagent (Sigma Chemical).

Determination of Pancreatic Injury

Serum amylase and lipase levels were determined by means of a Kodak Ektachem 700 automated analyzer standardized for these murine proteins (Eastman Kodak Company, Rochester, N.Y.). All samples were run in triplicate and averaged. Pancreatic tissue was collected and fixed in 10% buffered formalin. Fixed pancreatic tissues were embedded in paraffin and stained with hemotoxylin and eosin. Blind histologic grading of pancreatic edema, necrosis, and vacuolization (range 0 to 4) was performed on 10 high-power fields from each sample as previously described.[6]

Statistical Methods

Results are expressed as mean ± SEM. Statistical analysis was performed using the EPISTAT statistical program (Epistat Services, Richardson, Tex.) applying unpaired two-tailed Student's *t* test with significance being assigned to *P* values <0.05 unless otherwise stated.

RESULTS

Transfection in Healthy Animals

CAT transgene expression in the pancreas was highest 12 hours following transfection (803 ± 370 mEU/mg protein; Fig. 2, *A*). Pancreatic CAT activity decreased over the remainder of the experiment with detectable levels present even at 2 weeks. The lung tissue of healthy animals demonstrated peak CAT activity at 12 hours and 1 day (Fig. 2, *B*) with levels progressively declining over 2 weeks. Transgene

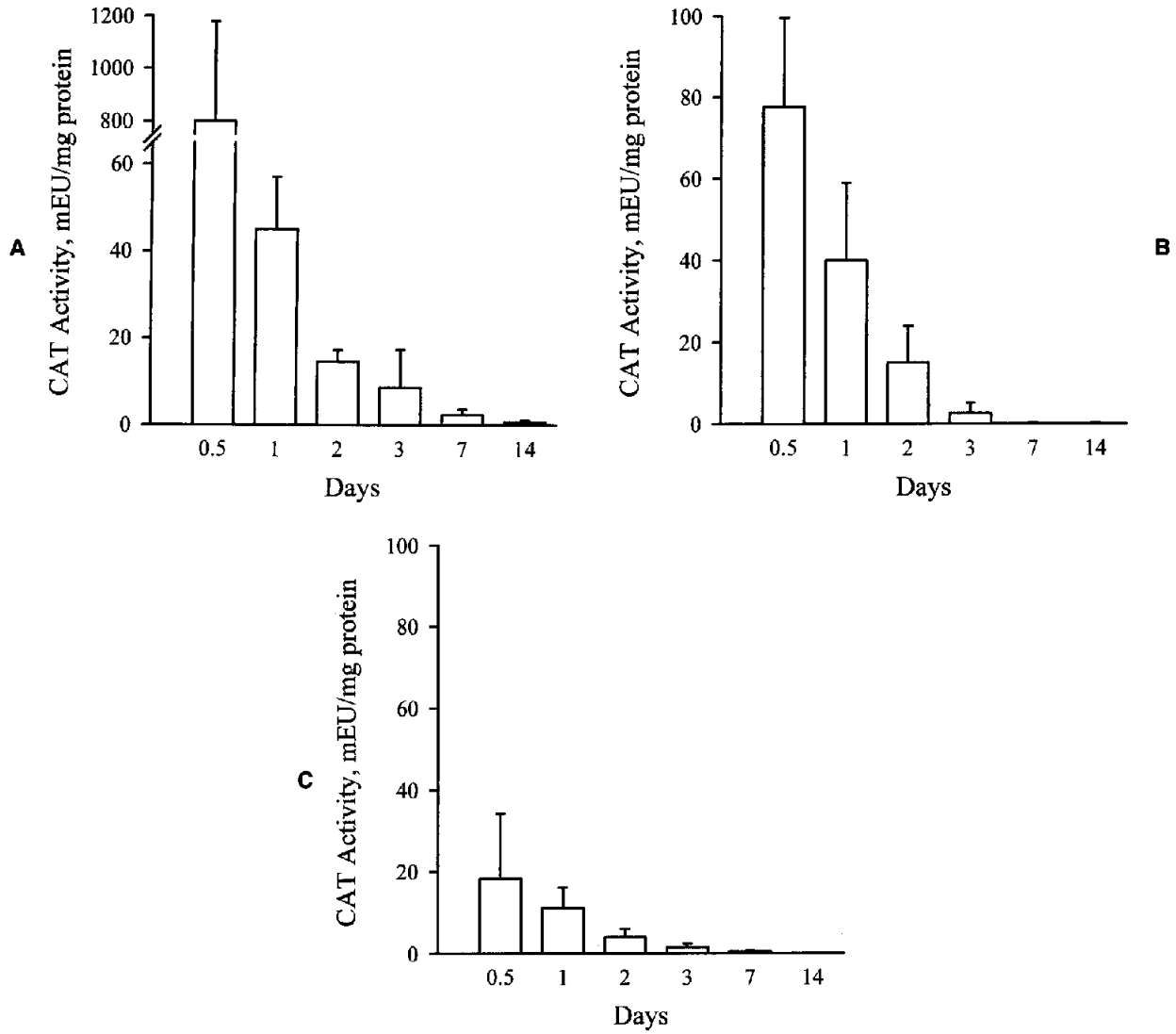

Fig. 2. Time course of pancreatic, pulmonary, and hepatic chloramphenicol acetyltransferase *(CAT)* activity following a single intraperitoneal injection of cationic liposomes and the pMP6-CAT plasmid. Healthy mice received 100 μg of pMP6-CAT and 200 nmol of cationic liposomes. Pancreatic CAT activity **(A)** is highest at 12 hours, and it declines over the 2-week time course. Maximal pulmonary **(B)** and hepatic **(C)** transfection is seen at 12 hours with a steady decrease in CAT activity over the following 14 days.

expression in the liver (Fig. 2, *C*) was demonstrated throughout the time course with the highest CAT activity measured at 12 hours (18 ± 16 mEU/mg protein).

Effect of Transfection on Pancreatic Enzymes and Histology

Serum amylase and lipase (Fig. 3) were unchanged over the entirety of the time course in healthy animals with effective transfection of the CAT gene into their pancreas (P = not significant [NS] vs. normal animals). Table I demonstrates pancreatic histology in normal mice and those that underwent gene transfection. There was no change in inflammation, necrosis, edema, or vacuolization in animals transfected with the CAT gene (P = NS vs. normal pancreata).

Effect of Pancreatic Inflammation on Transgene Expression

Cerulein pancreatitis was induced in two separate groups, one before and one after CAT transfection. CAT transfection with subsequent induction of pan-

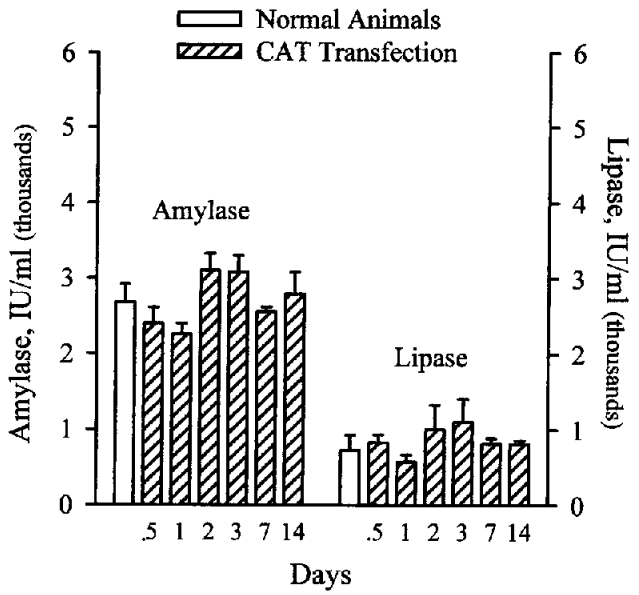

Fig. 3. Serum amylase and lipase from healthy animals transfected with the chloramphenicol acetyltransferase *(CAT)* reporter gene. Pancreatic enzyme secretion remains unchanged following successful gene transfer.

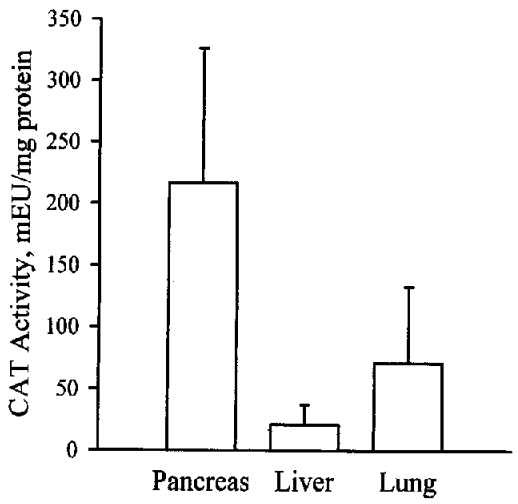

Fig. 4. Chloramphenicol acetyltransferase *(CAT)* activity in animals that underwent cationic-mediated gene transfer followed by cerulein pancreatitis 24 hours later. The tissue levels of CAT are fourfold greater when pancreatitis is induced after gene transfection in the pancreas, lungs, and liver.

creatitis 24 hours later resulted in CAT activity in the pancreas, lung, and liver (Fig. 4) that was fourfold greater compared to the activity in the organs of healthy animals. CAT transfection following acute pancreatitis induction resulted in CAT activity in the pancreas and liver (Fig. 5) that was 10-fold higher than the activity in healthy animals. The lungs of an-

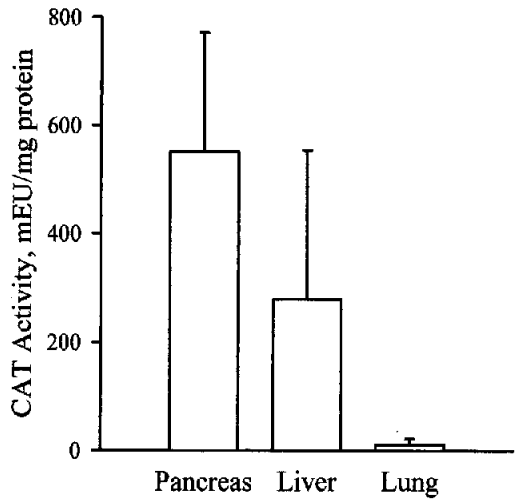

Fig. 5. Tissue activity of the reporter gene chloramphenicol acetyltransferase *(CAT)* in mice undergoing induction of pancreatitis 8 hours prior to cationic liposome-mediated gene transfection. Preexisting pancreatic inflammation results in a 10-fold increase in pancreatic and hepatic CAT activity compared to these tissues in animals that underwent transfection alone without the induction of pancreatitis.

Table I. Blind histologic scoring of pancreata*

	Normal	CAT transfection
Inflamation	0 ± 0	0 ± 0
Edema	0 ± 0	0.1 ± 0
Necrosis	0 ± 0	0 ± 0
Vacuolization	0 ± 0	0 ± 0

*Histologic sections of each pancreas were graded in a blinded fashion for inflammation, edema, necrosis, and vacuolization. Normal tissues were assigned a value of 0, whereas maximal severity for each parameter was assigned a value of 4. Values represent the mean ± standard error of the mean for 10 fields from each pancreatic specimen.

imals that were transfected after the induction of pancreatitis demonstrated CAT activity that was similar to that of lungs from normal animals.

DISCUSSION

The induction of acute pancreatitis is associated with a massive release of the inflammatory cytokines IL-1β and TNF-α from the pancreas, lungs, and to a lesser extent the liver.[1-4] By preventing the actions of these cytokines using pharmacologic blockade, pancreatic and lung injury is decreased and the mortality rate of these experimental pancreatitis models is cut in half.[5-9] In the hopes of finding a method by which we could regulate the pancreatitis-associated production of IL-1β and TNF-α in these tissues, this study was

designed to determine if a gene delivery system could be developed. We hypothesized that an ideal delivery system would allow active protein to be made in each of the organs known to produce cytokines during acute pancreatitis while having no ability to induce pancreatitis or tissue inflammation. Another ideal characteristic would include the ability to effectively transfect the pancreas, lung, and liver during the severe inflammation associated with acute pancreatitis.

Attempts to transfect the pancreas using direct injection of a first-generation recombinant adenoviral vector provided effective gene transfer but also resulted in pancreatic inflammation and tissue destruction.[12] This same vector was delivered by pancreatic duct injection, which again demonstrated gene transduction, but pancreatitis was induced.[13] Previous studies examining transfection by injection of cationic liposomes into the pancreatic duct resulted in occasional transfection of acinar cells, ductal epithelium, and blood vessels, but this transfer also resulted in interstitial edema.[14] Preliminary work in our laboratory[15] and experiments by others[17-20] have demonstrated effective transfection of a number of organs including the lung, lymph nodes, and spleen utilizing cationic liposomes. The ability to transfect the pancreas with this delivery system, however, has not been previously examined.

The pMP6-CAT expression vector used in these experiments has several novel characteristics that have been demonstrated to increase transgene expression. Liu et al.[20] have recently shown that the cytomegalovirus immediate-early promoter-enhancer is more active than vectors containing either SV40, adenovirus, or thymidine kinase promoters.[20] The cytomegalovirus immediate-early promoter-enhancer drives transgene expression at rates 5- to 1000-fold higher than these viral promoters. The pMP6-CAT plasmid does not contain viral origins of replication, per se, but does contain flanking terminal repeats obtained from adeno-associated virus, which are equivalent.[21] Philip et al.[21] have demonstrated under ex vivo conditions that transfection with a similar plasmid results in extended transgene expression for periods exceeding 30 days.

In the current study, CAT transgene expression in the normal, noninflamed pancreas was highest at 12 hours with activity decreasing over the ensuing 14 days. It is important to note that this study demonstrated that successful pancreatic transfection by means of an intraperitoneal injection of cationic liposomes did not result in an elevation in pancreatic enzyme levels, and there was no change in pancreatic edema, inflammation, vacuolization, or necrosis compared to values in normal animals that were not transfected. Our data differ from those of previous studies that used direct adenoviral injection into the pancreas or pancreatic duct and reported acute pancreatic inflammation with transfection.[12,13] Adenoviral vectors are known to induce inflammation in other organs.[22] In contrast, work by Canonico et al.[23] demonstrated that cationic-mediated gene transfer to the lung was not associated with adverse effects on pulmonary histology, lung compliance, lung resistance, or alveolar-arterial oxygen gradient. Our work is consistent with the later finding in that effective pancreatic transfection using intraperitoneal cationic liposomes did not induce pancreatic inflammation biochemically or histologically.

The lungs and to a lesser extent the liver produce IL-1β and TNF-α during pancreatitis. These organs are known to manifest dysfunction in severe pancreatitis, which is believed to be related to the production of these cytokines[1,4]; therefore the ability to transfect the lung and liver with this delivery system was investigated. Both of these organs are effectively transfected by means of the cationic liposome-mediated gene delivery system with pulmonary levels of CAT activity similar to those seen in the pancreas, whereas hepatic levels were slightly lower. In both organs, peak CAT activity was seen at 12 and 24 hours with declining levels over the 14-day experiment. These results are consistent with work by Philip et al.,[17] Zhu et al.,[18] Liu et al.,[20] and several of our coworkers[15] who examined transgene expression of the heart, lung, lymph nodes, and spleen using a cationic liposome delivery system.

Animals undergoing transfection of CAT followed 24 hours later by the induction of pancreatitis demonstrated nearly a fourfold increase in transgene expression in the pancreas, lungs, and liver compared to the same organs from animals following transfection alone in the absence of pancreatitis. Enhanced cationic-mediated gene transfer in the lung has previously been demonstrated during peritonitis secondary to cecal ligation and puncture and following intratracheal administration of endotoxin; however, the increased activity in the pancreas and liver during acute inflammation has not been previously reported.[15]

In a similar fashion, CAT activity in mice transfected 8 hours *after* the induction of acute pancreatitis was increased almost 10-fold in the liver and pancreas. The cationic liposomes employed in this study, which are based on equimolar quantities of DOPE and DDAB, appear to favor uptake by lymphocytes and inflammatory cell populations, which may account for the increase in CAT activity following the induction of pancreatitis, since numerous studies have demonstrated that the pancreas is infiltrated with white blood cells during acute pancreatitis.[3,4,17]

This study demonstrates that transfection of the

pancreas using a cationic liposome delivery system results in tissue expression of the reporter gene for up to 2 weeks with no induction of pancreatic inflammation. On a more clinically relevant note, the efficiency of pancreatic transfection is dramatically increased when gene transfer is performed during acute pancreatitis. These findings suggest that this is the most ideal mechanism to deliver gene-based therapies to the pancreas in the absence or presence of acute inflammation.

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Prospective Investigation of Complications, Reoperations, and Sustained Weight Loss With an Adjustable Gastric Banding Device for Treatment of Morbid Obesity

Cornelius Doherty, M.D., James W. Maher, M.D., Debra S. Heitshusen, R.N., B.S.N.

The purpose of this study was to determine prospectively the safety and efficacy of an adjustable silicone gastric band and reservoir system for the treatment of morbid obesity. Between 1992 and 1995, forty primary procedures were performed. Twenty-six females and 14 males entered the study. The mean age of the subjects was 34 years (range 19 to 51 years). Mean body mass index was 50 kg/m^2 (range 39 to 75 kg/m^2). There were no deaths. Mean body mass index (in kg/m^2) at follow-up visits was 38.4 at 1 year, 38.0 at 2 years, 40.2 at 3 years, and 40.4 at 4 years. These decreases were significant at $P < 0.001$. Thirty-two reoperations (12 intra-abdominal procedures and 20 abdominal wall procedures) have been necessary to maintain efficacy or correct complications. At the four-year interval, the reoperation rate of 80% was unsatisfactory. The excess weight loss has been 41% for those subjects who have an intact gastric band system and continue in the study. Improvements to the implantable band and/or operative technique must be implemented and studied long term if this procedure is to become an accepted surgical treatment for severe obesity. (J GASTROINTEST SURG 1998;2:102-108.)

The concept of banding the stomach to restrict food intake and provide an early sense of satiety as surgical treatment of morbid obesity is appealing. Desirable features include (1) no direct injury to the stomach, (2) access for endoscopic and radiographic studies, and (3) and ease of reversibility. Wilkinson and Peloso,[1] who used a 2 cm wide strip of polypropylene mesh, were the first surgeons to apply gastric banding. Preliminary reports from small series of patients who underwent Dacron gastric banding were promising.[2-4] Granstrom and Backman[5] were the first to measure the tension applied to the band by using a dynanometer. With endoscopic measurement Naslund[6] observed the widening of the stoma diameter over time. Kuzmak[7,8] used a 1 cm wide gastric band made of Dacron-reinforced silicone in conjunction with a special band-tightening instrument and a calibration tube connected to an electronic sensor to objectively measure pouch volume and outlet diameter. He later added a silicone balloon that was 4 cm long on the inner side of the band, which was connected by tubing to a self-sealing reservoir implanted in the rectus sheath to allow percutaneous adjustment of pouch outlet diameter by adding or removing normal saline solution under fluoroscopic guidance. This study was planned as a prospective long-term investigation of the safety and efficacy of this investigational device.

MATERIAL AND METHODS

The adjustable silicone gastric band (ASGB; Inamed Development Company, Carpinteria, Calif.) is a Dacron-reinforced silicone band, 22.5 cm long, 1 cm wide, and 3 mm thick (Fig. 1). The exterior surface of the band is made of soft silicone elastomer, chosen for its biocompatability and biologic inertness. The band is radiopaque. An inflatable section, 4 cm in length, is outlined on the band by radiographic markers. A radiopaque nonkinking 60 cm tube is attached to connect the inflatable portion to the subcutaneous reservoir. The self-sealing reservoir is for percutaneous

From the Department of Surgery, School of Medicine, University of Iowa, Iowa City, Iowa.
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Reprint requests: Cornelius Doherty, M.D., Department of Surgery, 4628 JCP, University of Iowa Hospitals and Clinics, 200 Hawkins Dr., Iowa City, IA 52242-1086.

band volume adjustment. A titanium plate at the base of the reservoir prevents penetration by the needle when adjusting the diameter of the stoma. Stoma adjustment may be performed without reoperation by accessing the reservoir under fluoroscopic guidance.

The calibration tube is a dual-lumen translucent silicone tube, 152 cm in length and 9 mm in external diameter (Fig. 2). Six centimeters from the proximal end is a 25 ml balloon for controlled sizing and positioning of the gastric pouch. It is inflated via an inflated port, which remains external during the procedure. Tightening of the band around the stomach tissue compresses the distal 3.5 cm of the calibration tube, which has a 13 mm external diameter. The change in pressure within the calibration tube is displayed on the attached gastrostenometer. This pressure-measuring device allows the surgeon to tighten the gastric band to a reproducible calibrated pouch outlet diameter.

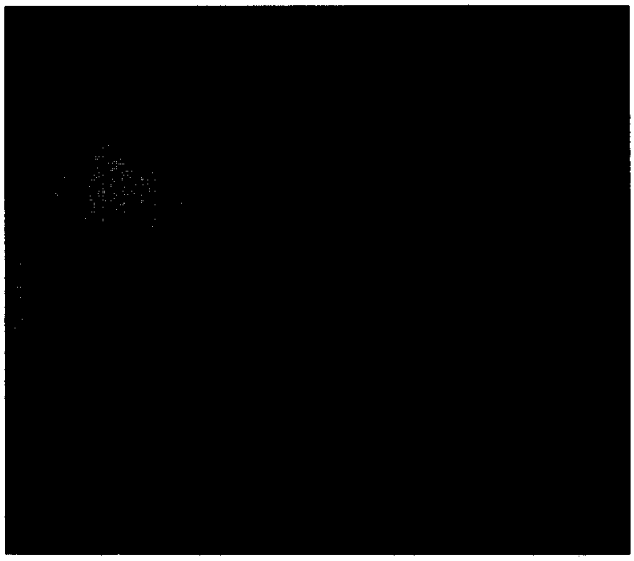

Fig. 1. Adjustable silicone gastric band, tubing, and injection port system showing adjustable part of band.

The gastrostenometer is a battery-powered electronic sensor designed to be used with the calibration tube of the ASGB system. The gastrostenometer and calibration tube are used to calibrate the stoma. The gastrostenometer has a display bar containing 10 lights. The stoma of the proximal pouch is reduced in diameter by compression of the stomach tissue within the band. Further tightening of the band applies pressure to the distal segment of the intragastric calibration tube and activates movement on the light scale of the gastrostenometer. Movement of the light to the fourth position from the left corresponds with an outlet diameter of 12.5 mm.

Procedure

This study has been subject to and is in compliance with all applicable governmental rules and regulations concerning the conduct of clinical trials of experimental devices. The study was approved by the University of Iowa Human Subjects Institutional Review Board, and informed consent was obtained from all subjects. Acceptance criteria for the study included the following: (1) Obese subjects who were 45 kg (100 pounds) overweight as defined by the Metropolitan Life Insurance tables of 1983; (2) failure to sustain

Fig. 2. Calibration tube and gastrostenometer showing inflatable portion to estimate pouch volume and air chamber to calibrate the stoma.

weight loss with nonoperative programs; (3) no previous history of surgical treatment for obesity; (4) age 18 to 51 years; and (5) history of severe obesity for 5 or more years. In addition, all subjects were required to complete two office visits, 30 days apart, prior to surgery. The purpose of these visits was to ensure informed consent and to document the medical history relationship to obesity. All subjects were enrolled in a diet and behavior modification program. Patients were dropped from the study for the following reasons: (1) pregnancy, (2) conversion to another bariatric procedure, or (3) unwillingness to continue in the study with or without band removal.

Operative Technique

An upper midline incision was made. At the midpoint of the wound, a small horizontal incision was made through the right anterior rectus sheath and the underlying muscle for later accommodation of the implanted reservoir. The calibration tube was passed through the mouth and inflated with 25 cc of air to aid in sizing the pouch. The calibration tube was then pulled back by the anesthesiologist until resistance from the esophagogastric junction was encountered. The gastrohepatic ligament was opened and a retrogastric tunnel was made through the lesser sac and a small opening was dissected through the gastrophrenic ligament. This site was cephalad to the first short gastric artery. A Penrose drain was placed to secure this tract. Another opening was then made through the anterior and posterior leaves of the gastrohepatic ligament. This location was 2 cm below the esophagogastric junction, adjacent to the lesser curvature, and verified by the calibration tube position. The Penrose drain was repositioned through this new opening. The ASGB was then prepared for implantation by irrigating the inflatable bladder segment with sterile normal saline solution to evacuate retained air. One milliliter of saline solution was injected into the connecting tubing to the inflatable bladder and then secured with a rubber-shod clamp. The gastric band was then positioned around the stomach in the tract secured by the Penrose drain. The Penrose drain was then removed. The gastric band was tightened by hand below the balloon and over the pressure-sensitive segment of the calibration tube. A specially designed band-tightening instrument was used to tighten the band until the fourth light from the left was illuminated on the gastrostenometer. This corresponded to a 12.5 mm stoma diameter. A special band-holding instrument was placed over the band to secure the measured diameter. The position of the band was secured by suturing the band to itself with four 2-0 nonabsorbable sutures (Fig. 3). The calibration tube and banding instrument were removed. To further secure the band position, three to five nonabsorbable 2-0 interrupted sutures were placed to oppose the wall of the distal stomach to the pouch over the band. The excess band and buckle were excised. The silicone tubing leading from the inflatable band was directed between the diaphragm and the superior surface of the liver for delivery through the right posterior rectus sheath to the site previously prepared for

Fig. 3. Adjustable silicone gastric band in situ following suturing of the band to itself, demonstrating the small pouch and inflatable band.

implantation of the reservoir. The reservoir was irrigated with sterile normal saline solution and then joined via a metal connector to the silicone tubing from the band. These junctures were secured with 2-0 nonabsorbable ligatures. The reservoir implantation was completed by placing four 2-0 nonabsorbable ligatures through the posterior right rectus sheath and the openings at the base of the reservoir. The incision in the anterior rectus sheath was closed with interrupted 2-0 polyglyconate sutures. The midline abdominal incision was closed with a running No. 1 polypropylene suture.

Postoperative Care

After the operation, patients were placed on a 900- to 1200-calorie balanced gastroplasty diet of liquid and soft foods. All patients received daily a chewable multiple vitamin tablet with iron. They were instructed in early recognition of satiety signals and proper eating behavior modifications by a registered dietitian. They were discharged from the hospital when their nutritional intake was adequate and their incisional pain was relieved with oral analgesics. Follow-up visits were scheduled at 1 month with subsequent visits every 2 months for 1 year, every 3 months for a second year, and then annually or as needed. Diet and behavior modification sessions were conducted at each visit. Weight loss was expressed as percentage of excess weight loss using the 1983 Metropolitan Life Insurance Company tables and also change in body mass index. Patients were considered for band tightening if their weight loss plateaued and they no longer had a sense of early satiety with meals. The band was loosened if they had symptoms of postprandial reflux, nausea and vomiting, excessive weight loss, or pregnancy. Band adjustment was carried out by visualizing the reservoir on fluoroscopy and accessing it with a spinal needle for volume adjustment.

Statistical Analysis

A paired *t*-test was used for statistical analysis of weight loss.

RESULTS

Forty consecutive patients underwent adjustable silicone gastric banding for severe obesity between March 1992 and May 1997. Follow-up was complete in all patients and ranged from 13 to 62 months (mean 38 months). There were 26 women (65%) and 14 men (35%). Mean age was 34 years (range 19 to 51 years). Mean height was 171 cm (range 152 to 190 cm). Mean weight was 147 kg (range 100 to 214 kg). Mean excess weight was 82 kg (range 46 to 137 kg). Mean percentage of excess weight was 226% (range 174%-326%). Mean body mass index was 50 kg/m^2 (range 39 to 75 kg/m^2). Gastric band placement was technically straightforward and without intraoperative complications. The calibration tube and gastrostenometer functioned well. The band-tightening tool was awkward to use and required preliminary tightening of the band by hand in order for it to function appropriately. There were no early postoperative complications and no operative deaths. All patients underwent upper gastrointestinal endoscopy at 1 year postoperatively to rule out band erosion. There have been no difficulties with band erosion through the wall of the stomach. The mean sustained weight loss observed at annual evaluation was 35 kg at 1 year, 36 kg at 2 years, 33 kg at 3 years, and 29 kg at 4 years. Mean body mass index decreased from 50 kg/m^2 prior to surgery to a low of 38 kg/m^2 at 2 years following the operation. There was a small increase in body mass index to 40 kg/m^2 at 3 and 4 years. Mean percentage of excess weight loss was 44% at 1 year, 47% at 2 years, 40% at 3 years, and 41% at 4 years. All of these decreases in body mass index, weight, and percentage of excess weight loss were significant at $P < 0.001$. It should be noted that these comparisons were made only for patients who remained in the study with an intact gastric band system. The percentages of excess weight loss at yearly intervals are shown in Table I.

It has not been possible to evaluate the benefit of the adjustable feature of the gastric band because of the unreliable performance of the reservoir. Twenty patients have required reoperations for failure of the reservoir. Fifteen of the procedures were required because of leakage of the reservoir secondary to failure of the seal of the titanium baseplate to the silicone reservoir or failure of the self-seal of the silicone dome. Two patients required removal of the reservoir because of infection. Two reservoirs flipped over precluding percutaneous access and required operative repositioning to reestablish access. There was one in-

Table I. Percentage of excess weight loss by year

Interval (yr)	>25% Excess weight loss Percent (No.)	>50% Excess weight loss Percent (No.)
1	74 (29/39)	42 (17/39)
2	76 (26/34)	44 (15/34)
3	62 (15/24)	33 (8/24)
4	75 (9/12)	25 (3/12)

cident of laceration of the reservoir tubing by the needle. Defective reservoirs were replaced with an improved substitute. There was no further occurrence of this complication.

Three patients began to regain weight and did not respond to narrowing of the band outlet. Subsequent radiologic evaluation of the integrity of the band system revealed aneurysmal deformity of the inflatable balloon portion of the band in all three. One of these patients has undergone operative removal of the band. Inspection confirmed the aneurysmal deformity of the balloon. The other two bands with aneurysmal deformity remain in place. Thirty-eight percent of patients (n = 15) have required at least one hospitalization for symptoms of postprandial nausea, vomiting, and severe gastroesophageal reflux. All of these patients were managed initially with deflation of the inflatable balloon of the gastric band, fasting, and parenteral fluids and nutrients. Radiologic evaluation revealed enlarged proximal pouches with delayed or absent pouch emptying and severe reflux. Four patients responded to nonoperative treatment and became asymptomatic. Narrowing of the pouch outlet channel and pouch enlargement both resolved. The remaining 11 patients have required laparotomy. Three of them were found to have only an enlarged pouch. The adjustable silicone gastric band was removed and a new smaller pouch was created and maintained with a LapBand (Bioenterics Corp., Carpinteria, Calif.). The mean time interval for occurrence of an enlarged pouch was 37 months (range 21 to 55 months).

Eight patients (20%) who had obstructive symptoms at reoperation had herniation of the posterior wall of the distal stomach through the band. The mean time interval after operation for occurrence of this complication was 24 months (range 14 to 33 months). These patients have been treated by band removal. One patient in this group admitted to bulimic behavior and no further procedure was performed. Four patients had their bands removed after which a new pouch was measured and another ASGB placed with posterior sutures to imbricate stomach tissue over the band. There has been no recurrence of posterior herniation in these patients, but two have developed an enlarged pouch. These patients remain in the study. Three patients have undergone band removal and concomitant vertical banded gastroplasty. Reasons for the 32 reoperations are summarized in Table II.

After 62 months, 24 subjects remain in the study. Sixteen subjects (40%) have withdrawn from the study. Five (13%) did not want to have an ineffective ASGB removed operatively. Two (5%) had their ASGBs removed operatively. Five (13%) had an ASGB removed and a vertical banded gastroplasty performed at the same operation. Two had an ASGB removed and replaced with a LapBand, which was implanted during the same operation. Two patients became pregnant. Their bands were deflated but remain in place. The mean time of withdrawal from the study was 25 months (range 13 to 38 months). Many of the earlier observations of Kuzmak[7,8] were confirmed in this study. Revision and removal of the ASGB was relatively easy to perform since the band was encapsulated and did not adhere to the stomach wall.

Table II. Summary of reoperations

Type of operation	No.	Reason for reoperation
Intra-abdominal		
	8	Posterior herniation
	3	Enlarged pouch
	1	Patient request
	1	Aneurysmal deformity
TOTAL	13	
Abdominal wall		
	15	Reservior failures
	1	Tube laceration
	2	Flipped reservoirs
	2	Infected reservoirs
TOTAL	20	

DISCUSSION

Gastric banding has been used in the surgical treatment of morbid obesity for 20 years. The goal has been to develop a procedure that is safe and effective. Some favorable trends have been noted. The incidence of early (<30 days) postoperative complications has decreased. Advances in materials and techniques of operative placement have evolved. The previously reported problem with band erosion through the gastric wall has been diminished as a result of several factors including: (1) use of the Dacron-reinforced silicone band, (2) stabilization of band placement by suture apposition of the stomach wall above and below the band, and (3) abandonment of the practice of suturing the band to the gastric wall.

Band placement has become more cephalad on the stomach to create a smaller proximal pouch. Objective methods for calibration of the pouch outlet diameter have been developed. The need for early reoperation because of a narrow outlet has become an

infrequent occurrence compared to what was previously the case.

Nevertheless, certain problems persist. Outlet widening occurs when the accordion-like folding of the gastric wall inside the band attenuates over time.[6-8] This leads to loss of early satiety with meals, less restricted eating, and weight regain. The inflatable balloon was added to the gastric band to correct for this change and to avoid the need for an intra-abdominal revisional reoperation. Unfortunately, in this study the benefit of the adjustable component of the band cannot be evaluated because of the high incidence of reservoir failure. The question remaining to be answered is this: Will regulation of the stoma diameter, if the proximal pouch is small, be a useful adjunct for weight loss maintenance?

Another distressing problem that has persisted is the herniation of the distal stomach through the band. Kirby et al.[9] described this as a reverse intussusception. Each episode of herniation in this study was preceded by an episode of vigorous vomiting. The end result of herniation is that the band constricts stomach tissue causing luminal obstruction. Emptying the reservoir system to decompress the inflatable balloon and relieve the obstruction has not corrected the problem. Operative intervention has been necessary. All patients with a gastric band remain at risk for this complication. It may be that suturing the posterior wall of the stomach over the band will reduce the incidence of this complication. This seems to have been effective anteriorly.

Gastric banding has reintroduced an old phenomenon. Vertical banded gastroplasty and Roux-en-Y gastric bypass have evolved to a lesser curvature pouch with exclusion of the distensible fundic tissue from the pouch. With these procedures, if the patient overeats, pouch pressure can be dissipated into other areas. In gastric bypass patients will experience dilatation of the gastrojejunostomy stoma, enlargement of the pouch, or disruption of the gastric partition. Gastroplasty patients may experience disruption of the stapled partition or enlargement of the proximal pouch. Gastric banding procedures, however, include fundic tissue. The fundus normally functions to accommodate large meals by receptive relaxation. A procedure that includes fundic tissue may have an innate propensity to cause dilation. The fundus may not be suitable for construction of a pouch that must remain small to maintain weight loss.

Others have also reported a high incidence of reoperation in their gastric banding procedures.[5,9-11] Hallberg and Forsell[12] and Forsell et al.[13,14] have been in the process of developing the Swedish adjustable gastric band and the Stockholm gastric restriction method since 1985. The lumen of the Swedish band can be varied within a wide range, 0 to 40 mm, compared to the 0 to 4 mm for the adjustable silicone gastric band designed by Kuzmak that was used in this study. Gastric banding with minimally invasive laparoscopic technology is widely available in Europe, Australia, and Latin America. A second generation of adjustable silicone gastric band, the LapBand, is presently being investigated at eight sites in the United States.

CONCLUSION

Thirty-two reoperations were necessary in the 40 patients studied over this 4-year period to maintain the ASGB system or correct major complications. Twenty reoperations were limited to the anterior abdominal wall to correct reservoir problems. An improved reservoir was placed and seems to have eliminated the problems associated with leakage. The efficacy of the adjustable feature of the ASGB could not be evaluated in this study because of the reservoir defects and a recently identified problem with aneurysmal deformity of the balloon. Twelve major intra-abdominal reoperations have been required to correct posterior herniation of the stomach through the ASGB and/or pouch enlargement. The long-term incidence of this complication is unknown at this time. A reoperation incidence of 80% is unacceptable. The challenge for future investigators is to present complete prospective long-term data on improvements in the band system or operative technique that would make the procedure acceptable.

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Discussion

Dr. R. Brolin (New Brunswick, N.J.). I know that you and your colleagues have performed more than 1000 gastroplasties and I would like you to comment briefly your impressions of the banding technique presented in this report relative to the gastroplasty technique, particularly with reference to postprandial vomiting, reoperation, and long-term weight loss. I would also like your opinion regarding the future of this evolving technology.

Dr. C. Doherty. This procedure mimics very closely the horizontal gastroplasties that are no longer standard practice. I think a procedure that includes much fundic tissue eventually will result in dilatation and lead to a need for reoperation. The present evolution of the Roux-en-Y gastric bypass and vertical banded gastroplasty is a small pouch close to the lesser curvature with inclusion of as little fundic tissue as possible. These changes have been accompanied by less postprandial vomiting, less need for reoperation, and improved maintenance of weight loss.

Dr. B. Schirmer (Charlottesville, Va.). I would like your opinion after your initial experience. Could you clarify for us the magnitude of weight loss? What percentage of excess weight loss was experienced?

Dr. Doherty. The initial excess weight loss is approximately 45% to 47% and then it drops to 42%. After studying this subject for 18 years, I am convinced that there are two major concerns. One is that it is difficult to select the proper patient for this type of surgery and provide the proper initial procedure. In addition, fundic tissue cannot be included in a gastric restrictive procedure. There is no question that there will be patients who will need a procedure that combines gastric restriction with malabsorption.