Abandonment

Josef E. Fischer, M.D.

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I am extraordinarily pleased to have served this organization over the past decade in a variety of positions, and I am especially pleased to have had the honor of being your president. This is a very different organization from the one I joined 30 years ago. It is vibrant, alive, forward-looking, and very active. We owe a lot to my predecessors in this position and the other volunteers who have served on the council and on the various committees to change the organization to what it now is. I am also profoundly indebted to my wife, Karen, and my children for their support, love, and understanding over the past years, now numbering almost 43 with respect to Karen. While I have been at work or traveling, she has been both mother and father to our children, Alexandra and Erich, as well as my own anchor to windward and has provided the stability and love that only a steadfast partner can offer. I love and respect her, and I want to once again acknowledge this debt publicly.

We now find ourselves in an interesting situation. I would have predicted that at this point the field of general surgery—and gastrointestinal surgery, in particular—would have been in an extraordinary situation, one that we really would have enjoyed. In the mid-1980s, a prominent leader of American surgery declared that general surgery was dead and prophesied the emergence of various specialties with the disappearance of the general surgeon. Happily, that prophecy has proved to be wrong, and in fact general surgery has been resuscitated by minimally invasive surgery and other factors in a way that few of us could have foreseen. This impetus has given great presence to general surgery, and despite the emergence of various subspecialties within general surgery, and even within gastrointestinal surgery, the future for general surgery and gastrointestinal surgery should have been extraordinarily bright.

But it is not. "We are surrounded by alarums and excursions," as Shakespeare said, particularly in the area of our lifeblood, the future, the young people whom we count on to pick up the banner and carry it forward with pride, innovation, and vibrancy. I am speaking about the "generation Xers"—those medical students who are products of a different society, have different priorities, and have said to us that although they enjoy surgery, in general, and gastrointestinal surgery, in particular, they have different priorities. They want to have a life. They want to do other things besides work. They want to watch their children grow up. They want to go to soccer games and, yes, perhaps even coach those soccer games. They want to go to PTA meetings. They want to read to their children before putting them to bed. This naturally assumes that their children would be awake by the time they get home. They want to have a family life. They do not want to spend their lives in the hospitals as we did. They do not necessarily want to operate from 7:30 AM to 8:00 PM, as we do. Yes, they say, operating is fun, and taking care of the patients is fun; they know that. They just do not want what has so intimately involved all of us to prevent them from doing other things that are their priorities. In short, they think we are crazy. We neglected our families, we were not there for our children, we were having fun; we were working, operating, traveling, drinking, making good friends on the national scene, many of them in this room, but neglecting some of the other priorities that the current generation, having watched, believes are inappropriate. While we were spending time away from our families and our children, abandoning them to their own devices,

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sometimes with disastrous results, what were we doing? Well, I suppose, we were "making it." Times were good, and money was plentiful. There were, and to a certain extent still are, financial rewards and social status. Families lived well; they had big houses, took trips and vacations, and perhaps even had vacation houses, to which some of us actually got. When we were there, we tried to make up for the times that we were not.

The new generation says to us: we like surgery, we think we understand what you are about, but we do not want to follow in your footsteps. Your lifestyle is not for us. In short, "you people are nuts." You had fun, yes, but at what price? Besides, the monetary rewards are harder to come by. So you can keep your lifestyle. We are going to have a different lifestyle, and if going into what you value as your profession is not open to us unless we emulate that lifestyle, thanks but no thanks. We would love to do it, but it makes us violate too much of what we believe is good.

I have thought a lot about this over the past several years. You know, as someone who has been called one of the last surgical dinosaurs, I am not certain they are totally wrong. Nor am I certain that the response of the surgical leadership to this crisis in our future has been correct—understandable perhaps, but maybe, just maybe, not entirely correct. It may be disappointing. Regardless of the argument as to the quality of the current medical students, and I personally believe that the quality has decreased despite the data, there are, in fact, good medical students. There may be worthy successors among these medical students. They like surgery and if they are properly exposed, if they can have mentors and people to welcome them, many will become surgeons. But our response to this change in priorities on the part of our potential successors has been to totally reject their priorities. Our approach has come from the standpoint of we were "the last of the iron interns." But I think the new generation may have stumbled onto something else. Times really are different.

1. The monetary rewards are different.
2. Although we think we have sacrificed our families and ourselves for our patients as well as other laudable goals, society in fact does not see it that way. Society does not believe that we are always interested in our patients and not in ourselves. As a result they have devalued our status and devalued us monetarily to the point that we have crossed what I call the red line. As individuals interested in education, as we all are, regardless of whether in private practice or academic practice, because of our own long educational commitment, the red line is that we cannot send our children to the school that we attended because of financial realities. I believe that in many cases we are either close to or over that line.
3. Physicians no longer control medical care. I differentiate between health care and medical care. Health care has not been controlled by physicians, perhaps for the past 20 or 30 years, but medical care was. Now physicians control neither medical care nor health care.
4. We do not control how long we can work. We prized our ability to stay up nights; we functioned while fatigued and trained our acolytes to do similarly. Society has rejected that, although the basis of the original accusations, the "Libby Zion" case, has proved to be not about fatigued residents but inappropriate coverage by faculty. Whether we like it or not and despite our protestations, almost exclusively from the surgical community, the 80-hour work week is here. In the future, the 80-hour work week may apply to faculty as well as practicing surgeons, whereas currently it applies only to residents. More about this later.

Are we correct? No. We may have really been entirely wrong about not welcoming this refreshing sensibility to our ranks. The generation Xers may have a better idea than we had. They may not be soft. They may not be weak. They may not be slothful. They may not be lazy. They are just interested in different things than we were. Should we note their point of view and welcome them? Should we overlook the difficulties in acquiring the requisite knowledge and experiential base in a decreased time frame, because the issues of the knowledge base and an experiential base needs to be dealt with separately and can, perhaps, within the diminished time in which to immerse those who will follow us in our craft? I am not certain. Maybe not with complete success but probably not with complete failure.

At this point, before returning to this theme, let me digress for a little while and discuss the 80-hour work week. The past 20 years have been unhappy ones for surgery. But of all the misfortunes and our responses thereto, the 80-hour work week has generated a response I have rarely seen. It is amazing to contemplate the remarks and the reaction from the surgical community to this particular innovation. With respect to money, the HMOs, and the tort system, I have participated in most of the debates for the past 15 or 20 years concerning our response to these areas or, in most cases, our lack of response to them. There has certainly been unhappiness with those various trials and tribulations. But the

angst in response to the 80-hour work week has a different quality to it, a more personal and deeply felt heartache. Why? The reason is, I think, that the 80-hour work week attacks one of the basic tenets of surgical practice and that is continuity of care. Our responsibility to the patient, an issue central to our surgical identity, but most important, our moral responsibility to patients, has been overlooked and as a matter of fact has even been negated.

How could this have happened? Despite adequate representation on the committees that dealt with the 80-hour work week, this cardinal tenet of surgical faith was not simply overlooked, it was abandoned. How, despite our efforts in all of our surgical programs to have someone in the house who knows a particular patient, because only you and the resident team that has operated on that patient know how happy you are with that anastomosis. Only you and the surgical team know how many extra sutures you placed because you were not happy with one corner. So, in the middle of the sixth postoperative night, when that patient complains of acute abdominal pain and spikes a fever to 103°, you would not, as someone who does not know that patient, go looking in every place other than the place you should be looking at—that is, has that anastomosis "blown?" No, control and the final solution to this issue is passed to the public. Congress, which threatened the medical establishment with the Conyers bill, OSHA in a suit brought by nonsurgical residents, who did not understand the cardinal tenet of the continuity of care, because we, the surgical community, could not, did not, or would not articulate in our particular lexicon and in our value system why continuity of care is so important. The internal medical services in academic medical centers have long since given up the concept of continuity of care. Deans of medical schools deemphasize clinical care in pursuit of the almighty NIH research dollar. Jordan Cohen, M.D., President of the Association of American Medical Colleges (AAMC), in recent testimony before congress on the effect of the 80-hour work week on the VA system, dismissively testified that in the VA system only surgical residents are affected. So much for AAMC representation for surgery. Here again, one of our centrally held beliefs has been abandoned.

One of the differences is that we like patients. We like to operate on them, but we also like to take care of them. We do not palliate patients. We do not give them chemotherapy; we excise cancers, often resulting in the only possible chance for cure. We like to get patients we can help when we are on call because we think an operation can make them better. That is why most of us went into surgery. If you listen to the way residents sign out to each other, on the medical service, they call the patients that they admitted "hits." We do not have a name for these patients; to us they are not "hits," they are patients. If you listen to the way the surgical teams sign out to each other there is usually a substantive discussion over what the patient's problems are, how the resident attempts to deal with them, what they think the problem may be, and what the initial therapeutic course has been. When I listen to medical residents sign out to each other in the frequent litany of the night float, the afternoon float, and the morning float to the float-float, so that you can never tell on a medical service who takes care of the patient, all they say is who is DNR, and who is CMO (comfort measures only).

All surgical residents, especially the senior surgical residents, (PGY-4′ and PGY-5′), understand that the 80-hour work week, whether we get a 10% increase or not, interferes with the continuity of care. They are unhappy. Yet it may be an advantage in that it causes us to rethink the efficiency of the training system that we ourselves have set up and imposed. The greatest difficulty with the 80-hour work week is the 6-hour transition. Whatever the fate of the 10% increase when and if you apply for it, and the Residency Review Committee has set the rules, it will not alter any of the proscriptions on hours and how these may evolve. In other words it will not change the 6-hour transition; it will not change the 24-hour on call; it will not change the 10-hour rest period; it won't change the one out of seven free and it will not change the every third night call system; and the 10% increase must have an educational rationale rather than the service rationale. So here we are with a new era, a new attitude, but also important, new limitations on a basic tenet that we hold very dear, and that is the continuity of care. Surgery alone values it, and surgery and its emphasis on continuity of care have been abandoned by the medical community. We are the last compleat physicians—but no one cares. Thus the aforementioned angst.

I have always said that if we want to see where our health care system is going we should look at the United Kingdom. One of the interesting features of the American medical scene is the reverence health economists are accorded. These health economists have been dead wrong 100% of the time over the past 30 years, and yet, they are still asked for their prescriptions on health care. The most important point health economists have missed is that if professionals are treated as employees they act as employees. In England, the National Health Service was organized in 1948, taking individuals who were trained as professionals and making them employees. These people remained professionals until they retired (generally in the mid-1970s) at which time individuals who had never been trained as professionals

but as employees picked up the banner. So you had an employee mentality taking control of an institution that had been set up to be run by professionals. They were physicians and surgeons but an employee physician or surgeon. Now if an accident occurs in London you may have to drive for an hour to find a neurosurgeon to take care of a serious head injury. It is not that these individuals, the physicians and surgeons of the National Health Service, haven't been offered money to keep accident floors open so that the injured would only have to drive for 15 minutes, but these "employees" said the money didn't appeal to them, it would be heavily taxed anyway and "no I don't have any responsibility as a professional to work overtime because I am not a professional. Quite honestly, rather than making more money and being taxed on it so I don't really ever have it, I would rather go to the pub with my friends; I would rather stay at home and watch television with my family."

In this country we are raising a group of professionals and instilling in them an employee mentality. The health economists do not mean for us to raise a group of employees, but in fact it is inevitable. Professionals are not constrained by hours; they work until the job is done and it is ironic that the very same people who have brought us professionalism as one of the six competencies have eliminated the central tenent of a profession—getting the job done without a temporal limitation. But this is not surprising given that most of the individuals involved have long since given up active patient care. The new professionals/employees will work 80 hours or less. They may not in the future decry the absence of continuity of care because they will have no experience with what continuity of care was like when there were unlimited amounts of time in which to take care of patients. And, yes, after completing the 80-hour situation with the residents, I believe that there will be 80-hour strictures on faculty and staff. It is logical. The results of that will mean that you will not be able to take care of patients by yourself as you heretofore have. You must come to another place in your profession. You may be able to have as much of a knowledge base as we had, particularly with the help of the Internet. There actually may be more time for reading, for scholarly activity, for leisure, and for time with your family. But what systems-based practice really means is that you will need the help of others to care for your patients—physician assistants, nurse practitioners, visiting nurses, and case managers. You will not work endlessly in the care of patients because you will not be allowed to because of time constraints.

Taken together, perhaps we should welcome this new era. It is the result of societal decisions over which, although we could have influenced them, we do not now have any control. We cannot do anything about it. When the system completely disintegrates, which it is well on its way to doing, society may in fact realize what they have missed, but I doubt it. If they do, they will probably blame the doctors anyway. We should encourage a more humane approach for the generation Xers. We should stop calling them names and we should welcome them. We should try to make a more humane environment for surgical residents and think about the positive aspects of their adopted lifestyle. I think that efforts in that regard and efforts to talk up surgery, specialty and subspecialty, will be greeted by an avalanche of persons wanting to go into surgery provided they are made to feel welcome. The older among us, however, will have difficulty. We will remember the "good old days." But the good old days are not here anymore. We do owe it to our trainees and the younger surgeons to improve our efficiency, to stop the nonsense aspects of surgical training, and to be more directed and to determine what is important to be passed on to the younger generation and what is not. It probably will not be enough, and there will inevitably be compromise in the quality of our care that many of us are concerned about but at this point in time cannot do anything about.

I do remember the president of a local university in Cincinnati that had a Nursing School and abandoned it for a baccalaureate program. We were talking at dinner one night and I said to him, "You know you have contributed to the nursing crisis by not producing diploma nurses in your nursing school. You have gone to a 4-year baccalaureate program." He said to me, with direct eye contact and unflinching, "I am not trying to prepare them for nursing. I am trying to prepare them for life." In a sense, we should be doing the same thing. We should be preparing our trainees for surgery as we know it to the best of our ability, but we also need to prepare them for life and since we have not, society has said we will do it for you.

So, to all of you I wish you well and I would urge you to welcome some of the changes that have been thrust upon us. They may very well be very good changes. They may enable people who are very talented who heretofore have looked at surgery, whether in general, or in specific, as an insane pursuit, to believe that surgery is in the realm of the feasible. And to those of you who are younger and about to reenter human society, good luck. But for those of us who believe we experienced "the golden age of surgery," this is bittersweet. Although we welcome the new, we do not really think it is right, but we no longer are empowered to choose. Some of our basic principles have been abandoned, to be sure, by others, but they have been abandoned nevertheless. We will miss the good old days.

Tissue and Organ Engineering: Can We Build Intestine and Vital Organs?

Joseph P. Vacanti, M.D.

The field of tissue engineering has emerged over the past 20 years. This paper presents a brief review of the conceptual framework of the field, its relationship to stem cell biology and regenerative medicine, and several examples of the state of the art including engineering of intestine as well as large vital organs such as the liver.

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RATIONALE FOR TISSUE ENGINEERING

The field of organ transplantation began in humans in December of 1954 when Dr. Joseph Murray performed the first successful human renal transplant with the donor being the identical twin of the recipient.[1] For this contribution and his contributions to immunosuppression, Dr. Murray received the Nobel Prize in 1990. However, the very success of organ transplantation has produced its most difficult problem—namely, the ever-worsening shortage of organ donors. More than 80,000 Americans are currently waiting for a vital organ, and many adults and children become severely ill while waiting and 10% die while waiting. In fact, in every field of reconstructive surgery the major problem is insufficient tissue for reconstruction. Currently, in my specialty of pediatric surgery, long-gap esophageal atresia is now repaired by placing a segment of colon in the chest from the upper pouch to the lower pouch based on its own vascular supply. Although this procedure has been successful, long-term complications such as stricture formation and even the formation of adenocarcinoma of the colon can develop. One can compare the way we currently perform transplantations to attempting to place a building on a site by ripping an old, abandoned building from its roots and "transplanting" it to the new location. Although this method would work, we all recognize that it is not optimal. We design and build the exact structure that is needed in this situation. By analogy, tissue engineering proposes to create living structures by designing and building them. Over the past 20 years, several approaches have evolved. Our approach involves combining cells on specially configured degradable polymer matrices in cell culture in three dimensions. The fundamental problem of mass transfer of oxygen and nutrition is solved by the design of the system. The system must have very large surface areas for exchange before angiogenesis occurs[1–4] (Fig. 1). This methodology has become the most common approach in the field of tissue engineering, combining cells of various tissues and types to matrices that can be either natural or synthetic but are degradable in combinations to produce living structures. Until 5 years ago, all approaches relied on angiogenesis to produce permanent vascularized new tissue.

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

The field of stem cell biology is very new and potentially of great importance. One can view the contributions of stem cell biology combined with

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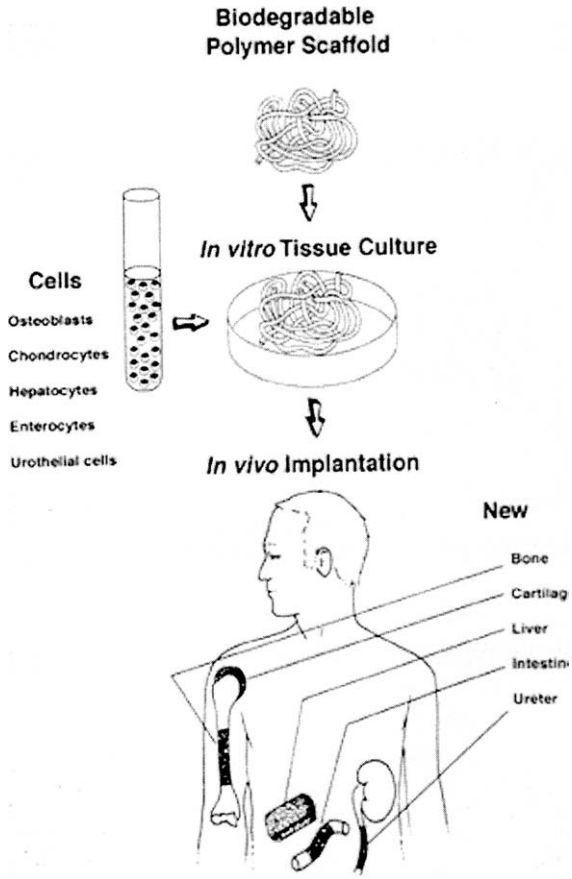

Fig. 1. Schema for the most frequently used approach in tissue engineering. (Reprinted with permission from Langer R, Vacanti J. Tissue engineering. Science 1993;260:920–926.)

tissue engineering to produce a new general field termed "regenerative medicine." Because stem cell biology is in its infancy, much remains to be discovered about the true biology of stem cells and their true potential. In addition, forms of stem cells are controversial including embryonic stem cells as well as cells made using therapeutic cloning techniques. At the present time, an operational definition of stem cells divides them into two distinct types: whole-body stem cells and organ-specific stem cells. Whole-body stem cells can undergo asymmetric doubling with one daughter cell being another stem cell and the other daughter cell having the potential to be differentiated into any cell type from the germ layers of endoderm, ectoderm, and mesoderm. Organ-specific stem cells also undergo asymmetric doubling with self-renewal and then the ability to produce all cell types of a given tissue or organ. Stem cells can give rise to progenitor or precursor cells, which then can give rise to mature cells. There is inherent plasticity in these systems with the ability of some cells to return to a less differentiated state including the possibility of returning to a stem cell state. It is important to note that any of the cells along this line, including mature cells, can be used in the field of tissue engineering, and all have the potential to make new, viable, functional tissue.

EXAMPLES OF TISSUE ENGINEERING

Over the past 17 years, groups at Harvard and MIT have been able to produce almost 30 tissues of the body, with many showing function in animal replacement models. Approximately five tissue-engineered living tissues have been used in humans. Several types of skin are commercially available for use in humans. Different forms of cartilage replacement have been implanted into humans with success, as well as bone and blood vessels. Urologic tissue is in its early phases.

We reported the first demonstration of engineered cartilage in the shape of a human ear in 1992.[5] Subsequently several cartilage and bone tissues have been produced and tested in animals.[6–9] We were also able to show that we could generate new cartilage from chondrocytes isolated from a 100-year-old man who was operated on for a fractured hip.[10]

Cardiovascular tissue was an early target of tissue engineering for small-caliber blood vessels. However, this work was not successful. We have replaced a single valve leaflet in a lamb model in the pulmonary artery position as an early demonstration of success in cardiovascular tissue engineering.[11] This work has now evolved into the demonstration of successful trileaflet valve replacement in the pulmonary valve position in lambs. Likewise, a segment of pulmonary artery replacement was demonstrated in animals and was recently reported in a child with a severe pulmonary artery stenosis and complex congenital heart disease[12] (Fig. 2).

TISSUE ENGINEERING OF INTESTINE

Short-bowel syndrome has been a difficult problem in pediatrics, in adult medicine, and in surgery. We reported the generation of intestine from minced pieces of fetal intestine in 1988.[2] Through the years we have investigated the potential in small animal models. We have turned to the development of what are termed isolated epithelial organoid units to produce all of the cellular elements of intestine.[13] We have demonstrated small intestine structure and function in a replacement model. Grikscheit et al.[14–16] have gone on to demonstrate good formation of functional colon tissue,[14] esophageal tissue,[15] and stomach tissue[16] (Fig. 3).

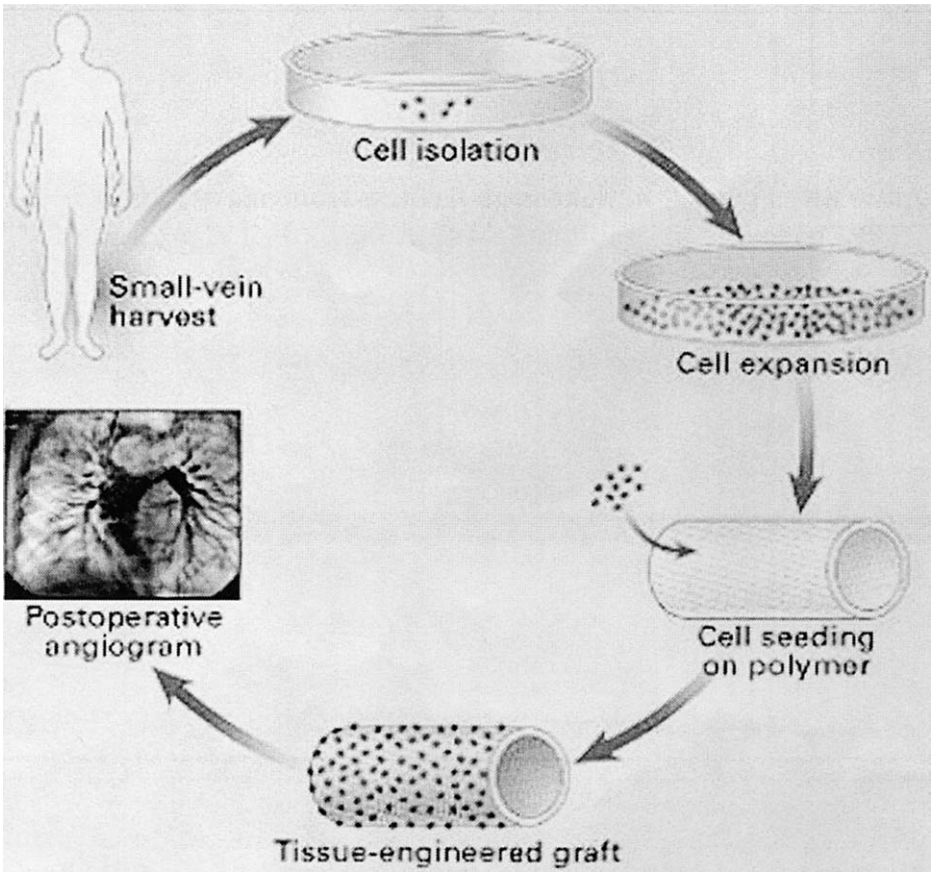

Fig. 2. Technique for engineering a pulmonary artery segment for a human trial. (Reprinted with permission from Shin'oka T, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. N Engl J Med 2001;344:532–533.)

THE PROBLEM OF VITAL ORGAN FABRICATION

Despite these advances in tissue engineering and regenerative medicine, the problem of whole-organ replacement remains as a major barrier to the field. The fundamental problem is mass of living tissue in three-dimensional space. Because the generation of living tissue is dependent on angiogenesis and because this process takes 3 to 5 days, the thickness of new living constructs has been limited to less than 1 cm. Clearly, sufficient liver mass for a patient's health cannot be supplied by a tissue that is only 1 cm thick. Therefore, in 1998, we began to design living systems that would include their own vascular supply.[17] As part of the Center for the Integration of Medicine and Innovative Technologies, which began at Massachusetts General Hospital, we have developed a multidisciplinary team of engineers, biologists, and clinical scientists. We have developed a robust computational model of the vascular circulation, which includes the fractal nature of network topology, the rheology of blood flow through this computational system, and the mass transfer of oxygen and nutrients across the vascular bed.[18] We have adapted the tools of microfabrication technology, which were originally invented to make electrical circuits for computer chips. We now have developed systems where we have been able to etch vascular channels including capillaries onto silicon wafers and transferred this to degradable polymer systems. Using a strategy of stacking these systems and alternating them with the hepatocyte compartment, we have been able to achieve a living hepatic system with its own vascular supply.[19–21] This work is in its early phases and, although scientifically complex, it has the promise of scalability and manufacturability should it be proven a successful strategy. This would allow it to be commercialized and used for all patients in need.

SUMMARY

In summary, the fields of regenerative medicine and tissue engineering hold enormous promise for

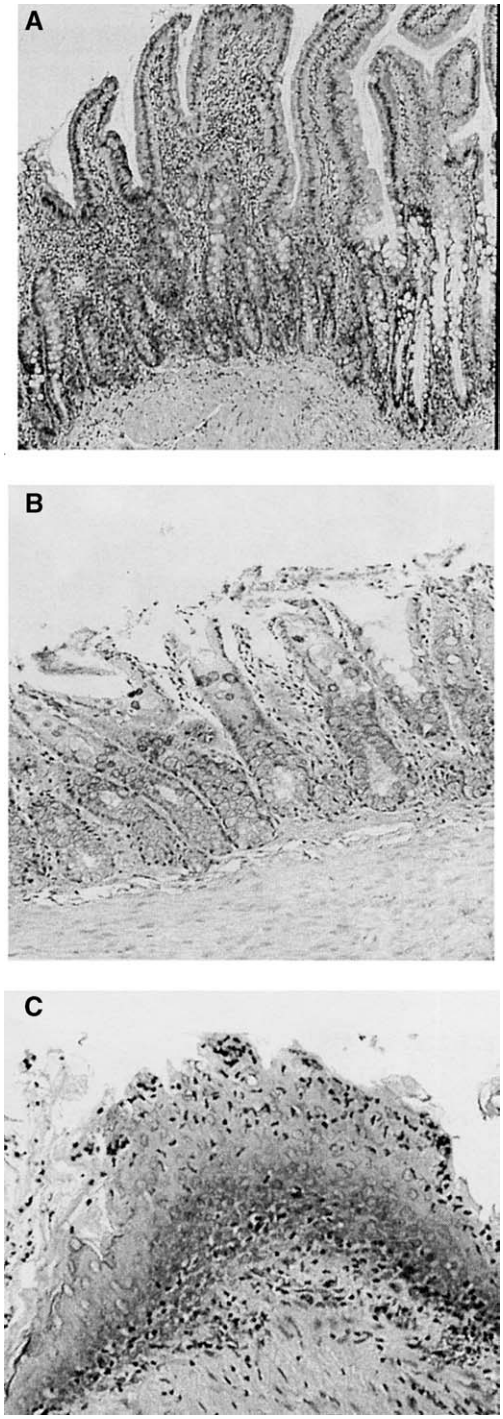

Fig. 3. Engineered segments of intestine (courtesy of Tracy C. Grikscheit, M.D.). **A**, Tissue-engineered rat intestine (original magnification × 10). **B**, Tissue-engineered rat colon (original magnification × 10). **C**, Tissue-engineered neonatal rat esophagus (original magnification × 10).

improving our ability to replace structures and improve function in patients in need. Much work remains but the first fruits of these efforts are now being demonstrated in patients with promising results.

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Joseph P. Vacanti, M.D.

RATIONALE FOR TISSUE ENGINEERING

The field of organ transplantation began in humans in December of 1954 when Dr. Joseph Murray performed the first successful human renal transplant with the donor being the identical twin of the recipient.[1] For this contribution and his contributions to immunosuppression, Dr. Murray received the Nobel Prize in 1990. However, the very success of organ transplantation has produced its most difficult problem—namely, the ever-worsening shortage of organ donors. More than 80,000 Americans are currently waiting for a vital organ, and many adults and children become severely ill while waiting and 10% die while waiting. In fact, in every field of reconstructive surgery the major problem is insufficient tissue for reconstruction. Currently, in my specialty of pediatric surgery, long-gap esophageal atresia is now repaired by placing a segment of colon in the chest from the upper pouch to the lower pouch based on its own vascular supply. Although this procedure has been successful, long-term complications such as stricture formation and even the formation of adenocarcinoma of the colon can develop. One can compare the way we currently perform transplantations to attempting to place a building on a site by ripping an old, abandoned building from its roots and "transplanting" it to the new location. Although this method would work, we all recognize that it is not optimal. We design and build the exact structure that is needed in this situation. By analogy, tissue engineering proposes to create living structures by designing and building them. Over the past 20 years, several approaches have evolved. Our approach involves combining cells on specially configured degradable polymer matrices in cell culture in three dimensions. The fundamental problem of mass transfer of oxygen and nutrition is solved by the design of the system. The system must have very large surface areas for exchange before angiogenesis occurs[1–4] (Fig. 1). This methodology has become the most common approach in the field of tissue engineering, combining cells of various tissues and types to matrices that can be either natural or synthetic but are degradable in combinations to produce living structures. Until 5 years ago, all approaches relied on angiogenesis to produce permanent vascularized new tissue.

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

The field of stem cell biology is very new and potentially of great importance. One can view the contributions of stem cell biology combined with

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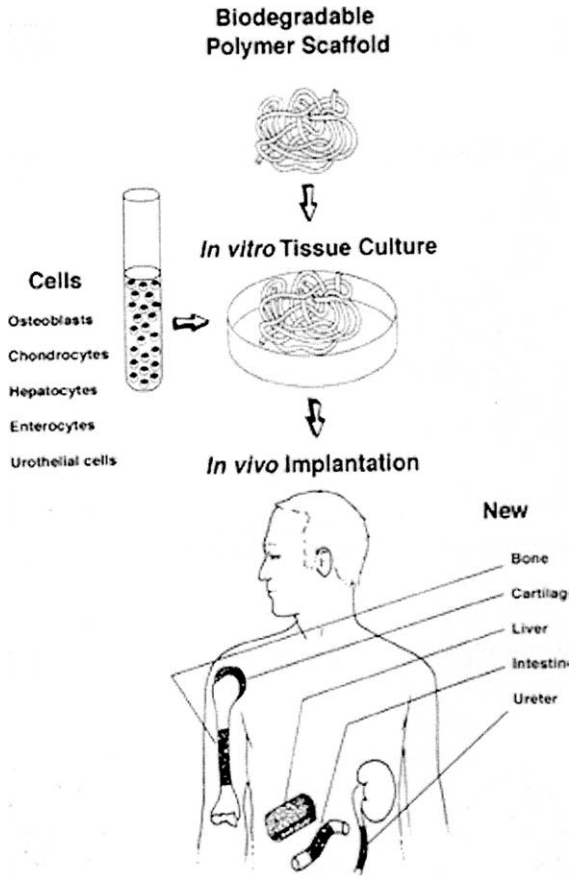

Fig. 1. Schema for the most frequently used approach in tissue engineering. (Reprinted with permission from Langer R, Vacanti J. Tissue engineering. Science 1993;260:920–926.)

tissue engineering to produce a new general field termed "regenerative medicine." Because stem cell biology is in its infancy, much remains to be discovered about the true biology of stem cells and their true potential. In addition, forms of stem cells are controversial including embryonic stem cells as well as cells made using therapeutic cloning techniques. At the present time, an operational definition of stem cells divides them into two distinct types: whole-body stem cells and organ-specific stem cells. Whole-body stem cells can undergo asymmetric doubling with one daughter cell being another stem cell and the other daughter cell having the potential to be differentiated into any cell type from the germ layers of endoderm, ectoderm, and mesoderm. Organ-specific stem cells also undergo asymmetric doubling with self-renewal and then the ability to produce all cell types of a given tissue or organ. Stem cells can give rise to progenitor or precursor cells, which then can give rise to mature cells. There is inherent plasticity in these systems with the ability of some cells to return to a less differentiated state including the possibility of returning to a stem cell state. It is important to note that any of the cells along this line, including mature cells, can be used in the field of tissue engineering, and all have the potential to make new, viable, functional tissue.

EXAMPLES OF TISSUE ENGINEERING

Over the past 17 years, groups at Harvard and MIT have been able to produce almost 30 tissues of the body, with many showing function in animal replacement models. Approximately five tissue-engineered living tissues have been used in humans. Several types of skin are commercially available for use in humans. Different forms of cartilage replacement have been implanted into humans with success, as well as bone and blood vessels. Urologic tissue is in its early phases.

We reported the first demonstration of engineered cartilage in the shape of a human ear in 1992.[5] Subsequently several cartilage and bone tissues have been produced and tested in animals.[6–9] We were also able to show that we could generate new cartilage from chondrocytes isolated from a 100-year-old man who was operated on for a fractured hip.[10]

Cardiovascular tissue was an early target of tissue engineering for small-caliber blood vessels. However, this work was not successful. We have replaced a single valve leaflet in a lamb model in the pulmonary artery position as an early demonstration of success in cardiovascular tissue engineering.[11] This work has now evolved into the demonstration of successful trileaflet valve replacement in the pulmonary valve position in lambs. Likewise, a segment of pulmonary artery replacement was demonstrated in animals and was recently reported in a child with a severe pulmonary artery stenosis and complex congenital heart disease[12] (Fig. 2).

TISSUE ENGINEERING OF INTESTINE

Short-bowel syndrome has been a difficult problem in pediatrics, in adult medicine, and in surgery. We reported the generation of intestine from minced pieces of fetal intestine in 1988.[2] Through the years we have investigated the potential in small animal models. We have turned to the development of what are termed isolated epithelial organoid units to produce all of the cellular elements of intestine.[13] We have demonstrated small intestine structure and function in a replacement model. Grikscheit et al.[14–16] have gone on to demonstrate good formation of functional colon tissue,[14] esophageal tissue,[15] and stomach tissue[16] (Fig. 3).

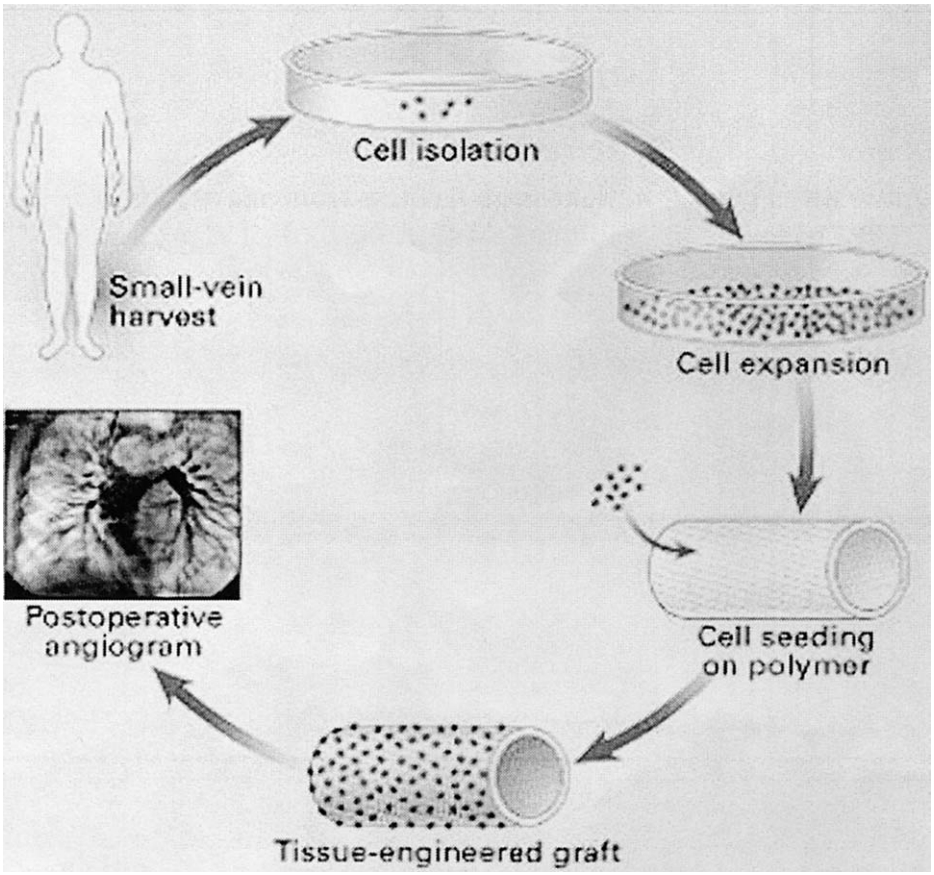

Fig. 2. Technique for engineering a pulmonary artery segment for a human trial. (Reprinted with permission from Shin'oka T, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. N Engl J Med 2001;344:532–533.)

THE PROBLEM OF VITAL ORGAN FABRICATION

Despite these advances in tissue engineering and regenerative medicine, the problem of whole-organ replacement remains as a major barrier to the field. The fundamental problem is mass of living tissue in three-dimensional space. Because the generation of living tissue is dependent on angiogenesis and because this process takes 3 to 5 days, the thickness of new living constructs has been limited to less than 1 cm. Clearly, sufficient liver mass for a patient's health cannot be supplied by a tissue that is only 1 cm thick. Therefore, in 1998, we began to design living systems that would include their own vascular supply.[17] As part of the Center for the Integration of Medicine and Innovative Technologies, which began at Massachusetts General Hospital, we have developed a multidisciplinary team of engineers, biologists, and clinical scientists. We have developed a robust computational model of the vascular circulation, which includes the fractal nature of network topology, the rheology of blood flow through this computational system, and the mass transfer of oxygen and nutrients across the vascular bed.[18] We have adapted the tools of microfabrication technology, which were originally invented to make electrical circuits for computer chips. We now have developed systems where we have been able to etch vascular channels including capillaries onto silicon wafers and transferred this to degradable polymer systems. Using a strategy of stacking these systems and alternating them with the hepatocyte compartment, we have been able to achieve a living hepatic system with its own vascular supply.[19–21] This work is in its early phases and, although scientifically complex, it has the promise of scalability and manufacturability should it be proven a successful strategy. This would allow it to be commercialized and used for all patients in need.

SUMMARY

In summary, the fields of regenerative medicine and tissue engineering hold enormous promise for

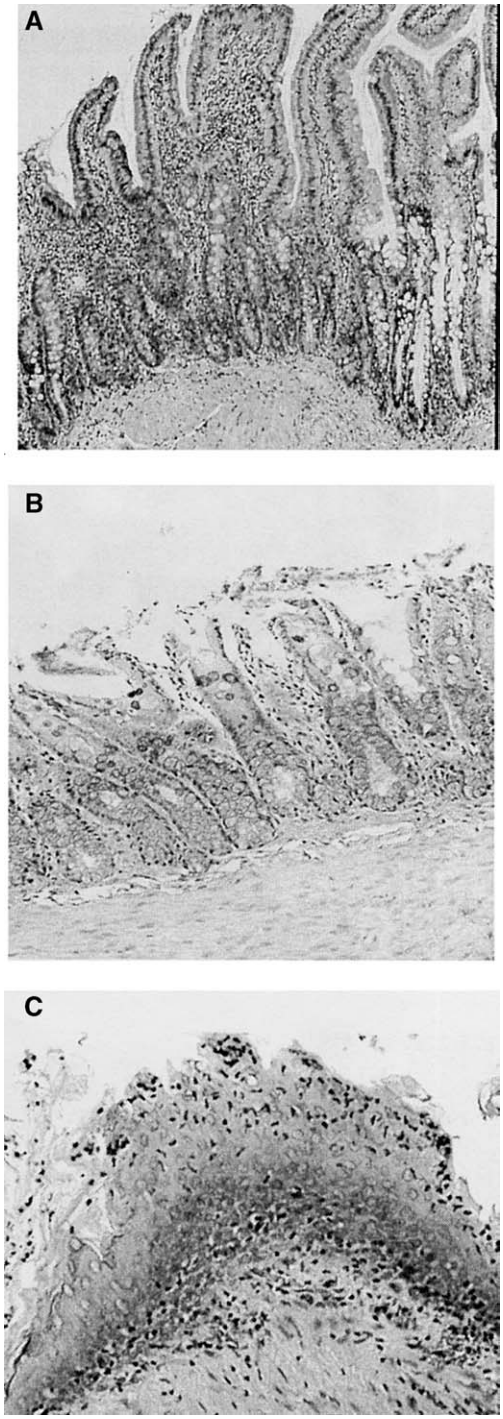

Fig. 3. Engineered segments of intestine (courtesy of Tracy C. Grikscheit, M.D.). **A**, Tissue-engineered rat intestine (original magnification × 10). **B**, Tissue-engineered rat colon (original magnification × 10). **C**, Tissue-engineered neonatal rat esophagus (original magnification × 10).

improving our ability to replace structures and improve function in patients in need. Much work remains but the first fruits of these efforts are now being demonstrated in patients with promising results.

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Etiology, Treatment, and Outcome of Esophageal Ulcers: A 10-Year Experience in an Urban Emergency Hospital

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Esophageal ulcers are a rare cause of upper gastrointestinal bleeding. This report describes the etiology, treatment, complications, and outcome of esophageal ulcers. An esophageal ulcer is defined as a discrete break in the esophageal mucosa with a clearly circumscribed margin; esophageal ulcers were seen in 88 patients from a total of 7564 esophagogastroduodenoscopies done by one surgeon at an urban hospital from 1991 to 2001. All hospital reports were reviewed. The etiology of esophageal ulcers included the following: gastrointestinal reflux disease (GERD) (n = 58, 65.9%), drug induced (n = 20, 22.7%), candidal (n = 3, 3.4%), caustic injury (n = 2, 2.3%), and herpes simplex virus (HSV), human immunodeficiency virus (HIV), marginal ulcer, foreign body, and unknown etiology (n = 1 of each, 1.1%). The mean size of GERD-induced esophageal ulcers and drug-induced esophageal ulcers was 2.78 and 2.92 cm, respectively; 80.3% of GERD-induced esophageal ulcers and 13.8% of drug-induced esophageal ulcers were located in the lower thoracic esophagus. Morbidity (n = 44, 50%) included hemorrhage (n = 30, 34%), esophageal stricture (n = 11, 12.5%), and esophageal perforation (n = 3, 3.4%). Nonoperative therapy sufficed in 81 patients (92%). Three patients (3.4%) had a recurrence of esophageal ulcers. Fifteen patients (17.0%) required endoscopic intervention including esophageal dilatation for stricture in 11 patients and endoscopic hemostasis for esophageal bleeding in four patients. Surgery (n = 7, 8.0%) was reserved for esophageal stricture and perforation. Two patients (2.3%) died from complications of esophageal ulcers: hemorrhage in one and perforation in one. Three patients died of their primary disease. GERD and drug ingestion are common causes of esophageal ulcers. Midesophageal ulcers have a greater tendency to hemorrhage compared with ulcers at the gastroesophageal junction; this may reflect the etiology. Strictures complicate GERD-induced esophageal ulcers but not drug-induced esophageal ulcers. Esophageal dilatation is an effective treatment for most strictures associated with esophageal ulcers. Esophageal ulcers rarely cause death. (J GASTROINTEST SURG 2003;7:836–842)

KEY WORDS: Esophageal ulcers, etiology, treatment outcome

Esophageal ulcers most commonly occur as a result of gastroesophageal reflux disease (GERD) with a reported prevalence of 2% to 7%.[1,2] Because of the rarity of these ulcers, there is little comprehensive literature regarding etiology and clinical course. Tileston,[3] in 1906, identified the causes of esophageal ulcers—namely, peptic ulcer disease, carcinoma, corrosive substances, foreign body, infectious disease, aneurysm, catarrhal, traction diverticula, tuberculosis, syphilis, esophageal varices, and thrush. Since then, the etiology has changed reflecting differences in demographics, diagnostic modalities, and therapeutic interventions. Esophageal ulcers secondary to nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, radiation therapy, Crohn's disease, and dermatologic diseases have also been reported.[1,2] More recently esophageal ulcers due to cytomegalovirus, herpes simplex virus, and human immunodeficiency virus have become more prevalent.[2,4,5] This report defines the incidence, etiology, treatment, and outcome of esophageal ulcers seen in a large urban medical center.

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METHODS

This study was performed at Detroit Receiving Hospital, a large urban teaching emergency trauma hospital. Review of 7564 consecutive esophagogastroduodenoscopies (EGDs) performed by a single surgical endoscopist from August 1991 to June 2001 identified 88 patients (1.2%) with esophageal ulcers. The records of all of these patients were reviewed. All 88 of these patients were part of a subgroup of 3520 patients who had endoscopically diagnosed esophagitis. Esophageal ulcer was defined as a discrete break in the esophageal mucosa with a clearly identifiable margin. The term erosion refers to a superficial lesion that remains confined to the lamina propria and muscularis mucosae. In contrast, necrosis, hemorrhage, and inflammation associated with ulcers extend deeper into the underlying submucosa or muscularis propria. Erosions or ulcers may appear isolated or confluent, and they commonly coexist with one another.[6] The etiology of esophageal ulcers was ascertained from clinical, endoscopic, and pathologic findings. Data recorded included: history of caustic ingestion, location of the ulcer, morphology of the ulcer, previous EGD findings and pictures, concurrent EGD findings and pictures, and comorbid conditions. Biopsy specimens were obtained from both the center and the margin of the ulcer. EGD examinations were performed with Olympus flexible video endoscopes. Esophageal ulcers after sclerotherapy and those associated with esophageal malignancy were excluded from this analysis.

RESULTS

Patient Profile

Of the 88 patients with esophageal ulcers, 56 were men (63.6%), and 32 were women (36.4%) with a mean age of 56.4 years (±16 years standard deviation [SD]). Comorbid conditions included: hypertension (n = 33, 37.5%), diabetes mellitus (n = 18, 20.5%), central nervous system disorders such as cerebrovascular accident and head trauma (n = 16, 18.2%), peptic ulcer disease including four patients with gastric ulcers, three patients with duodenal ulcers, and one patient with both gastric and duodenal ulcers (n = 8, 9.1%), congestive heart failure (n = 8, 9.1%), cirrhosis (n = 6, 6.8%), pneumonia (n = 5, 5.7%), asthma (n = 5, 5.7%), recurrent episodes of acute pancreatitis (n = 5, 5.7%), and renal failure (n = 4, 4.5%). Eight patients (9.1%) were bedridden. A history of chronic, moderate, daily drinking or heavy (more than 8 ounces of alcohol per day) alcohol consumption was given in 40 patients (45.5%), and recent heavy alcohol consumption within 24 hours of admission occurred in 32 patients (36.4%); 35 patients (39.8%) smoked cigarettes daily or used illicit street drugs such as cocaine or heroin mix daily (n = 4, 4.5%). Nonsteroidal anti-inflammatory agents or clycooxygenase-2 (COX-2) inhibitors had been recently used in 28 patients (31.8%). H_2 blockers or proton pump inhibitors had been used recently in 20 patients (22.7%).

Most patients (79, 89.8%) were initially seen in the emergency room for evaluation. Esophageal ulcers were found in 79 patients during the initial EGD. The esophageal ulcers in the remaining nine patients developed in the hospital while these patients were being treated for another condition. The duration of symptoms was less than 3 days in 43 patients, and more than 10 days in 20 patients. These signs and symptoms included the following: hematemesis (n = 36, 40.9%), nausea and vomiting with regurgitation (n = 35, 39.8%), epigastric (with or without substernal) pain (n = 27, 30.7%), melena (n = 22, 25%), dysphagia to solids (n = 16, 18.2%), "coffee ground" gastric aspirate (n = 15, 17.0%), and chest pain that was substernal with extension to the back (n = 10, 11.4%). The chest pain was thought to be a manifestation of heartburn due to regurgitation. Esophageal ulcers were found on the first endoscopic assessment in 79 patients, whereas esophageal ulcers were first seen on a follow-up endoscopy in nine patients. Most patients (n = 74, 84.1%) required in-hospital treatment at the time of the initial diagnostic EGD. Eight patients (9.1%) required repeat EGD evaluation for esophageal ulcers or their complications after discharge.

Etiology of Esophageal Ulcers

The etiology of esophageal ulcers (Table 1) was most commonly associated with GERD (n = 58). All patients with esophageal ulcers caused by GERD had a

Table 1. Etiology of esophageal ulcers

Etiology	No. of patients	%
GERD	58	65.9
Drug-induced	20	22.7
Candida	3	3.4
Caustic injury	2	2.3
AIDS	1	1.1
Herpes simplex virus	1	1.1
Marginal	1	1.1
Foreign body	1	1.1
Unknown	1	1.1
Total	88	100

GERD = gastrointestinal reflux disease; AIDS = acquired immune deficiency syndrome.

definite hiatal hernia. Hiatal hernia was diagnosed endoscopically when the squamocolumnar junction was more than 3 cm above the diaphragmatic impression.[7] In moderate-sized or large hiatal hernias, the gastric mucosal folds can be seen running proximally over the hiatal margin into the bulbous cavity of the distended hernia pouch. A hiatal hernia is also confirmed based on a retroflexed endoscopic view of the hernia pouch and the squamocolumnar junction from below.[7] All 58 patients with ulcer associated with hiatal hernia had moderate-sized or large hiatal hernias. The endoscopic grading of GERD depends on the endoscopist's interpretation of these visual images. Unfortunately there is no standard classification scheme for endoscopic findings.[7] Several classification systems have been devised to define or grade reflux changes by using characteristics seen at endoscopy. All 58 patients had grade III or IV esophagitis according to the Savary-Miller endoscopic grading system,[7,8] and also grade III or IV esophagitis according to the system devised by Hetzel et al.[7,8] Barrett's esophagus complicated GERD-induced esophageal ulcers in 10 patients and drug-induced esophageal ulcers in one patient. Other etiologies included drug-induced (n = 20), candidal (n = 3), and caustic injury (n = 2), in addition to acute human immunodeficiency virus (HIV), herpes simplex virus (HSV), marginal, foreign body, and obscure origin (n = 1 case of each). The offending medications of drug-induced esophageal ulcers included aspirin, ibuprophen, aspirin plus ibuprophen, ferrous sulfate, doxycycline, erythromycin, amoxicillin clavulanate, nifedipine, and cyclobenzaprine (Table 2).

Morphology of GERD-Induced and Drug-Induced Esophageal Ulcers

The mean size of all esophageal ulcers, GERD-induced esophageal ulcers, and drug-induced esophageal ulcers was 2.80 cm, 2.78 cm, and 2.92 cm, respectively (Table 3). Size was measured with the use of open biopsy forceps. The mean number of all esophageal ulcers, GERD-induced esophageal ulcers, and drug-induced esophageal ulcers was 2.48, 1.96, and 2.80, respectively (see Table 3). Most (91.4%) of the GERD-induced esophageal ulcers were located in the lower intrathoracic esophagus (35 to 40 cm from the incisors), whereas 80.0% of drug-induced esophageal ulcers were located in the middle intrathoracic esophagus (28 to 33 cm from the incisors).

Table 2. Drug-induced esophageal ulcers

Drug implicated	No.	%
NSAIDs	13	65
Aspirin only	7	35
Ibuprophen only	3	15
Aspirin + ibuprophen	3	15
Ferrous sulfate	2	10
Doxycycline	1	5
Erythromycin	1	5
Amoxicillin, clavulanate	1	5
Nifedipine	1	5
Cyclobenzaprine	1	5
Total	20	100

Table 3. Endoscopic morphology of esophageal ulcers

	GERD	Drug
Location		
Upper third	2 (3.4%)	9 (4.9%)
Middle third	11 (19.0%)	16 (80.0%)
Lower third	53 (91.4%)	4 (20.0%)
Total	66	29
Size		
Minimum	0.6 cm	0.6 cm
Maximum	10.0 cm	6.0 cm
Mean ± SD	2.78 cm ± 2.13	2.92 cm ± 1.98
Number		
Minimum	1	1
Maximum	12	16
Mean ± SD	1.96 ± 2.12	2.80 ± 4.19

SD = standard deviation.

Esophageal Ulcers: Inpatients

In-hospital esophageal ulcers developed in nine inpatients; this was due to GERD (n = 6), drugs (n = 1), Candida (n = 1), and AIDS (n = 1). Of the six patients with GERD, one had liver cirrhosis and five were bedridden because of a cerebral vascular occlusion (n = 4) or a right femoral neck fracture (n = 1). Concurrent diagnoses included pneumonia, decubitus ulcer, gas gangrene, seizure disorder and weakness, retroperitoneal mass, and tuberculosis peritonitis. Three patients died of primary disease, multisystem organ failure, and sepsis.

Esophageal Strictures

Eleven patients developed an esophageal stricture including 10 patients with GERD-induced esophageal ulcers and one of two patients with caustic injury. All esophageal strictures developed at the squamocolumnar junction on the distal esophagus and were associated with a hiatal hernia. Endoscopic dilatation was performed in all 11 of them; three patients underwent multiple endoscopic dilatations. Nissen fundoplication had been performed in four patients with esophageal stricture. Esophagectomy with colonic interposition was performed in one patient with caustic

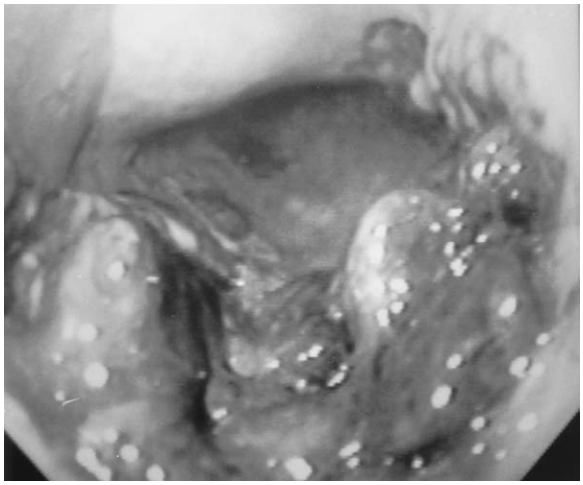

Fig. 1. A large ulcer with clot on the base is seen at midesophagus (25–30 cm from incisors). This patient had been taking 8–10 tablets of aspirin and Anacin daily for 3 weeks to treat her abdominal and chest pain. She chewed some pills before hematemesis. She did not have hiatal hernia or GERD.

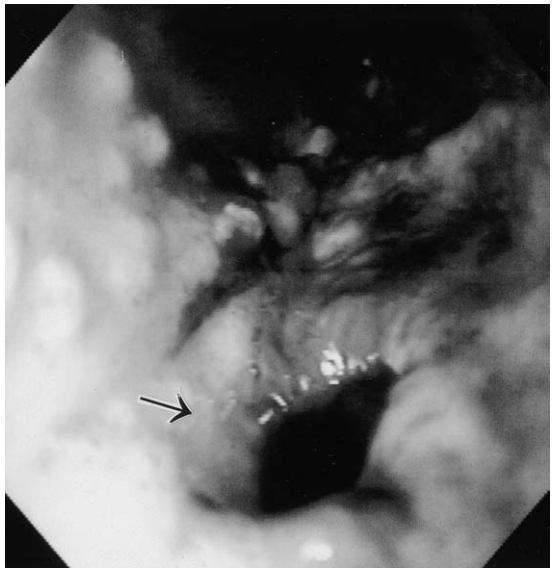

Fig. 2. A GERD-induced diffuse esophageal ulcer with perforation (see arrow).

injury. There were no complications related to dilatation. All five patients survived operative intervention.

Hemorrhage

Hemorrhage from esophageal ulcers was diagnosed by EGD in 30 patients including 17 with GERD, 10 with drug-induced esophageal ulcers (Fig. 1), and one patient each with HSV, idiopathic esophageal ulcers with HIV, and esophageal ulcers of unknown etiology. Twenty-one patients required blood transfusion including nine patients with GERD, nine patients with drug-induced esophageal ulcers, and one patient each with HSV, idiopathic esophageal ulcers of HIV, and esophageal ulcers of obscure origin. The mean amount of blood transfused was 5.38 ± 3.43 units (range 2 to 14 units). This included an average of 3.62 units in patients with GERD and 6.28 units in patients with drug-induced esophageal ulcers. Endoscopic hemostasis with epinephrine injection and heater probe application was required in three patients with drug-induced esophageal ulcers and in one patient with esophageal ulcers of unknown etiology. Endoscopic hemostasis was not necessary for bleeding from GERD-induced esophageal ulcers. No operative intervention was undertaken for esophageal bleeding secondary to esophageal ulcers.

Esophageal Perforation

Esophageal perforation occurred in three patients resulting in two deaths. One patient developed upper gastrointestinal bleeding 3 days after operative fixation of a right femoral neck fracture. EGD revealed GERD-induced diffuse esophageal ulcers and a 2×2 cm gastric polyp. A repeat EGD done 10 days later for recurrent bleeding showed esophageal perforation from the ulcer (Fig. 2). Emergency operation included cervical esophagostomy, gastrostomy, and feeding jejunostomy. This patient died of sepsis 3 weeks later. A second patient with drug-induced esophageal ulcers and an aneurysm of the thoracic aorta developed an esophagoaortic fistula and died rapidly from massive hemorrhage and aspiration. A third patient with GERD-induced esophageal ulcers diagnosed 3 months earlier was noncompliant with H_2 blocker therapy and developed a distal esophageal perforation, which was treated with segmental resection of the esophageal perforation, esophageal exclusion, cervical esophagostomy, feeding jejunostomy, and decompressive gastrostomy. The patient was discharged to a rehabilitation institute and eventually underwent esophageal reconstruction.

Multiple Presentations to the Hospital

One patient, a 63-year-old woman, was found to have drug (ibuprophen)–induced esophageal ulcers in the midesophagus with a moderate-sized hiatal hernia without GERD in 1991. Seven years later, at age 70, she developed GERD-induced esophageal ulcers with stricture at the squamocolumnar junction and a larger hiatal hernia than that previously seen. Another patient, a 41-year-old man with AIDS, presented with chest pain and melena and was diagnosed with an idiopathic esophageal ulcer in 1996. Three years later,

during hospitalization for AIDS encephalitis complicated by seizures, he developed coffee ground emesis and was diagnosed again with idiopathic esophageal ulcers. A third patient, 48 years of age, had been admitted for hematemesis in 1996 and was readmitted because of hematemesis 6 months later, despite taking proton pump inhibitors, and underwent a Nissen fundoplication. Four patients underwent multiple esophageal dilatations for esophageal stricture with esophageal ulcers; three were caused by GERD and one was due to caustic injury.

Treatment of Uncomplicated Esophageal Ulcers

Patients with uncomplicated esophageal ulcers were treated with H_2 blockers, proton pump inhibitors, or antifungal medication in the case of candidal esophageal ulcers. The mean length of stay for patients with uncomplicated esophageal ulcers (n = 50) was 6.08 ± 6.37 days (range 1 to 38 days). Endoscopically confirmed resolution of esophageal ulcers was seen in five patients with a mean resolution time of 49.8 days (range 4 to 150 days). This occurred in two patients with GERD-induced esophageal ulcers, two patients with drug-induced esophageal ulcers, and one patient with esophageal ulcers of unknown etiology. Long follow-up of these patients were not possible unless they returned to Detroit Receiving Hospital. Some patients with intractable esophageal ulcers or GERD may have had elective surgery at other hospitals.

Coexistent EGD Findings Associated With Esophageal Ulcers

Significant coexistent findings on EGD were noted in 50 (57%) of 88 patients with esophageal ulcers; these included: acute erosive gastritis (n = 29), doudenitis (n = 14), acute gastric ulcers (n = 12), and duodenal ulcers (n = 11). *Helicobacter pylori* testing was positive in 18 of 28 patients tested. Barrett's esophagus complicated GERD–induced esophageal ulcers in 10 patients and drug-induced esophageal ulcers in one patient. This latter patient developed a midesophageal ulcer surrounded by normal mucosa and had a strong history of NSAID use.

DISCUSSION

The EGD diagnosis of esophageal ulcers is rare; they were present in only 88 (1.2%) of 7564 patients undergoing upper endoscopic evaluation. The most common cause of esophageal ulcers in this series was GERD; esophageal ulcers complicated GERD in 2.5% (88 of 3520) of patients with endoscopically diagnosed esophagitis. The reported rate of esophageal ulcers in patients with esophagitis ranges from 2% to 7%.[2] All patients with GERD-induced esophageal ulcers will have esophagitis at the squamocolumnar junction.[9] Esophageal ulcers with no abnormality at the squamocolumnar juction are likely the result of a neoplasm because benign solitary esophageal ulcers related to acid reflux do not occur in normal squamous epithelium.[9]

The incidence of drug-induced esophageal ulcers has not been reported. Esophageal injury results from mucosal contact with the offending agent, thus the danger of administering known irritating agents by mouth to the bedridden patient.[10] Tablets and capsules may adhere to the esophageal wall and dissolve locally within the normal esophagus.[11–13] The characteristic appearance of NSAID-induced esophageal ulcers consists of large, shallow, discrete ulcers in the midesophagus near the aortic arch surrounded by normal mucosa.[10,12]

Differentiation between distal drug-induced esophageal ulcers and GERD-induced esophageal ulcers can be difficult. Patients with drug-induced esophageal ulcers may be more prone to develop GERD as a result of disorders of esophageal motility caused by the drug-induced esophageal ulcers. Five of 13 patients herein with NSAID-induced esophageal ulcers demonstrated GERD. One patient with a large NSAID-induced bleeding esophageal ulcer in the midesophagus developed a GERD-induced esophageal ulcer with esophageal stricture in the distal esophagus 7 years later. Possibly, drug-induced esophageal injuries are aggravated by GERD, and GERD-induced esophageal injuries progress with certain drugs.[14]

Complications of esophageal ulcers relate to etiology and include stricture, hemorrhage, and perforation. Esophageal stricture complicating GERD has been reported to occur in 4% to 20% of patients with GERD.[1,15] Esophageal stricture may occur in 2.6% to 7.0% of patients with NSAID-induced esophageal injury.[12,16] None of the patients in our study who developed stricture after drug-induced esophageal ulcers. Ten of the 11 patients diagnosed with stricture in this study had GERD-induced esophageal ulcers.

The reported rate of esophageal bleeding from GERD- and NSAID-induced esophageal injury is less than 2% and 30.1%, respectively.[2,16] Silverstein et al.[17] reported 1.7% of acute upper gastrointestinal hemorrhage is due to esophageal ulcers. The present study noted bleeding in 17 (29.3%) of 58 patients with GERD-induced esophageal ulcers and in 10 (50%) of 20 patients with drug-induced esophageal ulcers. Active bleeding of esophageal ulcers during EGD

was noted in 13.8% of patients with GERD-induced esophageal ulcers and in 45.0% of patients with drug-induced esophageal ulcers. Bleeding from drug-induced esophageal ulcers is always from the midesophagus and is more likely to be active bleeding than GERD-related bleeding (45.0% vs. 13.8%); patients with this bleeding require blood transfusions (45.0% vs. 13.8%) and endoscopic hemostasis (15.0% vs. 0%).

Anatomically there is a rich arterial and venous network at the level of the mucosa and submucosa throughout the esophagus, especially near the gastroesophageal junction.[18] Thus the bleeding tendency associated with drug-induced esophageal ulcers seems to be determined by factors other than vascular anatomy. Superficial ulceration of the squamous epithelium is typical of reflux esophagitis.[19] In some patients with GERD-induced esophageal ulcers, esophageal ulceration stimulates fibrous tissue production with collagen deposition and stricture formation.[1] The chronic nature of GERD, which is due to intermittent regurgitation of acid, may reduce the bleeding tendency of the epithelium lining the gastroesophageal junction. In contrast, the acute nature of drug-induced esophageal ulcers resulting from continuous contact of a caustic agent with previously normal mucosa may increase the bleeding tendency, particularly with NSAID-induced esophageal ulcers.[10]

NSAIDs are strikingly more likely to cause hemorrhage than other pill classes when they injure the esophagus.[10,16] Kikendall[16] noted that 22 of 154 NSAID-induced esophageal injuries were complicated by hemorrhage. In contrast, only 25 of 796 esophageal injuries induced by other medications resulted in hemorrhage. Furthermore, 8 of 19 esophageal injuries induced by aspirin were complicated by hemorrhage, compared to only one of five esophageal injuries caused by ibuprophen.[16]

The reported rate of esophageal perforation complicating GERD is less than 0.2%.[2] The rate of perforation of GERD-induced esophageal ulcers has not been previously reported. In the present study, two patients with perforation were seen in 58 patients with GERD induced esophageal ulcers (5.1%). In a review of 22 esophageal perforations reported by Nesbitt and Sawyers,[20] the etiology was barogenic transmural disruption (Boerhaave's syndrome) in 20 patients and distal esophageal ulcers in two.

No perforation complicated the 154 NSAID-induced esophageal injuries identified in Kikendall's series.[16] One of our patients subsequently died of hemorrhage from an aortoesophageal fistula through an underlying thoracic aortic aneurysm; this patient had been taking aspirin and ibuprophen for 2 years.

Approximately 70% of patients with GERD-induced esophageal ulcers show complete healing within several months with H_2 receptor–blocking agents administered in conventional doses.[1] Most ulcerations refractory to conventional treatment will heal with the intensive suppression of gastric acid secretion achieved by administering high doses of H_2 receptor–blocking agents or proton pump inhibitors.[1] The role of antireflux surgery in the treatment of esophageal ulcers is limited to those few patients with GERD-induced esophageal ulcers refractory to high-dose medical therapy.

The clinical course of hospitalized patients who develop esophageal ulcers is unique. The development of esophageal ulcers is influenced by organ failure, recumbency, and underlying disease. Five of the nine patients who developed esophageal ulcers while hospitalized for other diagnoses had an infectious process including pneumonia, decubitus ulceration, gas gangrene, infected retroperitoneal mass, and tuberculous peritonitis. Three of these five patients died of sepsis or multiple organ failure. Likewise, the patient who died after fixation of a right femoral neck fracture developed upper gastrointestinal bleeding from GERD-induced esophageal ulcers and a later perforation that caused his death.

CONCLUSION

GERD and drug ingestion are the most common causes of esophageal ulcers. Midesophageal ulcers caused by NSAIDs have a greater tendency toward hemorrhage that requires blood transfusion and endoscopic hemostasis. Stricture formation is very likely with GERD-induced esophageal ulcers and is effectively treated with dilatation in most patients. Esophageal ulcers rarely cause death.

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Etiology, Treatment, and Outcome of Esophageal Ulcers: A 10-Year Experience in an Urban Emergency Hospital

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Esophageal ulcers are a rare cause of upper gastrointestinal bleeding. This report describes the etiology, treatment, complications, and outcome of esophageal ulcers. An esophageal ulcer is defined as a discrete break in the esophageal mucosa with a clearly circumscribed margin; esophageal ulcers were seen in 88 patients from a total of 7564 esophagogastroduodenoscopies done by one surgeon at an urban hospital from 1991 to 2001. All hospital reports were reviewed. The etiology of esophageal ulcers included the following: gastrointestinal reflux disease (GERD) (n = 58, 65.9%), drug induced (n = 20, 22.7%), candidal (n = 3, 3.4%), caustic injury (n = 2, 2.3%), and herpes simplex virus (HSV), human immunodeficiency virus (HIV), marginal ulcer, foreign body, and unknown etiology (n = 1 of each, 1.1%). The mean size of GERD-induced esophageal ulcers and drug-induced esophageal ulcers was 2.78 and 2.92 cm, respectively; 80.3% of GERD-induced esophageal ulcers and 13.8% of drug-induced esophageal ulcers were located in the lower thoracic esophagus. Morbidity (n = 44, 50%) included hemorrhage (n = 30, 34%), esophageal stricture (n = 11, 12.5%), and esophageal perforation (n = 3, 3.4%). Nonoperative therapy sufficed in 81 patients (92%). Three patients (3.4%) had a recurrence of esophageal ulcers. Fifteen patients (17.0%) required endoscopic intervention including esophageal dilatation for stricture in 11 patients and endoscopic hemostasis for esophageal bleeding in four patients. Surgery (n = 7, 8.0%) was reserved for esophageal stricture and perforation. Two patients (2.3%) died from complications of esophageal ulcers: hemorrhage in one and perforation in one. Three patients died of their primary disease. GERD and drug ingestion are common causes of esophageal ulcers. Midesophageal ulcers have a greater tendency to hemorrhage compared with ulcers at the gastroesophageal junction; this may reflect the etiology. Strictures complicate GERD-induced esophageal ulcers but not drug-induced esophageal ulcers. Esophageal dilatation is an effective treatment for most strictures associated with esophageal ulcers. Esophageal ulcers rarely cause death. (J GASTROINTEST SURG 2003;7:836–842)

KEY WORDS: Esophageal ulcers, etiology, treatment outcome

Esophageal ulcers most commonly occur as a result of gastroesophageal reflux disease (GERD) with a reported prevalence of 2% to 7%.[1,2] Because of the rarity of these ulcers, there is little comprehensive literature regarding etiology and clinical course. Tileston,[3] in 1906, identified the causes of esophageal ulcers—namely, peptic ulcer disease, carcinoma, corrosive substances, foreign body, infectious disease, aneurysm, catarrhal, traction diverticula, tuberculosis, syphilis, esophageal varices, and thrush. Since then, the etiology has changed reflecting differences in demographics, diagnostic modalities, and therapeutic interventions. Esophageal ulcers secondary to nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, radiation therapy, Crohn's disease, and dermatologic diseases have also been reported.[1,2] More recently esophageal ulcers due to cytomegalovirus, herpes simplex virus, and human immunodeficiency virus have become more prevalent.[2,4,5] This report defines the incidence, etiology, treatment, and outcome of esophageal ulcers seen in a large urban medical center.

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METHODS

This study was performed at Detroit Receiving Hospital, a large urban teaching emergency trauma hospital. Review of 7564 consecutive esophagogastroduodenoscopies (EGDs) performed by a single surgical endoscopist from August 1991 to June 2001 identified 88 patients (1.2%) with esophageal ulcers. The records of all of these patients were reviewed. All 88 of these patients were part of a subgroup of 3520 patients who had endoscopically diagnosed esophagitis. Esophageal ulcer was defined as a discrete break in the esophageal mucosa with a clearly identifiable margin. The term erosion refers to a superficial lesion that remains confined to the lamina propria and muscularis mucosae. In contrast, necrosis, hemorrhage, and inflammation associated with ulcers extend deeper into the underlying submucosa or muscularis propria. Erosions or ulcers may appear isolated or confluent, and they commonly coexist with one another.[6] The etiology of esophageal ulcers was ascertained from clinical, endoscopic, and pathologic findings. Data recorded included: history of caustic ingestion, location of the ulcer, morphology of the ulcer, previous EGD findings and pictures, concurrent EGD findings and pictures, and comorbid conditions. Biopsy specimens were obtained from both the center and the margin of the ulcer. EGD examinations were performed with Olympus flexible video endoscopes. Esophageal ulcers after sclerotherapy and those associated with esophageal malignancy were excluded from this analysis.

RESULTS

Patient Profile

Of the 88 patients with esophageal ulcers, 56 were men (63.6%), and 32 were women (36.4%) with a mean age of 56.4 years (±16 years standard deviation [SD]). Comorbid conditions included: hypertension (n = 33, 37.5%), diabetes mellitus (n = 18, 20.5%), central nervous system disorders such as cerebrovascular accident and head trauma (n = 16, 18.2%), peptic ulcer disease including four patients with gastric ulcers, three patients with duodenal ulcers, and one patient with both gastric and duodenal ulcers (n = 8, 9.1%), congestive heart failure (n = 8, 9.1%), cirrhosis (n = 6, 6.8%), pneumonia (n = 5, 5.7%), asthma (n = 5, 5.7%), recurrent episodes of acute pancreatitis (n = 5, 5.7%), and renal failure (n = 4, 4.5%). Eight patients (9.1%) were bedridden. A history of chronic, moderate, daily drinking or heavy (more than 8 ounces of alcohol per day) alcohol consumption was given in 40 patients (45.5%), and recent heavy alcohol consumption within 24 hours of admission occurred in 32 patients (36.4%); 35 patients (39.8%) smoked cigarettes daily or used illicit street drugs such as cocaine or heroin mix daily (n = 4, 4.5%). Nonsteroidal anti-inflammatory agents or clycooxygenase-2 (COX-2) inhibitors had been recently used in 28 patients (31.8%). H_2 blockers or proton pump inhibitors had been used recently in 20 patients (22.7%).

Most patients (79, 89.8%) were initially seen in the emergency room for evaluation. Esophageal ulcers were found in 79 patients during the initial EGD. The esophageal ulcers in the remaining nine patients developed in the hospital while these patients were being treated for another condition. The duration of symptoms was less than 3 days in 43 patients, and more than 10 days in 20 patients. These signs and symptoms included the following: hematemesis (n = 36, 40.9%), nausea and vomiting with regurgitation (n = 35, 39.8%), epigastric (with or without substernal) pain (n = 27, 30.7%), melena (n = 22, 25%), dysphagia to solids (n = 16, 18.2%), "coffee ground" gastric aspirate (n = 15, 17.0%), and chest pain that was substernal with extension to the back (n = 10, 11.4%). The chest pain was thought to be a manifestation of heartburn due to regurgitation. Esophageal ulcers were found on the first endoscopic assessment in 79 patients, whereas esophageal ulcers were first seen on a follow-up endoscopy in nine patients. Most patients (n = 74, 84.1%) required in-hospital treatment at the time of the initial diagnostic EGD. Eight patients (9.1%) required repeat EGD evaluation for esophageal ulcers or their complications after discharge.

Etiology of Esophageal Ulcers

The etiology of esophageal ulcers (Table 1) was most commonly associated with GERD (n = 58). All patients with esophageal ulcers caused by GERD had a

Table 1. Etiology of esophageal ulcers

Etiology	No. of patients	%
GERD	58	65.9
Drug-induced	20	22.7
Candida	3	3.4
Caustic injury	2	2.3
AIDS	1	1.1
Herpes simplex virus	1	1.1
Marginal	1	1.1
Foreign body	1	1.1
Unknown	1	1.1
Total	88	100

GERD = gastrointestinal reflux disease; AIDS = acquired immune deficiency syndrome.

definite hiatal hernia. Hiatal hernia was diagnosed endoscopically when the squamocolumnar junction was more than 3 cm above the diaphragmatic impression.[7] In moderate-sized or large hiatal hernias, the gastric mucosal folds can be seen running proximally over the hiatal margin into the bulbous cavity of the distended hernia pouch. A hiatal hernia is also confirmed based on a retroflexed endoscopic view of the hernia pouch and the squamocolumnar junction from below.[7] All 58 patients with ulcer associated with hiatal hernia had moderate-sized or large hiatal hernias. The endoscopic grading of GERD depends on the endoscopist's interpretation of these visual images. Unfortunately there is no standard classification scheme for endoscopic findings.[7] Several classification systems have been devised to define or grade reflux changes by using characteristics seen at endoscopy. All 58 patients had grade III or IV esophagitis according to the Savary-Miller endoscopic grading system,[7,8] and also grade III or IV esophagitis according to the system devised by Hetzel et al.[7,8] Barrett's esophagus complicated GERD-induced esophageal ulcers in 10 patients and drug-induced esophageal ulcers in one patient. Other etiologies included drug-induced (n = 20), candidal (n = 3), and caustic injury (n = 2), in addition to acute human immunodeficiency virus (HIV), herpes simplex virus (HSV), marginal, foreign body, and obscure origin (n = 1 case of each). The offending medications of drug-induced esophageal ulcers included aspirin, ibuprophen, aspirin plus ibuprophen, ferrous sulfate, doxycycline, erythromycin, amoxicillin clavulanate, nifedipine, and cyclobenzaprine (Table 2).

Morphology of GERD-Induced and Drug-Induced Esophageal Ulcers

The mean size of all esophageal ulcers, GERD-induced esophageal ulcers, and drug-induced esophageal ulcers was 2.80 cm, 2.78 cm, and 2.92 cm, respectively (Table 3). Size was measured with the use of open biopsy forceps. The mean number of all esophageal ulcers, GERD-induced esophageal ulcers, and drug-induced esophageal ulcers was 2.48, 1.96, and 2.80, respectively (see Table 3). Most (91.4%) of the GERD-induced esophageal ulcers were located in the lower intrathoracic esophagus (35 to 40 cm from the incisors), whereas 80.0% of drug-induced esophageal ulcers were located in the middle intrathoracic esophagus (28 to 33 cm from the incisors).

Table 2. Drug-induced esophageal ulcers

Drug implicated	No.	%
NSAIDs	13	65
Aspirin only	7	35
Ibuprophen only	3	15
Aspirin + ibuprophen	3	15
Ferrous sulfate	2	10
Doxycycline	1	5
Erythromycin	1	5
Amoxicillin, clavulanate	1	5
Nifedipine	1	5
Cyclobenzaprine	1	5
Total	20	100

Table 3. Endoscopic morphology of esophageal ulcers

	GERD	Drug
Location		
Upper third	2 (3.4%)	9 (4.9%)
Middle third	11 (19.0%)	16 (80.0%)
Lower third	53 (91.4%)	4 (20.0%)
Total	66	29
Size		
Minimum	0.6 cm	0.6 cm
Maximum	10.0 cm	6.0 cm
Mean ± SD	2.78 cm ± 2.13	2.92 cm ± 1.98
Number		
Minimum	1	1
Maximum	12	16
Mean ± SD	1.96 ± 2.12	2.80 ± 4.19

SD = standard deviation.

Esophageal Ulcers: Inpatients

In-hospital esophageal ulcers developed in nine inpatients; this was due to GERD (n = 6), drugs (n = 1), Candida (n = 1), and AIDS (n = 1). Of the six patients with GERD, one had liver cirrhosis and five were bedridden because of a cerebral vascular occlusion (n = 4) or a right femoral neck fracture (n = 1). Concurrent diagnoses included pneumonia, decubitus ulcer, gas gangrene, seizure disorder and weakness, retroperitoneal mass, and tuberculosis peritonitis. Three patients died of primary disease, multisystem organ failure, and sepsis.

Esophageal Strictures

Eleven patients developed an esophageal stricture including 10 patients with GERD-induced esophageal ulcers and one of two patients with caustic injury. All esophageal strictures developed at the squamocolumnar junction on the distal esophagus and were associated with a hiatal hernia. Endoscopic dilatation was performed in all 11 of them; three patients underwent multiple endoscopic dilatations. Nissen fundoplication had been performed in four patients with esophageal stricture. Esophagectomy with colonic interposition was performed in one patient with caustic

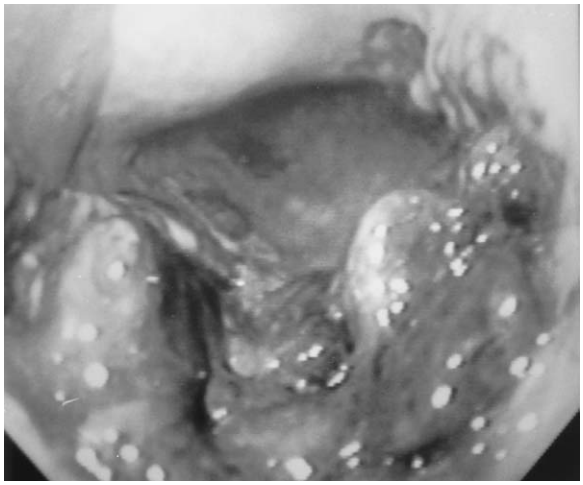

Fig. 1. A large ulcer with clot on the base is seen at midesophagus (25–30 cm from incisors). This patient had been taking 8–10 tablets of aspirin and Anacin daily for 3 weeks to treat her abdominal and chest pain. She chewed some pills before hematemesis. She did not have hiatal hernia or GERD.

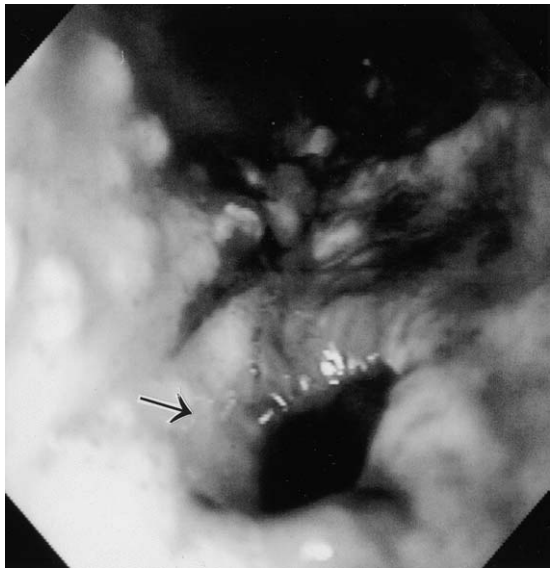

Fig. 2. A GERD-induced diffuse esophageal ulcer with perforation (see arrow).

injury. There were no complications related to dilatation. All five patients survived operative intervention.

Hemorrhage

Hemorrhage from esophageal ulcers was diagnosed by EGD in 30 patients including 17 with GERD, 10 with drug-induced esophageal ulcers (Fig. 1), and one patient each with HSV, idiopathic esophageal ulcers with HIV, and esophageal ulcers of unknown etiology. Twenty-one patients required blood transfusion including nine patients with GERD, nine patients with drug-induced esophageal ulcers, and one patient each with HSV, idiopathic esophageal ulcers of HIV, and esophageal ulcers of obscure origin. The mean amount of blood transfused was 5.38 ± 3.43 units (range 2 to 14 units). This included an average of 3.62 units in patients with GERD and 6.28 units in patients with drug-induced esophageal ulcers. Endoscopic hemostasis with epinephrine injection and heater probe application was required in three patients with drug-induced esophageal ulcers and in one patient with esophageal ulcers of unknown etiology. Endoscopic hemostasis was not necessary for bleeding from GERD-induced esophageal ulcers. No operative intervention was undertaken for esophageal bleeding secondary to esophageal ulcers.

Esophageal Perforation

Esophageal perforation occurred in three patients resulting in two deaths. One patient developed upper gastrointestinal bleeding 3 days after operative fixation of a right femoral neck fracture. EGD revealed GERD-induced diffuse esophageal ulcers and a 2×2 cm gastric polyp. A repeat EGD done 10 days later for recurrent bleeding showed esophageal perforation from the ulcer (Fig. 2). Emergency operation included cervical esophagostomy, gastrostomy, and feeding jejunostomy. This patient died of sepsis 3 weeks later. A second patient with drug-induced esophageal ulcers and an aneurysm of the thoracic aorta developed an esophagoaortic fistula and died rapidly from massive hemorrhage and aspiration. A third patient with GERD-induced esophageal ulcers diagnosed 3 months earlier was noncompliant with H_2 blocker therapy and developed a distal esophageal perforation, which was treated with segmental resection of the esophageal perforation, esophageal exclusion, cervical esophagostomy, feeding jejunostomy, and decompressive gastrostomy. The patient was discharged to a rehabilitation institute and eventually underwent esophageal reconstruction.

Multiple Presentations to the Hospital

One patient, a 63-year-old woman, was found to have drug (ibuprophen)–induced esophageal ulcers in the midesophagus with a moderate-sized hiatal hernia without GERD in 1991. Seven years later, at age 70, she developed GERD-induced esophageal ulcers with stricture at the squamocolumnar junction and a larger hiatal hernia than that previously seen. Another patient, a 41-year-old man with AIDS, presented with chest pain and melena and was diagnosed with an idiopathic esophageal ulcer in 1996. Three years later,

during hospitalization for AIDS encephalitis complicated by seizures, he developed coffee ground emesis and was diagnosed again with idiopathic esophageal ulcers. A third patient, 48 years of age, had been admitted for hematemesis in 1996 and was readmitted because of hematemesis 6 months later, despite taking proton pump inhibitors, and underwent a Nissen fundoplication. Four patients underwent multiple esophageal dilatations for esophageal stricture with esophageal ulcers; three were caused by GERD and one was due to caustic injury.

Treatment of Uncomplicated Esophageal Ulcers

Patients with uncomplicated esophageal ulcers were treated with H_2 blockers, proton pump inhibitors, or antifungal medication in the case of candidal esophageal ulcers. The mean length of stay for patients with uncomplicated esophageal ulcers (n = 50) was 6.08 ± 6.37 days (range 1 to 38 days). Endoscopically confirmed resolution of esophageal ulcers was seen in five patients with a mean resolution time of 49.8 days (range 4 to 150 days). This occurred in two patients with GERD-induced esophageal ulcers, two patients with drug-induced esophageal ulcers, and one patient with esophageal ulcers of unknown etiology. Long follow-up of these patients were not possible unless they returned to Detroit Receiving Hospital. Some patients with intractable esophageal ulcers or GERD may have had elective surgery at other hospitals.

Coexistent EGD Findings Associated With Esophageal Ulcers

Significant coexistent findings on EGD were noted in 50 (57%) of 88 patients with esophageal ulcers; these included: acute erosive gastritis (n = 29), doudenitis (n = 14), acute gastric ulcers (n = 12), and duodenal ulcers (n = 11). *Helicobacter pylori* testing was positive in 18 of 28 patients tested. Barrett's esophagus complicated GERD–induced esophageal ulcers in 10 patients and drug-induced esophageal ulcers in one patient. This latter patient developed a midesophageal ulcer surrounded by normal mucosa and had a strong history of NSAID use.

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Differentiation between distal drug-induced esophageal ulcers and GERD-induced esophageal ulcers can be difficult. Patients with drug-induced esophageal ulcers may be more prone to develop GERD as a result of disorders of esophageal motility caused by the drug-induced esophageal ulcers. Five of 13 patients herein with NSAID-induced esophageal ulcers demonstrated GERD. One patient with a large NSAID-induced bleeding esophageal ulcer in the midesophagus developed a GERD-induced esophageal ulcer with esophageal stricture in the distal esophagus 7 years later. Possibly, drug-induced esophageal injuries are aggravated by GERD, and GERD-induced esophageal injuries progress with certain drugs.[14]

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was noted in 13.8% of patients with GERD-induced esophageal ulcers and in 45.0% of patients with drug-induced esophageal ulcers. Bleeding from drug-induced esophageal ulcers is always from the midesophagus and is more likely to be active bleeding than GERD-related bleeding (45.0% vs. 13.8%); patients with this bleeding require blood transfusions (45.0% vs. 13.8%) and endoscopic hemostasis (15.0% vs. 0%).

Anatomically there is a rich arterial and venous network at the level of the mucosa and submucosa throughout the esophagus, especially near the gastroesophageal junction.[18] Thus the bleeding tendency associated with drug-induced esophageal ulcers seems to be determined by factors other than vascular anatomy. Superficial ulceration of the squamous epithelium is typical of reflux esophagitis.[19] In some patients with GERD-induced esophageal ulcers, esophageal ulceration stimulates fibrous tissue production with collagen deposition and stricture formation.[1] The chronic nature of GERD, which is due to intermittent regurgitation of acid, may reduce the bleeding tendency of the epithelium lining the gastroesophageal junction. In contrast, the acute nature of drug-induced esophageal ulcers resulting from continuous contact of a caustic agent with previously normal mucosa may increase the bleeding tendency, particularly with NSAID-induced esophageal ulcers.[10]

NSAIDs are strikingly more likely to cause hemorrhage than other pill classes when they injure the esophagus.[10,16] Kikendall[16] noted that 22 of 154 NSAID-induced esophageal injuries were complicated by hemorrhage. In contrast, only 25 of 796 esophageal injuries induced by other medications resulted in hemorrhage. Furthermore, 8 of 19 esophageal injuries induced by aspirin were complicated by hemorrhage, compared to only one of five esophageal injuries caused by ibuprophen.[16]

The reported rate of esophageal perforation complicating GERD is less than 0.2%.[2] The rate of perforation of GERD-induced esophageal ulcers has not been previously reported. In the present study, two patients with perforation were seen in 58 patients with GERD induced esophageal ulcers (5.1%). In a review of 22 esophageal perforations reported by Nesbitt and Sawyers,[20] the etiology was barogenic transmural disruption (Boerhaave's syndrome) in 20 patients and distal esophageal ulcers in two.

No perforation complicated the 154 NSAID-induced esophageal injuries identified in Kikendall's series.[16] One of our patients subsequently died of hemorrhage from an aortoesophageal fistula through an underlying thoracic aortic aneurysm; this patient had been taking aspirin and ibuprophen for 2 years.

Approximately 70% of patients with GERD-induced esophageal ulcers show complete healing within several months with H_2 receptor–blocking agents administered in conventional doses.[1] Most ulcerations refractory to conventional treatment will heal with the intensive suppression of gastric acid secretion achieved by administering high doses of H_2 receptor–blocking agents or proton pump inhibitors.[1] The role of antireflux surgery in the treatment of esophageal ulcers is limited to those few patients with GERD-induced esophageal ulcers refractory to high-dose medical therapy.

The clinical course of hospitalized patients who develop esophageal ulcers is unique. The development of esophageal ulcers is influenced by organ failure, recumbency, and underlying disease. Five of the nine patients who developed esophageal ulcers while hospitalized for other diagnoses had an infectious process including pneumonia, decubitus ulceration, gas gangrene, infected retroperitoneal mass, and tuberculous peritonitis. Three of these five patients died of sepsis or multiple organ failure. Likewise, the patient who died after fixation of a right femoral neck fracture developed upper gastrointestinal bleeding from GERD-induced esophageal ulcers and a later perforation that caused his death.

CONCLUSION

GERD and drug ingestion are the most common causes of esophageal ulcers. Midesophageal ulcers caused by NSAIDs have a greater tendency toward hemorrhage that requires blood transfusion and endoscopic hemostasis. Stricture formation is very likely with GERD-induced esophageal ulcers and is effectively treated with dilatation in most patients. Esophageal ulcers rarely cause death.

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Electrical Stimulation of the Vagus Nerve Restores Motility in an Animal Model of Achalasia

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Esophageal peristalsis generally does not return to normal after surgical treatment of achalasia. Direct electrical stimulation of the vagus nerve is known to stimulate antegrade peristalsis in the normal esophagus; however, it is not known whether electrical stimulation will induce return of peristalsis once an achalasia-like disorder has been established. The objective of this study was to perform quantitive and qualitative measurements of motility during electrical stimulation of the vagal nerve in an animal model of achalasia. An already established and verified animal achalasia model using adult North American opossums (*Didelphis virginiana*) was used. Fifteen opossums were divided into three groups. Sham surgery was performed on three animals (group 1). In group 2 (n = 6) a loose Gore-Tex band (110% of the esophageal circumference) was placed around the gastroesophageal junction to prevent relaxation of the lower esophageal sphincter during swallowing. In group 3 (n = 6) a relatively tighter band (90% of the esophageal circumference) was used to further elevate the lower esophageal sphincter pressure. At 6 weeks, after manometric and radiolologic confirmation of achalasia, electrical stimulation of the esophagus was performed before and after removal of the band using a graduated square-wave electrical stimulus. Changes in esophageal neural plexi were assessed histologically. Pre- and postoperative manometric data were compared using standard statistical techniques. No difference was observed in esophageal characteristics and motility after sham surgery in group 1. Animals in group 2 demonstrated a vigorous variety of achalasia (high-amplitude, simultaneous, repetitive contractions), moderate esophageal dilatation, and degeneration of 40% to 60% of intramuscular nerve plexi. Animals in group 3 developed amotile achalasia with typical low-amplitude simultaneous (mirror image) contractions, severely dilated ("bird beak") esophagus, and degeneration of 50% to 65% of nerve plexi. Vagal stimulation in group 2 demonstrated a significant increase in the amplitude of contractions ($P < 0.001$) and return of peristaltic activity in 49% of swallows before band removal. After band removal, all of the contractions were peristaltic. In group 3 vagal stimulation before and after removal of the band demonstrated a significant increase in amplitude of contractions ($P < 0.0001$) but no return of propagative peristalsis before band removal, however, 44% of contractions were progressive in the smooth portion of the esophagus after removal of the band. Electrical stimulation of the vagus nerve improved the force of esophageal contractions irrespective of the severity of the disease; however, peristaltic activity completely returned to normal only in the vigorous (early) variety of achalasia. Removal of the functional esophageal outlet obstruction, as with a surgical myotomy, may be necessary to obtain significant peristalsis with vagal pacing in severe achalasia. (J GASTROINTEST SURG 2003;7:843–849)

KEY WORDS: Esophagus, achalasia, vigorous achalasia, animal model, opossum, electrical stimulation, motility disorder, vagus nerve

Achalasia is the most common of the primary named esophageal motility disorders.[1] It is characterized manometrically by an incompletely relaxing lower esophageal sphincter (LES) and complete loss of primary peristalsis. A relatively uncommon variant, vigorous achalasia, is characterized by nonperistaltic

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spastic contractions. This variant has been theorized to represent an early phase in the natural history of this disease. Untreated, achalasia leads to progressive esophageal dilatation, pulmonary aspiration, malnutrition, and eventually death. There are no effective medical therapies for achalasia,[2] and surgical treatment of achalasia, which is directed toward relieving functional obstruction of the esophagus by disruption of the lower esophageal sphincter,[3] is not without risk to the patient from anesthesia or surgical complications.[4,5] Current literature suggests that esophageal peristalsis only rarely returns to normal after palliative treatment measures.[6,7] The failure to regain normal progressive peristalsis is more commonly observed in patients who present with complete loss of motility or a severely dilated esophagus, or those who have a long-standing disease.[6]

Electrical stimulation of the vagus nerve is known to produce antegrade peristalsis in the normal esophagus in animal models.[8,9] It is not known, however, whether electrical stimulation of the vagus nerve will induce return of progressive peristalsis after an achalasia-like disorder has been created in an animal model. The aim of the current study was to establish propagative antegrade peristalsis by electrical stimulation of the vagus nerve in an animal model after an achalasia-like motility disorder has been confirmed objectively.

METHODS

Fifteen adult opossums (*Didelphis virginiana*) were investigated in accordance with the protocol approved by the Legacy Institutional Animal Care and Use Committee. Esophageal studies were performed with the animals under conscious sedation and surgical interventions under general endotracheal anesthesia. Baseline esophageal manometry and contrast esophagography were performed in all animals using a specially designed four-channel water-perfused catheter. The LES was located by stationary pull-through technique. Resting LES pressure and relaxation were obtained for 10 swallows induced by hypopharyngeal stimulation using stationary pull-through technique. Esophageal body motility in the lower third, the middle third, and the upper third of the esophagus was assessed by positioning the lowermost channel of the catheter 1 cm above the upper border of the LES. Manometric tracings for the body were recorded for a total of 10 swallows.

Animals were divided into three groups at the time of surgery. Sham surgery was performed on animals in group 1 (n = 3). Six animals each were assigned to groups 2 and 3. A midline laparotomy was performed under general anesthesia. The gastroesophageal junction (GEJ) was gently mobilized, and the esophageal circumference at that level was measured with a tape. In group 2 (n = 6) a Gore-Tex (W.L. Gore & Associates, Newark, DE) band, 1 cm wide and 110% of the esophageal circumference in length, was placed around the GEJ to prevent relaxation of the LES during swallowing. In group 3 (n = 6) a relatively tighter band (1 cm wide and 90% of the esophageal circumference in length) was used to further elevate the resting LES pressure. No band was placed around the GEJ in group 1 animals (n = 3).

After banding, esophageal motility was assessed every week with the animals under light sedation. As soon as the animals had documented amotility, or at a maximum of 6 weeks after banding, bilateral electrical stimulation of the vagus nerves, accessed by a lateral cervicotomy, was performed under general anesthesia using a constant-current nerve stimulator (Grass model 544, Grass Instruments, Quincy, MA). A graduated square-wave electrical stimulus was used. Stimulus values were modulated over a wide range (frequency 1 to 20 Hz., a pulse width of 0.1 to 5 msec, and train duration of 0.1 to 10 seconds) until peristaltic activity was demonstrated.[8]

Contrast esophagograms were obtained before vagal nerve stimulation was performed. In groups 1 and 2, esophageal motility was assessed in response to vagal nerve stimulation both before and after surgical removal of the band. Animals were euthanized at the end of the experiment. The entire esophagus and the upper part of the stomach were harvested. Histologic changes were assessed in the lower third of the esophagus immediately proximal to the LES using 5 mm sections and hematoxylin and eosin staining under light microscopy. A quantitative analysis of the intramuscular ganglion cells was performed. Ten medium-power (× 200) fields were counted for the presence of degenerating plexi. Plexi were considered degenerating if more than 33% of neurons in the plexus demonstrated nuclear margination, chromatolysis, atrophy, or vacuolation.

Comparisons were made between the preoperative baseline manometric data and the manometric data obtained before and after the removal of the band. All of the values are presented as means (± standard deviations). Continuous variables were compared using Wilcoxon rank-sum or paired/unpaired *t* tests as appropriate. Proportions were compared using chi-square or Fisher exact tests. *P* values of 0.05 or less were considered statistically significant.

RESULTS

There were no statistically significant differences in the preoperative resting LES pressure, LES relaxation, and mean amplitude of distal body contractions

among the three groups. All of the animals had normal esophageal body motility preoperatively (Table 1).

In group 1 following sham surgery, no difference was observed in resting LES pressure (31.22 [± 4.52] mm Hg vs. 29.22 [± 5.58] mm Hg, $P = 0.56$), LES relaxation (101.8 [± 7.1]% vs. 98.8 [± 8]%), and esophageal motility at 6 weeks (see Table 1). Only 5% of nerve plexi showed degenerative changes in this group (Fig. 1, *A*) and the esophagogram did not show any esophageal dilatation (Fig. 2, *A*). Bilateral cervical vagal stimulation in this group resulted in a significant increase in the amplitude of distal contractions, (Fig. 3, *A*) and all contractions were progressive (Fig. 4, *A*).

In group 2 the preoperative mean resting LES pressure and mean percentage relaxation of the LES were 30.14 [± 6.35] mm Hg and 99.9 [± 6.5]%, respectively. Placement of a loose band around the GEJ did not produce any significant increase in the resting LES pressure (mean post-banding resting LES pressure 33.02 [± 8.36] mm Hg, $P = 0.13$); however, relaxation of the LES during swallows decreased significantly (mean relaxation pressure 20.55 [± 6.53] mm Hg, mean percentage relaxation 28.1 [± 14.9]%, $P < 0.001$). After a mean follow-up period of 4.6 weeks, animals in group 2 developed a vigorous variety of achalasia represented by high-amplitude repetitive simultaneous contractions (see Table 1 and Fig. 3, *B* and 4, *B*). A barium swallow in these animals demonstrated moderate dilation of the esophagus (see Fig. 2, *B*), and histologic sections showed degeneration of 40% of the nerve plexi in three animals (see Fig. 1, *B*), 60% of nerve plexi in one animal, and 5% to 10% of nerve plexi in one animal. Electrical stimulation of the vagus nerve before removal of the band demonstrated a significant increase in the amplitude of contractions (see Fig. 3, *B*). Forty-nine percent of the contractions showed normal propagative progression in the distal smooth portion of the esophagus (see Table 1). However, none of the animals demonstrated complete return of normal peristaltic activity. After removal of the band, four animals showed complete return of normal progressive peristalsis (all contractions progressive) with electrical stimulation of vagi (see Figs. 3, *B* and 4, *B*). One animal had 40% nonresponse to stimulation and another animal had 20% simultaneous contractions.

Animals in group 3 developed a hypertensive LES with banding. Mean preoperative resting LES pressure in this group was 29.22 [± 5.58] mm Hg. After placement of a relatively tighter band, the mean resting LES pressure increased to 50.41 [± 10.71] mm Hg ($P < 0.001$). The mean percentage relaxation decreased from 99.1 [± 3.8]% to 28.4 [± 16]%, and the mean post-banding LES relaxation pressure was 34.34 [± 9.81] mm Hg. Animals in this group demonstrated a severe amotile variety of esophageal peristalsis after a mean follow-up period of 3.3 weeks (see Table 1). The mean amplitude of contractions decreased more than 50% (see Fig. 3, *C*), and all stimulated swallows resulted in simultaneous mirror-image contractions (Fig. 4, *C*). Postoperative esophagography demonstrated a severely dilated esophagus with a typical "bird beak" appearance at the GEJ (see Fig. 2, *C*). Animals in this group demonstrated degeneration of 50% to 65% of the nerve plexi (see Fig. 3, *C*), except for two animals that developed dysphagia and esophageal dysmotility relatively rapidly (within 2 weeks) after banding of the GEJ. These two animals showed degeneration of 20% and 5% to 10% nerve plexi, respectively. Electrical stimulation of the vagus nerves before removal of the band demonstrated a significant ($P < 0.001$) increase in the amplitude of contractions (see Fig. 3, *C*); however, no return of progressive peristalsis was observed (see Fig. 4, *C*). After removal of the band, electrical stimulation was performed in five animals because one animal died intraoperatively. Only one animal (20%) showed complete return of progressive peristaltic activity, whereas 60% to 100% of the contractions were simultaneous in the remaining animals (see Figs. 3, *C* and 4, *C*).

Table 1. Relative proportion of propagative and nonpropagative contractions in the distal (smooth muscle) portion of esophagus

	Group 1 (n = 3)		Group 2 (n = 6)		Group 3 (n = 6)	
	Propagative peristalsis	Simultaneous (dropped)	Propagative peristalsis	Simultaneous (dropped)	Propagative peristalsis	Simultaneous (dropped)
Preoperative	94%	0% (6%)	100%	0% (0%)	100%	0% (0%)
Postoperative	100%	0% (0%)	2%	78% (20%)	0%	93% (7%)
Vagal stimulation before band removal	95%	5% (0%)	49%	44% (7%)	8%	89% (3%)
Vagal stimulation after band removal	—	—	90%	3% (7%)	44%	52% (4%)

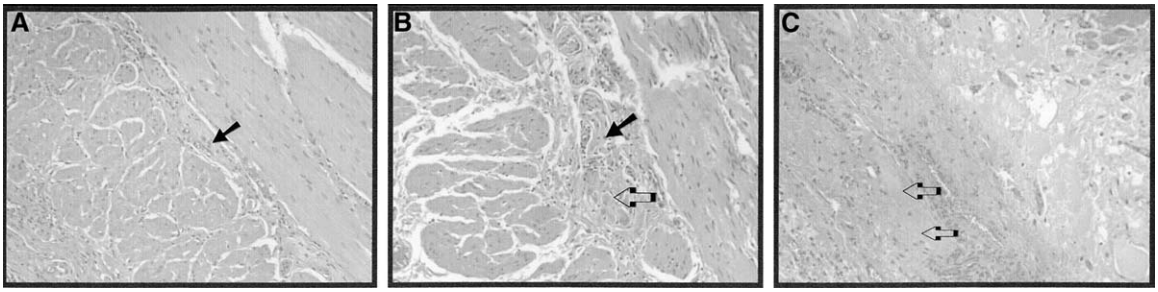

Fig. 1. Histopathologic sections in the lower esophagus after banding demonstrates normal ganglion cells (*black arrow*) in group 1 (**A**), degenerated ganglion cells (*clear arrow*) with some preserved normal ganglion cells (*black arrow*) as a result of loose banding in group 2 (**B**), and complete degeneration of ganglion cells (*clear arrow*) in group 3 (**C**). (Hematoxylin and eosin stain.)

DISCUSSION

The term achalasia, which means "failure to relax," was first used by Herz[10] in 1914 to describe a nonrelaxing LES. Although it is a rare disease, it causes a profound effect on the quality of life of those who have it, presenting as it does with dysphagia, weight loss, nocturnal regurgitation, and noncardiac chest pain. Left untreated it usually leads to more severe stasis-related complications (aspiration, squamous cell carcinoma, malnutrition, etc.). Although its precise etiology is still unknown, the functional and physiologic abnormalities associated with this disorder have been well documented since the introduction of intraluminal manometry.[11] A poorly functioning LES that fails to relax completely during swallowing and a complete absence of primary esophageal peristalsis are considered to be the essential manometric criteria for a diagnosis of achalasia.[12,13] Patients with this disorder may also have a hypertensive LES and other gastrointestinal tract motility abnormalities. The etiology and primary neuralgic defect responsible for the development of achalasia remains poorly defined at present. However, the most consistent pathologic findings have been noted in the distal esophagus and LES, where an absence or degeneration of ganglion cells in the myentric plexus (Auerbach's plexus) is seen.[14–16] The significance of the neural degeneration is unknown and may be a primary etiology of the achalasia, perhaps from an infectious or autoimmune cause, or a secondary finding resulting from the stasis and dilatation of the esophagus as the disease progresses. Our model, which by functional, histopathologic, and manometric criteria *is* similar to achalasia, would imply that the neural degeneration might be secondary to esophageal failure. Whether this phenomenon translates to human achalasia is unknown because the true initiator of this disorder is still unknown and may, in fact, end up being multifactorial. Our finding, however, should raise concerns about the ultimate effect of restrictive treatments of the human esophagus such as an overly tight or nonrelaxing fundoplication or perhaps the increasingly

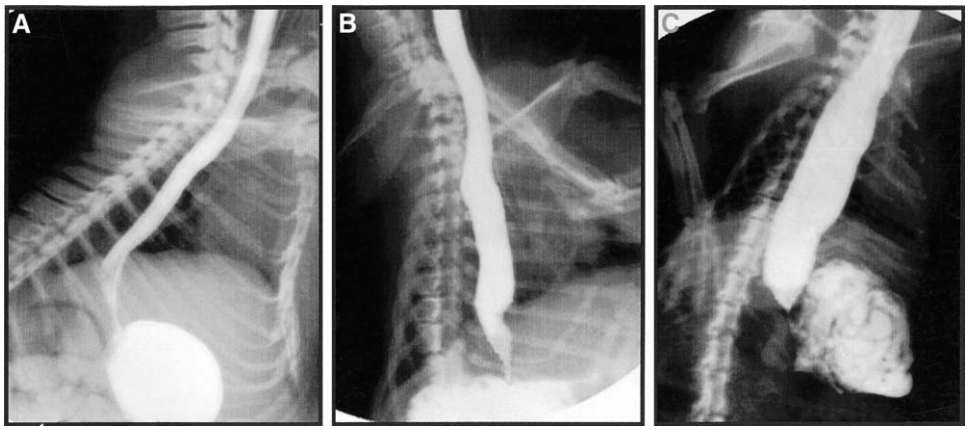

Fig. 2. Contrast esophagogram showing no esophageal dilatation in a group 1 animal (**A**), moderate dilatation of the esophagus in a group 2 animal (**B**), and severe esophageal dilatation with typical "bird-beak" appearance at the GEJ in a group 3 animal (**C**).

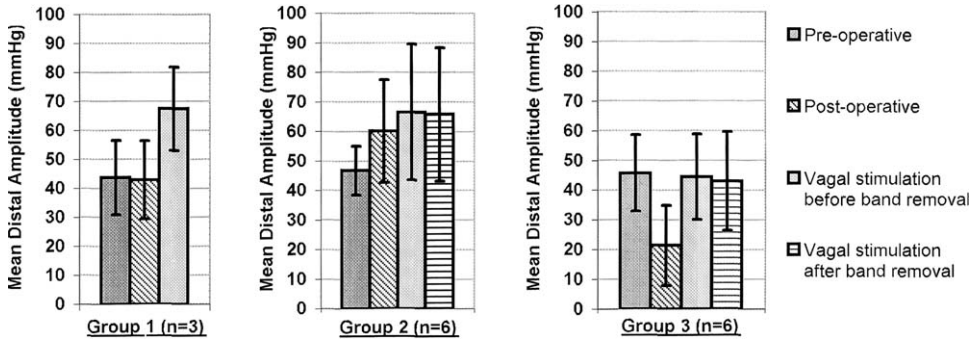

Fig. 3. Mean distal esophageal body amplitude in group 1 (**A**), group 2 (**B**), and group 3 (**C**).

popular laparoscopic restrictive bands for morbid obesity that are often placed very close to the GEJ.

The natural history of achalasia is not well defined either. Some have theorized that the spastic or "vigorous" type of achalasia is an early manifestation of the disease, which will later progress to the total noncontractility and massive esophageal dilatation of the classic form.

Treatment of achalasia is directed toward the relief of the functional obstruction at the LES. All of the current medical and endoscopic treatments are less satisfactory than surgical myotomy.[2] Surgery provides excellent palliation by facilitating esophageal emptying, which improves quality of life and possibly prevents stasis-related complications by preventing progressive dilatation.[17] Return of progressive peristalsis in the body of the esophagus is observed only in a very small proportion of patients who perhaps had a milder variety of achalasia and received intervention early in the course of their disease.[6] A vast majority of patients never regain normal esophageal function,[7] and some will ultimately require extreme measures including esophagectomy if their palliation is not successful.[18,19]

The primary focus of our research has been to determine whether the physiologic functioning of the esophagus can be improved by means such as electrical pacing of the esophagus. There has been a great deal of research and success in the modulation of gastrointestinal motility by using electrical pacing devices. Karlstrom and Kelly[20] demonstrated that reversal of peristalsis in the jejunal Roux limb is possible by external pacing. O' Connell et al.[21] succeeded in reducing jejunostomy output with retrograde external pacing in a canine model. Recently gastric pacing has become available, and reasonably effective, for therapy of chronic intractable gastroparesis.[22] Experimental studies involving decentralized vagal nerve stimulation of the normal esophagus have established antegrade peristalsis in opossum and feline models.[8,9] We have chosen the opossum model for our studies as it has an esophageal physiology similar to another omnivore, the human. This includes a proximal esophagus composed of striated muscle and distal two thirds composed of autonomically innervated smooth muscle. Previous studies using feline models, where the esophagus is completely composed of striated muscle, are questionable because of the difference in esophageal innervation. Previous studies in both cats and opossums have shown that altering various stimulus parameters could modulate all the direction, velocity of the peristaltic waves, and amplitude of the contractions. However, there has been no previous evidence that electrical stimulation of the vagus nerve can induce progressive peristalsis once an achalasia-like disorder has been established.

Our results show that esophageal motility can be improved by electrical stimulation of the vagus nerve in an animal model of achalasia-like disorder. We deliberately chose the timing of our intervention (6 weeks or less) so that there was a spectrum of dysmotility to study. We have previously discovered that beyond 6 weeks opossums uniformly develop end-stage dilatation which, much as in humans, has less of a response to any interventions. Overall 91% of the animals with achalasia-like disorder established 40% or greater primary peristalsis with vagal stimulation. Forty-five percent had 100% normal peristalsis. Complete return of normal progressive peristalsis after vagal stimulation was observed in the group of animals having a vigorous achalasia–like motility disorder following relief of obstruction at the GEJ. In the animals developing a severe amotile type of motility disorder, with massive dilatation of the esophagus, a significant improvement in the amplitude and morphology of the contractions was observed when electrical stimulation of the vagus nerve was performed. After band removal, our model's equivalent of a surgical myotomy, only 20% of the animals regained completely normal peristalsis. Another 20% had no improvement, whereas the majority reestablished around 40% of swallows with

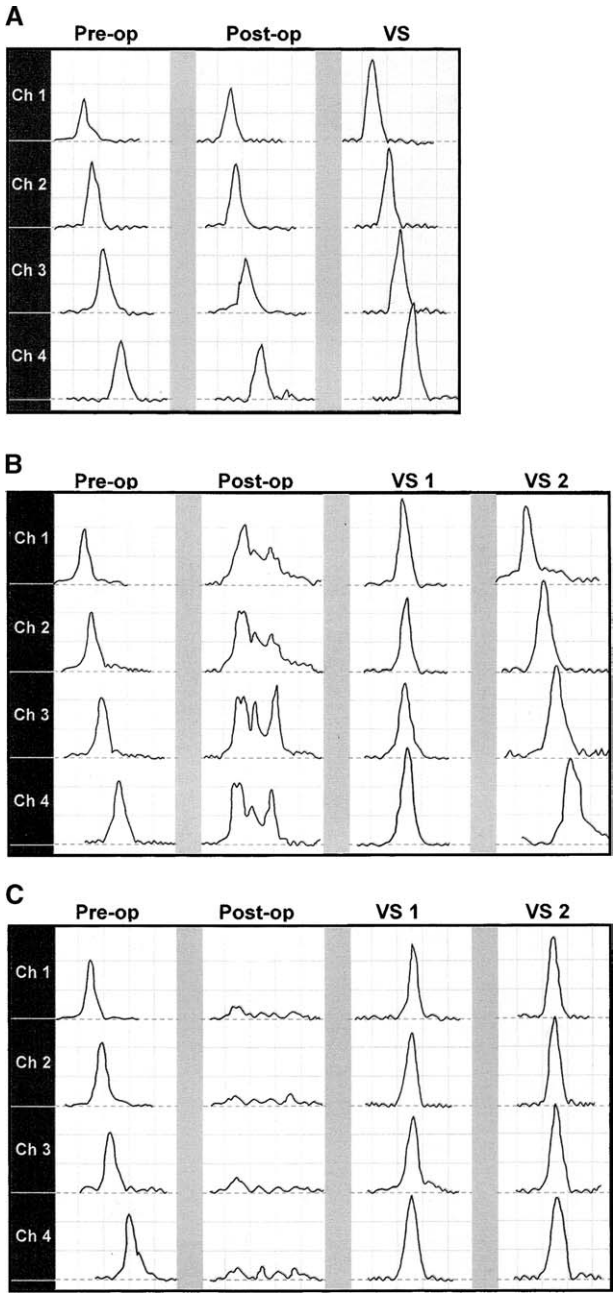

Fig. 4. Manometric tracings of the esophageal body motility in group 1 (**A**), group 2 (**B**), and group 3 (**C**). Pre-op = motility in response to swallow induced by hypopharyngeal stimulation before surgery; Post-op = motility in response to swallow induced by hypopharyngeal stimulation after sham surgery in group 1 (**A**), and after band placement in group 2 (**B**) and group 3 (**C**); VS = motility after vagal stimulation in group 1; VS1 and VS2 = motility following vagal stimulation before and after removal of the band, respectively, in group 2 and group 3.

primary peristalsis. These findings suggest that a point of irreversibility of the normal motility may exist in the natural history of the disease. This may be related to the degree of degeneration of the Auerbach's plexus nerves that we observed. However, a definitive histologic correlation was not possible between function, dilatation, and the percentage of nerve degeneration because of the small sample size and short duration of follow-up after banding.

It may be argued that esophageal pacing is unnecessary, that peristalsis may return to normal spontaneously after relief of obstruction as was suggested by Schneider et al.[23] These investigators, however, used an obstructed esophagus in a feline model. A similar response may not be reproducible in the human esophagus inasmuch as secondary peristalsis in cats appears to be independent of vagal control.[24] It is known that esophageal peristalsis in human beings appears to be influenced by vagal control as is evident by disordered primary peristalsis in diabetic neuropathy[25] and dysautonomia.[26] Additionally, partial return of normal primary peristalsis after surgical myotomy has only been reported in a very small number of patients.[6,7] Long-term survival studies will be needed in our model to prove that loss of peristalsis is a permanent condition in the absence of vagal pacing.

The increased force of contraction in all of the animals irrespective of severity of dysmotility, the induction of progressive peristalsis in 50% of animals with vigorous achalasia even before relief of obstruction, and the restoration of some peristaltic swallows in all but one animal with pacing and relief of outlet obstruction would indicate that vagal stimulation leads to improved esophageal motility in an achalasia-like disorder. The clinical effect of this and its long-term clinical effectiveness are unknown but will be the basis of further investigations.

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Invited Discussion—Expert Commentator

Carlos A. Pellegrini, M.D. (Seattle, WA): The authors of this paper designed this experiment to test the hypothesis that electrical stimulation of the vagus nerves may improve esophageal motility after treatment for achalasia. To this end they were apparently successful in developing an excellent animal model of achalasia. In fact, this model not only reproduces faithfully the manometric findings of achalasia but yields a histologic picture of achalasia given the damage to the myenteric plexus that results (apparently) from the banding of the distal esophagus. This, in and of itself, is an important advance that should allow us to study in more detail the pathogenesis of achalasia itself as well as the impact of different therapeutic maneuvers in an animal model that has been shown to relate to humans. Their study showed that electrical stimulation of the cervical vagus resulted in improvement or complete restitution of the peristaltic wave in these animals and that this effect was more prominent when the disease was treated at an earlier stage. Although these findings all make sense, one must wonder what in this model leads to the disruption of the myenteric plexus in the first place and whether the damage observed in these animals is permanent or reversible. Most important, it will be of great interest to determine whether these changes can be reproduced in humans.

Electrical Stimulation of the Vagus Nerve Restores Motility in an Animal Model of Achalasia

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Esophageal peristalsis generally does not return to normal after surgical treatment of achalasia. Direct electrical stimulation of the vagus nerve is known to stimulate antegrade peristalsis in the normal esophagus; however, it is not known whether electrical stimulation will induce return of peristalsis once an achalasia-like disorder has been established. The objective of this study was to perform quantitive and qualitative measurements of motility during electrical stimulation of the vagal nerve in an animal model of achalasia. An already established and verified animal achalasia model using adult North American opossums (*Didelphis virginiana*) was used. Fifteen opossums were divided into three groups. Sham surgery was performed on three animals (group 1). In group 2 (n = 6) a loose Gore-Tex band (110% of the esophageal circumference) was placed around the gastroesophageal junction to prevent relaxation of the lower esophageal sphincter during swallowing. In group 3 (n = 6) a relatively tighter band (90% of the esophageal circumference) was used to further elevate the lower esophageal sphincter pressure. At 6 weeks, after manometric and radiolologic confirmation of achalasia, electrical stimulation of the esophagus was performed before and after removal of the band using a graduated square-wave electrical stimulus. Changes in esophageal neural plexi were assessed histologically. Pre- and postoperative manometric data were compared using standard statistical techniques. No difference was observed in esophageal characteristics and motility after sham surgery in group 1. Animals in group 2 demonstrated a vigorous variety of achalasia (high-amplitude, simultaneous, repetitive contractions), moderate esophageal dilatation, and degeneration of 40% to 60% of intramuscular nerve plexi. Animals in group 3 developed amotile achalasia with typical low-amplitude simultaneous (mirror image) contractions, severely dilated ("bird beak") esophagus, and degeneration of 50% to 65% of nerve plexi. Vagal stimulation in group 2 demonstrated a significant increase in the amplitude of contractions ($P < 0.001$) and return of peristaltic activity in 49% of swallows before band removal. After band removal, all of the contractions were peristaltic. In group 3 vagal stimulation before and after removal of the band demonstrated a significant increase in amplitude of contractions ($P < 0.0001$) but no return of propagative peristalsis before band removal, however, 44% of contractions were progressive in the smooth portion of the esophagus after removal of the band. Electrical stimulation of the vagus nerve improved the force of esophageal contractions irrespective of the severity of the disease; however, peristaltic activity completely returned to normal only in the vigorous (early) variety of achalasia. Removal of the functional esophageal outlet obstruction, as with a surgical myotomy, may be necessary to obtain significant peristalsis with vagal pacing in severe achalasia. (J GASTROINTEST SURG 2003;7:843–849)

KEY WORDS: Esophagus, achalasia, vigorous achalasia, animal model, opossum, electrical stimulation, motility disorder, vagus nerve

Achalasia is the most common of the primary named esophageal motility disorders.[1] It is characterized manometrically by an incompletely relaxing lower esophageal sphincter (LES) and complete loss of primary peristalsis. A relatively uncommon variant, vigorous achalasia, is characterized by nonperistaltic

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spastic contractions. This variant has been theorized to represent an early phase in the natural history of this disease. Untreated, achalasia leads to progressive esophageal dilatation, pulmonary aspiration, malnutrition, and eventually death. There are no effective medical therapies for achalasia,[2] and surgical treatment of achalasia, which is directed toward relieving functional obstruction of the esophagus by disruption of the lower esophageal sphincter,[3] is not without risk to the patient from anesthesia or surgical complications.[4,5] Current literature suggests that esophageal peristalsis only rarely returns to normal after palliative treatment measures.[6,7] The failure to regain normal progressive peristalsis is more commonly observed in patients who present with complete loss of motility or a severely dilated esophagus, or those who have a long-standing disease.[6]

Electrical stimulation of the vagus nerve is known to produce antegrade peristalsis in the normal esophagus in animal models.[8,9] It is not known, however, whether electrical stimulation of the vagus nerve will induce return of progressive peristalsis after an achalasia-like disorder has been created in an animal model. The aim of the current study was to establish propagative antegrade peristalsis by electrical stimulation of the vagus nerve in an animal model after an achalasia-like motility disorder has been confirmed objectively.

METHODS

Fifteen adult opossums (*Didelphis virginiana*) were investigated in accordance with the protocol approved by the Legacy Institutional Animal Care and Use Committee. Esophageal studies were performed with the animals under conscious sedation and surgical interventions under general endotracheal anesthesia. Baseline esophageal manometry and contrast esophagography were performed in all animals using a specially designed four-channel water-perfused catheter. The LES was located by stationary pull-through technique. Resting LES pressure and relaxation were obtained for 10 swallows induced by hypopharyngeal stimulation using stationary pull-through technique. Esophageal body motility in the lower third, the middle third, and the upper third of the esophagus was assessed by positioning the lowermost channel of the catheter 1 cm above the upper border of the LES. Manometric tracings for the body were recorded for a total of 10 swallows.

Animals were divided into three groups at the time of surgery. Sham surgery was performed on animals in group 1 (n = 3). Six animals each were assigned to groups 2 and 3. A midline laparotomy was performed under general anesthesia. The gastroesophageal junction (GEJ) was gently mobilized, and the esophageal circumference at that level was measured with a tape. In group 2 (n = 6) a Gore-Tex (W.L. Gore & Associates, Newark, DE) band, 1 cm wide and 110% of the esophageal circumference in length, was placed around the GEJ to prevent relaxation of the LES during swallowing. In group 3 (n = 6) a relatively tighter band (1 cm wide and 90% of the esophageal circumference in length) was used to further elevate the resting LES pressure. No band was placed around the GEJ in group 1 animals (n = 3).

After banding, esophageal motility was assessed every week with the animals under light sedation. As soon as the animals had documented amotility, or at a maximum of 6 weeks after banding, bilateral electrical stimulation of the vagus nerves, accessed by a lateral cervicotomy, was performed under general anesthesia using a constant-current nerve stimulator (Grass model 544, Grass Instruments, Quincy, MA). A graduated square-wave electrical stimulus was used. Stimulus values were modulated over a wide range (frequency 1 to 20 Hz., a pulse width of 0.1 to 5 msec, and train duration of 0.1 to 10 seconds) until peristaltic activity was demonstrated.[8]

Contrast esophagograms were obtained before vagal nerve stimulation was performed. In groups 1 and 2, esophageal motility was assessed in response to vagal nerve stimulation both before and after surgical removal of the band. Animals were euthanized at the end of the experiment. The entire esophagus and the upper part of the stomach were harvested. Histologic changes were assessed in the lower third of the esophagus immediately proximal to the LES using 5 mm sections and hematoxylin and eosin staining under light microscopy. A quantitative analysis of the intramuscular ganglion cells was performed. Ten medium-power (× 200) fields were counted for the presence of degenerating plexi. Plexi were considered degenerating if more than 33% of neurons in the plexus demonstrated nuclear margination, chromatolysis, atrophy, or vacuolation.

Comparisons were made between the preoperative baseline manometric data and the manometric data obtained before and after the removal of the band. All of the values are presented as means (± standard deviations). Continuous variables were compared using Wilcoxon rank-sum or paired/unpaired *t* tests as appropriate. Proportions were compared using chi-square or Fisher exact tests. *P* values of 0.05 or less were considered statistically significant.

RESULTS

There were no statistically significant differences in the preoperative resting LES pressure, LES relaxation, and mean amplitude of distal body contractions

among the three groups. All of the animals had normal esophageal body motility preoperatively (Table 1).

In group 1 following sham surgery, no difference was observed in resting LES pressure (31.22 [± 4.52] mm Hg vs. 29.22 [± 5.58] mm Hg, $P = 0.56$), LES relaxation (101.8 [± 7.1]% vs. 98.8 [± 8]%), and esophageal motility at 6 weeks (see Table 1). Only 5% of nerve plexi showed degenerative changes in this group (Fig. 1, *A*) and the esophagogram did not show any esophageal dilatation (Fig. 2, *A*). Bilateral cervical vagal stimulation in this group resulted in a significant increase in the amplitude of distal contractions, (Fig. 3, *A*) and all contractions were progressive (Fig. 4, *A*).

In group 2 the preoperative mean resting LES pressure and mean percentage relaxation of the LES were 30.14 [± 6.35] mm Hg and 99.9 [± 6.5]%, respectively. Placement of a loose band around the GEJ did not produce any significant increase in the resting LES pressure (mean post-banding resting LES pressure 33.02 [± 8.36] mm Hg, $P = 0.13$); however, relaxation of the LES during swallows decreased significantly (mean relaxation pressure 20.55 [± 6.53] mm Hg, mean percentage relaxation 28.1 [± 14.9]%, $P < 0.001$). After a mean follow-up period of 4.6 weeks, animals in group 2 developed a vigorous variety of achalasia represented by high-amplitude repetitive simultaneous contractions (see Table 1 and Fig. 3, *B* and 4, *B*). A barium swallow in these animals demonstrated moderate dilation of the esophagus (see Fig. 2, *B*), and histologic sections showed degeneration of 40% of the nerve plexi in three animals (see Fig. 1, *B*), 60% of nerve plexi in one animal, and 5% to 10% of nerve plexi in one animal. Electrical stimulation of the vagus nerve before removal of the band demonstrated a significant increase in the amplitude of contractions (see Fig. 3, *B*). Forty-nine percent of the contractions showed normal propagative progression in the distal smooth portion of the esophagus (see Table 1). However, none of the animals demonstrated complete return of normal peristaltic activity. After removal of the band, four animals showed complete return of normal progressive peristalsis (all contractions progressive) with electrical stimulation of vagi (see Figs. 3, *B* and 4, *B*). One animal had 40% nonresponse to stimulation and another animal had 20% simultaneous contractions.

Animals in group 3 developed a hypertensive LES with banding. Mean preoperative resting LES pressure in this group was 29.22 [± 5.58] mm Hg. After placement of a relatively tighter band, the mean resting LES pressure increased to 50.41 [± 10.71] mm Hg ($P < 0.001$). The mean percentage relaxation decreased from 99.1 [± 3.8]% to 28.4 [± 16]%, and the mean post-banding LES relaxation pressure was 34.34 [± 9.81] mm Hg. Animals in this group demonstrated a severe amotile variety of esophageal peristalsis after a mean follow-up period of 3.3 weeks (see Table 1). The mean amplitude of contractions decreased more than 50% (see Fig. 3, *C*), and all stimulated swallows resulted in simultaneous mirror-image contractions (Fig. 4, *C*). Postoperative esophagography demonstrated a severely dilated esophagus with a typical "bird beak" appearance at the GEJ (see Fig. 2, *C*). Animals in this group demonstrated degeneration of 50% to 65% of the nerve plexi (see Fig. 3, *C*), except for two animals that developed dysphagia and esophageal dysmotility relatively rapidly (within 2 weeks) after banding of the GEJ. These two animals showed degeneration of 20% and 5% to 10% nerve plexi, respectively. Electrical stimulation of the vagus nerves before removal of the band demonstrated a significant ($P < 0.001$) increase in the amplitude of contractions (see Fig. 3, *C*); however, no return of progressive peristalsis was observed (see Fig. 4, *C*). After removal of the band, electrical stimulation was performed in five animals because one animal died intraoperatively. Only one animal (20%) showed complete return of progressive peristaltic activity, whereas 60% to 100% of the contractions were simultaneous in the remaining animals (see Figs. 3, *C* and 4, *C*).

Table 1. Relative proportion of propagative and nonpropagative contractions in the distal (smooth muscle) portion of esophagus

	Group 1 (n = 3)		Group 2 (n = 6)		Group 3 (n = 6)	
	Propagative peristalsis	Simultaneous (dropped)	Propagative peristalsis	Simultaneous (dropped)	Propagative peristalsis	Simultaneous (dropped)
Preoperative	94%	0% (6%)	100%	0% (0%)	100%	0% (0%)
Postoperative	100%	0% (0%)	2%	78% (20%)	0%	93% (7%)
Vagal stimulation before band removal	95%	5% (0%)	49%	44% (7%)	8%	89% (3%)
Vagal stimulation after band removal	—	—	90%	3% (7%)	44%	52% (4%)

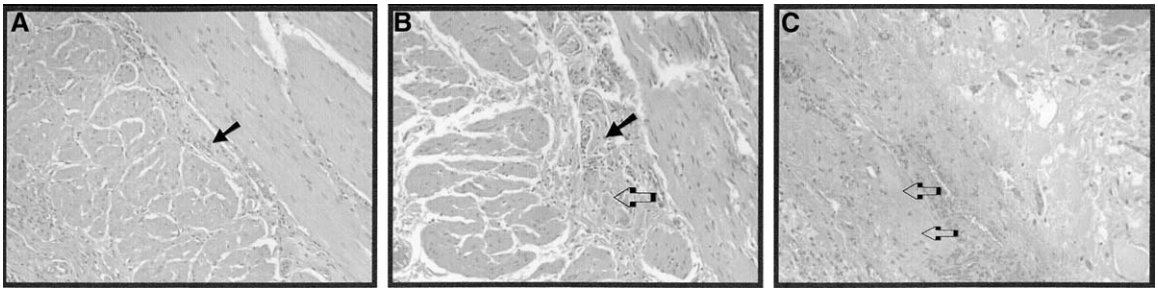

Fig. 1. Histopathologic sections in the lower esophagus after banding demonstrates normal ganglion cells (*black arrow*) in group 1 (**A**), degenerated ganglion cells (*clear arrow*) with some preserved normal ganglion cells (*black arrow*) as a result of loose banding in group 2 (**B**), and complete degeneration of ganglion cells (*clear arrow*) in group 3 (**C**). (Hematoxylin and eosin stain.)

DISCUSSION

The term achalasia, which means "failure to relax," was first used by Herz[10] in 1914 to describe a nonrelaxing LES. Although it is a rare disease, it causes a profound effect on the quality of life of those who have it, presenting as it does with dysphagia, weight loss, nocturnal regurgitation, and noncardiac chest pain. Left untreated it usually leads to more severe stasis-related complications (aspiration, squamous cell carcinoma, malnutrition, etc.). Although its precise etiology is still unknown, the functional and physiologic abnormalities associated with this disorder have been well documented since the introduction of intraluminal manometry.[11] A poorly functioning LES that fails to relax completely during swallowing and a complete absence of primary esophageal peristalsis are considered to be the essential manometric criteria for a diagnosis of achalasia.[12,13] Patients with this disorder may also have a hypertensive LES and other gastrointestinal tract motility abnormalities. The etiology and primary neuralgic defect responsible for the development of achalasia remains poorly defined at present. However, the most consistent pathologic findings have been noted in the distal esophagus and LES, where an absence or degeneration of ganglion cells in the myentric plexus (Auerbach's plexus) is seen.[14–16] The significance of the neural degeneration is unknown and may be a primary etiology of the achalasia, perhaps from an infectious or autoimmune cause, or a secondary finding resulting from the stasis and dilatation of the esophagus as the disease progresses. Our model, which by functional, histopathologic, and manometric criteria *is* similar to achalasia, would imply that the neural degeneration might be secondary to esophageal failure. Whether this phenomenon translates to human achalasia is unknown because the true initiator of this disorder is still unknown and may, in fact, end up being multifactorial. Our finding, however, should raise concerns about the ultimate effect of restrictive treatments of the human esophagus such as an overly tight or nonrelaxing fundoplication or perhaps the increasingly

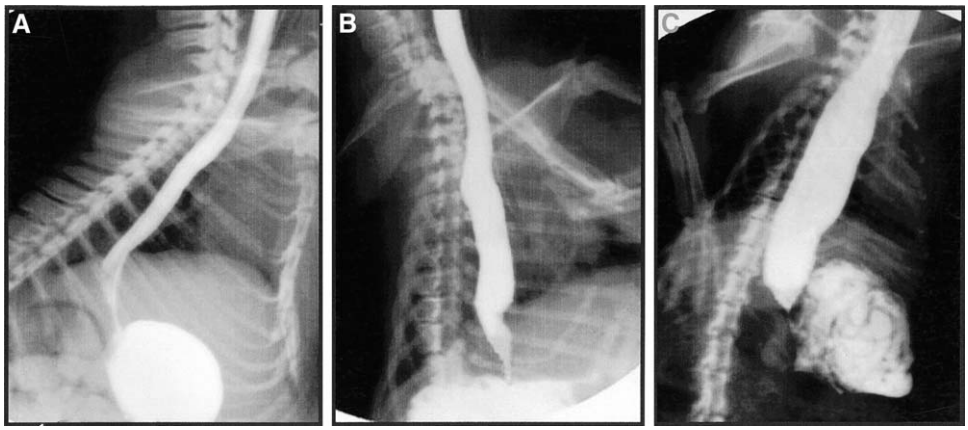

Fig. 2. Contrast esophagogram showing no esophageal dilatation in a group 1 animal (**A**), moderate dilatation of the esophagus in a group 2 animal (**B**), and severe esophageal dilatation with typical "bird-beak" appearance at the GEJ in a group 3 animal (**C**).

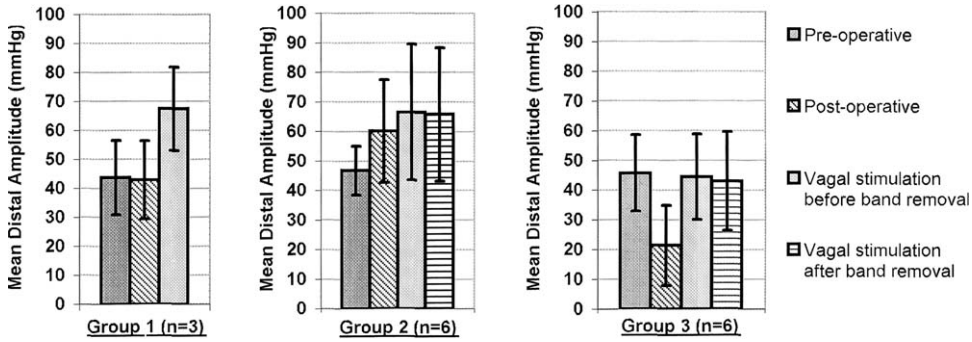

Fig. 3. Mean distal esophageal body amplitude in group 1 (**A**), group 2 (**B**), and group 3 (**C**).

popular laparoscopic restrictive bands for morbid obesity that are often placed very close to the GEJ.

The natural history of achalasia is not well defined either. Some have theorized that the spastic or "vigorous" type of achalasia is an early manifestation of the disease, which will later progress to the total noncontractility and massive esophageal dilatation of the classic form.

Treatment of achalasia is directed toward the relief of the functional obstruction at the LES. All of the current medical and endoscopic treatments are less satisfactory than surgical myotomy.[2] Surgery provides excellent palliation by facilitating esophageal emptying, which improves quality of life and possibly prevents stasis-related complications by preventing progressive dilatation.[17] Return of progressive peristalsis in the body of the esophagus is observed only in a very small proportion of patients who perhaps had a milder variety of achalasia and received intervention early in the course of their disease.[6] A vast majority of patients never regain normal esophageal function,[7] and some will ultimately require extreme measures including esophagectomy if their palliation is not successful.[18,19]

The primary focus of our research has been to determine whether the physiologic functioning of the esophagus can be improved by means such as electrical pacing of the esophagus. There has been a great deal of research and success in the modulation of gastrointestinal motility by using electrical pacing devices. Karlstrom and Kelly[20] demonstrated that reversal of peristalsis in the jejunal Roux limb is possible by external pacing. O' Connell et al.[21] succeeded in reducing jejunostomy output with retrograde external pacing in a canine model. Recently gastric pacing has become available, and reasonably effective, for therapy of chronic intractable gastroparesis.[22] Experimental studies involving decentralized vagal nerve stimulation of the normal esophagus have established antegrade peristalsis in opossum and feline models.[8,9] We have chosen the opossum model for our studies as it has an esophageal physiology similar to another omnivore, the human. This includes a proximal esophagus composed of striated muscle and distal two thirds composed of autonomically innervated smooth muscle. Previous studies using feline models, where the esophagus is completely composed of striated muscle, are questionable because of the difference in esophageal innervation. Previous studies in both cats and opossums have shown that altering various stimulus parameters could modulate all the direction, velocity of the peristaltic waves, and amplitude of the contractions. However, there has been no previous evidence that electrical stimulation of the vagus nerve can induce progressive peristalsis once an achalasia-like disorder has been established.

Our results show that esophageal motility can be improved by electrical stimulation of the vagus nerve in an animal model of achalasia-like disorder. We deliberately chose the timing of our intervention (6 weeks or less) so that there was a spectrum of dysmotility to study. We have previously discovered that beyond 6 weeks opossums uniformly develop end-stage dilatation which, much as in humans, has less of a response to any interventions. Overall 91% of the animals with achalasia-like disorder established 40% or greater primary peristalsis with vagal stimulation. Forty-five percent had 100% normal peristalsis. Complete return of normal progressive peristalsis after vagal stimulation was observed in the group of animals having a vigorous achalasia–like motility disorder following relief of obstruction at the GEJ. In the animals developing a severe amotile type of motility disorder, with massive dilatation of the esophagus, a significant improvement in the amplitude and morphology of the contractions was observed when electrical stimulation of the vagus nerve was performed. After band removal, our model's equivalent of a surgical myotomy, only 20% of the animals regained completely normal peristalsis. Another 20% had no improvement, whereas the majority reestablished around 40% of swallows with

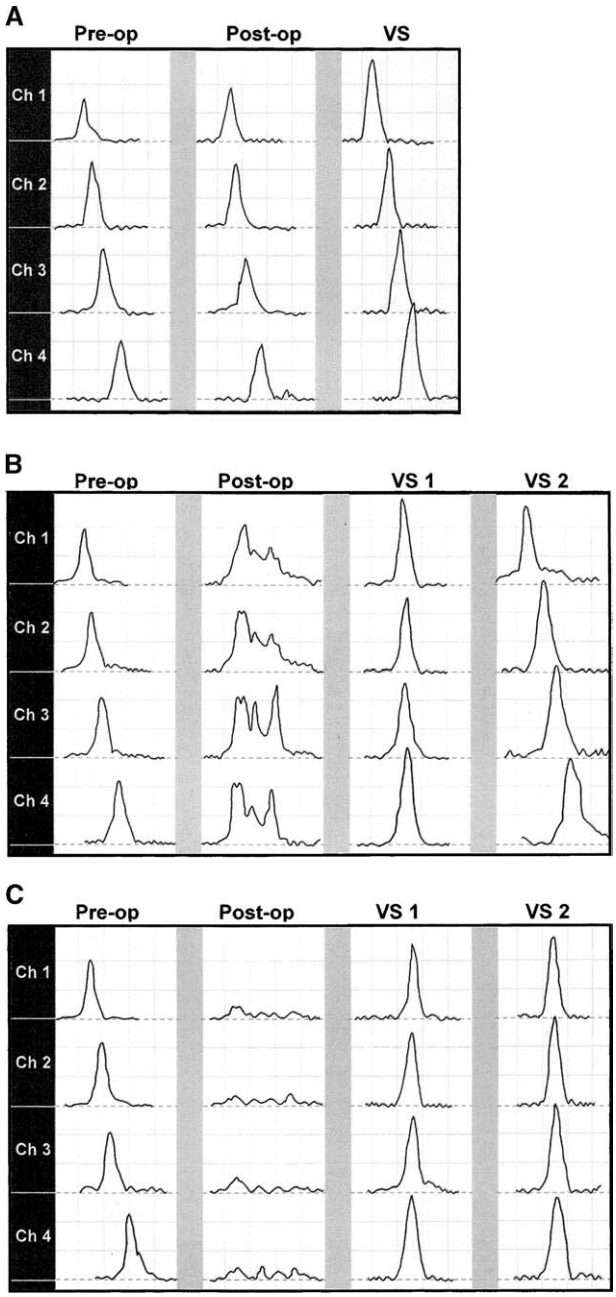

Fig. 4. Manometric tracings of the esophageal body motility in group 1 (**A**), group 2 (**B**), and group 3 (**C**). Pre-op = motility in response to swallow induced by hypopharyngeal stimulation before surgery; Post-op = motility in response to swallow induced by hypopharyngeal stimulation after sham surgery in group 1 (**A**), and after band placement in group 2 (**B**) and group 3 (**C**); VS = motility after vagal stimulation in group 1; VS1 and VS2 = motility following vagal stimulation before and after removal of the band, respectively, in group 2 and group 3.

primary peristalsis. These findings suggest that a point of irreversibility of the normal motility may exist in the natural history of the disease. This may be related to the degree of degeneration of the Auerbach's plexus nerves that we observed. However, a definitive histologic correlation was not possible between function, dilatation, and the percentage of nerve degeneration because of the small sample size and short duration of follow-up after banding.

It may be argued that esophageal pacing is unnecessary, that peristalsis may return to normal spontaneously after relief of obstruction as was suggested by Schneider et al.[23] These investigators, however, used an obstructed esophagus in a feline model. A similar response may not be reproducible in the human esophagus inasmuch as secondary peristalsis in cats appears to be independent of vagal control.[24] It is known that esophageal peristalsis in human beings appears to be influenced by vagal control as is evident by disordered primary peristalsis in diabetic neuropathy[25] and dysautonomia.[26] Additionally, partial return of normal primary peristalsis after surgical myotomy has only been reported in a very small number of patients.[6,7] Long-term survival studies will be needed in our model to prove that loss of peristalsis is a permanent condition in the absence of vagal pacing.

The increased force of contraction in all of the animals irrespective of severity of dysmotility, the induction of progressive peristalsis in 50% of animals with vigorous achalasia even before relief of obstruction, and the restoration of some peristaltic swallows in all but one animal with pacing and relief of outlet obstruction would indicate that vagal stimulation leads to improved esophageal motility in an achalasia-like disorder. The clinical effect of this and its long-term clinical effectiveness are unknown but will be the basis of further investigations.

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Invited Discussion—Expert Commentator

Carlos A. Pellegrini, M.D. (Seattle, WA): The authors of this paper designed this experiment to test the hypothesis that electrical stimulation of the vagus nerves may improve esophageal motility after treatment for achalasia. To this end they were apparently successful in developing an excellent animal model of achalasia. In fact, this model not only reproduces faithfully the manometric findings of achalasia but yields a histologic picture of achalasia given the damage to the myenteric plexus that results (apparently) from the banding of the distal esophagus. This, in and of itself, is an important advance that should allow us to study in more detail the pathogenesis of achalasia itself as well as the impact of different therapeutic maneuvers in an animal model that has been shown to relate to humans. Their study showed that electrical stimulation of the cervical vagus resulted in improvement or complete restitution of the peristaltic wave in these animals and that this effect was more prominent when the disease was treated at an earlier stage. Although these findings all make sense, one must wonder what in this model leads to the disruption of the myenteric plexus in the first place and whether the damage observed in these animals is permanent or reversible. Most important, it will be of great interest to determine whether these changes can be reproduced in humans.

Patterns of Regional Lymph Node Involvement in Intrahepatic Cholangiocarcinoma of the Left Lobe

Jiro Okami, M.D., Keizo Dono, M.D., Masato Sakon, M.D., Masanori Tsujie, M.D., Nobuyasu Hayashi, M.D., Yoshiyuki Fujiwara, M.D., Hiroaki Nagano, M.D., Koji Umeshita, M.D., Shoji Nakamori, M.D., Morito Monden, M.D.

Lymph node involvement is an important prognostic factor in intrahepatic cholangiocarcinoma. Besides the nodes in the hepatoduodenal ligament, recent studies have suggested that the nodes around the cardiac portion of the stomach or along the gastric lesser curvature can be affected when the primary tumor is located in the left hepatic lobe. However, the distribution of metastatic nodes has not been well described in this disease. Thirteen patients with intrahepatic cholangiocarcinoma in the left hepatic lobe were enrolled in this study. Lymphatic mapping was performed by means of both histologic examination and reverse transcriptase–polymerase chain reaction assays. Nodal involvement around the cardiac portion of the stomach or along the lesser gastric curvature (left pathway) was found in 7 (54%) of 13 patients by histologic examination or reverse transcriptase–polymerase chain reaction, whereas positive nodes in the hepatoduodenal ligament (right pathway) were found in 6 (46%) of 13 patients. Two patients (15%) had positive nodes only in the left pathway. Therefore, for a more accurate clinical staging of intrahepatic cholangiocarcinoma in the hepatic left lobe, lymph nodes around the cardiac portion of the stomach and along the lesser gastric curvature should be examined in addition to nodes in the hepatoduodenal ligament.
(J GASTROINTEST SURG 2003;7:850–856)

KEY WORDS: Intrahepatic cholangiocarcinoma, lymph node, metastasis

Intrahepatic cholangiocarcinoma, a primary adenocarcinoma of the liver originating from the intrahepatic biliary epithelium, is the second most common primary hepatic malignancy, next to hepatocellular carcinoma.[1] The incidence of this malignancy is increasing rapidly worldwide.[2] In the United States, the age-adjusted mortality rate per 100,000 persons has increased from 0.15 to 0.66 in the past two decades.[3] For patients with this malignancy, only complete surgical resection provides the opportunity for cure and longer survival. However, despite recent advances in hepatobiliary surgery including safe major hepatectomy and extended lymphadenectomy with low perioperative mortality, outcomes with intrahepatic cholangiocarcinoma have been poor with 3-year survival ranging from 16% to 61% even in the patients who underwent curative resection.[4–8] Because the outcomes of patients with cholangiocarcinoma may be attributed to not only the difficulty of early detection but also the failure to select patients appropriately, precise staging of the disease should be emphasized to allow a better prognostic stratification of patients, and thus a better therapeutic approach in planning optimal management for patients with cholangiocarcinoma.

Lymph node involvement, which differentiates intrahepatic cholangiocarcinoma from hepatocellular carcinoma, is associated with poor prognosis in cholangiocarcinoma.[5–8] Precise knowledge of the mode of lymphatic spread is imperative to determine the

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extent of lymphadenectomy and to allow a better prognostic evaluation. It is well known that lymphatic drainage of the liver flows along the hepatoduodenal ligament and that nodes in this area are defined as regional.[9] Recently another lymphatic pathway across the lesser omentum from the hepatic bed to the stomach has been recognized.[10] Two reports have described metastatic lymph nodes around the cardiac portion of the stomach or along the gastric lesser curvature in patients with intrahepatic cholangiocarcinoma in the hepatic left lobe.[5,10] In these reports, however, lymphadenectomy around the cardia and gastric lesser curvature was not systemically performed, and the frequency of positive nodes in these areas remains obscure.

The present study was conducted to assess the pattern of lymphogenous tumor cell spread in patients with intrahepatic cholangiocarcinoma in the left hepatic lobe. We particularly focused on the incidence of nodal metastasis around the cardiac portion of the stomach and along the lesser gastric curvature. Not to ignore any minimum metastatic foci, we used molecular-based analysis for diagnosis of lymph node status in addition to the histopathologic approach. This previously established genetic detection system,[11] which uses carcinoembryonic antigen (CEA) and mammaglobin B (MMGB) as genetic markers, is an assay with a high sensitivity and a lower false negative rate for the detection of lymph node micrometastasis in cancer of the biliary tract.

PATIENTS AND METHODS

Patients and Surgical Samples

Thirteen consecutive patients with intrahepatic cholangiocarcinoma in the left hepatic lobe were enrolled in this study; written informed consent was obtained from all of them. All patients were treated with curative intent between 1997 and 2001 at Osaka University Hospital (Osaka, Japan) and Moriguchi Keijinkai Hospital (Osaka, Japan) by left lobectomy or extended left lobectomy of the liver; additional caudate lobectomy, if necessary, and lymphadenectomy along the hepatoduodenal ligament, around the cardiac portion of the stomach, and along the gastric lesser curvature were also performed. We collected 13 primary tumor tissues and 275 lymph nodes, and documented the location of each lymph node. Each lymph node was cut into two pieces. One piece was fixed in formalin and embedded in paraffin for routine histologic examination using hematoxylin and eosin staining, and the other piece was stored for reverse transcriptase–polymerase chain reaction (RT-PCR) assay. Tissue samples for molecular analysis were immediately frozen in liquid nitrogen after surgical resection at −80° C until ribonucleic acid (RNA) extraction.

RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction

RNA extraction was carried out with the use of TRIZOL reagent (Life Technologies, Vienna, Austria) in a single-step method, and purified total cellular RNA was quantitated and assessed for purity by means of ultraviolet spectrophotometry. Complementary deoxyribonucleic acid (cDNA) was generated from 1 μg RNA with avian myeloblastosis virus reverse transcriptase (Promega Corp., Madison, WI). The amplification of each specific RNA was performed in a 25 μl reaction mixture containing 2 μl of cDNA template, 1 × Perkin-Elmer (Norwalk, CT) polymerase chain reaction buffer, 1.5 mmol/L of $MgCl_2$, 0.8 mmol/L of deoxynucleotide triphosphate, 5 pmol of each primer, and 1 unit of Taq DNA polymerase (AmpliTaq Gold; Roche Molecular Systems, Inc., NJ). The polymerase chain reaction primers used for detection of porphobilinogen deaminase (PBGD), MMGB, and CEA have been previously described.[11–13] These primers were designed to flank intronic sequences in order to avoid false positive results due to amplification of contaminated genomic DNA. The polymerase chain reaction cDNA products of PBGD, MMGB, and CEA were 127, 245, and 160 base-pairs, respectively. The annealing temperature and cycles for the polymerase chain reaction were set up as follows: one cycle of denaturing at 95° C for 12 minutes, followed by 40 cycles (95° C for 1 minute, 62° C for 1 minute, and 72° C for 1 minute for PBGD and 95° C for 1 minute, 58° C for 1 minute, and 72° C for 1 minute for mammaglobin B) or 35 cycles (95° C for 1 minute and 72° C for 1.5 minutes for CEA) before a final extension at 72° C for 10 minutes. These polymerase chain reaction conditions were set up in a GeneAmp PCR System 9600 (Perkin-Elmer). Aliquots (8 μl) from each reaction mixture were size fractionated on 2% agarose gel and visualized with ethidium bromide staining. To verify the integrity of each RNA sample, PBGD as the housekeeping gene was amplified. Specimens that failed to amplify PBGD were not considered.

Evaluation of the Mode of Lymphatic Tumor Cell Spread

Each lymph node was evaluated by histologic analysis and RT-PCR assay separately. The results were marked on an anatomic map of each patient. To assess lymphogenous tumor cell spread from the primary lesion, we categorized the site of lymph nodes into the following three groups: (1) lymph nodes along the

right pathway; (2) lymph nodes along the left pathway; and (3) lymph nodes in any distant areas. Lymph nodes located in the hepatoduodenal ligament were considered nodes along the right pathway. Lymph nodes around the cardiac portion of the stomach and along the gastric lesser curvature were considered nodes along the left pathway (Fig. 1). The group of distant areas includes all nodes collected from retroperitoneal tissue along the celiac artery, superior mesenteric artery, aorta, inferior vena cava, or common hepatic artery.

RESULTS

Patient Characteristics

Patient characteristics are presented in Table 1. The median age of the 13 patients accrued for this study was 61 years (range 34 to 77 years). There were five men and eight women. The median tumor size was 4.5 cm (range 1.5 to 9.0 cm). On the basis of histologic findings, 12 tumors were confirmed to be adenocarcinoma and one tumor (in patient 7; see Table 1) was a mixed type of intrahepatic adenocarcinoma with hepatocellular carcinoma. Left lobectomy was performed in seven patients, left and caudate lobectomy in four patients, and extended left hepatectomy in two patients. None of the patients had any major surgical complications.

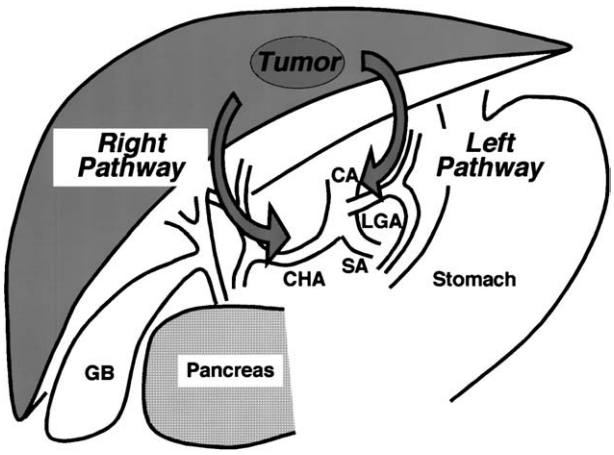

Fig. 1. Schematic of two possible drainage pathways. Two lymphatic pathways from the hepatic left lobe are shown: the right pathway through the hepatoduodenal ligament and the left pathway through the lesser omentum to the cardiac portion of the stomach and the gastric lesser curvature. CA = celiac artery; CHA = common hepatic artery; GB = gallbladder; LGA = left gastric artery; SA = splenic artery.

Lymph Node Metastasis

A total of 275 lymph nodes were harvested from 13 patients, ranging from 5 to 57 nodes per patient with a median value of 20 lymph nodes. Metastases were found in 27 nodes by means of histologic examination and in 51 nodes by RT-PCR assay (see Table 1). All 27 histologically positive nodes were also positive by RT-PCR assay. In addition to these 27 histologically metastasis-positive nodes, another 24 lymph nodes were positive by RT-PCR assay in lymph nodes that were negative according to histologic examination (Fig. 2). The genetic analysis, however, was not applicable for one patient (No. 10; see Table 1) because the primary tumor did not express any of two genetic markers required for RT-PCR. Two of the seven patients with node-negative disease by histologic examination were positive by RT-PCR assay (Nos. 1 and 6; see Table 1). In a patient-based analysis, 6 of 13 patients were node positive by histologic examination and 8 of 12 patients whose primary tumors were positive for either of two genetic markers were node positive by RT-PCR assay.

Anatomic Distribution of Lymph Node Metastases

As described in Patients and Methods, we drew the map of positive and negative nodes in each patient and then analyzed anatomic distribution of lymph node metastases. The summarized anatomic distribution of lymph node metastasis in each patient is shown in Table 1. Positive nodes in the right pathway were found in 5 (38%) of 13 patients by histologic examination and 6 (50%) of 12 patients by RT-PCR assay. In the left pathway, histologic nodal involvement was found in 4 (31%) of 13 patients and in 7 (58%) of 12 patients by RT-PCR assay. The number of patients with lymph nodes containing metastases in both pathways included three (23%) shown by histologic examination and five (38%) by molecular assay (Table 2). Metastasis-positive lymph nodes of distant areas were found in 3 (23%) of 13 patients by histologic examination and in 5 (42%) of 12 patients by RT-PCR assay (see Table 2). In the patients with positive lymph nodes in distant areas, positive nodes were also found in either the right or left pathway. Of all 13 patients, two had positive nodes only within the area along the left pathway by histologic or RT-PCR examination. Detailed anatomic mapping of lymph node metastasis in three patients whose positive nodes were limited to the area along the right and/or left pathway(s) are shown in Fig. 3.

Table 1. Summary of clinical features and nodal status in each patient

Patient	Age (yrs)	Sex	Tumor size (cm)	Operation	No. of lymph nodes sampled	No. of histologically positive nodes	No. of RT-PCR–positive nodes	Right pathway		Left pathway		Distant area	
								Histology	RT-PCR	Histology	RT-PCR	Histology	RT-PCR
1	58	F	5.0	Left and caudate lobectomy	57	0	13	(−)	(+)	(−)	(+)	(−)	(+)
2	77	F	2.5	Left and caudate lobectomy	10	0	0	(−)	(−)	(−)	(−)	(−)	(−)
3	70	M	1.5	Left lobectomy	5	0	0	(−)	(−)	(−)	(−)	NS	NS
4	60	F	7.0	Left lobectomy	10	7	9	(+)	(+)	(+)	(+)	(+)	(+)
5	67	M	3.0	Left lobectomy	20	1	1	(−)	(−)	(+)	(+)	(−)	(−)
6	65	F	6.2	Left lobectomy	31	0	1	(−)	(−)	(−)	(+)	(−)	(−)
7	61	M	9.0	Left lobectomy	14	7	10	(+)	(+)	(+)	(+)	(+)	(+)
8	72	F	3.5	Left and caudate lobectomy	20	0	0	(−)	(−)	(−)	(−)	(−)	(−)
9	67	M	4.5	Left lobectomy	20	0	0	(−)	(−)	(−)	(−)	(−)	(−)
10	71	M	3.5	Extended left lobectomy	34	0	NA	(−)	NA	(−)	NA	(−)	NA
11	34	F	8.0	Left and caudate lobectomy	13	8	10	(+)	(+)	(+)	(+)	(+)	(+)
12	60	F	6.5	Extended left lobectomy	25	3	4	(+)	(+)	(−)	(−)	(−)	(+)
13	63	F	4.2	Left lobectomy	16	1	3	(+)	(+)	(−)	(+)	(−)	(−)

NA = not applicable; NS = not sampled; RT-PCR = reverse transcriptase–polymerase chain reaction.

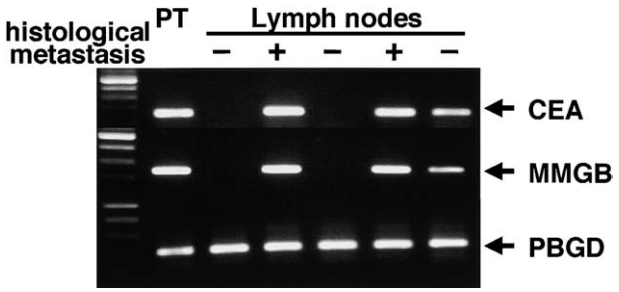

Fig. 2. Typical profile of detection of carcinoembryonic antigen (*CEA*) and mammaglobin B (*MMGB*) reverse transcriptase–polymerase chain reaction (RT-PCR) products in lymph nodes. *Upper*, CEA RT-PCR; *B*, MMGB RT-PCR; *lower*, Porphobilinogen deaminase (*PBGD*) RT-PCR. PT = primary tumor; − = histologically metastasis-negative node; + = histologically metastasis-positive node.

Table 2. Summary of patients with lymph node metastasis

	Histology (n = 13)	RT-PCR (n = 12)
Nodal metastasis (−)	7 (54%)	4 (33%)
Nodal metastasis (+)	6 (46%)	8 (67%)
Right pathway (+)	5 (38%)	6 (50%)
Left pathway (+)	4 (31%)	7 (58%)
Distant area (+)	3 (23%)	5 (42%)

RT-PCR = reverse transcriptase–polymerase chain reaction.

DISCUSSION

Negative nodal status for metastasis is one of the important favorable prognostic factors after hepatectomy for intrahepatic cholangiocarcinoma.[4–8] However, the distribution of metastatic nodes has not been well described in this disease. In the present study, we clearly demonstrated that the nodes around the cardiac portion of the stomach or along the gastric lesser curvature were common sites of lymphatic metastases in patients with left intrahepatic cholangiocarcinoma. Lymph node metastasis in these regions were detected in 4 (31%) of 13 patients by histologic examination and in 7 (58%) of 12 patients by molecular examination, whereas metastases in the right pathway were detected in 5 (38%) of 13 patients by histologic examination and 6 (50%) of 12 patients by molecular examination. The frequency of the lymph node metastasis in the left pathway seems to

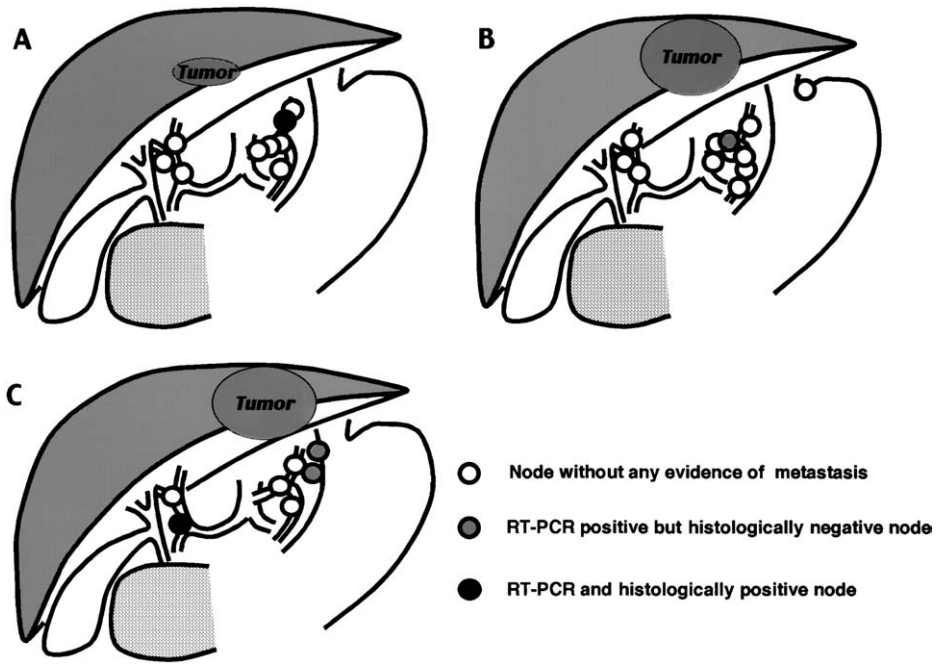

Fig. 3. Lymphatic maps of positive and negative nodes in three representative cases. **A**, Patient 5 in Table 1 had one positive node detected by both histopathologic examination and reverse transcriptase–polymerase chain reaction (*RT-PCR*) assay along the lesser gastric curvature. **B**, Patient 6 in the Table 1 had one positive node that was not detected by histologic examination but could be detected by RT-PCR. This node was also located in the left pathway. **C**, Patient 13 had three positive nodes. One was detected in the hepatoduodenal ligament by histologic examination and the others were detected in the connective tissue around the gastric cardia by RT-PCR.

be nearly equal to that in the right pathway, a pathway considered to be the primary regional nodal pathway in left intrahepatic cholangiocarcinoma.[14] Of note, two (29%) of seven patients with metastatic nodes had a positive node only along the left pathway. If no attention had been paid to the nodal status in that region, the stage of these patients would have been underestimated as metastasis-free disease. Furthermore, lymph nodes in distant areas, such as nodes in retroperitoneal tissue along the celiac artery, aorta, inferior vena cava, or common hepatic artery, were also affected in 3 (25%) of 13 patients by histologic examination and in 5 (42%) of 12 patients by molecular examination. All patients with positive nodes in any of these distant areas also had metastatic nodes in the right and/or left pathway, suggesting that tumor cells passed through either of these two pathways to spread to the distant area. On the basis of our findings, we propose that both the nodes along the left pathway and those along the right pathway should be classified as regional lymph nodes of intrahepatic cholangiocarcinoma arising in the left hepatic lobe.

According to the TNM staging system, which is applied to intrahepatic cholangiocarcinoma, the regional site of this disease is limited to the hepatoduodenal ligament regardless of where in the liver the primary tumor is located.[14] Nozaki et al.[10] had previously reported that intrahepatic cholangiocarcinoma in the left hepatic lobe displayed a different distribution of metastatic nodes from intrahepatic cholangiocarcinoma in the right lobe. The most important point in their article was that the lymph nodes around the cardiac portion and along the lesser curvature of the stomach were affected, as well as nodes in the hepatoduodenal ligament, if the primary tumors were located in the left lobe, as shown in the present study. However, as mentioned in their article, it was possible that they missed small metastatic foci within the clinically unremarkable lymph nodes because only enlarged lymph nodes were sampled and examined histopathologically. In the present study we evaluated patients who had intrahepatic cholangiocarcinoma that was limited to the left hepatic lobe and offered the same manner of lymphadenectomy to all patients. To avoid missing any metastatic lymph nodes, we removed the entire connective tissue in the hepatododenal ligament area and the lesser omentum, along the lesser gastric curvature, and around the cardiac portion of the stomach and then sampled all lymph nodes in the resected specimen. Furthermore, to detect metastasis more accurately, we used not only a histologic examination but also a molecular-based analysis.[11] Thus the present findings may be more reliable than those in some previous studies.[5,10]

In recent years many analyses based on molecular techniques have been developed to evaluate minimal residual cancer.[15] We applied a RT-PCR assay with two molecular markers[11] to assess the presence of small metastatic foci (micrometastasis) in lymph nodes that were not detected by histologic examination. Indeed, of the 214 histologically negative nodes in six patients, we detected lymph node "micrometastatses" in 24 nodes from two patients by RT-PCR assay. Thus we believe that our highly sensitive RT-PCR assay with two molecular markers enabled us to more accurately analyze the lymphatic spread of cancer cells from the left hepatic lobe.

From a clinical point of view, whether the presence of positive nodes along the left pathway correlates with postoperative prognosis remains unknown. Despite the short follow-up period, five of the seven patients with lymph node metastasis either in the right or left pathway had a recurrence within 1 year after surgery, whereas only one of five patients without any evidence of lymph node metastasis had a recurrence. This observation suggests the importance of lymph node staging for optimal adjuvant treatment after surgery. Further study with more patients and long-term analyses are needed to reveal the clinical implications of nodal status in patients with left intrahepatic cholangiocarcinomas.

CONCLUSION

For more accurate clinical staging of the patients with intrahepatic cholangiocarcinoma in the left hepatic lobe, lymph nodes around the cardia and along the gastric lesser curvature should be considered regional lymph nodes in addition to those along the hepatoduodenal ligament.

We thank all of the surgeons in our department and Moriguchi Keijinkai Hospital for helping to collect the surgical specimens and Kyoko Iwao and Yurika Sugita for technical assistance.

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extent of lymphadenectomy and to allow a better prognostic evaluation. It is well known that lymphatic drainage of the liver flows along the hepatoduodenal ligament and that nodes in this area are defined as regional.[9] Recently another lymphatic pathway across the lesser omentum from the hepatic bed to the stomach has been recognized.[10] Two reports have described metastatic lymph nodes around the cardiac portion of the stomach or along the gastric lesser curvature in patients with intrahepatic cholangiocarcinoma in the hepatic left lobe.[5,10] In these reports, however, lymphadenectomy around the cardia and gastric lesser curvature was not systemically performed, and the frequency of positive nodes in these areas remains obscure.

The present study was conducted to assess the pattern of lymphogenous tumor cell spread in patients with intrahepatic cholangiocarcinoma in the left hepatic lobe. We particularly focused on the incidence of nodal metastasis around the cardiac portion of the stomach and along the lesser gastric curvature. Not to ignore any minimum metastatic foci, we used molecular-based analysis for diagnosis of lymph node status in addition to the histopathologic approach. This previously established genetic detection system,[11] which uses carcinoembryonic antigen (CEA) and mammaglobin B (MMGB) as genetic markers, is an assay with a high sensitivity and a lower false negative rate for the detection of lymph node micrometastasis in cancer of the biliary tract.

PATIENTS AND METHODS

Patients and Surgical Samples

Thirteen consecutive patients with intrahepatic cholangiocarcinoma in the left hepatic lobe were enrolled in this study; written informed consent was obtained from all of them. All patients were treated with curative intent between 1997 and 2001 at Osaka University Hospital (Osaka, Japan) and Moriguchi Keijinkai Hospital (Osaka, Japan) by left lobectomy or extended left lobectomy of the liver; additional caudate lobectomy, if necessary, and lymphadenectomy along the hepatoduodenal ligament, around the cardiac portion of the stomach, and along the gastric lesser curvature were also performed. We collected 13 primary tumor tissues and 275 lymph nodes, and documented the location of each lymph node. Each lymph node was cut into two pieces. One piece was fixed in formalin and embedded in paraffin for routine histologic examination using hematoxylin and eosin staining, and the other piece was stored for reverse transcriptase–polymerase chain reaction (RT-PCR) assay. Tissue samples for molecular analysis were immediately frozen in liquid nitrogen after surgical resection at −80° C until ribonucleic acid (RNA) extraction.

RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction

RNA extraction was carried out with the use of TRIZOL reagent (Life Technologies, Vienna, Austria) in a single-step method, and purified total cellular RNA was quantitated and assessed for purity by means of ultraviolet spectrophotometry. Complementary deoxyribonucleic acid (cDNA) was generated from 1 μg RNA with avian myeloblastosis virus reverse transcriptase (Promega Corp., Madison, WI). The amplification of each specific RNA was performed in a 25 μl reaction mixture containing 2 μl of cDNA template, 1 × Perkin-Elmer (Norwalk, CT) polymerase chain reaction buffer, 1.5 mmol/L of $MgCl_2$, 0.8 mmol/L of deoxynucleotide triphosphate, 5 pmol of each primer, and 1 unit of Taq DNA polymerase (AmpliTaq Gold; Roche Molecular Systems, Inc., NJ). The polymerase chain reaction primers used for detection of porphobilinogen deaminase (PBGD), MMGB, and CEA have been previously described.[11–13] These primers were designed to flank intronic sequences in order to avoid false positive results due to amplification of contaminated genomic DNA. The polymerase chain reaction cDNA products of PBGD, MMGB, and CEA were 127, 245, and 160 base-pairs, respectively. The annealing temperature and cycles for the polymerase chain reaction were set up as follows: one cycle of denaturing at 95° C for 12 minutes, followed by 40 cycles (95° C for 1 minute, 62° C for 1 minute, and 72° C for 1 minute for PBGD and 95° C for 1 minute, 58° C for 1 minute, and 72° C for 1 minute for mammaglobin B) or 35 cycles (95° C for 1 minute and 72° C for 1.5 minutes for CEA) before a final extension at 72° C for 10 minutes. These polymerase chain reaction conditions were set up in a GeneAmp PCR System 9600 (Perkin-Elmer). Aliquots (8 μl) from each reaction mixture were size fractionated on 2% agarose gel and visualized with ethidium bromide staining. To verify the integrity of each RNA sample, PBGD as the housekeeping gene was amplified. Specimens that failed to amplify PBGD were not considered.

Evaluation of the Mode of Lymphatic Tumor Cell Spread

Each lymph node was evaluated by histologic analysis and RT-PCR assay separately. The results were marked on an anatomic map of each patient. To assess lymphogenous tumor cell spread from the primary lesion, we categorized the site of lymph nodes into the following three groups: (1) lymph nodes along the

right pathway; (2) lymph nodes along the left pathway; and (3) lymph nodes in any distant areas. Lymph nodes located in the hepatoduodenal ligament were considered nodes along the right pathway. Lymph nodes around the cardiac portion of the stomach and along the gastric lesser curvature were considered nodes along the left pathway (Fig. 1). The group of distant areas includes all nodes collected from retroperitoneal tissue along the celiac artery, superior mesenteric artery, aorta, inferior vena cava, or common hepatic artery.

RESULTS

Patient Characteristics

Patient characteristics are presented in Table 1. The median age of the 13 patients accrued for this study was 61 years (range 34 to 77 years). There were five men and eight women. The median tumor size was 4.5 cm (range 1.5 to 9.0 cm). On the basis of histologic findings, 12 tumors were confirmed to be adenocarcinoma and one tumor (in patient 7; see Table 1) was a mixed type of intrahepatic adenocarcinoma with hepatocellular carcinoma. Left lobectomy was performed in seven patients, left and caudate lobectomy in four patients, and extended left hepatectomy in two patients. None of the patients had any major surgical complications.

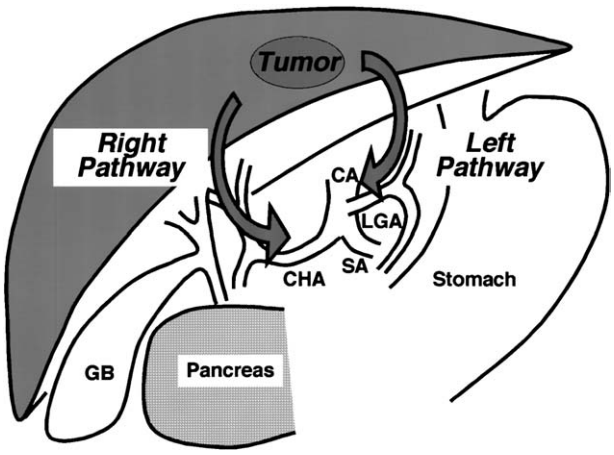

Fig. 1. Schematic of two possible drainage pathways. Two lymphatic pathways from the hepatic left lobe are shown: the right pathway through the hepatoduodenal ligament and the left pathway through the lesser omentum to the cardiac portion of the stomach and the gastric lesser curvature. CA = celiac artery; CHA = common hepatic artery; GB = gallbladder; LGA = left gastric artery; SA = splenic artery.

Lymph Node Metastasis

A total of 275 lymph nodes were harvested from 13 patients, ranging from 5 to 57 nodes per patient with a median value of 20 lymph nodes. Metastases were found in 27 nodes by means of histologic examination and in 51 nodes by RT-PCR assay (see Table 1). All 27 histologically positive nodes were also positive by RT-PCR assay. In addition to these 27 histologically metastasis-positive nodes, another 24 lymph nodes were positive by RT-PCR assay in lymph nodes that were negative according to histologic examination (Fig. 2). The genetic analysis, however, was not applicable for one patient (No. 10; see Table 1) because the primary tumor did not express any of two genetic markers required for RT-PCR. Two of the seven patients with node-negative disease by histologic examination were positive by RT-PCR assay (Nos. 1 and 6; see Table 1). In a patient-based analysis, 6 of 13 patients were node positive by histologic examination and 8 of 12 patients whose primary tumors were positive for either of two genetic markers were node positive by RT-PCR assay.

Anatomic Distribution of Lymph Node Metastases

As described in Patients and Methods, we drew the map of positive and negative nodes in each patient and then analyzed anatomic distribution of lymph node metastases. The summarized anatomic distribution of lymph node metastasis in each patient is shown in Table 1. Positive nodes in the right pathway were found in 5 (38%) of 13 patients by histologic examination and 6 (50%) of 12 patients by RT-PCR assay. In the left pathway, histologic nodal involvement was found in 4 (31%) of 13 patients and in 7 (58%) of 12 patients by RT-PCR assay. The number of patients with lymph nodes containing metastases in both pathways included three (23%) shown by histologic examination and five (38%) by molecular assay (Table 2). Metastasis-positive lymph nodes of distant areas were found in 3 (23%) of 13 patients by histologic examination and in 5 (42%) of 12 patients by RT-PCR assay (see Table 2). In the patients with positive lymph nodes in distant areas, positive nodes were also found in either the right or left pathway. Of all 13 patients, two had positive nodes only within the area along the left pathway by histologic or RT-PCR examination. Detailed anatomic mapping of lymph node metastasis in three patients whose positive nodes were limited to the area along the right and/or left pathway(s) are shown in Fig. 3.

Table 1. Summary of clinical features and nodal status in each patient

Patient	Age (yrs)	Sex	Tumor size (cm)	Operation	No. of lymph nodes sampled	No. of histologically positive nodes	No. of RT-PCR–positive nodes	Right pathway		Left pathway		Distant area	
								Histology	RT-PCR	Histology	RT-PCR	Histology	RT-PCR
1	58	F	5.0	Left and caudate lobectomy	57	0	13	(−)	(+)	(−)	(+)	(−)	(+)
2	77	F	2.5	Left and caudate lobectomy	10	0	0	(−)	(−)	(−)	(−)	(−)	(−)
3	70	M	1.5	Left lobectomy	5	0	0	(−)	(−)	(−)	(−)	NS	NS
4	60	F	7.0	Left lobectomy	10	7	9	(+)	(+)	(+)	(+)	(+)	(+)
5	67	M	3.0	Left lobectomy	20	1	1	(−)	(−)	(+)	(+)	(−)	(−)
6	65	F	6.2	Left lobectomy	31	0	1	(−)	(−)	(−)	(+)	(−)	(−)
7	61	M	9.0	Left lobectomy	14	7	10	(+)	(+)	(+)	(+)	(+)	(+)
8	72	F	3.5	Left and caudate lobectomy	20	0	0	(−)	(−)	(−)	(−)	(−)	(−)
9	67	M	4.5	Left lobectomy	20	0	0	(−)	(−)	(−)	(−)	(−)	(−)
10	71	M	3.5	Extended left lobectomy	34	0	NA	(−)	NA	(−)	NA	(−)	NA
11	34	F	8.0	Left and caudate lobectomy	13	8	10	(+)	(+)	(+)	(+)	(+)	(+)
12	60	F	6.5	Extended left lobectomy	25	3	4	(+)	(+)	(−)	(−)	(−)	(+)
13	63	F	4.2	Left lobectomy	16	1	3	(+)	(+)	(−)	(+)	(−)	(−)

NA = not applicable; NS = not sampled; RT-PCR = reverse transcriptase–polymerase chain reaction.

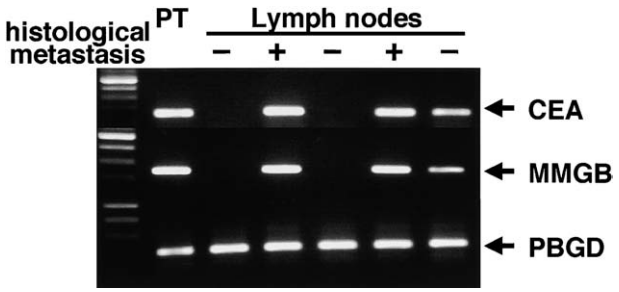

Fig. 2. Typical profile of detection of carcinoembryonic antigen (*CEA*) and mammaglobin B (*MMGB*) reverse transcriptase–polymerase chain reaction (RT-PCR) products in lymph nodes. *Upper*, CEA RT-PCR; *B*, MMGB RT-PCR; *lower*, Porphobilinogen deaminase (*PBGD*) RT-PCR. PT = primary tumor; − = histologically metastasis-negative node; + = histologically metastasis-positive node.

Table 2. Summary of patients with lymph node metastasis

	Histology (n = 13)	RT-PCR (n = 12)
Nodal metastasis (−)	7 (54%)	4 (33%)
Nodal metastasis (+)	6 (46%)	8 (67%)
Right pathway (+)	5 (38%)	6 (50%)
Left pathway (+)	4 (31%)	7 (58%)
Distant area (+)	3 (23%)	5 (42%)

RT-PCR = reverse transcriptase–polymerase chain reaction.

DISCUSSION

Negative nodal status for metastasis is one of the important favorable prognostic factors after hepatectomy for intrahepatic cholangiocarcinoma.[4–8] However, the distribution of metastatic nodes has not been well described in this disease. In the present study, we clearly demonstrated that the nodes around the cardiac portion of the stomach or along the gastric lesser curvature were common sites of lymphatic metastases in patients with left intrahepatic cholangiocarcinoma. Lymph node metastasis in these regions were detected in 4 (31%) of 13 patients by histologic examination and in 7 (58%) of 12 patients by molecular examination, whereas metastases in the right pathway were detected in 5 (38%) of 13 patients by histologic examination and 6 (50%) of 12 patients by molecular examination. The frequency of the lymph node metastasis in the left pathway seems to

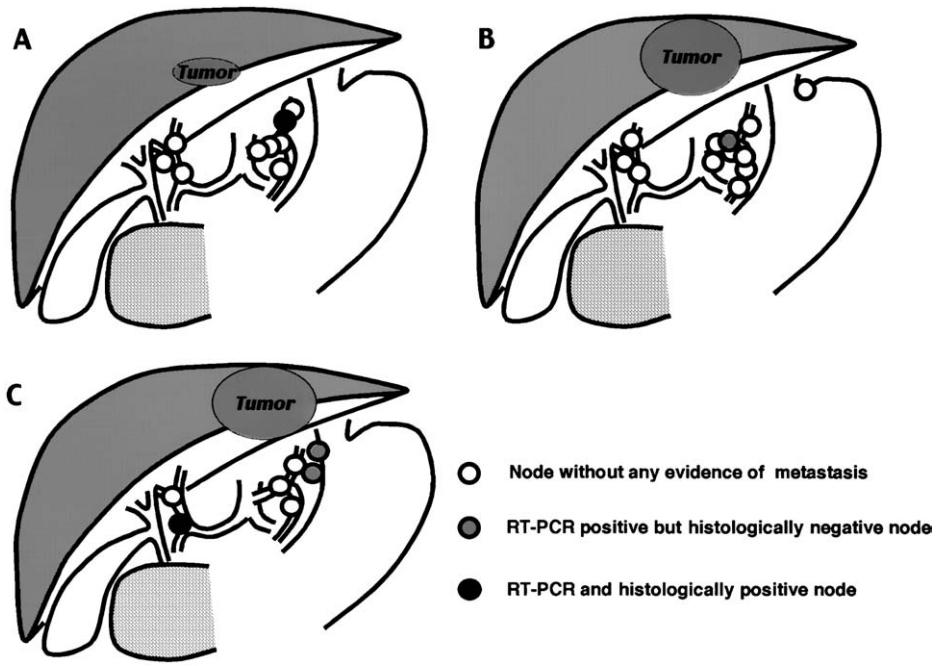

Fig. 3. Lymphatic maps of positive and negative nodes in three representative cases. **A**, Patient 5 in Table 1 had one positive node detected by both histopathologic examination and reverse transcriptase–polymerase chain reaction (*RT-PCR*) assay along the lesser gastric curvature. **B**, Patient 6 in the Table 1 had one positive node that was not detected by histologic examination but could be detected by RT-PCR. This node was also located in the left pathway. **C**, Patient 13 had three positive nodes. One was detected in the hepatoduodenal ligament by histologic examination and the others were detected in the connective tissue around the gastric cardia by RT-PCR.

be nearly equal to that in the right pathway, a pathway considered to be the primary regional nodal pathway in left intrahepatic cholangiocarcinoma.[14] Of note, two (29%) of seven patients with metastatic nodes had a positive node only along the left pathway. If no attention had been paid to the nodal status in that region, the stage of these patients would have been underestimated as metastasis-free disease. Furthermore, lymph nodes in distant areas, such as nodes in retroperitoneal tissue along the celiac artery, aorta, inferior vena cava, or common hepatic artery, were also affected in 3 (25%) of 13 patients by histologic examination and in 5 (42%) of 12 patients by molecular examination. All patients with positive nodes in any of these distant areas also had metastatic nodes in the right and/or left pathway, suggesting that tumor cells passed through either of these two pathways to spread to the distant area. On the basis of our findings, we propose that both the nodes along the left pathway and those along the right pathway should be classified as regional lymph nodes of intrahepatic cholangiocarcinoma arising in the left hepatic lobe.

According to the TNM staging system, which is applied to intrahepatic cholangiocarcinoma, the regional site of this disease is limited to the hepatoduodenal ligament regardless of where in the liver the primary tumor is located.[14] Nozaki et al.[10] had previously reported that intrahepatic cholangiocarcinoma in the left hepatic lobe displayed a different distribution of metastatic nodes from intrahepatic cholangiocarcinoma in the right lobe. The most important point in their article was that the lymph nodes around the cardiac portion and along the lesser curvature of the stomach were affected, as well as nodes in the hepatoduodenal ligament, if the primary tumors were located in the left lobe, as shown in the present study. However, as mentioned in their article, it was possible that they missed small metastatic foci within the clinically unremarkable lymph nodes because only enlarged lymph nodes were sampled and examined histopathologically. In the present study we evaluated patients who had intrahepatic cholangiocarcinoma that was limited to the left hepatic lobe and offered the same manner of lymphadenectomy to all patients. To avoid missing any metastatic lymph nodes, we removed the entire connective tissue in the hepatododenal ligament area and the lesser omentum, along the lesser gastric curvature, and around the cardiac portion of the stomach and then sampled all lymph nodes in the resected specimen. Furthermore, to detect metastasis more accurately, we used not only a histologic examination but also a molecular-based analysis.[11] Thus the present findings may be more reliable than those in some previous studies.[5,10]

In recent years many analyses based on molecular techniques have been developed to evaluate minimal residual cancer.[15] We applied a RT-PCR assay with two molecular markers[11] to assess the presence of small metastatic foci (micrometastasis) in lymph nodes that were not detected by histologic examination. Indeed, of the 214 histologically negative nodes in six patients, we detected lymph node "micrometastatses" in 24 nodes from two patients by RT-PCR assay. Thus we believe that our highly sensitive RT-PCR assay with two molecular markers enabled us to more accurately analyze the lymphatic spread of cancer cells from the left hepatic lobe.

From a clinical point of view, whether the presence of positive nodes along the left pathway correlates with postoperative prognosis remains unknown. Despite the short follow-up period, five of the seven patients with lymph node metastasis either in the right or left pathway had a recurrence within 1 year after surgery, whereas only one of five patients without any evidence of lymph node metastasis had a recurrence. This observation suggests the importance of lymph node staging for optimal adjuvant treatment after surgery. Further study with more patients and long-term analyses are needed to reveal the clinical implications of nodal status in patients with left intrahepatic cholangiocarcinomas.

CONCLUSION

For more accurate clinical staging of the patients with intrahepatic cholangiocarcinoma in the left hepatic lobe, lymph nodes around the cardia and along the gastric lesser curvature should be considered regional lymph nodes in addition to those along the hepatoduodenal ligament.

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Diabetes and Hyperlipidemia Correlate With Gallbladder Contractility in Leptin-Related Murine Obesity

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Obesity is associated with many comorbid conditions including diabetes, hyperlipidemia, and gallstones. However, the interaction among these modalities remains unclear. We recently demonstrated that both leptin-deficient and leptin-resistant obese mice have impaired biliary motility. These obese mice also are diabetic and hyperlipidemic. Therefore, we tested the hypothesis that serum glucose, insulin, cholesterol, and triglyceride levels would correlate with gallbladder contractility. Thirty-four lean control, 10 lean heterozygous leptin-deficient, 18 obese homozygous leptin-deficient, and 12 obese homozygous leptin-resistant mice were fed a nonlithogenic chow diet while nine lean control and nine obese homozygous leptin-deficient mice were fed a high-cholesterol diet for 4 weeks. In vitro gallbladder responses to cholecystokinin (CCK; 10^{-8} mol/L), acetylcholine (ACh; 10^{-5} mol/L), and neuropeptide Y (NPY; 10^{-6} mol/L) were measured. Serum glucose, insulin, cholesterol, and triglyceride levels were measured from pooled serum from an additional 704 animals. Gallbladder responses were greatest for CCK, intermediate for ACh, and least for NPY. Serum glucose, insulin, cholesterol, and triglyceride levels and body weight all correlated similarly, negatively, and significantly ($P < 0.001$) with gallbladder contractility. Hyperglycemia, insulin-resistance, hyperlipidemia, and body weight in obese mice with leptin dysfunction are associated with poor gallbladder contractility, which in turn may contribute to the association between obesity and gallstone formation. (J GASTROINTEST SURG 2003;7:857–863)

KEY WORDS: Cholesterol, diabetes, gallbladder, leptin, obesity

Gallstone disease is a major health problem in industrialized countries. In the United States gallstone disease is estimated to affect 14 million women and 6 million men.[1] Classical risk factors for cholesterol gallstone formation include female sex, obesity, age, parity, and family history.[2] Other factors that have been associated with gallstone disease include diabetes and hyperlipidemia. De Santis et al.[3] reported a higher prevalence of diabetes in patients with gallbladder disease compared to a matched control group. Thijs and Knipschild[4] noted that patients with gallstone disease have higher serum triglyceride and total cholesterol levels than matched control subjects. However, the evidence for these observations remains controversial, as other researchers have reported contradictory observations.[5,6] Moreover, the mechanisms leading to gallstone formation and the interactions among the risk factors are still not well understood.

Previous studies from our laboratory have demonstrated a relationship between gallbladder dysfunction and obesity in mice with leptin dysfunction.[7,8] Leptin is a hormone that controls satiety and activity through its actions on the hypothalamus with either leptin deficiency or leptin receptor dysfunction leading to obesity.[9] Both obese mice with absent (leptin-deficient; Lep^{ob}) and very high (leptin-resistant; Lep^{db}) serum leptin have impaired gallbladder contractility. However, recent work from this and

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another laboratory has suggested that obesity alone may not fully explain the risk of gallstone formation in these mice.[10,11] Because obese mice with leptin dysfunction also have diabetes and hyperlipidemia, we tested the hypothesis that serum glucose, insulin, cholesterol, and triglyceride levels would correlate with gallbladder contractility in leptin-related murine obesity.

MATERIAL AND METHODS

Animals and Diets

To determine in vitro gallbladder contractility, 43 lean control C57BL/6J, 10 lean heterozygous leptin-deficient (Lep^{het}), 27 obese homozygous leptin-deficient (Lep^{ob}), and 12 obese leptin-resistant (Lep^{db}) female mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 8 weeks of age (Table 1). The mice were housed four to five per cage in a light- (6 AM to 6 PM) and temperature (22° C)–controlled room. Nine lean control and nine obese homozygous leptin-deficient mice received a high-cholesterol diet (No. 180-024; Dyets, Inc., Bethlehem, PA) while the rest of the mice received a laboratory chow diet with trace amounts of cholesterol (Ralston Purina, St. Louis, MO) for 4 weeks. The high-cholesterol diet contained 1.0% cholesterol, 15% butterfat, and 0.5% cholic acid. When animals are fed this diet for 4 weeks, their bile becomes supersaturated with cholesterol and some animals form crystals, but none have gallstones.[10,12]

To measure serum levels of glucose, insulin, cholesterol, and triglycerides, 349 lean control, 160 lean heterozygous leptin-deficient, 117 obese homozygous leptin-deficient, and 78 obese leptin-resistant female mice were obtained at the same age from the same source and were maintained under identical conditions as the animals used to determine muscle contractility (see Table 1). One hundred twenty-eight lean control and 49 obese homozygous leptin-deficient mice received the high-cholesterol diet while the rest received the laboratory chow diet.

In Vitro Muscle Bath

At 12 weeks of age, the animals were fasted overnight. The next morning they were anesthetized with xylazine (15 mg/kg) and ketamine (50 mg/kg) and underwent cholecystectomy. The gallbladders were then placed in ice-cold modified Krebs solution consisting (in mmol/L) of the following: NaCl, 116.6; $NaCO_3$, 21.9; KH_2PO_4, 1.2; glucose, 5.4; $MgCl_2$, 1.2; KCl, 3.4; and $ClCl_2$, 2.5. The modified Krebs solution was bubbled with 95% O_2/5% CO_2 prior to use. The gallbladders were suspended from their longitudinal poles with 7-0 polypropylene sutures in muscle bath chambers containing modified Krebs solution and kept at 37° C. The gallbladders were allowed to equilibrate under 0.025 g of tension for 45 minutes.

Optimal length was then achieved by exposure to 10^{-5} mol/L acetylcholine (ACh; Sigma Chemical, St. Louis, MO) under a stepwise increase in tension (0.025 g). Next the gallbladders were exposed to 10^{-6} mol/L neuropeptide Y (NPY; Sigma Chemical) and 10^{-8} mol/L cholecystokinin (CCK; Sigma Chemical). Gallbladder responses were measured using the computer program WinDaq (DATAQ Instruments, Akron, OH). The muscle baths were rinsed with modified Krebs solution, and the gallbladders were allowed to return to baseline after each treatment. The gallbladder length and weight were measured and used to calculate cross-sectional area. Gallbladder contractile responses were then calculated and expressed as newtons per centimeter squared (N/cm^2). The responses among the various groups were then normalized with the response of lean control mice on a laboratory chow diet to CCK equaling 1.00.

Serum Analysis

At 12 weeks of age, the animals were fasted overnight. The following morning all animals were anesthetized with xylazine (15 mg/kg) and ketamine (50 mg/kg) and weighed. Whole blood was aspirated from the hearts and centrifuged to isolate serum. Serum was then pooled for further analysis. The number of pools from each study group is presented

Table 1. Mouse strains, diets, weights, number of animals, and serum pools

Strain	Diet	Weight (g)	GB response (No. of animals)	Serum (No. of animals)	Serum (No. of pools)
Lean control	Chow	18.1 ± 0.1	34	221	17
Heterozygous leptin-deficient	Chow	20.8 ± 0.2	10	160	13
Homozygous leptin-deficient	Chow	49.8 ± 0.7	18	68	11
Homozygous leptin-resistant	Chow	42.0 ± 0.4	12	78	13
Lean control	Cholesterol	18.3 ± 0.1	9	128	10
Homozygous leptin-deficient	Cholesterol	45.9 ± 0.7	9	49	8

GB = gallbladder.

in Table 1. Serum glucose was determined by the o-toluidine method (635-6; Sigma). Spectrophotometric assays were used to determine serum insulin (No. 960; Crystal Chem, Inc., Downers Grove, IL), total cholesterol (No. 401-100P; Sigma), and triglycerides (No. 23666410; Fisher Scientific, Hanover Park, IL).

Statistical Analysis

Data analyses were performed using SPSS software (SPSS, Inc., Chicago, IL). Data are expressed as mean ± standard error of the mean (SEM). Gallbladder responses to CCK, ACh, and NPY were correlated with serum glucose, insulin, cholesterol, and triglyceride levels and body weight using Pearson's correlation. Significance was determined and P values less than 0.05 were considered statistically significant.

RESULTS

Gallbladder Responses

In vitro gallbladder responses to CCK, ACh, and NPY are shown in Table 2. For each strain, gallbladder responses were CCK > ACh > NPY. Lean control mice fed a laboratory chow diet had the highest contractile responses to all three neurotransmitters. Heterozygous leptin-deficient mice on a laboratory chow diet had somewhat lower responses, and homozygous leptin-deficient and leptin-resistant mice on a laboratory chow diet had significantly lower responses ($P < 0.01$). When lean control mice were fed a high-cholesterol diet, gallbladder responses were significantly lower ($P < 0.01$) than those of the same mice on a laboratory chow diet. However, the high-cholesterol diet did not cause further diminution of gallbladder contractility in homozygous leptin-deficient mice.

Serum Levels

Serum levels of glucose, insulin, cholesterol, and triglycerides are also presented in Table 2. When fed a laboratory chow diet, obese animals (homozygous leptin-deficient and homozygous leptin-resistant) have significantly higher ($P < 0.05$) serum glucose, insulin, and cholesterol levels than lean animals (lean control, lean heterozygous leptin-deficient). All three strains with leptin dysfunction have significantly higher ($P < 0.05$) triglyceride levels than lean control mice when fed a laboratory chow diet.

When fed a high-cholesterol diet, lean control mice had slight increases in serum levels of glucose, insulin, and cholesterol compared to their laboratory chow counterparts. On the other hand, homozygous leptin-deficient mice on a high-cholesterol diet had significantly lower ($P < 0.01$) serum glucose and insulin levels, yet their cholesterol levels were significantly higher ($P < 0.01$) than those on a laboratory chow diet. Interestingly, serum triglyceride levels were not affected by the high-cholesterol diet.

Correlations

Correlations between gallbladder responses and serum glucose, insulin, cholesterol, and triglyceride levels are presented in Figs. 1 and 2, respectively. The correlation between gallbladder response and body weight is presented in Fig. 3. For each neurotransmitter, gallbladder contractility correlated negatively with serum glucose, insulin, cholesterol, and triglyceride levels and body weight. All correlation coefficients were similar and all were significant at $P < 0.001$.

DISCUSSION

In this study serum levels of glucose, insulin, cholesterol, and triglycerides in obese mice with leptin

Table 2. Gallbladder (GB) responses to neurotransmitters and serum levels

Strain	Diet	GB response			Serum level			
		CCK	ACh	NPY	Glucose (mg/dl)	Insulin (pg/ml)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Lean control	Chow	1.00 ± 0.08	0.42 ± 0.03	0.19 ± 0.02	193 ± 9	2.1 ± 0.7	68 ± 2	61 ± 3
Heterozygous leptin-deficient	Chow	0.69 ± 0.10	0.36 ± 0.07	0.13 ± 0.03	116 ± 9	2.9 ± 0.5	63 ± 4	78 ± 4
Homozygous leptin-deficient	Chow	0.43 ± 0.06	0.18 ± 0.02	0.07 ± 0.01	428 ± 23	33.1 ± 9	145 ± 5	78 ± 5
Homozygous leptin-resistant	Chow	0.42 ± 0.11	0.15 ± 0.05	0.04 ± 0.01	496 ± 25	8.8 ± 1.6	122 ± 4	98 ± 6
Lean control	Cholesterol	0.36 ± 0.08	0.18 ± 0.04	0.04 ± 0.01	221 ± 8	3.3 ± 0.8	89 ± 11	61 ± 5
Homozygous leptin-deficient	Cholesterol	0.47 ± 0.15	0.22 ± 0.07	0.08 ± 0.02	277 ± 19	13.5 ± 0.9	222 ± 15	67 ± 5

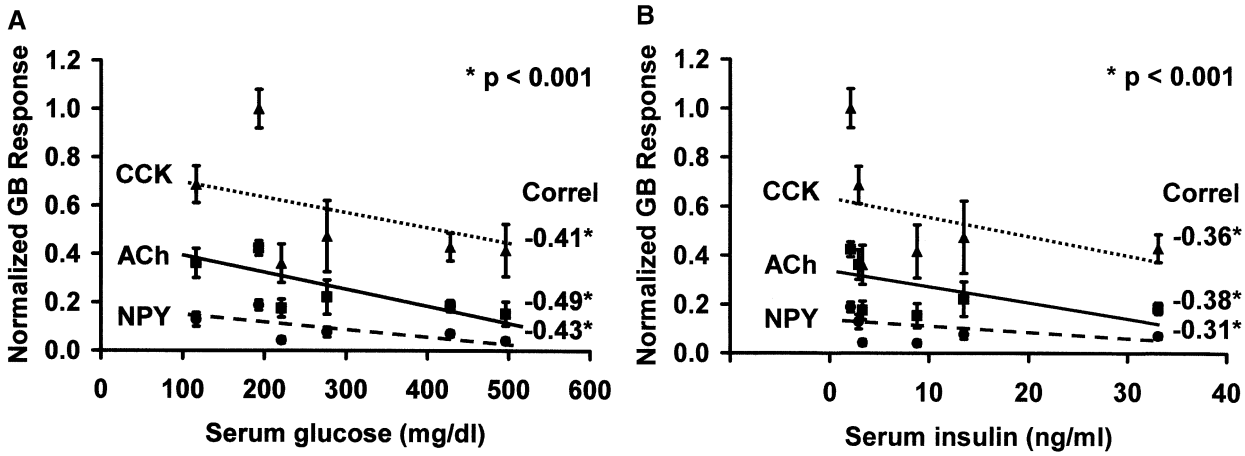

Fig. 1. A, Correlation of serum glucose with gallbladder response to cholecystokinin (CCK-▲), acetylcholine (ACh-■), and neuropeptide Y (NPY-●). **B**, Correlation of serum insulin with gallbladder response to cholecystokinin (CCK-▲), acetylcholine (ACh-■), and neuropeptide Y (NPY-●).

dysfunction were measured and correlated with gallbladder responses to CCK, ACh, and NPY. Although the patterns of these serum levels within the different strains varied, they all correlated negatively with gallbladder responses to neurotransmitters. Animal weights also were measured and, as expected, they too correlated negatively with gallbladder responses. Furthermore, all of the correlations and their significances were similar. These observations suggest that hyperglycemia, hyperinsulinemia, hyperlipidemia, and obesity are all associated with gallbladder contractility.

Cholesterol gallstone formation requires the following three essential elements: cholesterol supersaturation of bile, pronucleators, and biliary stasis. When fed a high-cholesterol diet, homozygous leptin-deficient obese mice form significantly more cholesterol crystals in gallbladder bile than similarly treated lean control mice.[12] Furthermore, bile isolated from homozygous leptin-deficient obese mice on a laboratory chow diet forms cholesterol crystals more rapidly in vitro than bile from lean control mice despite a normal cholesterol saturation of bile.[13–15] Previous studies from our laboratory also have

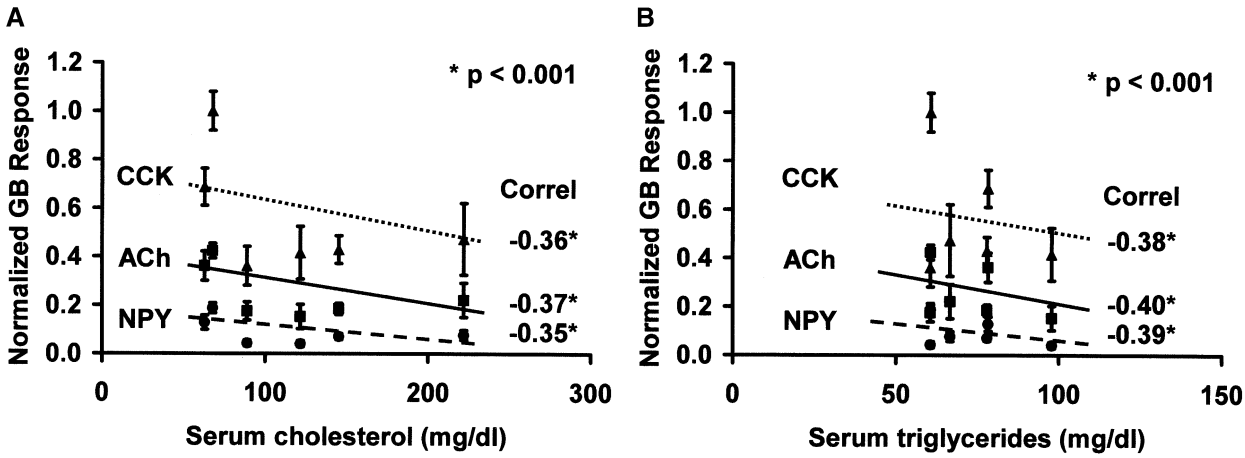

Fig. 2. A, Correlation of serum cholesterol with gallbladder response to cholecystokinin (CCK-▲), acetylcholine (ACh-■), and neuropeptide Y (NPY-●). **B**, Correlation of serum triglycerides with gallbladder response to cholecystokinin (CCK-▲), acetylcholine (ACh-■), and neuropeptide Y (NPY-●).

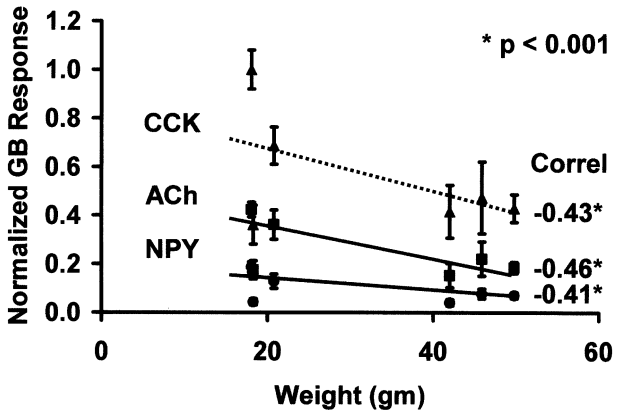

Fig. 3. Correlation of body weight with gallbladder response to cholecystokinin (CCK-▲), acetylcholine (ACh-■), and neuropeptide Y (NPY-●).

demonstrated that obese mice with leptin dysfunction have an impaired gallbladder response to neurotransmitters.[7,8] Finally, administration of leptin to homozygous leptin-deficient obese mice ameliorates this impaired gallbladder motility.[7,16] These observations suggest that decreased gallbladder contractility is a link between obesity and gallstone formation. However, the mechanism underlying this phenomenon has not been elucidated.

Classical risk factors for gallstone disease in humans include obesity, age, female sex, parity, and family history. However, other factors such as diabetes and hyperlipidemia have also been linked to the pathogenesis of gallstone disease in humans. De Santis et al.[3] reported a higher prevalence of diabetes in patients with gallstone disease, whereas Chapman et al.[5] reported a higher prevalence of gallstone disease in patients with diabetes. Moreover, Ruhl and Everhart[17] noted that hyperinsulinemia increased the risk for gallstones. Finally, Hahm et al.[18] reported that gallbladder volume in patients with diabetes was greater than that in control animals. These observations suggest that biliary stasis plays an important role in diabetes-related gallstone formation in humans. In this study both leptin-deficient and leptin-resistant obese mice were diabetic, hyperinsulinemic, and had diminished gallbladder contractility.

Leptin-deficient and leptin-resistant obese mice serve as murine models for human obesity. These mice have also provided insights into the association between gallstones and obesity. However, recent observations suggest that obesity alone does not explain susceptibility to gallstone formation. Bouchard et al.[11] reported that leptin-deficient and leptin-resistant obese mice are less susceptible to gallstone formation than nonobese diabetic mice. In another study from our laboratory, we found that homozygous leptin-resistant obese mice have low biliary cholesterol levels and decreased in vitro cholesterol crystal formation.[10] These observations, along with results of previously mentioned studies implicating diabetes as a significant factor in gallstone pathogenesis, led us to perform the present experiment. Because hyperglycemia and hyperinsulinemia have been observed to result in poor gallbladder contractility, we surmised that diabetes contributes to gallstone susceptibility through impairment of gallbladder myocyte and/or neurotransmitter function.

The adverse effects of increased glucose and insulin levels on gallbladder and antropyloric function have been well documented.[19,20] Both hyperglycemia and euglycemic hyperinsulinemia have been shown to inhibit CCK-stimulated gallbladder motility.[19] Hyperinsulinemia may also be a key factor in these observations because insulin regulates the Na^+,K^+ pump, which may adversely affect the ionic and osmotic homeostasis of smooth muscle cells including gallbladder myocytes.[21] The Na^+,K^+ pump of presynaptic nerve terminals is also regulated by insulin.[21] Moreover, decreased Na^+,K^+ pump activity can result in increased intracellular Na^+, which in turn increases the Na^+/Ca^{++} exchange, thereby increasing intracellular calcium. Increased intracellular calcium will alter both smooth muscle tone and release of neurotransmitters. Finally, leptin has also been recently demonstrated to inhibit Na^+,K^+ pump function.[21] Thus, the combined effect of hyperinsulinemia and altered leptin function may explain the dramatic decreases in gallbladder muscle response observed in this study.

Hypertriglyceridemia and hypercholesterolemia also have been previously associated with gallstones. Scragg et al.[22] reported in 1984 that triglyceridemia contributes to gallstone risk in a case-control study. In 1990 Thijs and Knipschild[4] found the same association and suggested that triglycerides may be a more important risk factor than obesity. The data on serum cholesterol are more controversial, with some researchers noting weak associations between gallstones and serum total cholesterol as well as high-density lipoproteins (HDLs).[4,22] In the present study serum total cholesterol and triglyceride levels were negatively correlated with gallbladder responses to CCK, ACh, and NPY in obese mice with leptin dysfunction. In addition, serum HDLs were negatively correlated ($P < 0.01$) with gallbladder responses to neurotransmitters (data not reported). Interestingly, this observation contradicts human data, which suggest that high HDL levels are protective against gallbladder disease, perhaps because they alter biliary

lipid composition.[22] The mechanism by which elevated serum lipids alter gallbladder motor function may be through lipid-laden myocytes as has previously been demonstrated in guinea pigs fed a high-cholesterol diet.[23]

The data presented in this report correlated serum glucose, insulin, cholesterol, and triglyceride levels with gallbladder contractility. An inherent weakness of the study is the collection of serum data from one set of animals and gallbladder responses from another set. Although both sets of animals were identically obtained and treated, a correlation among data gathered from a single set of animals would have been better because individual serum values could have then been matched to the corresponding gallbladder response. However, because of the very small amount of serum available from each mouse, we were forced to pool the collected serum to obtain volumes large enough for analysis. Thus, serum data came from 704 mice, whereas gallbladder responses were measured in 73 mice. Despite this potential flaw, all correlations were highly statistically significant ($P < 0.001$).

Although obesity is a major risk factor for gallstone disease in humans, recent independent studies in multiple models of murine obesity have suggested that obesity alone may not lead to gallbladder disease. Obesity is a polygenic disorder that is associated with multiple comorbidities that have been implicated in gallstone pathogenesis. In this study we examined biliary stasis, an essential element for cholesterol gallstone formation, by measuring gallbladder contractility. Our data suggest that serum glucose, insulin, cholesterol, and triglyceride levels are inversely correlated with gallbladder responses to the neurotransmitters CCK, ACh, and NPY. Thus, high serum levels of glucose, insulin, cholesterol, and triglycerides are all associated with poor gallbladder motility. This experiment and other recent reports suggest that both diabetes and hyperlipidemia may be independent risk factors for gallbladder stasis, and therefore, for gallstone formation.

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Discussion

Dr. K. Lillemoe (Baltimore, MD): It is your conclusion that these findings were solely related to leptin deficiency, since you actually observed significant changes in contractility associated with abnormal biochemistry levels and in the lean control mice when fed the high-cholesterol diet. Could you then translate these findings into human gallstone disease, which, again, as you noted, is more frequent in diabetics, a group of patients in which leptin deficiency has not been defined?

Finally, can you tie your model of leptin deficiency into the major problem of morbid obesity, which we are facing in this country?

Dr. K. Tran: To answer the first question, it is true that our model amonogenic applies to obese mice with leptin deficiency, and obviously human obesity is polygenic. We are actually planning to include mice with polygenic obesity in our next set of studies because, based on studies from other laboratories, some of these mice are more prone to gallstone formation than leptin-deficient or leptin-resistant mice.

Trying the leptin model to obesity, again I think your point is valid. Obesity is a polygenic disorder, and in our model we have been studying mice with single defects. Of course this strategy has the advantage of knowing the effects of leptin on gallstone pathogenesis.

To explain the findings from the present analysis, we have looked at how leptin and insulin may be acting on the gallbladder myocyte. Based on results of recent studies that have been reported in the literature, both of those hormones influence the sodium-potassium channel, which regulates osmolarity and electrolyte composition of the cell. Our current working hypothesis is that these two hormones affect the sodium-potassium channel, which then may affect both gallbladder contractility directly and via nerve transmission.

Dr. B. Schirmer (Charlottesville, VA): Do the leptin-resistant mice get gallstones if you feed them a high-cholesterol diet?

Dr. Tran: We have recently completed a study in leptin-resistant mice on a high-cholesterol diet for 4 weeks, and they formed fewer cholesterol crystals than the control mice. A similar finding was observed by investigators in another laboratory, who fed the leptin-resistant mice for 12 weeks, and these mice formed fewer gallstones than control mice.

Invited Discussion—Expert Commentator

Bruce D. Shirmer, M.D. (Charlottesville, VA): The authors have made observations of the in vitro response of murine gallbladders of various strains of leptin obese mice to neurotransmitters and compared that with data of pooled blood samples of other mice of a similar strain that were measured for glucose. These investigators demonstrated that obese mice with leptin dysfunction have poor gallbladder contractility and that they have hyperglycemia. No causal relationship has been demonstrated.

Why did the authors not use blood samples from the mice undergoing cholecystectomy instead of comparing the gallbladder responses to pooled blood sample data from other mice? I wonder if the authors found any gallstones in the gallbladders of the obese mice when they were removed? This would be greater evidence that the gallbladder dysfunction shown in the in vitro studies is important.

The leptin-resistent mice are known to have low biliary lipid levels. Why would they be expected to develop gallstones, even if their gallbladders do not contract well? The difference in biliary lipid levels may be more important in the incidence of gallstone formation, and do the authors have any data as to the incidence of gallstone formation in the leptin-deficient vs. leptin-resistent strains?

Finally, extrapolating these data to humans has a long way to go. This may be especially true in obese humans, where factors such as the number of crash diets and weight lost during these diets may be more significant factors as to the incidence of gallstone formation in the obese human population then serum leptin levels.

Butyrate Inhibits Pancreatic Cancer Invasion

Buckminster Farrow, M.D., Piotr Rychahou, M.D., Kathleen L. O'Connor, Ph.D., B. Mark Evers, M.D.

Pancreatic cancer is the most deadly gastrointestinal malignancy because of its propensity for local invasion and early metastasis. Integrin chains, in particular β4, can promote invasion in other cancers. The effect of sodium butyrate (NaBT), which induces differentiation in transformed cells, on integrin expression is unknown. The purpose of this study was to determine patterns of integrin expression in pancreatic cancer cells and investigate the effect of NaBT on integrin expression and invasion. Integrin expression was assessed in the less invasive MIA-PaCa-2 and PANC-1 and more invasive L3.6, AsPC-1, and SUIT-2 human pancreatic cancer cell lines by ribonuclease (RNase) protection assay. Western blotting and immunofluorescent staining for β4 expression was determined after NaBT treatment. Matrigel invasion chambers were used to assess pancreatic cancer cell invasion. β4 and β7 integrin expression was highest in L3.6, AsPC-1, and SUIT-2 cells. NaBT reduced the expression of β4 integrin in AsPC-1 cells including less cell surface β4. Invasion of AsPC-1 cells was also reduced by NaBT. Expression of β4 is higher in more aggressive pancreatic cancer cells; NaBT inhibits β4 expression and invasion. NaBT may represent a novel strategy to inhibit pancreatic cancer invasion and improve the prognosis of this deadly disease. (J GASTROINTEST SURG 2003;7:864–870)

KEY WORDS: Pancreatic cancer, invasion, integrins, sodium butyrate

More than 30,000 patients will die of pancreatic cancer in 2003,[1] making it the fourth leading cause of cancer death in the United States. Pancreatic cancer is the most lethal abdominal malignancy; 99% of patients diagnosed with adenocarcinoma of the pancreas will be dead within 5 years of diagnosis.[2] The lethality of this cancer is related to its rapid growth and propensity to invade adjacent organs and metastasize. Surgical resection offers the only possibility for cure, yet fewer than 15% of patients are candidates for tumor resection at the time of diagnosis, and conventional chemotherapy has shown only a minimal survival benefit when combined with surgical resection.[3] Additionally, most patients who have undergone potentially curative surgical therapy eventually have recurrent metastatic disease. Specific therapies targeted to factors that facilitate invasion may increase the tumor resectability and improve the overall prognosis of pancreatic cancer.

The process of cancer cell invasion requires multiple steps. In epithelial-derived cancers, tumor cells must degrade and then traverse the basement membrane to invade local structures and disseminate through lymphatic channels and the bloodstream.[4] Tumor-related adhesion molecules, such as integrins, facilitate the motility of cancer cells and allow them to migrate into adjacent organs or into the bloodstream.[5] Integrins are dimers, composed of α and β subunits; each pairing of α and β chains binds to a specific ligand on cell membranes or within the extracellular matrix.[6] Ligand binding to the integrin dimer can facilitate adhesion, migration, and activation of intracellular signaling pathways.[7] In pancreatic cancer, expression of several integrin chains including α6,[8] αv,[9] and β1[10] have been implicated as mediators of invasion. Developing strategies to inhibit the expression of these proinvasive integrins has lagged

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behind their discovery; thus the feasibility of decreasing integrin expression and inhibiting pancreatic cancer cell invasion is relatively unknown.

Sodium butyrate (NaBT) is a short-chain fatty acid that is produced by naturally occurring bacteria in the human colon.[11] Our laboratory has previously shown that NaBT can induce differentiation and cell cycle arrest in colorectal cancer cells,[12] thus indicating its potential role as an effective antitumor agent. Other investigators have demonstrated that NaBT induces differentiation of pancreatic cancer cells[13] and may possess antitumor activity against chemoresistant pancreatic cancers in vivo.[14] NaBT may also alter the expression of integrins that are associated with a highly metastatic and poorly differentiated phenotype. Therefore the purpose of this study was to determine the following: (1) whether expression of specific integrins correlates with known invasiveness of different pancreatic cancer cells and (2) whether NaBT can inhibit invasion-associated integrin expression and pancreatic cancer cell invasion.

METHODS

Cell Lines and Reagents

Five different human pancreatic cancer cell lines were used in this study. AsPC-1 (American Type Culture Collection, Manassass, VA) and SUIT-2 (Dr. Takeshi Iwamura, Miyazaki Medical College, Miyazaki, Japan) cells were grown in RPMI 1640 with 10% to 20% fetal bovine serum (FBS) and penicillin/streptomycin. L3.6 cells (Dr. Isiah Fidler, M.D. Anderson Cancer Center, Houston, TX) were grown in Dulbecco's modified Eagle medium (DMEM) with 10% FBS and 200 mmol/L L-glutamine, 100 mmol/L sodium pyruvate, and nonessential amino acids. MIA PaCa-2 and PANC-1 (American Type Culture Collection) were grown in DMEM with 10% FBS, and penicillin/streptomycin. NaBT (Sigma, St. Louis, MO) was dissolved in water and used at the concentrations indicated.

Ribonuclease Protection Assay

Total RNA was extracted from MIA PaCa-2, PANC-1, L3.6, AsPC-1, and SUIT-2 cells or from NaBT-treated and control (untreated) AsPC-1 cells using Ultraspec (Biotecx Laboratories, Houston, TX). The hITG-2 multiprobe (BD Pharmingen, San Diego, CA) was radiolabeled using the Maxiscript T7 kit (Ambion, Austin, TX) and hybridized to 40 μg of total RNA overnight. Digestion of nonhybridized fragments was performed, and samples were resolved on a polyacrylamide denaturing gel. Results were obtained using autoradiography.

Western Blotting

Whole-cell protein was resolved on a 10% polyacrylamide gel and transferred to PVDF membranes as previously described.[15] Filters were incubated for 30 minutes at room temperature in blocking solution (Tris-buffered saline solution with 0.1% TWEEN 20 (TBST) containing 5% nonfat dried milk) followed by 1-hour incubation with primary antibodies to β4 integrin (a generous gift from Dr. Arthur Mercurio, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA). Filters were washed three times in TBST and incubated with peroxidase-conjugated goat antimouse antibody (Upstate Biotechnology, Lake Placid, NY) for 1 hour. After three final washes, immune complexes were visualized by the enhanced chemiluminescence (ECL) detection system. Membranes were stripped and reprobed with β-actin to ensure equal loading.

Immunofluorescence Staining

AsPC-1 cells were plated on coverslips, treated with NaBT (5 mmol/L) for 24, 48, and 72 hours and then fixed briefly in ice-cold methanol. Coverslips were blocked in 3% dry milk in phosphate-buffered saline solution and immunostained with monoclonal mouse β4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Staining was detected using Alexa-488 labeled antimouse antibody (Molecular Probes, Eugene, OR) and standard fluorescence microscopy.

Invasion Assays

AsPC-1 cells were pretreated for 8 hours with 5 mmol/L NaBT. Matrigel invasion chambers with 8 μmol/L pores were reconstituted with 1% FBS medium for 2 hours at 37° C prior to the addition of cells. After pretreatment, AsPC-1 cells (5×10^4) were trypsinized and resuspended in 1% FBS medium alone (control) or with fresh 5 mmol/L NaBT added just prior to placement into the invasion chambers. Laminin (Invitrogen, Carlsbad, CA), 20 μg/ml, was added to the upper membrane surface with the cells and recombinant human epidermal growth factor (EGF) (Invitrogen) 25 ng/ml was placed in the bottom of each well as a chemoattractant. After 48 hours, cells that had not invaded were removed from the upper surface using cotton swabs, and the invading cells were fixed with 100% methanol and stained with 0.2% crystal violet in 2% ethanol. Invasion was quantitated by visual counting, and the sum of invading cells in five individual high-powered fields for each membrane was obtained. Assays were performed in triplicate for each treatment group. Results

are expressed as the mean number of cells invading per treatment group.

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). The effect of NaBT on invasion was analyzed using the one-sided paired *t* test. A *P* value of less than 0.05 was considered significant.

RESULTS

Expression of β4 and β7 Integrin Chains Correlates With Invasiveness

Despite pancreatic cancer being a highly invasive malignancy, human pancreatic cancer cell lines differ in their metastatic potential because of unique patterns of gene expression and degrees of differentiation. For this study we selected the following five human pancreatic cancer cell lines: MIA PaCa-2 and PANC-1, which are nonmetastatic; and three aggressive metastatic cell lines, L3.6, AsPC-1, and SUIT-2. Other investigators have shown the aggressiveness of L3.6, AsPC-1, and SUIT-2 cell lines in vivo as demonstrated by the development of peritoneal metastases following splenic injection into nude mice.[16,17] To confirm the invasiveness of the more aggressive cell lines, we injected AsPC-1 and SUIT-2 cells subcutaneously into the flanks or into the spleens of athymic nude mice. Subcutaneous injection of both AsPC-1 and SUIT-2 cells produced local invasion into the surrounding tissues, whereas splenic injection resulted in multiple liver metastases within 4 weeks (data not shown).

To establish whether integrin expression is different between the more aggressive L3.6, AsPC-1, and SUIT-2 cells and the less aggressive MIA PaCa-2 and PANC-1 cells, a ribonuclease (RNase) protection assay was performed. Expression of most α and β chains was not consistently different between the two groups of cells; however, expression of both β4 and β7 was stronger in L3.6, AsPC-1, and SUIT-2 cells, compared to the less aggressive cells (Fig. 1). These results suggest that the expression of β4 and/or β7 may be important factors related to pancreatic cancer invasion.

NaBT Inhibits β4 Integrin Expression

NaBT has been described as a potent inducer of differentiation in both pancreatic[13] and colon cancer[18] cell lines. RNase protection analysis demonstrated expression of both β4 and β7 integrins in the metastatic cell lines, which suggests that these are markers of a less differentiated and more invasive phenotype. To determine whether NaBT inhibits the expression of the invasion-related integrin β4, AsPC-1 cells were treated with NaBT (5 mmol/L) for 24 hours and an RNase protection assay was performed. NaBT decreased the expression of both β4 and β7; L32 and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) expression was unaffected indicating equal loading of RNA (Fig. 2, *A*). To confirm that NaBT inhibited protein expression of β4, AsPC-1 cells were again treated with NaBT, and whole-cell lysates immunoblotted for β4 protein. NaBT (5 mmol/L) inhibited the expression of β4 protein (Fig. 2, *B*). Taken together, RNase protection and Western blot analysis indicate that NaBT is a potent inhibitor of β4 integrin expression, which may have profound effects on pancreatic cancer cell invasion.

Cell Surface Expression of β4 Is Decreased by NaBT

To be functional, integrins must be expressed on the surface of cells to bind appropriate ligands and

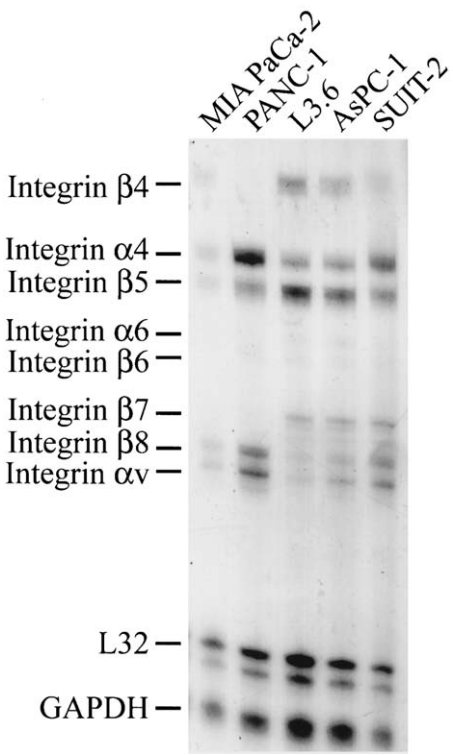

Fig. 1. Expression of β4 and β7 integrin chains correlates with invasiveness. Total RNA was extracted from nonmetastatic MIA PaCa-2 and PANC-1 cells or metastatic L3.6, AsPC-1, and SUIT-2 cells. RNase protection analysis was used to determine integrin chain expression with the hITG-2 multiprobe as described in Methods. L32 and GAPDH expression was measured to ensure equal loading of RNA.

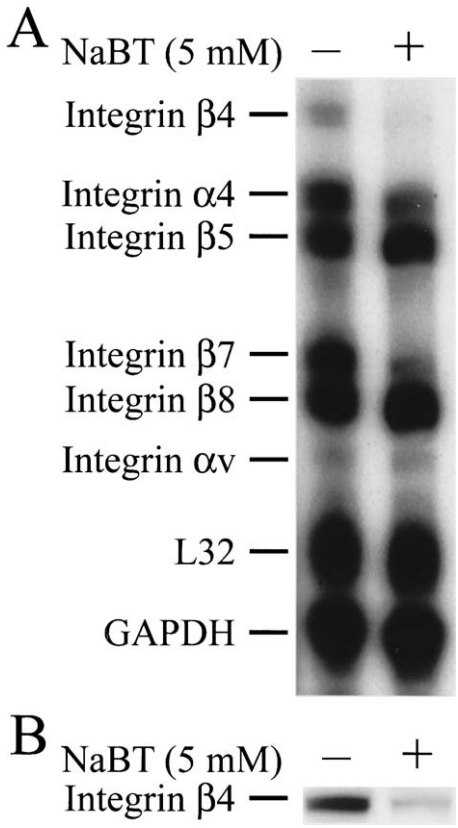

Fig. 2. NaBT inhibits β4 integrin expression. AsPC-1 cells were treated for 24 hours with NaBT (5 mmol/L). **A**, Total RNA was extracted from control (untreated) and NaBT-treated cells and subjected to RNase protection analysis with the hITG-2 multiprobe as described in Methods. L32 and GAPDH expression was measured to ensure equal loading of RNA. **B**, Whole-cell protein was also obtained and resolved on a 10% sodium dodecyl sulfate–polyacrylamide gel before transfer to PVDF membranes. Membranes were immunoblotted for β4 integrin; membranes were stripped and reprobed for β-actin to ensure equal loading of protein (data not shown).

activate signaling pathways.[6] The turnover of integrins on the membrane of pancreatic cancer cells is unknown; thus we sought to confirm that decreases in β4 expression, observed by RNase protection and Western blotting, also resulted in reduced β4 integrin expression on the cell surface. In AsPC-1 cells, β4 immunofluorescent staining on the cell surface was greatly reduced by NaBT within 24 hours (Fig. 3). This reduction in membrane β4 was less pronounced after 48 hours, and β4 expression was nearly restored within 72 hours after NaBT treatment (data not shown). The decreased β4 expression at the level of the membrane suggests that NaBT may be a useful strategy to inhibit pancreatic cancer cell invasion.

NaBT Inhibits Pancreatic Cancer Invasion

The expression of β4 integrin has been shown to be critical for invasion of breast[19] and other cancer cells.[20] To determine whether NaBT could inhibit pancreatic cancer cell invasion, similar to its inhibition of β4 integrin expression, AsPC-1 cells were treated with NaBT and placed into Matrigel invasion chambers. The ligand for the β4 integrin, laminin, was added to the invasion chambers to facilitate β4-dependent invasion. After 48 hours, NaBT significantly reduced the number of invading cells compared to control treatment (Fig. 4). Because NaBT nearly silences expression of β4 integrin, its negative effect on pancreatic cancer cell invasion is likely due to reduced β4 expression.

DISCUSSION

The prognosis for patients with pancreatic cancer is dismal; 5-year survival is less than 5% despite optimal surgical and chemotherapeutic treatments.[2] Identifying mechanisms of pancreatic cancer invasion may facilitate the development of more effective adjunctive treatments to improve outcomes following surgical resection. In this study we demonstrate that expression of the β4 integrin chain closely correlates with known metastatic potential of pancreatic cancer cells. Additionally, the naturally occurring fatty acid, NaBT, potently inhibits β4 integrin expression and pancreatic cancer cell invasion.

The importance of β4 integrin expression in facilitating invasion has been extensively analyzed. Shaw et al.[21] demonstrated that the α6β4 integrin binding to laminin can facilitate cell movement and activation of the phosphatidylinositol 3-kinase (PI3K) pathway whose downstream effectors facilitate invasion. One factor activated by PI3K is Akt, which is overexpressed in pancreatic cancer[22] and facilitates invasion of pancreatic cancer cells.[23] Interestingly, the ability of α6β4 to activate PI3K and stimulate invasion may not require binding to laminin; thus expression of β4, in the absence of its ligand laminin, may enhance carcinoma invasion. Therefore integrin α6β4 can facilitate invasion by providing mechanical support to migrating cells and by changing the intracellular milieu to favor expression of other proinvasive factors. The role of β4 in carcinoma invasion has also been shown using another differentiating agent, retinoic acid, to inhibit β4 expression and metastasis formation in lung cancer cell lines.[20] Our finding that NaBT significantly inhibits pancreatic cancer invasion and β4 expression is consistent with results of previous studies,[19–21] which describe the importance

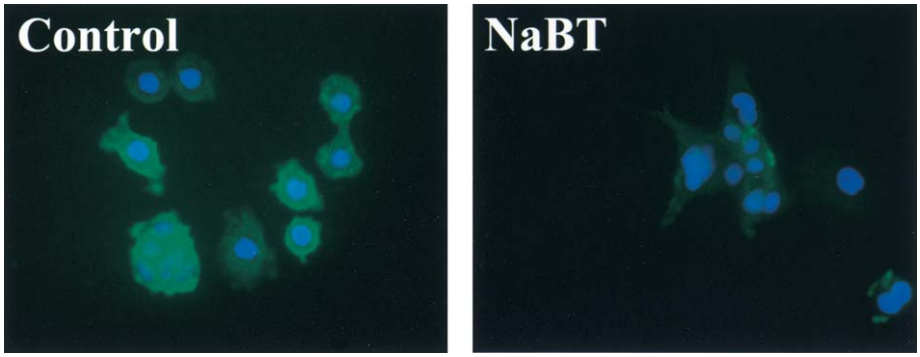

Fig. 3. Cell surface expression of β4 is decreased by NaBT. AsPC-1 cells were grown on coverslips and treated with 5 mmol/L NaBT or medium alone. After 24 hours, cells were fixed with methanol, blocked in phosphate-buffered saline solution with 3% milk, and incubated with β4 antibody for 45 minutes. β4 staining was detected using Alexa 488–labeled goat antimouse antibody with a standard fluorescence microscope and appropriate filters.

of β4 and underscore the significance of this effect in aggressive pancreatic cancer cell lines.

Other integrins may also be important for pancreatic cancer cell invasion. The expression of another laminin-binding integrin, β3, correlates with lymph node metastasis and high matrix metalloproteinase-2 (MMP-2) expression. Investigators have also shown a relationship between expression of β1 and invasiveness by demonstrating a reduction in the metastatic rate in vivo using β1-blocking antibodies[8] and enhanced invasiveness when β1 expression is increased by the cytokine interleukin-1.[10] The relative importance of these integrins, compared to α6β4, is unclear. In most of these studies, β1 expression was examined only when dimerized with the α6 integrin chain; however, the β1 chain can dimerize with 11 other α chains, each of which binds a unique set of ligands. Therefore blocking β1 expression alone may affect the function of many other integrins, which suggests that the observed effects may not be specific for β1. Second, blocking antibodies intended to "block" β1 function may prevent mechanical interactions between β1 and extracellular matrix proteins but may also simultaneously activate signaling pathways as if the integrin were binding to its ligand. Last, α6β4 activates PI3K more efficiently than α6β1 or other β1 integrins, and this α6β4 activation of PI3K is required for the formation of lamellae that facilitate carcinoma cell motility.[21] The abundance of evidence supports a critical role for α6β4 in carcinoma invasion. Furthermore, the effectiveness of NaBT in reducing β4 expression and invasion in pancreatic cancer cells suggests α6β4 is also a key facilitator of pancreatic cancer invasion.

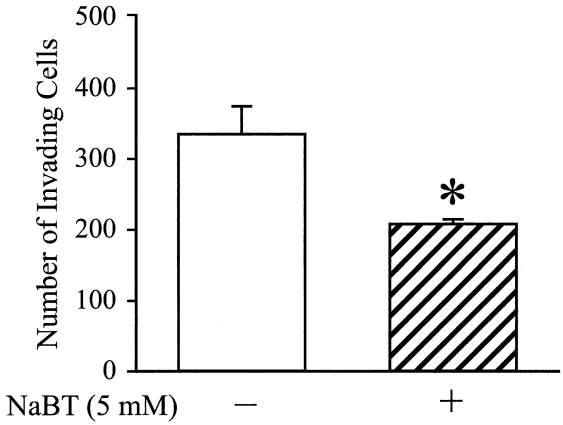

Fig. 4. NaBT inhibits pancreatic cancer invasion. AsPC-1 cells were pretreated with 5 mmol/L NaBT for 8 hours, then trypsinized and resuspended in 1% FBS medium with or without 5 mmol/L NaBT. Cells (5 × 104) were then placed in the upper well of Matrigel invasion chambers with laminin (20 μg/ml), and EGF-1 (50 ng/ml) was used as a chemoattractant in the lower well. Invasion was assessed by cell counting after 48 hours as described in Methods. (Data are expressed as mean ± SEM; $^*P < 0.05$ vs. control values.)

NaBT, a short-chain fatty acid produced by the fermentation of dietary fiber in the colon,[24] exerts its nonmetabolic effects by inhibiting the enzyme histone deacetylase,[25] which subsequently alters the expression of numerous target genes. NaBT is a potent inducer of apoptosis and cell cycle arrest in other gastrointestinal tract cancer cells, most notably colon cancer[26]; however, these effects have not been reported for pancreatic cancer. More generally, NaBT is a differentiation agent, with effectiveness in colon[18] and prostate[27] cancer cell lines. NaBT can induce differentiation in pancreatic cancer cells, as demonstrated by an increase in the epithelial

marker keratin 23[13] or the mucin marker M1.[28] Here we extend the scope of NaBT-induced differentiation to demonstrate that NaBT reduces expression of the invasion promoting integrin β4 and, moreover, inhibits pancreatic cancer cell invasion. We have shown previously that NaBT can inhibit the invasion of hepatocellular carcinoma cells,[29] whereas other investigators have demonstrated similar anti-invasive effects in both breast[30] and colon cancer[31] cells. We demonstrate, for the first time, that NaBT can also inhibit invasion of pancreatic cancer cells. NaBT also reduced the expression of the β7 integrin chain, which was expressed only in the more aggressive pancreatic cancer cell lines. The β7 integrin chain was known previously to be expressed only in lymphocytes where it is believed to facilitate lymphocyte homing in the gastrointestinal tract.[32] Because β7 integrin expression and pancreatic cancer cell invasion were both inhibited by NaBT, it is intriguing to hypothesize whether β7 integrin may facilitate the spread of metastatic pancreatic cancer cells in the gastrointestinal tract. Detailed studies to fully understand the role of the β7 integrin chain in pancreatic cancer cell invasion are required.

CONCLUSION

In this study we demonstrate that expression of the β4 and β7 integrin chains is closely linked to the metastatic potential of pancreatic cancer cells. Additionally, using the fatty acid NaBT, we have shown a reduction of β4 expression and inhibition of pancreatic cancer cell invasion. The efficacy of NaBT suggests that this may be an effective strategy to target β4 integrin expression in patients with pancreatic cancer and improve the prognosis of this lethal disease. Based on these findings, we believe further investigation into the role of NaBT as an inhibitor of invasion and an adjuvant therapy against pancreatic cancer is warranted.

We thank Drs. Takeshi Iwamura for the SUIT-2 cells, Isiah Fidler for the L3.6 cells, and Arthur Mercurio for the β4 antibody. We also thank Tatsuo Uchida for performing the statistical analysis and Eileen Figueroa and Karen Martin for manuscript preparation.

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Butyrate Inhibits Pancreatic Cancer Invasion

Buckminster Farrow, M.D., Piotr Rychahou, M.D., Kathleen L. O'Connor, Ph.D., B. Mark Evers, M.D.

Pancreatic cancer is the most deadly gastrointestinal malignancy because of its propensity for local invasion and early metastasis. Integrin chains, in particular β4, can promote invasion in other cancers. The effect of sodium butyrate (NaBT), which induces differentiation in transformed cells, on integrin expression is unknown. The purpose of this study was to determine patterns of integrin expression in pancreatic cancer cells and investigate the effect of NaBT on integrin expression and invasion. Integrin expression was assessed in the less invasive MIA-PaCa-2 and PANC-1 and more invasive L3.6, AsPC-1, and SUIT-2 human pancreatic cancer cell lines by ribonuclease (RNase) protection assay. Western blotting and immunofluorescent staining for β4 expression was determined after NaBT treatment. Matrigel invasion chambers were used to assess pancreatic cancer cell invasion. β4 and β7 integrin expression was highest in L3.6, AsPC-1, and SUIT-2 cells. NaBT reduced the expression of β4 integrin in AsPC-1 cells including less cell surface β4. Invasion of AsPC-1 cells was also reduced by NaBT. Expression of β4 is higher in more aggressive pancreatic cancer cells; NaBT inhibits β4 expression and invasion. NaBT may represent a novel strategy to inhibit pancreatic cancer invasion and improve the prognosis of this deadly disease. (J GASTROINTEST SURG 2003;7:864–870)

KEY WORDS: Pancreatic cancer, invasion, integrins, sodium butyrate

More than 30,000 patients will die of pancreatic cancer in 2003,[1] making it the fourth leading cause of cancer death in the United States. Pancreatic cancer is the most lethal abdominal malignancy; 99% of patients diagnosed with adenocarcinoma of the pancreas will be dead within 5 years of diagnosis.[2] The lethality of this cancer is related to its rapid growth and propensity to invade adjacent organs and metastasize. Surgical resection offers the only possibility for cure, yet fewer than 15% of patients are candidates for tumor resection at the time of diagnosis, and conventional chemotherapy has shown only a minimal survival benefit when combined with surgical resection.[3] Additionally, most patients who have undergone potentially curative surgical therapy eventually have recurrent metastatic disease. Specific therapies targeted to factors that facilitate invasion may increase the tumor resectability and improve the overall prognosis of pancreatic cancer.

The process of cancer cell invasion requires multiple steps. In epithelial-derived cancers, tumor cells must degrade and then traverse the basement membrane to invade local structures and disseminate through lymphatic channels and the bloodstream.[4] Tumor-related adhesion molecules, such as integrins, facilitate the motility of cancer cells and allow them to migrate into adjacent organs or into the bloodstream.[5] Integrins are dimers, composed of α and β subunits; each pairing of α and β chains binds to a specific ligand on cell membranes or within the extracellular matrix.[6] Ligand binding to the integrin dimer can facilitate adhesion, migration, and activation of intracellular signaling pathways.[7] In pancreatic cancer, expression of several integrin chains including α6,[8] αv,[9] and β1[10] have been implicated as mediators of invasion. Developing strategies to inhibit the expression of these proinvasive integrins has lagged

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behind their discovery; thus the feasibility of decreasing integrin expression and inhibiting pancreatic cancer cell invasion is relatively unknown.

Sodium butyrate (NaBT) is a short-chain fatty acid that is produced by naturally occurring bacteria in the human colon.[11] Our laboratory has previously shown that NaBT can induce differentiation and cell cycle arrest in colorectal cancer cells,[12] thus indicating its potential role as an effective antitumor agent. Other investigators have demonstrated that NaBT induces differentiation of pancreatic cancer cells[13] and may possess antitumor activity against chemoresistant pancreatic cancers in vivo.[14] NaBT may also alter the expression of integrins that are associated with a highly metastatic and poorly differentiated phenotype. Therefore the purpose of this study was to determine the following: (1) whether expression of specific integrins correlates with known invasiveness of different pancreatic cancer cells and (2) whether NaBT can inhibit invasion-associated integrin expression and pancreatic cancer cell invasion.

METHODS

Cell Lines and Reagents

Five different human pancreatic cancer cell lines were used in this study. AsPC-1 (American Type Culture Collection, Manassass, VA) and SUIT-2 (Dr. Takeshi Iwamura, Miyazaki Medical College, Miyazaki, Japan) cells were grown in RPMI 1640 with 10% to 20% fetal bovine serum (FBS) and penicillin/streptomycin. L3.6 cells (Dr. Isiah Fidler, M.D. Anderson Cancer Center, Houston, TX) were grown in Dulbecco's modified Eagle medium (DMEM) with 10% FBS and 200 mmol/L L-glutamine, 100 mmol/L sodium pyruvate, and nonessential amino acids. MIA PaCa-2 and PANC-1 (American Type Culture Collection) were grown in DMEM with 10% FBS, and penicillin/streptomycin. NaBT (Sigma, St. Louis, MO) was dissolved in water and used at the concentrations indicated.

Ribonuclease Protection Assay

Total RNA was extracted from MIA PaCa-2, PANC-1, L3.6, AsPC-1, and SUIT-2 cells or from NaBT-treated and control (untreated) AsPC-1 cells using Ultraspec (Biotecx Laboratories, Houston, TX). The hITG-2 multiprobe (BD Pharmingen, San Diego, CA) was radiolabeled using the Maxiscript T7 kit (Ambion, Austin, TX) and hybridized to 40 μg of total RNA overnight. Digestion of nonhybridized fragments was performed, and samples were resolved on a polyacrylamide denaturing gel. Results were obtained using autoradiography.

Western Blotting

Whole-cell protein was resolved on a 10% polyacrylamide gel and transferred to PVDF membranes as previously described.[15] Filters were incubated for 30 minutes at room temperature in blocking solution (Tris-buffered saline solution with 0.1% TWEEN 20 (TBST) containing 5% nonfat dried milk) followed by 1-hour incubation with primary antibodies to β4 integrin (a generous gift from Dr. Arthur Mercurio, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA). Filters were washed three times in TBST and incubated with peroxidase-conjugated goat antimouse antibody (Upstate Biotechnology, Lake Placid, NY) for 1 hour. After three final washes, immune complexes were visualized by the enhanced chemiluminescence (ECL) detection system. Membranes were stripped and reprobed with β-actin to ensure equal loading.

Immunofluorescence Staining

AsPC-1 cells were plated on coverslips, treated with NaBT (5 mmol/L) for 24, 48, and 72 hours and then fixed briefly in ice-cold methanol. Coverslips were blocked in 3% dry milk in phosphate-buffered saline solution and immunostained with monoclonal mouse β4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Staining was detected using Alexa-488 labeled antimouse antibody (Molecular Probes, Eugene, OR) and standard fluorescence microscopy.

Invasion Assays

AsPC-1 cells were pretreated for 8 hours with 5 mmol/L NaBT. Matrigel invasion chambers with 8 μmol/L pores were reconstituted with 1% FBS medium for 2 hours at 37° C prior to the addition of cells. After pretreatment, AsPC-1 cells (5×10^4) were trypsinized and resuspended in 1% FBS medium alone (control) or with fresh 5 mmol/L NaBT added just prior to placement into the invasion chambers. Laminin (Invitrogen, Carlsbad, CA), 20 μg/ml, was added to the upper membrane surface with the cells and recombinant human epidermal growth factor (EGF) (Invitrogen) 25 ng/ml was placed in the bottom of each well as a chemoattractant. After 48 hours, cells that had not invaded were removed from the upper surface using cotton swabs, and the invading cells were fixed with 100% methanol and stained with 0.2% crystal violet in 2% ethanol. Invasion was quantitated by visual counting, and the sum of invading cells in five individual high-powered fields for each membrane was obtained. Assays were performed in triplicate for each treatment group. Results

are expressed as the mean number of cells invading per treatment group.

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). The effect of NaBT on invasion was analyzed using the one-sided paired *t* test. A *P* value of less than 0.05 was considered significant.

RESULTS

Expression of β4 and β7 Integrin Chains Correlates With Invasiveness

Despite pancreatic cancer being a highly invasive malignancy, human pancreatic cancer cell lines differ in their metastatic potential because of unique patterns of gene expression and degrees of differentiation. For this study we selected the following five human pancreatic cancer cell lines: MIA PaCa-2 and PANC-1, which are nonmetastatic; and three aggressive metastatic cell lines, L3.6, AsPC-1, and SUIT-2. Other investigators have shown the aggressiveness of L3.6, AsPC-1, and SUIT-2 cell lines in vivo as demonstrated by the development of peritoneal metastases following splenic injection into nude mice.[16,17] To confirm the invasiveness of the more aggressive cell lines, we injected AsPC-1 and SUIT-2 cells subcutaneously into the flanks or into the spleens of athymic nude mice. Subcutaneous injection of both AsPC-1 and SUIT-2 cells produced local invasion into the surrounding tissues, whereas splenic injection resulted in multiple liver metastases within 4 weeks (data not shown).

To establish whether integrin expression is different between the more aggressive L3.6, AsPC-1, and SUIT-2 cells and the less aggressive MIA PaCa-2 and PANC-1 cells, a ribonuclease (RNase) protection assay was performed. Expression of most α and β chains was not consistently different between the two groups of cells; however, expression of both β4 and β7 was stronger in L3.6, AsPC-1, and SUIT-2 cells, compared to the less aggressive cells (Fig. 1). These results suggest that the expression of β4 and/or β7 may be important factors related to pancreatic cancer invasion.

NaBT Inhibits β4 Integrin Expression

NaBT has been described as a potent inducer of differentiation in both pancreatic[13] and colon cancer[18] cell lines. RNase protection analysis demonstrated expression of both β4 and β7 integrins in the metastatic cell lines, which suggests that these are markers of a less differentiated and more invasive phenotype. To determine whether NaBT inhibits the expression of the invasion-related integrin β4, AsPC-1 cells were treated with NaBT (5 mmol/L) for 24 hours and an RNase protection assay was performed. NaBT decreased the expression of both β4 and β7; L32 and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) expression was unaffected indicating equal loading of RNA (Fig. 2, *A*). To confirm that NaBT inhibited protein expression of β4, AsPC-1 cells were again treated with NaBT, and whole-cell lysates immunoblotted for β4 protein. NaBT (5 mmol/L) inhibited the expression of β4 protein (Fig. 2, *B*). Taken together, RNase protection and Western blot analysis indicate that NaBT is a potent inhibitor of β4 integrin expression, which may have profound effects on pancreatic cancer cell invasion.

Cell Surface Expression of β4 Is Decreased by NaBT

To be functional, integrins must be expressed on the surface of cells to bind appropriate ligands and

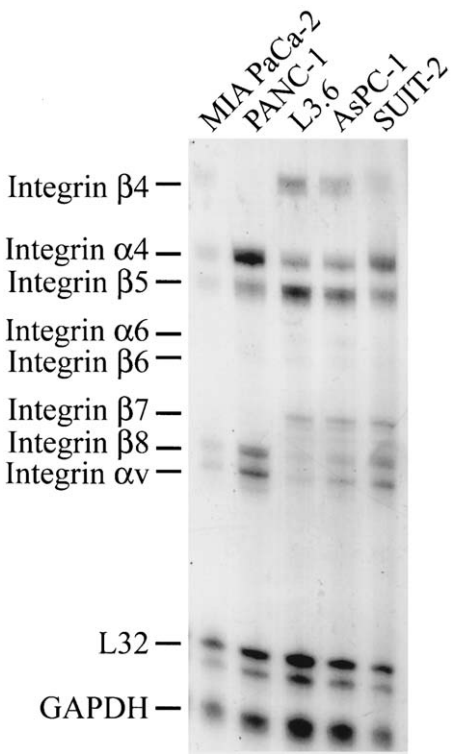
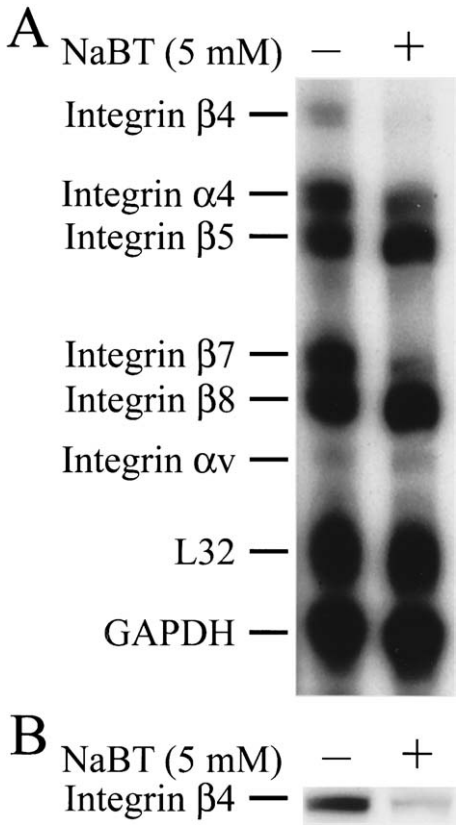


Fig. 1. Expression of β4 and β7 integrin chains correlates with invasiveness. Total RNA was extracted from nonmetastatic MIA PaCa-2 and PANC-1 cells or metastatic L3.6, AsPC-1, and SUIT-2 cells. RNase protection analysis was used to determine integrin chain expression with the hITG-2 multiprobe as described in Methods. L32 and GAPDH expression was measured to ensure equal loading of RNA.



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activate signaling pathways.[6] The turnover of integrins on the membrane of pancreatic cancer cells is unknown; thus we sought to confirm that decreases in β4 expression, observed by RNase protection and Western blotting, also resulted in reduced β4 integrin expression on the cell surface. In AsPC-1 cells, β4 immunofluorescent staining on the cell surface was greatly reduced by NaBT within 24 hours (Fig. 3). This reduction in membrane β4 was less pronounced after 48 hours, and β4 expression was nearly restored within 72 hours after NaBT treatment (data not shown). The decreased β4 expression at the level of the membrane suggests that NaBT may be a useful strategy to inhibit pancreatic cancer cell invasion.

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DISCUSSION

The prognosis for patients with pancreatic cancer is dismal; 5-year survival is less than 5% despite optimal surgical and chemotherapeutic treatments.[2] Identifying mechanisms of pancreatic cancer invasion may facilitate the development of more effective adjunctive treatments to improve outcomes following surgical resection. In this study we demonstrate that expression of the β4 integrin chain closely correlates with known metastatic potential of pancreatic cancer cells. Additionally, the naturally occurring fatty acid, NaBT, potently inhibits β4 integrin expression and pancreatic cancer cell invasion.

The importance of β4 integrin expression in facilitating invasion has been extensively analyzed. Shaw et al.[21] demonstrated that the α6β4 integrin binding to laminin can facilitate cell movement and activation of the phosphatidylinositol 3-kinase (PI3K) pathway whose downstream effectors facilitate invasion. One factor activated by PI3K is Akt, which is overexpressed in pancreatic cancer[22] and facilitates invasion of pancreatic cancer cells.[23] Interestingly, the ability of α6β4 to activate PI3K and stimulate invasion may not require binding to laminin; thus expression of β4, in the absence of its ligand laminin, may enhance carcinoma invasion. Therefore integrin α6β4 can facilitate invasion by providing mechanical support to migrating cells and by changing the intracellular milieu to favor expression of other proinvasive factors. The role of β4 in carcinoma invasion has also been shown using another differentiating agent, retinoic acid, to inhibit β4 expression and metastasis formation in lung cancer cell lines.[20] Our finding that NaBT significantly inhibits pancreatic cancer invasion and β4 expression is consistent with results of previous studies,[19–21] which describe the importance

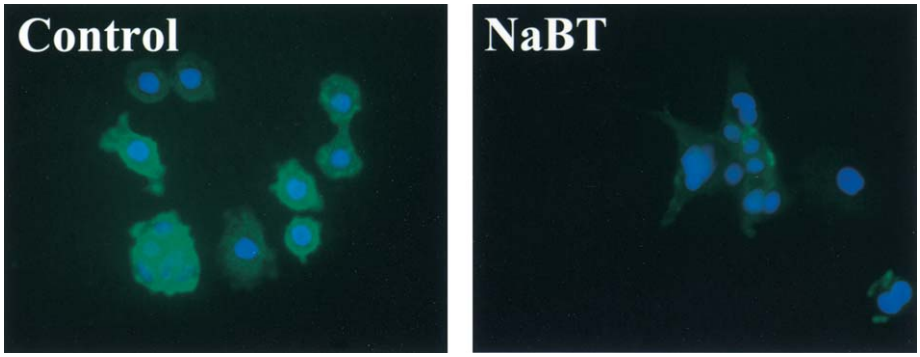

Fig. 3. Cell surface expression of β4 is decreased by NaBT. AsPC-1 cells were grown on coverslips and treated with 5 mmol/L NaBT or medium alone. After 24 hours, cells were fixed with methanol, blocked in phosphate-buffered saline solution with 3% milk, and incubated with β4 antibody for 45 minutes. β4 staining was detected using Alexa 488–labeled goat antimouse antibody with a standard fluorescence microscope and appropriate filters.

of β4 and underscore the significance of this effect in aggressive pancreatic cancer cell lines.

Other integrins may also be important for pancreatic cancer cell invasion. The expression of another laminin-binding integrin, β3, correlates with lymph node metastasis and high matrix metalloproteinase-2 (MMP-2) expression. Investigators have also shown a relationship between expression of β1 and invasiveness by demonstrating a reduction in the metastatic rate in vivo using β1-blocking antibodies[8] and enhanced invasiveness when β1 expression is increased by the cytokine interleukin-1.[10] The relative importance of these integrins, compared to α6β4, is unclear. In most of these studies, β1 expression was examined only when dimerized with the α6 integrin chain; however, the β1 chain can dimerize with 11 other α chains, each of which binds a unique set of ligands. Therefore blocking β1 expression alone may affect the function of many other integrins, which suggests that the observed effects may not be specific for β1. Second, blocking antibodies intended to "block" β1 function may prevent mechanical interactions between β1 and extracellular matrix proteins but may also simultaneously activate signaling pathways as if the integrin were binding to its ligand. Last, α6β4 activates PI3K more efficiently than α6β1 or other β1 integrins, and this α6β4 activation of PI3K is required for the formation of lamellae that facilitate carcinoma cell motility.[21] The abundance of evidence supports a critical role for α6β4 in carcinoma invasion. Furthermore, the effectiveness of NaBT in reducing β4 expression and invasion in pancreatic cancer cells suggests α6β4 is also a key facilitator of pancreatic cancer invasion.

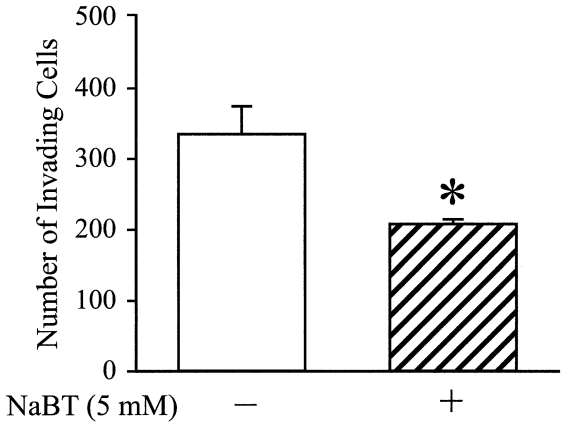

Fig. 4. NaBT inhibits pancreatic cancer invasion. AsPC-1 cells were pretreated with 5 mmol/L NaBT for 8 hours, then trypsinized and resuspended in 1% FBS medium with or without 5 mmol/L NaBT. Cells (5 × 104) were then placed in the upper well of Matrigel invasion chambers with laminin (20 μg/ml), and EGF-1 (50 ng/ml) was used as a chemoattractant in the lower well. Invasion was assessed by cell counting after 48 hours as described in Methods. (Data are expressed as mean ± SEM; $^*P < 0.05$ vs. control values.)

NaBT, a short-chain fatty acid produced by the fermentation of dietary fiber in the colon,[24] exerts its nonmetabolic effects by inhibiting the enzyme histone deacetylase,[25] which subsequently alters the expression of numerous target genes. NaBT is a potent inducer of apoptosis and cell cycle arrest in other gastrointestinal tract cancer cells, most notably colon cancer[26]; however, these effects have not been reported for pancreatic cancer. More generally, NaBT is a differentiation agent, with effectiveness in colon[18] and prostate[27] cancer cell lines. NaBT can induce differentiation in pancreatic cancer cells, as demonstrated by an increase in the epithelial

marker keratin 23[13] or the mucin marker M1.[28] Here we extend the scope of NaBT-induced differentiation to demonstrate that NaBT reduces expression of the invasion promoting integrin β4 and, moreover, inhibits pancreatic cancer cell invasion. We have shown previously that NaBT can inhibit the invasion of hepatocellular carcinoma cells,[29] whereas other investigators have demonstrated similar anti-invasive effects in both breast[30] and colon cancer[31] cells. We demonstrate, for the first time, that NaBT can also inhibit invasion of pancreatic cancer cells. NaBT also reduced the expression of the β7 integrin chain, which was expressed only in the more aggressive pancreatic cancer cell lines. The β7 integrin chain was known previously to be expressed only in lymphocytes where it is believed to facilitate lymphocyte homing in the gastrointestinal tract.[32] Because β7 integrin expression and pancreatic cancer cell invasion were both inhibited by NaBT, it is intriguing to hypothesize whether β7 integrin may facilitate the spread of metastatic pancreatic cancer cells in the gastrointestinal tract. Detailed studies to fully understand the role of the β7 integrin chain in pancreatic cancer cell invasion are required.

CONCLUSION

In this study we demonstrate that expression of the β4 and β7 integrin chains is closely linked to the metastatic potential of pancreatic cancer cells. Additionally, using the fatty acid NaBT, we have shown a reduction of β4 expression and inhibition of pancreatic cancer cell invasion. The efficacy of NaBT suggests that this may be an effective strategy to target β4 integrin expression in patients with pancreatic cancer and improve the prognosis of this lethal disease. Based on these findings, we believe further investigation into the role of NaBT as an inhibitor of invasion and an adjuvant therapy against pancreatic cancer is warranted.

We thank Drs. Takeshi Iwamura for the SUIT-2 cells, Isiah Fidler for the L3.6 cells, and Arthur Mercurio for the β4 antibody. We also thank Tatsuo Uchida for performing the statistical analysis and Eileen Figueroa and Karen Martin for manuscript preparation.

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Objective Psychomotor Skills Assessment of Experienced and Novice Flexible Endoscopists With a Virtual Reality Simulator

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The objective of this study was to determine whether the GI Mentor II virtual reality simulator can distinguish the psychomotor skills of intermediately experienced endoscopists from those of novices, and do so with a high level of consistency and reliability. A total of five intermediate and nine novice endoscopists were evaluated using the EndoBubble abstract psychomotor task. Each subject performed three repetitions of the task. Performance and error data were recorded for each trial. The intermediate group performed better than the novice group in each trial. The differences were significant in trial 1 for balloons popped ($P = .001$), completion time ($P = .04$), and errors ($P = .03$). Trial 2 showed significance only for balloons popped ($P = .002$). Trial 3 showed significance for balloons popped ($P = .004$) and errors ($P = .008$). The novice group showed significant improvement between trials 1 and 3 ($P < 0.05$). No improvement was noted in the intermediate group. Measures of consistency and reliability were greater than 0.8 in both groups with the exception of novice completion time where test-retest reliability was 0.74. The GI Mentor II simulator can distinguish between novice and intermediate endoscopists. The simulator assesses skills with levels of consistency and reliability required for high-stakes assessment.
(J Gastrointest Surg 2003;7:871–878)

Key words: Endoscopy, computer simulation, educational measurement, validation studies

Skill in flexible endoscopy is essential for the evaluation and treatment of numerous gastrointestinal conditions. In fact, flexible endoscopic procedures ranked second only to cholecystectomy as the most frequently performed category of procedure reported to the American Board of Surgery by surgeons applying for recertification in the year 2000.[1] The frequency of flexible endoscopy in surgical practice roughly approximates the frequency of hernia repair, comprising 13% of all reported procedures. Currently the American Board of Surgery requires that all general surgical residents be exposed to a wide variety of endoscopic procedures and techniques,[2] but no objective standard for surgical endoscopic training has been set. Residents from many programs complete their training with little or no endoscopic experience. In fact, statistics for residents completing training in 2002 compiled by the Residency Review Committee for Surgery for more than 1000 residents from 254 different programs showed that the mean number of colonoscopies, esophagogastroduodenoscopies (EGDs), and flexible sigmoidoscopies completed by graduating residents was 35, 23, and 9, respectively.[3] Because most published recommendations regarding minimum procedure numbers for competency suggest a minimum of 100 to 400 colonoscopies and 100 to 300 EGDs,[4–9] it is clear that most surgical residents do not come out of training with competency in flexible gastrointestinal endoscopy. This mismatch between exposure during surgical training and the requirements of surgical practice emphasizes the need to increase the role of flexible gastrointestinal endoscopic training in general surgical residency programs.

The need for surgeons to improve their training in flexible endoscopy is further highlighted by technologic advances now visible on the surgical horizon.

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Advances in robotics technology have produced prototypes of flexible endoscopic robots that will allow more advanced procedures to be performed via the endoscopic approach. If surgeons do not take steps to improve flexible endoscopy training now, we risk losing patients in the future.

This need for increased endoscopy training comes at a difficult time. Increased public awareness of medical error secondary to high-profile events such as the "Libby Zion" case[10] in New York and the release of the "To Err Is Human" report[11] have led to changes in the traditional surgical training paradigm, such as the 80-hour work week limit and 24-hour continuous workday limitations for residents. These new limitations have placed training time at a premium, and many programs are struggling to simply maintain their current levels of training while complying with these new limitations. Increasing exposure to flexible endoscopy via traditional Halsteadian "see one, do one, teach one" methodology will not be successful in this new climate.

One possible solution to the conundrum of how to accomplish more training in less time with improved safety is the use of simulation. Although bench-top simulation of gastrointestinal endoscopy has existed for years,[12] it has traditionally lacked both realism and the ability to objectively assess performance. Use of animals for training adds some realism but is expensive, remains difficult to objectively assess, and raises ethical issues for many persons. Recently several virtual reality simulators for flexible endoscopy have come on the market.[12,13] These computer-based simulators provide a safe, realistic, inanimate environment where skills can potentially be both assessed and learned. However, before a simulator is used for assessment or training, it must be validated to show that it first assesses and then teaches the correct set of psychomotor skills. Recently the Minimally Invasive Surgery Trainer–Virtual Reality (MIST-VR) was validated both for its ability to objectively assess performance of novice, junior, and experienced minimally invasive surgeons[14,15] and to improve performance in the operating room when used as a training tool for surgical residents.[16]

Based on the work done on the MIST-VR, the purpose of the study reported herein was to evaluate the construct validity of a virtual reality simulator for flexible gastrointestinal endoscopy—the GI Mentor II (Simbionix USA Corp., Cleveland, OH). Although others have reported the ability of the GI Mentor virtual reality simulator to discriminate between novices with no endoscopic experience and experts who have performed more than 1000 colonoscopies,[17] we hypothesized that similar differences would be seen between novices and a group of intermediately experienced endoscopists still on their learning curves. If proved true, this hypothesis would demonstrate that both assessment and training using the simulator in its current configuration should be focused on those subjects early in their endoscopic experience.

MATERIAL AND METHODS

Subjects

Five intermediately experienced endoscopists (3 men and 2 women) with a total number of completed flexible gastrointestinal endoscopies ranging from 100 to 250 (esophagogastroduodenoscopies, flexible sigmoidoscopies, and colonoscopies) and six novice endoscopists (2 men and 4 women) who had completed less than 10 total flexible gastrointestinal endoscopies participated in the study. The demographics of these groups are summarized in Table 1. The intermediately experienced group was composed of fellows and junior attending physicians in minimally invasive surgery. The novice group was composed of junior surgical residents, medical students, veterinary medicine residents, and veterinary technicians who had performed no more than 10 flexible gastrointestinal endoscopies. All subjects were right hand dominant.

Apparatus

All subjects were evaluated using the GI Mentor II virtual reality endoscopic simulator (Fig. 1). This simulator features an actual Pentax endoscope and improved force-feedback system allowing for realistic simulation of flexible endoscopy. The software package allows users to perform EGD, colonoscopy, endoscopic retrograde cholangiopancreatography, or CyberScopy. CyberScopy consists of two abstract psychomotor skills tasks: the EndoBubble and EndoBasket. The EndoBubble task (Fig. 2) requires users

Table 1. Demographic and flexible endoscopic experience data for the two groups

Mean	Experienced (std)	Novice (std)	*P* value
Age (yr)	33 (2)	30 (6)	NS
Glove size	7 (1)	7.5 (1)	NS
Colonoscopies	48 (33)	1 (2)	0.006
EGD	81 (47)	1 (2)	0.001
Flexible sigmoidoscopies	9 (13)	0 (0)	N/A
Total GI endoscopies	139 (47)	2 (4)	0.003

NS = not significant; N/A = not available.

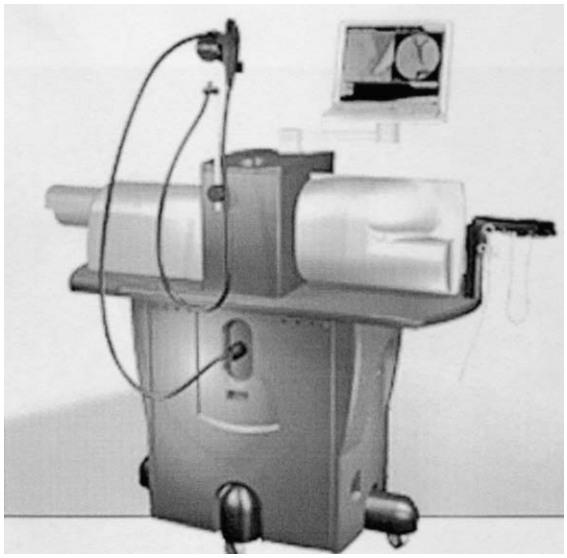

Fig. 1. Simbionix GI Mentor II virtual reality flexible gastrointestinal endoscopy simulator.

to navigate a virtual pipe and burst balloons using a virtual injection needle. On level 1 the balloons are fixed and permanent. On level 2 the balloons move and will disappear if not popped in a finite period of time. The EndoBasket task requires users to navigate the same virtual pipe as in the EndoBubble task, but now they must pick up virtual spheres using a grasper and place them into a basket located further down the pipe. Because it required no previous cognitive knowledge of the stomach or colon, and had the most robust scoring matrix, the EndoBubble task was used for this study.

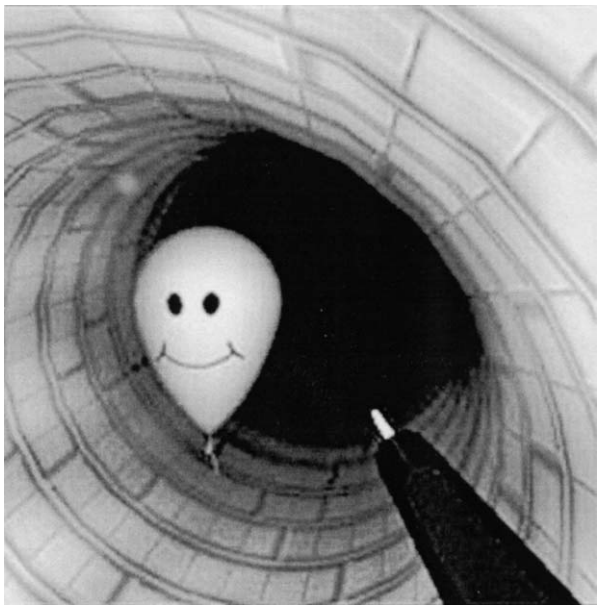

Fig. 2. The EndoBubble task requires the subject to navigate a virtual pipe and burst balloons using an injection needle.

Procedure

Subjects from both groups were oriented to the simulator by a brief demonstration of the function of the endoscope, and were then allowed to perform a single practice trial of the EndoBubble task on level 1. All questions as to function of the endoscope or the simulator were answered, but no instruction was given concerning ways to optimize performance. Each subject then completed three consecutive trials of the EndoBubble task on level 2. Recorded data included the number of balloons popped (maximum 40), time to completion, and number of times the injection needle struck the wall of the virtual pipe. These data were provided by the scoring matrix incorporated into the GI Mentor II software, and were displayed and recorded at the conclusion of each trial.

RESULTS

Differences in the means for each group and each trial were examined for statistical significance using a Mann-Whitney U test; the results are summarized in Table 2. The mean number of balloons popped per trial for each group is shown in Fig. 3. The intermediate group popped significantly more balloons than the novice group for each of the three trials (trial 1, $P = 0.001$; trial 2, $P = 0.002$; trial 3, $P = 0.004$). The mean time to completion and number of wall strikes for each group are presented in Figs. 4 and 5, respectively. The intermediate group performed consistently faster than the novice group for all three trials, and this difference was statistically significant for trial 1 ($P = 0.042$). The time differences in trials 2 and 3 approached statistical significance ($P = 0.072$, and $P = 0.083$, respectively). Similarly, the intermediate group had fewer wall strikes than the novice group for all three trials, with these differences reaching statistical significance in trials 1 and 3 ($P = 0.032$, and $P = 0.008$, respectively). Statistical significance was not reached in trial 2 because of the large performance variability in the novice group. This variability in wall strike error rate is an added notable aspect of the differences observed between the intermediate and novice groups. The mean variability in wall strike errors for the two groups over all three trials is shown in Fig. 6. The novice group variability score was more than five times greater than the score for the intermediate group, demonstrating that not only did the novice group make significantly more errors than the intermediate group as shown earlier, but their performance with respect to errors was highly variable

Table 2. Summary of mean balloons popped, completion time, and wall strike errors per group for each of the three trials

	Balloons popped (mean [std])			Completion time (sec) (mean [std])			No. of wall strikes (mean [std])		
Trial	Exp	(*P* value)	Novice	Exp	(*P* value)	Novice	Exp	(*P* value)	Novice
1	22 (5)	0.001	7.5 (4)	104 (14)	0.042	131 (25)	0.2 (0.4)	0.032	4 (3)
	NS		NS	NS		NS	NS		NS
2	25 (10)	0.002	8 (3)	103 (13)	0.072	117 (11)	0.4 (0.9)	0.11	3 (4)
	NS		NS	NS		NS	NS		NS
3	23 (7)	0.004	11 (4)	99 (7)	0.083	110 (11)	0.4 (0.5)	0.008	3 (2)
Trials 1 to 3	NS		$P < 0.05$	NS		$P < 0.01$	NS		NS

Exp = experienced; NS = not significant.

and inconsistent. In contrast, the intermediate group consistently minimized errors during task performance showing that they not only had enough psychomotor skill to quickly pop the balloons, but they also had enough control to prevent errors from occurring.

To test for a learning effect, differences within groups were evaluated using one-way analysis of variance (ANOVA) for repeated measures with a Friedman test for nonparametric data. Post hoc analysis was performed using Dunn's multiple-comparison test. The intermediately experienced group showed no significant difference in performance between trials 1 and 3 for number of balloons popped, completion time, or wall strikes. The novice group showed statistically significant improvement between trials 1 and 3 for number of balloons popped and completion time ($P < 0.05$, and $P < 0.01$, respectively). The decrease in wall strikes for the novice group was not statistically significant.

The internal consistency of the GI Mentor II virtual reality simulator to reliably test repeated performance was assessed for balloons popped, completion time, and wall strikes for all 14 subjects with standardized coefficient alpha. Test-retest reliability for trials 1 and 2 was established with Pearson's product moment correlation coefficient. Table 3 summarizes the results of these analyses. Scientific standards for acceptable internal consistency and test-retest reliability are levels greater than 0.8, and all measures except time reached this level.

DISCUSSION

The study reported here was designed to determine whether the Simbionix GI Mentor II virtual

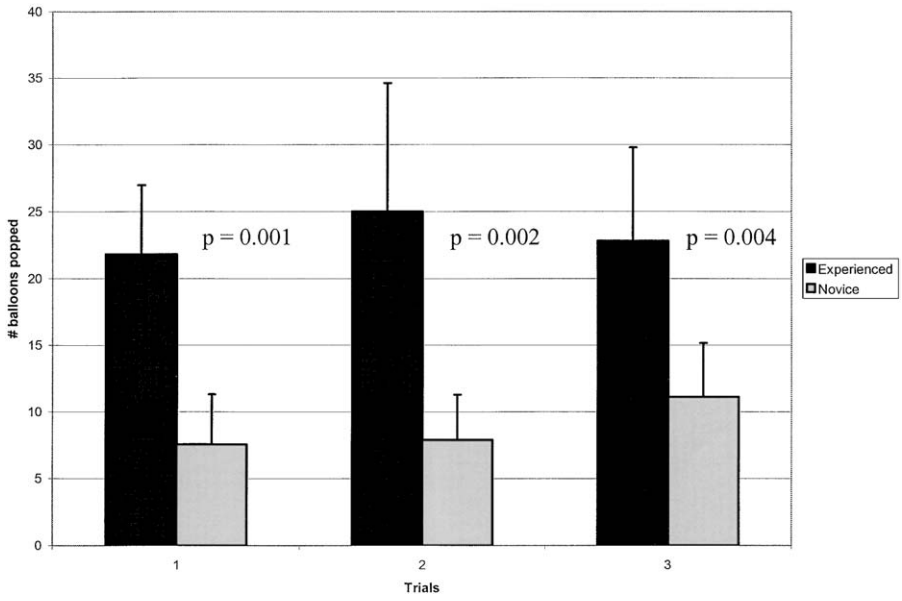

Fig. 3. Mean number of balloons popped per trial for the experienced and novice groups.

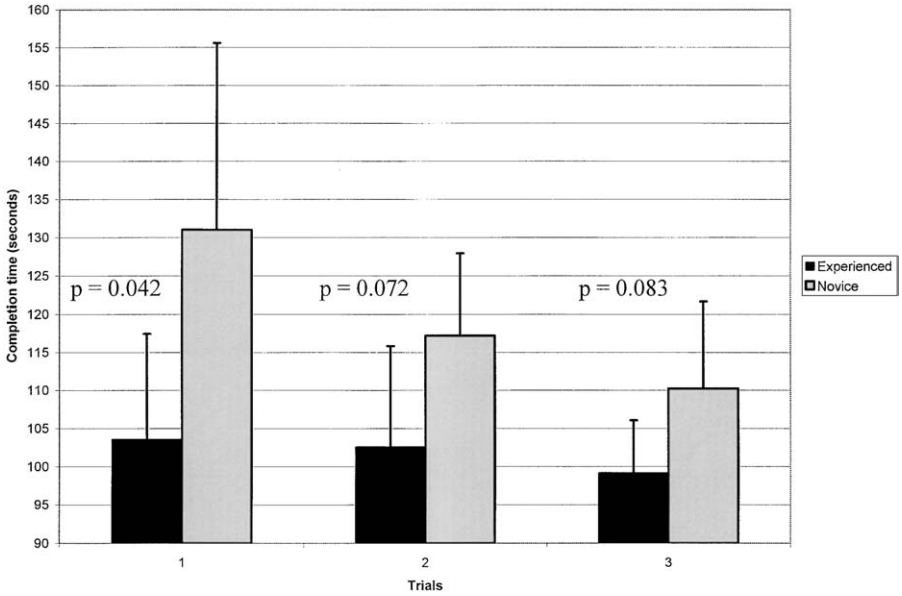

Fig. 4. Mean completion time per trial for the experienced and novice groups.

endoscopic simulator could differentiate between a group of intermediately experienced endoscopists and a group of novice endoscopists in performance of a basic endoscopic psychomotor task. Much like novice minimally invasive surgeons learning to adapt to the fulcrum effect,[18] neophyte flexible endoscopists must adapt the psychomotor pathways in their brain to allow them to negotiate the gastrointestinal tract. During flexible endoscopy the cognitive and psychomotor demands of the procedure combined with the stress of performance on an actual patient in a time-sensitive environment often leads to failure and frustration for both mentor and protégé. This combination of factors likely contributes to the difficulty in becoming competent in flexible endoscopy and results in the large minimum number of procedures recommended by many investigators and endoscopic societies to be considered competent.[4–8]

The use of a virtual reality simulator allows reduction in the number of variables in the training equation. By removing the patient and the endoscopy suite time from the training equation, stress is considerably reduced, allowing the trainee to focus on developing the necessary psychomotor skills. By using a simple,

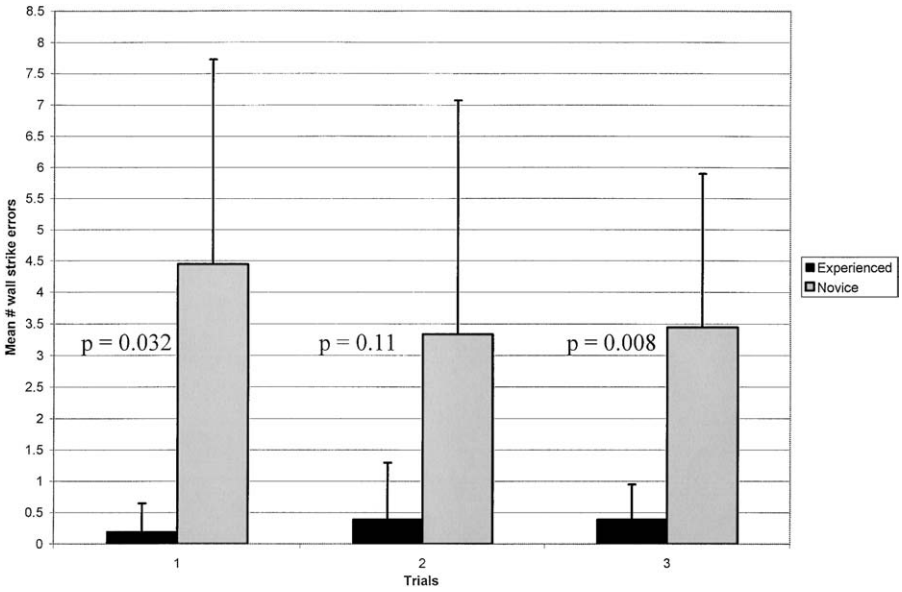

Fig. 5. Mean number of wall strike errors per trial for the experienced and novice groups.

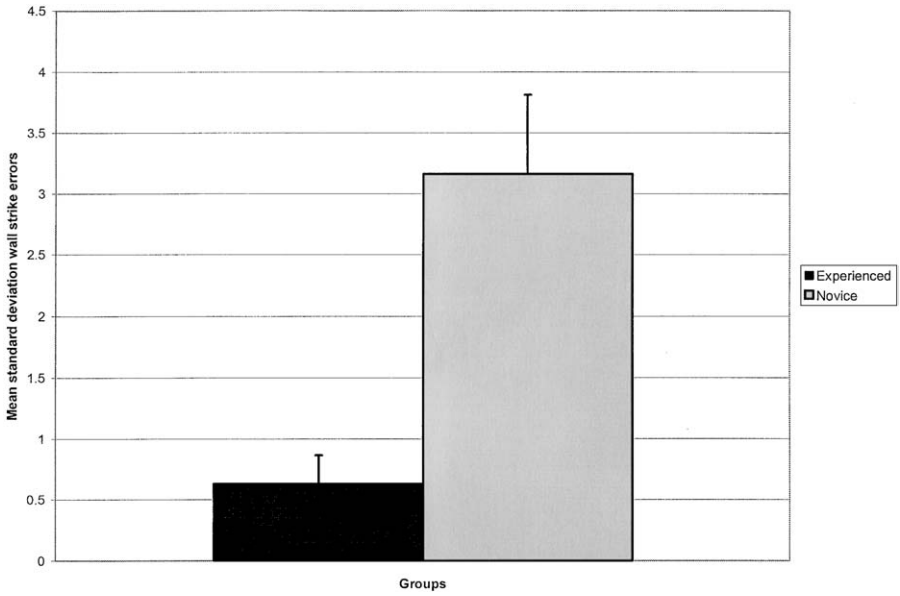

Fig. 6. Mean variability of wall strike errors made by the two groups over all three trials.

abstract task, the cognitive knowledge of the anatomy and physiology of the gastrointestinal tract are also eliminated. All that remains to be assessed is psychomotor performance.

The results of the assessment of the psychomotor performance of groups of intermediately experienced and novice endoscopists show that the intermediately experienced group performed consistently better than the novice group in a shorter amount of time and with consistently fewer errors. The scores of our intermediate group for number of balloons popped compares favorably with the previously published results of experts with more than 1000 colonoscopies each,[17] and although it is impossible to statistically analyze, no significant difference would be expected. The difference in performance variability seen between the novice and experienced groups also makes an important point. The importance of performance consistency is highlighted by examining the game of golf. Most golfers at some point in their career have hit the perfect tee shot—long and right down the middle. However, those who can hit that shot consistently find themselves on the PGA tour. The results of this study add to the growing support for use of variability as an important measure of performance. In either golf or endoscopy, consistently good performance is probably the best indicator of true skills mastery. These differences in performance and performance variability validate the construct that the GI Mentor II simulator can objectively assess the difference in psychomotor skills between novice endoscopists and those with intermediate endoscopic experience. The role of the GI Mentor II simulator as a tool to assess endoscopists still on their learning curves is further supported by the high internal consistency and test-retest reliability coefficients reported. These measures show how well an assessment tool will identify a given level of skill during repeated testing. Because only the correlation coefficient for time fell below the scientifically acceptable level of 0.8, the GI Mentor II simulator can be considered a consistent assessment tool.

Table 3. Internal consistency and test-retest reliability for 14 subjects for three performance measures

	Standardized coefficient alpha	Test-retest reliability (*r*)
Balloons popped	0.97	0.93
Completion time	0.82	0.74
Wall strike errors	0.89	0.86

Analysis within the groups also yielded encouraging results. The GI Mentor II simulator demonstrated consistent performance across all three trials for the intermediately experienced group as evidenced by no significant improvement in performance with respect to balloons popped, time, or wall strike errors between trials 1 and 3. This implies that from a psychomotor standpoint, those in the IE group have reached the plateau of their learning curve. In contrast, the novice group showed significant improvement in balloons popped and completion time between trials 1 and 3. This improvement suggests that the GI Mentor II simulator would be useful as a training tool, but its role in training remains to be demonstrated.

The ability to assess psychomotor performance is the most essential construct of any procedural simulator. Many simulators, including the GI Mentor II, incorporate cognitive assessment components into their simulated tasks. Although it is essential for the trainee to master and be assessed on the knowledge required to safely perform a task, this knowledge can be gained and assessed through a variety of means that do not require virtual reality simulation. Validated assessment tools such as the American Board of Surgery In-Training Examination and the qualifying and certifying examinations are well-established measures of cognitive knowledge. It is the ability to simulate the psychomotor demands of a procedure that will build the foundation for use of simulation in training and certifying physicians in the future. Once the psychomotor construct is well established, addition of cognitive and judgment components will quickly follow.

CONCLUSION

We have shown that the GI Mentor II simulator is capable of objectively assessing differences in performance between novice and intermediately experienced endoscopists. We have also demonstrated that this assessment can be made with levels of internal consistency and test-retest reliability required for high-stakes assessment such as licensure and certification. Based on these data, use of the GI Mentor II simulator in its current configuration for psychomotor assessment or training should focus on those still on the learning curve. The data supporting the use of the GI Mentor II as a training tool are encouraging, but its role in training remains to be empirically demonstrated.

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Discussion

Dr. H. Reber (Los Angeles, CA): You obviously focused on the ability of this technology to assess skill, and at the end of your presentation you touched on its role as a teaching tool. Maybe you could go a little bit further with that and tell us, just to focus on the colonoscopy issue, what has been done along those lines? Teaching colonoscopy is a painful experience for the patient as well as for the person who is learning to do it.

Dr. E. Ritter: Within the realm of using virtual reality and simulation in general for training skills, a great deal of work is being done, and it is still being sorted out as how best to apply simulation to training. The most common current application is to have trainees perform a certain number of procedures or spend a certain amount of time on the simulator.

One option that was reported at last year's meeting, and that we are currently exploring, is using objectively defined goals for simulator training. These goals are established by the performance of experts in flexible endoscopy, and are used to set a minimum standard for training on the simulator before trainees move on to in vivo training. This helps adjust for

differences in baseline abilities between subjects to ensure that each trainee reaches at least the same baseline skill level.

Dr. K. Lillemoe (Baltimore, MD): This was a very nice presentation. For those of us who do not have those simulators, could you tell us a little bit more about how you have incorporated them into your residency training program and specifically how much they cost? Have you seen any benefit when trainees have started out on the simulator and then gone into the clinical situation?

Dr. Ritter: I will address the cost issue first because I truly do not know how much this simulator costs, to be perfectly honest.

The issue of using the simulator in training is in the process of being evaluated. We only recently obtained this machine, and we have employed it for training in a class that we offer for medical students. Anecdotally, seeing their performance on the simulator improve over time, as well as improvement in performance in the animal laboratory, I would say that it does make a difference. We are planning to conduct a prospective randomized trial for training in upper endoscopy this next academic year to evaluate the training benefit, but we do not yet have any data on that.

Dr. J. Bender (Oklahoma City, OK): As any of us with small kids know, any time they get a new video game, their performance improves markedly the second and third time they try it. Yes, they can pop more balloons, but do you have any data at all that this correlates with better performance of endoscopy?

Dr. Ritter: Right now, no.

Dr. M. Stelzner (Seattle, WA): I would like to know if you have made efforts to identify certain factors that would predict less skillful performance. For example, are there certain motions that if they were corrected would enable a novice to accelerate his or her learning?

We know that in laparoscopy we can successfully identify, let us say, incorrect hand motions or an inappropriate speed of action and factors like that. Would your simulator be able to give the mentor information as to what factors would need to be corrected for a novice to learn endoscopy more efficiently?

Dr. Ritter: The simulator does record quite a number of metrics, including several of the things you mentioned. When using upper and lower endoscopy case simulations, the simulator does measure things such as overinsufflation, excess local pressure, and forming loops, so that the mentor can review and identify specific repeated errors in a specific student. Identification of these errors should increase the rate of learning, but right now we have no good objective data to prove that.

Objective Psychomotor Skills Assessment of Experienced and Novice Flexible Endoscopists With a Virtual Reality Simulator

E. Matt Ritter, M.D., David A. McClusky III, M.D., Andrew B. Lederman, M.D., Anthony G. Gallagher, Ph.D., C. Daniel Smith, M.D.

The objective of this study was to determine whether the GI Mentor II virtual reality simulator can distinguish the psychomotor skills of intermediately experienced endoscopists from those of novices, and do so with a high level of consistency and reliability. A total of five intermediate and nine novice endoscopists were evaluated using the EndoBubble abstract psychomotor task. Each subject performed three repetitions of the task. Performance and error data were recorded for each trial. The intermediate group performed better than the novice group in each trial. The differences were significant in trial 1 for balloons popped ($P = .001$), completion time ($P = .04$), and errors ($P = .03$). Trial 2 showed significance only for balloons popped ($P = .002$). Trial 3 showed significance for balloons popped ($P = .004$) and errors ($P = .008$). The novice group showed significant improvement between trials 1 and 3 ($P < 0.05$). No improvement was noted in the intermediate group. Measures of consistency and reliability were greater than 0.8 in both groups with the exception of novice completion time where test-retest reliability was 0.74. The GI Mentor II simulator can distinguish between novice and intermediate endoscopists. The simulator assesses skills with levels of consistency and reliability required for high-stakes assessment.
(J Gastrointest Surg 2003;7:871–878)

Key words: Endoscopy, computer simulation, educational measurement, validation studies

Skill in flexible endoscopy is essential for the evaluation and treatment of numerous gastrointestinal conditions. In fact, flexible endoscopic procedures ranked second only to cholecystectomy as the most frequently performed category of procedure reported to the American Board of Surgery by surgeons applying for recertification in the year 2000.[1] The frequency of flexible endoscopy in surgical practice roughly approximates the frequency of hernia repair, comprising 13% of all reported procedures. Currently the American Board of Surgery requires that all general surgical residents be exposed to a wide variety of endoscopic procedures and techniques,[2] but no objective standard for surgical endoscopic training has been set. Residents from many programs complete their training with little or no endoscopic experience. In fact, statistics for residents completing training in 2002 compiled by the Residency Review Committee for Surgery for more than 1000 residents from 254 different programs showed that the mean number of colonoscopies, esophagogastroduodenoscopies (EGDs), and flexible sigmoidoscopies completed by graduating residents was 35, 23, and 9, respectively.[3] Because most published recommendations regarding minimum procedure numbers for competency suggest a minimum of 100 to 400 colonoscopies and 100 to 300 EGDs,[4–9] it is clear that most surgical residents do not come out of training with competency in flexible gastrointestinal endoscopy. This mismatch between exposure during surgical training and the requirements of surgical practice emphasizes the need to increase the role of flexible gastrointestinal endoscopic training in general surgical residency programs.

The need for surgeons to improve their training in flexible endoscopy is further highlighted by technologic advances now visible on the surgical horizon.

Presented at the Forty-Fourth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Florida, May 18–22, 2003 (oral presentation).
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Advances in robotics technology have produced prototypes of flexible endoscopic robots that will allow more advanced procedures to be performed via the endoscopic approach. If surgeons do not take steps to improve flexible endoscopy training now, we risk losing patients in the future.

This need for increased endoscopy training comes at a difficult time. Increased public awareness of medical error secondary to high-profile events such as the "Libby Zion" case[10] in New York and the release of the "To Err Is Human" report[11] have led to changes in the traditional surgical training paradigm, such as the 80-hour work week limit and 24-hour continuous workday limitations for residents. These new limitations have placed training time at a premium, and many programs are struggling to simply maintain their current levels of training while complying with these new limitations. Increasing exposure to flexible endoscopy via traditional Halsteadian "see one, do one, teach one" methodology will not be successful in this new climate.

One possible solution to the conundrum of how to accomplish more training in less time with improved safety is the use of simulation. Although bench-top simulation of gastrointestinal endoscopy has existed for years,[12] it has traditionally lacked both realism and the ability to objectively assess performance. Use of animals for training adds some realism but is expensive, remains difficult to objectively assess, and raises ethical issues for many persons. Recently several virtual reality simulators for flexible endoscopy have come on the market.[12,13] These computer-based simulators provide a safe, realistic, inanimate environment where skills can potentially be both assessed and learned. However, before a simulator is used for assessment or training, it must be validated to show that it first assesses and then teaches the correct set of psychomotor skills. Recently the Minimally Invasive Surgery Trainer–Virtual Reality (MIST-VR) was validated both for its ability to objectively assess performance of novice, junior, and experienced minimally invasive surgeons[14,15] and to improve performance in the operating room when used as a training tool for surgical residents.[16]

Based on the work done on the MIST-VR, the purpose of the study reported herein was to evaluate the construct validity of a virtual reality simulator for flexible gastrointestinal endoscopy—the GI Mentor II (Simbionix USA Corp., Cleveland, OH). Although others have reported the ability of the GI Mentor virtual reality simulator to discriminate between novices with no endoscopic experience and experts who have performed more than 1000 colonoscopies,[17] we hypothesized that similar differences would be seen between novices and a group of intermediately experienced endoscopists still on their learning curves. If proved true, this hypothesis would demonstrate that both assessment and training using the simulator in its current configuration should be focused on those subjects early in their endoscopic experience.

MATERIAL AND METHODS

Subjects

Five intermediately experienced endoscopists (3 men and 2 women) with a total number of completed flexible gastrointestinal endoscopies ranging from 100 to 250 (esophagogastroduodenoscopies, flexible sigmoidoscopies, and colonoscopies) and six novice endoscopists (2 men and 4 women) who had completed less than 10 total flexible gastrointestinal endoscopies participated in the study. The demographics of these groups are summarized in Table 1. The intermediately experienced group was composed of fellows and junior attending physicians in minimally invasive surgery. The novice group was composed of junior surgical residents, medical students, veterinary medicine residents, and veterinary technicians who had performed no more than 10 flexible gastrointestinal endoscopies. All subjects were right hand dominant.

Apparatus

All subjects were evaluated using the GI Mentor II virtual reality endoscopic simulator (Fig. 1). This simulator features an actual Pentax endoscope and improved force-feedback system allowing for realistic simulation of flexible endoscopy. The software package allows users to perform EGD, colonoscopy, endoscopic retrograde cholangiopancreatography, or CyberScopy. CyberScopy consists of two abstract psychomotor skills tasks: the EndoBubble and EndoBasket. The EndoBubble task (Fig. 2) requires users

Table 1. Demographic and flexible endoscopic experience data for the two groups

Mean	Experienced (std)	Novice (std)	*P* value
Age (yr)	33 (2)	30 (6)	NS
Glove size	7 (1)	7.5 (1)	NS
Colonoscopies	48 (33)	1 (2)	0.006
EGD	81 (47)	1 (2)	0.001
Flexible sigmoidoscopies	9 (13)	0 (0)	N/A
Total GI endoscopies	139 (47)	2 (4)	0.003

NS = not significant; N/A = not available.

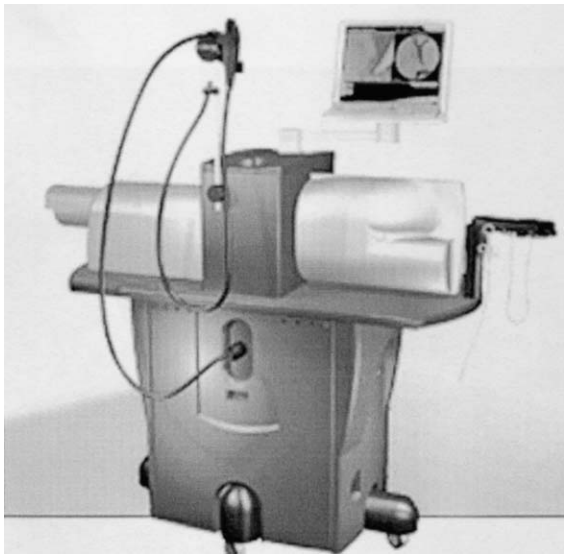

Fig. 1. Simbionix GI Mentor II virtual reality flexible gastrointestinal endoscopy simulator.

to navigate a virtual pipe and burst balloons using a virtual injection needle. On level 1 the balloons are fixed and permanent. On level 2 the balloons move and will disappear if not popped in a finite period of time. The EndoBasket task requires users to navigate the same virtual pipe as in the EndoBubble task, but now they must pick up virtual spheres using a grasper and place them into a basket located further down the pipe. Because it required no previous cognitive knowledge of the stomach or colon, and had the most robust scoring matrix, the EndoBubble task was used for this study.

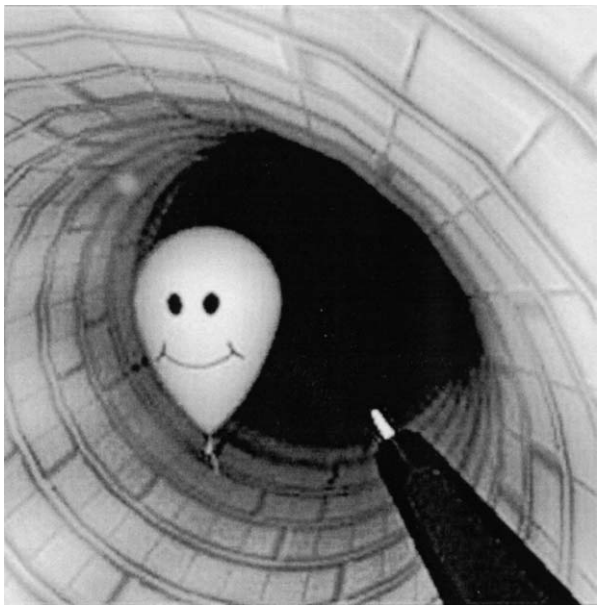

Fig. 2. The EndoBubble task requires the subject to navigate a virtual pipe and burst balloons using an injection needle.

Procedure

Subjects from both groups were oriented to the simulator by a brief demonstration of the function of the endoscope, and were then allowed to perform a single practice trial of the EndoBubble task on level 1. All questions as to function of the endoscope or the simulator were answered, but no instruction was given concerning ways to optimize performance. Each subject then completed three consecutive trials of the EndoBubble task on level 2. Recorded data included the number of balloons popped (maximum 40), time to completion, and number of times the injection needle struck the wall of the virtual pipe. These data were provided by the scoring matrix incorporated into the GI Mentor II software, and were displayed and recorded at the conclusion of each trial.

RESULTS

Differences in the means for each group and each trial were examined for statistical significance using a Mann-Whitney U test; the results are summarized in Table 2. The mean number of balloons popped per trial for each group is shown in Fig. 3. The intermediate group popped significantly more balloons than the novice group for each of the three trials (trial 1, $P = 0.001$; trial 2, $P = 0.002$; trial 3, $P = 0.004$). The mean time to completion and number of wall strikes for each group are presented in Figs. 4 and 5, respectively. The intermediate group performed consistently faster than the novice group for all three trials, and this difference was statistically significant for trial 1 ($P = 0.042$). The time differences in trials 2 and 3 approached statistical significance ($P = 0.072$, and $P = 0.083$, respectively). Similarly, the intermediate group had fewer wall strikes than the novice group for all three trials, with these differences reaching statistical significance in trials 1 and 3 ($P = 0.032$, and $P = 0.008$, respectively). Statistical significance was not reached in trial 2 because of the large performance variability in the novice group. This variability in wall strike error rate is an added notable aspect of the differences observed between the intermediate and novice groups. The mean variability in wall strike errors for the two groups over all three trials is shown in Fig. 6. The novice group variability score was more than five times greater than the score for the intermediate group, demonstrating that not only did the novice group make significantly more errors than the intermediate group as shown earlier, but their performance with respect to errors was highly variable

Table 2. Summary of mean balloons popped, completion time, and wall strike errors per group for each of the three trials

	Balloons popped (mean [std])			Completion time (sec) (mean [std])			No. of wall strikes (mean [std])		
Trial	Exp	(*P* value)	Novice	Exp	(*P* value)	Novice	Exp	(*P* value)	Novice
1	22 (5)	0.001	7.5 (4)	104 (14)	0.042	131 (25)	0.2 (0.4)	0.032	4 (3)
	NS		NS	NS		NS	NS		NS
2	25 (10)	0.002	8 (3)	103 (13)	0.072	117 (11)	0.4 (0.9)	0.11	3 (4)
	NS		NS	NS		NS	NS		NS
3	23 (7)	0.004	11 (4)	99 (7)	0.083	110 (11)	0.4 (0.5)	0.008	3 (2)
Trials 1 to 3	NS		$P < 0.05$	NS		$P < 0.01$	NS		NS

Exp = experienced; NS = not significant.

and inconsistent. In contrast, the intermediate group consistently minimized errors during task performance showing that they not only had enough psychomotor skill to quickly pop the balloons, but they also had enough control to prevent errors from occurring.

To test for a learning effect, differences within groups were evaluated using one-way analysis of variance (ANOVA) for repeated measures with a Friedman test for nonparametric data. Post hoc analysis was performed using Dunn's multiple-comparison test. The intermediately experienced group showed no significant difference in performance between trials 1 and 3 for number of balloons popped, completion time, or wall strikes. The novice group showed statistically significant improvement between trials 1 and 3 for number of balloons popped and completion time ($P < 0.05$, and $P < 0.01$, respectively). The decrease in wall strikes for the novice group was not statistically significant.

The internal consistency of the GI Mentor II virtual reality simulator to reliably test repeated performance was assessed for balloons popped, completion time, and wall strikes for all 14 subjects with standardized coefficient alpha. Test-retest reliability for trials 1 and 2 was established with Pearson's product moment correlation coefficient. Table 3 summarizes the results of these analyses. Scientific standards for acceptable internal consistency and test-retest reliability are levels greater than 0.8, and all measures except time reached this level.

DISCUSSION

The study reported here was designed to determine whether the Simbionix GI Mentor II virtual

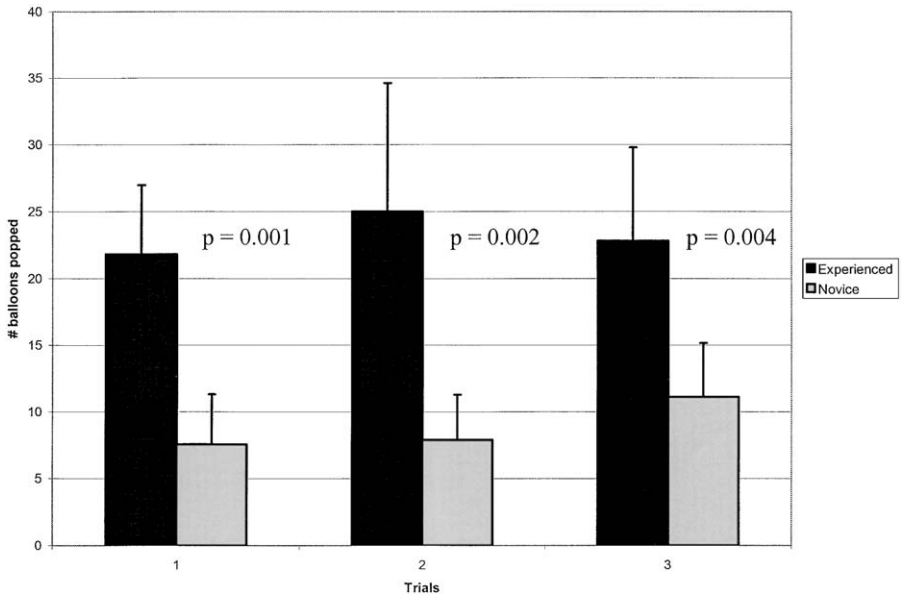

Fig. 3. Mean number of balloons popped per trial for the experienced and novice groups.

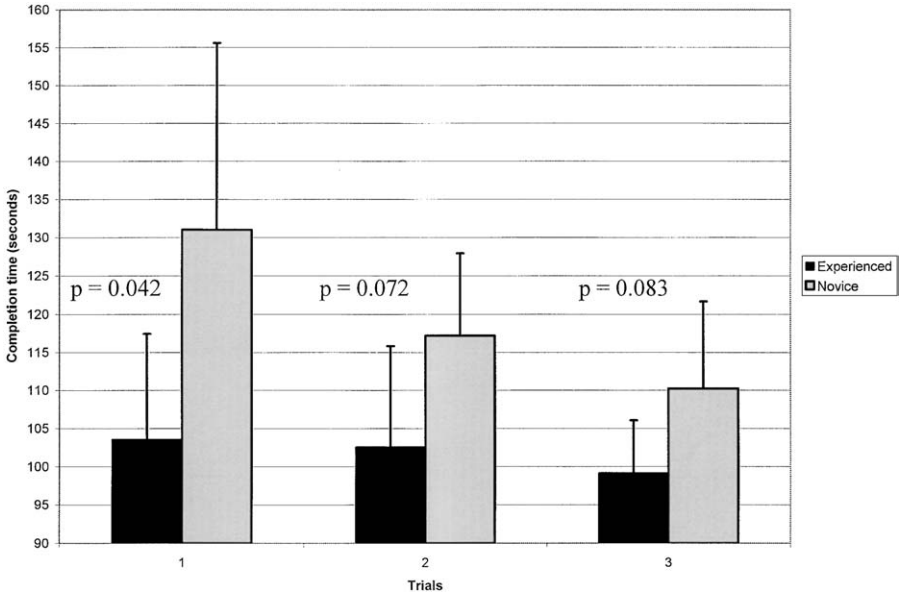

Fig. 4. Mean completion time per trial for the experienced and novice groups.

endoscopic simulator could differentiate between a group of intermediately experienced endoscopists and a group of novice endoscopists in performance of a basic endoscopic psychomotor task. Much like novice minimally invasive surgeons learning to adapt to the fulcrum effect,[18] neophyte flexible endoscopists must adapt the psychomotor pathways in their brain to allow them to negotiate the gastrointestinal tract. During flexible endoscopy the cognitive and psychomotor demands of the procedure combined with the stress of performance on an actual patient in a time-sensitive environment often leads to failure and frustration for both mentor and protégé. This combination of factors likely contributes to the difficulty in becoming competent in flexible endoscopy and results in the large minimum number of procedures recommended by many investigators and endoscopic societies to be considered competent.[4–8]

The use of a virtual reality simulator allows reduction in the number of variables in the training equation. By removing the patient and the endoscopy suite time from the training equation, stress is considerably reduced, allowing the trainee to focus on developing the necessary psychomotor skills. By using a simple,

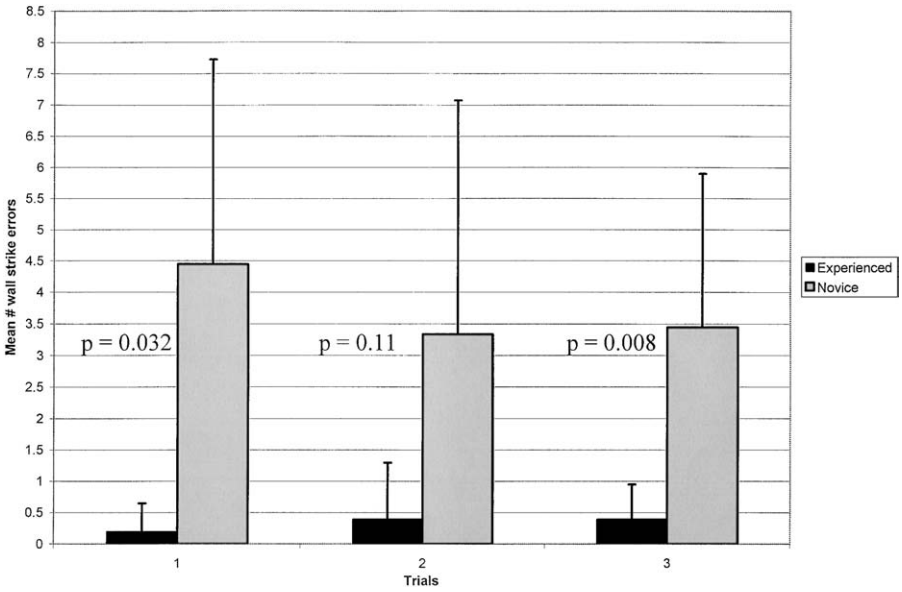

Fig. 5. Mean number of wall strike errors per trial for the experienced and novice groups.

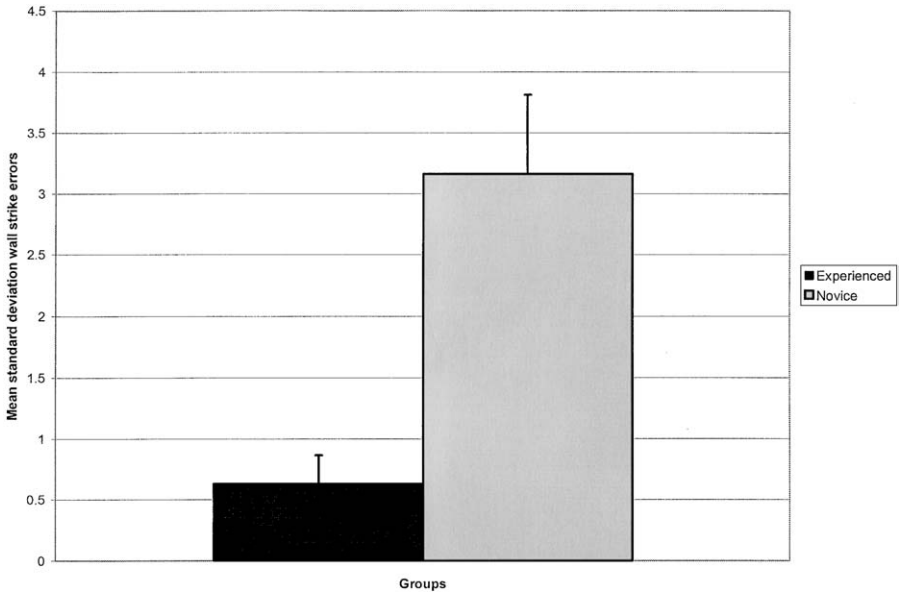

Fig. 6. Mean variability of wall strike errors made by the two groups over all three trials.

abstract task, the cognitive knowledge of the anatomy and physiology of the gastrointestinal tract are also eliminated. All that remains to be assessed is psychomotor performance.

The results of the assessment of the psychomotor performance of groups of intermediately experienced and novice endoscopists show that the intermediately experienced group performed consistently better than the novice group in a shorter amount of time and with consistently fewer errors. The scores of our intermediate group for number of balloons popped compares favorably with the previously published results of experts with more than 1000 colonoscopies each,[17] and although it is impossible to statistically analyze, no significant difference would be expected. The difference in performance variability seen between the novice and experienced groups also makes an important point. The importance of performance consistency is highlighted by examining the game of golf. Most golfers at some point in their career have hit the perfect tee shot—long and right down the middle. However, those who can hit that shot consistently find themselves on the PGA tour. The results of this study add to the growing support for use of variability as an important measure of performance. In either golf or endoscopy, consistently good performance is probably the best indicator of true skills mastery. These differences in performance and performance variability validate the construct that the GI Mentor II simulator can objectively assess the difference in psychomotor skills between novice endoscopists and those with intermediate endoscopic experience. The role of the GI Mentor II simulator as a tool to assess endoscopists still on their learning curves is further supported by the high internal consistency and test-retest reliability coefficients reported. These measures show how well an assessment tool will identify a given level of skill during repeated testing. Because only the correlation coefficient for time fell below the scientifically acceptable level of 0.8, the GI Mentor II simulator can be considered a consistent assessment tool.

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Analysis within the groups also yielded encouraging results. The GI Mentor II simulator demonstrated consistent performance across all three trials for the intermediately experienced group as evidenced by no significant improvement in performance with respect to balloons popped, time, or wall strike errors between trials 1 and 3. This implies that from a psychomotor standpoint, those in the IE group have reached the plateau of their learning curve. In contrast, the novice group showed significant improvement in balloons popped and completion time between trials 1 and 3. This improvement suggests that the GI Mentor II simulator would be useful as a training tool, but its role in training remains to be demonstrated.

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Discussion

Dr. H. Reber (Los Angeles, CA): You obviously focused on the ability of this technology to assess skill, and at the end of your presentation you touched on its role as a teaching tool. Maybe you could go a little bit further with that and tell us, just to focus on the colonoscopy issue, what has been done along those lines? Teaching colonoscopy is a painful experience for the patient as well as for the person who is learning to do it.

Dr. E. Ritter: Within the realm of using virtual reality and simulation in general for training skills, a great deal of work is being done, and it is still being sorted out as how best to apply simulation to training. The most common current application is to have trainees perform a certain number of procedures or spend a certain amount of time on the simulator.

One option that was reported at last year's meeting, and that we are currently exploring, is using objectively defined goals for simulator training. These goals are established by the performance of experts in flexible endoscopy, and are used to set a minimum standard for training on the simulator before trainees move on to in vivo training. This helps adjust for

differences in baseline abilities between subjects to ensure that each trainee reaches at least the same baseline skill level.

Dr. K. Lillemoe (Baltimore, MD): This was a very nice presentation. For those of us who do not have those simulators, could you tell us a little bit more about how you have incorporated them into your residency training program and specifically how much they cost? Have you seen any benefit when trainees have started out on the simulator and then gone into the clinical situation?

Dr. Ritter: I will address the cost issue first because I truly do not know how much this simulator costs, to be perfectly honest.

The issue of using the simulator in training is in the process of being evaluated. We only recently obtained this machine, and we have employed it for training in a class that we offer for medical students. Anecdotally, seeing their performance on the simulator improve over time, as well as improvement in performance in the animal laboratory, I would say that it does make a difference. We are planning to conduct a prospective randomized trial for training in upper endoscopy this next academic year to evaluate the training benefit, but we do not yet have any data on that.

Dr. J. Bender (Oklahoma City, OK): As any of us with small kids know, any time they get a new video game, their performance improves markedly the second and third time they try it. Yes, they can pop more balloons, but do you have any data at all that this correlates with better performance of endoscopy?

Dr. Ritter: Right now, no.

Dr. M. Stelzner (Seattle, WA): I would like to know if you have made efforts to identify certain factors that would predict less skillful performance. For example, are there certain motions that if they were corrected would enable a novice to accelerate his or her learning?

We know that in laparoscopy we can successfully identify, let us say, incorrect hand motions or an inappropriate speed of action and factors like that. Would your simulator be able to give the mentor information as to what factors would need to be corrected for a novice to learn endoscopy more efficiently?

Dr. Ritter: The simulator does record quite a number of metrics, including several of the things you mentioned. When using upper and lower endoscopy case simulations, the simulator does measure things such as overinsufflation, excess local pressure, and forming loops, so that the mentor can review and identify specific repeated errors in a specific student. Identification of these errors should increase the rate of learning, but right now we have no good objective data to prove that.

Epidemiology of Surgically Treated Gastric Cancer in the United States, 1988–2000

Reid M. Wainess, B.S., Justin B. Dimick, M.D., Gilbert R. Upchurch, Jr., M.D., John A. Cowan, Jr., M.D., Michael W. Mulholland, M.D., Ph.D.

The incidence of gastric cancer and the need for subsequent surgery has been decreasing in the United States. However, very few population-based studies on the magnitude of these changes are available. The objective of the present study was to characterize temporal trends in the use of gastric resection in the treatment of gastric cancer. Patients with a primary diagnosis code for gastric cancer (N = 105,887) and a procedure code for gastric resection (N = 23,690) in the Nationwide Inpatient Sample for 1988–2000 were included. The Nationwide Inpatient Sample represents a 20% stratified random sample representative of all United States hospitals. Outcome variables included the overall incidence, in-hospital mortality rate, and length of stay. Rates of surgery are shown as the number of cases per 100,000 hospital discharges. Hospital volume was defined as follows: low volume (1 to 4 cases per year), medium volume (5 to 8 cases per year), and high volume (9 or more cases per year). Rates of gastric resection have shown a 20% decline from 30 cases per 100,000 (1988–1989) to 24 cases per 100,000 (1999–2000) ($P = 0.001$). In-hospital mortality has not changed over the 13-year period and remains at 7.4%. There was significant variation in mortality across hospitals, with very low–volume centers having an 8.9% mortality rate, whereas very high–volume centers had a 6.4% mortality rate ($P < 0.001$). The market share of gastric resections performed at high-volume centers increased a small amount from 43% (1988–1989) to 48% (1999–2000) ($P = 0.023$). Over the 13-year period, length of stay decreased from 15 days (interquartile range [IQR] 11–23) in 1988 to 11 days (interquartile range [IQR] 8–16) in 2000 ($P < 0.001$). Rates of gastric resection for cancer have shown a modest decline over the past 13 years in the United States. Although the length of stay for these patients has decreased, no significant changes to in-hospital mortality have occurred. Given the declining rates of gastric cancer surgery, and the superior outcomes at high-volume centers, regionalization of care may improve mortality rates for this high-risk surgical procedure. (J GASTROINTEST SURG 2003;7:879–883)

KEY WORDS: Gastric cancer, hospital volume, outcomes

Epidemiologic studies have shown that gastric cancer has decreased in incidence over the past two decades.[1] Despite the reduction in incidence, gastric cancer remains one of the most common cancer diagnoses in the United States.[1,2] Surgical resection of early-stage gastric cancer offers the only chance of cure and, for patients presenting in later stages, surgical intervention often provides significant palliation.[3,4] In recent years the need for benign gastric surgery for indications such as peptic ulcer disease has also decreased given more effective medical and pharmacologic management.[5] As a result, an increasing proportion of gastric surgery is performed for gastric cancer.

Patients undergoing gastric resection for cancer are at a high risk of operative morbidity and mortality. Surgical experience has been associated with improved short- and long-term outcomes for several gastrointestinal operations. Given the decreased incidence of gastric cancer, it is likely that surgeons are performing fewer resections. With these changes in surgeon experience profiles for gastric surgery, it is important to understand the relationship of procedural volume to outcome. However, little is known regarding the epidemiology of surgically treated gastric cancer. Much of the current literature focuses on case series from tertiary centers of excellence, and little data remain available on nationwide trends in

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the utilization and outcomes of surgery for gastric cancer. The objective of the present study was to characterize these population-based trends for gastric resection for cancer from 1988 to 2000 in the United States.

METHODS

Data Source

The Nationwide Inpatient Sample (NIS) is a 20% stratified random sample of all hospital discharges in the United States. It is maintained by the Agency for Healthcare Research and Quality (AHRQ) as part of the Healthcare Cost and Utilization Project. The data for this study were derived from 1988–2000 versions of the NIS. All patients who were discharged during these years with an International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnostic code for malignant neoplasm of the stomach (ICD9-CM code 1510 to 1516, 1518, or 1519) were included in the study. In addition, a procedure code for partial gastrectomy with anastomosis to the esophagus (ICD-9-CM code 435), partial gastrectomy with anastomosis to the duodenum (ICD-9-CM code 436), partial gastrectomy with anastomosis to the jejunum (ICD-9-CM code 437), other partial gastrectomy (ICD-9-CM code 438), or total gastrectomy (ICD-9-CM codes 439, 4391, and 4399) was used to indicate gastric resection.

Outcome Variables

The primary outcome variable was in-hospital mortality. Hospital volume was categorized into three equal-sized groups based on terciles of the number of cases performed per year, thus placing approximately one third of the cases into each volume group. Low-volume hospitals performed four or fewer cases per year, medium-volume hospitals performed five to eight cases per year, and high-volume hospitals performed nine or more cases per year. Length of stay (LOS) was also assessed as an outcome to ascertain changes in resources over time. Prolonged LOS was considered a hospital stay longer than the seventy-fifth percentile of cases.

Statistical Analysis

Univariate analyses were performed to assess differences over time in mortality rates and LOS using linear regression and chi-square tests. Multivariate analyses of mortality and prolonged LOS were performed by multiple logistic regression. For modeling purposes, calendar years were divided into the following three time periods: time period 1, 1988 to 1992; time period 2, 1993 to 1996; and time period 3, 1997 to 2000. For the sake of analysis, race was analyzed as white vs. nonwhite. Comorbid diseases were used as a marker of illness in accordance with previously established standards.[6–9] The incidence rates of surgery were obtained by dividing the number of cases per year by the total number of discharges in the NIS for each year. True population-based rates were obtained by using sampling weights to find the estimated number of total procedures performed in the United States each year. This estimate was then divided by the total United States population for each year to obtain an approximation of the true population-based rates. $P < 0.05$ was considered significant in all final analyses. SPSS version 11.0 (SPSS, Chicago, IL) was used for all statistical analyses.

RESULTS

Patient Characteristics

From 1988 to 2000, a total of 105,887 patients were discharged from hospitals in the NIS with a diagnostic code for gastric cancer. In the same time period, 23,690 patients were discharged after undergoing gastric resection. The average age for patients undergoing gastric resection was 68 years (standard deviation 12.7), with 63% of the patients being male. Patients were most often admitted as elective (59.1%) admissions and 73.6% of patients were white (Table 1).

Incidence Trends

The number of patients with a discharge diagnosis of gastric cancer decreased from 25.4 cases per

Table 1. Patient characteristics for those undergoing gastric resection

Characteristics	N
Total patients	23,690
Age (yr)	68 ± 12.7 (mean ± SD)
Male	14,988 (63%)
Nonwhite	4277 (26.4%)
Elective admission	12,475 (59.1%)
Urgent admission	4857 (23.0%)
Emergent admission	3779 (17.9%)
Chronic obstructive pulmonary disease	2266 (9.6%)
Diabetes mellitus	2127 (9.0%)
Metastases from solid tumor	13,502 (57.0%)
History of myocardial infarction	526 (2.2%)
Peripheral vascular disease	463 (2.0%)
Chronic renal disease	24 (0.1%)
Mild liver disease	259 (1.1%)
Severe liver disease	25 (0.1%)

SD = standard deviation.

100,000 United States adults (1988) to 19.8 cases per 100,000 United States adults (2000) (Fig. 1) ($P < 0.001$). In addition, rates of gastric resection have shown a 29% decline from 5.6 cases per 100,000 United States adults (1988) to 4.0 cases per 100,000 United States adults (2000) (see Fig. 1) ($P < 0.001$). The overall proportion of gastric cancer patients undergoing gastric resection has remained constant at approximately 22%.

In-hospital Mortality

In-hospital mortality did not significantly change over the 13-year period, with an overall mortality rate of 7.4% for the sample group (Fig. 2). There was significant variation in mortality across hospitals, with low-volume centers having an 8.3% mortality rate, medium-volume hospitals having a 7.1% morality rate, and high-volume centers having a 6.5% mortality rate (Fig. 3) ($P < 0.001$). The market share of gastric resection performed at high-volume centers increased from 28.3% (1988–1989) to 34.0% (1999–2000) ($P < 0.001$). The mortality rate declined significantly over time at high-volume centers, where it decreased from 7.1% in 1988–1992 to 6.5% in 1993–1996 to 5.8% in 1997–2000. There was not a significant decline in mortality at low- or medium-volume centers.

By univariate analysis, patients older than 65 years old were three times more likely than younger patients to die after gastric resection (9.6% vs. 3.4%, $P < 0.001$). Male patients had a higher mortality rate than female patients (7.7% vs. 6.7%, $P = 0.004$). White patients had a higher mortality than nonwhite patients (8.0% vs. 5.8%, $P < 0.001$). An emergent or urgent admission was associated with higher mortality (12.3% and 9.1%) than an elective admission (5.3%) ($P < 0.001$). Patients from areas where the average income was greater than $45,000 per year had a lower mortality (6.5% vs. 7.8%, $P < 0.001$) than those from lower income areas.

In a multivariate analysis, significant predictors included age, race, admission type, and total number of comorbid diagnoses (Table 2). Hospital volume and time period were not significant predictors of mortality.

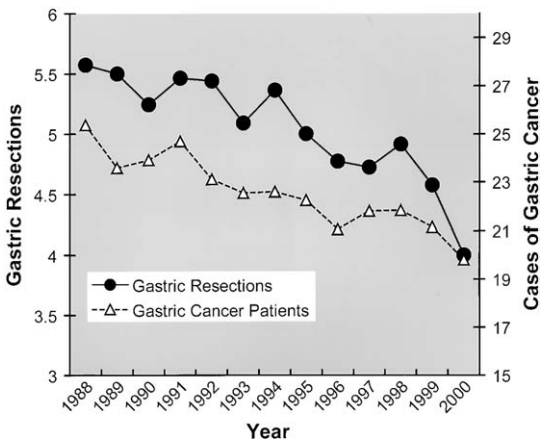

Fig. 1. Decreasing incidence of gastric cancer and gastric resection in the United States per 100,000 adults ($P < 0.001$).

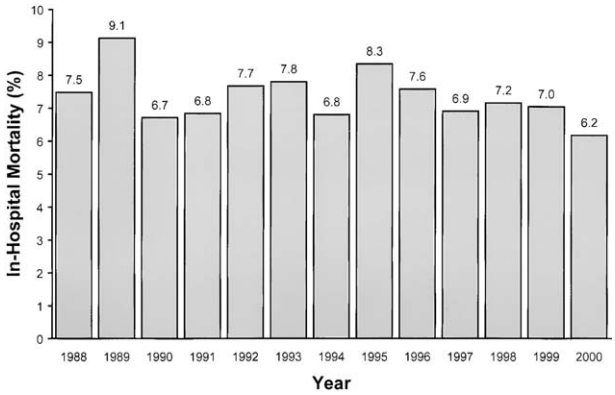

Fig. 2. Between 1988 and 2000, no significant changes for in-hospital mortality after gastric resection were demonstrated ($P = 0.134$ by linear regression).

Length of Hospital Stay

Over the 13-year period, the LOS decreased from 15 days [IQR 11–23] in 1988 to 11 days [IQR 8–16] in 2000 (Fig. 4) ($P < 0.001$). Variations in LOS across hospital volume were minimal with all three volume

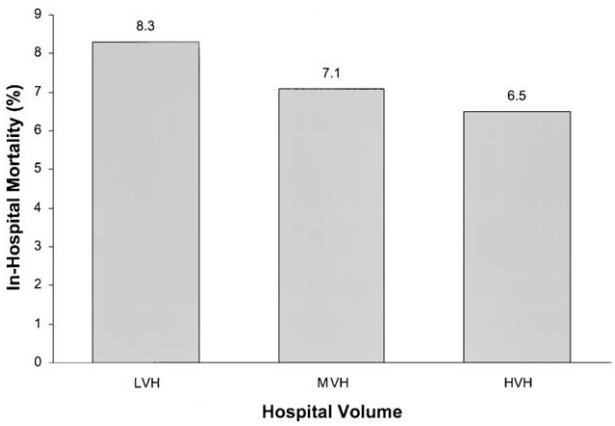

Fig. 3. In-hospital mortality inversely correlates with varying hospital volume ($P < 0.001$ by chi-square analysis). Low-volume hospitals (*LVH*) performed four or fewer resections per year. Medium-volume hospitals (*MVH*) performed five to eight resections per year. High-volume hospitals (*HVH*) performed nine or more resections per year.

Table 2. Multivariate analysis of mortality: Independent predictors of in-hospital mortality following gastric resection

Independent variable	Risk of mortality, odds ratio (95% CI)	*P* value
Age (yr)		
51 to 60	2.0 (1.3 to 3.2)	0.002
61 to 70	2.4 (1.6 to 3.6)	<0.001
71 to 80	4.5 (3.0 to 6.6)	<0.001
>80	7.3 (4.9 to 11.0)	<0.001
Female	0.7 (0.6 to 0.8)	<0.001
Nonwhite	0.8 (0.7 to 0.9)	0.005
Emergent admission	2.3 (2.0 to 2.8)	<0.001
Urgent admission	1.7 (1.5 to 2.0)	<0.001
Low-volume hospital	1.1 (1.0 to 1.3)	0.113
Medium-volume hospital	1.0 (0.9 to 1.2)	0.648
Median income < $45,000	1.0 (0.9 to 1.2)	0.530
Time period 2 (1993 to 1996)	1.0 (0.8 to 1.2)	0.826
Time period 3 (1997 to 2000)	1.0 (0.9 to 1.2)	0.553
No. of comorbid diseases		
One	1.2 (1.1 to 1.4)	0.008
Two	1.4 (1.1 to 1.7)	0.003
Three or more	1.4 (0.9 to 2.3)	0.167

CI = confidence interval.

categories having a median LOS of 13 days. Prolonged LOS was greater for patients over 65 years of age (20.6% vs. 28.0%, $P < 0.001$). Differences in prolonged LOS were not significant for gender or race. Patients with an emergent (45.0%) or urgent (30.6%) admission were more likely than those with an elective admission (18.1%) to have a prolonged LOS ($P < 0.001$). Patients from areas where the average income was greater than $45,000 per year had a slightly lower incidence of prolonged LOS (24.1% vs. 26.0%, $P = 0.003$) than those from lower income areas. In a multivariate analysis of prolonged LOS, significant predictors included age, admission type, and time period (Table 3).

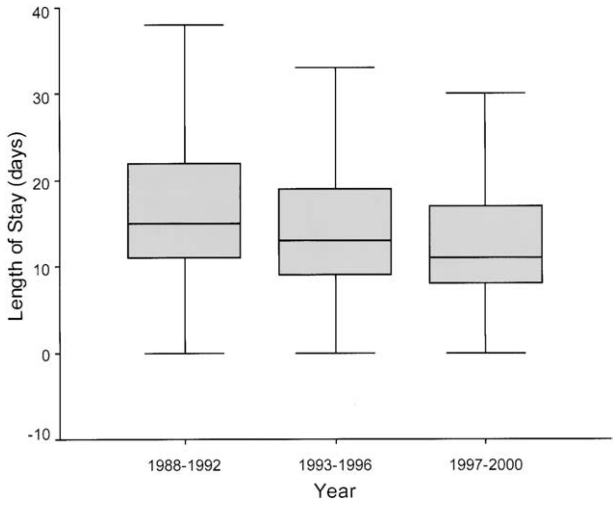

Fig. 4. Decreased length of stay after gastric resection over time ($P < 0.001$ by chi-square analysis).

DISCUSSION

The overall incidence of gastric cancer and subsequent gastric resection has declined from 1988 to 2000. Despite this decline in the frequency of the disease, the proportion of gastric cancer cases undergoing gastric resection has remained relatively constant. In the present study, improvements in short-term outcomes following gastric resection were not demonstrated. However, variation in outcomes across varying hospital volumes, with higher volume hospitals having lower mortality rates, was noted. In addition, despite stagnant overall mortality rates, patients are spending less time in the hospital after gastric resection. This decrease in LOS, coupled with the unchanging mortality rates, suggests more efficient use of resources over time. However, although shorter hospital stays may be, at least in part, the result of improvements in postoperative care, such reductions could also be a result of cost-saving initiatives implemented by health care payers during the same time period.

Gastric resection for cancer, similar to other complex gastrointestinal procedures, has a relatively high rate of postoperative complications and operative mortality. Rates for these adverse outcomes are not uniform across medical centers and vary in relation to the surgeon's and the hospital's experience with

Table 3. Multivariate analysis of length of stay: Independent predictors of prolonged length of stay for gastric resection

Independent variable	Risk of mortality, odds ratio (95% CI)	*P* value
Age (yr)		
51 to 60	1.1 (1.0 to 1.3)	0.086
61 to 70	1.4 (1.2 to 1.5)	<0.001
71 to 80	1.6 (1.4 to 1.8)	<0.001
>80	1.9 (1.7 to 2.2)	<0.001
Emergent admission	3.6 (3.3 to 3.9)	<0.001
Urgent admission	1.8 (1.7 to 2.0)	<0.001
Time period 2 (1988 to 1992)	1.8 (1.7 to 2.0)	<0.001
Time period 3 (1993 to 1996)	1.4 (1.3 to 1.5)	<0.001
Low-volume hospital	1.0 (0.9 to 1.1)	0.727
Medium-volume hospital	1.0 (0.9 to 1.1)	0.417
Medium income < $35,000	1.0 (0.9 to 1.1)	0.794

the operation. Health policy efforts should focus on addressing these apparent variations in the quality of care received across centers. Previous studies have confirmed a relationship between hospital volume and improved outcomes.[10–12] As a result of these data, private and professional organizations have suggested regionalizing high-risk surgery in order to improve outcomes after some complex surgical procedures.[13]

The findings that provider experience is an important determinant in outcomes are particularly relevant given the decline in the rate of surgery for gastric cancer. As fewer surgeons perform this type of surgery, the population-based outcomes may worsen in the United States. In this setting, selective referral strategies based on volume standards are particularly relevant and may improve the quality of care for this relatively high-risk surgical procedure. The data from the current study are in agreement with previously published reports[14] and suggest that, in general, higher volume hospitals have superior outcomes for gastric resection compared to lower volume centers. Although these variations were not significant in the multivariate model, they still persist throughout the current literature and thus should not be deemed insignificant, but should instead be cautiously accepted. Further, the current study demonstrates that some degree of regionalization is occurring, with patients more likely to undergo surgery at higher volume hospitals over time. Yet the overall mortality rates have not declined. This suggests that more patients undergoing gastric resection should be offered referral to higher volume centers in order to improve outcomes from a population perspective.

There are several specific limitations to the current study, the majority of which are related to the use of administrative databases.[15,16] For instance, the NIS lacks clinical data that would help directly control for severity of disease, such as the stage of a patient's gastric cancer. This appears to be especially relevant in gastric cancer, where patient prognosis is much better with early-stage disease.[17] However, the main outcome variables for the current study focus on the short-term and cancer stage primarily affects long-term survival. The NIS database also does not provide laboratory variables such as serum albumin and liver function tests, which might give us a better idea about the patient's functional status.

In summary, the current study demonstrates a decreasing incidence of surgery for gastric cancer in the United States over the past 13 years. Although a relationship between operative mortality and hospital volume was demonstrated, overall mortality rates for gastric cancer resection have not improved significantly during the study period. In contrast, LOS has shown a significant steady decline over the study period. The implications of this study suggest that further improvements are possible after gastric resection. The process variables associated with improved outcomes need to be further investigated to improve the overall care for patients undergoing operations for gastric cancer.

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METHODS

Data Source

The Nationwide Inpatient Sample (NIS) is a 20% stratified random sample of all hospital discharges in the United States. It is maintained by the Agency for Healthcare Research and Quality (AHRQ) as part of the Healthcare Cost and Utilization Project. The data for this study were derived from 1988–2000 versions of the NIS. All patients who were discharged during these years with an International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnostic code for malignant neoplasm of the stomach (ICD9-CM code 1510 to 1516, 1518, or 1519) were included in the study. In addition, a procedure code for partial gastrectomy with anastomosis to the esophagus (ICD-9-CM code 435), partial gastrectomy with anastomosis to the duodenum (ICD-9-CM code 436), partial gastrectomy with anastomosis to the jejunum (ICD-9-CM code 437), other partial gastrectomy (ICD-9-CM code 438), or total gastrectomy (ICD-9-CM codes 439, 4391, and 4399) was used to indicate gastric resection.

Outcome Variables

The primary outcome variable was in-hospital mortality. Hospital volume was categorized into three equal-sized groups based on terciles of the number of cases performed per year, thus placing approximately one third of the cases into each volume group. Low-volume hospitals performed four or fewer cases per year, medium-volume hospitals performed five to eight cases per year, and high-volume hospitals performed nine or more cases per year. Length of stay (LOS) was also assessed as an outcome to ascertain changes in resources over time. Prolonged LOS was considered a hospital stay longer than the seventy-fifth percentile of cases.

Statistical Analysis

Univariate analyses were performed to assess differences over time in mortality rates and LOS using linear regression and chi-square tests. Multivariate analyses of mortality and prolonged LOS were performed by multiple logistic regression. For modeling purposes, calendar years were divided into the following three time periods: time period 1, 1988 to 1992; time period 2, 1993 to 1996; and time period 3, 1997 to 2000. For the sake of analysis, race was analyzed as white vs. nonwhite. Comorbid diseases were used as a marker of illness in accordance with previously established standards.[6–9] The incidence rates of surgery were obtained by dividing the number of cases per year by the total number of discharges in the NIS for each year. True population-based rates were obtained by using sampling weights to find the estimated number of total procedures performed in the United States each year. This estimate was then divided by the total United States population for each year to obtain an approximation of the true population-based rates. $P < 0.05$ was considered significant in all final analyses. SPSS version 11.0 (SPSS, Chicago, IL) was used for all statistical analyses.

RESULTS

Patient Characteristics

From 1988 to 2000, a total of 105,887 patients were discharged from hospitals in the NIS with a diagnostic code for gastric cancer. In the same time period, 23,690 patients were discharged after undergoing gastric resection. The average age for patients undergoing gastric resection was 68 years (standard deviation 12.7), with 63% of the patients being male. Patients were most often admitted as elective (59.1%) admissions and 73.6% of patients were white (Table 1).

Incidence Trends

The number of patients with a discharge diagnosis of gastric cancer decreased from 25.4 cases per

Table 1. Patient characteristics for those undergoing gastric resection

Characteristics	N
Total patients	23,690
Age (yr)	68 ± 12.7 (mean ± SD)
Male	14,988 (63%)
Nonwhite	4277 (26.4%)
Elective admission	12,475 (59.1%)
Urgent admission	4857 (23.0%)
Emergent admission	3779 (17.9%)
Chronic obstructive pulmonary disease	2266 (9.6%)
Diabetes mellitus	2127 (9.0%)
Metastases from solid tumor	13,502 (57.0%)
History of myocardial infarction	526 (2.2%)
Peripheral vascular disease	463 (2.0%)
Chronic renal disease	24 (0.1%)
Mild liver disease	259 (1.1%)
Severe liver disease	25 (0.1%)

SD = standard deviation.

100,000 United States adults (1988) to 19.8 cases per 100,000 United States adults (2000) (Fig. 1) ($P < 0.001$). In addition, rates of gastric resection have shown a 29% decline from 5.6 cases per 100,000 United States adults (1988) to 4.0 cases per 100,000 United States adults (2000) (see Fig. 1) ($P < 0.001$). The overall proportion of gastric cancer patients undergoing gastric resection has remained constant at approximately 22%.

In-hospital Mortality

In-hospital mortality did not significantly change over the 13-year period, with an overall mortality rate of 7.4% for the sample group (Fig. 2). There was significant variation in mortality across hospitals, with low-volume centers having an 8.3% mortality rate, medium-volume hospitals having a 7.1% morality rate, and high-volume centers having a 6.5% mortality rate (Fig. 3) ($P < 0.001$). The market share of gastric resection performed at high-volume centers increased from 28.3% (1988–1989) to 34.0% (1999–2000) ($P < 0.001$). The mortality rate declined significantly over time at high-volume centers, where it decreased from 7.1% in 1988–1992 to 6.5% in 1993–1996 to 5.8% in 1997–2000. There was not a significant decline in mortality at low- or medium-volume centers.

By univariate analysis, patients older than 65 years old were three times more likely than younger patients to die after gastric resection (9.6% vs. 3.4%, $P < 0.001$). Male patients had a higher mortality rate than female patients (7.7% vs. 6.7%, $P = 0.004$). White patients had a higher mortality than nonwhite patients (8.0% vs. 5.8%, $P < 0.001$). An emergent or urgent admission was associated with higher mortality (12.3% and 9.1%) than an elective admission (5.3%) ($P < 0.001$). Patients from areas where the average income was greater than $45,000 per year had a lower mortality (6.5% vs. 7.8%, $P < 0.001$) than those from lower income areas.

In a multivariate analysis, significant predictors included age, race, admission type, and total number of comorbid diagnoses (Table 2). Hospital volume and time period were not significant predictors of mortality.

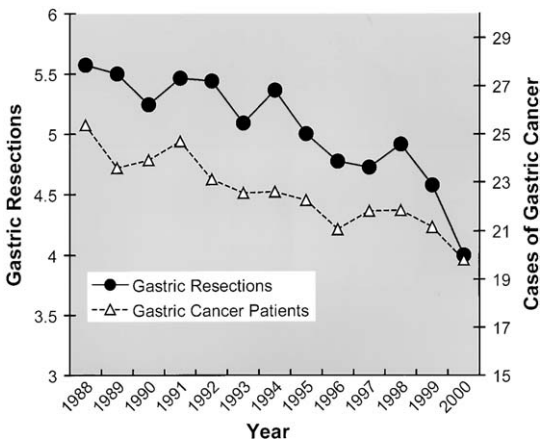

Fig. 1. Decreasing incidence of gastric cancer and gastric resection in the United States per 100,000 adults ($P < 0.001$).

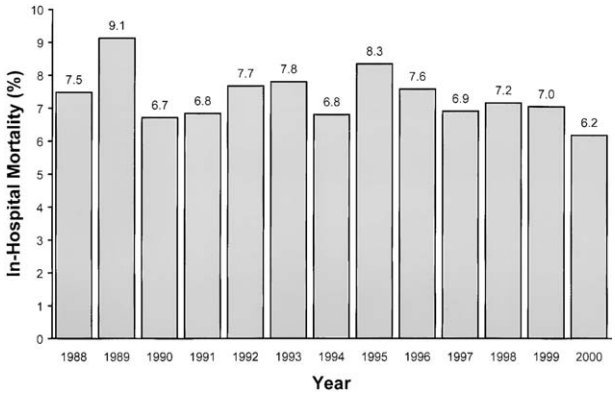

Fig. 2. Between 1988 and 2000, no significant changes for in-hospital mortality after gastric resection were demonstrated ($P = 0.134$ by linear regression).

Length of Hospital Stay

Over the 13-year period, the LOS decreased from 15 days [IQR 11–23] in 1988 to 11 days [IQR 8–16] in 2000 (Fig. 4) ($P < 0.001$). Variations in LOS across hospital volume were minimal with all three volume

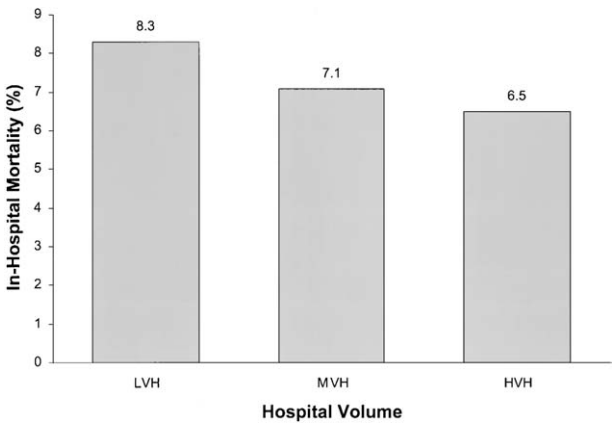

Fig. 3. In-hospital mortality inversely correlates with varying hospital volume ($P < 0.001$ by chi-square analysis). Low-volume hospitals (*LVH*) performed four or fewer resections per year. Medium-volume hospitals (*MVH*) performed five to eight resections per year. High-volume hospitals (*HVH*) performed nine or more resections per year.

Table 2. Multivariate analysis of mortality: Independent predictors of in-hospital mortality following gastric resection

Independent variable	Risk of mortality, odds ratio (95% CI)	*P* value
Age (yr)		
51 to 60	2.0 (1.3 to 3.2)	0.002
61 to 70	2.4 (1.6 to 3.6)	<0.001
71 to 80	4.5 (3.0 to 6.6)	<0.001
>80	7.3 (4.9 to 11.0)	<0.001
Female	0.7 (0.6 to 0.8)	<0.001
Nonwhite	0.8 (0.7 to 0.9)	0.005
Emergent admission	2.3 (2.0 to 2.8)	<0.001
Urgent admission	1.7 (1.5 to 2.0)	<0.001
Low-volume hospital	1.1 (1.0 to 1.3)	0.113
Medium-volume hospital	1.0 (0.9 to 1.2)	0.648
Median income < $45,000	1.0 (0.9 to 1.2)	0.530
Time period 2 (1993 to 1996)	1.0 (0.8 to 1.2)	0.826
Time period 3 (1997 to 2000)	1.0 (0.9 to 1.2)	0.553
No. of comorbid diseases		
One	1.2 (1.1 to 1.4)	0.008
Two	1.4 (1.1 to 1.7)	0.003
Three or more	1.4 (0.9 to 2.3)	0.167

CI = confidence interval.

categories having a median LOS of 13 days. Prolonged LOS was greater for patients over 65 years of age (20.6% vs. 28.0%, $P < 0.001$). Differences in prolonged LOS were not significant for gender or race. Patients with an emergent (45.0%) or urgent (30.6%) admission were more likely than those with an elective admission (18.1%) to have a prolonged LOS ($P < 0.001$). Patients from areas where the average income was greater than $45,000 per year had a slightly lower incidence of prolonged LOS (24.1% vs. 26.0%, $P = 0.003$) than those from lower income areas. In a multivariate analysis of prolonged LOS, significant predictors included age, admission type, and time period (Table 3).

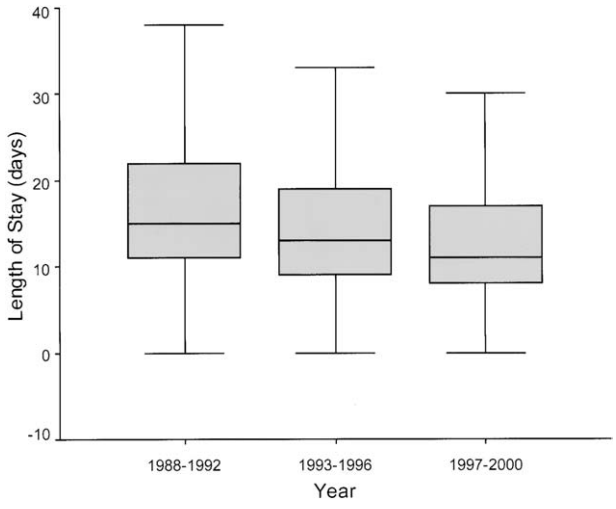

Fig. 4. Decreased length of stay after gastric resection over time ($P < 0.001$ by chi-square analysis).

DISCUSSION

The overall incidence of gastric cancer and subsequent gastric resection has declined from 1988 to 2000. Despite this decline in the frequency of the disease, the proportion of gastric cancer cases undergoing gastric resection has remained relatively constant. In the present study, improvements in short-term outcomes following gastric resection were not demonstrated. However, variation in outcomes across varying hospital volumes, with higher volume hospitals having lower mortality rates, was noted. In addition, despite stagnant overall mortality rates, patients are spending less time in the hospital after gastric resection. This decrease in LOS, coupled with the unchanging mortality rates, suggests more efficient use of resources over time. However, although shorter hospital stays may be, at least in part, the result of improvements in postoperative care, such reductions could also be a result of cost-saving initiatives implemented by health care payers during the same time period.

Gastric resection for cancer, similar to other complex gastrointestinal procedures, has a relatively high rate of postoperative complications and operative mortality. Rates for these adverse outcomes are not uniform across medical centers and vary in relation to the surgeon's and the hospital's experience with

Table 3. Multivariate analysis of length of stay: Independent predictors of prolonged length of stay for gastric resection

Independent variable	Risk of mortality, odds ratio (95% CI)	*P* value
Age (yr)		
51 to 60	1.1 (1.0 to 1.3)	0.086
61 to 70	1.4 (1.2 to 1.5)	<0.001
71 to 80	1.6 (1.4 to 1.8)	<0.001
>80	1.9 (1.7 to 2.2)	<0.001
Emergent admission	3.6 (3.3 to 3.9)	<0.001
Urgent admission	1.8 (1.7 to 2.0)	<0.001
Time period 2 (1988 to 1992)	1.8 (1.7 to 2.0)	<0.001
Time period 3 (1993 to 1996)	1.4 (1.3 to 1.5)	<0.001
Low-volume hospital	1.0 (0.9 to 1.1)	0.727
Medium-volume hospital	1.0 (0.9 to 1.1)	0.417
Medium income < $35,000	1.0 (0.9 to 1.1)	0.794

the operation. Health policy efforts should focus on addressing these apparent variations in the quality of care received across centers. Previous studies have confirmed a relationship between hospital volume and improved outcomes.[10–12] As a result of these data, private and professional organizations have suggested regionalizing high-risk surgery in order to improve outcomes after some complex surgical procedures.[13]

The findings that provider experience is an important determinant in outcomes are particularly relevant given the decline in the rate of surgery for gastric cancer. As fewer surgeons perform this type of surgery, the population-based outcomes may worsen in the United States. In this setting, selective referral strategies based on volume standards are particularly relevant and may improve the quality of care for this relatively high-risk surgical procedure. The data from the current study are in agreement with previously published reports[14] and suggest that, in general, higher volume hospitals have superior outcomes for gastric resection compared to lower volume centers. Although these variations were not significant in the multivariate model, they still persist throughout the current literature and thus should not be deemed insignificant, but should instead be cautiously accepted. Further, the current study demonstrates that some degree of regionalization is occurring, with patients more likely to undergo surgery at higher volume hospitals over time. Yet the overall mortality rates have not declined. This suggests that more patients undergoing gastric resection should be offered referral to higher volume centers in order to improve outcomes from a population perspective.

There are several specific limitations to the current study, the majority of which are related to the use of administrative databases.[15,16] For instance, the NIS lacks clinical data that would help directly control for severity of disease, such as the stage of a patient's gastric cancer. This appears to be especially relevant in gastric cancer, where patient prognosis is much better with early-stage disease.[17] However, the main outcome variables for the current study focus on the short-term and cancer stage primarily affects long-term survival. The NIS database also does not provide laboratory variables such as serum albumin and liver function tests, which might give us a better idea about the patient's functional status.

In summary, the current study demonstrates a decreasing incidence of surgery for gastric cancer in the United States over the past 13 years. Although a relationship between operative mortality and hospital volume was demonstrated, overall mortality rates for gastric cancer resection have not improved significantly during the study period. In contrast, LOS has shown a significant steady decline over the study period. The implications of this study suggest that further improvements are possible after gastric resection. The process variables associated with improved outcomes need to be further investigated to improve the overall care for patients undergoing operations for gastric cancer.

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A Better Cell Cycle Target for Gene Therapy of Colorectal Cancer: Cyclin G

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The purpose of this study was to evaluate the overexpression of cyclin G in colorectal neoplasia, which may be a more frequent event than cyclin D1 during the cell cycle and thus may have a more enhanced therapeutic potential in treating colorectal cancer. Ninety formalin-fixed, paraffin-embedded human colon and rectal specimens were obtained from the Pathology Department of Norris Cancer Center/ University of Southern California. The tissues had been obtained after surgical resection between 1995 and 2001, and had been processed by routine clinical histopathologic methods. Ninety-one percent of colorectal tumors had cyclin G overexpression. These cyclin-positive patients were evenly distributed between men and women, and between tumor locations, that is, 36% rectal tumors and 34% right-sided tumors. Thirty-two percent were well differentiated, and 66% were moderately differentiated. Thirty patients (38%) had stage I disease, 16 (20%) had stage II disease, 25 (32%) had stage III, and seven (9%) had stage IV disease. Eight patients (10%) in this group had recurrent disease during follow-up. There was no correlation between cyclin G overexpression and clinical and pathologic characteristics. Cyclin D1 overexpression was found to be present in only 42% of colorectal adenocarcinomas. There was no correlation between cyclin D1 overexpression and clinical and pathologic characteristics. The present study demonstrates that cyclin G overexpression is a frequent event in colorectal cancer. This frequent event in colorectal carcinogenesis may facilitate new therapeutic approaches acting as a target for gene therapy, possibly directed at downregulating cyclin G in colorectal cancer. (J GASTROINTEST SURG 2003;7:884–889)

KEY WORDS: Colon cancer, cancer epidemiology

Human cancer is the end result of multiple molecular events determined by genetic alterations giving rise to a cell or a population of cells with altered proliferation, regulation of cell cycle, and differentiation characteristics.[1]

The cell cycle is a highly organized process regulated by cyclins, cyclin-dependent kinases (CDK), and cyclin-dependent kinase inhibitors (CKI).[2] These are prime cell cycle regulators and control the major checkpoints in cell cycle transition of eukaryotic cells. This complex mechanism ensures that there is complete and accurate replication of the cell before division by maintaining the orderly progression of cells through the various phases of the cell cycle. Mutations and/or altered expression of cyclins may be involved in states of altered proliferation, such as neoplasia.[2–4]

Currently 14 cyclins have been identified (cyclins A to J), and they share a common 100 amino acid series known as the cyclin box. The concentrations of different cyclins oscillate throughout the cell cycle activating specific kinases involved in the various phases of the cell cycle.[2,4]

Cyclin D1 appears to be involved in the early phase of cell cycle progression. As a result of stimulation of the cell in the G0 or early G1 phase to initiate progression through the cycle, there is a rise in the concentration of cyclins D and E known as the G1 cyclins (because of their location within the cell cycle).[2]

Overexpression of cyclin D1 is observed in several types of tumors, such as esophageal, liver, breast, and colorectal. In colorectal cancer, increased expression of cyclin D1 appears to be an early event in the

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process of multistage carcinogenesis occurring in approximately 30% to 50%.[5] Furthermore, mutations in the APC gene, often found in cancers of various organs including the large bowel, may result in cytoplasmic accumulation of β-catenin. In the cytoplasm, β-catenin binds to a transcription factor (TCF/LEF) and is transferred to the nucleus. The cyclin D1 gene may be a target for β-catenin resulting in its overexpression in colon cancer.[5–7]

Cyclin G, a recent addition to the cyclin family, is the only known cyclin that is transcriptionally activated by the p53 tumor suppressor gene, suggesting that it may play a role in p53-mediated cell growth control.[8] In contrast to most p53 target genes such as p21/Waf1/Cip1/Sdi1, Bax1 and others, cyclin G does not seem to exert a tumor-suppressive role but rather, similar to other cyclins or protooncogenes, plays a growth-promoting role. Recently observations of cyclin G overexpression in breast and prostate cancer were reported.[8] In these tumors, cyclin G was overexpressed independent of p53 status, and there were no changes in the level of their expression throughout the cell cycle. However, like other members of the cyclin family, cyclin G expression is gradually increased with cell cycle progression, peaking in the late G2/M phase as observed in normal human mammary epithelial cells.[8]

To determine the presence and frequency of cyclin G and D overexpression in colorectal cancer, we used immunohistochemistry to analyze 87 previously resected human colorectal adenocarcinomas.

MATERIAL AND METHODS

Tissue Samples

Eighty-seven formalin-fixed, paraffin-embedded human colon and rectal adenocarcinoma specimens were obtained from the Department of Pathology, Norris Cancer Center/Keck School of Medicine (Los Angeles, CA). These tissues had been obtained after surgical resections performed between 1995 and 2001 for sporadic colorectal cancer and had been processed at that time by routine clinical histopathologic methods. All patients with hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, or other hereditary syndromes for colorectal cancer were excluded.

Previous pathologic reports were reviewed for degree of differentiation, tumor node metastasis (TNM) stage, lymphovascular invasion, and location within the colon (right, left, and rectum). Tumor and patient characteristics are summarized in Tables 1 and 2. Normal colonic or rectal tissues were also reviewed from each tumor sample for immunostaining comparison.

Antibodies

The following antibodies were used for immunohistochemical analyses in this study: mouse monoclonal antibody against human cyclin G (Vector Laboratories, Burlingame, CA), and mouse monoclonal anti-human cyclin D1 antibody (Vector Laboratories).

Immunohistochemical Analysis

Formalin-fixed and paraffin-embedded tumor samples were sliced 5 μm thick on positively charged glass slides, deparaffinized in xylene, rehydrated, and then subjected to antigen retrieval using 0.01 mol/L citrate buffer solution at pH 5-6 (Biogenex, San Ramon, CA). For cyclin D1, it is heated in a microwave for 5 + 5 minutes. For cyclin G, it is heated in a pressure cooker inside the microwave for 30 minutes (15 minutes on high power; 15 minutes at 40% power). After antigen retrieval, the slides are cooled down to room temperature. Slides intended

Table 1. Patient characteristics and cyclin D1 status

	Cyclin D (−)	Cyclin D (+)	*P* value
% of positive cells (mean)	3.3	23.5	<0.001
Characteristics			
N	49 (56%)	38 (44%)	
Mean age (yr)	63.8 (range 25–92)	64.8 (range 37–86)	
Sex			
Female	27 (54%)	17 (46%)	0.29
Male	23 (46%)	20 (54%)	
Tumor location			
Rectum	24 (48%)	5 (13.5%)	0.002
Left colon	11 (22%)	17 (46%)	
Right colon	15 (30%)	15 (40.5%)	
Tumor differentiation			
Well	23 (46%)	30 (81%)	0.67
Moderate	25 (50%)	7 (9%)	
Poor or undifferentiated	2 (4%)	0	
Staging			
Stage I	16 (32%)	15 (39%)	0.39
Stage II	13 (24%)	11 (29%)	
Stage III	14 (30%)	11 (29%)	
Stage IV	6 (12%)	1 (3%)	
TOTAL	49 (100%)	38 (100%)	

Table 2. Patient characteristics and cyclin G status

	Cyclin G (−)	Cyclin G (+)	*P* value
% of positive Cells (mean)	2.1	73.1	<0.001
Characteristics			
N	8 (9%)	79 (91%)	
Mean age (yr)	64.1 (range 49–91)	64.2 (range 25–92)	
Sex			
Female	4 (50%)	39 (49%)	0.63
Male	4 (50%)	40 (50%)	
Tumor location			
Rectum	1 (12.5%)	28 (35%)	0.38
Left colon	4 (50%)	24 (30%)	
Right colon	3 (37.5%)	27 (35%)	
Tumor differentiation			
Well	5 (62.5%)	25 (31%)	0.99
Moderate	3 (37.5%)	52 (66%)	
Poor or undifferentiated	0	2 (3%)	
Staging			
Stage I	1 (12.5%)	30 (38%)	0.005
Stage II	6 (75%)	16 (20%)	
Stage III	1 (12.5%)	26 (33%)	
Stage IV	0	7 (9%)	
TOTAL	8 (100%)	79 (100%)	

for cyclin D1 are then pretreated with proteinase (IP Enzyme, Ventana Medical Systems, Inc., Tucson, AZ) for 10 minutes. Immunostaining of sections was performed by the streptavidin-biotin peroxidase complex (ABC) method using the Vectastain Elite ABC kit (Vector Laboratories). In each stain the primary antibody was employed at 1:20 dilution. The incubation time for primary antibody was 1 hour for cyclin D1 and overnight for cyclin G, both at room temperature. After incubation with primary antibody, staining was completed using the ABC-based system (biotinylated horse antimouse antibody was used as secondary antibody with diaminobenzidine [DAB] as chromogen). Finally, the slides were counterstained with hematoxylin and mounted for examination.

Interpretation of Immunohistochemical Staining

Normal prostate tissue was used as positive control specimens, whereas normal colonic mucosa was used as negative control samples (Figs. 1 and 2). Tumors were classified as positive (+) when more than 10% of cancer cells showed nuclear staining for cyclin G or cyclin D1; tumors were classified as negative (−) when less than 10% of cancer cells showed such a staining pattern. The cutoff lines for these antibodies were commonly used in previous studies.[5,6] All immunostained sections were evaluated in a coded manner without knowledge of the clinical and pathologic background of the patients. Two separate observers (R.P. and N.W.) performed the assessment of the staining independently.

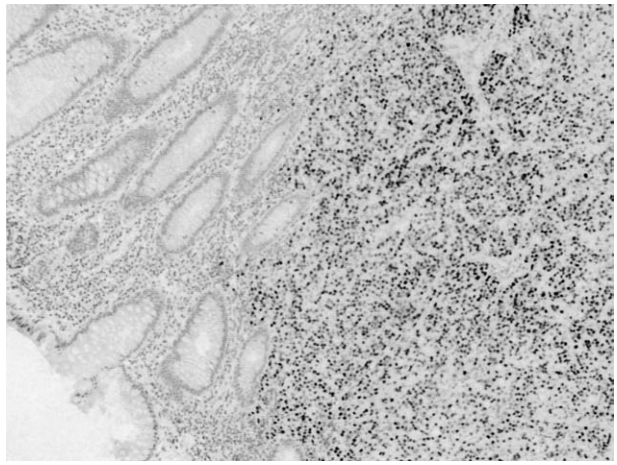

Fig. 1. Cyclin D1–staining tumor with adjacent normal mucosa.

Statistical Analysis

Pearson's chi-square test was used to compare frequencies among the groups to determine the association between immunohistologic results and clinicopathologic features in categorical variables.

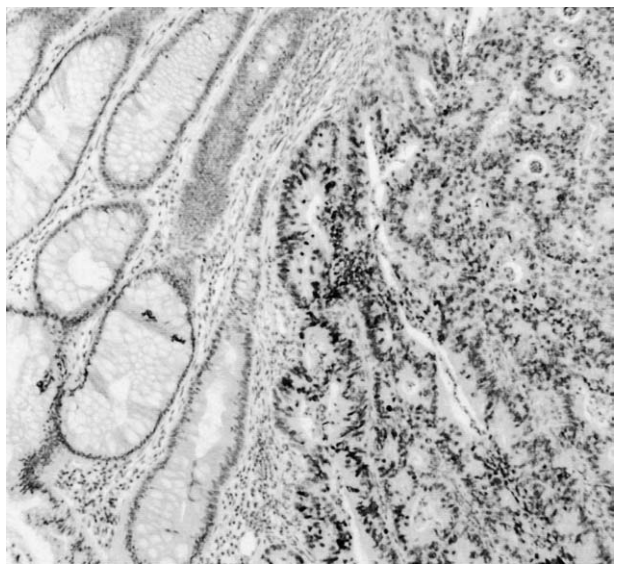

Fig. 2. Cyclin G–staining tumor with adjacent normal mucosa.

Student's *t* test was used to compare mean immunohistologic results and numerical variables. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Ninety pathology reports from patients with previously resected colorectal adenocarcinomas were reviewed. In three cases there was insufficient tumor tissue for immunohistologic study, and these were excluded. Eighty-seven patients were eligible for immunohistologic studies using cyclin G and D1 antibodies.

Cyclin D

Forty-nine tumors (56%) were considered negative (<10% of tumor cells with nuclear staining) for cyclin D1, and 38 (44%) were considered positive (> 10%). In the cyclin D1 (negative) group the mean age was 63.8 years (range 25–92 years); 23 patients (46%) were male and 27 (54%) were female. Twenty-four patients (48%) had rectal tumors, 11 (22%) had left-sided tumors, and 15 (30%) had right-sided tumors. Twenty-three tumors (46%) were well differentiated, 25 (50%) were moderately differentiated, and two (4%) were poorly or undifferentiated. Sixteen patients (32%) had stage I disease (T1-2N0), 12 (24%) had stage II disease (T3-4N0), 15 (30%) had stage III (anyTN1-2), and six (12%) had stage IV disease (any T, any N, M1). One patient refused radical surgery and underwent local excision of his rectal tumor; for this reason he could not be completely staged because there was no lymph node resection. Nine patients (18%) had recurrence of disease during follow-up.

In the cyclin D1 (positive) group, the mean age was 64.8 years (range 37 to 86 years); 20 patients (54%) were male and 17 (46%) were female. Five patients (13.5%) had rectal tumors, 17 (46%) had left-sided tumors, and 15 (40.5%) had right-sided tumors. Thirty tumors (81%) were well differentiated and seven (9%) were moderately differentiated. Fifteen patients (40.5%) had stage I disease (T1-2N0), 10 (27%) had stage II disease (T3-4N0), 11 (30%) had stage III disease (anyTN1-2), and six (2.5%) had stage IV disease (any T, any N, M1). None of the patients in this group had a recurrence of disease during follow-up.

Results of immunostaining were categorized as positive or negative but also as an approximate percentage of positive tumor cells within the tumor. In the group of patients considered cyclin D1 negative, the mean number of positive cells was 3.3%. In the group of patients considered cyclin D1 positive, the mean number of positive cells at immunostaining was 23.5%. These differences were statistically significant ($P < 0.001$).

When results from both groups were compared, there was no significant statistical difference in mean age, sex distribution, tumor grade differentiation, presence of lymphovascular invasion, TNM distribution, or recurrence rates. Curiously our results showed a trend in cyclin D1–positive tumors toward proximal locations (nonrectal tumors) (see Table 1).

Cyclin G

Eight tumors (9%) were considered negative (<10% of tumor cells with nuclear staining) for cyclin G and 79 (91%) were considered positive (>10%) (see Table 2). In the cyclin G (negative) group the mean age was 64.1 years (range 49 to 91 years); four patients (50%) were male and four (50%) were female. One patient (12.5%) had a rectal tumor, four (50%) had left-sided tumors, and three (37.5%) had right-sided tumors. Five tumors (62.5%) were well differentiated and three (37.5%) were moderately differentiated. One patient (12.5%) had stage I disease (T1-2N0), six (75%) had stage II disease (T3-4N0), and one (12.5%) had stage III disease (anyTN1-2). One patient (1.2%) had a recurrence of disease during follow-up.

In the cyclin G (positive) group the mean age was 64.8 years (range 37 to 86 years); 40 patients (50.5%) were male and 39 (49.5%) were female. Twenty-eight patients (35.5%) had rectal tumors, 24 (30.5%) had left-sided tumors, and 27 (34%) had right-sided tumors. Twenty-five tumors (31.5%) were well differentiated, 52 (66%) were moderately differentiated, and two were poorly or undifferentiated. Thirty patients (38%) had stage I disease (T1-2N0), 16 (20%) had stage II disease (T3-4N0), 25 (32%) had stage III disease (anyTN1-2), and seven (9%) had stage IV disease (any T, any N, M1). One patient refused radical surgery and underwent local excision of a rectal tumor; this patient could not be completely staged because there was no lymph node resection. Eight patients (10%) in this group had recurrent disease during follow-up.

Immunostaining results were categorized as positive or negative but also as approximate percentages of positive tumor cells within the tumor. In the group of patients considered cyclin G negative, the mean number of positive cells was 2.1%. In the group of patients considered cyclin G positive, the mean number of positive cells at immunostaining was 73.1%. These differences were statistically significant ($P < 0.001$).

When the results of the two groups were compared, there was no significant statistical difference in mean age, sex distribution, tumor grade differentiation,

presence of lymphovascular invasion, TNM distribution, or recurrence rates. Curiously our results showed a significant trend in cyclin G–negative tumors toward stage II disease (6 patients, 75%), even though there were no siginifcant differences between T, N, and M results separately. Furthermore, it should be emphasized that cyclin G–negative staining was a very infrequent event (9% of all cases), and thus even a small number of patients may be responsible for this difference. In a study with a greater number of patients, this trend may or may not be reproduced.

Comparison of the two cyclins together shows that 33 patients (38%) were positive for cyclins D1 and G, whereas 83 patients (95.4%) were positive for cyclin D1 or G (Table 3).

DISCUSSION

Recent studies suggest that altered expression of cyclins may be involved in human cancer development, including cyclins A, D1, E, and G.[2,3] Cyclin D1 is a major regulator of cell cycle progression, especially at the G1 checkpoint, and is regarded as an oncogene that induces malignant transformation.[9–11] This cyclin appears to be important in overcoming the restriction point at the G1-S transition by activating cyclin D–dependent kinases, which in turn phosphorylates the retinoblastoma protein.[12] Clinical observations have revealed that cyclin D1 overexpression is present in human cancers such as breast, esophageal, and colon cancers.[5,13–15] Previous studies have reported that cyclin D1 overexpression appears to be present in 20% to 30% of colon adenocarcinomas.[5] Furthermore, overexpression of cyclin D may be an early event in multistage colorectal carcinogenesis.[5]

Cyclin G was recently identified as being overexpressed in breast and prostate cancer cells, including early or in situ carcinomas.[8] Identification of cyclin G overexpression in a variety of cancer types suggests that cyclin G may possess or correlate with oncogenic potential.[16–18] Furthermore, cyclin G antisense appears to inhibit tumor growth in vitro and in a nude mouse model in vivo.[18] However, cyclin G has also been identified as a transcriptional target for p53, but in contrast to other p53-regulated genes it appears to behave as others cyclins or protooncogenes rather than as a tumor suppressor gene.[19,20] Despite its homology to other cyclins such as cyclin A and I, cyclin G has not been matched with its cyclin-dependent kinase partner, and its biological function remains elusive.[8]

To determine the relevance of cyclin G and D1 overexpression in human colorectal cancer, we reviewed surgically resected specimens and examined the pattern of their expression by means of immunohistochemical analysis. Staining of both antibodies appeared to be restricted to the nuclei in the malignant cells of the specimen, whereas normal cells exhibited scant or even absence of staining.

In our study cyclin D1 overexpression was found to be present in approximately 44% of colorectal adenocarcinomas, which is even higher than in other reported studies. We observed a significantly higher rate of overexpression in tumors located in the colon (right and left) compared to rectal tumors. There was no correlation between cyclin D1 overexpression and other clinicopathologic characteristics such as age, sex, tumor differentiation, TNM or stage classification, recurrence, and survival.

Approximately 91% of colorectal tumors had cyclin G overexpression. Furthermore, it appears that this may be an early event in colorectal carcinogenesis because it was present in early cancers and even in tubulovillous adenomas. This impressive rate of overexpression in transformed human colorectal specimens suggests that cyclin G is deregulated during oncogenesis. There was no correlation between cyclin G overexpression and clinicopathologic characteristics such as age, sex, tumor location, tumor differentiation, recurrence, and survival. Because cyclin G overexpression was a frequent event in this study, the cyclin-negative group was very small. For this reason it may be inadequate to assume that there is no association between cyclin G overexpression or normal expression with clinical and pathologic features.

We observed a curious association between cyclin G (−) staining and stage II disease, but the same argument of small numbers applies, because six out of eight patients in this group had stage II disease. There was no association between cyclin G overexpression pattern, and T, N, and M status were considered separately. Once cyclin G overexpression appears to be frequent in colorectal cancer, it may be very difficult to establish these associations with clinical and pathologic results, and thus cyclin G may not be very useful as a prognostic and diagnostic tool. However, such a frequent event in colorectal tumors may be useful for therapeutic measures such as a gene target for gene therapy, implying that the great majority of patients with the disease could benefit from this treatment strategy.

Table 3. Comparison of cyclin G and D1 status

	Cyclin D (−)	Cyclin D(+)	N
Cyclin G−	3 (3%)	5 (6%)	8 (9%)
Cyclin G+	46 (53%)	33 (38%)	79 (91%)
N	49 (56%)	38 (44%)	87 (100%)

CONCLUSION

The present study demonstrates that cyclin G overexpression is a very frequent event in colorectal cancer tissues. Observation of cyclin G overexpression in early tumors or even in colorectal adenomas may prove to be an important marker for tumor initiation. This very frequent event in colorectal carcinogenesis may pose additional options for new therapeutic approaches. Additional therapies could be directed at downregulating cyclin G in colorectal cancer, or using cyclin G as a target for site-specific gene therapy aimed at colorectal cancer.

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A Better Cell Cycle Target for Gene Therapy of Colorectal Cancer: Cyclin G

Rodrigo Perez, M.D., Nancy Wu, M.D., Adam A. Klipfel, M.D., Robert W. Beart, Jr., M.D.

The purpose of this study was to evaluate the overexpression of cyclin G in colorectal neoplasia, which may be a more frequent event than cyclin D1 during the cell cycle and thus may have a more enhanced therapeutic potential in treating colorectal cancer. Ninety formalin-fixed, paraffin-embedded human colon and rectal specimens were obtained from the Pathology Department of Norris Cancer Center/ University of Southern California. The tissues had been obtained after surgical resection between 1995 and 2001, and had been processed by routine clinical histopathologic methods. Ninety-one percent of colorectal tumors had cyclin G overexpression. These cyclin-positive patients were evenly distributed between men and women, and between tumor locations, that is, 36% rectal tumors and 34% right-sided tumors. Thirty-two percent were well differentiated, and 66% were moderately differentiated. Thirty patients (38%) had stage I disease, 16 (20%) had stage II disease, 25 (32%) had stage III, and seven (9%) had stage IV disease. Eight patients (10%) in this group had recurrent disease during follow-up. There was no correlation between cyclin G overexpression and clinical and pathologic characteristics. Cyclin D1 overexpression was found to be present in only 42% of colorectal adenocarcinomas. There was no correlation between cyclin D1 overexpression and clinical and pathologic characteristics. The present study demonstrates that cyclin G overexpression is a frequent event in colorectal cancer. This frequent event in colorectal carcinogenesis may facilitate new therapeutic approaches acting as a target for gene therapy, possibly directed at downregulating cyclin G in colorectal cancer. (J GASTROINTEST SURG 2003;7:884–889)

KEY WORDS: Colon cancer, cancer epidemiology

Human cancer is the end result of multiple molecular events determined by genetic alterations giving rise to a cell or a population of cells with altered proliferation, regulation of cell cycle, and differentiation characteristics.[1]

The cell cycle is a highly organized process regulated by cyclins, cyclin-dependent kinases (CDK), and cyclin-dependent kinase inhibitors (CKI).[2] These are prime cell cycle regulators and control the major checkpoints in cell cycle transition of eukaryotic cells. This complex mechanism ensures that there is complete and accurate replication of the cell before division by maintaining the orderly progression of cells through the various phases of the cell cycle. Mutations and/or altered expression of cyclins may be involved in states of altered proliferation, such as neoplasia.[2–4]

Currently 14 cyclins have been identified (cyclins A to J), and they share a common 100 amino acid series known as the cyclin box. The concentrations of different cyclins oscillate throughout the cell cycle activating specific kinases involved in the various phases of the cell cycle.[2,4]

Cyclin D1 appears to be involved in the early phase of cell cycle progression. As a result of stimulation of the cell in the G0 or early G1 phase to initiate progression through the cycle, there is a rise in the concentration of cyclins D and E known as the G1 cyclins (because of their location within the cell cycle).[2]

Overexpression of cyclin D1 is observed in several types of tumors, such as esophageal, liver, breast, and colorectal. In colorectal cancer, increased expression of cyclin D1 appears to be an early event in the

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process of multistage carcinogenesis occurring in approximately 30% to 50%.[5] Furthermore, mutations in the APC gene, often found in cancers of various organs including the large bowel, may result in cytoplasmic accumulation of β-catenin. In the cytoplasm, β-catenin binds to a transcription factor (TCF/LEF) and is transferred to the nucleus. The cyclin D1 gene may be a target for β-catenin resulting in its overexpression in colon cancer.[5–7]

Cyclin G, a recent addition to the cyclin family, is the only known cyclin that is transcriptionally activated by the p53 tumor suppressor gene, suggesting that it may play a role in p53-mediated cell growth control.[8] In contrast to most p53 target genes such as p21/Waf1/Cip1/Sdi1, Bax1 and others, cyclin G does not seem to exert a tumor-suppressive role but rather, similar to other cyclins or protooncogenes, plays a growth-promoting role. Recently observations of cyclin G overexpression in breast and prostate cancer were reported.[8] In these tumors, cyclin G was overexpressed independent of p53 status, and there were no changes in the level of their expression throughout the cell cycle. However, like other members of the cyclin family, cyclin G expression is gradually increased with cell cycle progression, peaking in the late G2/M phase as observed in normal human mammary epithelial cells.[8]

To determine the presence and frequency of cyclin G and D overexpression in colorectal cancer, we used immunohistochemistry to analyze 87 previously resected human colorectal adenocarcinomas.

MATERIAL AND METHODS

Tissue Samples

Eighty-seven formalin-fixed, paraffin-embedded human colon and rectal adenocarcinoma specimens were obtained from the Department of Pathology, Norris Cancer Center/Keck School of Medicine (Los Angeles, CA). These tissues had been obtained after surgical resections performed between 1995 and 2001 for sporadic colorectal cancer and had been processed at that time by routine clinical histopathologic methods. All patients with hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, or other hereditary syndromes for colorectal cancer were excluded.

Previous pathologic reports were reviewed for degree of differentiation, tumor node metastasis (TNM) stage, lymphovascular invasion, and location within the colon (right, left, and rectum). Tumor and patient characteristics are summarized in Tables 1 and 2. Normal colonic or rectal tissues were also reviewed from each tumor sample for immunostaining comparison.

Antibodies

The following antibodies were used for immunohistochemical analyses in this study: mouse monoclonal antibody against human cyclin G (Vector Laboratories, Burlingame, CA), and mouse monoclonal anti-human cyclin D1 antibody (Vector Laboratories).

Immunohistochemical Analysis

Formalin-fixed and paraffin-embedded tumor samples were sliced 5 μm thick on positively charged glass slides, deparaffinized in xylene, rehydrated, and then subjected to antigen retrieval using 0.01 mol/L citrate buffer solution at pH 5-6 (Biogenex, San Ramon, CA). For cyclin D1, it is heated in a microwave for 5 + 5 minutes. For cyclin G, it is heated in a pressure cooker inside the microwave for 30 minutes (15 minutes on high power; 15 minutes at 40% power). After antigen retrieval, the slides are cooled down to room temperature. Slides intended

Table 1. Patient characteristics and cyclin D1 status

	Cyclin D (−)	Cyclin D (+)	*P* value
% of positive cells (mean)	3.3	23.5	<0.001
Characteristics			
N	49 (56%)	38 (44%)	
Mean age (yr)	63.8 (range 25–92)	64.8 (range 37–86)	
Sex			
Female	27 (54%)	17 (46%)	0.29
Male	23 (46%)	20 (54%)	
Tumor location			
Rectum	24 (48%)	5 (13.5%)	0.002
Left colon	11 (22%)	17 (46%)	
Right colon	15 (30%)	15 (40.5%)	
Tumor differentiation			
Well	23 (46%)	30 (81%)	0.67
Moderate	25 (50%)	7 (9%)	
Poor or undifferentiated	2 (4%)	0	
Staging			
Stage I	16 (32%)	15 (39%)	0.39
Stage II	13 (24%)	11 (29%)	
Stage III	14 (30%)	11 (29%)	
Stage IV	6 (12%)	1 (3%)	
TOTAL	49 (100%)	38 (100%)	

Table 2. Patient characteristics and cyclin G status

	Cyclin G (−)	Cyclin G (+)	*P* value
% of positive Cells (mean)	2.1	73.1	<0.001
Characteristics			
N	8 (9%)	79 (91%)	
Mean age (yr)	64.1 (range 49–91)	64.2 (range 25–92)	
Sex			
Female	4 (50%)	39 (49%)	0.63
Male	4 (50%)	40 (50%)	
Tumor location			
Rectum	1 (12.5%)	28 (35%)	0.38
Left colon	4 (50%)	24 (30%)	
Right colon	3 (37.5%)	27 (35%)	
Tumor differentiation			
Well	5 (62.5%)	25 (31%)	0.99
Moderate	3 (37.5%)	52 (66%)	
Poor or undifferentiated	0	2 (3%)	
Staging			
Stage I	1 (12.5%)	30 (38%)	0.005
Stage II	6 (75%)	16 (20%)	
Stage III	1 (12.5%)	26 (33%)	
Stage IV	0	7 (9%)	
TOTAL	8 (100%)	79 (100%)	

for cyclin D1 are then pretreated with proteinase (IP Enzyme, Ventana Medical Systems, Inc., Tucson, AZ) for 10 minutes. Immunostaining of sections was performed by the streptavidin-biotin peroxidase complex (ABC) method using the Vectastain Elite ABC kit (Vector Laboratories). In each stain the primary antibody was employed at 1:20 dilution. The incubation time for primary antibody was 1 hour for cyclin D1 and overnight for cyclin G, both at room temperature. After incubation with primary antibody, staining was completed using the ABC-based system (biotinylated horse antimouse antibody was used as secondary antibody with diaminobenzidine [DAB] as chromogen). Finally, the slides were counterstained with hematoxylin and mounted for examination.

Interpretation of Immunohistochemical Staining

Normal prostate tissue was used as positive control specimens, whereas normal colonic mucosa was used as negative control samples (Figs. 1 and 2). Tumors were classified as positive (+) when more than 10% of cancer cells showed nuclear staining for cyclin G or cyclin D1; tumors were classified as negative (−) when less than 10% of cancer cells showed such a staining pattern. The cutoff lines for these antibodies were commonly used in previous studies.[5,6] All immunostained sections were evaluated in a coded manner without knowledge of the clinical and pathologic background of the patients. Two separate observers (R.P. and N.W.) performed the assessment of the staining independently.

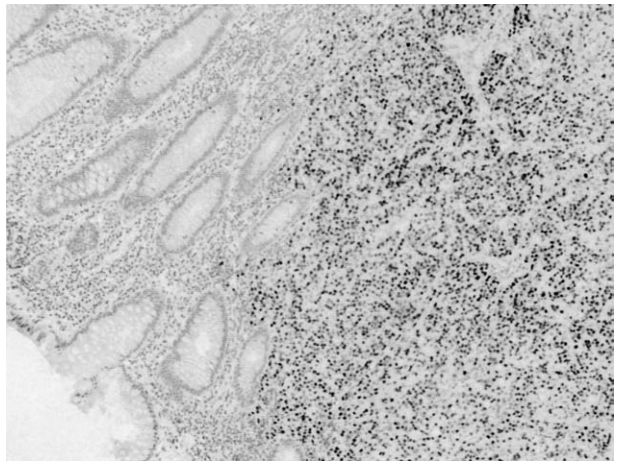

Fig. 1. Cyclin D1–staining tumor with adjacent normal mucosa.

Statistical Analysis

Pearson's chi-square test was used to compare frequencies among the groups to determine the association between immunohistologic results and clinicopathologic features in categorical variables.

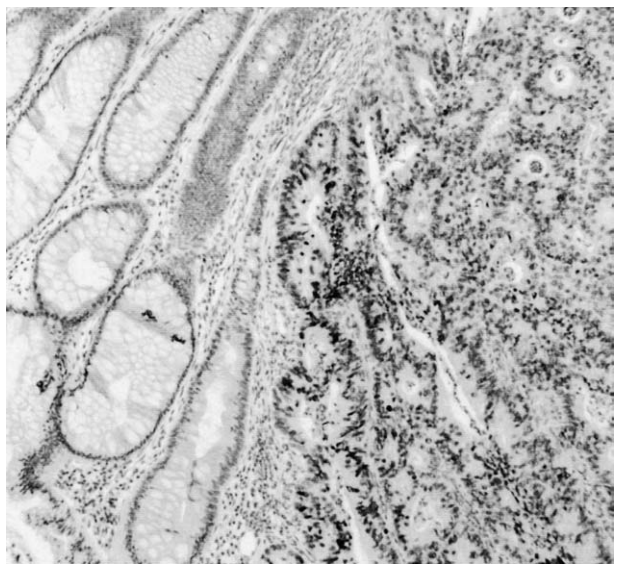

Fig. 2. Cyclin G–staining tumor with adjacent normal mucosa.

Student's *t* test was used to compare mean immunohistologic results and numerical variables. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Ninety pathology reports from patients with previously resected colorectal adenocarcinomas were reviewed. In three cases there was insufficient tumor tissue for immunohistologic study, and these were excluded. Eighty-seven patients were eligible for immunohistologic studies using cyclin G and D1 antibodies.

Cyclin D

Forty-nine tumors (56%) were considered negative (<10% of tumor cells with nuclear staining) for cyclin D1, and 38 (44%) were considered positive (> 10%). In the cyclin D1 (negative) group the mean age was 63.8 years (range 25–92 years); 23 patients (46%) were male and 27 (54%) were female. Twenty-four patients (48%) had rectal tumors, 11 (22%) had left-sided tumors, and 15 (30%) had right-sided tumors. Twenty-three tumors (46%) were well differentiated, 25 (50%) were moderately differentiated, and two (4%) were poorly or undifferentiated. Sixteen patients (32%) had stage I disease (T1-2N0), 12 (24%) had stage II disease (T3-4N0), 15 (30%) had stage III (anyTN1-2), and six (12%) had stage IV disease (any T, any N, M1). One patient refused radical surgery and underwent local excision of his rectal tumor; for this reason he could not be completely staged because there was no lymph node resection. Nine patients (18%) had recurrence of disease during follow-up.

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Cyclin G

Eight tumors (9%) were considered negative (<10% of tumor cells with nuclear staining) for cyclin G and 79 (91%) were considered positive (>10%) (see Table 2). In the cyclin G (negative) group the mean age was 64.1 years (range 49 to 91 years); four patients (50%) were male and four (50%) were female. One patient (12.5%) had a rectal tumor, four (50%) had left-sided tumors, and three (37.5%) had right-sided tumors. Five tumors (62.5%) were well differentiated and three (37.5%) were moderately differentiated. One patient (12.5%) had stage I disease (T1-2N0), six (75%) had stage II disease (T3-4N0), and one (12.5%) had stage III disease (anyTN1-2). One patient (1.2%) had a recurrence of disease during follow-up.

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DISCUSSION

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To determine the relevance of cyclin G and D1 overexpression in human colorectal cancer, we reviewed surgically resected specimens and examined the pattern of their expression by means of immunohistochemical analysis. Staining of both antibodies appeared to be restricted to the nuclei in the malignant cells of the specimen, whereas normal cells exhibited scant or even absence of staining.

In our study cyclin D1 overexpression was found to be present in approximately 44% of colorectal adenocarcinomas, which is even higher than in other reported studies. We observed a significantly higher rate of overexpression in tumors located in the colon (right and left) compared to rectal tumors. There was no correlation between cyclin D1 overexpression and other clinicopathologic characteristics such as age, sex, tumor differentiation, TNM or stage classification, recurrence, and survival.

Approximately 91% of colorectal tumors had cyclin G overexpression. Furthermore, it appears that this may be an early event in colorectal carcinogenesis because it was present in early cancers and even in tubulovillous adenomas. This impressive rate of overexpression in transformed human colorectal specimens suggests that cyclin G is deregulated during oncogenesis. There was no correlation between cyclin G overexpression and clinicopathologic characteristics such as age, sex, tumor location, tumor differentiation, recurrence, and survival. Because cyclin G overexpression was a frequent event in this study, the cyclin-negative group was very small. For this reason it may be inadequate to assume that there is no association between cyclin G overexpression or normal expression with clinical and pathologic features.

We observed a curious association between cyclin G (−) staining and stage II disease, but the same argument of small numbers applies, because six out of eight patients in this group had stage II disease. There was no association between cyclin G overexpression pattern, and T, N, and M status were considered separately. Once cyclin G overexpression appears to be frequent in colorectal cancer, it may be very difficult to establish these associations with clinical and pathologic results, and thus cyclin G may not be very useful as a prognostic and diagnostic tool. However, such a frequent event in colorectal tumors may be useful for therapeutic measures such as a gene target for gene therapy, implying that the great majority of patients with the disease could benefit from this treatment strategy.

Table 3. Comparison of cyclin G and D1 status

	Cyclin D (−)	Cyclin D(+)	N
Cyclin G−	3 (3%)	5 (6%)	8 (9%)
Cyclin G+	46 (53%)	33 (38%)	79 (91%)
N	49 (56%)	38 (44%)	87 (100%)

CONCLUSION

The present study demonstrates that cyclin G overexpression is a very frequent event in colorectal cancer tissues. Observation of cyclin G overexpression in early tumors or even in colorectal adenomas may prove to be an important marker for tumor initiation. This very frequent event in colorectal carcinogenesis may pose additional options for new therapeutic approaches. Additional therapies could be directed at downregulating cyclin G in colorectal cancer, or using cyclin G as a target for site-specific gene therapy aimed at colorectal cancer.

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Cystic Pancreatic Neoplasms: Enucleate or Resect?

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Asymptomatic cystic pancreatic neoplasms are being detected by abdominal imaging with increasing frequency. Enucleation of small cystic neoplasms can be performed without recurrence but has been associated with a higher incidence of pancreatic fistula. Thus the procedure has been modified to include intraoperative ultrasound imaging and closure of the pancreatic defect. This analysis was performed to determine whether these modifications have improved operative outcome. Thirty patients with mucinous cystic neoplasms (n = 16), serous cystadenomas (n = 10), and cystic islet cell tumors (n = 4) were studied. Enucleation was performed in 11 patients (7 with mucinous cystic neoplasms, 2 with serous cystadenomas and 2 with islet cell tumors), whereas 19 underwent resection of cystic tumors (pancreatoduodenectomy in 8 and distal pancreatectomy in 11). The mean groups did not differ with regard to age (57 years), gender (73% female), presentation (63% incidental), or site (43% head, neck, or uncinate). Patients undergoing enucleation had smaller tumors (2.2 vs. 4.7 cm, $P < 0.01$) that were less likely to be in the tail (9% vs. 42%). Operative time was significantly shorter in the enucleation group (199 vs. 298 minutes, $P < 0.01$). Blood loss also was significantly reduced in the enucleation group (114 vs. 450 ml, $P < 0.001$). Pancreatic fistula rates (27% vs. 26%) and length of hospital stay (12.6 vs. 15.7 days) were similar in the two groups. Enucleation of benign cystic pancreatic neoplasms reduces operative time and blood loss without increasing postoperative complications or length of stay. Therefore enucleation should be the standard operation for small benign cystic neoplasms in the uncinate, head, neck, and body of the pancreas.
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Key words: Cystadenoma, islet cell tumor, pancreatic cyst

With the introduction of newer sophisticated abdominal imaging techniques, cystic pancreatic neoplasms are being detected with increasing frequency.[1] In the past, most of these patients presented with abdominal pain and/or pancreatitis.[2–4] However, most cystic pancreatic neoplasms are now being detected as incidental findings on abdominal ultrasound imaging, computerized tomography, (CT) scans, and magnetic resonance imaging (MRI). Nevertheless, the nonoperative differentiation among the various pathologic entities including serous cystadenomas, mucinous cystic neoplasms, intraductal papillary mucinous neoplasms, cystic islet cell tumors, and solid and cystic papillary (Hamoudi) tumors remains difficult.[2–8]

Many cystic pancreatic tumors have malignant potential, and therefore resection is recommended by most experts.[2–8] Resection is clearly the treatment of choice for larger lesions, but a potentially more effective method for dealing with small cystic lesions is provided by enucleation. The senior author (H.A.P.) and his colleagues have previously reported that enucleation of mucinous cystic neoplasms can be performed safely without recurrence.[3] When compared to resection, however, enucleation was associated with a higher incidence of pancreatic fistula (50% vs. 12%, $P < 0.05$) and a longer length of hospital stay (19.5 vs. 10.0 days, $P < 0.02$). Over the past 5 years, the indications for enucleation have expanded, and the procedure has been modified to include intraoperative ultrasound imaging and, when possible, closure of the pancreatic defect. This study was performed, therefore, to determine (1) whether these operative modifications have improved outcome and (2) which lesions are most amenable to enucleation.

MATERIAL AND METHODS

Patients

A retrospective analysis of patients undergoing surgery for cystic pancreatic neoplasms at Froedtert Memorial Hospital/Medical College of Wisconsin over

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an 11-year period from March 1992 through February 2003 was performed. Only tumors pathologically determined to be benign were included in this study. Thirty patients were identified: 16 with mucinous cystic neoplasms, 10 with serous cystadenomas, and four with cystic islet cell tumors. Patients with benign or malignant intraductal papillary mucinous neoplasms were not included in this analysis because enucleation was not thought to be adequate therapy. The mean age of the patients was 56.8 years, and 73% were female (Table 1). Enucleation was performed in 11 patients (37%), whereas 19 patients (63%) had their tumors resected. Age and gender were similar in the patients undergoing enucleation and resection. Twenty-three of the operations were performed within the last 4 years including all 11 enucleations (48%).

Presentation and Evaluation

Most of the patients undergoing surgery in this series were asymptomatic (see Table 1). In fact, in two thirds of the patients undergoing resection the cyst was discovered incidentally on abdominal imaging, as compared to 55% in the enucleation group. In the enucleation group, four patients presented with abdominal pain, and one had nausea and fullness. In the resected group, five patients presented with abdominal pain, and one patient with an insulinoma and a history of hypoglycemic episodes presented in a coma. One patient from each group had a history of pancreatitis. Diabetes was evident in four patients, one of whom had enucleation and three of whom underwent resection. One of the patients in the resected group had previously undergone an attempted Whipple procedure. Another patient in the resection group had a history of pseudocyst and had undergone a cyst jejunostomy.

Table 1. Patient characteristics, symptoms, and surgery

	Enucleation	Resection	Total
Number of patients	11	19	30
Mean age (yr)	53.0	58.8	56.8
Range	38–65	30–82	30–82
Female (%)	73	74	73
Symptoms			
Asymptomatic (%)	55	68	63
Abdominal pain (%)	36	26	30
Resection type			
Enucleation	11	0	37
Distal pancreatectomy	0	11	37
Pancreatoduodenectomy	0	8	26

All 30 patients had CT scans (Figs. 1 and 2), whereas MRI was performed in only two patients. Seven patients underwent preoperative endoscopic retrograde cholangiopancreatography (ERCP): five from the enucleation group and two from the resected group. Five patients underwent preoperative endoscopic ultrasonography and fine-needle aspiration, two from the enucleation group and three from the resected group. One patient from the resected group also underwent preoperative angiography early in the series.

Surgery

In the enucleation group, intraoperative ultrasound imaging was employed routinely to assess the relationship between the pancreatic duct and the cystic lesion. In addition, the pancreatic defect after enucleation was closed in 8 (73%) of the 11 patients. Closure was usually performed with one or two "figure-of-eight" 3-0 absorbable sutures, taking care not to injure the pancreatic duct. One defect was closed with fibrin glue. A closed suction drain was placed near the defect or closure in all patients. One surgeon (H.A.P.) performed 9 (82%) of the 11 enucleation procedures. One patient in the enucleation group underwent a concurrent cholecystectomy.

Of the 19 patients who underwent resection, 11 had a distal pancreatectomy (7 [64%] with splenic preservation), seven had a pylorus-preserving pancreatoduodenectomy, and one had a standard pancreatoduodenectomy (see Table 1). Six of these patients (32%) had concurrent procedures including splenectomy in four, sigmoid colectomy for recurrent diverticulitis in one, and resection of a splenic artery aneurysm in one patient who required a pancreatoduodenectomy. One distal pancreatectomy and splenectomy was performed as a laparoscopic hand-assisted procedure, whereas one distal pancreatectomy and colectomy was performed laparoscopically. Intraoperative ultrasonography was employed in 6 (32%) of 19 resected patients. Closed suction drains were used routinely. Octreotide or vapreotide was used at the discretion of the surgeon or in accordance with a randomized trial. Approximately one third of the patients in each group received a somatostatin analogue.

Cyst Characteristics

Information on cyst size, location, and type is presented in Table 2. Cysts that were enucleated were significantly smaller ($P < 0.01$) when measured either by CT or by pathologic examination. Ten (91%) of 11 enucleated cysts were in the uncinate, head, neck, or body of the pancreas compared to 13 (58%) of 19 resected cysts. This difference did not quite reach

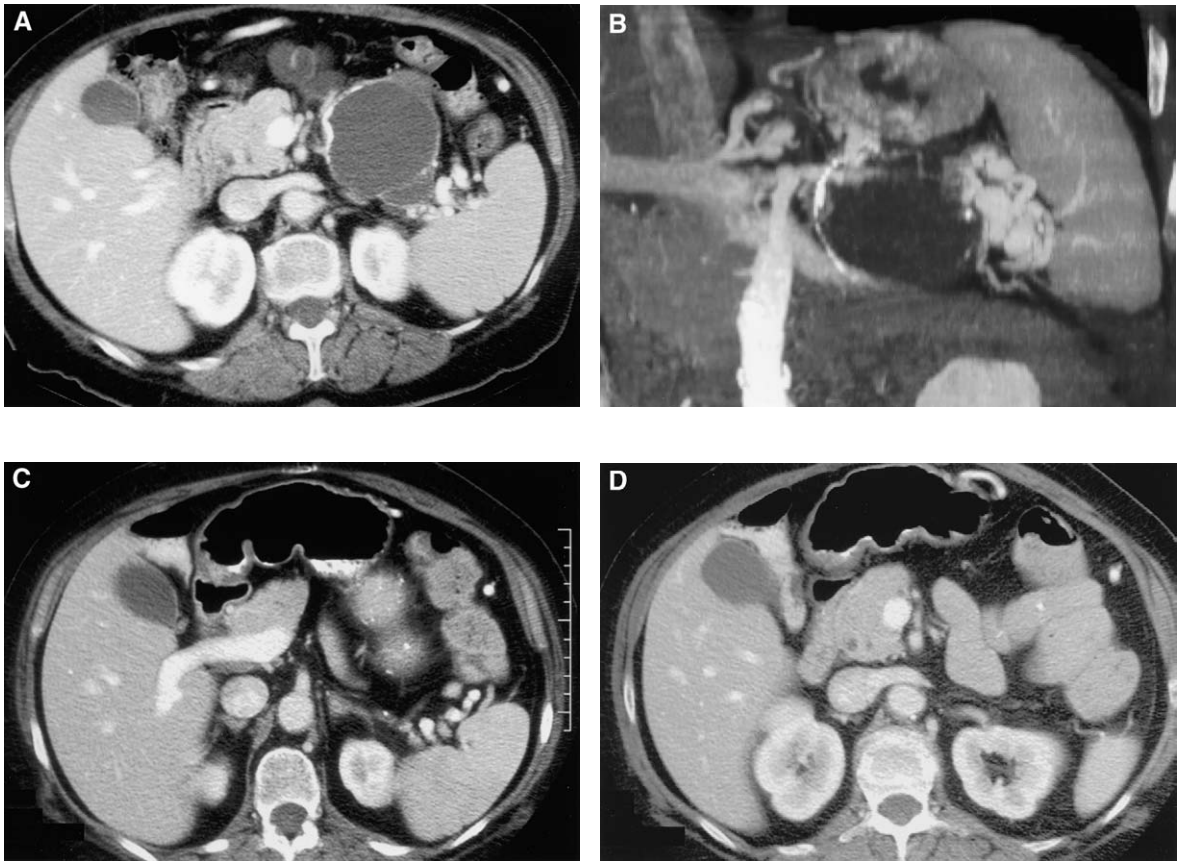

Fig. 1. A, CT scan demonstrating a 6.0 cm calcified mucinous cystic neoplasm in the tail of the pancreas in a 63-year-old woman. **B,** Three-dimensional reconstruction in the same patient demonstrating splenic vein compression and multiple venous collateral vessels. **C,** CT scan 3 years after distal pancreatectomy demonstrating pancreatic neck and preserved spleen. **D,** Lower cut from the same CT scan 3 years after surgery demonstrating a normal head of the pancreas.

statistical significance ($P < 0.08$). All specimens were reviewed by one pathologist (R.A.K.). Sixteen (53%) of the 30 cysts were mucinous cystic neoplasms, 10 were serous cystadenomas (33%), and four were cystic islet cell tumors (13%). Relatively more of the enucleated cysts (64%) were mucinous cystic neoplasms, but the distribution of cyst type between the two groups was not statistically different.

Morbidity

Morbidity was characterized as pancreatic fistula formation, postoperative pancreatitis, or any other major complication. Pancreatic fistula was defined as drainage of more than 50 ml of amylase-rich fluid through operatively placed drains on or after postoperative day 7. Postoperative pancreatitis was defined as more than threefold elevation of serum amylase over the upper limit of normal as well as CT evidence of peripancreatic inflammation.

Follow-Up and Statistics

Follow-up and survival information was obtained from clinic notes, hospital records, and the social security database. Data are presented as means ± standard error of the mean (SEM). Statistical analyses were performed using Student's *t* test or the Mann-Whitney rank sum test where appropriate, with statistical significance achieved at $P < 0.05$.

RESULTS

Operative Data

The mean operative time in the enucleation group was significantly shorter than in those who had resections (199 ± 16 vs. 298 ± 27 minutes) ($P < 0.01$; Fig. 3). Also, significantly less blood loss occurred in patients who underwent enucleation (114 ± 14 vs. 450 ± 120 ml, $P < 0.01$; see Fig. 3).

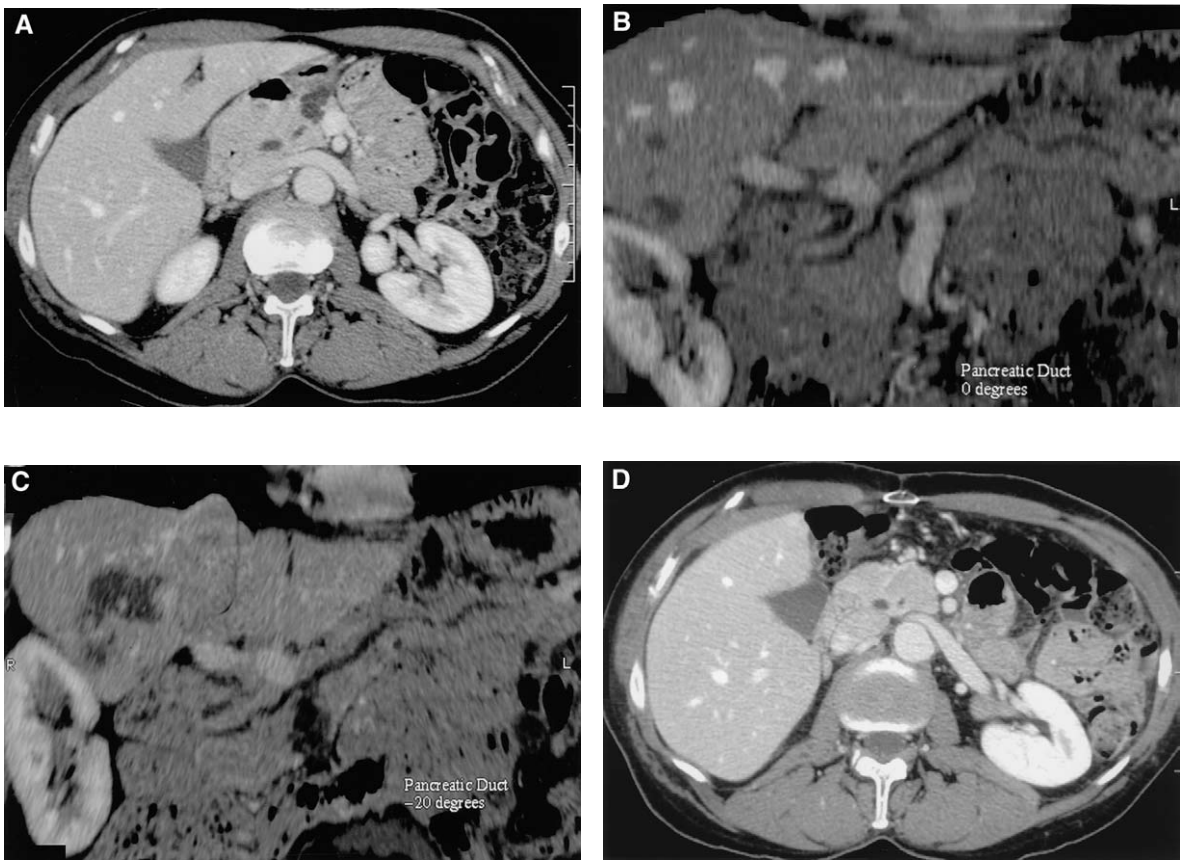

Fig. 2. A, CT scan demonstrating a 2.4 cm cystic mass in the neck of the pancreas in a 56-year-old woman. **B,** CT pancreatogram (0 degrees) showing the cyst adjacent to the superior mesenteric vein. **C,** CT pancreatogram (−20 degrees) showing the cyst in communication with the pancreatic duct. **D,** CT scan 7 months after enucleation demonstrating the preserved pancreas and a normal pancreatic duct.

Morbidity

The incidence of pancreatic fistulas and all complications are presented in Fig. 4, which demonstrates no significant differences between the enucleation and resection groups. The incidence of pancreatic fistulas was 27% and 26%, respectively. In the enucleation group, three patients developed pancreatic fistulas, and one had a partial small bowel obstruction for an overall complication rate of 36%. In the resection group five patients developed pancreatic fistulas, and four additional patients had an abdominal abscess, gastric outlet obstruction, pneumonia, and a urinary tract infection. Thus, the overall complication rate in the resected patients was 47%, which was not significantly greater than in the enucleated patients.

The median and mean postoperative length of stay in the enucleated group were 9 days and 12.6 ± 2.8 days, respectively. The median and mean length of stay in the resected patients were 14 days and 15.7 ± 2.5 days, respectively. These differences between groups were not statistically different. One patient in the enucleation group with a pancreatic fistula had a pancreatic stent placed after discharge. Two patients in the resected group had late complications including a small bowel obstruction at 4 months and a pseudocyst at 9 months postoperatively. Both of these patients were managed with open operations and have now been well for 7 months and 7 years, respectively.

Follow-Up

The mean follow-up time for all patients was 38.4 months and ranged from 1 to 122 months. All patients, except for the most recently enucleated patient, underwent follow-up CT scanning. None of the patients have had a recurrence of their benign cystic tumors on follow-up CT scans (see Figs. 1 and 2, and Fig. 5). All 30 patients are alive, and none of the patients have developed pancreatic exocrine or new endocrine insufficiency.

Table 2. Cyst size, location, and type

	Enucleation	Resection	Total
Size (cm)			
CT	2.2 ± 0.31*	4.7 ± 0.64	3.7 ± 0.47
Pathologic examination	1.8 ± 0.32*	4.5 ± 0.84	3.5 ± 0.59
Location (%)			
Head†	45	42	43
Body	45	16	27
Tail	9	42	30
Type (%)			
Mucinous cystic neoplasm	64	47	53
Serous cystoadenoma	18	37	33
Islet cell tumor	18	11	13

*$P < 0.01$ vs. resection.
†Head = uncinate, head, and neck.

DISCUSSION

In this series of 30 patients who underwent surgery for benign pancreatic tumors, 11 patients were managed by enucleation, whereas 19 had pancreatic resection. The two groups were similar with respect to age, gender, and presenting symptoms. According to CT scan measurements cysts that were enucleated were smaller (2.2 vs. 4.7 cm, $P < 0.01$). Ten (91%) of 11 enucleated cysts were in the uncinate, head, neck, or body of the pancreas compared to 13 (58%) of 19 resected cysts ($P < 0.08$). Cyst type was similar in the two groups with the majority (53%) being mucinous cystic neoplasms. Both operative time ($P < 0.01$) and blood loss ($P < 0.001$) were less in the enucleated patients. Pancreatic fistula rates and length of hospital stay were similar in the two groups. This analysis confirms that operative modifications have improved outcomes compared to an earlier report[3] and further defines which lesions are most amenable to enucleation.

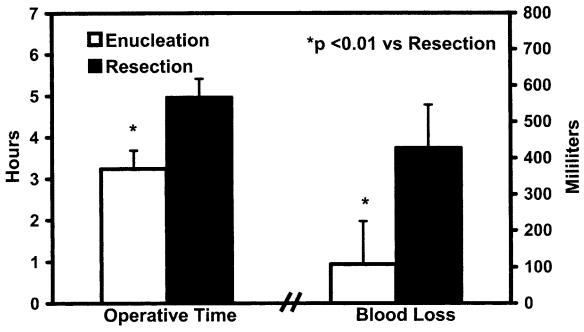

Fig. 3. Operative time and blood loss in patients undergoing enucleation and resection.

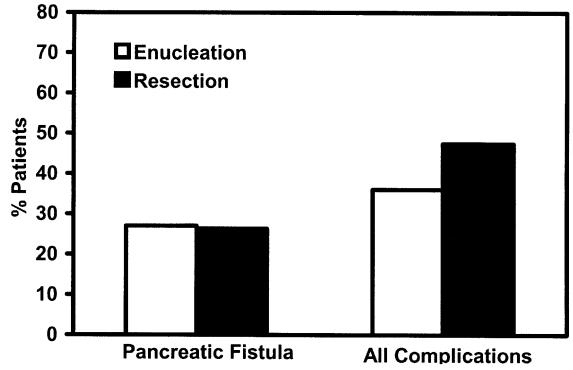

Fig. 4. Pancreatic fistula and all complications in patients undergoing enucleation and resection.

In the 1998 report by Talamini et al.,[3] the patients who were studied were similar to those in the present analysis with respect to age, gender, and cyst location. However, in the earlier series all cysts were mucinous cystic neoplasms, whereas in the present analysis 46% of the cysts were serous cystadenomas (33%) or cystic islet cell tumors (13%). In addition, in the present series enucleated cysts were smaller (2.2 vs. 2.8 cm), whereas resected cysts were larger (4.7 vs. 3.6 cm). Interestingly, more patients in the present series were asymptomatic and had their cysts discovered incidentally on abdominal imaging (63% vs. 14%).

The major operative modifications from the earlier series to the present study were the introduction of intraoperative ultrasound imaging to identify the pancreatic duct and closure of the pancreatic defect after enucleation. In both analyses, enucleation was associated with a shorter operative time than resection. In the present series, the operative time for enucleation decreased 73 minutes from the earlier report (199 vs. 272 minutes). In addition, in the present report operative blood loss for enucleation decreased 95 ml compared to the earlier report (114 vs. 209 ml). Moreover, the pancreatic fistula rate in the recent series decreased by 23% compared to the initial report (27% vs. 50%). As a result, the hospital stay was decreased by 6.9 days (12.6 vs. 19.5 days), and the median hospital stay in the present report was only 9 days. These many improvements in outcome suggest that a learning curve is associated with this operation, which may be on the order of 10 pancreatic cyst enucleations. Finally, none of the enucleated cysts have recurred in the two series with follow-up now extending more than 12 years in the initial patient.

For cystic tumors in the tail of the pancreas, resection remains the operation of choice. In our opinion

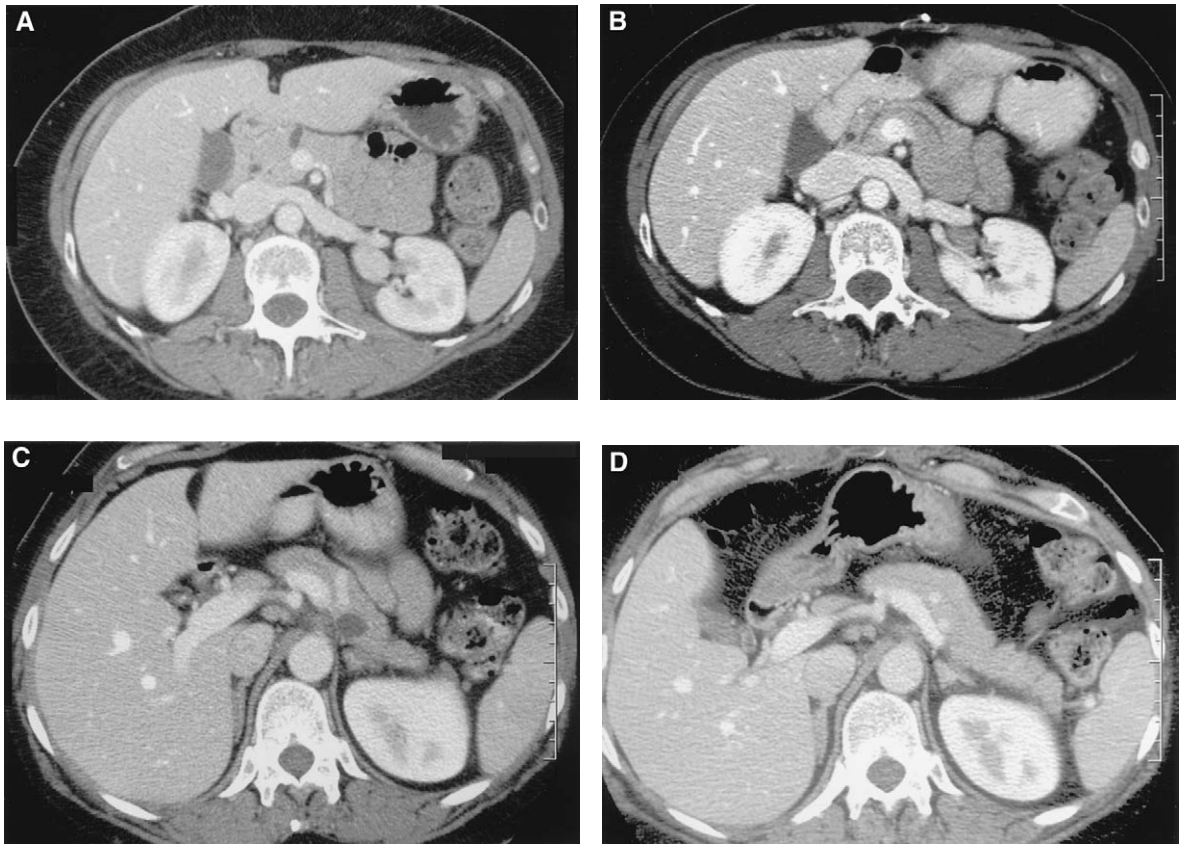

Fig. 5. A, CT scan demonstrating a 2.0 cm mucinous cystic neoplasm in the neck of the pancreas in a 51-year-old woman. **B,** CT scan 14 months after enucleation demonstrating the preserved pancreas without recurrence. **C,** CT scan demonstrating a 1.5 cm mucinous cystic neoplasm in the body of the pancreas in a 47-year-old woman. **D,** CT scan 12 months after enucleation demonstrating preserved pancreas and spleen.

splenic preservation should be attempted in patients suspected to have a benign lesion in the pancreatic tail.[9] In the present series, splenic preservation was achieved in 50% of the patients compared to only 26% in the earlier report.[3] Splenic preservation can be carried out even when the splenic vein is compromised by the cyst (see Fig. 1). Laparoscopic distal pancreatectomy is another good option for small cystic lesions in the tail.[10] Again, splenic preservation should be attempted and avoids the need to extend a port incision to remove the spleen.

For small cystic tumors in the uncinate, head, neck, and body of the pancreas, we believe that enucleation has advantages over pancreatic resection with respect to operative time, blood loss, and preservation of pancreatic parenchyma. Because the pancreas is otherwise normal in these patients, the risk of pancreatic fistula is high. However, the morbidity associated with a pure pancreatic fistula that may occur after an enucleation is generally less than that of a fistula from a pancreatic-enteric anastomosis after a pancreatoduodenectomy. Segmental resection of the neck or body of the pancreas is another option for some of these patients but also has the disadvantage of a pancreatojejunostomy or pancreatogastrostomy with a normal pancreas. Similarly, a Beger[11] or a Frey[12] operation might be considered for lesions in the uncinate, head, or neck, but performing these procedures in a normal pancreas is likely to be fraught with difficulty as well as a high rate of pancreatic fistula. Moreover, performance of a pancreatoduodenectomy with a normal pancreas and bile duct has an increased risk of leakage at the biliary anastomoses.[4,13–16]

Most of the tumors that were enucleated in the present and in the earlier series[3] were mucinous cystic neoplasms. All of these mucinous cystic neoplasms have been benign on frozen section and final pathologic examination. One potential concern is that an enucleation might be performed for a malignant mucinous cystic neoplasm. However, in our experience,

when enucleation is possible, the lesion is benign and enucleation has not been feasible for malignant mucinous cystic neoplasms or cystadenocarcinomas. Another potential dilemma is whether a side-branch intraductal papillary mucinous neoplasm might be enucleated, and if so, would this operation be adequate?[5–7] This possibility is real because both side-branch intraductal papillary mucinous neoplasms and mucinous cystic neoplasms may communicate with the main pancreatic duct (see Fig. 2, *C*). In our experience, most side-branch intraductal papillary mucinous neoplasms have been deeper in the pancreas and not easily enucleable. As a result, neither a malignant mucinous cystic neoplasm nor a side-branch intraductal papillary mucinous neoplasm has been enucleated with the subsequent need for a resection.

Noninvasive imaging techniques such as CT and MRI are unreliable to accurately distinguish among the different pancreatic cysts.[2–4,17,18] As a result, many experts recommend surgical removal, especially in younger, fit patients. Others have recommended percutaneous aspiration with fluid analysis.[19,20] More recently, endoscopic ultrasound with fine-needle aspiration has been suggested as a method to differentiate between benign and premalignant or malignant lesions.[21] However, percutaneous or endoscopic aspiration has the potential to spill malignant cells into the peritoneum with subsequent seeding and reduced survival. For this reason, as well as concerns about accuracy, we do not recommend preoperative aspiration. ERCP and magnetic resonance cholangiopancreatography may be quite helpful in defining cyst and pancreatic duct anatomy. However, CT pancreatography (see Fig. 2, *B* and *C*) and intraoperative ultrasonography may be just as useful, less invasive than ERCP, and potentially less expensive.

Another issue is whether these smaller cysts in asymptomatic patients should be removed as opposed to being observed. Serous cystadenomas have little malignant potential and may be observed when asymptomatic.[22] However, in the present series only one third of the patients had serous cystadenomas. Similarly, in an analysis that focused on large (>10 cm) cystic lesions, only 7 (29%) of 24 patients had serous cystadenomas.[4] In addition, many serous cystadenomas will have macroscopic as well as microscopic cysts, which are indistinguishable from mucinous cystic neoplasms on CT, MRI, or endoscopic ultrasonography. Although serous cystadenomas do not communicate with the pancreatic duct on ERCP, many mucinous cystic neoplasms and islet cell tumors, which have malignant potential, also do not communicate.

In the present report, 82% of the lesions that were enucleated were premalignant. The mean cyst size was 2.2 cm. The mean age of these patients was 53.0 years. Had these patients been observed with yearly CT scans with an initial endoscopic ultrasound and fine needle aspiration, which has false negative findings, the overall cost and potential for decreased survival certainly would have been greater. Thus, we currently recommend an exploratory operation with enucleation when appropriate in younger patients with cystic pancreatic lesions that are 1.5 cm or larger. This strategy also may be appropriate for otherwise healthy patients in their 70s and early 80s with cysts that are 2.0 cm or larger. Enucleation of small pancreatic cysts is safe, preserves pancreatic function, and has not been associated with recurrence. Therefore, we conclude that enucleation should be the standard operation for small, benign cystic neoplasms in the uncinate, head, neck, and body of the pancreas.

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Cystic Pancreatic Neoplasms: Enucleate or Resect?

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Asymptomatic cystic pancreatic neoplasms are being detected by abdominal imaging with increasing frequency. Enucleation of small cystic neoplasms can be performed without recurrence but has been associated with a higher incidence of pancreatic fistula. Thus the procedure has been modified to include intraoperative ultrasound imaging and closure of the pancreatic defect. This analysis was performed to determine whether these modifications have improved operative outcome. Thirty patients with mucinous cystic neoplasms (n = 16), serous cystadenomas (n = 10), and cystic islet cell tumors (n = 4) were studied. Enucleation was performed in 11 patients (7 with mucinous cystic neoplasms, 2 with serous cystadenomas and 2 with islet cell tumors), whereas 19 underwent resection of cystic tumors (pancreatoduodenectomy in 8 and distal pancreatectomy in 11). The mean groups did not differ with regard to age (57 years), gender (73% female), presentation (63% incidental), or site (43% head, neck, or uncinate). Patients undergoing enucleation had smaller tumors (2.2 vs. 4.7 cm, $P < 0.01$) that were less likely to be in the tail (9% vs. 42%). Operative time was significantly shorter in the enucleation group (199 vs. 298 minutes, $P < 0.01$). Blood loss also was significantly reduced in the enucleation group (114 vs. 450 ml, $P < 0.001$). Pancreatic fistula rates (27% vs. 26%) and length of hospital stay (12.6 vs. 15.7 days) were similar in the two groups. Enucleation of benign cystic pancreatic neoplasms reduces operative time and blood loss without increasing postoperative complications or length of stay. Therefore enucleation should be the standard operation for small benign cystic neoplasms in the uncinate, head, neck, and body of the pancreas.
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Key words: Cystadenoma, islet cell tumor, pancreatic cyst

With the introduction of newer sophisticated abdominal imaging techniques, cystic pancreatic neoplasms are being detected with increasing frequency.[1] In the past, most of these patients presented with abdominal pain and/or pancreatitis.[2–4] However, most cystic pancreatic neoplasms are now being detected as incidental findings on abdominal ultrasound imaging, computerized tomography, (CT) scans, and magnetic resonance imaging (MRI). Nevertheless, the nonoperative differentiation among the various pathologic entities including serous cystadenomas, mucinous cystic neoplasms, intraductal papillary mucinous neoplasms, cystic islet cell tumors, and solid and cystic papillary (Hamoudi) tumors remains difficult.[2–8]

Many cystic pancreatic tumors have malignant potential, and therefore resection is recommended by most experts.[2–8] Resection is clearly the treatment of choice for larger lesions, but a potentially more effective method for dealing with small cystic lesions is provided by enucleation. The senior author (H.A.P.) and his colleagues have previously reported that enucleation of mucinous cystic neoplasms can be performed safely without recurrence.[3] When compared to resection, however, enucleation was associated with a higher incidence of pancreatic fistula (50% vs. 12%, $P < 0.05$) and a longer length of hospital stay (19.5 vs. 10.0 days, $P < 0.02$). Over the past 5 years, the indications for enucleation have expanded, and the procedure has been modified to include intraoperative ultrasound imaging and, when possible, closure of the pancreatic defect. This study was performed, therefore, to determine (1) whether these operative modifications have improved outcome and (2) which lesions are most amenable to enucleation.

MATERIAL AND METHODS

Patients

A retrospective analysis of patients undergoing surgery for cystic pancreatic neoplasms at Froedtert Memorial Hospital/Medical College of Wisconsin over

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an 11-year period from March 1992 through February 2003 was performed. Only tumors pathologically determined to be benign were included in this study. Thirty patients were identified: 16 with mucinous cystic neoplasms, 10 with serous cystadenomas, and four with cystic islet cell tumors. Patients with benign or malignant intraductal papillary mucinous neoplasms were not included in this analysis because enucleation was not thought to be adequate therapy. The mean age of the patients was 56.8 years, and 73% were female (Table 1). Enucleation was performed in 11 patients (37%), whereas 19 patients (63%) had their tumors resected. Age and gender were similar in the patients undergoing enucleation and resection. Twenty-three of the operations were performed within the last 4 years including all 11 enucleations (48%).

Presentation and Evaluation

Most of the patients undergoing surgery in this series were asymptomatic (see Table 1). In fact, in two thirds of the patients undergoing resection the cyst was discovered incidentally on abdominal imaging, as compared to 55% in the enucleation group. In the enucleation group, four patients presented with abdominal pain, and one had nausea and fullness. In the resected group, five patients presented with abdominal pain, and one patient with an insulinoma and a history of hypoglycemic episodes presented in a coma. One patient from each group had a history of pancreatitis. Diabetes was evident in four patients, one of whom had enucleation and three of whom underwent resection. One of the patients in the resected group had previously undergone an attempted Whipple procedure. Another patient in the resection group had a history of pseudocyst and had undergone a cyst jejunostomy.

Table 1. Patient characteristics, symptoms, and surgery

	Enucleation	Resection	Total
Number of patients	11	19	30
Mean age (yr)	53.0	58.8	56.8
Range	38–65	30–82	30–82
Female (%)	73	74	73
Symptoms			
Asymptomatic (%)	55	68	63
Abdominal pain (%)	36	26	30
Resection type			
Enucleation	11	0	37
Distal pancreatectomy	0	11	37
Pancreatoduodenectomy	0	8	26

All 30 patients had CT scans (Figs. 1 and 2), whereas MRI was performed in only two patients. Seven patients underwent preoperative endoscopic retrograde cholangiopancreatography (ERCP): five from the enucleation group and two from the resected group. Five patients underwent preoperative endoscopic ultrasonography and fine-needle aspiration, two from the enucleation group and three from the resected group. One patient from the resected group also underwent preoperative angiography early in the series.

Surgery

In the enucleation group, intraoperative ultrasound imaging was employed routinely to assess the relationship between the pancreatic duct and the cystic lesion. In addition, the pancreatic defect after enucleation was closed in 8 (73%) of the 11 patients. Closure was usually performed with one or two "figure-of-eight" 3-0 absorbable sutures, taking care not to injure the pancreatic duct. One defect was closed with fibrin glue. A closed suction drain was placed near the defect or closure in all patients. One surgeon (H.A.P.) performed 9 (82%) of the 11 enucleation procedures. One patient in the enucleation group underwent a concurrent cholecystectomy.

Of the 19 patients who underwent resection, 11 had a distal pancreatectomy (7 [64%] with splenic preservation), seven had a pylorus-preserving pancreatoduodenectomy, and one had a standard pancreatoduodenectomy (see Table 1). Six of these patients (32%) had concurrent procedures including splenectomy in four, sigmoid colectomy for recurrent diverticulitis in one, and resection of a splenic artery aneurysm in one patient who required a pancreatoduodenectomy. One distal pancreatectomy and splenectomy was performed as a laparoscopic hand-assisted procedure, whereas one distal pancreatectomy and colectomy was performed laparoscopically. Intraoperative ultrasonography was employed in 6 (32%) of 19 resected patients. Closed suction drains were used routinely. Octreotide or vapreotide was used at the discretion of the surgeon or in accordance with a randomized trial. Approximately one third of the patients in each group received a somatostatin analogue.

Cyst Characteristics

Information on cyst size, location, and type is presented in Table 2. Cysts that were enucleated were significantly smaller ($P < 0.01$) when measured either by CT or by pathologic examination. Ten (91%) of 11 enucleated cysts were in the uncinate, head, neck, or body of the pancreas compared to 13 (58%) of 19 resected cysts. This difference did not quite reach

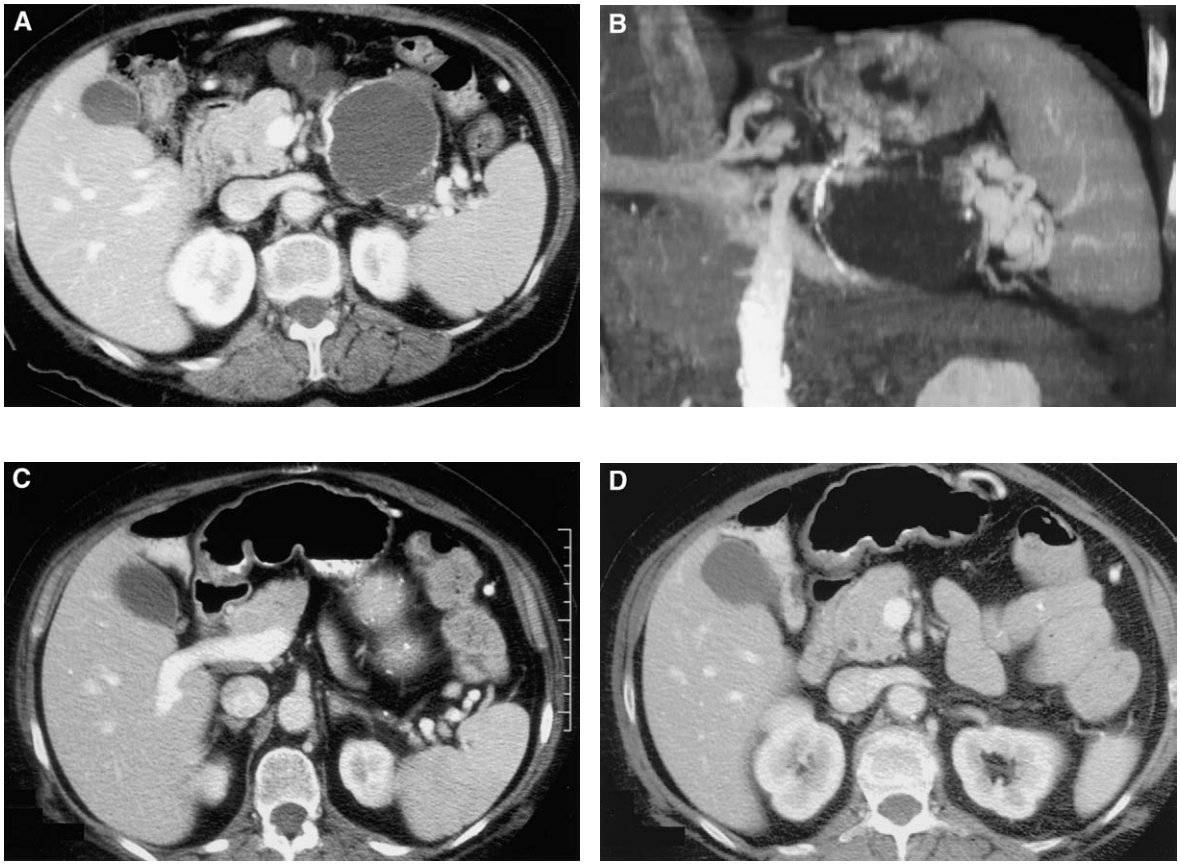

Fig. 1. A, CT scan demonstrating a 6.0 cm calcified mucinous cystic neoplasm in the tail of the pancreas in a 63-year-old woman. **B,** Three-dimensional reconstruction in the same patient demonstrating splenic vein compression and multiple venous collateral vessels. **C,** CT scan 3 years after distal pancreatectomy demonstrating pancreatic neck and preserved spleen. **D,** Lower cut from the same CT scan 3 years after surgery demonstrating a normal head of the pancreas.

statistical significance ($P < 0.08$). All specimens were reviewed by one pathologist (R.A.K.). Sixteen (53%) of the 30 cysts were mucinous cystic neoplasms, 10 were serous cystadenomas (33%), and four were cystic islet cell tumors (13%). Relatively more of the enucleated cysts (64%) were mucinous cystic neoplasms, but the distribution of cyst type between the two groups was not statistically different.

Morbidity

Morbidity was characterized as pancreatic fistula formation, postoperative pancreatitis, or any other major complication. Pancreatic fistula was defined as drainage of more than 50 ml of amylase-rich fluid through operatively placed drains on or after postoperative day 7. Postoperative pancreatitis was defined as more than threefold elevation of serum amylase over the upper limit of normal as well as CT evidence of peripancreatic inflammation.

Follow-Up and Statistics

Follow-up and survival information was obtained from clinic notes, hospital records, and the social security database. Data are presented as means ± standard error of the mean (SEM). Statistical analyses were performed using Student's *t* test or the Mann-Whitney rank sum test where appropriate, with statistical significance achieved at $P < 0.05$.

RESULTS

Operative Data

The mean operative time in the enucleation group was significantly shorter than in those who had resections (199 ± 16 vs. 298 ± 27 minutes) ($P < 0.01$; Fig. 3). Also, significantly less blood loss occurred in patients who underwent enucleation (114 ± 14 vs. 450 ± 120 ml, $P < 0.01$; see Fig. 3).

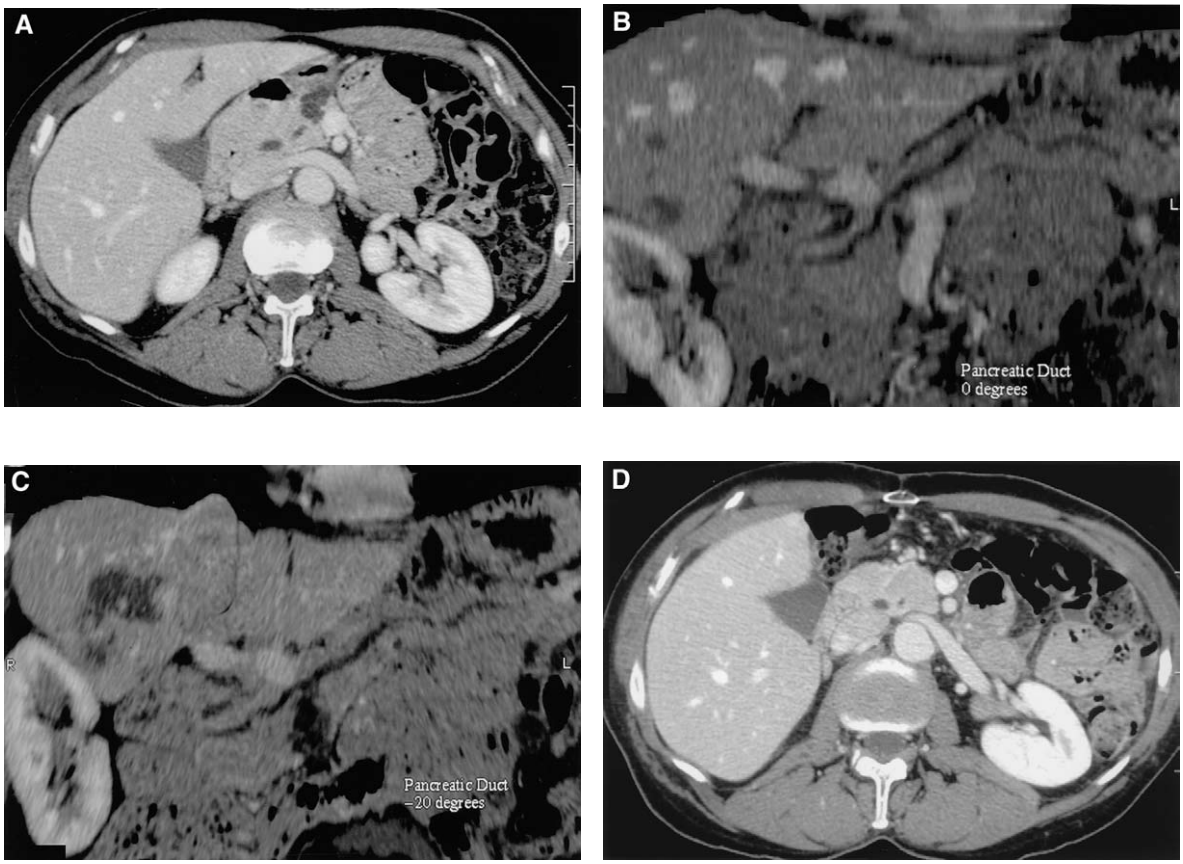

Fig. 2. A, CT scan demonstrating a 2.4 cm cystic mass in the neck of the pancreas in a 56-year-old woman. **B,** CT pancreatogram (0 degrees) showing the cyst adjacent to the superior mesenteric vein. **C,** CT pancreatogram (−20 degrees) showing the cyst in communication with the pancreatic duct. **D,** CT scan 7 months after enucleation demonstrating the preserved pancreas and a normal pancreatic duct.

Morbidity

The incidence of pancreatic fistulas and all complications are presented in Fig. 4, which demonstrates no significant differences between the enucleation and resection groups. The incidence of pancreatic fistulas was 27% and 26%, respectively. In the enucleation group, three patients developed pancreatic fistulas, and one had a partial small bowel obstruction for an overall complication rate of 36%. In the resection group five patients developed pancreatic fistulas, and four additional patients had an abdominal abscess, gastric outlet obstruction, pneumonia, and a urinary tract infection. Thus, the overall complication rate in the resected patients was 47%, which was not significantly greater than in the enucleated patients.

The median and mean postoperative length of stay in the enucleated group were 9 days and 12.6 ± 2.8 days, respectively. The median and mean length of stay in the resected patients were 14 days and 15.7 ± 2.5 days, respectively. These differences between groups were not statistically different. One patient in the enucleation group with a pancreatic fistula had a pancreatic stent placed after discharge. Two patients in the resected group had late complications including a small bowel obstruction at 4 months and a pseudocyst at 9 months postoperatively. Both of these patients were managed with open operations and have now been well for 7 months and 7 years, respectively.

Follow-Up

The mean follow-up time for all patients was 38.4 months and ranged from 1 to 122 months. All patients, except for the most recently enucleated patient, underwent follow-up CT scanning. None of the patients have had a recurrence of their benign cystic tumors on follow-up CT scans (see Figs. 1 and 2, and Fig. 5). All 30 patients are alive, and none of the patients have developed pancreatic exocrine or new endocrine insufficiency.

Table 2. Cyst size, location, and type

	Enucleation	Resection	Total
Size (cm)			
CT	2.2 ± 0.31*	4.7 ± 0.64	3.7 ± 0.47
Pathologic examination	1.8 ± 0.32*	4.5 ± 0.84	3.5 ± 0.59
Location (%)			
Head†	45	42	43
Body	45	16	27
Tail	9	42	30
Type (%)			
Mucinous cystic neoplasm	64	47	53
Serous cystoadenoma	18	37	33
Islet cell tumor	18	11	13

*$P < 0.01$ vs. resection.
†Head = uncinate, head, and neck.

DISCUSSION

In this series of 30 patients who underwent surgery for benign pancreatic tumors, 11 patients were managed by enucleation, whereas 19 had pancreatic resection. The two groups were similar with respect to age, gender, and presenting symptoms. According to CT scan measurements cysts that were enucleated were smaller (2.2 vs. 4.7 cm, $P < 0.01$). Ten (91%) of 11 enucleated cysts were in the uncinate, head, neck, or body of the pancreas compared to 13 (58%) of 19 resected cysts ($P < 0.08$). Cyst type was similar in the two groups with the majority (53%) being mucinous cystic neoplasms. Both operative time ($P < 0.01$) and blood loss ($P < 0.001$) were less in the enucleated patients. Pancreatic fistula rates and length of hospital stay were similar in the two groups. This analysis confirms that operative modifications have improved outcomes compared to an earlier report[3] and further defines which lesions are most amenable to enucleation.

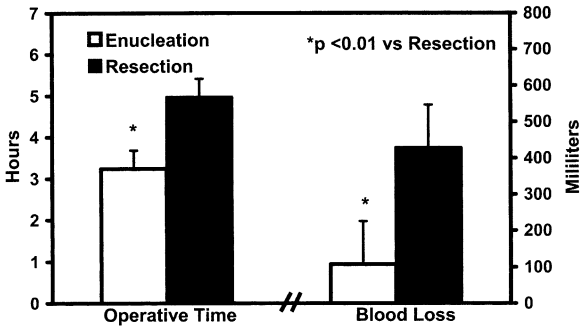

Fig. 3. Operative time and blood loss in patients undergoing enucleation and resection.

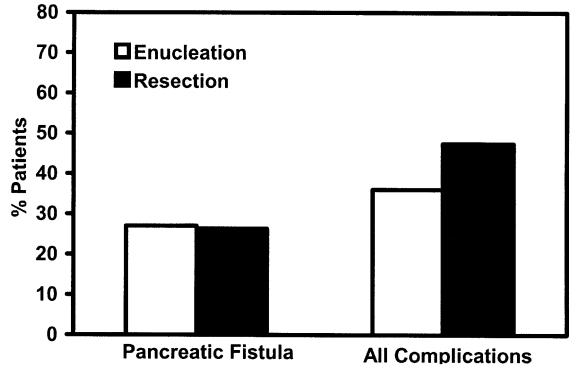

Fig. 4. Pancreatic fistula and all complications in patients undergoing enucleation and resection.

In the 1998 report by Talamini et al.,[3] the patients who were studied were similar to those in the present analysis with respect to age, gender, and cyst location. However, in the earlier series all cysts were mucinous cystic neoplasms, whereas in the present analysis 46% of the cysts were serous cystadenomas (33%) or cystic islet cell tumors (13%). In addition, in the present series enucleated cysts were smaller (2.2 vs. 2.8 cm), whereas resected cysts were larger (4.7 vs. 3.6 cm). Interestingly, more patients in the present series were asymptomatic and had their cysts discovered incidentally on abdominal imaging (63% vs. 14%).

The major operative modifications from the earlier series to the present study were the introduction of intraoperative ultrasound imaging to identify the pancreatic duct and closure of the pancreatic defect after enucleation. In both analyses, enucleation was associated with a shorter operative time than resection. In the present series, the operative time for enucleation decreased 73 minutes from the earlier report (199 vs. 272 minutes). In addition, in the present report operative blood loss for enucleation decreased 95 ml compared to the earlier report (114 vs. 209 ml). Moreover, the pancreatic fistula rate in the recent series decreased by 23% compared to the initial report (27% vs. 50%). As a result, the hospital stay was decreased by 6.9 days (12.6 vs. 19.5 days), and the median hospital stay in the present report was only 9 days. These many improvements in outcome suggest that a learning curve is associated with this operation, which may be on the order of 10 pancreatic cyst enucleations. Finally, none of the enucleated cysts have recurred in the two series with follow-up now extending more than 12 years in the initial patient.

For cystic tumors in the tail of the pancreas, resection remains the operation of choice. In our opinion

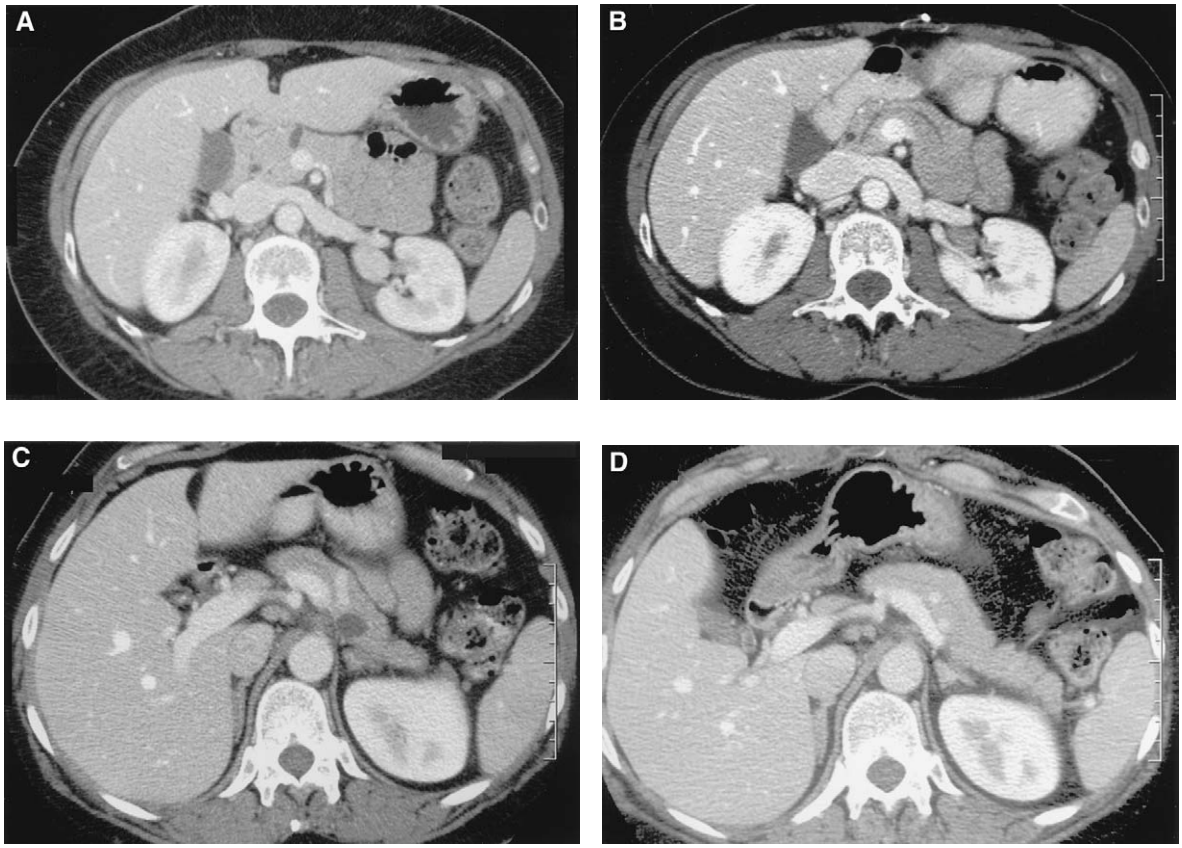

Fig. 5. A, CT scan demonstrating a 2.0 cm mucinous cystic neoplasm in the neck of the pancreas in a 51-year-old woman. **B,** CT scan 14 months after enucleation demonstrating the preserved pancreas without recurrence. **C,** CT scan demonstrating a 1.5 cm mucinous cystic neoplasm in the body of the pancreas in a 47-year-old woman. **D,** CT scan 12 months after enucleation demonstrating preserved pancreas and spleen.

splenic preservation should be attempted in patients suspected to have a benign lesion in the pancreatic tail.[9] In the present series, splenic preservation was achieved in 50% of the patients compared to only 26% in the earlier report.[3] Splenic preservation can be carried out even when the splenic vein is compromised by the cyst (see Fig. 1). Laparoscopic distal pancreatectomy is another good option for small cystic lesions in the tail.[10] Again, splenic preservation should be attempted and avoids the need to extend a port incision to remove the spleen.

For small cystic tumors in the uncinate, head, neck, and body of the pancreas, we believe that enucleation has advantages over pancreatic resection with respect to operative time, blood loss, and preservation of pancreatic parenchyma. Because the pancreas is otherwise normal in these patients, the risk of pancreatic fistula is high. However, the morbidity associated with a pure pancreatic fistula that may occur after an enucleation is generally less than that of a fistula from a pancreatic-enteric anastomosis after a pancreatoduodenectomy. Segmental resection of the neck or body of the pancreas is another option for some of these patients but also has the disadvantage of a pancreatojejunostomy or pancreatogastrostomy with a normal pancreas. Similarly, a Beger[11] or a Frey[12] operation might be considered for lesions in the uncinate, head, or neck, but performing these procedures in a normal pancreas is likely to be fraught with difficulty as well as a high rate of pancreatic fistula. Moreover, performance of a pancreatoduodenectomy with a normal pancreas and bile duct has an increased risk of leakage at the biliary anastomoses.[4,13–16]

Most of the tumors that were enucleated in the present and in the earlier series[3] were mucinous cystic neoplasms. All of these mucinous cystic neoplasms have been benign on frozen section and final pathologic examination. One potential concern is that an enucleation might be performed for a malignant mucinous cystic neoplasm. However, in our experience,

when enucleation is possible, the lesion is benign and enucleation has not been feasible for malignant mucinous cystic neoplasms or cystadenocarcinomas. Another potential dilemma is whether a side-branch intraductal papillary mucinous neoplasm might be enucleated, and if so, would this operation be adequate?[5–7] This possibility is real because both side-branch intraductal papillary mucinous neoplasms and mucinous cystic neoplasms may communicate with the main pancreatic duct (see Fig. 2, *C*). In our experience, most side-branch intraductal papillary mucinous neoplasms have been deeper in the pancreas and not easily enucleable. As a result, neither a malignant mucinous cystic neoplasm nor a side-branch intraductal papillary mucinous neoplasm has been enucleated with the subsequent need for a resection.

Noninvasive imaging techniques such as CT and MRI are unreliable to accurately distinguish among the different pancreatic cysts.[2–4,17,18] As a result, many experts recommend surgical removal, especially in younger, fit patients. Others have recommended percutaneous aspiration with fluid analysis.[19,20] More recently, endoscopic ultrasound with fine-needle aspiration has been suggested as a method to differentiate between benign and premalignant or malignant lesions.[21] However, percutaneous or endoscopic aspiration has the potential to spill malignant cells into the peritoneum with subsequent seeding and reduced survival. For this reason, as well as concerns about accuracy, we do not recommend preoperative aspiration. ERCP and magnetic resonance cholangiopancreatography may be quite helpful in defining cyst and pancreatic duct anatomy. However, CT pancreatography (see Fig. 2, *B* and *C*) and intraoperative ultrasonography may be just as useful, less invasive than ERCP, and potentially less expensive.

Another issue is whether these smaller cysts in asymptomatic patients should be removed as opposed to being observed. Serous cystadenomas have little malignant potential and may be observed when asymptomatic.[22] However, in the present series only one third of the patients had serous cystadenomas. Similarly, in an analysis that focused on large (>10 cm) cystic lesions, only 7 (29%) of 24 patients had serous cystadenomas.[4] In addition, many serous cystadenomas will have macroscopic as well as microscopic cysts, which are indistinguishable from mucinous cystic neoplasms on CT, MRI, or endoscopic ultrasonography. Although serous cystadenomas do not communicate with the pancreatic duct on ERCP, many mucinous cystic neoplasms and islet cell tumors, which have malignant potential, also do not communicate.

In the present report, 82% of the lesions that were enucleated were premalignant. The mean cyst size was 2.2 cm. The mean age of these patients was 53.0 years. Had these patients been observed with yearly CT scans with an initial endoscopic ultrasound and fine needle aspiration, which has false negative findings, the overall cost and potential for decreased survival certainly would have been greater. Thus, we currently recommend an exploratory operation with enucleation when appropriate in younger patients with cystic pancreatic lesions that are 1.5 cm or larger. This strategy also may be appropriate for otherwise healthy patients in their 70s and early 80s with cysts that are 2.0 cm or larger. Enucleation of small pancreatic cysts is safe, preserves pancreatic function, and has not been associated with recurrence. Therefore, we conclude that enucleation should be the standard operation for small, benign cystic neoplasms in the uncinate, head, neck, and body of the pancreas.

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Binding Pancreaticojejunostomy: 150 Consecutive Cases Without Leakage

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The objective of this study was to verify the safety of a new technique termed "binding pancreaticojejunostomy" in a prospective cohort study. Pancreaticojejunal anastomostic leakage is a major cause of morbidity and mortality after pancreaticoduodenectomy. To prevent the development of pancreatic fistulas, we designed a special technique that we termed binding pancreaticojejunostomy. Binding pancreaticojejunostomy entails binding 3 cm of the serosamuscular sheath of the jejunum to the intussuscepted pancreatic stump. From January 1996 to May 2001, a total of 150 consecutive patients were treated with this type of pancreaticojejunostomy, including typical pancreaticoduodenectomy in 120, hepatopancreaticoduodenectomy in 17, pylorus-preserving pancreaticoduodenectomy in 10, and duodenal-preserving resection of the head of the pancreas in three. None of the patients developed pancreatic fistulas. The overall morbidity was 31.3%. The following complications occurred: gastrointestinal bleeding in six, pulmonary infection in 12, wound infection in 20, delayed gastric emptying in three, incision dehiscence in four, and hepatic insufficiency in two. The mean postoperative hospital stay was 19.8 ± 5 days. Binding pancreaticojejunostomy is a safe, simple, and effective technique. (J GASTROINTEST SURG 2003;7:898–900)

KEY WORDS: Pancreas, surgery, pancreatic leakage, pancreaticoduodenectomy, pancreaticojejunostomy, binding

Anastomotic leakage from pancreaticojejunostomy is the most feared complication of the Whipple operation. Several methods have been advocated in an effort to prevent anastomotic leakage, but none is perfect.[1–3] To prevent pancreatic fistulas, we designed a special technique that we termed "binding pancreaticojejunostomy." The preliminary results are encouraging.[4,5] This is a large-cohort series to verify our preliminary results.

PATIENTS AND METHODS

From January 1996 through May 2001, a total of 150 patients undergoing pancreaticoduodenectomy at the Second Affiliated Hospital and Sir Run Run Shaw Hospital of Zhejiang University were treated with binding pancreaticojejunostomy. There were 107 men and 43 women, and the patients ranged in age from 36 to 78 years (mean 62 years).

The surgical techniques of binding pancreaticojejunostomy have been described previously.[4,5] In brief, this procedure is performed as follows: First, the cut end of the pancreatic remnant is isolated for a distance of 3 cm. Three centimeters of the distal cut end of the jejunum is everted. The exposed jejunal mucosa is destroyed either by electrical coagulation, or by 10% carbolic acid followed by rinsing immediately with 75% alcohol and normal saline solution (Fig. 1). The pancreatic stump and the everted jejunum are brought together and sutured with silk, intermittently or continuously. Care is taken to suture the mucosa only, and to avoid penetrating the serosa and muscular layer of the jejunum (Fig. 2). The everted jejunum is then turned down to its normal position to wrap over the pancreatic stump, and it is sutured to the pancreas with a few stitches for fixation. Last, 1 cm

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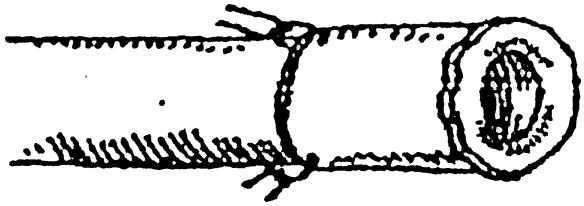

Fig. 1. Three centimeters of the distal cut end of the jejunum is everted. The exposed jejunal mucosa is destroyed by electrical coagulation.

from the cut end of the jejunum, a 2/0 Vicryl suture is looped around the entire circumference of the anastomosis. A bundle of vessels is spared for maintaining the blood supply to the jejunal cut end distal to the binding ligature (Fig. 3). In the most recent 11 cases, we did not suture the mucosa of the jejunum to the cut end of pancreas, and we only bound the serosamuscular sheath of the jejunum to the pancreatic remnant.

A tube is inserted into the jejunal lumen, and saline solution is injected to test for a watertight closure. Then a Jackson-Pratt drainage tube is placed near the anastomosis. The volume and amylase contents of the drainage are measured every day. Pancreatic leaks were defined as a significant increase in the volume or a change in the nature of the effluent from the surgical drain, or the persistence of amylase-rich drainage output in excess of 50 ml/day and amylase more than 1000 IU/L. The tubes were removed when the volume was less than 20 ml/day. Prophylactic octreotide was not used.

RESULTS

From January 1996 through October 2001, a total of 150 consecutive pancreaticojejunostomies were performed using this technique, which included the following: typical pancreaticoduodenectomy (PD) in 120 patients, hepatopancreaticoduodenectomy (HPD)

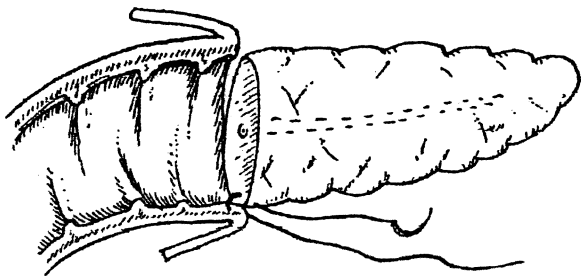

Fig. 2. The pancreatic stump and the everted jejunum are brought together and sutured with silk. Care is taken to suture the mucosa only.

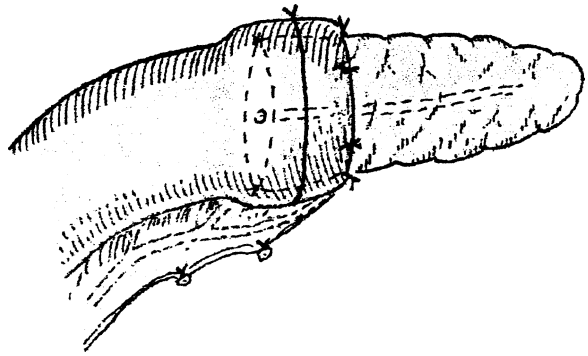

Fig. 3. Remnant of pancreas in the lumen of the jejunum; both are looped around and ligated together. A bundle of vessels is spared to maintain an intact blood supply to the cut end of the jejunum.

in 17, pylorus-preserving pancreaticoduodenectomy (PPPD) in 10, and duodenal-preserving resection of the head of the pancreas (DPRHD) in three. Pathologic examination showed pancreatic cancer (n = 56), ampullary cancer (n = 32), distal common bile duct cancer (n = 18), duodenal cancer (n = 12), chronic pancreatitis (n = 11), gallbladder cancer (n = 16), and gastric cancer (n = 5). The pancreatic jejunal anastomoses were all watertight during the surgical procedure. None of the patients developed pancreatic leakage. The overall morbidity was 31.3% (gastrointestinal bleeding in 6, pulmonary infection in 12, wound infection in 20, delayed gastric emptying in 3, incision dehiscence in 4, and hepatic insufficiency in 2). The mean postoperative hospital stay was 19.8 ± 5 days.

DISCUSSION

Although the first pancreaticoduodenectomy was performed in 1904, it did not become the standard treatment for periampullary and pancreatic carcinoma until after it was reintroduced by Dr. Allen Whipple in 1935. It has been used increasingly to treat a variety of malignant and benign diseases of the pancreas and periampullary region.

After pancreaticoduodenectomy, leakage or fistula from the pancreatic anastomosis is the leading cause of morbidity and mortality. Many preoperative and intraoperative parameters may be the risk factors associated with the development of pancreatic fistulas. According to one recent retrospective study, no preoperative parameters were found to have a significant association with the risk of pancreatic leakage. The following three intraoperative parameters were identified as significant by means of univariate analysis:

anastomotic technique, pancreatic duct size, and texture of the remnant pancreas. Multivariate analysis revealed that only anastomotic technique turned out to be an independent risk factor.[6] But another study showed that the degree of pancreatic fibrosis, type of resection, anastomosis technique, anastomosis site, and the presence of congestion at the anastomosis site significantly influenced pancreatic leakage.[7]

The primary approach to pancreatic fistulas should be prevention. According to a review of the Medline database from 1990 to 2000 concerning studies on the prevention of pancreatic anastomotic leakage, the routine use of octreotide in pancreaticoduodenectomies cannot be recommended. However, some retrospective or nonrandomized prospective studies suggested that technical modifications could reduce the leakage rate.[3] Reported techniques cover a spectrum of complexity ranging from simple "parachuting" of the pancreatic stump into the jejunum to a more elaborate four-layer end-to-side ductal mucosa anastomosis.[8–15]

We employ a binding method to seal the jejunum to the pancreatic stump rather than using multiple anchoring sutures. The results are very encouraging. The success of our technique may be attributed to three safety measures. First, the serosamuscular sheath of the jejunum is bound to the pancreatic remnant; second, the anastomotic suture needle penetrates only the inner mucosal layer, with care being taken to keep the muscular and serosal layer intact; third, the jejunal mucosa covering the pancreas is destroyed to avoid its secretions and promote healing. In the most recent 11 cases, we did not suture the mucosa of the jejunum to the cut end of pancreas; we only bound the serosamuscular sheath of the jejunum to the pancreatic remnant. All of the patients recovered well. Consequently we consider the first step to be the essential measure of preventing pancreatic juices and jejunal contents from leaking out, which leads to good primary healing.

An ideal pancreaticojejunal anastomosis after pancreaticoduodenectomy should be safe and simple. Moreover, it should be applicable to all kinds of pancreatic remnants. The use of binding pancreaticojejunostomy ensures a tight seal around any pancreatic stump. This technique is helpful particularly in patients with a "small" duct or a "soft" pancreas, because these patients are believed to be at greater risk of developing pancreatic leaks.[6,10]

CONCLUSION

Binding pancreaticojejunostomy is a safe, simple, and effective technique that avoids the primary complication of anastomotic leakage.

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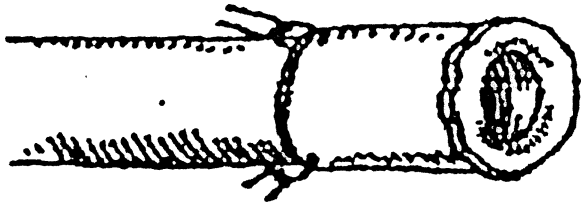

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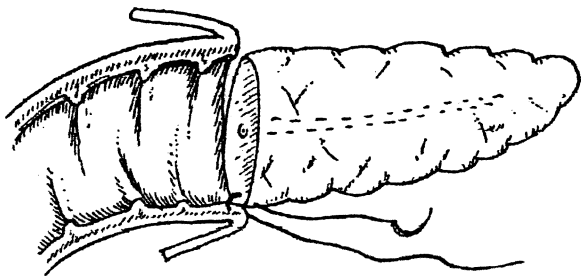

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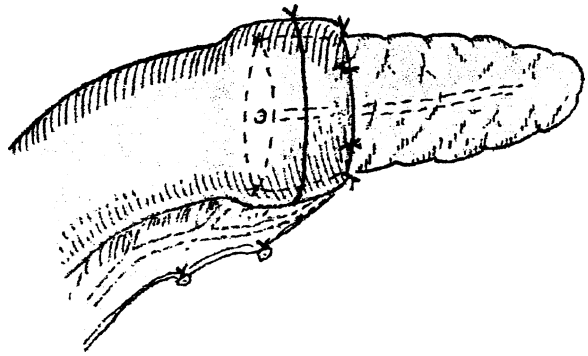

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Induction of MIC-1/Growth Differentiation Factor-15 Following Bile Duct Injury

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Macrophage inflammatory peptide-1 (MIC-1)/growth/differentiation factor-15 (GDF-15) is a divergent member of the transforming growth factor-β superfamily cloned by others and us. MIC-1/GDF-15 is expressed in the liver, breast, and colon. Studies have demonstrated a growth-inhibiting effect of MIC-1/GDF-15 on colon and breast cancer cell lines in vitro and on tumor growth in vivo. We previously reported that MIC-1 expression is rapidly induced after a wide variety of murine acute and chronic liver injuries including aniline dye administration. I hypothesized, therefore, that MIC-1/GDF-15 may be a mediator of biliary tract injury and could play a role in regulation of bile duct proliferation. C57BL/6 mice underwent surgical ligation of the common bile duct. Northern blot analysis revealed a time-dependent induction of MIC-1/GDF-15 mRNA in the liver. In situ hybridization of liver sections for MIC-1/GDF-15 expression after bile duct ligation demonstrated a zone 1 or periportal expression pattern, consistent with expression of MIC-1 in periductular hepatocytes. Northern blot analysis of liver mRNA from patients with sclerosing cholangitis or cirrhosis also demonstrated enhanced expression of MIC-1/GDF-15. MIC-1/GDF-15 is expressed after bile duct injury in mice and humans. Taken together with the previously demonstrated growth inhibitory effects of MIC-1/GDF-15 on normal and transformed cells, MIC-1/GDF-15 may play a role in regulation of bile duct proliferation and biliary tumor formation. (J Gastrointest Surg 2003;7:901–905)

Key words: Cytokines, inflammation, growth substances, cholangiocarcinoma

Human macrophage inhibitory cytokine-1 (MIC-1) and murine growth differentiation factor-15 (GDF-15) are divergent members of the transforming growth factor-beta (TGF-β) superfamily.[1–3] Like all members of the TGF-β superfamily, they are secreted as pre-proproteins and undergo post-translational modification and cleavage. The biologically active portion of the protein is a disulfide-linked dimer of the C-terminal domain, which is the region of the protein most conserved across members of this family. Pairwise comparison of the C-terminal regions of GDF-15 and MIC-1 demonstrate 67% amino acid identity and 72% nucleotide identity. Previously we reported that Southern blot analysis of mouse DNA with the use of an MIC-1 probe revealed hybridization to bands corresponding the GDF-15 gene. Furthermore, radiation hybrid mapping linked GDF-15 to the mouse framework marker D8Mit233. This region on mouse chromosome 8 is syntenic to a portion of human chromosome 19p13.1, an interval that contains the MIC-1 locus. Thus, based on results of our previous studies, it appears that GDF-15 is the murine ortholog of MIC-1 and hence will be referred to herein as MIC-1/GDF-15.[3]

MIC-1/GDF-15 (also called hPDF, hPLAB, hPTGFB, and NAG-1) has been independently identified by a number of groups searching for novel secreted factors.[2,4–8] By Northern blot analysis, murine MIC-1/GDF-15 is expressed in normal colon, liver, breast, prostate, and kidney.[2,3] More sensitive techniques of RNA detection have demonstrated MIC-1/GDF-15 expression in normal choroid plexus,[9] placenta,[4] prostate,[3] and vascular endothelium.[10,11] Studies have identified rodent MIC-1/GDF-15 expression in injured liver and brain, and more recently serum MIC-1 concentrations have

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been demonstrated to be an independent risk factor for arthrosclerotic vascular disease in humans.[10]

MIC-1/GDF-15 functions are under active investigation and currently appear to fall into two broad categories: growth control and inflammation. With regard to the former, MIC-1/GDF-15 has been identified as a p53-inducible gene and has been implicated in growth inhibition following p53 activation.[2,12] Furthermore, stable transfection of various transformed cell lines with MIC-1/GDF-15 prevents tumor formation in vivo. MIC-1/GDF-15 potently inhibits colon carcinogenesis[13] and can induce apoptosis of transformed colonic and breast cancer cells.[14,15] Interestingly, MIC-1/GDF-15 is induced after administration of nonsteroidal anti-inflammatory drugs (NSAIDS), diallyl disulfides (an antitumor agent found in garlic), and resveratrol (an antitumor agent found in grape skins), and has been implicated in the antitumor mechanism of each of these agents.[16–18] In addition to possessing growth inhibitory activities on epithelial cells, MIC-1/GDF-15 also has been implicated as a potent survival factor for cultured aminergic and cholinergic neurons.[19]

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liver RNA after methylene dianiline (DAPM) injection. Marked induction was noted at 1 hour after DAPM administration and persisted for up to 6 hours after injury. Given the observed induction of MIC-1/GDF-15 after DAPM, we then examined its regulation after a second model of bile duct injury, surgical ligation of the common bile duct. Induction of MIC-1/GDF-15 was observed at 1 hour after bile duct ligation (Fig. 1, *A*). A probe for aldolase was used as loading control.[21] Densitometry demonstrated a 3.0-fold induction of MIC-1/GDF-15 after bile duct ligation (Fig. 1, *B*).

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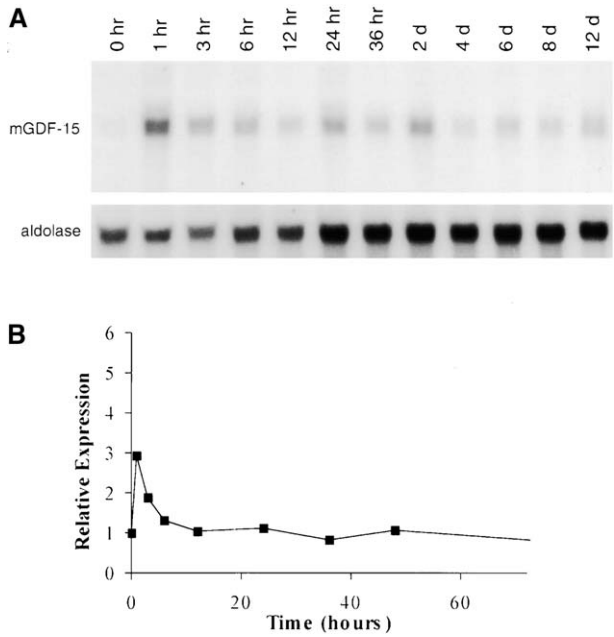
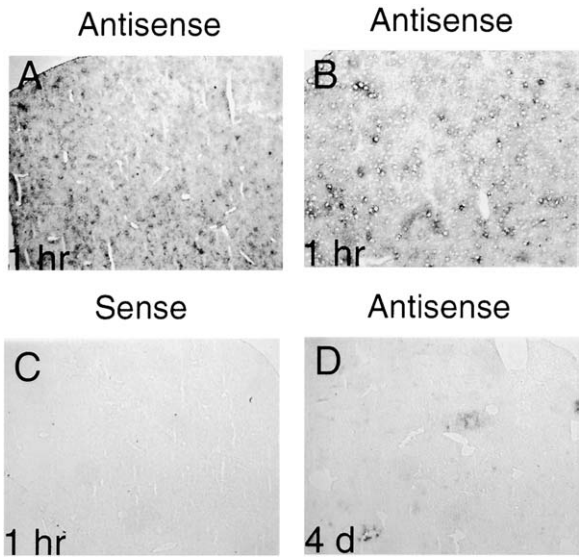


Fig. 1. Induction of MIC-1/GDF-15 after bile duct injuries. **A**, Northern blot demonstrating induction of MIC-1/GDF-15 after bile duct ligation. Aldolase probe of each experiment demonstrates loading control. **B**, Densitometry demonstrating time-course dependent MIC-1/GDF-15 induction. Relative MIC-1/GDF-15 induction is normalized to aldolase. Data presented are a representative time-course of three experiments.



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DISCUSSION

MIC-1/GDF-15 is a newly identified member of the TGF-β superfamily with growth inhibitory and immune regulatory activities on a wide variety of tissues. Transfection of tumor cells with MIC-1/GDF-15 has been demonstrated to prevent tumor growth in vivo. Recently dysregulation of MIC-1/GDF-15 has been implicated in colon carcinogenesis and in breast carcinogenesis.[13,14] Many studies examining the role of MIC-1/GDF-15 in carcinogenesis and recovery from organ injury suggest that one pathway for MIC-1/GDF-15 induction is in response to the tumor suppressor p53. MIC-1/GDF-15, similar to other members of the TGF-β superfamily, in turn appear to regulate tumor cell growth by binding to TGF-β RI and II and activating SMAD4 signaling.[24]

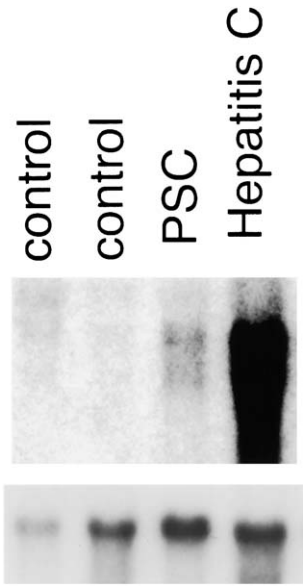

Fig. 3. Northern blot of human liver samples for MIC-1/GDF-15 expression. Aldolase probe of each experiment demonstrates loading control.

Here I report that MIC-1/GDF-15 is strongly induced both in murine models of bile duct injury and in damaged human liver. This report is the first identification of MIC-1/GDF-15 expression in chronic human liver disease. These data suggest a role for MIC-1/GDF-15 in both acute and chronic bile duct injury. Taken together with the published literature, these data also suggest a role for MIC-1/GDF-15 in mediating the inflammatory response after bile duct injury and identify MIC-1/GDF-15 as a potential negative growth regulatory factor in chronic liver diseases. Moreover, the observed induction both in murine and in human hepatobiliary disease suggests a commonality of function in vivo. Of note, however, it remains unclear whether acute biliary obstruction alone will cause GDF-15 induction in humans because concomitant cirrhosis was observed in the samples examined.

On the basis of these data, it is believed that MIC-1/GDF-15 may play a role in growth regulation and repair of human biliary and liver tissue after acute or chronic injury. Interestingly, Bootcov et al.[2] found that MIC-1/GDF-15 decreased tumor necrosis factor secretion by macrophages, which is known to be a critical mediator for the reparative response to liver injury.[25] These functions of tumor necrosis factor are known to involve both a priming component, mediated through NF-κB, and a potential proapoptotic action through the activation of the tumor necrosis factor receptor–associated death domain (TRADD). Interestingly, examination of concanavalin A–mediated liver injury demonstrates relative protection from injury for MIC-1/GDF-15-null mice (L.G.K., unpublished observation). This suggests both a growth regulatory and a potential proapoptotic function for MIC-1/GDF-15 after liver injury. Preliminary studies have also suggested that MIC-/GDF-15 is induced as an immediate early response inasmuch as cycloheximide-pretreated animals demonstrate upregulation after injury. Future studies are directed toward further defining the mechanistic basis for these observations.

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Induction of MIC-1/Growth Differentiation Factor-15 Following Bile Duct Injury

Leonidas G. Koniaris, M.D.

Macrophage inflammatory peptide-1 (MIC-1)/growth/differentiation factor-15 (GDF-15) is a divergent member of the transforming growth factor-β superfamily cloned by others and us. MIC-1/GDF-15 is expressed in the liver, breast, and colon. Studies have demonstrated a growth-inhibiting effect of MIC-1/GDF-15 on colon and breast cancer cell lines in vitro and on tumor growth in vivo. We previously reported that MIC-1 expression is rapidly induced after a wide variety of murine acute and chronic liver injuries including aniline dye administration. I hypothesized, therefore, that MIC-1/GDF-15 may be a mediator of biliary tract injury and could play a role in regulation of bile duct proliferation. C57BL/6 mice underwent surgical ligation of the common bile duct. Northern blot analysis revealed a time-dependent induction of MIC-1/GDF-15 mRNA in the liver. In situ hybridization of liver sections for MIC-1/GDF-15 expression after bile duct ligation demonstrated a zone 1 or periportal expression pattern, consistent with expression of MIC-1 in periductular hepatocytes. Northern blot analysis of liver mRNA from patients with sclerosing cholangitis or cirrhosis also demonstrated enhanced expression of MIC-1/GDF-15. MIC-1/GDF-15 is expressed after bile duct injury in mice and humans. Taken together with the previously demonstrated growth inhibitory effects of MIC-1/GDF-15 on normal and transformed cells, MIC-1/GDF-15 may play a role in regulation of bile duct proliferation and biliary tumor formation. (J Gastrointest Surg 2003;7:901–905)

Key words: Cytokines, inflammation, growth substances, cholangiocarcinoma

Human macrophage inhibitory cytokine-1 (MIC-1) and murine growth differentiation factor-15 (GDF-15) are divergent members of the transforming growth factor-beta (TGF-β) superfamily.[1–3] Like all members of the TGF-β superfamily, they are secreted as pre-proproteins and undergo post-translational modification and cleavage. The biologically active portion of the protein is a disulfide-linked dimer of the C-terminal domain, which is the region of the protein most conserved across members of this family. Pairwise comparison of the C-terminal regions of GDF-15 and MIC-1 demonstrate 67% amino acid identity and 72% nucleotide identity. Previously we reported that Southern blot analysis of mouse DNA with the use of an MIC-1 probe revealed hybridization to bands corresponding the GDF-15 gene. Furthermore, radiation hybrid mapping linked GDF-15 to the mouse framework marker D8Mit233. This region on mouse chromosome 8 is syntenic to a portion of human chromosome 19p13.1, an interval that contains the MIC-1 locus. Thus, based on results of our previous studies, it appears that GDF-15 is the murine ortholog of MIC-1 and hence will be referred to herein as MIC-1/GDF-15.[3]

MIC-1/GDF-15 (also called hPDF, hPLAB, hPTGFB, and NAG-1) has been independently identified by a number of groups searching for novel secreted factors.[2,4–8] By Northern blot analysis, murine MIC-1/GDF-15 is expressed in normal colon, liver, breast, prostate, and kidney.[2,3] More sensitive techniques of RNA detection have demonstrated MIC-1/GDF-15 expression in normal choroid plexus,[9] placenta,[4] prostate,[3] and vascular endothelium.[10,11] Studies have identified rodent MIC-1/GDF-15 expression in injured liver and brain, and more recently serum MIC-1 concentrations have

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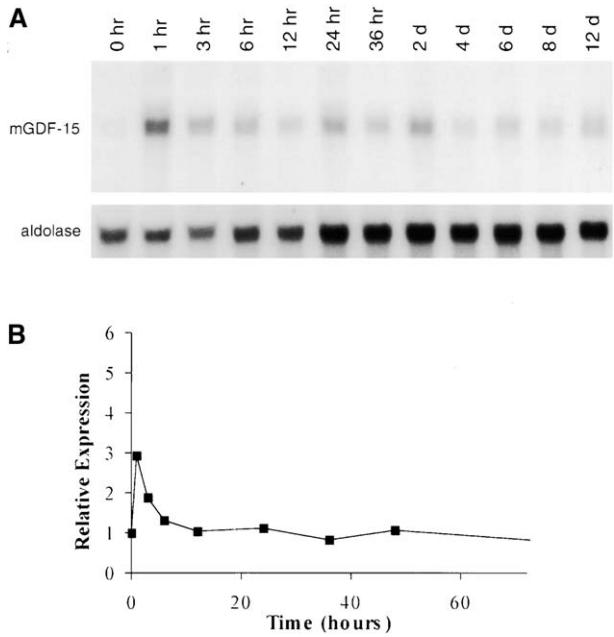

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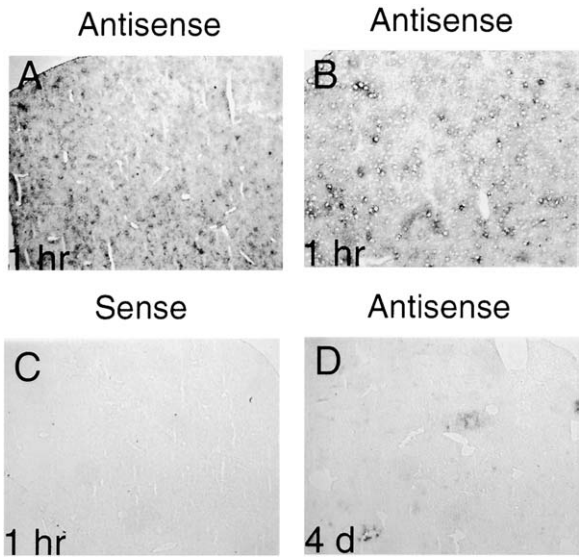

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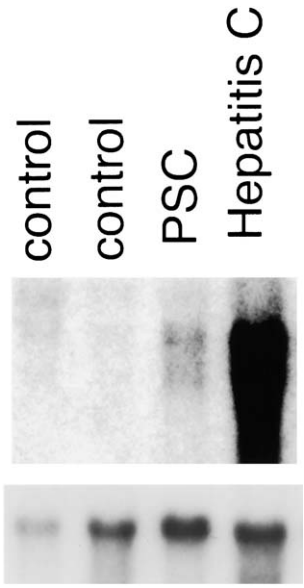

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Management of Epiphrenic Diverticula

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Epiphrenic diverticula are very rarely seen and are often associated with achalasia, esophageal body dysmotility, and a high resting lower esophageal sphincter pressure. The aim of this study was to evaluate the different treatment options for patients with epiphrenic diverticula. Patients with an epiphrenic diverticulum were divided into two treatment groups: surgical and nonsurgical. Retrospective chart review was performed, and a symptom questionnaire was created. There were six patients in the nonsurgical group and 11 patients in the surgical group. The mean follow-up was 26.4 months. Ten patients had a laparoscopic operation performed. One patient was operated on thoracoscopically and had to be converted to a thoracotomy. Two diverticula were inverted with good results. There was one postoperative esophageal leak where no myotomy was added. An empyema developed in another patient at 4 weeks after surgery. One patient, in whom no antireflux procedure was performed, reported postoperative heartburn. Patients in the nonsurgical group had smaller diverticula, were not good candidates for surgery, or were asymptomatic. Esophageal diverticula are very rarely seen. Asymptomatic patients may not require therapy. If surgery is performed and the diverticulum is large, it should be removed. The laparoscopic approach is the surgical treatment of choice. A long myotomy and an antireflux procedure should be added to avoid esophageal leakage at the line of repair and gastroesophageal reflux. (J GASTROINTEST SURG 2003;7:906–911)

KEY WORDS: Esophageal diverticulum, epiphrenic diverticulum, laparoscopic surgery, achalasia

Opinions differ as to whether epiphrenic diverticula are true[1,2] or false[3–5] diverticula, and their origin remains speculative. Furthermore, the reason for diverticula to increase in size is thought to be due to an outflow obstruction. Epiphrenic diverticula occur in the distal 10 cm of the esophagus and are frequently associated with spastic esophageal dysmotility and a high resting pressure in the lower esophageal sphincter (LES),[6] suggesting that they are pulsion diverticula. Streitz et al.[7] reported the occurrence of epiphrenic diverticula with achalasia and drew a line between these two diseases in terms of the pathophysiology that causes epiphrenic diverticula and esophageal body motility abnormalities. The true incidence of epiphrenic diverticula is unknown because they are often asymptomatic and usually go undiagnosed. Therefore most patients with an epiphrenic diverticulum do not undergo surgery. The decision to operate and which operation to perform depends on the presence of symptoms, which may include dysphagia, regurgitation, weight loss, and chest pain. The preoperative workup should include an upper gastrointestinal contrast study, upper endoscopy, and esophageal manometry. However, manometry may not always be successful in these patients because of the inability to pass the probe past the diverticulum.[8] If the patient has symptoms of reflux disease, pH monitoring should be done to diagnose true gastroesophageal reflux as opposed to reflux from the diverticulum into the midesophagus.[9]

The surgical approach may be via a left or right thoracotomy, a thoracoscopy, a laparotomy, or laparoscopy. The undetermined etiology of epiphrenic diverticula has led to controversy regarding the routine use of a myotomy in addition to the diverticulectomy.[10,11] Also, the required length of the myotomy remains controversial. The surgical therapeutic possibilities include diverticulectomy with a long myotomy with or without an antireflux procedure, diverticulectomy with selective myotomy for those with a motor abnormality found on manometry, or diverticulectomy alone if no motor abnormality is detected. The

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latter is controversial because case reports suggest a lower recurrence rate, a reduced risk of suture line leakage, and better symptom resolution when a myotomy is performed. To evaluate the outcome of patients with epiphrenic diverticula, we investigated all patients in our institutional records between 1996 and 2000 who are known to have had an esophageal diverticulum and compared the different surgical and nonsurgical treatment options.

MATERIAL AND METHODS

Study Group

All patients undergoing surgery for epiphrenic diverticula at the Mayo Clinic Jacksonville, Florida, from May 1996 to December 2000 were included in the study group.

Control Group

All patients seen at the Mayo Clinic Jacksonville, Florida, from March 1996 to December 2000 who were known to have an epiphrenic diverticulum were included in the control group. These patients were identified by examining all esophageal manometric and radiologic records for this period of time.

Study Design

A retrospective chart review was done to determine preoperative symptoms, manometric findings, radiologic findings, type of surgical procedure, postoperative outcome, and type of conservative therapy. A questionnaire was answered by all patients via a telephone call from a blinded investigator. Patients were asked to rate their symptoms on a scale of 1 to 10 with 1 indicating no symptoms and 10 indicating severe symptoms. Questions were asked concerning dysphagia, regurgitation, chest pain, heartburn, other symptoms, and current treatment. All patients had a barium esophagram. Esophageal manometry and 24-hour pH monitoring were performed according to the method described by DeMeester et al.[10]

Surgical Procedures

In six patients the diverticulum was excised, in two it was inverted into the esophagus, and in three it was included in a fundoplication. The surgical approach was either laparoscopic or through the chest, depending on the size of the diverticulum or its accessibility from the abdomen. In 10 patients the laparoscopic approach was used. After the establishment of a pneumoperitoneum superior to the umbilicus and insertion of four subcostal ports, as is customary for laparoscopic Nissen fundoplication, the diaphragmatic hiatus was dissected and the esophagogastric junction encircled. The lower esophagus was dissected into the mediastinum until the diverticulum was evident on the right or left side of the esophagus. This was then dissected off the mediastinum using blunt and sharp dissection. Most were quite densely adherent to the mediastinum by fibrous tissue around the diverticulum. The neck was then identified and encircled until the esophageal muscle was evident all around the neck of the diverticulum. The diverticulum was then excised by firing an Endo GIA stapler (U.S. Surgical, Norwalk, CT) several times across its neck, or it was inverted into the esophagus and the muscle layer was closed with a few interrupted sutures. In all but one patient a Heller myotomy (anterior esophagomyotomy 6 to 8 cm in length) was performed after the diverticulectomy. The hiatus was approximated with several interrupted sutures, if required, and the short gastric vessels were divided. The fundus was brought behind the esophagus and a fundoplication was created. In one patient the thoracoscopic approach was attempted. This patient required conversion to a thoracotomy because of difficulty in dissecting the adherent diverticulum. The thoracoscopy was begun by placing four thoracoports after which the diaphragm and lung were retracted, and the pulmonary ligament was divided. An attempt was made to dissect the esophagus, which was surrounded by a large amount of fibrous tissue. It was then decided to convert to a thoracotomy. The diverticulum was fully mobilized in the mediastinum down to a narrow neck, which was stapled, and the diverticulum was removed. All but one patient who underwent surgery had an antireflux procedure performed. The choice of antireflux procedure, a 360-degree Nissen fundoplication or a 270-degree Toupet fundoplication, was based on the results of esophageal manometry. Patients with poor esophageal manometric findings (peristaltic pressure <30 mm Hg in the distal esophagus or 20% simultaneous or nontransmitted contractions) underwent a 270-degree fundoplication, whereas patients with normal esophageal body motility had a 360-degree Nissen fundoplication performed.

RESULTS

Demographics

Table 1 presents the demographic data from all patients in both groups. The total number of patients who underwent surgery during the study period was 11. Seven patients who had nonsurgical therapy constituted the control group. Surgical data are summarized in Table 2. The mean follow-up time was

Table 1. Demographic data in surgical and nonsurgical patients prior to therapy

	Treatment	
	Surgical	**Nonsurgical**
No. of patients	11	6
Sex ratio (females:males)	5:6	4:3
Mean age (yr)		
Females	65	60
Males	70	75
Mean weight (kg)		
Females	56.2	66.2
Males	82.2	74.4
Mean duration of symptoms prior to surgical or medical therapy (mo)		
Females	62	27
Males	18	75
Symptoms		
Heartburn	8	1
Regurgitation	7	1
Acid reflux	3	4
Dysphagia	6	3
Pulmonary symptoms	6	0
Vomiting	1	0
Chest pain	0	1

26.4 months with a range of 2 to 48 months. There was one conversion to an open procedure. Two patients had two complications. One patient had an esophageal leak at the staple line on day 4 after a laparoscopic diverticulectomy and required a right-sided thoracotomy to oversew the leak and to add a myotomy. A jejunal feeding tube was inserted via a minilaparotomy. This was the one patient in whom a Heller myotomy was not performed after diverticulectomy. Another patient had an empyema in the right chest 4 weeks after surgery. A Gastrografin swallow test showed no evidence of esophageal leakage of contrast medium. The empyema was successfully drained and the patient did well. All other operative procedures were successfully completed laparoscopically. Table 3 presents the details of patients after nonsurgical treatment. All of them had a small diverticulum (up to 2 cm) or a broad-based diverticulum. One patient died during follow-up due to progression of esophageal cancer. The other patients are either asymptomatic under conservative treatment or they were not operated on because of concomitant diseases that made surgery too risky.

Esophageal Manometry

The results of esophageal manometry in all patients is presented in Table 4. If no data are given,

Table 2. Patients treated with surgery

Patient	Diverticulum	Additional diagnosis	Diverticulectomy	Antireflux surgery	Myotomy	Approach	Complication	Conversion	Duration (min)	Blood loss (ml)	Length of hospital stay (days)
C.P.	2 diverticula <2 cm, midthoracic esophagus		No	Nissen	–	Lap	–	No	60	50	6
E.B.	Distal diverticulum <2 cm		No	Nissen	–	Lap	–	No	60	40	1
I.C.	Distal diverticulum <2 cm	Achalasia	No	Toupet	+	Lap	–	No	60	50	1
C.W.	Distal diverticulum <2 cm	Achalasia	No	Toupet	+	Lap	–	No	60	75	1
M.F.	7 cm Ø, distal diverticulum		Yes	None	–	Lap	Leak	No	175	100	29
J.D.	8 cm Ø, distal diverticulum		Yes	Toupet	+	Thorac	–	Yes	240	500	5
R.A.	8 cm Ø, distal diverticulum		Yes	Toupet	+	Lap	–	No	340	250	5
R.M.	10 cm Ø, distal diverticulum		Yes	Toupet	+	Lap	–	No	240	100	3
M.M.	10 cm Ø, distal diverticulum		Yes	Toupet	+	Lap	–	No	255	100	6
D.H.	Distal diverticulum <2 cm		No	Nissen	–	Lap	–	No	60	100	1
C.C.	10 cm Ø, distal diverticulum		Yes	Nissen	+	Lap	Empyema	No	300	200	2

Lap = laparoscopic; Ø = diameter; Thorac = thoracoscopic.

Table 3. Patients with esophageal diverticula and nonsurgical therapy

Patient	Diverticulum	Additional diagnosis	Therapy	Reason for conservative therapy
S.H.	2 cm distal diverticulum	Irritable bowel syndrome	Bethanecol	Normal 24-hour pH, normal EGD, asymptomatic
R.M.†	Small distal diverticulum	Esophageal cancer	Chemo and radiation therapy	Metastatic esophageal cancer
C.E.	Small distal diverticulum	Nutcracker esophagus	H_2 blocker	Asymptomatic
D.O.	Small diverticulum, midthoracic esophagus	Fibromyalgia, spastic esophagus	Botulinum injection	Spastic esophagus
M.M.	Small diverticulum	Severe Parkinson's disease, esophageal spasm	Dilatation	Surgery not indicated, esophageal spasm
G.M.	Small diverticulum, midthoracic esophagus	Distal esophageal ring	Proton pump inhibitor, esophageal dilation	Asymptomatic after dilation

EGD = esophagogastroduodenoscopy.
†Diseased.

this means that manometry could not be performed because of inability to pass the probe into the esophagus or into the LES.

Questionnaire

Eight of the 11 patients in the surgical group answered the questionnaire. One patient lives outside of the United States, and two patients were lost to follow-up. The data are graphically depicted in Fig. 1. None of the patients had significant dysphagia, regurgitation, chest pain, or heartburn. There was no statistically significant difference between the two groups. Only one patient required modification of his diet after surgery. Two patients were on proton pump inhibitors for mild reflux symptoms. One of these patients did not undergo an antireflux procedure. In the nonsurgical treatment group, four of the six patients were able to answer the questionnaire. One patient died of progression of esophageal cancer, and the second patient was unable to answer the questionnaire because of severe Parkinson's disease. Fig. 1 shows the results of the questionnaire. Two patients were asymptomatic while on medication, and two had mild symptoms according to the scoring system.

DISCUSSION

Epiphrenic diverticula are very rarely seen, and the pathogenesis is poorly understood. Esophageal

Table 4. Manometric characteristics of all patients

Patient	Therapy	LESP	OL	AL	rel	rP	Simult	Nontra
C.P.	Surgery	11.7	3.5	2	—	—	0	0
E.B.	Surgery	4.6	6.8	2	100	0	0	0
L.C.	Surgery	28.8	7	2.3	50	14.3	100	0
C.W.	Surgery	17.6	4.25	3.25	<85	2.94	100	100
M.F.	Surgery	13	—	—	100	0	100	0
J.D.	Surgery	—	5	5	100	0	0	30
R.A.	Surgery	25	5.7	3	100	0	0	30
R.M.	Surgery	—	—	—	—	—	10	10
M.M.	Surgery	—	—	—	—	—	—	—
D.H.	Surgery	10.2	10.3	4.5	100	0	0	10
C.C.	Surgery	—	—	—	—	—	0	10
S.H.	Conservative	33.1	3	2	100	0	10	20
R.M.†	Conservative	13.7	5	4	65.6	4.37	0	0
C.E.	Conservative	22.9	—	—	88	3.8	0	0
D.O.	Conservative	—	5	5	—	—	50	0
M.M.	Conservative	39.2	5	—	—	0	0	0

LESP = lower esophageal sphincter pressure (mm Hg), (normal 5–28 mm Hg); OL = overall sphincter length (cm), (normal >2 cm); AL = abdominal sphincter length (cm), (Normal >1 cm); Nontra = nontransmitted contractions (%), (normal <20%); rel = relaxation (%), (normal >85%); rP = residual sphincter pressure (mm Hg), (normal <2 mm Hg); Simult = simultaneous contractions (%), (normal <20%).
†Diseased.

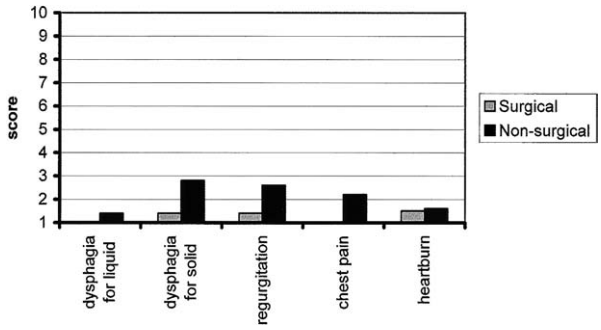

Fig. 1. Mean scores for postoperative symptoms in the surgical group and symptoms after conservative treatment in the non-surgical group.

motility abnormalities are commonly associated with esophageal diverticula; however, they may also arise in the absence of esophageal motility disturbances. Because esophageal epiphrenic diverticula are of varying sizes and symptomatology, the treatment must be individually adapted for each patient. Generally speaking, small diverticula are less symptomatic and can be treated nonsurgically. Patients who are asymptomatic and have their diverticula incidentally diagnosed do not require specific therapy. Symptomatic patients who are not suitable surgical candidates may benefit from medical therapy such as proton pump inhibitors or H_2 blockers. In our series we had two patients who were asymptomatic while on these medications. Alternatives other than surgical treatment are endoscopic dilation or botulinum toxin injection because epiphrenic diverticula are often associated with achalasia or achalasia-like conditions.

For symptomatic patients with large diverticula, the surgical treatment options are still controversial. Large epiphrenic diverticula should be removed. Since the introduction of minimally invasive surgery, the laparoscopic approach has been favored. In our series, laparoscopy was found to be safe and effective with a low mortality, acceptable blood loss, and rapid postoperative recovery, provided that there were no complications. On the other hand, thoracoscopy or thoracotomy was found to be more invasive and more difficult. The gastroesophageal junction may be more accessible by laparoscopy than by thoracosopy, as has been shown in anitreflux surgery. It is easier to dissect the diverticulum through the diaphragmatic hiatus and add a fundoplication from the abdomen than through the chest. In all of our 10 patients, the diverticulectomy was successfully performed laparoscopically without intraoperative complications and without the need for conversion to open surgery. Altorki et al.[11] reported their experience with the open transthoracic approach for epiphrenic diverticula in 17 patients. They did not encounter any morbidity; however, one patient died as a result of rupture of the mucosa through the myotomy site. Long-term outcome in their patient population was good, and all but one was symptom free during a follow-up of 2 years. Another large series has been reported by Benacci et al.[12] There were 112 patients in that series, 35 of whom had severe symptoms and underwent open surgical repair. These investigators also chose the open thoracic approach. Compared to our results, their perioperative and postoperative complications seem to be high. Six patients had esophageal leakage, four of whom had spontaneous resolution of the leaks and remained asymptomatic. There were three operative deaths. Two of these patients had esophageal leakage and one died of cardiac arrhythmia. This strengthens our opinion that the laparoscopic approach for patients with epiphrenic diverticula is associated with lower morbidity and mortality when compared to the open approach.

There is still controversy regarding the addition of a myotomy and the length of the myotomy. Because the pathogenesis of epiphrenic diverticula is thought to be due to esophageal dysmotility or an elevated LES pressure,[13] we added a long myotomy in all but one case after diverticulectomy. This patient, who had normal manometric findings and did not have an esophageal myotomy, had a postoperative leak and required a thoracotomy to oversew a leak at the staple line. This experience has strengthened our decision to perform a long myotomy in every patient to reduce the intraluminal pressure at the staple line. Nehra et al.[14] demonstrated similar findings and reported that resection of the diverticulum and a surgical myotomy of the manometrically defined abnormal segment results in relief of symptoms and protection from aspiration.

If a myotomy is added, a fundoplication should be done to prevent gastroesophageal reflux. Only one of our patients reported heartburn after surgery. We had not performed an antireflux procedure in this patient after the myotomy. The choice of antireflux procedure should be based on the results of esophageal motility testing. A Nissen fundoplication should be performed in patients who have normal esophageal motility and a partial Toupet fundoplication should be used in patients with altered esophageal motility.

CONCLUSION

We have shown that esophageal diverticula are very uncommon, and symptoms are related to the size of these diverticula. Asymptomatic patients, who usually have small diverticula, may not require specific therapy, and those patients who are not good candidates for surgery can be successfully treated nonsurgically.

Surgery should be considered in those with diverticula larger than 2 to 5 cm. The laparoscopic approach is the best surgical approach. A long myotomy and an antireflux procedure should be added to avoid esophageal leakage or gastroesophageal reflux.

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The surgical approach may be via a left or right thoracotomy, a thoracoscopy, a laparotomy, or laparoscopy. The undetermined etiology of epiphrenic diverticula has led to controversy regarding the routine use of a myotomy in addition to the diverticulectomy.[10,11] Also, the required length of the myotomy remains controversial. The surgical therapeutic possibilities include diverticulectomy with a long myotomy with or without an antireflux procedure, diverticulectomy with selective myotomy for those with a motor abnormality found on manometry, or diverticulectomy alone if no motor abnormality is detected. The

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latter is controversial because case reports suggest a lower recurrence rate, a reduced risk of suture line leakage, and better symptom resolution when a myotomy is performed. To evaluate the outcome of patients with epiphrenic diverticula, we investigated all patients in our institutional records between 1996 and 2000 who are known to have had an esophageal diverticulum and compared the different surgical and nonsurgical treatment options.

MATERIAL AND METHODS

Study Group

All patients undergoing surgery for epiphrenic diverticula at the Mayo Clinic Jacksonville, Florida, from May 1996 to December 2000 were included in the study group.

Control Group

All patients seen at the Mayo Clinic Jacksonville, Florida, from March 1996 to December 2000 who were known to have an epiphrenic diverticulum were included in the control group. These patients were identified by examining all esophageal manometric and radiologic records for this period of time.

Study Design

A retrospective chart review was done to determine preoperative symptoms, manometric findings, radiologic findings, type of surgical procedure, postoperative outcome, and type of conservative therapy. A questionnaire was answered by all patients via a telephone call from a blinded investigator. Patients were asked to rate their symptoms on a scale of 1 to 10 with 1 indicating no symptoms and 10 indicating severe symptoms. Questions were asked concerning dysphagia, regurgitation, chest pain, heartburn, other symptoms, and current treatment. All patients had a barium esophagram. Esophageal manometry and 24-hour pH monitoring were performed according to the method described by DeMeester et al.[10]

Surgical Procedures

In six patients the diverticulum was excised, in two it was inverted into the esophagus, and in three it was included in a fundoplication. The surgical approach was either laparoscopic or through the chest, depending on the size of the diverticulum or its accessibility from the abdomen. In 10 patients the laparoscopic approach was used. After the establishment of a pneumoperitoneum superior to the umbilicus and insertion of four subcostal ports, as is customary for laparoscopic Nissen fundoplication, the diaphragmatic hiatus was dissected and the esophagogastric junction encircled. The lower esophagus was dissected into the mediastinum until the diverticulum was evident on the right or left side of the esophagus. This was then dissected off the mediastinum using blunt and sharp dissection. Most were quite densely adherent to the mediastinum by fibrous tissue around the diverticulum. The neck was then identified and encircled until the esophageal muscle was evident all around the neck of the diverticulum. The diverticulum was then excised by firing an Endo GIA stapler (U.S. Surgical, Norwalk, CT) several times across its neck, or it was inverted into the esophagus and the muscle layer was closed with a few interrupted sutures. In all but one patient a Heller myotomy (anterior esophagomyotomy 6 to 8 cm in length) was performed after the diverticulectomy. The hiatus was approximated with several interrupted sutures, if required, and the short gastric vessels were divided. The fundus was brought behind the esophagus and a fundoplication was created. In one patient the thoracoscopic approach was attempted. This patient required conversion to a thoracotomy because of difficulty in dissecting the adherent diverticulum. The thoracoscopy was begun by placing four thoracoports after which the diaphragm and lung were retracted, and the pulmonary ligament was divided. An attempt was made to dissect the esophagus, which was surrounded by a large amount of fibrous tissue. It was then decided to convert to a thoracotomy. The diverticulum was fully mobilized in the mediastinum down to a narrow neck, which was stapled, and the diverticulum was removed. All but one patient who underwent surgery had an antireflux procedure performed. The choice of antireflux procedure, a 360-degree Nissen fundoplication or a 270-degree Toupet fundoplication, was based on the results of esophageal manometry. Patients with poor esophageal manometric findings (peristaltic pressure <30 mm Hg in the distal esophagus or 20% simultaneous or nontransmitted contractions) underwent a 270-degree fundoplication, whereas patients with normal esophageal body motility had a 360-degree Nissen fundoplication performed.

RESULTS

Demographics

Table 1 presents the demographic data from all patients in both groups. The total number of patients who underwent surgery during the study period was 11. Seven patients who had nonsurgical therapy constituted the control group. Surgical data are summarized in Table 2. The mean follow-up time was

Table 1. Demographic data in surgical and nonsurgical patients prior to therapy

	Treatment	
	Surgical	**Nonsurgical**
No. of patients	11	6
Sex ratio (females:males)	5:6	4:3
Mean age (yr)		
Females	65	60
Males	70	75
Mean weight (kg)		
Females	56.2	66.2
Males	82.2	74.4
Mean duration of symptoms prior to surgical or medical therapy (mo)		
Females	62	27
Males	18	75
Symptoms		
Heartburn	8	1
Regurgitation	7	1
Acid reflux	3	4
Dysphagia	6	3
Pulmonary symptoms	6	0
Vomiting	1	0
Chest pain	0	1

26.4 months with a range of 2 to 48 months. There was one conversion to an open procedure. Two patients had two complications. One patient had an esophageal leak at the staple line on day 4 after a laparoscopic diverticulectomy and required a right-sided thoracotomy to oversew the leak and to add a myotomy. A jejunal feeding tube was inserted via a minilaparotomy. This was the one patient in whom a Heller myotomy was not performed after diverticulectomy. Another patient had an empyema in the right chest 4 weeks after surgery. A Gastrografin swallow test showed no evidence of esophageal leakage of contrast medium. The empyema was successfully drained and the patient did well. All other operative procedures were successfully completed laparoscopically. Table 3 presents the details of patients after nonsurgical treatment. All of them had a small diverticulum (up to 2 cm) or a broad-based diverticulum. One patient died during follow-up due to progression of esophageal cancer. The other patients are either asymptomatic under conservative treatment or they were not operated on because of concomitant diseases that made surgery too risky.

Esophageal Manometry

The results of esophageal manometry in all patients is presented in Table 4. If no data are given,

Table 2. Patients treated with surgery

Patient	Diverticulum	Additional diagnosis	Diverticulectomy	Antireflux surgery	Myotomy	Approach	Complication	Conversion	Duration (min)	Blood loss (ml)	Length of hospital stay (days)
C.P.	2 diverticula <2 cm, midthoracic esophagus		No	Nissen	–	Lap	–	No	60	50	6
E.B.	Distal diverticulum <2 cm		No	Nissen	–	Lap	–	No	60	40	1
I.C.	Distal diverticulum <2 cm	Achalasia	No	Toupet	+	Lap	–	No	60	50	1
C.W.	Distal diverticulum <2 cm	Achalasia	No	Toupet	+	Lap	–	No	60	75	1
M.F.	7 cm Ø, distal diverticulum		Yes	None	–	Lap	Leak	No	175	100	29
J.D.	8 cm Ø, distal diverticulum		Yes	Toupet	+	Thorac	–	Yes	240	500	5
R.A.	8 cm Ø, distal diverticulum		Yes	Toupet	+	Lap	–	No	340	250	5
R.M.	10 cm Ø, distal diverticulum		Yes	Toupet	+	Lap	–	No	240	100	3
M.M.	10 cm Ø, distal diverticulum		Yes	Toupet	+	Lap	–	No	255	100	6
D.H.	Distal diverticulum <2 cm		No	Nissen	–	Lap	–	No	60	100	1
C.C.	10 cm Ø, distal diverticulum		Yes	Nissen	+	Lap	Empyema	No	300	200	2

Lap = laparoscopic; Ø = diameter; Thorac = thoracoscopic.

Table 3. Patients with esophageal diverticula and nonsurgical therapy

Patient	Diverticulum	Additional diagnosis	Therapy	Reason for conservative therapy
S.H.	2 cm distal diverticulum	Irritable bowel syndrome	Bethanecol	Normal 24-hour pH, normal EGD, asymptomatic
R.M.†	Small distal diverticulum	Esophageal cancer	Chemo and radiation therapy	Metastatic esophageal cancer
C.E.	Small distal diverticulum	Nutcracker esophagus	H_2 blocker	Asymptomatic
D.O.	Small diverticulum, midthoracic esophagus	Fibromyalgia, spastic esophagus	Botulinum injection	Spastic esophagus
M.M.	Small diverticulum	Severe Parkinson's disease, esophageal spasm	Dilatation	Surgery not indicated, esophageal spasm
G.M.	Small diverticulum, midthoracic esophagus	Distal esophageal ring	Proton pump inhibitor, esophageal dilation	Asymptomatic after dilation

EGD = esophagogastroduodenoscopy.
†Diseased.

this means that manometry could not be performed because of inability to pass the probe into the esophagus or into the LES.

Questionnaire

Eight of the 11 patients in the surgical group answered the questionnaire. One patient lives outside of the United States, and two patients were lost to follow-up. The data are graphically depicted in Fig. 1. None of the patients had significant dysphagia, regurgitation, chest pain, or heartburn. There was no statistically significant difference between the two groups. Only one patient required modification of his diet after surgery. Two patients were on proton pump inhibitors for mild reflux symptoms. One of these patients did not undergo an antireflux procedure. In the nonsurgical treatment group, four of the six patients were able to answer the questionnaire. One patient died of progression of esophageal cancer, and the second patient was unable to answer the questionnaire because of severe Parkinson's disease. Fig. 1 shows the results of the questionnaire. Two patients were asymptomatic while on medication, and two had mild symptoms according to the scoring system.

DISCUSSION

Epiphrenic diverticula are very rarely seen, and the pathogenesis is poorly understood. Esophageal

Table 4. Manometric characteristics of all patients

Patient	Therapy	LESP	OL	AL	rel	rP	Simult	Nontra
C.P.	Surgery	11.7	3.5	2	—	—	0	0
E.B.	Surgery	4.6	6.8	2	100	0	0	0
L.C.	Surgery	28.8	7	2.3	50	14.3	100	0
C.W.	Surgery	17.6	4.25	3.25	<85	2.94	100	100
M.F.	Surgery	13	—	—	100	0	100	0
J.D.	Surgery	—	5	5	100	0	0	30
R.A.	Surgery	25	5.7	3	100	0	0	30
R.M.	Surgery	—	—	—	—	—	10	10
M.M.	Surgery	—	—	—	—	—	—	—
D.H.	Surgery	10.2	10.3	4.5	100	0	0	10
C.C.	Surgery	—	—	—	—	—	0	10
S.H.	Conservative	33.1	3	2	100	0	10	20
R.M.†	Conservative	13.7	5	4	65.6	4.37	0	0
C.E.	Conservative	22.9	—	—	88	3.8	0	0
D.O.	Conservative	—	5	5	—	—	50	0
M.M.	Conservative	39.2	5	—	—	0	0	0

LESP = lower esophageal sphincter pressure (mm Hg), (normal 5–28 mm Hg); OL = overall sphincter length (cm), (normal >2 cm); AL = abdominal sphincter length (cm), (Normal >1 cm); Nontra = nontransmitted contractions (%), (normal <20%); rel = relaxation (%), (normal >85%); rP = residual sphincter pressure (mm Hg), (normal <2 mm Hg); Simult = simultaneous contractions (%), (normal <20%).
†Diseased.

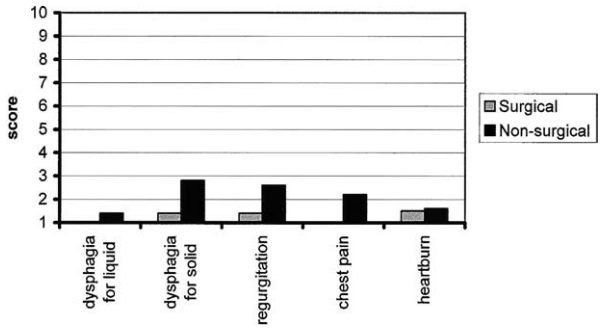

Fig. 1. Mean scores for postoperative symptoms in the surgical group and symptoms after conservative treatment in the non-surgical group.

motility abnormalities are commonly associated with esophageal diverticula; however, they may also arise in the absence of esophageal motility disturbances. Because esophageal epiphrenic diverticula are of varying sizes and symptomatology, the treatment must be individually adapted for each patient. Generally speaking, small diverticula are less symptomatic and can be treated nonsurgically. Patients who are asymptomatic and have their diverticula incidentally diagnosed do not require specific therapy. Symptomatic patients who are not suitable surgical candidates may benefit from medical therapy such as proton pump inhibitors or H_2 blockers. In our series we had two patients who were asymptomatic while on these medications. Alternatives other than surgical treatment are endoscopic dilation or botulinum toxin injection because epiphrenic diverticula are often associated with achalasia or achalasia-like conditions.

For symptomatic patients with large diverticula, the surgical treatment options are still controversial. Large epiphrenic diverticula should be removed. Since the introduction of minimally invasive surgery, the laparoscopic approach has been favored. In our series, laparoscopy was found to be safe and effective with a low mortality, acceptable blood loss, and rapid postoperative recovery, provided that there were no complications. On the other hand, thoracoscopy or thoracotomy was found to be more invasive and more difficult. The gastroesophageal junction may be more accessible by laparoscopy than by thoracosopy, as has been shown in anitreflux surgery. It is easier to dissect the diverticulum through the diaphragmatic hiatus and add a fundoplication from the abdomen than through the chest. In all of our 10 patients, the diverticulectomy was successfully performed laparoscopically without intraoperative complications and without the need for conversion to open surgery. Altorki et al.[11] reported their experience with the open transthoracic approach for epiphrenic diverticula in 17 patients. They did not encounter any morbidity; however, one patient died as a result of rupture of the mucosa through the myotomy site. Long-term outcome in their patient population was good, and all but one was symptom free during a follow-up of 2 years. Another large series has been reported by Benacci et al.[12] There were 112 patients in that series, 35 of whom had severe symptoms and underwent open surgical repair. These investigators also chose the open thoracic approach. Compared to our results, their perioperative and postoperative complications seem to be high. Six patients had esophageal leakage, four of whom had spontaneous resolution of the leaks and remained asymptomatic. There were three operative deaths. Two of these patients had esophageal leakage and one died of cardiac arrhythmia. This strengthens our opinion that the laparoscopic approach for patients with epiphrenic diverticula is associated with lower morbidity and mortality when compared to the open approach.

There is still controversy regarding the addition of a myotomy and the length of the myotomy. Because the pathogenesis of epiphrenic diverticula is thought to be due to esophageal dysmotility or an elevated LES pressure,[13] we added a long myotomy in all but one case after diverticulectomy. This patient, who had normal manometric findings and did not have an esophageal myotomy, had a postoperative leak and required a thoracotomy to oversew a leak at the staple line. This experience has strengthened our decision to perform a long myotomy in every patient to reduce the intraluminal pressure at the staple line. Nehra et al.[14] demonstrated similar findings and reported that resection of the diverticulum and a surgical myotomy of the manometrically defined abnormal segment results in relief of symptoms and protection from aspiration.

If a myotomy is added, a fundoplication should be done to prevent gastroesophageal reflux. Only one of our patients reported heartburn after surgery. We had not performed an antireflux procedure in this patient after the myotomy. The choice of antireflux procedure should be based on the results of esophageal motility testing. A Nissen fundoplication should be performed in patients who have normal esophageal motility and a partial Toupet fundoplication should be used in patients with altered esophageal motility.

CONCLUSION

We have shown that esophageal diverticula are very uncommon, and symptoms are related to the size of these diverticula. Asymptomatic patients, who usually have small diverticula, may not require specific therapy, and those patients who are not good candidates for surgery can be successfully treated nonsurgically.

Surgery should be considered in those with diverticula larger than 2 to 5 cm. The laparoscopic approach is the best surgical approach. A long myotomy and an antireflux procedure should be added to avoid esophageal leakage or gastroesophageal reflux.

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Old and New TNM in Carcinoma of the Gastric Antrum: Analysis of Our Personal Experience

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Various tumor node metastasis (TNM) classifications have been proposed for staging of gastric carcinoma, including the fourth edition of the TNM classification and the Japanese Research Society for Gastric Cancer (JRSGC) system. In 1997 the fifth edition of TNM classification introduced the concept of the number of metastatic lymph nodes. We review our experience with staging gastric cancer in light of both the fourth and fifth editions of the TNM classification system. From January 1986 to December 1997, we performed subtotal resection in 193 patients with carcinoma of the gastric antrum. A total of 147 patients presented with criteria from the fifth TNM edition. We compared data from these patients with data from the fourth TNM edition. We analyzed 84 females and 63 males whose average age was 68.9 years. The average number of lymph nodes removed was 16.7. We used the Kaplan-Meier method to analyze survival. In accordance with the fourth TNM edition, we recorded 82 patients who were pN0, 36 who were pN1, and 29 who were pN2; according to the fifth edition, 82 patients were pN0, 33 were pN1, 17 were pN2, and 15 were pN3. Average follow-up was 26.7 months, and average survival was 56.9 months for N0 patients, 38.7 months for N1 patients, and 24.5 months for N2 patients staged according to the fourth edition. According to the fifth edition, survival was 39.3 months for N1 patients, 33.6 months for N2 patients, and 10.3 months for N3 patients. The survival curve was statistically different ($P < 0.001$) between N0 and N1 patients according to the fourth edition; there was no significant difference between N1 and N2 patients. According to the fifth edition, the difference in survival probability was $P < 0.001$ between N0 and N1 patients and N2 and N3 patients. The fifth TNM edition presents a greater ease of stratification in bringing together and mediating diverse cultural experiences between West and East. This staging lays the basis for a more accurate comparison between the groups. (J GASTROINTEST SURG 2003;7:912–916)

KEY WORDS: Gastric cancer, TNM, survival, antrum

The two most important prognostic factors after curative surgery for gastric carcinoma are tumor invasion of the stomach wall and the presence of lymph node metastases. Various TNM classifications have been proposed for staging gastric carcinoma, including the fourth edition of the TNM classification and the Japanese Research Society for Gastric Cancer (JRSGC) system; both rely on an anatomic classification of lymph node involvement to stage the neoplasm. There is, however, real difficulty in comparing results obtained by means of different methods of classification. In 1997 the fifth edition of the TNM classification introduced the concept of the overall number of lymph nodes with a neoplasm, independent of their localization or distance from the primary tumor. We review the experience at our institution in the surgical treatment of carcinoma of the gastric antrum in light of both the 1987 (fourth edition) and the 1997 (fifth edition) TNM classifications by verifying their practical usefulness and prognostic value. Currently we are using the latest TNM classification; we analyze only the gastric antrum because it is at this site that we encounter the major difficulty in performing lymphadenectomy (≥15 lymph nodes).

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MATERIAL AND METHODS

From January 1986 to December 1997, we performed subtotal resections in 193 patients with carcinoma of the gastric antrum. Cure was attempted in 147 patients who met the criteria set forth in the fifth TNM edition. We subdivided these patients into groups according to the fifth edition of the TNM classification published in 1997.[1] For each neoplasm, we evaluated the relationship between the various parameters on the basis of diffusion, lymph node involvement, and the eventual presence of distant metastases, and compared these findings with the data from the previous TNM edition (fourth edition, 1987).

The study group consisted of 84 female and 63 male patients whose average age was 68.9 years. During subtotal gastric resection we removed between 15 to 29 lymph nodes, with an average of 16.7. All lesions were adenocarcinomas. We excluded patients who had undergone a lymphadenectomy with removal of less than 15 lymph nodes, as specified in the fifth edition of the TNM (46 patients), which analyzed the number but not the extent of lymphadenectomy. Survival curves were based on the results of Kaplan-Meier analysis with *P* values based on the Yates correction.

RESULTS

Using the 1987 classification, we recorded the neoplasms as follows: 82 pN0, 36 pN1, and 29 pN2. When we restaged these cancers according to the 1997 classification criteria, we identified the neoplasms as follows: 82 pN0, 33 pN1, 17 pN2, and 15 pN3 (Table 1) (Figs. 1 and 2). Similar to findings in other groups reported in the Western literature, the number of patients with stage T3 disease was equal to almost half of the patients evaluated.[2,3]

In order to have a minimum follow-up of 5 years, we considered patients who underwent subtotal gastric resection for carcinoma of the antrum from January 1986 to December 1997. Average follow-up was 26.7 months (range 6 to 72 months) with twice-yearly checkups. Average survival at 5 years from surgery on the basis of lymph node involvement (N) was as follows: 56.9 months for N0, 38.7 months for N1, and 24.5 months for N2 for patients staged according to the fourth edition of the TNM (1987). When the more recent version (fifth edition, 1997) was used for restaging, average survival was 39.3 months for N1, 33.6 months for N2, and 10.3 months for N3 patients.

Table 1. Tumor (T) and node (N) classification, 1987–1997, of 147 cases of gastric cancer

	T	N (1987)	N (1997)
0	2 (1.4%)	82 (55.7%)	82 (55.8%)
1	47 (31.9%)	36 (24.5%)	33 (22.4%)
2	23 (15.7%)	29 (19.8%)	17 (11.6%)
3	64 (43.5%)		15 (10.2%)
4	11 (7.5%)		

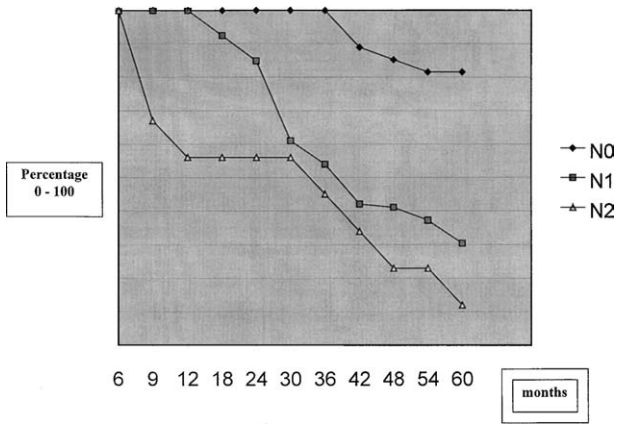

Fig. 1. Survival as determined by the fourth edition of the TNM classification.

We then examined the survival curves on the basis of N staging from both versions of TNM classification, verifying the eventual presence of a statistically significant difference between them. A statistically significant ($P < 0.001$) difference was evidenced between the N0 and N1 survival curves, whereas no significant difference was noted between the N1 and N2 survival curves according to the 1987 TNM classification ($P < 0.2$).

Again, when the fifth TNM edition was used, a statistically significant difference between N0 and N1 survival ($P < 0.001$) and no significance between

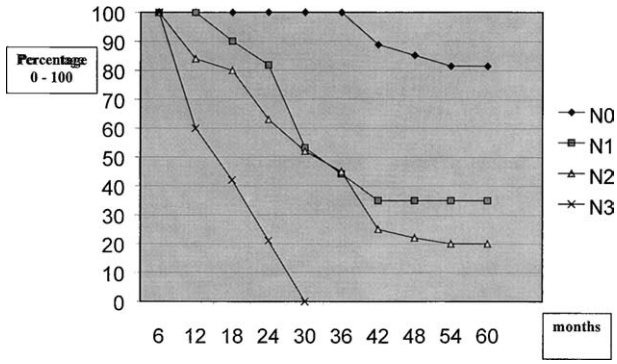

Fig. 2. Survival as determined by the fifth edition of the TNM classification.

N1 and N2 survival was confirmed, whereas the difference between the N2 and N3 survival curves was sharply positive ($P < 0.001$; P [Yates] < 0.005).

Comparison of the two classification systems—that is, the N1 category from the fourth edition and N1 from the fifth, as well as comparison of the N2 category—did not show any evidence of a real significant difference in survival curves. Moreover, we subdivided our patients further into stages on the basis of classification of both the fourth and fifth TNM editions. According to the 1987 classification, comparison of survival in relation to stage demostrated the presence of a significant difference between patients with stage Ia and patients with stage Ib disease ($P < 0.005$; P [Yates] < 0.02). When we carried out a sequential examination of this classification (i.e., between stages Ib and II, between stages II and IIIa, and between stages IIIa and IIIb), we did not find significant differences in P values.

When the 1997 staging was considered, a statistical positivity emerged between stages Ia and Ib ($P < 0.05$) and between stages IIIb and IV ($P < 0.002$; P [Yates] < 0.007). We also compared survival, with staging being equal, between the two TNM classification systems, and we did not observe any statistically significant differences between the curves examined.

DISCUSSION

Recent data from Japanese study groups have evidenced a constant decrease in gastric cancer during the past 35 years,[4] which most probably can be attributed to changes in dietary habits, the introduction of food conservation through refrigeration, and a decrease in the incidence of *Helicobacter pylori* infection.[4–10]

Kaneko and Yoshimura[4] analyzed 161,067 cases of gastric carcinoma recorded in the Japanese tumor registry from 1975 to 1989 and subdivided them according to age and histologic type. According to the Lauren classification,[11] they found evidence of a considerable decrease in the proportion of patients with intestinal tumors in their earlier years, but the incidence of diffuse-type tumors remained stable. At the same time, a decrease in mortality from gastric carcinoma was also observed, which can be attributed chiefly to the large-scale diagnostic use of digestive endoscopy and refinements in surgical and reanimation techniques.

As for surgery, particularly up to the recent past, doubts persisted regarding the most correct approach for managing neoplasms located in the gastric antrum. In particular, the appropriateness of performing an initial total gastrectomy or limiting the resection to a subtotal gastrectomy has been discussed. This debate arose at the end of the nineteenth century when Connor[12] unsuccessfully performed the first total gastrectomy; 10 years later Langenbuch, who performed the first subtotal gastrectomy, maintained that complete removal of the stomach was technically impossible.

In the past, this problem has been analyzed in numerous studies, and findings have indicated that subtotal gastrectomy is the operation of choice for tumors located at the antrum. Over the years numerous investigators have compared the two surgical options, taking into consideration the radical natures of the two operations with regard to the viscera, lymph nodes, and long-term results expressed in terms of quality of life and long-term survival.[13–21]

Some objections have been raised concerning the possible nonradical nature of this technique because of reports in the literature of tumor multi-focality. In reality, this occurrence has proven to be minimal (<4% in the literature; <3.2% in our experience), and some histopathologic studies have found evidence that an adequate safety margin, controlled intraoperatively, with extemporaneous histologic examination of frozen sections, constitutes the best guarantee for verifying the state of intramural tumor diffusion.

In a large case study, Bozzetti et al.[22] and Hornig et al.[23] observed that the risk of an "infiltrated" margin was nonexistent if examination of the proximal slice was carried out beyond 3 cm in tumors involving the muscle and beyond 6 cm in tumors extending to the serosa and proved to be negative. Hornig et al.[23] then reported that for gastric resections in which the visceral section was removed beyond the 5 cm of the neoplasm margin, survival was distinctly greater with respect to survival in cases where this level was not maintained. These data were confirmed 10 years later by Maruyama,[24] who reported that subtotal gastrectomy associated with D2 radical lymphectomy enabled good long-term survival.[24–26]

In numerous investigations, morbidity and mortality rates for this surgery were found to be lower compared to total gastrectomy, even though the use of mechanical anastomoses for the latter operation has resulted in greatly reduced morbidity and mortality. Finally, reports from expert nutritionists have demonstrated that weight trends and nutrition indexes are significantly lower in patients who undergo total gastrectomy.[27–34]

We thus decided to review our group of 147 patients with carcinomas of the gastric antrum treated by subtotal gastrectomy and radical lymphadenectomy between 1986 and 1997. We wanted to compare the results obtained through stratification of patients

according to the 1987 TNM modalities, and review them by following the restaging method set forth in the subsequent (1997) edition to verify possible differences in prognostic potential. For about 20 years, TNM staging has remained unchanged with regard to tumor (T) classification—that is, involvement of the visceral wall—while there has been unquestionable evolution concerning consideration attributed to the role that lymph node staging plays in long-term prognosis. In fact, the criterion of merely *anatomic* lymph node involvement has changed to a criterion that considers the *number* of lymph nodes removed during surgery, which provides decisive information regarding the extent of the cancer.

The advantages of the new TNM system may be summarized as follows: (1) increased reproducibilty of this classification; (2) the possibility of allowing the pathologist to study the disease directly from the piece removed without depending on a third party for preparation or recognition of the lymph nodes; (3) the process of histopathologic "reading" appears simplified because the problem of verification of the distance between the removed lymph nodes and the primary tumor no longer exists.

Moreover, the literature appears to be unanimous in reporting that the new classification system more accurately identifies the patient group with the worst prognosis as N3. In fact, by comparing survival curves on the basis of lymph node staging, the 1987 TNM classification stratifies patients into only two groups, N1 and N2, whereas the improved distribution of the sample into three distinct prognostic classes (N1, N2, and N3) in the 1997 TNM system is evident. This allows us to understand that the old TNM system, which was based on the topographic classification of lymph nodes, created objective comparative difficulties between Western and Japanese patient groups, based on anatomic criteria, with enormous problems involving identification of the site itself. Recent studies have demonstrated the possible overlapping between the 1997 TNM system and the JRSGC classification in order to provide prognostic information.

Negative aspects of the new staging method include the necessity of removing a conspicuous number of lymph nodes as an essential requirement to avoid substaging of the patient when a sufficient number of lymph nodes is obtained (a minimum number of 15 nodes is the cutoff point required for analysis). In fact, some investigators have indicated that it can be difficult to remove the minimum of 15 lymph nodes necessary for the present staging in many Western centers, rendering it impossible to obtain correct prognostic classes. Thus, in the majority of surgical programs at Western medical schools[35–38] a minimum number of 5 to 10 nodes is considered optimal.

In this sense it should be remembered that in subtotal gastrectomy, the number of lymph nodes involved seems to be an important prognostic factor: the significance of radical lymphadenectomy emerges not only in staging procedures but also as an important component of curative surgery. However, a well-founded objection remains—that is, such wide-ranging removal of local and regional lymph nodes can significantly increase the morbidity and mortality of surgery for gastric carcinoma.

Our experience is in line with results obtained by other investigators and confirms the greater ease of stratification in the fifth edition of the TNM classification, which combines and distinguishes between the diverse cultural experiences in medicine in the West and the East. This staging system lays the basis for more accurate comparisons between patient groups, which at the present time is difficult to achieve.

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KEY WORDS: Gastrostomy, PEG, feeding tube, endoscopic gastrostomy

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CASE REPORTS

Case 1

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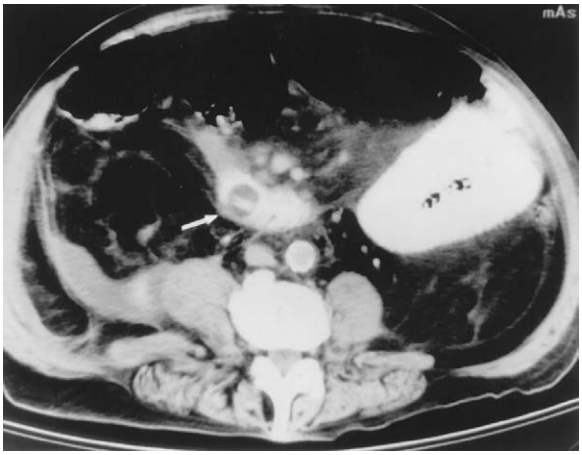

Fig. 1. CT scan of the abdomen with extravasation of contrast medium through a jejunal perforation adjacent to the Foley balloon (*arrow*).

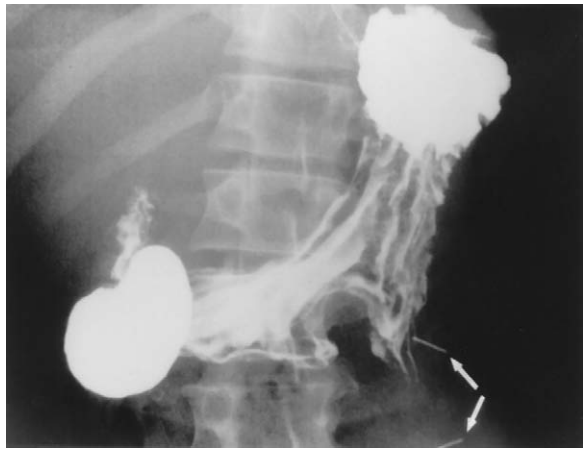

Fig. 2. Upper gastrointestinal series with T-fasteners (*arrows*) embedded in the gastric and anterior abdominal walls. (Reprinted with permission. Collure D, Bumpers H, Hoover E. A complication of T-fasteners in percutaneous endoscopic gastrostomy. Surg Endosc 1996;10:939. Figure 2, Springer-Verlag.)

and the jejunum was repaired after debridement of the edges of the perforation. A feeding jejunostomy was placed distal to the repair. The postoperative recovery was stormy and complicated by pneumonia.

Cases 2 and 3

Patients R.H. (an 18-year-old male) and A.K. (a 24-year-old female) were victims of unrelated auto accidents and had suffered severe closed-head injuries. They had both undergone PEG placement using Flexiflo gastrostomy kits with T-fasteners (Ross Laboratories, Columbus, OH). In both patients the external pledgets and the nylon sutures holding the T-fasteners had been divided at the skin level at 14 days after the procedure. After recovery and satisfactory oral intake, the PEG tubes had been removed at 3 months (patient R.H.) and 4 months (patient A.K.) after their insertion.

Patient R.H. returned 18 months later with purulent discharge near the PEG site. The site of insertion of the PEG tube was well healed, but there were two sinuses corresponding to points of insertion of the T-fasteners. An upper gastrointestinal series showed no evidence of a gastrocutaneous fistula. It did show that one T-fastener was embedded in the wall of the stomach, whereas another was in the anterior abdominal wall (Fig. 2). The sinus tracts were explored under local anesthesia, and the ends of the two nylon sutures were identified. These were divided in the depths of the wounds. The sinuses completely healed without incident.

Patient A.K. returned 12 months after removal of the PEG tube with two sinus tracts adjacent to the healed PEG tube site. These were explored and the metal T-fasteners were removed along with the nylon sutures. Uneventful healing of the wounds followed this procedure.

Case 4

Patient M.M. was a 52-year-old woman with severe mental retardation and a seizure disorder who was confined to a nursing home. She had had a "pull-through"–type gastrostomy inserted 4 years previously, and she was seen in the emergency department for its replacement. The outside stem of the PEG tube had deteriorated and was stiff with multiple dilatations along its length. While attempting to remove the tube, it separated from the internal flange that was retained within the stomach. A replacement gastrostomy tube was inserted through the track, and the retained flange was removed endoscopically without incident.

Case 5

Patient A.D. was an 80-year-old female resident of a nursing home who had a pull-through–type PEG tube placed approximately 3 weeks prior to admission. The patient by tugging at it had dislodged the original tube, and a Foley catheter had been inserted through the tract. Attempts to feed her through the new catheter were met with resistance. She was seen in the emergency department with erythema and swelling around the PEG tube site. A Gastrografin study through the catheter showed extravasation of the contrast medium, with the Foley balloon wedged between the stomach and the anterior abdominal wall

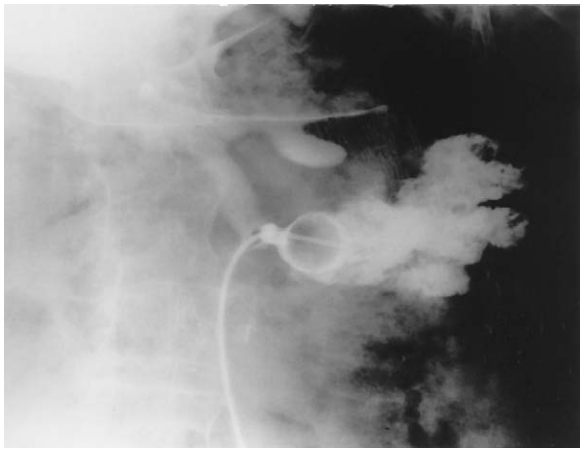

Fig. 3. A Gastrografin study through the PEG tube with extravasation of contrast medium between the anterior abdominal wall and the stomach.

(Fig. 3). Exploration and irrigation of the peritoneal cavity with repair of the anterior gastric wall at the gastrostomy site was carried out. A Dobhoff feeding tube was inserted into the proximal jejunum. The patient had a protracted postoperative course with a wound infection that subsequently resolved.

DISCUSSION

Morbidity secondary to PEG tubes has been reported to be 16%,[2,3] and a major complication rate of approximately 8% has also been reported.[4] Unusual complications may result from gastrostomy tubes remaining in place for an extended period of time. Many of these problems can be avoided by instructing the caregivers on proper maintenance of these tubes. When a balloon-tipped gastrostomy is used, it should always be distended with sterile water. With long-term gastrostomy placement, the tube should be evaluated for cracks, proper fit, and other signs of wear on a regular basis. Worn tubes should be replaced with an appropriate replacement tube. Gastrostomy replacement tubes generally have the balloon centered around the end of the tube. This makes for a snug and even fit once the tube is anchored. The Foley catheter is considered only a temporary measure to prevent closure of the ostomy site until the proper replacement is available. Normal saline solution tends to crystallize over time and clog the narrow balloon port. Even though the balloon and the tube are intact, the fluid volume tends to decrease over a period of months. This may result in the tube slipping out of position, and thus leakage around the tube will occur. Although leakage around the tube is not an uncommon complication, it can be a nuisance and may lead to cutaneous inflammation at the catheter site. Inserting a properly fitting balloon-tipped catheter and using a topical antibiotic ointment can easily remedy this. It is important to test the integrity of the balloon at intervals and reinflate it as necessary. Even though the capacity of the Foley balloon is given as 30 ml, it need not be distended with more than 8 to 10 ml of water.

If the external bumper anchoring the feeding tube is not secure, it may allow the tube to slip further into the stomach causing the balloon tip to be carried by gastric motility to its outlet. A distended balloon may occlude the pylorus, and the patient will have emesis. A properly placed percutaneous gastrostomy tube should be pulled back snugly against the inner aspect of the stomach and abdominal wall and an external bumper pushed into position against the skin and anchored with a 0 silk tie. Each bumper should only slightly depress its respective surfaces. Neither the mucosa nor the skin should be left in a state of blanching. Permanent blanching may lead to pressure necrosis at the ostomy site. The 0 silk tie anchors the bumper to the tube, and we use 2-0 monofilament sutures to anchor the bumper to the skin at the time of initial placement. The skin under the bumper is cleansed daily with an antiseptic solution, and the skin sutures are removed within 5 to 7 days. An external bumper is necessary to prevent migration of the tube with peristalsis, which may allow it to travel as far as the fourth part of the duodenum at the duodenojejunal flexure where it gets stuck. The limiting factor for more distant migration of a tube without an external bumper is the length of the tube between the balloon tip and the forked hub of the catheter. A complication of the balloon tip of the catheter remaining in contact with the small bowel for a long period of time is pressure necrosis and perforation (patient G.M.). It is preferable to replace the gastrostomy tube with a commercially available kit with an external bumper included. If a Foley urinary catheter is used as a replacement tube, an external bumper can be fashioned by inserting the doughnut-shaped cut end nipple of a 24 F de Pezzar catheter.[5]

It has been reported that the internal anchoring device, frequently a plastic disc, may erode into the gastric wall. In a series of 148 patients, Ma et al.[6] noted a 6.1% incidence of the so-called "buried bumper syndrome." This usually results from a tube being too firmly fixed in place for a long time. It is recommended that the tube be checked daily and 1 cm of tubing be present between the external bumper and the abdominal wall. The T-fasteners used as anchoring devices in the Flexiflo gastrostomy kits that we used may get buried in the gastric wall

in a similar fashion (patients R.H. and A.K.). The T-fasteners hold the stomach snugly against the anterior abdominal wall until the gastrostomy tract is well healed in approximately 2 weeks. The recommendation is to divide the external pledgets at skin level at this time, allowing the T-fasteners to fall into the gastric lumen and be carried along by peristaltic activity. Although the retained T-fasteners by themselves may not be a problem, patients are seen with persistent sinuses surrounding the gastrostomy site many months after their placement. In such situations the sinus tracts need to be explored and the T-fasteners removed or the nylon sutures divided deep in the wound and removed.[7] If a metal T-fastener did not appear to be the nidus of infection, no attempt was made to remove it. The metal portion of the T-fasteners did not hinder healing once the nylon portion was removed. Although mostly innocuous, T-fasteners have been associated with major late complications.[8]

While attempting to extract an old pull-through–type gastrostomy tube, the internal head may get separated from a deteriorated stem and be retained within the stomach. This head can travel through the pylorus into the small bowel where it may become stuck and cause intestinal obstruction.[9] This would then require an exploratory laparotomy and enterotomy to retrieve the bumper head. The common site for such obstruction is the distal ileum approximately 2 feet from the ileocecal junction. This is the site of attachment of the omphalomesenteric duct, and the bowel lumen is narrowest in this area. Once the bumper head becomes separated and is retained within the stomach, it is prudent to retrieve it by means of endoscopy (patient M.M.).

A PEG tube that has been placed recently or one that shows no return of gastric contents after reinsertion of the catheter indicates the need for a Gastrografin study prior to using it. This is further underscored if the PEG tube was of the pull-through type with no anchoring device such as T-fasteners to keep the stomach firmly attached to the anterior abdominal wall. It is unwise to reintubate the ostomy site during the immediate postoperative period. If reinsertion of the tube cannot be confirmed by radiologic studies and the stomach has actually moved away from the anterior abdominal wall, laparotomy is performed to repair the opening in the stomach wall (patient A.D.). Laparoscopy has proved to be useful in managing this complication and avoids some of the surgical morbidity in these debilitated patients.[10] It should also be emphasized that even a mature tract will seal over if the tube has been left out for more than 24 hours. Reinsertion may require gentle dilatation of the tract. It may sometimes be necessary to insert a guidewire and carry out serial dilation using the dilators from a gastrostomy kit. Multiple Foley catheters may also be used in a graded fashion to enlarge the tract to accommodate an 18 F replacement gastrostomy tube. Gastrocolic and colocutaneous fistulas are quite unusual as complications of PEG tube insertions, but they do occur. One such patient was evaluated with feces draining from the feeding tube. Colonoscopy revealed the tube to be in the lumen of the left colon. The tube was removed transanally by cutting it at the external abdominal wall and using the colonoscope and a snare to retrieve it. Because the patient had a well-formed tract, the fistula drained and subsequently healed without incident. This most likely occurred as a result of the colon being trapped between the stomach and abdominal wall during PEG insertion and gastric insufflation. To prevent this problem, the light intensity of the endoscope is maximized while it is in the stomach, and the abdominal wall is transilluminated. PEG tube placement can be a very simple procedure. Long-term complications can be minimized by care and attention to technical details at the time of PEG tube insertion, as well as proper maintenance of the tube.

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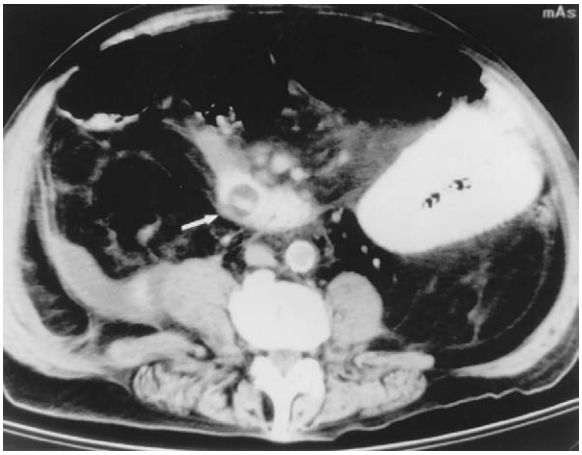

Fig. 1. CT scan of the abdomen with extravasation of contrast medium through a jejunal perforation adjacent to the Foley balloon (*arrow*).

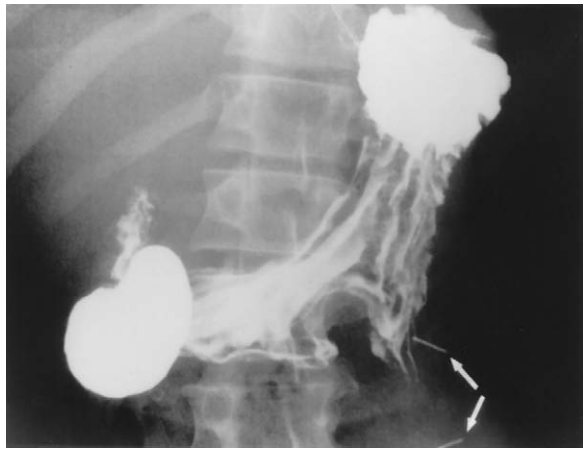

Fig. 2. Upper gastrointestinal series with T-fasteners (*arrows*) embedded in the gastric and anterior abdominal walls. (Reprinted with permission. Collure D, Bumpers H, Hoover E. A complication of T-fasteners in percutaneous endoscopic gastrostomy. Surg Endosc 1996;10:939. Figure 2, Springer-Verlag.)

and the jejunum was repaired after debridement of the edges of the perforation. A feeding jejunostomy was placed distal to the repair. The postoperative recovery was stormy and complicated by pneumonia.

Cases 2 and 3

Patients R.H. (an 18-year-old male) and A.K. (a 24-year-old female) were victims of unrelated auto accidents and had suffered severe closed-head injuries. They had both undergone PEG placement using Flexiflo gastrostomy kits with T-fasteners (Ross Laboratories, Columbus, OH). In both patients the external pledgets and the nylon sutures holding the T-fasteners had been divided at the skin level at 14 days after the procedure. After recovery and satisfactory oral intake, the PEG tubes had been removed at 3 months (patient R.H.) and 4 months (patient A.K.) after their insertion.

Patient R.H. returned 18 months later with purulent discharge near the PEG site. The site of insertion of the PEG tube was well healed, but there were two sinuses corresponding to points of insertion of the T-fasteners. An upper gastrointestinal series showed no evidence of a gastrocutaneous fistula. It did show that one T-fastener was embedded in the wall of the stomach, whereas another was in the anterior abdominal wall (Fig. 2). The sinus tracts were explored under local anesthesia, and the ends of the two nylon sutures were identified. These were divided in the depths of the wounds. The sinuses completely healed without incident.

Patient A.K. returned 12 months after removal of the PEG tube with two sinus tracts adjacent to the healed PEG tube site. These were explored and the metal T-fasteners were removed along with the nylon sutures. Uneventful healing of the wounds followed this procedure.

Case 4

Patient M.M. was a 52-year-old woman with severe mental retardation and a seizure disorder who was confined to a nursing home. She had had a "pull-through"–type gastrostomy inserted 4 years previously, and she was seen in the emergency department for its replacement. The outside stem of the PEG tube had deteriorated and was stiff with multiple dilatations along its length. While attempting to remove the tube, it separated from the internal flange that was retained within the stomach. A replacement gastrostomy tube was inserted through the track, and the retained flange was removed endoscopically without incident.

Case 5

Patient A.D. was an 80-year-old female resident of a nursing home who had a pull-through–type PEG tube placed approximately 3 weeks prior to admission. The patient by tugging at it had dislodged the original tube, and a Foley catheter had been inserted through the tract. Attempts to feed her through the new catheter were met with resistance. She was seen in the emergency department with erythema and swelling around the PEG tube site. A Gastrografin study through the catheter showed extravasation of the contrast medium, with the Foley balloon wedged between the stomach and the anterior abdominal wall

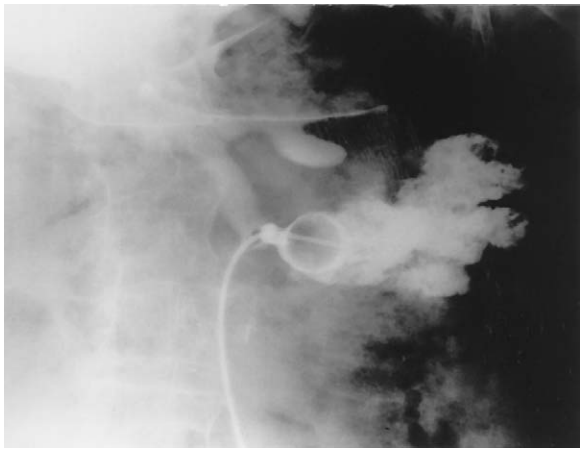

Fig. 3. A Gastrografin study through the PEG tube with extravasation of contrast medium between the anterior abdominal wall and the stomach.

(Fig. 3). Exploration and irrigation of the peritoneal cavity with repair of the anterior gastric wall at the gastrostomy site was carried out. A Dobhoff feeding tube was inserted into the proximal jejunum. The patient had a protracted postoperative course with a wound infection that subsequently resolved.

DISCUSSION

Morbidity secondary to PEG tubes has been reported to be 16%,[2,3] and a major complication rate of approximately 8% has also been reported.[4] Unusual complications may result from gastrostomy tubes remaining in place for an extended period of time. Many of these problems can be avoided by instructing the caregivers on proper maintenance of these tubes. When a balloon-tipped gastrostomy is used, it should always be distended with sterile water. With long-term gastrostomy placement, the tube should be evaluated for cracks, proper fit, and other signs of wear on a regular basis. Worn tubes should be replaced with an appropriate replacement tube. Gastrostomy replacement tubes generally have the balloon centered around the end of the tube. This makes for a snug and even fit once the tube is anchored. The Foley catheter is considered only a temporary measure to prevent closure of the ostomy site until the proper replacement is available. Normal saline solution tends to crystallize over time and clog the narrow balloon port. Even though the balloon and the tube are intact, the fluid volume tends to decrease over a period of months. This may result in the tube slipping out of position, and thus leakage around the tube will occur. Although leakage around the tube is not an uncommon complication, it can be a nuisance and may lead to cutaneous inflammation at the catheter site. Inserting a properly fitting balloon-tipped catheter and using a topical antibiotic ointment can easily remedy this. It is important to test the integrity of the balloon at intervals and reinflate it as necessary. Even though the capacity of the Foley balloon is given as 30 ml, it need not be distended with more than 8 to 10 ml of water.

If the external bumper anchoring the feeding tube is not secure, it may allow the tube to slip further into the stomach causing the balloon tip to be carried by gastric motility to its outlet. A distended balloon may occlude the pylorus, and the patient will have emesis. A properly placed percutaneous gastrostomy tube should be pulled back snugly against the inner aspect of the stomach and abdominal wall and an external bumper pushed into position against the skin and anchored with a 0 silk tie. Each bumper should only slightly depress its respective surfaces. Neither the mucosa nor the skin should be left in a state of blanching. Permanent blanching may lead to pressure necrosis at the ostomy site. The 0 silk tie anchors the bumper to the tube, and we use 2-0 monofilament sutures to anchor the bumper to the skin at the time of initial placement. The skin under the bumper is cleansed daily with an antiseptic solution, and the skin sutures are removed within 5 to 7 days. An external bumper is necessary to prevent migration of the tube with peristalsis, which may allow it to travel as far as the fourth part of the duodenum at the duodenojejunal flexure where it gets stuck. The limiting factor for more distant migration of a tube without an external bumper is the length of the tube between the balloon tip and the forked hub of the catheter. A complication of the balloon tip of the catheter remaining in contact with the small bowel for a long period of time is pressure necrosis and perforation (patient G.M.). It is preferable to replace the gastrostomy tube with a commercially available kit with an external bumper included. If a Foley urinary catheter is used as a replacement tube, an external bumper can be fashioned by inserting the doughnut-shaped cut end nipple of a 24 F de Pezzar catheter.[5]

It has been reported that the internal anchoring device, frequently a plastic disc, may erode into the gastric wall. In a series of 148 patients, Ma et al.[6] noted a 6.1% incidence of the so-called "buried bumper syndrome." This usually results from a tube being too firmly fixed in place for a long time. It is recommended that the tube be checked daily and 1 cm of tubing be present between the external bumper and the abdominal wall. The T-fasteners used as anchoring devices in the Flexiflo gastrostomy kits that we used may get buried in the gastric wall

in a similar fashion (patients R.H. and A.K.). The T-fasteners hold the stomach snugly against the anterior abdominal wall until the gastrostomy tract is well healed in approximately 2 weeks. The recommendation is to divide the external pledgets at skin level at this time, allowing the T-fasteners to fall into the gastric lumen and be carried along by peristaltic activity. Although the retained T-fasteners by themselves may not be a problem, patients are seen with persistent sinuses surrounding the gastrostomy site many months after their placement. In such situations the sinus tracts need to be explored and the T-fasteners removed or the nylon sutures divided deep in the wound and removed.[7] If a metal T-fastener did not appear to be the nidus of infection, no attempt was made to remove it. The metal portion of the T-fasteners did not hinder healing once the nylon portion was removed. Although mostly innocuous, T-fasteners have been associated with major late complications.[8]

While attempting to extract an old pull-through–type gastrostomy tube, the internal head may get separated from a deteriorated stem and be retained within the stomach. This head can travel through the pylorus into the small bowel where it may become stuck and cause intestinal obstruction.[9] This would then require an exploratory laparotomy and enterotomy to retrieve the bumper head. The common site for such obstruction is the distal ileum approximately 2 feet from the ileocecal junction. This is the site of attachment of the omphalomesenteric duct, and the bowel lumen is narrowest in this area. Once the bumper head becomes separated and is retained within the stomach, it is prudent to retrieve it by means of endoscopy (patient M.M.).

A PEG tube that has been placed recently or one that shows no return of gastric contents after reinsertion of the catheter indicates the need for a Gastrografin study prior to using it. This is further underscored if the PEG tube was of the pull-through type with no anchoring device such as T-fasteners to keep the stomach firmly attached to the anterior abdominal wall. It is unwise to reintubate the ostomy site during the immediate postoperative period. If reinsertion of the tube cannot be confirmed by radiologic studies and the stomach has actually moved away from the anterior abdominal wall, laparotomy is performed to repair the opening in the stomach wall (patient A.D.). Laparoscopy has proved to be useful in managing this complication and avoids some of the surgical morbidity in these debilitated patients.[10] It should also be emphasized that even a mature tract will seal over if the tube has been left out for more than 24 hours. Reinsertion may require gentle dilatation of the tract. It may sometimes be necessary to insert a guidewire and carry out serial dilation using the dilators from a gastrostomy kit. Multiple Foley catheters may also be used in a graded fashion to enlarge the tract to accommodate an 18 F replacement gastrostomy tube. Gastrocolic and colocutaneous fistulas are quite unusual as complications of PEG tube insertions, but they do occur. One such patient was evaluated with feces draining from the feeding tube. Colonoscopy revealed the tube to be in the lumen of the left colon. The tube was removed transanally by cutting it at the external abdominal wall and using the colonoscope and a snare to retrieve it. Because the patient had a well-formed tract, the fistula drained and subsequently healed without incident. This most likely occurred as a result of the colon being trapped between the stomach and abdominal wall during PEG insertion and gastric insufflation. To prevent this problem, the light intensity of the endoscope is maximized while it is in the stomach, and the abdominal wall is transilluminated. PEG tube placement can be a very simple procedure. Long-term complications can be minimized by care and attention to technical details at the time of PEG tube insertion, as well as proper maintenance of the tube.

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Laparoscopic Enucleation of Solitary True Pancreatic Cyst in an Adult

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Solitary or true pancreatic cyst is a very rare pathologic condition; only a few cases are reported in literature. We report a case of a 22-year-old woman with a symptomatic true pancreatic cyst located in proximity to the pancreatic head, duodenum, vena cava, biliary tree, and right kidney, which was enucleated through a laparoscopic approach. Laparoscopic ultrasound imaging allowed the surgeon to better identify the morphology of the cyst and its relationship with the adjacent structures. The treatment is briefly reviewed and discussed. (J GASTROINTEST SURG 2003;7:921–924)

KEY WORDS: Pancreas, true cyst, laparoscopy, surgical enucleation

Cystic lesions of the pancreas are now being recognized more frequently because of the widespread use of advanced imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasonography, and laparoscopic ultrasonography.[1–3] Benign pancreatic cysts are rare entities; they are subclassified as solitary or simple true cysts, pseudocysts, and serous adenomas. Pseudocysts account for 80% to 90% of all pancreatic cystic lesions.[4] Solitary true cyst of the pancreas is a very rare pathologic condition in adults; fewer than 20 cases are reported in the literature. In fact, most cases are diagnosed in infants and children.[5] Until now, little has been known about the clinical and pathologic features of these cysts making their diagnosis difficult and their treatment selected on an individual basis according to the surgeon's experience. Only a few reports of laparoscopic treatment of benign pancreatic disorder are present in the literature; thus its role remains controversial and unclear.[6,7]

Reported herein is a case of a solitary pancreatic cyst that was enucleated using a laparoscopic approach.

CASE REPORT

A 22-year-old woman was admitted to our hospital for intermittent epigastric pain and dyspepsia due to the presence of a cystic mass located in the head of the pancreas detected by abdominal ultrasonography. The patient had no history of trauma, alcohol abuse, or gallbladder stones. Results of routine biochemical and hematologic tests were normal. Serum amylase, carcinoembryonic antigen (CEA), and CA 19-9 levels were normal. Results of an upper gastrointestinal tract endoscopy were normal. CT scan revealed a low attenuation and a well-demarcated cystic area, 4 cm in diameter, located in the head of the pancreas. The cystic mass was unilocular and its wall was thin (Fig. 1). Endoscopic ultrasonography showed a hypoanechoic structure (35 × 40 mm in diameter) located between the right kidney and the duodenal wall without any sign of duodenal encasement. The presumptive diagnosis was true pancreatic cyst. Because of the persistence of symptoms, without any other evident causative factors, the patient was referred for surgery. With the surgeon standing between the patient's legs, a 10 mm port was placed in the umbilical ring for the camera; a 10 mm working port and two 5 mm ports were respectively placed in the left subcostal area, the right subcostal area, and the subxiphoid region. Before the operation was begun, laparoscopic ultrasonography was performed. First, the probe was inserted through the umbilical trocar to obtain longitudinal scanning of the hepatoduodenal ligament and

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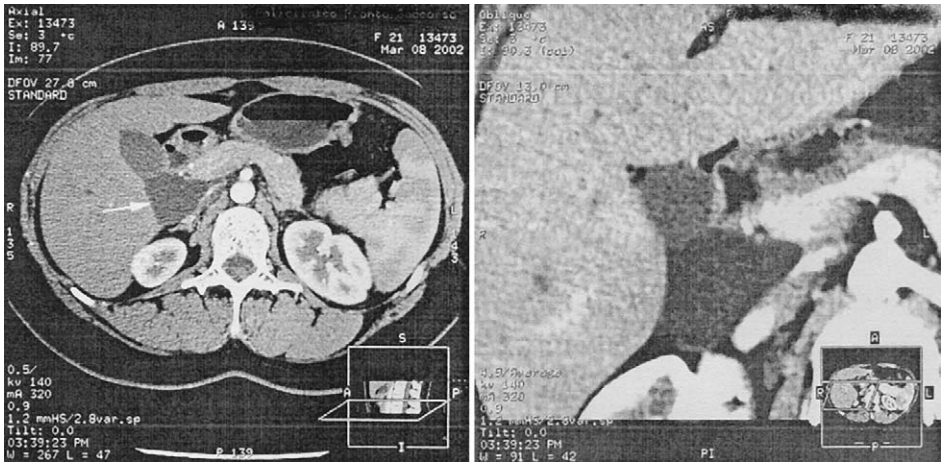

Fig. 1. A, CT scan showing a low-density area, 4 cm in diameter, located in the head of the pancreas. This cyst (*arrow*) has a different attenuation value compared to the gallbladder. **B**, A coronal CT reconstruction showing the relationship of the cyst with the gallbladder. The cyst determines secondary compression of the vena cava.

the pancreas to assess the precise relationship between the cyst and the common bile duct, the pancreatic head, and the vascular structures. Subsequently the probe was passed through the right trocar to achieve oblique scanning of the pancreatic area.

Through a transgastric exploratory operation, the relationship between the cyst and the hepatoduodenal structures was easily examined. To optimize acoustic coupling between the transducer and the tissues, a warm crystalloid solution was injected into the peritoneal cavity. Images showed an anechoic lesion with a clearly defined border. The cyst appeared uniloculated with a thin uniform wall. It appeared to take origin from the posterior part of the head of the pancreas. Laparoscopic ultrasonography showed the persistence of the hyperechoic vessel–tumor interface among the cyst wall and the inferior vena cava and portal vein. The operation started with a window in the lesser omentum. The stomach was elevated and displaced to the left, and the cystic bulge was then visualized. The cystic fluid was aspirated using a 22-gauge spinal needle, and a sample was taken for bacteriologic, biochemical, and cytologic analysis. After that, the cyst was slowly excised with meticulous dissection and removed after being placed in an endobag. Hemostasis was achieved using electrocautery. Macroscopically the cystic lesion was 3.7 cm in major diameter with a fibrotic wall and an inner layer of granular aspect Fig. 2). The cyst contained clear serous fluid. Amylase and lipase levels were comparable to those in normal serum, and the CEA and CA 19-9 levels were also within the normal range. No malignant epithelial cells were found. Histologically the cystic lesion was composed of a fibrotic wall with diffuse disepithelization. Where the epithelium was maintained, it consisted of a monolayer of cuboidal-cylindric epithelium without dysplastic features (Fig. 3).

Around the fibrotic tissue, residual exocrine pancreatic acinar structures were evident. The final histologic diagnosis was a true pancreatic cyst. The postoperative course was uneventful, and the patient

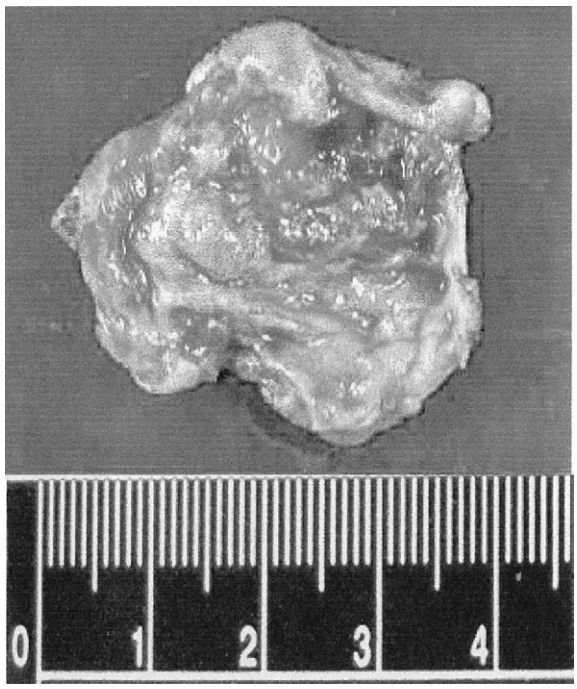

Fig. 2. Macroscopically the cyst was 3.7 cm in major diameter with a fibrotic wall and an inner layer of granular aspect.

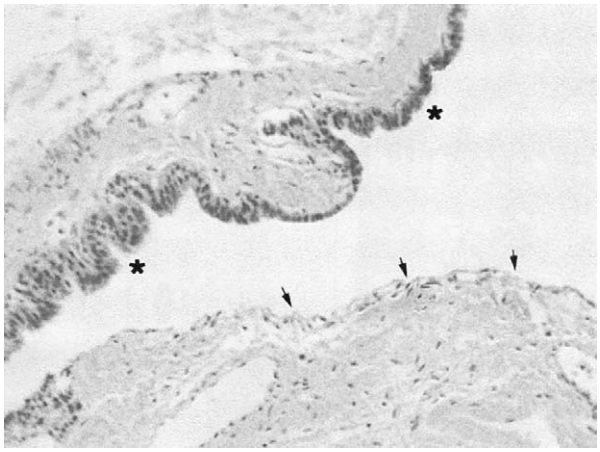

Fig. 3. Histologic findings. The lesion is composed of a fibrotic wall with diffusely disepithelization (*black arrows*). The inner part is constituted by a monolayer of cuboidal-cylindrical epithelium without dysplastic features (*asterisks*).

was discharged on postoperative day 3 in good general condition. At 1-year follow-up, the patient is well without any abdominal symptoms. Ultrasound images and spiral CT scans revealed no cyst recurrence.

DISCUSSION

The etiology of true pancreatic cysts remains unknown. As the observation of these rare entities is verified in infants and children, the congenital nature is suggested.[4] In most patients the symptoms are related to the size and location of the mass, and include epigastric pain, nausea, vomiting, biliary obstruction, duodenal obstruction, and so forth. However, even as adults some patients may be asymptomatic. With the use of ultrasonography, CT, or MRI alone, it may be difficult to differentiate between true pancreatic cysts and pseudocysts, and to distinguish benign from malignant pancreatic lesions. Use of endoscopic ultrasonography and endoscopic ultrasound with fine-needle aspiration enhance the visualization of the pancreatic cystic lesions as well as aspiration of cystic fluid with a reported sensitivity up to 90% and specificity up to 70% to 80%.[1–3] At endoscopic ultrasonography, images of a uniform thin regular wall are suggestive of a lesion with a benign nature, whereas an irregular thickened wall suggests a cystic lesion with malignant potential.[1] Moreover, endoscopic ultrasonography makes drainage of the cyst possible even in nonbulging detected lesions of 1 cm.[8] Determination of the neoplastic markers in the cystic fluid, mucin, and other biochemical agents is useful to differentiate true cysts from pseudocysts and benign lesions from malignant lesions. When a definite preoperative diagnosis cannot be reached, laparoscopic ultrasound is found to significantly improve the diagnosis of pancreatic cystic lesions.[1] This was the case in our patient where laparoscopic ultrasound allowed us to define the morphology of the cyst and its origin.

Surgical intervention for pancreatic cysts is recommended for two reasons: (1) the possibility that the mass will enlarge and compress adjacent organs with concomitant symptoms, and (2) to exclude cystic malignancy, even if this is not a frequent event.

Percutaneous CT or ultrasound drainage, endoscopic transgastric drainage, and transpapillary procedures for the treatment of pancreatic cystic lesions are associated with low mortality, but a high rate of complications such as cystic recurrence, infection, and bleeding.[6,7] More radical approaches include cyst excision or distal pancreatectomy, which are usually performed via laparotomy. Minimally invasive surgery for pancreatic diseases is gaining interest among surgeons. However, this treatment modality is not yet commonplace because of the technical difficulties associated with it.[9,10] Laparoscopic approaches for successful management of pancreatic cysts and pseudocysts, such as pancreatic cyst–gastrostomy, cyst-duodenostomy, cyst-jejunostomy, and laparoscopic pancreatic excision, have been reported in the literature.[6,7,9–11] The present report is the first case in the literature, to our knowledge, in which an enucleation of the true cyst was accomplished through a totally laparoscopic approach. Laparoscopic ultrasound allowed us to clearly delineate the entire wall of the cyst and the neighboring structures, and to aspirate the contents completely, thus facilitating the enucleation of the cyst. The patient was discharged on postoperative day 3 free of any symptoms or complications, and with a great cosmetic result.

Our study demonstrates that CT and endoscopic ultrasonography are sensitive for preoperative identification of this lesion, and for the differential diagnosis of other cystic pancreatic lesions. In this regard it must be remembered that true pancreatic cysts are quite rare, and other lesions with potential malignant degeneration must be excluded. A laparoscopic exploration in association with laparoscopic ultrasound can be very helpful in establishing the correct diagnosis, allowing improved identification of the morphology of the cyst and its relationship to adjacent structures. Having excluded other potentially malignant pancreatic cystic lesions, laparoscopic surgery may represent a sound and effective approach for these patients.

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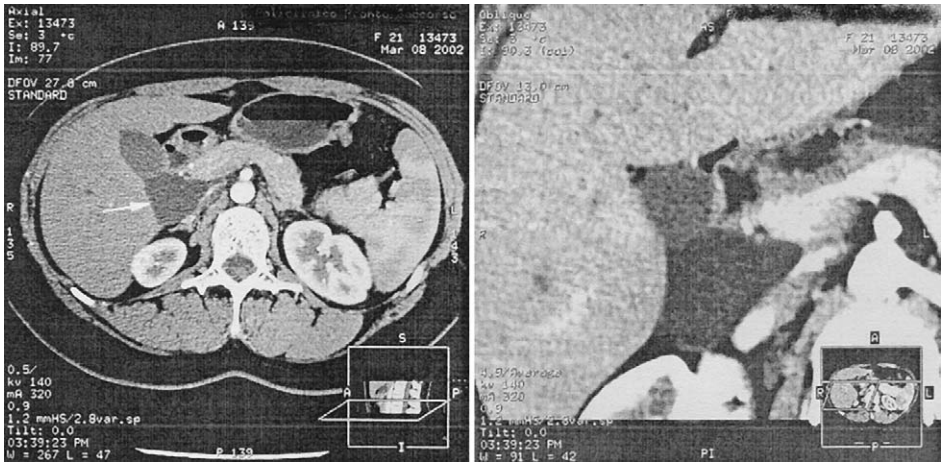

Fig. 1. A, CT scan showing a low-density area, 4 cm in diameter, located in the head of the pancreas. This cyst (*arrow*) has a different attenuation value compared to the gallbladder. **B**, A coronal CT reconstruction showing the relationship of the cyst with the gallbladder. The cyst determines secondary compression of the vena cava.

the pancreas to assess the precise relationship between the cyst and the common bile duct, the pancreatic head, and the vascular structures. Subsequently the probe was passed through the right trocar to achieve oblique scanning of the pancreatic area.

Through a transgastric exploratory operation, the relationship between the cyst and the hepatoduodenal structures was easily examined. To optimize acoustic coupling between the transducer and the tissues, a warm crystalloid solution was injected into the peritoneal cavity. Images showed an anechoic lesion with a clearly defined border. The cyst appeared uniloculated with a thin uniform wall. It appeared to take origin from the posterior part of the head of the pancreas. Laparoscopic ultrasonography showed the persistence of the hyperechoic vessel–tumor interface among the cyst wall and the inferior vena cava and portal vein. The operation started with a window in the lesser omentum. The stomach was elevated and displaced to the left, and the cystic bulge was then visualized. The cystic fluid was aspirated using a 22-gauge spinal needle, and a sample was taken for bacteriologic, biochemical, and cytologic analysis. After that, the cyst was slowly excised with meticulous dissection and removed after being placed in an endobag. Hemostasis was achieved using electrocautery. Macroscopically the cystic lesion was 3.7 cm in major diameter with a fibrotic wall and an inner layer of granular aspect Fig. 2). The cyst contained clear serous fluid. Amylase and lipase levels were comparable to those in normal serum, and the CEA and CA 19-9 levels were also within the normal range. No malignant epithelial cells were found. Histologically the cystic lesion was composed of a fibrotic wall with diffuse disepithelization. Where the epithelium was maintained, it consisted of a monolayer of cuboidal-cylindric epithelium without dysplastic features (Fig. 3).

Around the fibrotic tissue, residual exocrine pancreatic acinar structures were evident. The final histologic diagnosis was a true pancreatic cyst. The postoperative course was uneventful, and the patient

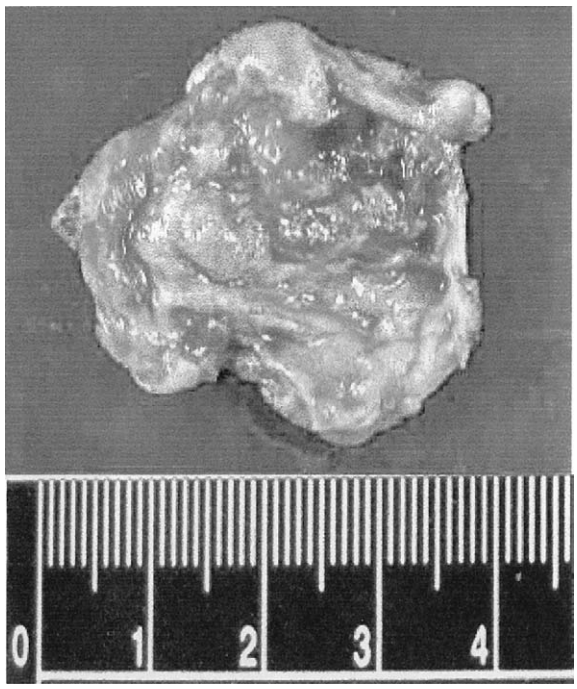

Fig. 2. Macroscopically the cyst was 3.7 cm in major diameter with a fibrotic wall and an inner layer of granular aspect.

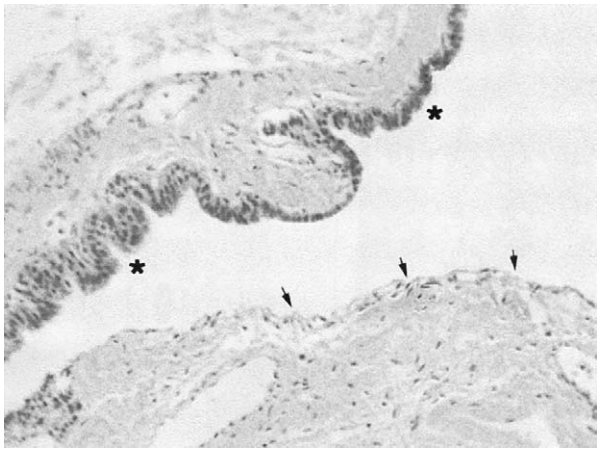

Fig. 3. Histologic findings. The lesion is composed of a fibrotic wall with diffusely disepithelization (*black arrows*). The inner part is constituted by a monolayer of cuboidal-cylindrical epithelium without dysplastic features (*asterisks*).

was discharged on postoperative day 3 in good general condition. At 1-year follow-up, the patient is well without any abdominal symptoms. Ultrasound images and spiral CT scans revealed no cyst recurrence.

DISCUSSION

The etiology of true pancreatic cysts remains unknown. As the observation of these rare entities is verified in infants and children, the congenital nature is suggested.[4] In most patients the symptoms are related to the size and location of the mass, and include epigastric pain, nausea, vomiting, biliary obstruction, duodenal obstruction, and so forth. However, even as adults some patients may be asymptomatic. With the use of ultrasonography, CT, or MRI alone, it may be difficult to differentiate between true pancreatic cysts and pseudocysts, and to distinguish benign from malignant pancreatic lesions. Use of endoscopic ultrasonography and endoscopic ultrasound with fine-needle aspiration enhance the visualization of the pancreatic cystic lesions as well as aspiration of cystic fluid with a reported sensitivity up to 90% and specificity up to 70% to 80%.[1–3] At endoscopic ultrasonography, images of a uniform thin regular wall are suggestive of a lesion with a benign nature, whereas an irregular thickened wall suggests a cystic lesion with malignant potential.[1] Moreover, endoscopic ultrasonography makes drainage of the cyst possible even in nonbulging detected lesions of 1 cm.[8] Determination of the neoplastic markers in the cystic fluid, mucin, and other biochemical agents is useful to differentiate true cysts from pseudocysts and benign lesions from malignant lesions. When a definite preoperative diagnosis cannot be reached, laparoscopic ultrasound is found to significantly improve the diagnosis of pancreatic cystic lesions.[1] This was the case in our patient where laparoscopic ultrasound allowed us to define the morphology of the cyst and its origin.

Surgical intervention for pancreatic cysts is recommended for two reasons: (1) the possibility that the mass will enlarge and compress adjacent organs with concomitant symptoms, and (2) to exclude cystic malignancy, even if this is not a frequent event.

Percutaneous CT or ultrasound drainage, endoscopic transgastric drainage, and transpapillary procedures for the treatment of pancreatic cystic lesions are associated with low mortality, but a high rate of complications such as cystic recurrence, infection, and bleeding.[6,7] More radical approaches include cyst excision or distal pancreatectomy, which are usually performed via laparotomy. Minimally invasive surgery for pancreatic diseases is gaining interest among surgeons. However, this treatment modality is not yet commonplace because of the technical difficulties associated with it.[9,10] Laparoscopic approaches for successful management of pancreatic cysts and pseudocysts, such as pancreatic cyst–gastrostomy, cyst-duodenostomy, cyst-jejunostomy, and laparoscopic pancreatic excision, have been reported in the literature.[6,7,9–11] The present report is the first case in the literature, to our knowledge, in which an enucleation of the true cyst was accomplished through a totally laparoscopic approach. Laparoscopic ultrasound allowed us to clearly delineate the entire wall of the cyst and the neighboring structures, and to aspirate the contents completely, thus facilitating the enucleation of the cyst. The patient was discharged on postoperative day 3 free of any symptoms or complications, and with a great cosmetic result.

Our study demonstrates that CT and endoscopic ultrasonography are sensitive for preoperative identification of this lesion, and for the differential diagnosis of other cystic pancreatic lesions. In this regard it must be remembered that true pancreatic cysts are quite rare, and other lesions with potential malignant degeneration must be excluded. A laparoscopic exploration in association with laparoscopic ultrasound can be very helpful in establishing the correct diagnosis, allowing improved identification of the morphology of the cyst and its relationship to adjacent structures. Having excluded other potentially malignant pancreatic cystic lesions, laparoscopic surgery may represent a sound and effective approach for these patients.

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Surgical Treatment of Small Bowel Cancer: A 20-Year Single Institution Experience

Hiromichi Ito, M.D., Alexander Perez, M.D., David C. Brooks, M.D., Robert T. Osteen, M.D., Michael J. Zinner, M.D., Francis D. Moore, Jr., M.D., Stanley W. Ashley, M.D., Edward E. Whang, M.D.

Small bowel malignancies are rare. The aims of this study were to evaluate the outcomes associated with surgical therapy for small bowel cancers and to define prognostic factors. The medical records of 96 consecutive patients with primary small bowel cancer (excluding lymphoma) treated at our institution over a 20 year period were reviewed. Survival was analyzed using the Kaplan-Meier method (mean follow-up period 57 months). Mean patient age was 56 years, and 58% of patients were male. Sixty percent of patients had an adenocarcinoma, 21% had a sarcoma, and 19% had a carcinoid tumor. The percentages of patients who underwent complete (curative) resection were 51%, 90%, and 50% for those with adenocarcinoma, sarcoma, and carcinoid tumor, respectively. For patients with adenocarcinoma who underwent curative resection, tumor (T) and node (N) stages were significant prognostic factors predicting overall survival. For patients with sarcomas who underwent curative resection, tumor grade was a significant prognostic factor predicting overall survival. The prognosis for patients with small intestinal carcinoid tumors is uniformly favorable. The prognosis for patients with sarcomas and adenocarcinomas is generally poor, although long-term survival is achieved by patients with favorable prognostic factors.
(J GASTROINTEST SURG 2003;7:925–930)

KEY WORDS: Small bowel cancer, adenocarcinoma, sarcoma, carcinoid tumor

Small bowel cancers are rare. The incidence of small bowel cancer in the United States is estimated to be only 5300 cases per year, with 1100 deaths attributable to this cancer per year.[1] For comparison, the incidences of colorectal cancer and stomach cancer in the United States are estimated to be 147,500 and 22,400 per year, respectively.[1]

Because of the low incidence of small bowel cancers, data on their natural history and treatment-associated outcomes are limited. The aim of this study was to characterize the clinical presentation and outcomes associated with surgical therapy in patients with small bowel cancer treated at our institution during the past two decades. A second goal was to define prognostic factors associated with improved survival.

PATIENTS AND METHOD

The study was approved by the Brigham and Women's Hospital Institutional Review Board. The medical records of all patients with small bowel cancer admitted to the inpatient unit of Brigham and Women's Hospital during the period spanning January 1981 through November 2001 were analyzed. Patients were identified using the International Classification of Disease-9 (ICD-9) codes for small bowel neoplasm (codes 152.x) and the computer-assisted hospitalization analysis for the study of efficacy (CHASE) management system. Patients with lymphomas involving the small bowel were excluded. Parameters obtained from the medical records included patient demographic data (age and sex), signs and symptoms at the time of diagnosis, tumor location, the surgical procedure and whether it was curative (no gross residual cancer present at the completion of surgery) or palliative (gross residual cancer present at the completion of surgery), and pathologic findings.

Tumors were categorized into one of three histologic types: adenocarcinoma, carcinoid tumor, and

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sarcoma. Pathologic parameters analyzed for adenocarcinomas were histologic differentiation, depth of tumor invasion, regional lymph node status, margin status, and overall stage according to the American Joint Committee on Cancer (AJCC) staging system.[2] Those for sarcoma were histologic grade, tumor size, regional lymph node status, and overall stage according to the AJCC staging system.[2] Parameters for carcinoid tumors were tumor size, depth of tumor invasion, regional lymph node status, and margin status.

Patient survival data were obtained from the United States Social Security Administration's Death Master File. The survival duration was calculated from the time of operation or time of diagnosis, if patients did not undergo any surgery, through time of death. Survival curves for selected patient groups were determined using the Kaplan-Meier method and compared by means of the log-rank test and Mantel test. Overall survival estimates were derived from the corresponding Kaplan-Meier curves. Chi-square analyses were used for other parameters unless specified otherwise.

RESULTS

Demographic and Tumor Data

During the study period, 96 patients with small bowel cancer were admitted to the hospital. The median age of this cohort was 64 years (range 26–88 years). Fifty-six patients (58%) were male.

The frequency of tumor of types in this cohort and the distribution of their locations within the small bowel are summarized in Table 1. Fifty-eight patients (60%) had an adenocarcinoma, 20 (21%) had a sarcoma, and 18 (19%) had a carcinoid tumor. Adenocarcinomas were located most frequently in the duodenum (55%), sarcomas in the jejunum (75%), and carcinoid tumors in the ileum (55%).

Table 1. Tumor histologic type and location

	Tumor type			
Location	Adenocarcinoma	Sarcoma	Carcinoid tumor	Total
Duodenum	32 (55)	3 (15)	1 (6)	36 (38)
Jejunum	14 (24)	15 (75)	7 (39)	36 (38)
Ileum	12 (21)	2 (10)	10 (56)	24 (25)

Numbers in parentheses are percentages.

Symptoms and Signs

Symptoms and signs that were present at the time of diagnosis are summarized in Table 2. The most frequent symptoms were abdominal pain, present in 41% of patients, and nausea and vomiting, present in 34% of patients. Twenty-two percent of patients had gastrointestinal bleeding. Nausea and vomiting and pain were more prevalent ($P < 0.05$) among patients with adenocarcinomas than in those with carcinoid tumors or sarcomas. Gastrointestinal bleeding was less prevalent ($P < 0.05$) among patients with carcinoid tumors than those with adenocarcinomas or sarcomas. Twenty-two percent of patients with carcinoid tumors presented with symptoms of the carcinoid syndrome.

Staging

The AJCC stage distributions for patients with adenocarcinomas or sarcomas are shown in Table 3. Forty-five percent of patients with adenocarcinomas and 35% of those with sarcomas had stage IV disease at the time of diagnosis. Among patients with carcinoid

Table 2. Symptoms and signs at presentation

	Tumor type			
Symptom/sign	Adenocarcinoma (%)	Sarcoma (%)	Carcinoid tumor (%)	Total (%)
Nausea/vomiting	45*	15	22	34
Pain	46*	25	39	41
Gastrointestinal bleeding	26†	30†	0	22
Weight loss	9	5	11	8
Palpable mass	0	10	0	2
Anemia, occult fecal blood	3	0	0	2
Diarrhea	2	5	22	6
Jaundice	9	0	6	6
Carcinoid tumor syndrome	0	0	22	4

Numbers indicate percentage of patients with each type of tumor.
*$P < 0.05$ vs. sarcoma or carcinoid tumor.
†$P < 0.05$ vs. carcinoid tumor.

Table 3. Stage distribution of adenocarcinoma and sarcoma

Stage	Adenocarcinoma (%)	Sarcoma (%)
I	5	20
II	24	25
III	26	20
IV	45	35

tumors, 14 (78%) had metastatic disease detected either preoperatively or intraoperatively.

Surgical Therapy

Among study patients, 24 (25%) did not undergo tumor resection because of the presence of advanced disease. Of these 24 patients, 11 did not undergo laparotomy because metastases were detected preoperatively, four underwent laparotomy and tumor biopsy alone, and nine underwent palliative intestinal bypass.

Fifteen patients (16%) underwent incomplete (palliative) tumor resection. The percentages of the patients who underwent complete (curative) tumor resection were 51%, 90%, and 50% for those with adenocarcinomas, sarcomas, and carcinoid tumors, respectively. The nature of these curative procedures was related to tumor location. Among patients with tumors located in the duodenum, 11 underwent pancreaticoduodenectomy, and seven underwent segmental (sleeve) duodenal resection. Each of the patients with tumors located in the jejunum or ileum underwent segmental intestinal resection with regional lymph node excision.

Eleven percent of patients who underwent curative resection had en bloc resection of organs adjacent to the intestinal primary tumor (this percentage excludes pancreaticoduodenectomies for duodenal cancers); one patient (with an adenocarcinoma) underwent partial cystectomy and salpingoopherectomy, two patients (one with a sarcoma and one with a carcinoid tumor) underwent sigmoidectomy, and two patients (one with a sarcoma and one with a carcinoid tumor) underwent wedge resection of the liver.

Pathologic Findings

Pathologic parameters evaluated for specimens from patients who underwent complete resection are summarized in Table 4. Among the 38 resected adenocarcinomas, 80% of tumors were T3 or T4, 53% had associated regional lymph node metastases, and 93% were moderately or poorly differentiated. Of the 18 resected sarcomas, 83% of tumors were T2 (tumor size greater than 5 cm in diameter), none had associated regional lymph node metastases, and 44% were high grade. Of the nine resected carcinoid tumors, 55% of tumors were T3 or T4, 67% had associated regional lymph node metastases, and 44% were larger than 1.5 cm in diameter. Of the 18 resected sarcomas, eight specimens were diagnosed as GIST, gastrointestinal stromal tumors by KIT (CD117) positivity on immunohistochemical analysis.

Outcomes Associated With Surgical Treatment and Prognostic Factors

The mean follow-up period for study patients was 57 months. The 30-day postoperative mortality rate

Table 4. Pathologic findings in specimens from patients who underwent complete resection

	Adenocarcinoma	Sarcoma	Carcinoid tumor
T			
1	3 (10%)	3 (17%)	1 (11%)
2	3 (10%)	15 (83%)	3 (33%)
3	18 (60%)		2 (22%)
4	6 (20%)		3 (33%)
N			
0	14 (47%)	18 (100%)	3 (33%)
1	16 (53%)	0	6 (67%)
Margin			
Positive	5 (17%)	3 (17%)	1 (11%)
Grade			
Well differentiated/low grade	2 (7%)	5 (28%)	
Moderate/intermediate	16 (53%)	5 (28%)	
Poor/high	12 (40%)	8 (44%)	
Tumor size			
$<$1.5 cm			5 (56%)
$\geq$1.5 cm			4 (44%)

was 1.2%. The overall 5-year survival rates for all study patients with adenocarcinomas, sarcomas, and carcinoid tumors were 26%, 22%, and 70%, respectively (Fig. 1). Survival data for patients who underwent complete or incomplete resection and for those who presented with unresectable disease are shown in Table 5. For all tumor types, median survival and 5-year survival rates were greater ($P < 0.05$) among patients who underwent curative resection than among those who underwent incomplete resection or no resection.

To identify prognostic factors associated with the surgical treatment of small bowel cancers, the following factors were analyzed for patients who underwent complete tumor resection: age, sex, margin involvement, and tumor site for all three types of tumors; tumor grade, tumor node metastasis (TNM) factors, and stage according to the AJCC staging system for adenocarcinomas and sarcomas; and tumor size, depth of tumor invasion, and lymph node involvement for carcinoid tumors.

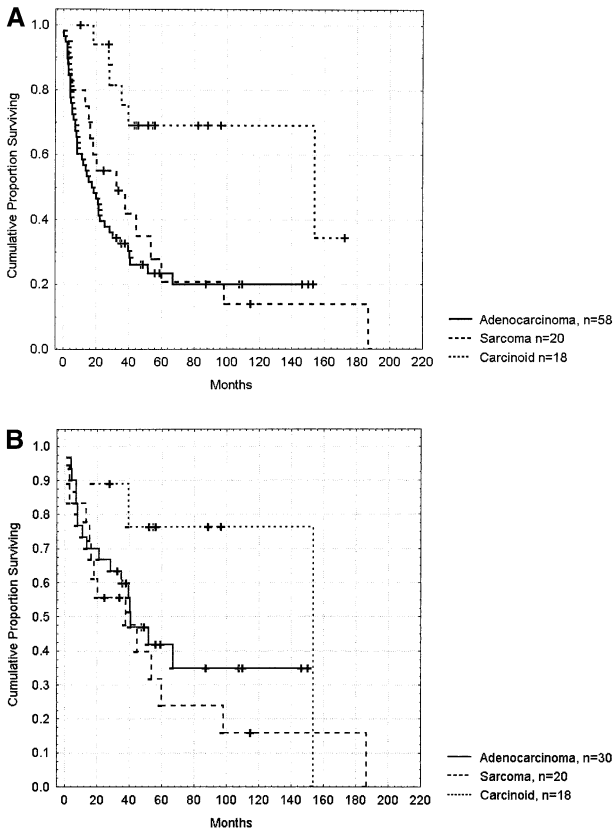

Fig. 1. Overall survival for patients with adenocarcinoma, sarcoma, and carcinoid tumor, and survival for selected patients who underwent curative resection. *A*, Kaplan-Meier estimates of overall survival for patients with adenocarcinoma, sarcoma, and carcinoid tumor. *B*, Kaplan-Meier estimates of survival for patients with adenocarcinoma, sarcoma and carcinoid tumor who underwent curative resection.

Table 5. Median survival and 5-year survival rates after surgical resection of small bowel cancers

Cancer type and procedure type	Median survival (mos)	5-yr survival (%)
Adenocarcinoma Curative resection (n = 30)	36.5	46.9
Adenocarcinoma Palliative resection (n = 8)	10.2	12.5*
Adenocarcinoma Unresectable (n = 20)	6.9	0*
GIST/sarcoma Curative resection (n = 18)	42.8	24.5
GIST/sarcoma Palliative resection (n = 1)	32.5	0
GIST/sarcoma Unresectable (n = 1)	5.1	0
Carcinoid tumor Curative resection (n = 9)	54.7	75.6
Carcinoid tumor Palliative resection (n = 6)	44.1	59.5*
Carcinoid tumor Unresectable (n = 3)	27.9	50*

GIST = gastrointestinal stromal tumor.
*$P < 0.05$ vs. curative resection.

For patients with adenocarcinoma who underwent curative resection, T and N stage were significant prognostic factors associated with overall survival. Five-year survival rates for patients with T1/T2 tumors and those with T3/T4 tumors were 82% and 58%, respectively ($P < 0.05$) (Fig. 2, *A*). Five-year survival rates for patients with uninvolved lymph nodes and those with lymph node metastases were 58% and 29%, respectively ($P < 0.05$) (Fig. 2, *B*). For patients with sarcomas who underwent curative resection, tumor grade was a significant prognostic factor predicting overall survival. Five-year survival rates for patients with low-grade, intermediate-grade, and high-grade tumors were 67%, 20%, and 19%, respectively ($P < 0.05$) (Fig. 3). None of the factors examined were significantly associated with survival for patients with carcinoid tumors who underwent curative resection.

DISCUSSION

The small bowel accounts for 75% of the length of the gastrointestinal tract and contains more than 90% of its mucosal surface area. Yet, only 2% of primary gastrointestinal cancers arise in the small bowel. Difficulty in obtaining prognostic information for patients who have undergone surgical resection of

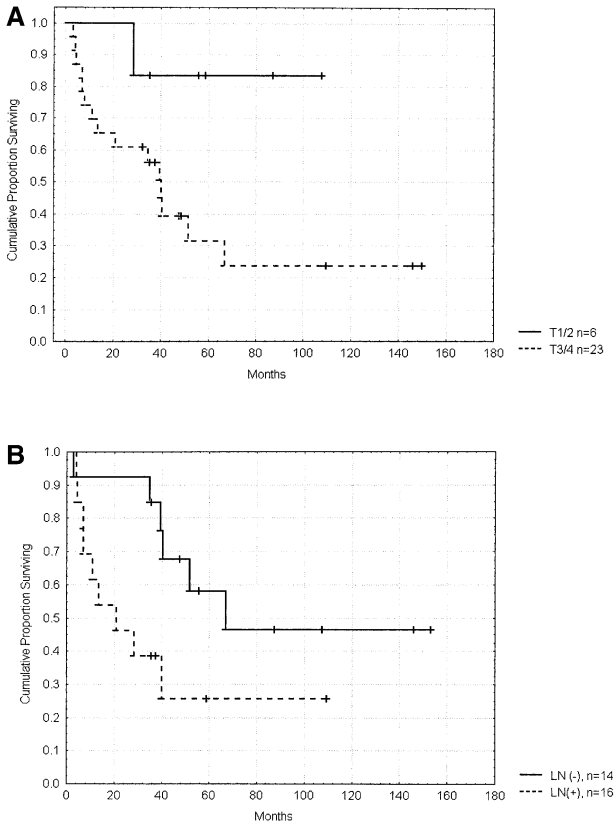

Fig. 2. Survival of patients with adenocarcinoma who underwent curative resection. *A*, Kaplan-Meier estimates of survival for patients with T1/2 and T3/4 adenocarcinomas. Differences between groups were significant ($P < 0.05$ by log-rank test). *B*, Kaplan-Meier estimates of survival for patients with N0 and N1/2 adenocarcinomas. Differences between groups were significant ($P < 0.05$ by log-rank test).

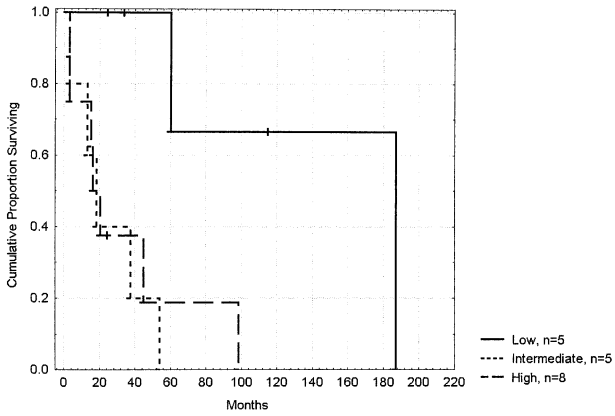

Fig. 3. Survival of patients with sarcoma who underwent curative resection. Kaplan-Meier estimates of survival for patients with G1 (low-grade), G2 (intermediate-grade) and G3 (high-grade). Differences between groups were significant ($P < 0.05$ by Mantel test).

these rare cancers has been compounded by the heterogeneity of tumor types included in series of patients with small bowel cancers.

In our study, the three tumor types examined were associated with distinct behaviors and responses to surgical therapy. Half of the patients with adenocarcinoma were able to undergo curative resection; almost half of patients who underwent curative resection survived at least 5 years. In contrast, prognosis among patients with sarcomas was poor. Less than one fourth of patients with sarcomas who underwent curative resection survived at least 5 years, even though 90% of patients with sarcomas were able to undergo such resection. Patients with carcinoid tumors had the best prognosis. Three fourths of the patients with carcinoid tumors who underwent curative resection survived at least 5 years. Even among patients with unresectable carcinoid tumors, half survived for least 5 years.

In our study, T and N stages were identified as prognostic factors associated with differences in survival among patients who had undergone curative resection of adenocarcinomas. Similarly, grade was identified as a prognostic factor associated with differences in survival among patients who had undergone curative resection of sarcomas. No such prognostic factors were identifiable for patients with carcinoid tumors.

There are relatively few reported analyses of large series of patients with small bowel cancers with which to compare ours. In the study reported by Veyrieres et al.,[3] anemia at the time of diagnosis was the only factor identified to be associated with improved survival among patients with small bowel adenocarcinoma. In the study reported by Abrahams et al.,[4] depth of cancer invasion, presence of lymph node metastases, tumor differentiation, surgical margin status, extramural venous spread, and a history of Crohn's disease emerged as factors associated with significant differences in survival. For small bowel sarcomas, T stage has been reported to be associated with significant differences in survival,[5] in addition to tumor grade.[6,7] For small bowel carcinoid tumors, patient age and tumor site have been reported to be associated with significant differences in survival.[8]

These single-institution studies, as well as ours, share an important limitation — that is, small sample size. Analyses of comprehensive multi-institutional databases will be required to better define the natural histories and prognostic factors associated with rare diseases such as small bowel cancers. An analysis of 4995 cases of small bowel adenocarcinoma contained in the National Cancer Data Base has been reported.[9] However, this analysis is limited by potential case

selection bias and limitations in what information is contained in that database. For example, detailed pathologic information evaluated in our study was not available for analysis in that study.

In addition to multi-institutional collaborative efforts, further investigation into the fundamental mechanisms driving the initiation and progression of small bowel cancers is needed. Although such investigations are considered to be of low priority, given the low incidence of this cancer, findings in these studies may have important implications for more prevalent cancers. An important case in point is the discovery of therapy targeted against the KIT protein for gastrointestinal stromal tumors.[10,11] This finding has stimulated great interest in and hope for the feasibility of targeted therapies as a general strategy for treating cancer.

In conclusion, most patients with small bowel cancer have a poor prognosis; however, selected patients who undergo complete resection can achieve long-term survival.

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Because of the low incidence of small bowel cancers, data on their natural history and treatment-associated outcomes are limited. The aim of this study was to characterize the clinical presentation and outcomes associated with surgical therapy in patients with small bowel cancer treated at our institution during the past two decades. A second goal was to define prognostic factors associated with improved survival.

PATIENTS AND METHOD

The study was approved by the Brigham and Women's Hospital Institutional Review Board. The medical records of all patients with small bowel cancer admitted to the inpatient unit of Brigham and Women's Hospital during the period spanning January 1981 through November 2001 were analyzed. Patients were identified using the International Classification of Disease-9 (ICD-9) codes for small bowel neoplasm (codes 152.x) and the computer-assisted hospitalization analysis for the study of efficacy (CHASE) management system. Patients with lymphomas involving the small bowel were excluded. Parameters obtained from the medical records included patient demographic data (age and sex), signs and symptoms at the time of diagnosis, tumor location, the surgical procedure and whether it was curative (no gross residual cancer present at the completion of surgery) or palliative (gross residual cancer present at the completion of surgery), and pathologic findings.

Tumors were categorized into one of three histologic types: adenocarcinoma, carcinoid tumor, and

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sarcoma. Pathologic parameters analyzed for adenocarcinomas were histologic differentiation, depth of tumor invasion, regional lymph node status, margin status, and overall stage according to the American Joint Committee on Cancer (AJCC) staging system.[2] Those for sarcoma were histologic grade, tumor size, regional lymph node status, and overall stage according to the AJCC staging system.[2] Parameters for carcinoid tumors were tumor size, depth of tumor invasion, regional lymph node status, and margin status.

Patient survival data were obtained from the United States Social Security Administration's Death Master File. The survival duration was calculated from the time of operation or time of diagnosis, if patients did not undergo any surgery, through time of death. Survival curves for selected patient groups were determined using the Kaplan-Meier method and compared by means of the log-rank test and Mantel test. Overall survival estimates were derived from the corresponding Kaplan-Meier curves. Chi-square analyses were used for other parameters unless specified otherwise.

RESULTS

Demographic and Tumor Data

During the study period, 96 patients with small bowel cancer were admitted to the hospital. The median age of this cohort was 64 years (range 26–88 years). Fifty-six patients (58%) were male.

The frequency of tumor of types in this cohort and the distribution of their locations within the small bowel are summarized in Table 1. Fifty-eight patients (60%) had an adenocarcinoma, 20 (21%) had a sarcoma, and 18 (19%) had a carcinoid tumor. Adenocarcinomas were located most frequently in the duodenum (55%), sarcomas in the jejunum (75%), and carcinoid tumors in the ileum (55%).

Table 1. Tumor histologic type and location

	Tumor type			
Location	Adenocarcinoma	Sarcoma	Carcinoid tumor	Total
Duodenum	32 (55)	3 (15)	1 (6)	36 (38)
Jejunum	14 (24)	15 (75)	7 (39)	36 (38)
Ileum	12 (21)	2 (10)	10 (56)	24 (25)

Numbers in parentheses are percentages.

Symptoms and Signs

Symptoms and signs that were present at the time of diagnosis are summarized in Table 2. The most frequent symptoms were abdominal pain, present in 41% of patients, and nausea and vomiting, present in 34% of patients. Twenty-two percent of patients had gastrointestinal bleeding. Nausea and vomiting and pain were more prevalent ($P < 0.05$) among patients with adenocarcinomas than in those with carcinoid tumors or sarcomas. Gastrointestinal bleeding was less prevalent ($P < 0.05$) among patients with carcinoid tumors than those with adenocarcinomas or sarcomas. Twenty-two percent of patients with carcinoid tumors presented with symptoms of the carcinoid syndrome.

Staging

The AJCC stage distributions for patients with adenocarcinomas or sarcomas are shown in Table 3. Forty-five percent of patients with adenocarcinomas and 35% of those with sarcomas had stage IV disease at the time of diagnosis. Among patients with carcinoid

Table 2. Symptoms and signs at presentation

	Tumor type			
Symptom/sign	Adenocarcinoma (%)	Sarcoma (%)	Carcinoid tumor (%)	Total (%)
Nausea/vomiting	45*	15	22	34
Pain	46*	25	39	41
Gastrointestinal bleeding	26†	30†	0	22
Weight loss	9	5	11	8
Palpable mass	0	10	0	2
Anemia, occult fecal blood	3	0	0	2
Diarrhea	2	5	22	6
Jaundice	9	0	6	6
Carcinoid tumor syndrome	0	0	22	4

Numbers indicate percentage of patients with each type of tumor.
*$P < 0.05$ vs. sarcoma or carcinoid tumor.
†$P < 0.05$ vs. carcinoid tumor.

Table 3. Stage distribution of adenocarcinoma and sarcoma

Stage	Adenocarcinoma (%)	Sarcoma (%)
I	5	20
II	24	25
III	26	20
IV	45	35

tumors, 14 (78%) had metastatic disease detected either preoperatively or intraoperatively.

Surgical Therapy

Among study patients, 24 (25%) did not undergo tumor resection because of the presence of advanced disease. Of these 24 patients, 11 did not undergo laparotomy because metastases were detected preoperatively, four underwent laparotomy and tumor biopsy alone, and nine underwent palliative intestinal bypass.

Fifteen patients (16%) underwent incomplete (palliative) tumor resection. The percentages of the patients who underwent complete (curative) tumor resection were 51%, 90%, and 50% for those with adenocarcinomas, sarcomas, and carcinoid tumors, respectively. The nature of these curative procedures was related to tumor location. Among patients with tumors located in the duodenum, 11 underwent pancreaticoduodenectomy, and seven underwent segmental (sleeve) duodenal resection. Each of the patients with tumors located in the jejunum or ileum underwent segmental intestinal resection with regional lymph node excision.

Eleven percent of patients who underwent curative resection had en bloc resection of organs adjacent to the intestinal primary tumor (this percentage excludes pancreaticoduodenectomies for duodenal cancers); one patient (with an adenocarcinoma) underwent partial cystectomy and salpingoopherectomy, two patients (one with a sarcoma and one with a carcinoid tumor) underwent sigmoidectomy, and two patients (one with a sarcoma and one with a carcinoid tumor) underwent wedge resection of the liver.

Pathologic Findings

Pathologic parameters evaluated for specimens from patients who underwent complete resection are summarized in Table 4. Among the 38 resected adenocarcinomas, 80% of tumors were T3 or T4, 53% had associated regional lymph node metastases, and 93% were moderately or poorly differentiated. Of the 18 resected sarcomas, 83% of tumors were T2 (tumor size greater than 5 cm in diameter), none had associated regional lymph node metastases, and 44% were high grade. Of the nine resected carcinoid tumors, 55% of tumors were T3 or T4, 67% had associated regional lymph node metastases, and 44% were larger than 1.5 cm in diameter. Of the 18 resected sarcomas, eight specimens were diagnosed as GIST, gastrointestinal stromal tumors by KIT (CD117) positivity on immunohistochemical analysis.

Outcomes Associated With Surgical Treatment and Prognostic Factors

The mean follow-up period for study patients was 57 months. The 30-day postoperative mortality rate

Table 4. Pathologic findings in specimens from patients who underwent complete resection

	Adenocarcinoma	Sarcoma	Carcinoid tumor
T			
1	3 (10%)	3 (17%)	1 (11%)
2	3 (10%)	15 (83%)	3 (33%)
3	18 (60%)		2 (22%)
4	6 (20%)		3 (33%)
N			
0	14 (47%)	18 (100%)	3 (33%)
1	16 (53%)	0	6 (67%)
Margin			
Positive	5 (17%)	3 (17%)	1 (11%)
Grade			
Well differentiated/low grade	2 (7%)	5 (28%)	
Moderate/intermediate	16 (53%)	5 (28%)	
Poor/high	12 (40%)	8 (44%)	
Tumor size			
$<$1.5 cm			5 (56%)
$\geq$1.5 cm			4 (44%)

was 1.2%. The overall 5-year survival rates for all study patients with adenocarcinomas, sarcomas, and carcinoid tumors were 26%, 22%, and 70%, respectively (Fig. 1). Survival data for patients who underwent complete or incomplete resection and for those who presented with unresectable disease are shown in Table 5. For all tumor types, median survival and 5-year survival rates were greater ($P < 0.05$) among patients who underwent curative resection than among those who underwent incomplete resection or no resection.

To identify prognostic factors associated with the surgical treatment of small bowel cancers, the following factors were analyzed for patients who underwent complete tumor resection: age, sex, margin involvement, and tumor site for all three types of tumors; tumor grade, tumor node metastasis (TNM) factors, and stage according to the AJCC staging system for adenocarcinomas and sarcomas; and tumor size, depth of tumor invasion, and lymph node involvement for carcinoid tumors.

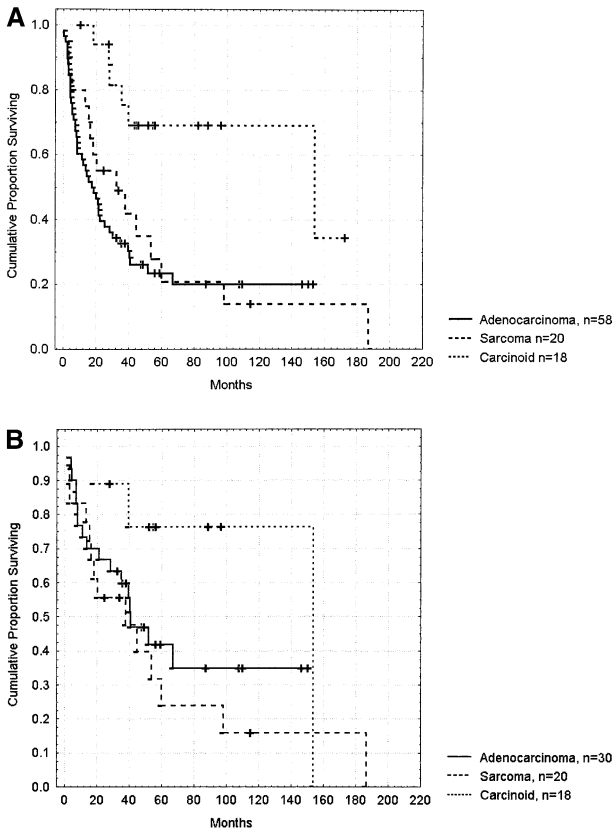

Fig. 1. Overall survival for patients with adenocarcinoma, sarcoma, and carcinoid tumor, and survival for selected patients who underwent curative resection. *A*, Kaplan-Meier estimates of overall survival for patients with adenocarcinoma, sarcoma, and carcinoid tumor. *B*, Kaplan-Meier estimates of survival for patients with adenocarcinoma, sarcoma and carcinoid tumor who underwent curative resection.

Table 5. Median survival and 5-year survival rates after surgical resection of small bowel cancers

Cancer type and procedure type	Median survival (mos)	5-yr survival (%)
Adenocarcinoma Curative resection (n = 30)	36.5	46.9
Adenocarcinoma Palliative resection (n = 8)	10.2	12.5*
Adenocarcinoma Unresectable (n = 20)	6.9	0*
GIST/sarcoma Curative resection (n = 18)	42.8	24.5
GIST/sarcoma Palliative resection (n = 1)	32.5	0
GIST/sarcoma Unresectable (n = 1)	5.1	0
Carcinoid tumor Curative resection (n = 9)	54.7	75.6
Carcinoid tumor Palliative resection (n = 6)	44.1	59.5*
Carcinoid tumor Unresectable (n = 3)	27.9	50*

GIST = gastrointestinal stromal tumor.
*$P < 0.05$ vs. curative resection.

For patients with adenocarcinoma who underwent curative resection, T and N stage were significant prognostic factors associated with overall survival. Five-year survival rates for patients with T1/T2 tumors and those with T3/T4 tumors were 82% and 58%, respectively ($P < 0.05$) (Fig. 2, *A*). Five-year survival rates for patients with uninvolved lymph nodes and those with lymph node metastases were 58% and 29%, respectively ($P < 0.05$) (Fig. 2, *B*). For patients with sarcomas who underwent curative resection, tumor grade was a significant prognostic factor predicting overall survival. Five-year survival rates for patients with low-grade, intermediate-grade, and high-grade tumors were 67%, 20%, and 19%, respectively ($P < 0.05$) (Fig. 3). None of the factors examined were significantly associated with survival for patients with carcinoid tumors who underwent curative resection.

DISCUSSION

The small bowel accounts for 75% of the length of the gastrointestinal tract and contains more than 90% of its mucosal surface area. Yet, only 2% of primary gastrointestinal cancers arise in the small bowel. Difficulty in obtaining prognostic information for patients who have undergone surgical resection of

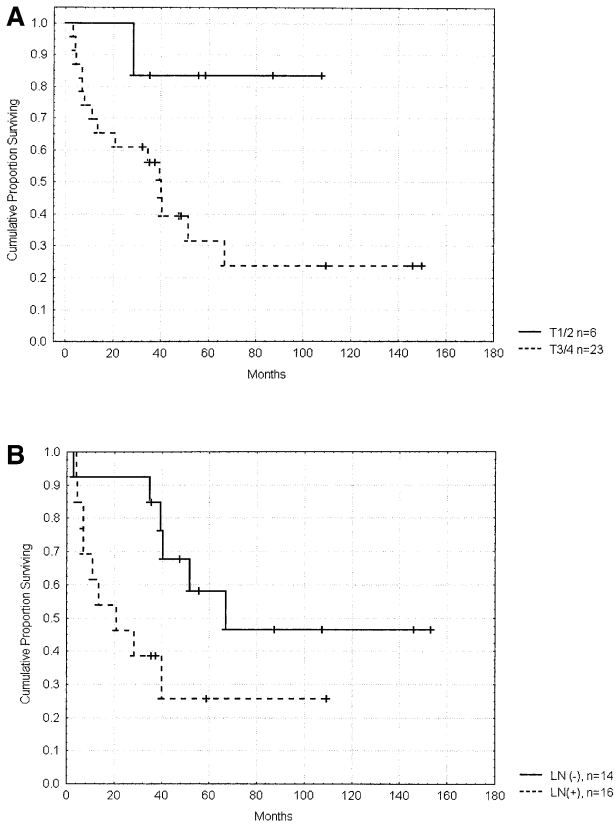

Fig. 2. Survival of patients with adenocarcinoma who underwent curative resection. *A*, Kaplan-Meier estimates of survival for patients with T1/2 and T3/4 adenocarcinomas. Differences between groups were significant ($P < 0.05$ by log-rank test). *B*, Kaplan-Meier estimates of survival for patients with N0 and N1/2 adenocarcinomas. Differences between groups were significant ($P < 0.05$ by log-rank test).

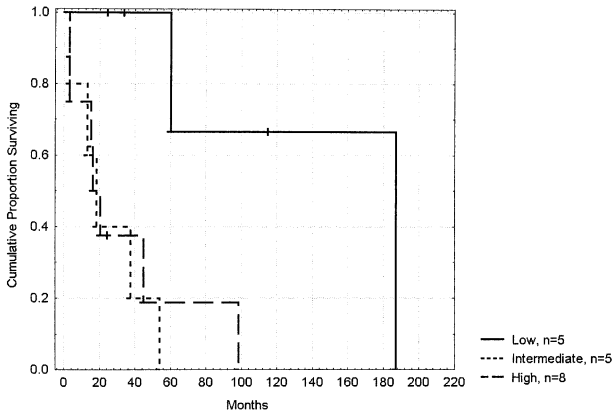

Fig. 3. Survival of patients with sarcoma who underwent curative resection. Kaplan-Meier estimates of survival for patients with G1 (low-grade), G2 (intermediate-grade) and G3 (high-grade). Differences between groups were significant ($P < 0.05$ by Mantel test).

these rare cancers has been compounded by the heterogeneity of tumor types included in series of patients with small bowel cancers.

In our study, the three tumor types examined were associated with distinct behaviors and responses to surgical therapy. Half of the patients with adenocarcinoma were able to undergo curative resection; almost half of patients who underwent curative resection survived at least 5 years. In contrast, prognosis among patients with sarcomas was poor. Less than one fourth of patients with sarcomas who underwent curative resection survived at least 5 years, even though 90% of patients with sarcomas were able to undergo such resection. Patients with carcinoid tumors had the best prognosis. Three fourths of the patients with carcinoid tumors who underwent curative resection survived at least 5 years. Even among patients with unresectable carcinoid tumors, half survived for least 5 years.

In our study, T and N stages were identified as prognostic factors associated with differences in survival among patients who had undergone curative resection of adenocarcinomas. Similarly, grade was identified as a prognostic factor associated with differences in survival among patients who had undergone curative resection of sarcomas. No such prognostic factors were identifiable for patients with carcinoid tumors.

There are relatively few reported analyses of large series of patients with small bowel cancers with which to compare ours. In the study reported by Veyrieres et al.,[3] anemia at the time of diagnosis was the only factor identified to be associated with improved survival among patients with small bowel adenocarcinoma. In the study reported by Abrahams et al.,[4] depth of cancer invasion, presence of lymph node metastases, tumor differentiation, surgical margin status, extramural venous spread, and a history of Crohn's disease emerged as factors associated with significant differences in survival. For small bowel sarcomas, T stage has been reported to be associated with significant differences in survival,[5] in addition to tumor grade.[6,7] For small bowel carcinoid tumors, patient age and tumor site have been reported to be associated with significant differences in survival.[8]

These single-institution studies, as well as ours, share an important limitation — that is, small sample size. Analyses of comprehensive multi-institutional databases will be required to better define the natural histories and prognostic factors associated with rare diseases such as small bowel cancers. An analysis of 4995 cases of small bowel adenocarcinoma contained in the National Cancer Data Base has been reported.[9] However, this analysis is limited by potential case

selection bias and limitations in what information is contained in that database. For example, detailed pathologic information evaluated in our study was not available for analysis in that study.

In addition to multi-institutional collaborative efforts, further investigation into the fundamental mechanisms driving the initiation and progression of small bowel cancers is needed. Although such investigations are considered to be of low priority, given the low incidence of this cancer, findings in these studies may have important implications for more prevalent cancers. An important case in point is the discovery of therapy targeted against the KIT protein for gastrointestinal stromal tumors.[10,11] This finding has stimulated great interest in and hope for the feasibility of targeted therapies as a general strategy for treating cancer.

In conclusion, most patients with small bowel cancer have a poor prognosis; however, selected patients who undergo complete resection can achieve long-term survival.

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Coexpression of Carcinoembryonic Antigen and E-cadherin in Colorectal Adenocarcinoma With Liver Metastasis

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Carcinoembryonic antigen (CEA) has been suggested as a metastatic activator in colorectal carcinoma, whereas the E-cadherin expression is downregulated in a variety of carcinomas. CEA and E-cadherin expressions were simultaneously assessed with regard to tumor progression in the various sites of colorectal carcinomas with liver metastasis. Twenty-six consecutive patients who had colorectal carcinoma with liver metastasis underwent curative surgery for primary tumor and liver metastasis. CEA and E-cadherin expression were identified on immunohistochemical staining using the labeled streptavidin-biotin method. Their mRNA expression was also detected by RT in situ PCR using one-step reverse transcription–polymerase chain reaction (RT-PCR). CEA and E-cadherin expression scores in the tumor center were greater than those in the tumor margin in both primary tumor and liver metastasis ($P < 0.001$ to 0.006). CEA expression scores were closely associated with E-cadherin expression scores on the corresponding tumor site ($P < 0.001$ to 0.017). CEA and E-cadherin mRNA expression was greatest in the hepatocytes adjacent to liver metastasis, next greatest in the primary tumor, and least in the liver metastasis ($P < 0.001$ to 0.002). CEA mRNA expression was also closely correlated with E-cadherin mRNA expression in the primary tumor ($P < 0.001$) and in the adjacent hepatocytes of the liver metastasis ($P = 0.018$). Patients with a lesser CEA expression score in the liver metastasis margin appeared to have a longer disease-free survival period than did those with a greater CEA expression score. Expression of CEA and E-cadherin was closely correlated with the mRNA levels. Furthermore, these correlations may be implicated in the tumor progression of colorectal carcinoma considering their biological properties. (J GASTROINTEST SURG 2003;7:931–938)

KEY WORDS: CEA, E-cadherin, mRNA, colorectal cancer, liver metastasis

Carcinoembryonic antigen (CEA) has been known to facilitate tumor progression and metastasis in colorectal carcinoma. CEA is a well-characterized cell surface antigen expressed in large quantities in approximately 95% of colorectal carcinomas.[1] Postoperative CEA monitoring is especially valuable for detecting metastatic colorectal carcinoma, especially liver metastasis. On the other hand, decreased membranous E-cadherin expression has been reported in a variety of carcinomas, including colorectal carcinoma, and has been suggested as a major determinant in the differentiation of colorectal carcinoma cell lines.[2,3]

CEA appears to provide a variety of cellular functions, that is, adhesion both mediating intercellular as well as CEA-matrix interactions,[4,5] signal transduction,[6] and cellular migration.[7] These functions suggest a possible explanation for the role of CEA as a facilitator of tumor invasion and metastasis. E-cadherin is a calcium-dependent transmembrane cell-cell adhesion and signal-transducing molecule linked by its cytoplasmic part via β-catenin or plakoglobin and α-catenin to the actin skeleton.[8] Downregulation of E-cadherin expression is known to correlate with a strong invasive potential, providing a poor prognosis in human carcinoma.[9,10] CEA and E-cadherin

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thereby appear to present different behaviors in tumor growth and metastasis.

The distribution of CEA in tumors and adjacent tissue appeared to correlate with invasiveness and metastatic behavior of colorectal carcinomas in our previous study.[11] E-cadherin expression was increased with cytoplasmic accumulation in more than 85% of sporadic colorectal cancers[12] and resumed at the metastatic site.[13] Post-transcriptional events, including glycosylation, apical targeting, and protein half-life, have been implicated in CEA and E-cadherin expression in human colorectal carcinoma.[14,15] In our study both mRNA and protein expressions of CEA and E-cadherin were examined together in colorectal adenocarcinomas with liver metastasis, and they were evaluated with regard to tumor progression based on clinicopathologic characteristics. As current study did not include in vitro biological consequences of these molecules, they were indirectly evaluated in terms of clinicopathologic characteristics.

MATERIAL AND METHODS

Patients

Twelve male and 14 female consecutive colorectal cancer patients with isolated liver metastasis were included in the study; their mean age was 57 years (range 38 to 75 years). As the metastatic lesions were confined to the liver and identified on preoperative evaluation and surgical findings, all study patients underwent curative surgery for liver metastasis as well as for primary tumor. Liver metastasis was found synchronously in 24 patients and metachronously in two. The median follow-up period was 33 months (10 to 57 months). Archival paraffin blocks of primary tumor and liver metastasis were prepared in 4 μm sections on DAKO glass slides (DAKO Corp., Carpinteria, CA) and on silane-coated glass slides (Perkin-Elmer, Norwalk, CT). The blocks used included tumor tissue as well as adjacent normal tissue.

Clinicopathologic Characteristics

Twenty-four tumors were classified as T3 and two as T4 according to the American Joint Committee on Cancer staging system (6th ed.). The lymph node status was as follows: four tumors were N0, 13 were N1, and nine were N2. There were seven right colon cancers and 19 left colon cancers including rectal cancers. All tumors showed moderately differentiated histology except for two poorly differentiated tumors. Twenty-one patients had a single liver metastasis; the remainder had two to four liver metastases. Twenty-one liver metastases were located in the right lobe of the liver and seven were in the left lobe. The mean serum CEA level was 34.4 ng/ml preoperatively (range 1~352 ng/ml), 8.8 ng/ml 1 month postoperatively (range 1.9~49.1 ng/ml), and 20.3 ng/ml 6 months postoperatively (range 1.9~173 ng/ml). The serum CEA level was within normal range (<6 ng/ml) in 12 patients preoperatively, 21 patients 1 month postoperatively, and 19 patients 6 months postoperatively. All patients received postoperative adjuvant chemotherapy consisting of 5-fluorouracil and leucovorin, and radiotherapy was also administered to patients with middle or lower rectal cancer.

CEA and E-cadherin Expression on Immunohistochemical Staining

Immunohistochemical staining was based on the labeled streptavidin-biotin method and carried out using a DAKO LSAB kit (DAKO Corp.) and standard protocol. In brief, slides were deparaffinized with xylene and dehydrated in graded ethanol. The antigen was retrieved by dipping into boiled citrate buffer (10 mmol/L, pH = 6) for 10 minutes in a microwave oven. Endogenous peroxidase activity was quenched by incubating in 3% hydrogen peroxide for 10 minutes. Slides were then incubated with appropriately characterized and diluted mouse anti-CEA (T84.66; American Type Culture Collection, Rockville, MD) and anti–E-cadherin (HECD-1; Takara, Shiga, Japan) monoclonal antibodies for 2 hours. T84.66 binds to CEA-specific epitopes on the A3 subdomain classified as Gold group 1.[16] HECD-1 is originated from the human breast cancer cell line MCF-7 showing specific inhibition of E-cadherin–dependent cell-cell contact. After cells were washed with Tris buffer for 10 minutes, they were incubated sequentially for 10 minutes with biotinylated-link antibody and peroxidase-labeled streptavidin. Immune staining was completed after incubation with substrate-chromogen solution. Counterstaining was done with hematoxylin solution. The percentage proportion of cells with positive staining was divided into the following five groups: 0%; >0 to $\leqslant 25\%$; >25 to $\leqslant 50\%$; >50 to $\leqslant 75\%$, and $>75\%$. The intensity of the positive staining was classified into three groups according to the standard we had previously used, that is, light brown, brown, and dark brown.[11] The expression score was determined by multiplying the grade of the percent-positive staining by the intensity score. The greater and the lesser expression scores were defined as $\geqslant 6$ and <6, respectively. Results were adopted when more than two of three observers (J.C.K., Y.M.K., and J.S.K) showed the same interpretation.

CEA and E-cadherin mRNA Detection Using Reverse Transcription In Situ Polymerase Chain Reaction

Reverse transcription (RT) in situ polymerase chain reaction (PCR) consisted of the following three steps: protease digestion, digestion with RNase-free DNase, and cDNA synthesis and PCR amplification. The negative and positive control specimens were prepared on the same glass slide with the actual test. Deparaffinized tissue sections were digested with 2 mg/ml pepsin (Roche, Mannheim, Germany) for 30 minutes at room temperature. Pepsin was inactivated using 0.1% DEPC (Sigma, St. Louis, MO) for 1 minute and was then washed in 100% ethanol. Two of the three tissue sections were digested with 1 μl RNase-free DNase in 1 μl of 10×X buffer and 8 μl of DEPC water in a humidity chamber at 37C for 30 minutes and were then washed for 1 minute using DEPC water and 100% ethanol. cDNA synthesis and PCR amplification were easily performed by one-step RT in situ PCR using an EZ rTth RNA PCR kit (Perkin-Elmer) and a standard protocol. Primer sets for CEA were selected, including a 3′-untranslated region (sense, 5′-TTCTGATACC ACTGCACTGT-3′ antisense, 5′-GAGCGACCA CATAGGGAGAA-3′),[17] and those for E-cadherin were adapted including exon 16 on a cytoplasmic domain (sense, 5′-AGATGACAGGTGTGCCCT TC-3′; antisense, 5′-ATTTCTGCATTTCCCAG-CAC).[18] The negative control includes DNase treatment without RT step and the positive control excludes DNase treatment. cDNA synthesis was made using primers in a standard solution including 0.6 μl digoxigenin dUTP at 65C for 30 minutes followed by 20 cycles of PCR amplification (60C for 1 minute and 94C for 30 seconds). After the detection step incubated with alkaline phosphatase-antidigoxigenin conjugate, the tissue sections were finally stained using 2.3 ml of nitroblue tetrazolium (NBT) and 1.8 ml of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in a dark humid chamber for 30 minutes and counterstained using nuclear fast red. Digoxigenin-labeled dUTP was incorporated into the RT-PCR product, and a positive signal in the nucleus was observed under a light microscope. No signal was evident in the negative control sample, whereas at least 50% of the cells showed an intense nuclear signal in the positive control sample. The percentage proportion of cells with positive staining was divided into five groups as in the immunohistochemical staining.

Statistical Analysis

The protein expression among the various sites and the mRNA expression grades were tested using the paired *t* test or one-way analysis of variance (ANOVA) according to the number of groups. The protein expression between the primary tumor and the liver metastasis, or between CEA and E-cadherin, was compared using the regression analysis of the Pearson product-moment correlation. The incidence of two or more groups, that is, CEA vs. E-cadherin mRNA expression grades, and protein or mRNA expression grades vs. disease-free survival, was compared by contingency table analysis using the Pearson chi-square test. Disease-free survival time was analyzed using either the Kaplan-Meier plot and log-rank test or a multivariate analysis using the Cox proportional hazards regression model with regard to independent variables of clinicopathologic characteristics. The significance level was determined at 5% for each analysis, and all calculations were performed with the use of IBM PC SPSS software (version 11, SPSS Inc., Chicago, IL).

RESULTS

CEA and E-cadherin Protein Expression

The tumor to stroma ratio was 72 ± 11% (mean ± standard deviation [SD] in the primary tumor and 76 ± 14% in the liver metastasis. Both CEA and E-cadherin expression scores in the tumor center were greater than those in the tumor margin regardless of primary tumor or liver metastasis ($P < 0.001$ to 0.006) (Table 1). The expression pattern of CEA was similar to that of E-cadherin (Fig. 1). Furthermore, CEA expression scores correlated closely with E-cadherin expression scores in their corresponding tumor sites ($P < 0.001$ to 0.017). The CEA and E-cadherin expression scores in the tumor center also correlated closely with those in the tumor margin in both the primary tumor and liver metastasis ($P < 0.001$ to 0.028). The CEA

Table 1. CEA and E-cadherin expression scores in the primary tumor and liver metastasis

Site	Expression score (N = 26), mean ± SD	
	CEA	E-cadherin
Primary tumor center	6.8 ± 2.6	6.8 ± 2.3
Primary tumor margin	4.5 ± 2.6	5.0 ± 2.3
Liver metastasis center	7.8 ± 2.9	7.1 ± 2.2
Liver metastasis margin	4.7 ± 2.2	4.3 ± 1.8

The expression score was determined by multiplying the grade of the percent-positive staining by the intensity score on immunohistochemical staining. Primary tumor center vs. margin, CEA ($P < 0.001$), E-cadherin ($P = 0.006$); liver metastasis center vs. margin, CEA ($P < 0.001$), E-cadherin ($P < 0.001$).

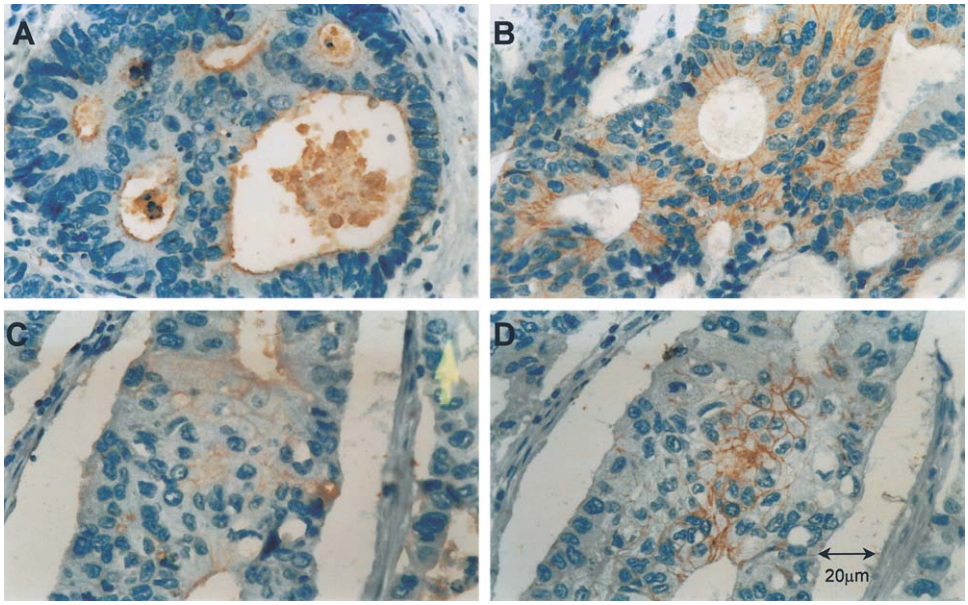

Fig. 1. CEA (**A** and **C**) and E-cadherin (**B** and **D**) expression in the primary tumor (*upper*) and liver metastasis (*lower*); counter staining using hematoxylin. The expression pattern of CEA was similar to that of E-cadherin on the same site.

expression scores of the primary tumor center correlated closely with those in the liver metastasis ($P = 0.019$), whereas the E-cadherin expression scores did not.

CEA and E-cadherin mRNA Expression

The CEA and E-cadherin mRNA expression was greatest in the adjacent hepatocytes of the liver metastasis, next greatest in the primary tumor, and least in the liver metastasis in descending order ($P < 0.001$ to 0.002) (Table 2). The CEA and E-cadherin mRNA expression in the primary tumor was closely associated with those in the liver metastasis and adjacent hepatocytes, respectively ($P \leqslant 0.001$) (Fig. 2). The CEA mRNA expression was closely correlated with E-cadherin mRNA expression in the primary tumor ($P < 0.001$) and in the adjacent hepatocytes of the liver metastasis ($P = 0.018$). Neither CEA nor E-cadherin mRNA expression was associated with its respective protein expression scores in the corresponding site. However, the E-cadherin mRNA expression of adjacent hepatocytes of the liver metastasis appeared to be associated with the E-cadherin expression score in the liver metastasis center ($P = 0.040$). Interestingly, the E-cadherin expression score in the liver metastasis center was greater in the lesser E-cadherin mRNA expression than in the greater (mean ± SD, 8.0 ± 2.4 vs. 6.2 ± 1.4; $P = 0.034$).

Table 2. CEA and E-cadherin mRNA expression in the primary tumor and liver metastasis

Site	mRNA expression (N = 26), mean ± SD	
	CEA	E-cadherin
Primary tumor	1.6 ± 0.7	1.6 ± 0.8
Liver metastasis	0.8 ± 0.5	0.9 ± 0.6
Adjacent hepatocytes	2.5 ± 0.9	2.5 ± 1

Adjacent hepatocytes vs. liver metastasis (CEA and E-cadherin, $P < 0.001$) and primary tumor (CEA, and E-cadherin, $P < 0.001$); primary tumor vs. liver metastasis (CEA, $P < 0.001$; E-cadherin, $P = 0.002$).

Clinicopathologic Correlation

The preoperative serum CEA level was positively associated with CEA expression scores in both primary tumor and liver metastasis centers ($P = 0.03$ and 0.049, respectively). The CEA expression score in the tumor margin was inversely correlated with the number of metastatic lymph nodes ($P = 0.026$). The E-cadherin mRNA expression in the primary tumor also showed an inverse correlation with the number of metastatic lymph nodes ($P = 0.036$). The CEA expression scores were less in tumors with lymphovascular invasion than in those without it, whereas E-cadherin expression scores showed no significant difference between them (Table 3).

CEA and E-cadherin Expression Scores as Related to Survival

Disease-free survival was not found in the seven patients with continuously elevated serum CEA

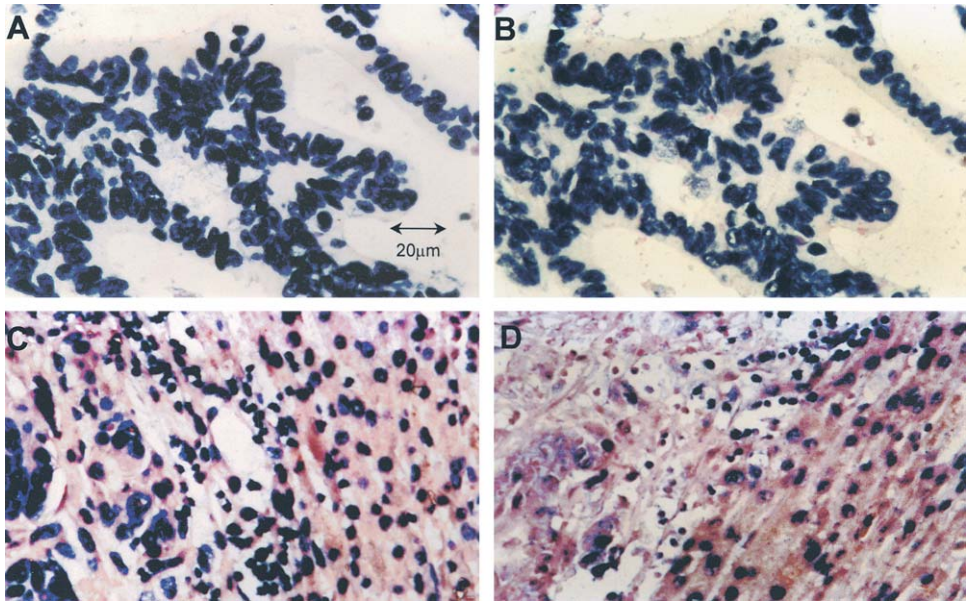

Fig. 2. CEA mRNA (**A** and **C**) and E-cadherin mRNA (**B** and **D**) expression in the primary tumor (*upper*) and liver metastasis (*lower*); counterstaining using nuclear fast red. Both CEA and E-cadherin mRNA expression in the primary tumor were closely associated with those in the liver metastasis and adjacent hepatocytes.

levels 6 months postoperatively, whereas 8 (42.2%) of 19 patients showed disease-free survival with a normal serum CEA level ($P = 0.002$). Patients with less CEA expression score in the liver metastasis margin appeared to have a longer disease-free survival than those with a greater CEA expression score (mean ± standard error [SE], 36 ± 8 m vs. 15 ± 4 m; $P = 0.041$). However, E-cadherin expression scores did not show statistically significant correlation with survival probably because of the limited number of the less E-cadherin expression group. A significant survival difference was not identified with regard to tumor CEA or E-cadherin expression scores on Cox regression analysis using various covariates, that is, tumor location, histologic differentiation, lymphovascular tumor invasion, N-stage, and number of liver metastases.

Table 3. CEA and E-cadherin expression scores in the primary tumor regarding lymphovascular invasion

Primary tumor site	Expression score, Mean ± SD		
	Lymphovascular invasion (−) N = 20	Lymphovascular invasion (+) N = 6	*P*
CEA			
Tumor center	7.4 ± 2.7	5.0 ± 1.1	0.049
Tumor margin	5.2 ± 2.6	2.5 ± 1.2	0.026
E-cadherin			
Tumor center	7.1 ± 2	6.0 ± 3.1	0.308
Tumor margin	5.5 ± 2.1	3.5 ± 2.2	0.057

The expression score was determined by multiplying the grade of the percent-positive staining by the intensity score on immunohistochemical staining. *P*, lymphovascular invasion (−) vs. (+) by LSD test.

DISCUSSION

The DNA sequences of 12 CEA subfamilies were compared in order to identify regions with a low degree of homology among the different members. As the 3′-untranslated region showed the lowest degree of sequence homology compared with other members of the subfamily showing a 40% to 60% sequence identity,[19] primer sets in this study were selected including a 3′-untranslated region. Several cadherin-like molecules share commonly with their putative Ca^{2+}-binding motifs in the repeated ectodomains, but diverge strikingly in various regions, particularly in the cytoplasmic domains. For example, extracellular domains of the desmogleins are very similar to those of classical cadherins, but their cytoplasmic domains are quite different.[20] Therefore our study used primers amplifying exon 16 on a cytoplasmic domain including least homology with other cadherins. Nonspecific signal possibly caused by surrounding tumor tissues and nonspecific DNA syntheses might be reliably eliminated with overnight digestion in RNase-free DNase in our study.

Functional roles of CEA and E-cadherin in colorectal carcinomas have been investigated with regard to their biological behavior in several animal models.

A significant increase in liver metastasis was observed in the colorectal cancer cell lines transfected with full-length CEA cDNA after intrasplenic injection into nude mice.[21,22] MDA-231 breast cancer cell lines transfected with E-cadherin cDNA showed a dramatically impaired capacity to form osteolytic metastasies.[23] In the other study, E-cadherin–negative colon cancer cells transfected with E-cadherin exhibited a great increase in adhesion strength when the expressed protein was appropriately linked to the cytoskeleton.[24] The protein and mRNA coexpression of CEA and E-cadherin in our study may provide possible interaction of these molecules in the implantation and proliferation of tumor growth.

One investigation suggested that changes in the expression of a single cell adhesion molecule such as CEA can lead to alterations in the expression of unrelated cell adhesion molecules, thereby contributing to the general derangement of the adhesive interactions frequently observed in tumor cells.[25] Thus, in our study, CEA expression may have triggered E-cadherin expression with regard to CEA and E-cadherin coexpression. Another study demonstrated that Th2 type cytokines, that is, IL-4 and IL-14, inhibited colon cancer cell-cell adhesion by downregulation of both CEA and E-cadherin.[26] Similarly, the logarithmic concentration of serum E-cadherin was reported to correlate with CEA concentration in gastric cancer.[27] As coexpression of CEA and E-cadherin was identified from their mRNA levels, this phenomenon may also be determined from pretranscriptional or transcriptional levels. Both up- and downregulation can be assumed in their coexpression. In cases of upregulation of both molecules, the migratory property of CEA is suppressed by E-cadherin, whereas the adhesive property is exaggerated. Their downregulation seems to produce a loose intercellular adhesion among tumor cells prone to migrate. Coexpression may thereby provide either intercellular binding or loosening, resulting in either progression or metastasis in colorectal carcinoma.

The genomic organization of the CEA gene was shown to be stable in both liver metastasis and adjacent liver tissues in colorectal carcinoma.[28] However, the epigenetic changes including CEA promoter methylation were controversial in numerous studies.[28–30] Otherwise, the detection of a 5′ high-density CpG island in the E-cadherin gene suggests that transcriptional downregulation by DNA methylation might be an inactivating mechanism of E-cadherin expression.[31] Inconsistent expressions between protein and mRNA in our study might occur from posttranscriptional regulation. The lack of direct proportionality between mRNA and the protein expression of CEA[14] and E-cadherin[3] has been reported as posttranscriptional and post-translational control. In our study CEA protein and mRNA expression was also observed in adjacent hepatocytes. CEA gene expression was identified in adjacent hepatocytes as well as in the liver metastasis.[32] CEA is regionally distributed in normal liver tissue more than 3 cm apart from the metastatic liver tumor in colorectal carcinoma, possibly from CEA in circulating tumor cells, hepatocytes, and Kupffer cells.[21,33]

Colorectal carcinoma cell lines with E-cadherin mutation represented 70% of the lymph node metastases after tumor resection in mice.[34] In our study, lymph node metastasis seemed to occur in tumors with less E-cadherin mRNA expression, suggesting an inactivation of this important adhesion molecule from the transcriptional level. CEA expression was also downregulated in tumors with multiple lymph node metastases or lymphovascular invasion. These downregulations may enable loosening of intercellular binding in terms of adhesive properties of these molecules providing invasiveness of tumor cells. Greater CEA expression scores in the liver metastasis margin and elevated serum CEA level after the metastatic resection showed less or no disease-free survival, respectively, which was similar to findings in other studies.[11,35,36] Otherwise, E-cadherin might not always act as a tumor suppressor in advanced colorectal carcinoma because expression scores did not affect postoperative survival regardless of sites. Similarly, some investigators have reported elevated levels of soluble E-cadherin in gastric cancer patients with advanced-stage and metastatic disease.[27] Although the CEA expression score was not an independent prognostic factor in our study, it needs to be interpreted with caution considering the limited number of cases and interactive variables, that is, histologic differentiation and tumor progression. Although there have been some controversial correlations between CEA expression and histologic differentiation of colorectal carcinoma, CEA expression is more pronounced in well-differentiated than in poorly differentiated tumors.[3,37]

CONCLUSION

Coexpression of CEA and E-cadherin in primary tumors and liver metastasis was identified and was closely correlated with their mRNA levels. This possibly is a suggestion of their impact on the implantation and proliferation of tumor progression in primary colorectal carcinoma and liver metastasis.

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Coexpression of Carcinoembryonic Antigen and E-cadherin in Colorectal Adenocarcinoma With Liver Metastasis

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Carcinoembryonic antigen (CEA) has been suggested as a metastatic activator in colorectal carcinoma, whereas the E-cadherin expression is downregulated in a variety of carcinomas. CEA and E-cadherin expressions were simultaneously assessed with regard to tumor progression in the various sites of colorectal carcinomas with liver metastasis. Twenty-six consecutive patients who had colorectal carcinoma with liver metastasis underwent curative surgery for primary tumor and liver metastasis. CEA and E-cadherin expression were identified on immunohistochemical staining using the labeled streptavidin-biotin method. Their mRNA expression was also detected by RT in situ PCR using one-step reverse transcription–polymerase chain reaction (RT-PCR). CEA and E-cadherin expression scores in the tumor center were greater than those in the tumor margin in both primary tumor and liver metastasis ($P < 0.001$ to 0.006). CEA expression scores were closely associated with E-cadherin expression scores on the corresponding tumor site ($P < 0.001$ to 0.017). CEA and E-cadherin mRNA expression was greatest in the hepatocytes adjacent to liver metastasis, next greatest in the primary tumor, and least in the liver metastasis ($P < 0.001$ to 0.002). CEA mRNA expression was also closely correlated with E-cadherin mRNA expression in the primary tumor ($P < 0.001$) and in the adjacent hepatocytes of the liver metastasis ($P = 0.018$). Patients with a lesser CEA expression score in the liver metastasis margin appeared to have a longer disease-free survival period than did those with a greater CEA expression score. Expression of CEA and E-cadherin was closely correlated with the mRNA levels. Furthermore, these correlations may be implicated in the tumor progression of colorectal carcinoma considering their biological properties. (J GASTROINTEST SURG 2003;7:931–938)

KEY WORDS: CEA, E-cadherin, mRNA, colorectal cancer, liver metastasis

Carcinoembryonic antigen (CEA) has been known to facilitate tumor progression and metastasis in colorectal carcinoma. CEA is a well-characterized cell surface antigen expressed in large quantities in approximately 95% of colorectal carcinomas.[1] Postoperative CEA monitoring is especially valuable for detecting metastatic colorectal carcinoma, especially liver metastasis. On the other hand, decreased membranous E-cadherin expression has been reported in a variety of carcinomas, including colorectal carcinoma, and has been suggested as a major determinant in the differentiation of colorectal carcinoma cell lines.[2,3]

CEA appears to provide a variety of cellular functions, that is, adhesion both mediating intercellular as well as CEA-matrix interactions,[4,5] signal transduction,[6] and cellular migration.[7] These functions suggest a possible explanation for the role of CEA as a facilitator of tumor invasion and metastasis. E-cadherin is a calcium-dependent transmembrane cell-cell adhesion and signal-transducing molecule linked by its cytoplasmic part via β-catenin or plakoglobin and α-catenin to the actin skeleton.[8] Downregulation of E-cadherin expression is known to correlate with a strong invasive potential, providing a poor prognosis in human carcinoma.[9,10] CEA and E-cadherin

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thereby appear to present different behaviors in tumor growth and metastasis.

The distribution of CEA in tumors and adjacent tissue appeared to correlate with invasiveness and metastatic behavior of colorectal carcinomas in our previous study.[11] E-cadherin expression was increased with cytoplasmic accumulation in more than 85% of sporadic colorectal cancers[12] and resumed at the metastatic site.[13] Post-transcriptional events, including glycosylation, apical targeting, and protein half-life, have been implicated in CEA and E-cadherin expression in human colorectal carcinoma.[14,15] In our study both mRNA and protein expressions of CEA and E-cadherin were examined together in colorectal adenocarcinomas with liver metastasis, and they were evaluated with regard to tumor progression based on clinicopathologic characteristics. As current study did not include in vitro biological consequences of these molecules, they were indirectly evaluated in terms of clinicopathologic characteristics.

MATERIAL AND METHODS

Patients

Twelve male and 14 female consecutive colorectal cancer patients with isolated liver metastasis were included in the study; their mean age was 57 years (range 38 to 75 years). As the metastatic lesions were confined to the liver and identified on preoperative evaluation and surgical findings, all study patients underwent curative surgery for liver metastasis as well as for primary tumor. Liver metastasis was found synchronously in 24 patients and metachronously in two. The median follow-up period was 33 months (10 to 57 months). Archival paraffin blocks of primary tumor and liver metastasis were prepared in 4 μm sections on DAKO glass slides (DAKO Corp., Carpinteria, CA) and on silane-coated glass slides (Perkin-Elmer, Norwalk, CT). The blocks used included tumor tissue as well as adjacent normal tissue.

Clinicopathologic Characteristics

Twenty-four tumors were classified as T3 and two as T4 according to the American Joint Committee on Cancer staging system (6th ed.). The lymph node status was as follows: four tumors were N0, 13 were N1, and nine were N2. There were seven right colon cancers and 19 left colon cancers including rectal cancers. All tumors showed moderately differentiated histology except for two poorly differentiated tumors. Twenty-one patients had a single liver metastasis; the remainder had two to four liver metastases. Twenty-one liver metastases were located in the right lobe of the liver and seven were in the left lobe. The mean serum CEA level was 34.4 ng/ml preoperatively (range 1~352 ng/ml), 8.8 ng/ml 1 month postoperatively (range 1.9~49.1 ng/ml), and 20.3 ng/ml 6 months postoperatively (range 1.9~173 ng/ml). The serum CEA level was within normal range (<6 ng/ml) in 12 patients preoperatively, 21 patients 1 month postoperatively, and 19 patients 6 months postoperatively. All patients received postoperative adjuvant chemotherapy consisting of 5-fluorouracil and leucovorin, and radiotherapy was also administered to patients with middle or lower rectal cancer.

CEA and E-cadherin Expression on Immunohistochemical Staining

Immunohistochemical staining was based on the labeled streptavidin-biotin method and carried out using a DAKO LSAB kit (DAKO Corp.) and standard protocol. In brief, slides were deparaffinized with xylene and dehydrated in graded ethanol. The antigen was retrieved by dipping into boiled citrate buffer (10 mmol/L, pH = 6) for 10 minutes in a microwave oven. Endogenous peroxidase activity was quenched by incubating in 3% hydrogen peroxide for 10 minutes. Slides were then incubated with appropriately characterized and diluted mouse anti-CEA (T84.66; American Type Culture Collection, Rockville, MD) and anti–E-cadherin (HECD-1; Takara, Shiga, Japan) monoclonal antibodies for 2 hours. T84.66 binds to CEA-specific epitopes on the A3 subdomain classified as Gold group 1.[16] HECD-1 is originated from the human breast cancer cell line MCF-7 showing specific inhibition of E-cadherin–dependent cell-cell contact. After cells were washed with Tris buffer for 10 minutes, they were incubated sequentially for 10 minutes with biotinylated-link antibody and peroxidase-labeled streptavidin. Immune staining was completed after incubation with substrate-chromogen solution. Counterstaining was done with hematoxylin solution. The percentage proportion of cells with positive staining was divided into the following five groups: 0%; >0 to $\leqslant 25\%$; >25 to $\leqslant 50\%$; >50 to $\leqslant 75\%$, and $>75\%$. The intensity of the positive staining was classified into three groups according to the standard we had previously used, that is, light brown, brown, and dark brown.[11] The expression score was determined by multiplying the grade of the percent-positive staining by the intensity score. The greater and the lesser expression scores were defined as $\geqslant 6$ and <6, respectively. Results were adopted when more than two of three observers (J.C.K., Y.M.K., and J.S.K) showed the same interpretation.

CEA and E-cadherin mRNA Detection Using Reverse Transcription In Situ Polymerase Chain Reaction

Reverse transcription (RT) in situ polymerase chain reaction (PCR) consisted of the following three steps: protease digestion, digestion with RNase-free DNase, and cDNA synthesis and PCR amplification. The negative and positive control specimens were prepared on the same glass slide with the actual test. Deparaffinized tissue sections were digested with 2 mg/ml pepsin (Roche, Mannheim, Germany) for 30 minutes at room temperature. Pepsin was inactivated using 0.1% DEPC (Sigma, St. Louis, MO) for 1 minute and was then washed in 100% ethanol. Two of the three tissue sections were digested with 1 μl RNase-free DNase in 1 μl of 10×X buffer and 8 μl of DEPC water in a humidity chamber at 37C for 30 minutes and were then washed for 1 minute using DEPC water and 100% ethanol. cDNA synthesis and PCR amplification were easily performed by one-step RT in situ PCR using an EZ rTth RNA PCR kit (Perkin-Elmer) and a standard protocol. Primer sets for CEA were selected, including a 3′-untranslated region (sense, 5′-TTCTGATACC ACTGCACTGT-3′ antisense, 5′-GAGCGACCA CATAGGGAGAA-3′),[17] and those for E-cadherin were adapted including exon 16 on a cytoplasmic domain (sense, 5′-AGATGACAGGTGTGCCCT TC-3′; antisense, 5′-ATTTCTGCATTTCCCAG-CAC).[18] The negative control includes DNase treatment without RT step and the positive control excludes DNase treatment. cDNA synthesis was made using primers in a standard solution including 0.6 μl digoxigenin dUTP at 65C for 30 minutes followed by 20 cycles of PCR amplification (60C for 1 minute and 94C for 30 seconds). After the detection step incubated with alkaline phosphatase-antidigoxigenin conjugate, the tissue sections were finally stained using 2.3 ml of nitroblue tetrazolium (NBT) and 1.8 ml of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in a dark humid chamber for 30 minutes and counterstained using nuclear fast red. Digoxigenin-labeled dUTP was incorporated into the RT-PCR product, and a positive signal in the nucleus was observed under a light microscope. No signal was evident in the negative control sample, whereas at least 50% of the cells showed an intense nuclear signal in the positive control sample. The percentage proportion of cells with positive staining was divided into five groups as in the immunohistochemical staining.

Statistical Analysis

The protein expression among the various sites and the mRNA expression grades were tested using the paired *t* test or one-way analysis of variance (ANOVA) according to the number of groups. The protein expression between the primary tumor and the liver metastasis, or between CEA and E-cadherin, was compared using the regression analysis of the Pearson product-moment correlation. The incidence of two or more groups, that is, CEA vs. E-cadherin mRNA expression grades, and protein or mRNA expression grades vs. disease-free survival, was compared by contingency table analysis using the Pearson chi-square test. Disease-free survival time was analyzed using either the Kaplan-Meier plot and log-rank test or a multivariate analysis using the Cox proportional hazards regression model with regard to independent variables of clinicopathologic characteristics. The significance level was determined at 5% for each analysis, and all calculations were performed with the use of IBM PC SPSS software (version 11, SPSS Inc., Chicago, IL).

RESULTS

CEA and E-cadherin Protein Expression

The tumor to stroma ratio was 72 ± 11% (mean ± standard deviation [SD] in the primary tumor and 76 ± 14% in the liver metastasis. Both CEA and E-cadherin expression scores in the tumor center were greater than those in the tumor margin regardless of primary tumor or liver metastasis ($P < 0.001$ to 0.006) (Table 1). The expression pattern of CEA was similar to that of E-cadherin (Fig. 1). Furthermore, CEA expression scores correlated closely with E-cadherin expression scores in their corresponding tumor sites ($P < 0.001$ to 0.017). The CEA and E-cadherin expression scores in the tumor center also correlated closely with those in the tumor margin in both the primary tumor and liver metastasis ($P < 0.001$ to 0.028). The CEA

Table 1. CEA and E-cadherin expression scores in the primary tumor and liver metastasis

Site	Expression score (N = 26), mean ± SD	
	CEA	E-cadherin
Primary tumor center	6.8 ± 2.6	6.8 ± 2.3
Primary tumor margin	4.5 ± 2.6	5.0 ± 2.3
Liver metastasis center	7.8 ± 2.9	7.1 ± 2.2
Liver metastasis margin	4.7 ± 2.2	4.3 ± 1.8

The expression score was determined by multiplying the grade of the percent-positive staining by the intensity score on immunohistochemical staining. Primary tumor center vs. margin, CEA ($P < 0.001$), E-cadherin ($P = 0.006$); liver metastasis center vs. margin, CEA ($P < 0.001$), E-cadherin ($P < 0.001$).

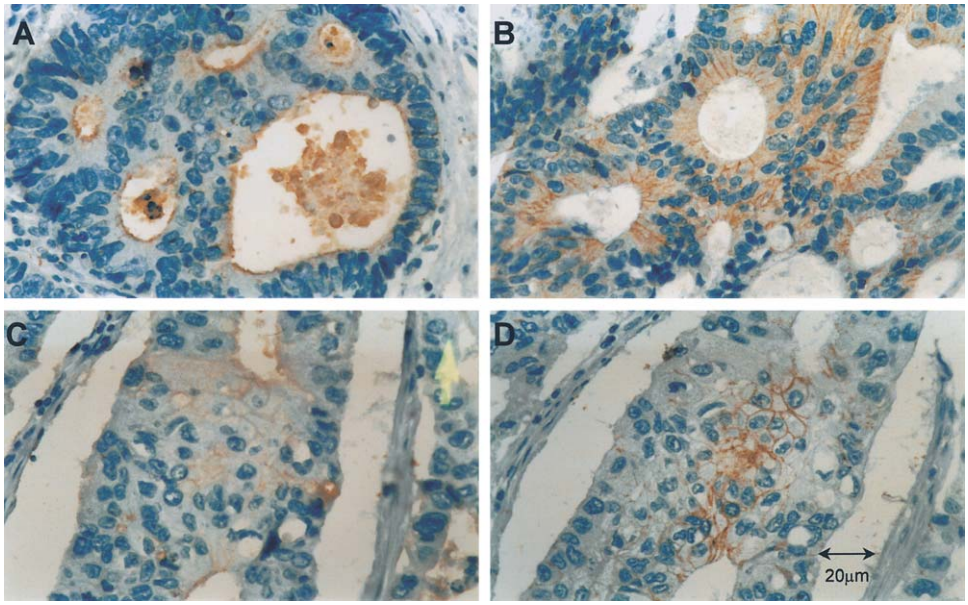

Fig. 1. CEA (**A** and **C**) and E-cadherin (**B** and **D**) expression in the primary tumor (*upper*) and liver metastasis (*lower*); counter staining using hematoxylin. The expression pattern of CEA was similar to that of E-cadherin on the same site.

expression scores of the primary tumor center correlated closely with those in the liver metastasis ($P = 0.019$), whereas the E-cadherin expression scores did not.

CEA and E-cadherin mRNA Expression

The CEA and E-cadherin mRNA expression was greatest in the adjacent hepatocytes of the liver metastasis, next greatest in the primary tumor, and least in the liver metastasis in descending order ($P < 0.001$ to 0.002) (Table 2). The CEA and E-cadherin mRNA expression in the primary tumor was closely associated with those in the liver metastasis and adjacent hepatocytes, respectively ($P \leqslant 0.001$) (Fig. 2). The CEA mRNA expression was closely correlated with E-cadherin mRNA expression in the primary tumor ($P < 0.001$) and in the adjacent hepatocytes of the liver metastasis ($P = 0.018$). Neither CEA nor E-cadherin mRNA expression was associated with its respective protein expression scores in the corresponding site. However, the E-cadherin mRNA expression of adjacent hepatocytes of the liver metastasis appeared to be associated with the E-cadherin expression score in the liver metastasis center ($P = 0.040$). Interestingly, the E-cadherin expression score in the liver metastasis center was greater in the lesser E-cadherin mRNA expression than in the greater (mean ± SD, 8.0 ± 2.4 vs. 6.2 ± 1.4; $P = 0.034$).

Table 2. CEA and E-cadherin mRNA expression in the primary tumor and liver metastasis

Site	mRNA expression (N = 26), mean ± SD	
	CEA	E-cadherin
Primary tumor	1.6 ± 0.7	1.6 ± 0.8
Liver metastasis	0.8 ± 0.5	0.9 ± 0.6
Adjacent hepatocytes	2.5 ± 0.9	2.5 ± 1

Adjacent hepatocytes vs. liver metastasis (CEA and E-cadherin, $P < 0.001$) and primary tumor (CEA, and E-cadherin, $P < 0.001$); primary tumor vs. liver metastasis (CEA, $P < 0.001$; E-cadherin, $P = 0.002$).

Clinicopathologic Correlation

The preoperative serum CEA level was positively associated with CEA expression scores in both primary tumor and liver metastasis centers ($P = 0.03$ and 0.049, respectively). The CEA expression score in the tumor margin was inversely correlated with the number of metastatic lymph nodes ($P = 0.026$). The E-cadherin mRNA expression in the primary tumor also showed an inverse correlation with the number of metastatic lymph nodes ($P = 0.036$). The CEA expression scores were less in tumors with lymphovascular invasion than in those without it, whereas E-cadherin expression scores showed no significant difference between them (Table 3).

CEA and E-cadherin Expression Scores as Related to Survival

Disease-free survival was not found in the seven patients with continuously elevated serum CEA

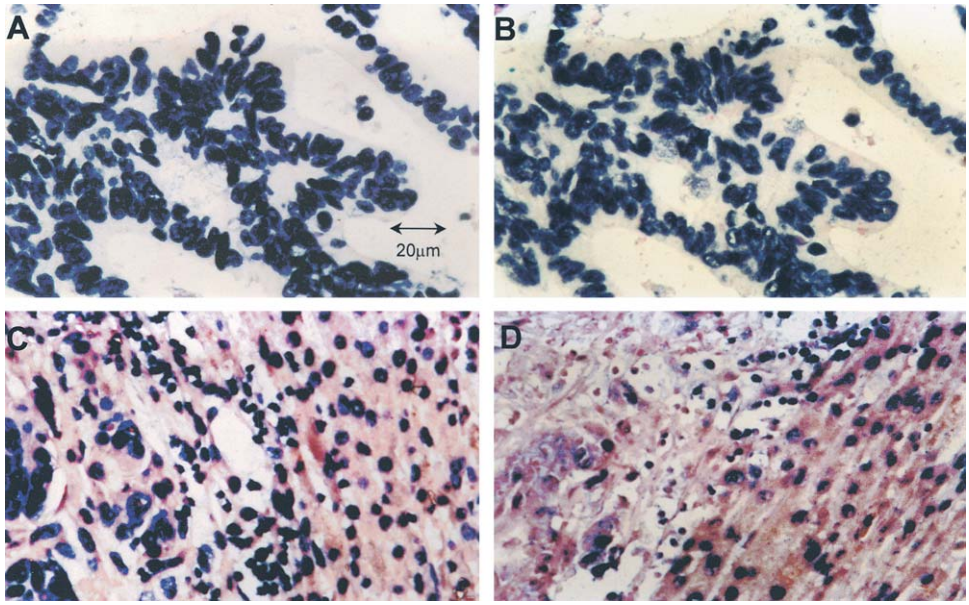

Fig. 2. CEA mRNA (**A** and **C**) and E-cadherin mRNA (**B** and **D**) expression in the primary tumor (*upper*) and liver metastasis (*lower*); counterstaining using nuclear fast red. Both CEA and E-cadherin mRNA expression in the primary tumor were closely associated with those in the liver metastasis and adjacent hepatocytes.

levels 6 months postoperatively, whereas 8 (42.2%) of 19 patients showed disease-free survival with a normal serum CEA level ($P = 0.002$). Patients with less CEA expression score in the liver metastasis margin appeared to have a longer disease-free survival than those with a greater CEA expression score (mean ± standard error [SE], 36 ± 8 m vs. 15 ± 4 m; $P = 0.041$). However, E-cadherin expression scores did not show statistically significant correlation with survival probably because of the limited number of the less E-cadherin expression group. A significant survival difference was not identified with regard to tumor CEA or E-cadherin expression scores on Cox regression analysis using various covariates, that is, tumor location, histologic differentiation, lymphovascular tumor invasion, N-stage, and number of liver metastases.

Table 3. CEA and E-cadherin expression scores in the primary tumor regarding lymphovascular invasion

Primary tumor site	Expression score, Mean ± SD		
	Lymphovascular invasion (−) N = 20	Lymphovascular invasion (+) N = 6	*P*
CEA			
Tumor center	7.4 ± 2.7	5.0 ± 1.1	0.049
Tumor margin	5.2 ± 2.6	2.5 ± 1.2	0.026
E-cadherin			
Tumor center	7.1 ± 2	6.0 ± 3.1	0.308
Tumor margin	5.5 ± 2.1	3.5 ± 2.2	0.057

The expression score was determined by multiplying the grade of the percent-positive staining by the intensity score on immunohistochemical staining. *P*, lymphovascular invasion (−) vs. (+) by LSD test.

DISCUSSION

The DNA sequences of 12 CEA subfamilies were compared in order to identify regions with a low degree of homology among the different members. As the 3′-untranslated region showed the lowest degree of sequence homology compared with other members of the subfamily showing a 40% to 60% sequence identity,[19] primer sets in this study were selected including a 3′-untranslated region. Several cadherin-like molecules share commonly with their putative Ca^{2+}-binding motifs in the repeated ectodomains, but diverge strikingly in various regions, particularly in the cytoplasmic domains. For example, extracellular domains of the desmogleins are very similar to those of classical cadherins, but their cytoplasmic domains are quite different.[20] Therefore our study used primers amplifying exon 16 on a cytoplasmic domain including least homology with other cadherins. Nonspecific signal possibly caused by surrounding tumor tissues and nonspecific DNA syntheses might be reliably eliminated with overnight digestion in RNase-free DNase in our study.

Functional roles of CEA and E-cadherin in colorectal carcinomas have been investigated with regard to their biological behavior in several animal models.

A significant increase in liver metastasis was observed in the colorectal cancer cell lines transfected with full-length CEA cDNA after intrasplenic injection into nude mice.[21,22] MDA-231 breast cancer cell lines transfected with E-cadherin cDNA showed a dramatically impaired capacity to form osteolytic metastasies.[23] In the other study, E-cadherin–negative colon cancer cells transfected with E-cadherin exhibited a great increase in adhesion strength when the expressed protein was appropriately linked to the cytoskeleton.[24] The protein and mRNA coexpression of CEA and E-cadherin in our study may provide possible interaction of these molecules in the implantation and proliferation of tumor growth.

One investigation suggested that changes in the expression of a single cell adhesion molecule such as CEA can lead to alterations in the expression of unrelated cell adhesion molecules, thereby contributing to the general derangement of the adhesive interactions frequently observed in tumor cells.[25] Thus, in our study, CEA expression may have triggered E-cadherin expression with regard to CEA and E-cadherin coexpression. Another study demonstrated that Th2 type cytokines, that is, IL-4 and IL-14, inhibited colon cancer cell-cell adhesion by downregulation of both CEA and E-cadherin.[26] Similarly, the logarithmic concentration of serum E-cadherin was reported to correlate with CEA concentration in gastric cancer.[27] As coexpression of CEA and E-cadherin was identified from their mRNA levels, this phenomenon may also be determined from pretranscriptional or transcriptional levels. Both up- and downregulation can be assumed in their coexpression. In cases of upregulation of both molecules, the migratory property of CEA is suppressed by E-cadherin, whereas the adhesive property is exaggerated. Their downregulation seems to produce a loose intercellular adhesion among tumor cells prone to migrate. Coexpression may thereby provide either intercellular binding or loosening, resulting in either progression or metastasis in colorectal carcinoma.

The genomic organization of the CEA gene was shown to be stable in both liver metastasis and adjacent liver tissues in colorectal carcinoma.[28] However, the epigenetic changes including CEA promoter methylation were controversial in numerous studies.[28–30] Otherwise, the detection of a 5′ high-density CpG island in the E-cadherin gene suggests that transcriptional downregulation by DNA methylation might be an inactivating mechanism of E-cadherin expression.[31] Inconsistent expressions between protein and mRNA in our study might occur from posttranscriptional regulation. The lack of direct proportionality between mRNA and the protein expression of CEA[14] and E-cadherin[3] has been reported as posttranscriptional and post-translational control. In our study CEA protein and mRNA expression was also observed in adjacent hepatocytes. CEA gene expression was identified in adjacent hepatocytes as well as in the liver metastasis.[32] CEA is regionally distributed in normal liver tissue more than 3 cm apart from the metastatic liver tumor in colorectal carcinoma, possibly from CEA in circulating tumor cells, hepatocytes, and Kupffer cells.[21,33]

Colorectal carcinoma cell lines with E-cadherin mutation represented 70% of the lymph node metastases after tumor resection in mice.[34] In our study, lymph node metastasis seemed to occur in tumors with less E-cadherin mRNA expression, suggesting an inactivation of this important adhesion molecule from the transcriptional level. CEA expression was also downregulated in tumors with multiple lymph node metastases or lymphovascular invasion. These downregulations may enable loosening of intercellular binding in terms of adhesive properties of these molecules providing invasiveness of tumor cells. Greater CEA expression scores in the liver metastasis margin and elevated serum CEA level after the metastatic resection showed less or no disease-free survival, respectively, which was similar to findings in other studies.[11,35,36] Otherwise, E-cadherin might not always act as a tumor suppressor in advanced colorectal carcinoma because expression scores did not affect postoperative survival regardless of sites. Similarly, some investigators have reported elevated levels of soluble E-cadherin in gastric cancer patients with advanced-stage and metastatic disease.[27] Although the CEA expression score was not an independent prognostic factor in our study, it needs to be interpreted with caution considering the limited number of cases and interactive variables, that is, histologic differentiation and tumor progression. Although there have been some controversial correlations between CEA expression and histologic differentiation of colorectal carcinoma, CEA expression is more pronounced in well-differentiated than in poorly differentiated tumors.[3,37]

CONCLUSION

Coexpression of CEA and E-cadherin in primary tumors and liver metastasis was identified and was closely correlated with their mRNA levels. This possibly is a suggestion of their impact on the implantation and proliferation of tumor progression in primary colorectal carcinoma and liver metastasis.

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