Presidential Address

Who's Going to Do My Operation? Expectations for the Next Generation of Surgeons

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Now in my sixth decade of life, the odds that I will require another operation increase with each passing year. Surrounded by surgeons, patients, residents, students, and all the people who make a busy hospital run, I'm surprised that I have not more often considered the fact that it is quite likely that I, and in fact most of us in this room, will at some point or another have the opportunity to use our services. Faced with that very real possibility and given this opportunity to ponder about the future of our specialty, not just in preparation for this address but also on various national boards in recent years, I've given a great deal of thought to what I would hope to expect from those surgeons currently in practice and those in the future who I may need to ask for help. I'm trusting my next surgical experience will be some good long time from now, but you never know.

I've had the chance to engage the services of a surgeon on two occasions in my life. The first was as a 19-year-old college student with the classic lump in her breast found while bathing. Not alarmed, but concerned given a grandmother who no longer had breasts, I was referred to a surgeon in a small town in suburban Boston by the physician at the college health service. After sending me for a mammogram, presumably indicated in those days, he proceeded to advise me that removal was essential and that the operation would include a frozen section analysis of the tumor. I was admitted to a quaint little hospital in the same suburb one evening where I was thoroughly shaved and scrubbed and signed a consent for possible mastectomy. This was beginning to get creepy. The next morning I rolled into the freezing cold operating room, where under general anesthetic the surgeon kindly removed a small fibroadenoma and, except for a more uncomfortable recovery than I would have anticipated from a 2-cm incision, that was the end of the story. I have no idea of this surgeon's training, the scope of his practice, or even for that matter if he was in fact a board-certified physician of any type. Nonetheless, the experience on balance was, at least from my perspective successful, error free and now a remote memory.

Fig. 1. Barbara Lee Bass

However, by the time of my second surgery, another major procedure, the repair of an itsy bitsy umbilical hernia acquired during my first pregnancy with my son Wes, I was much better informed and approached the system quite differently. Now a surgical resident, with an "outy" instead of an "iny," I asked my most valued surgical professor, the man

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who actually taught me more about surgical decision making and judgment and compassionate surgical care than anyone else in my training, Dr. Paul Shorb, to perform my surgery. He kindly agreed and only chuckled about my malady once—that I'm aware of. He allowed my favorite buddy in residency who had been my right-hand junior resident for many years, Steve Teich, now a pediatric surgeon in Columbus, Ohio, to serve as his assistant. Steve saved my career as a surgeon by sending me home for a few hours one night when we were on call together. It was Wes' first birthday, the day I came closer to quitting than any other in my surgical career, and I needed to see that baby—and Steve figured that out. I presume Steve actually repaired my hernia with Dr. Shorb, although I never asked. I similarly assembled the remainder of my personal surgical dream-team. I hand-selected the anesthesiologist to provide my intravenous sedation, who I knew to be highly competent; selected my operating room nurses as those I knew to be competent and compassionate (and friends); and arranged to have the surgery done in an environment that I knew cared deeply about me personally. Unfortunately, I do not remember much about the case except that as I complained about the local injection in a somewhat groggy state, I recall Dr. Shorb saying to the anesthesiologist in a slightly frustrated tone, "I think you better put her to sleep." Again, all went well, and furthermore, I have had a durable repair.

But contrasting these two situations made it very clear to me the difference between an informed and an uninformed patient. While both procedures went well, by the time of my second, I was aware that our systems are in fact highly variable and often error—or at least bad luck—prone. As I've anticipated the future, not just for my own health care, but obviously for all of those people I love and by professional responsibility for all of our surgical patients, I think those premises that guided my choices in my second operation may be valuable to us as we consider what we really want not only for ourselves but also for our patients in the future. I think if we make these considerations personal, we may perhaps enlighten our perspective on what we as surgeons bring to the equation. These people—our patients—really trust us. So allow me to ponder my future experience as a surgical patient.

WHO WILL MY SURGEON BE?

Only 22 years ago, when I had my last surgery, I would have been relatively confident that my future surgeon would be the same gender and cultural origin as my first two—white men, likely born, raised, and educated in the United States. But, I'd have been wrong, for clearly, that demographic is changing. Women now comprise approximately 9% of the surgical workforce in the general surgical specialties and received 23% of new American Board of Surgery certificates last year; international graduates received 12%.[1]

However, those surgical disciplines with roots in general surgery training remain relatively unfavored career choices for our medical students. In contrast to a decade ago, when 92% of categorical general surgery residents were graduates of U.S. allopathic medical schools, in 2005 18% of categorical residents were graduates of international medical schools.[2] Although the number of women residents in our programs has increased approximately 2-fold in the last 20 years, they still comprise only 28% of the entering class of general surgery residents, despite being the majority gender in our medical school graduating classes. This imbalance in medical school gender distribution will progress based on enrollment figures in undergraduate colleges and universities, where women now are approaching 60% of the student body. Might we anticipate the demographics of the United Kingdom, admittedly faced with a different set of societal drivers for the phenomenon, where women currently make up between 70% of 80% of medical students? The impact of this gender shift will, no doubt, have a major effect on surgical training programs and on surgical practice—and we will need to adapt our profession, both in training and in practice—to incorporate this badly needed surgical workforce for the future.

Why do women not choose surgery at the same rate as their male student colleagues? So many reasons—different for each one. I will choose to dismiss the recent survey report suggesting a major deterrent is that they simply do not like us—our "surgical personality": but only dismiss this recognizing that what we perceive to be "personality" may in fact reflect a culture that in some fashion remains unattractive to many women.[3] However, based on the innumerable discussions I have with students every year, I propose that these women students more commonly have tremendous difficulty simply foreseeing how they will mesh their anticipated personal lives, rich with longstanding gender roles that come with real joys and rewards, with a surgical career that comes with a different set of joys and rewards. They want both. It is simply true that we women do bear children and that in most families women typically nurse the sick, care for the elderly, and generally continue to provide the major contribution to home and household functions. These things are fun, important, satisfying, and consuming. These roles may

be changing in some families and respects, but this perceived impossible balancing act I think remains our most powerful deterrent to women who might otherwise relish a surgical career.

Life balance is one factor; a lack of successful women surgeon role models is another. Thankfully, more young women are joining our academic faculties, where they are visible and positive role models and mentors to our medical students. The positive effect of a critical mass of women surgical faculty on women medical students choosing to enter surgical training has been amply demonstrated.[4] Women surgical faculty, however, remain clustered at the junior academic ranks with only 20% achieving that critical step of promotion to associate professor, a distribution that has not changed in 20 years.[5] This is more than a pipeline issue. Many of our medical schools still do not have a single woman full professor of surgery or women in positions of leadership in their departments. Furthermore, women faculty who choose to leave academics, given their relatively small number, are more visible in their departures. And, regrettably, so often, this decision to leave academics is driven by the dawning realization in often brilliant young women that there are only so many hours in the day to practice surgery, punch scholarship buttons, teach, nurture a life, and maintain a household. Coupled to the reality that women surgeons do not as readily develop a sense of belonging in our profession, it begins to seem not worth the trouble.[6] These women fortunately rarely leave surgery altogether; rather they decide that academic surgery is the wrong path. Unfortunately, our students see only the loss of a role model.

While these young women faculty are highly admired by their senior surgical colleagues: " I don't know how she does it all"—while meant to be a compliment may at times be heard as a reminder to those young women faculty on what a thin sheet of ice they skate. We need to develop better networks for this cohort, better resources to reassure them that there are different phases in their lives for different priorities and that while their pathway may seem different and hard, it is actually appropriate and may ultimately lead to a happy and successful career and life.

The U.S. surgical training programs have long been highly sought by international graduates from around the world. To achieve success in our system requires incredible courage, resilience, and intellect and often personal sacrifice of family and home. But, for so many it is worth the remarkably tough journey associated with successful access to out surgical profession. As interest in surgery by our own allopathic medical students remains low for both men and women, we find that access to American training is increasingly available to the best and the brightest of the international medical school graduates. We now collectively glean through many thousands of applicants per year, selecting the top 250 to 300 to enter our training programs.[2] Once facing an uphill battle to successfully complete training and then to achieve success on the American Board of Surgery examinations, international graduates now statistically perform equally or better than graduates of surgical training programs who attended U.S. medical schools. We now have an increasing cohort of international graduates who bring the intellectual rigor and work ethic required to achieve success. Yet, once here they find themselves caring for patients in communities and cultures often very different from those in which they were raised. They must acquire a new language rife with indecipherable medical acronyms. They must learn to understand the values of the patients who they care for in this country, to embrace the values of our profession, and to become effective communicators—difficult challenges even for our own students. Nonetheless, it is our job as teachers and colleagues to ensure that we incorporate these surgeons into our communities and profession in a most complete fashion.

One area of diversity that remains woefully lacking, however, is that of racial diversity. In our country with 13% African Americans, only 3% of our surgical workforce is black. Hispanic enrollment in medical schools is similarly widely disparate from population demographics. Why? The pipeline to medical school remains a low-flow conduit for many young African American and Hispanic students starting long before the undergraduate education level. While we have had and continue to have brilliant surgeons to serve as role models for young African American surgeons—Lasalle Lefall, Claude Organ, Haile Debas, L. D. Britt, Steve Stain, Henri Ford, and many others—they remain rare stars in the galaxy of surgery. African American and Hispanic surgical faculty are spread thinly through most academic departments where our students might find them. We must continue to work to reverse this imbalance. We need to start early—really early, when these children are still in awe of the future—and we can instill dreams by inviting them to visit us on an ongoing basis, show them who we are and what we do and that they can do this, too—that we need them. We must work on this for our patients, for they will benefit by having a surgical workforce that reflects their own composition—racially and culturally. It will also enrich us as a profession to know that all members of our society can participate in this rare opportunity we have.

So while I was once certain my surgeon would be a white man—no more. My surgeon might be a man or woman, a U.S. or international graduate; and if we manage to address social inequities, I may even have a good chance at having a surgeon of some other racial background perform my surgery.

HOW WILL I CHOOSE MY SURGEON?

Many stakeholders these days are vying to be part of the quality oversight system for health care. Payors, government regulatory bodies, purchasers of health care, patient care advocacy groups, and, yes, thankfully at last some might suggest, our own surgical profession is beginning the difficult task of defining metrics for quality in surgery. As principles articulated in the Institute of Medicine study "Crossing the Quality Chasm" stated, we all recognize that *the right operation at the right time with an effective result delivered in a patient-focused environment with compassion and without error is the goal we seek.*[7] However, our ability to assess the real quality of the care we provide is hampered on many fronts—not the least of which is the expense and rigor required to collect the data that will inform us of the processes of care we use and the outcomes we achieve. No single quality measurement system will serve all purposes given this vast surgical enterprise that we have created.

But, many are offering surrogate—often expedient surrogates for quality—volume of procedures, the number of lymph nodes in a surgical specimen, length of stay. In marked contrast, however, there are several programs developed by surgeons with the intent of examining the quality of the care we provide that are now on the cusp of real-time application on a broad and meaningful level. The National Surgical Quality Improvement Program, now in its second year of operation by the American College of Surgeons, is enrolling hospitals at an increasingly rapid rate. The program, developed over 20 years as a labor of love in the VA health care system by the founding investigators, Shukri Khury, M.D., William Henderson, Ph.D., and Jennifer Daly, M.D., was at its start a research study—a VA cooperative study.[8] It has now matured into the only prospectively accrued, risk-adjusted surgical outcomes program in the disciplines of general and vascular surgery in the world. Is it labor intense? Yes. Is it costly? Perhaps, in the short term, although there is nothing more cost-effective than developing systems that can prevent surgical complications, not to mention the rewards to our patients.

I lived through the birthing of the NSQIP in the VA health care system and I like to think that I have contributed in some measure to the process. It was a fantastic experience—emotional, embattled, principled, and political. But in the end, it worked, and it has contributed substantially to improved surgical care in the VA system. While currently just a rudimentary program tracking morbidity and mortality, it offers an exceptional platform to develop more specific and meaningful metrics. I applaud Drs. Khury, Daly, and Henderson for taking this mission on for us. Further, I applaud the American College of Surgeons with the leadership of Drs. Scott Jones, Skip Campbell, Tom Russell, and Shukri Khury as they continue to leas the development of this program to a more meaningful and expansive level throughout our health care systems.

It is now time for surgeons with expertise in specific areas to define more meaningful and actionable outcomes. This is a great opportunity for the SSAT to contribute to the development of disease-specific metrics. During this meeting I will appoint the first SSAT working committee to work in partnership with the ACS NSQIP to start the process of defining specific metrics of quality in gastrointestinal, hepatobiliary, and pancreatic surgical procedures. I will seek the participation of our sister societies, AHPBA, SAGES, and ASCRS, to start the construction of gastrointestinal surgical outcome metrics within the framework of the ACS NSQIP. We have important work to do.

The NSQIP is but one tool we as a profession can use in quality measurement. As a profession, we need to train not only clinical surgeons but surgeons who can contribute to the science of surgical outcome measurement and improvement. This will require real investment in scholarship and education by a special group of surgeons with passion for this work. We must train surgeons capable of partnering with nonsurgeon scholars in the field to truly advance this science. Again, more work for us to do.

As I have become a truly informed patient, I recognize that my surgeon's performance is integrally linked to the quality of the system he works in. Efficient, integrated systems of care linked to a technically accomplished and knowledgeable surgeon is required for truly excellent care. Optimizing treatment for patients with conditions as diverse as inflammatory bowel disease, gastrointestinal malignancy, and hepatic disorders we know well requires a collaborative approach involving surgeons, radiologists, gastroenterologists, pathologists, and oncologists. Each contributes to the management and care of these patients with a unique, but ultimately unified, perspective.

No patient typifies this more clearly than a patient with rectal cancer. Steps in this patient's care include staging with transrectal ultrasound, colonoscopic evaluation of the colon, neoadjuvant chemoradiation therapy, molecular pathologic diagnosis to determine optimal chemotherapeutic regimens, surgical resection guided by appropriate imaging, staging, and patient comorbidities, and possibly genetic testing to assess family members at risk. A surgeon determines if transanal excision, transanal microscopic endoscopic mucosal resection, low anterior resection, or abdominoperineal resection is the optimal procedure but in the setting of collaborative management with multiple adjunctive therapies. Things clearly are not as simple as they used to be.

One has to consider that as our disciplines become more merged and treatment modalities blend, we will see greater cross-training between our current silo-like disciplines. As technologies advance, the line between interventionalists of each variety, be it radiology, gastroenterology, or surgery, will further blur. Natural orifice surgery is surely just off the horizon. One can only hope that we will have the vision to modify our training programs to offer optimal cross-fertilization among our disciplines to optimally train the gastrointestinal physicians of the future.

WHERE WILL MY SURGERY BE PERFORMED?

Depending on how complex my surgical problem may be, I anticipate my care will be provided at only certain high-volume medical centers. Practice at the process of integrated practice really does make perfect for these more challenging clinical conditions. Furthermore, the costly resources required to deliver the most sophisticated care, be it imaging, surgical technologies, access to novel therapeutics, or simply the gathering of the required professional expertise, justifies regionalization of care. I would be more than pleased to travel to a different site to have access to the sophisticated and expensive care I need to treat my complex disorder. With knowledge or valid quality metrics, our patients will be informed regarding this distinction as well. While this may be personally less convenient for me and my family, this is a small inconvenience to achieve enhanced quality. Mortality is clearly a bad outcome, but we now know that complications, while not deadly in the short term, translate into shortened life even from what would appear to be modest complications.[9]

While we focus now on relatively rudimentary measures of quality, such as complication rates and mortality, by the time I have my surgery we will have programmatic assessments of quality for integrated patient care systems. Programs will not only be required to have structural components essential for delivering comprehensive care but will also have transparent metrics for the outcomes achieved by the health care delivered by these groups. The processes of care will be carefully tracked and optimized based on evidence-based therapeutics. These teams will be better poised to incorporate new evidence-based therapies, in contrast to out current state when a decade may pass before new proved therapeutics are incorporated into practice. Many of these teams will be positioned in academic medical centers to test and introduce new therapies with programs in translational and clinical research. These centers will be the drivers of development of new therapeutics and will enhance our speed of discovery and implementation. Personally, if I have something wrong with me, that is where you'll find me for my surgery.

WHAT TYPE OF SURGERY WILL I HAVE?

No doubt the operating room of 2026 and the procedures performed therein will bear little resemblance to our current suites. Driven by advances in technology, our currently rudimentary minimally invasive surgical tools will develop greater flexibility, nimbleness, and potential for remote manipulation with guidance by sophisticated imaging. While our fingers may still enjoy the occasional sensual warm softness associated with palpating the liver or pancreas, of carefully examining the loops of the small bowel, or of palpating the colon, these tactile moments are likely ours for only a few more years. Our manipulations will be increasingly through more and more remote access sites with increasingly sophisticated miniaturized devices. I do believe this is a good thing for our patients; they will suffer less and likely recover more quickly from our efforts to heal them. As long as our technology can offer equivalent and ever-improving modalities to let us achieve our technical goals, the more we should embrace and prepare for this future.

It is true, however, that I think we lose something when we lose this tactile contact. There is truly something remarkable about placing one's hands in another person's abdomen to cure them of great misery. What a rare opportunity and what a rare sense of trust you share with that patient. To me it is quite a personal event and a reminder of the warmth of human life. While I know it is a bit crazed, I worry that as we detach from that human touch, with our instruments at arm's length, with only our cold tools penetrating into that warm spot, that we are in

a way disconnecting from our patients in other ways, or at least at risk of doing so. I do not, of course, advocate preserving old surgical ways to maintain a sense of personal connection; I just view this as another challenge to our profession to ensure that we recognize that technology has the potential to separate us from our patients just as it paradoxically has offered us new tools to improve the procedures we have to offer them. When push comes to shove, I will want my surgery done in the safest, most technologically advanced environment with the finest surgical tools with the expectation that my surgeon will be as passionate about the care he will provide to me regardless of whether his hands are in my belly or sitting on a console across the room or some more distant place. Technology is a wonderful thing, but as healers we must ensure that we preserve humanism in our relationships with our patients when all other factors tend to diminish the opportunity to express that value.

Odds are my surgery will, in fact, be quite different from those procedures performed today. Should I have established disease, a tumor, or an inflammatory process, odds are that my primary surgical procedure will be one of ablation rather than resection. Molecular fingerprinting will allow selection of the appropriate therapy, be it an adjuvant medical therapy, a primary surgical option, or a mixture of these and many other therapeutic modalities. In fact, resective procedures may well become the domain of prophylactic procedures. Molecular predictors for malignancy, be I familial gastric cancer, pre malignant conditions of the colon, or high-risk cancer syndromes, will allow us to identify those patients where the field defect is sufficiently great to warrant prophylactic removal of the end organ. In these procedures, preventive rather than resective operations, the expectations for my surgeons will be even the higher. Death or major complications in preventive surgery are clearly not acceptable. It is interesting and true that surgical stakes go up as disease expression goes down. When disease expression is simply a genetic marker predictive of phenotype in some years to come, the stakes are exceptionally high—for patients and surgeons.

HOW SKILLED WILL MY SURGEON BE?

Perhaps the most notable feature of surgery in the last decade is the remarkable pace of change. Surely one of the most transformative events in the discipline of surgery has been the introduction of minimally invasive approaches. From endocrine disorders to breast cancer to chest and abdominal procedures, we have learned that minimal access procedures can offer the same diagnostic and therapeutic benefit as their more extensive and invasive procedural counterparts. But how do you maintain a surgical workforce at a high level of technical expertise when the technical landscape changes so quickly? How are we to ensure that a surgeon stays if not at the top of his game, then at least well enough prepared to deliver current and timely surgical management to his patients?

While our system of primary surgical training certainly can use some fine tuning in regard to more appropriately focusing training to one's eventual practice, our current model using general surgical training as a platform for advanced surgical training currently delivers to us a very well trained surgical workforce. However, while surgical judgment may mature with years of experience, and technical expertise may similarly improve with practice, there are substantial data that this peak in performance may well fall relatively early in one's professional career, perhaps as early as 10 years into what is often a 30-or-more-year career.[10] Unlike our medical colleagues, surgeons are required not only to incorporate new paradigms of patient management but additionally to maintain technical expertise in rapidly changing technologies.

So how are we as a profession going to make good on our commitment to society that we have a technically up-to-date surgical workforce? Interestingly, most of our patients assume that we surgeons are tested on a regular basis, that we practice on simulators, and that we rehearse and are observed by other surgeons in our surgical practices, just like pilots who execute similarly potentially harmful but mandatory service for the public good. We, of course, do none of these things. Fortunately, by and large, our surgeons are in fact well trained in their craft and do offer to out patients valuable capable care. Nonetheless, there is room for improvement.

Re-tooling in technical procedures is a particularly great challenge, however. The drivers for premature incorporation of new procedures into one's surgical repertoire are many. Our patients demand the latest technologies, understandably wishing to have less invasive options to presumably achieve the same end. We are also pushed by our partners in industry who wish to introduce new products that on occasion are truly revolutionary in their capability but at other times, represent modest changes that nonetheless may be sufficiently different in operation to require readjustment of established routines—an opportunity for error. We have all experienced learning curves in our surgical practices—and it is not uncommonly our patients who unknowingly contribute to our acquisition of skill mastery.

Here again is another opportunity for us as a profession. It is our responsibility, as a profession, to ensure that our workforce is appropriately trained throughout their careers. This is not the responsibility of industry, the government, or payors—it is ours. But how to achieve this? The Committee on Evolving Surgical Technologies and Education (CESTE) of the American College of Surgeons has recently defined the components of acquiring a new surgical procedure into one's repertoire. This five-step process moves from understanding the rationale and indications for a procedure, to acquisition of a new skill in a simulated environment, to demonstration of the skill in a patient in a proctored environment, to mastery with successful outcome assessment in practice. We have rare opportunities to achieve this cycle outside of residency training in our country these days. The weekend course, see one, do one, obviously does not meet these criteria. This quandary is creating havoc for credentialing committees around the country who are struggling to define criteria for surgeons to incorporate new procedures into practice.

The new American College of Surgeons Surgical Education and Training Center programs is an excellent first step. In this program, a network of sophisticated educational centers will be developed around the country. The educational content will be deep and technical skills will be taught and tested in safe learning environments. A great contribution, but as a profession we can take this process a step further. I propose that we advocate that all surgeons enter a cycle of education and training with periodic retraining sabbaticals over the course of a surgeon's career. The pace of change is sufficient that a surgeon could benefit from an intense re-training/re-tooling and assessment sabbatical each 7 years. We need to develop educational structures and faculty capable of delivering these intense 2-week cognitive and skills-focused programs for surgeons in practice. I contend we would substantially augment the quality and preparedness of our surgical workforce if we did so. Surgeons would have the opportunity to acquire new skills relevant to their current practice and become familiar with the latest technologies. These sessions would allow surgeons to establish links to surgeons at the re-training sites for later consultation. Once back home, real-time transmission of surgical procedures to the remote proctor could allow interactive consultation for the operative surgeon. Not only is surgical technology allowing this intervention, but of course our informatic connectivity makes this a real, not virtual, reality.

The centers will also be sites for surgeons to demonstrate technical competence by a means not previously available to us. The goal of such appraisal is not necessarily to find that incompetent surgeon, although perhaps that may occasionally be the end result. The real goal is to provide feedback and appraisal to surgeons in practice for the procedures they actually do to allow themselves to benchmark their own performance and to identify areas where they might perform better.

Opportunities for acquiring new knowledge and skill during practice are changing. For decades, surgeons have been buoyed by the collective wisdom of their partners to mutually advance the care they provide to their patients. The experience of senior surgeons was invaluable to the continued maturation of junior surgeons. Now, those relationships have in many respects been reversed. Senior surgeons look to their junior colleagues to bring advances in technology, in multidisciplinary management, in image-guided and minimally invasive procedures. But again, further changes in the surgical professional landscape confound these processes. First, surgeons are dispersed to many different practice sites. Partners may operate one with another only on rare occasions, given pressures for productivity and lack of compensation for first assistants. The repertoires of senior and junior surgeons these days are often so disparate that re-tooling of the senior partner seems to be a daunting, if not low return, investment of the young surgeon's time. Hence, the senior surgeon persists with familiar approaches, forgoing the opportunity for novel, more contemporary patient management strategies. Additionally, more and more surgeons find themselves employed in large health care organizations and subject to relocation. Longstanding surgical relationships and the counsel and support that come with these relationships are lost. The pace of change, the complexity of care, the explosion of science simply make these more comfortable and traditional means of updating in surgery insufficient options to maintain competence.

This is a chance for us as a profession to take the lead. We should not resist, but rather demand, re-training and re-tooling. We should insist on the opportunity to demonstrate our skills in surgical environments or on simulators. We should look for ways to codify the commitment of our surgeons to their own ongoing education to enhance the care they provide to their patients. For some, this will be viewed as a requirement, perhaps onerous; for others, it will be a welcome opportunity. I would also propose that the financial burden of these surgical sabbaticals be subsidized by our payors and call on them to support the infrastructure facilities for this process. This would seem to be a valuable investment to maintain a high-quality surgical workforce.

SO, WHAT DO I EXPECT FROM MY SURGEON?

I expect my surgeon will have a good chance of sharing my chromosome complement. She will have specific expertise in my surgical disorder and will be an integral member, and ideally a leader, of the multidisciplinary treatment team that takes care of me. I expect to find her at an academic medical center, for her to know far more about the scientific basis of my disorder than I do about any disorder that I currently treat. The therapies she offers will be based on my own genotype as well as the aberrant molecular features of my disease. I expect my operation will be intricately planned in advance with sophisticated imagery to precisely identify the site of my problem and the optimal surgical approach. If this is a complex procedure, it may well be rehearsed in advance and that my surgeon will have demonstrated competence in the procedure. I anticipate she will perform my procedure with tools that pierce, penetrate, and carefully dissect and repair with relatively little tissue injury and minimal tactile contact. I'll miss that part.

Moreover, I hope that she will place my safety and comfort and well-being as her highest priority and that she will be happy in her work. I'd like to think that being an informed or uninformed patient will not have to matter, that our profession will have built into our system the guarantees that my surgeon will be knowledgeable, and skillful, compassionate, and honest—whether or not I know anything at all about my disease or surgery.

And last, I trust that she will provide to me the right operation, at the right time, in the best possible environment and that she will consider this to be a rare and wonderful opportunity to help me.

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1423 Pancreaticoduodenectomies for Pancreatic Cancer: A Single-Institution Experience

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Pancreaticoduodenectomy (PD) with the possible addition of neoadjuvant or adjuvant therapy is the standard of care in the United States for adenocarcinoma originating in the pancreatic head, neck, and uncinate process. We reviewed 1423 patients who underwent a PD for a malignancy originating in the pancreas at our institution between 1970 and 2006. We examined 1175 PDs for ductal adenocarcinomas in greater detail. Eighteen different histological types of pancreatic cancer were identified; the most common diagnoses included ductal adenocarcinoma, neuroendocrine carcinoma, and IPMN with invasive cancer. Patients with ductal adenocarcinoma were analyzed in detail. The median age was 66 years, with patients in the present decade significantly older (68 years), on average, than patients in the three prior decades (e.g., 60 years in 1970, $P = 0.02$). The median tumor diameter was 3 cm; 42% of the resections had positive margins and 78% had positive lymph nodes. The perioperative morbidity was 38%. The median postoperative stay declined over time, from 16 days in the 1980s to 8 days in the 2000s ($P < 0.001$). The perioperative mortality declined from 30% in the 1970s to 1% in the 2000s ($P < 0.001$). The median survival for all patients with ductal adenocarcinoma was 18 months (1-year survival = 65 %, 2-year survival = 37%, 5-year survival = 18%). In a Cox proportional hazards model, pathological factors having a significant impact on survival included tumor diameter, resection margin status, lymph node status, and histologic grade. This is the largest single-institution experience with PD for pancreatic cancer. Patients who have cancers with favorable pathological features have a statistically significant improved long-term survival. (J GASTROINTEST SURG 2006;10:1199–1211) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Pancreaticoduodenectomy, pancreatic cancer, ductal adenocarcinoma, cancer, Whipple

Pancreatic cancer is the tenth most common cancer in the United States and the fourth most common cause of cancer-related death, trailing cancers of the lung, colon, and breast[1]. Pancreatic cancer affects 11 per 100,000 people, or roughly 33,000 individuals annually in the United States. The accumulated lifetime risk of developing pancreatic cancer for individuals born today is estimated at 1.3[2] Men and woman have roughly equivalent risk[1].

Although there have been great advances in the surgical management of pancreatic cancer and in the understanding of the genetic and molecular events that underlie pancreatic carcinogenesis, the 5-year survival for all patients with the disease is

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only 5%[1] The incidence and the death rate of pancreatic cancer are virtually identical, suggesting that the cure rate is exceedingly low.

Patients with localized pancreatic cancer amenable to surgical resection stand the best chance at long-term survival. According to a recently published population-based study of 10,612 individuals from California, patients who underwent resection for cancer in the head of the pancreas between 1994 and 2000 had a median survival of 13 months, compared to just 4 months for patients who did not undergo resection[3]. In the same study, 22% of all patients with cancer in the head of the pancreas underwent a resection.

Pancreaticoduodenectomy (PD) is considered one of the most complex operations of the alimentary tract and the primary treatment for patients with resectable, right-sided pancreatic cancers. When Dr. Allen O. Whipple described his first three cases of PD in 1935, he wrote that the operation was considered "prohibitive in the minds of even the ablest surgeons."[4] However, through dedicated attention to surgical technique and anatomy, improvements in critical care management and nutritional support, advances in interventional radiology and endoscopic services, and increased experience of surgeons at high-volume centers, PD can now be performed safely at hospitals having a particular interest in the surgical management of pancreatic cancer.

Studies from the 1980s demonstrated decreased morbidity and mortality rates at our institution as surgeon volume increased. Improved outcomes were directly related to decreased operative blood loss and shorter operative times.[5,6] A later study from our institution observed that hospitals in Maryland that performed fewer than five pancreatic resections per year had a 19-fold risk increase for perioperative death compared to hospitals that performed more than 20 resections per year; hospitals that performed between 5 and 19 resections had an 8-fold risk increase[7]. A Pancreatic Cancer Web site (http://pathology.jhu.edu/pancreas/) was established at our institution in 1995 to help disseminate information to the public.[8] In 1995, our institution reported its experience with 201 PDs for pancreatic cancer.[9] In 2000, 616 resected pancreatic cancers were described, which included 564 PDs and 52 distal pancreatectomies.[10] The present study is an update of the Johns Hopkins experience with PD for pancreatic cancer. To our knowledge, this is the largest single-institution series on this subject to date.

MATERIALS AND METHODS

The analysis was based on data from the IRB-approved Johns Hopkins PD database and from electronic patient records. Data were reviewed for all 2943 patients who underwent PD (partial or total PD) between April 1970 and March 2006 at the Johns Hopkins Hospital. A total of 1423 patients who underwent a PD for a malignancy originating in the pancreas (herein referred to as a pancreatic cancer) were identified. The distribution of the different histological types of pancreatic cancers, including the rare variants of pancreatic adenocarcinoma, were determined according to the recommended nomenclature of pancreatic neoplasia described by Hruban et al.[11] The classification of specimens was based on the dominant histological pattern described in the original pathology reports. Those patients with tubuloglandular ductal adenocarcinoma, the most common histological type of pancreatic cancer, were analyzed in greater detail.

The parameters that were evaluated included past medical history, preoperative symptoms, preoperative procedures, intraoperative data (including estimated blood loss, transfusion requirements, and operative time), pathological data, surgical complications, perioperative mortality (30 day or in-hospital mortality), and long-term survival. Specific definitions for complications such as delayed gastric emptying and pancreatic fistula have been described elsewhere.[12–15]

There were 32 surgeons over the 36-year period who performed a PD for pancreatic cancer. Three surgeons performed 80% of the resections (J.L.C., K.D.L., C.J.Y.) and 11 surgeons performed 93% of the operations. Most of the patients underwent a partial pancreatectomy with pylorus preservation, as previously described.[15] A distal gastrectomy was performed when a pylorus-preserving procedure would have compromised the margin status of the specimen, when the duodenum was considered ischemic, or, in a minority of cases, as part of a clinical trial comparing stardard to radical PD.[16] Routine vagotomy, tube gastrostomy, and feeding jejunostomy were not performed. In general, prophylactic octreotide was not administered. Intraoperatively placed drains near the pancreatic and biliary anastomoses were left in place for at least 4 postoperative days. Five percent of the PDs for ductal adenocarcinoma of the pancreas were performed on patients who had undergone a prior laparotomy but were considered to have an unresectable cancer. Four percent of the patients with ductal adenocarcinoma received pre-resection neoadjuvant therapy.

Comparison of continuous variables was performed using the Mann-Whitney rank sum test, and comparison of categorical variables was performed using the χ^2 test or logistic regression.

Long-term survival data were computed using the Kaplan-Meier method, and multivariate survival analysis was performed by Cox proportional hazard regression. Results are reported as median values, unless indicated otherwise. Statistical significance was accepted for $P < 0.05$. Data analyses were performed using Intercooled Stata Version 8.0 (Chicago, IL).

RESULTS

Series Overview

There were 1423 pancreatic cancers resected by PD over the past 35 years, which comprised 48% of all PDs (1423 of 2943) and 65% of all PDs performed for malignant disease (1423 of 2194) during the same time period. The annual distribution of PDs for pancreatic cancer appears in Figure 1. In the recent years, approximately 120 pancreatic cancers were resected annually. There were 446 patients who resided in the state of Maryland (38.8%) in this series and 830 patients (72.2%) who came from either Maryland or one of its four border (West Virginia, Virginia, Pennsylvania, and Delaware). Twenty-two (2%) patients came from outside of the United States. The geographic distribution of PDs for pancreatic cancer, according to state or country of residence, is provided in Table 1.

Table 1. Distribution of PDs for pancreatic cancer by state/country

State/country*	n (% total)	State/country*	n (% total)
MD	446 (38.8)	NM	5 (0.4)
PA	197 (17.2)	KY	4 (0.4)
VA	122 (10.6)	OK	4 (0.4)
NJ	54 (4.7)	AZ	3 (0.3)
FL	40 (3.5)	Argentina	3 (0.3)
DE	38 (3.3)	Bermuda	3 (0.3)
NY	38 (3.3)	Canada	2 (0.2)
WV	27 (2.4)	Greece	2 (0.2)
NC	13 (1.1)	MA	2 (0.2)
DC	12 (1.0)	MO	2 (0.2)
MI	12 (1.0)	MS	2 (0.2)
CA	11 (1.0)	NE	2 (0.2)
GA	10 (0.9)	Phillipines	2 (0.2)
OH	10 (0.9)	PR	2 (0.2)
TN	10 (0.9)	Turkey	2 (0.2)
CO	8 (0.7)	AK	1 (0.1)
CT	8 (0.7)	AR	1 (0.1)
IL	8 (0.7)	Chile	1 (0.1)
SC	8 (0.7)	Guatamala	1 (0.1)
TX	8 (0.7)	IA	1 (0.1)
AL	5 (0.4)	IN	1 (0.1)
LA	5 (0.4)	Israel	1 (0.1)

*United States of America states/possessions are indicated by their USPS abbreviations.

The distribution of malignant pathologies originating in the pancreas, including rare variants of invasive ductal adenocarcinoma, is provided in Table 2. Eight-three percent of the resected pancreatic cancers (1175 of 1423) were of the typical tubuloglandular ductal adenocarcinoma variety. Seven percent were neuorendocrine carcinomas (n = 98), 6% were IPMNs with invasive cancer (n = 90), and 4% were other malignant pathologies (n = 60). Since the various types of pancreatic cancer differ from each other in biology, genetics, and clinical behavior, the remainder of this study (unless otherwise indicated) will focus on the most common type of pancreatic cancer—the classic from of ductal adenocarcinoma.

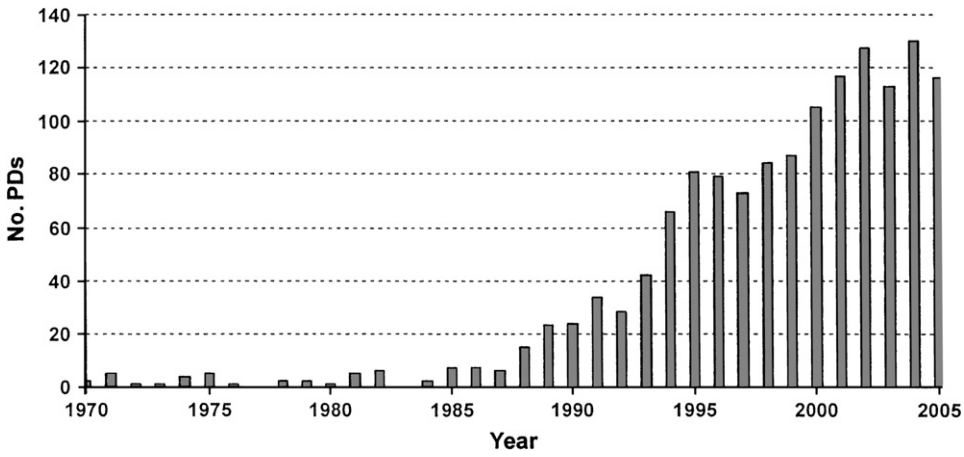

Fig. 1. The annual distribution of PDs performed for pancreatic cancer at Johns Hopkins between 1970 and 2005.

Table 2. Pathologic diagnoses: PD for pancreatic cancer

Pancreatic cancer	n (%)
Ductal adenocarcinoma	1175 (83%)
Neuroendocrine carcinoma	98 (7%)
IPMN with invasive cancer	90 (6%)
Adenosquamous carcinoma	15 (1%)
Cystadenocarcinoma	11 (0.8%)
Acinar cell carcinoma	7 (0.5%)
Clear cell carcinoma	5 (0.4%)
Undifferentiated carcinoma with anaplastic features	4 (0.3%)
Signet ring cell carcinoma	3 (0.2%)
Undifferentiated carcinoma with osteoclast-like giant cells	3 (0.2%)
Mixed ductal-endocrine carcinoma	3 (0.2%)
Small cell carcinoma	2 (0.1%)
Undifferentiated carcinoma with sarcomatoid features	2 (0.1%)
Small round cell tumor	1 (0.1%)
Giant cell carcinoma	1 (0.1%)
Extragastrointestinal stromal tumor	1 (0.1%)
Pancreatoblastoma	1 (0.1%)
Angiosarcoma	1 (0.1%)

Preoperative Data

Preoperative data on 1175 patients undergoing PD for a ductal adenocarcinoma in the head of the pancreas are presented in Table 3. The median age was 66 years (range, 32–92 years). Twenty-two patients were under 40 years (2% of patients with ductal adenocarcinoma) and 107 patients were over 80 years (9% of all patients with ductal adenocarcinoma). Two patients who were 90 years or older underwent a PD for ductal adenocarcinoma in the head of the pancreas in the present decade. Patients who underwent PD for ductal adenocarcinoma were significantly younger in previous decades compared to patients in the present decade. Slightly more than half of the patients were male and 88% were White.

The most common medical comorbidites included hypertension (40%), tobacco use (24%), diabetes (24%), and coronary artery disease (21%). Jaundice was the most common preoperative sign or reported symptm (75%). Other commonly observed signs or reported symptoms included weight loss (51%), abdominal pain (39%), nausea or vomiting (13%), and pruritis (11%). Eighty-eight percent of the patients underwent an invasive diagnostic or therapeutic procedure prior to undergoing a PD. Thirty-two percent of the patients had a preoperative biopsy, 77% had an ERCP, 38% had a percutaneous cholangiogram with biliary drain placement, and 17% of the patients had an endoscopic ultrasound. Seventy-six percent of patients had either an endostent or a percutaneous transhepatic biliary drain placed for biliary decompression.

The median laboratory value for 475 patients with a documented CA 19-9 levels was 139 U/mL (range 0.9–14,000 U/mL; normal 0–36 U/mL). Of note, 21% of the patients with a documented preoperative CA 19-9 level had a test result in the normal range in the setting of subsequently proven ductal adenocarcinoma of the pancreas.

Table 3. Preoperative data for patients with ductal adenocarcinoma (n = 1175)

Patient demographics	
Age (yr), median (range)	66 (32–92)
1970s	60 (37–73)*
1980s	61 (33–76)*
1990s	66 (34–89)*
2000s	68 (32–92)
Gender, male	628 (54)
Race, caucasian	1030 (88)
Past medical history	
Hypertension	430 (40)
History of tobacco use	252 (24)
Diabetes mellitus	260 (24)
Coronary artery disease	154 (21)
History of alcohol abuse	127 (12)
Myocardial infarction	70 (7)
Peripheral vascular disease	60 (6)
COPD	57 (5)
Peptic ulcer disease	36 (3)
Acute pancreatitits	36 (3)
Chronic pancreatitits	29 (3)
Inflammatory bowel disease	5 (0.5)
Pancreatic pseudocyst	3 (0.3)
Preoperative signs or reported symptoms	
Jaundice	800 (75)
Weight loss	545 (51)
Weight (lb), median (range)	15 (1–100)
Abdominal pain	408 (39)
Nausea or vomiting	133 (13)
Pruritis	115 (11)
Fevers	27 (3)
Gastrointestinal bleeding	11 (1)
Invasive preoperative procedures	
Any procedure	961 (88)
Biopsy	305 (32)
ERCP	601 (77)
Endostent	468 (45)
PTC/PBD	390 (38)
EUS	82 (17)

Values in the table are n (%), unless otherwise specified.
*$P < 0.5$ compared to the present decade.

Intraoperative Data

Intraoperative data are provided in Table 4. Seventy-one percent of the PDs were pylorus sparing, while the remainder included a distal gastrectomy. The larage majority of the PDs were partial pancreatectomies, while 7% were total pancreatectomies. Four percent of the operations included a resection of a portion of a major visceral vessel (e.g., the SMV, portal vein, or hepatic artery). The median blood loss was 800 mL (range 150–15,000 mL), the median number of intraoperative transfused units of packed red blood cells was 0 (range 0–27 units), and the median operatie time was 380 minutes or 6.3 hours (range 200–790 minutes).

The median tumor diameter was 3 cm (range 0.1–15.5 cm). Forty-two percent of the resections had a positive margin and 78% had positive lymph nodes. Most of the ductal adenocarcinomas were stage II (90%) according to the staging classification outlined in the *AJCC Cancer Staging Manual* sixth Edition.[17] There were three patients in the series of 1175 patients who underwent PD for ductal adenocarcinoma of the pancreas with distant disease at the time of surgery: two patients with solitary liver metastases and one patient with a skin lesion at the exit site of a percutaneous biliary drain. In each of the three cases, the metastasis was resected at the time of the PD. All three patients died of their disease less than 1 year after surgery. Just over half of the ductal adenocarcinomas were moderately differentiated (56%), with the remainder having mostly poor differentiation (40%). Small blood vessel invasion was observed in 53% of the ductal adenocarcinomas and perineural invasion in 91%.

Table 4. Intraoperative data for patients with ductal adenocarcinoma (n = 1175)

Technical factors	
Pyloric preserving PD (versus distal gastrectomy)	834 (71)
Total pancreatectomy (versus partial pancreatectomy)	79 (7)
Resection of major visceral vessel	47 (4)
Other intraoperative factors	
Estimated blood loss (ml), median (range)	800 (150–15,000)
Packed red blood cells (units), median (range)	0 (0–27)
Operating room time (minutes), median (range)	380 (200–790)
Pathology	
Primary tumor diameter (cm), median (range)	3 (0.1–15.5)
Positive resection margins	361 (42)
Positive lymph nodes	919 (78)
TNM stage*	
Stage I	22 (6)
Stage II	319 (90)
Stage III	13 (4)
Stage IV	1 (0.3)
Grade of differentiation	
Well	38 (3)
Moderate	649 (56)
Poor	466 (40)
Undifferentiated/anaplastic	2 (0.2)
Vascular (small vessel) invasion	383 (53)
Perineural invasion	721 (91)

Values in the table are n (%) unless otherwise specified.
*Staging information pertains to just the 355 patients undergoing PD for ductal adenocarcinoma of the pancreas subsequent to the publication of the AJCC *Cancer Staging Manual*, Sixth Edition (May 2002).

Postoperative Data

There were 26 deaths in the entire cohort of 1175 ductal adenocarcinomas, yielding a perioperative mortality rate of 2% (Table 5). The mortality rate declined steadily and significantly over time, with rates in the 1970s, 1980s, 1990s, and 2000s being 30% ($P < 0.001$ compared to the 2000s), 5% ($P = 0.02$ compared to the 2000s), 2%, and 1%, respectively. The overall morbidity rate was 38%; 3% of the patients required a return to the operating room during the index admission. The morbidity rates for the 1980s, 1990s, and 2000s were 30%, 31% ($P < 0.001$ compared to the 2000s), and 45%, respectively (the perioperative morbidity was not tracked well for patients undergoing PD in the 1970s). The most common complication following PD for ductal adenocarcinoma of the pancreas was delayed gastric emptying (15%). Other complications encountered less frequently included superficial wound infection (8%), pancreatic fistula (5%), a cardiac event (myocardial infarction or a new arrhythmia, 4%), and intra-abdominal abscess (4%). The median postoperative length of stay for the entire cohort of ductal adenocarcinomas was 9 days. The length of stay in the 1980s, 1990s, and 2000s was 16 days ($P < 0.001$ compared to 2000s), 11 days ($P < 0.001$ compared to 2000s) and 8 days, respectively. Postoperative adjuvant therapy data were available for 60% of the cohort (715 of 1175 patients). Eighty-four percent of the evaluable patients received adjuvant therapy, and the proportion receiving adjuvant therapy was greater in patients who underwent PD after 2000, compared to patients who underwent PD prior to 2000 (93% versus 79%, respectively; $P < 0.001$).

Table 5. Postoperative data for patients with ductal adenocarcinoma (n = 1175)

Perioperative mortality	26 (2)
1970s (n = 23)	7 (30)*
1980s (n = 65)	3 (5)*
1990s (n = 514)	10 (2)
2000s (n = 573)	6 (1)
Perioperative morbidity	415 (38)
1970s (n = 23)	No data
1980s (n = 65)	7 (30)
1990s (n = 514)	158 (31)*
2000s (n = 573)	250 (45)
Reoperation rate during index admission	35 (3)
Specific complications	
Delayed gastric emptying	161 (15)
Wound infection	91 (8)
Pancreatic fistula	52 (5)
Cardiac morbidity	27 (4)
Abdominal abscess	38 (4)
Cholangitis	26 (2)
Sepsis	19 (2)
Bile leak	16 (2)
Lymph leak	11 (1)
UTI	11 (1)
Peptic ulcer	10 (1)
Pneumonia	10 (1)
Acute pancreatitis	5 (1)
Small bowel obstruction	3 (0.3)
Postoperative length of stay (days), median (range)	9 (4–375)
1980s	16 (10–51)*
1990s	11 (7–373)*
2000s	8 (4–375)

Values in the table are n (%) unless otherwise specified.
*$P < 0.05$ compared to the present decade.

Survival Data

The Kaplan-Meier survival curves for all the 1175 patients with ductal adenocarcinoma (median survival = 18 months), 98 patients with neuroendocrine carcinoma (median survival = 139 months), and 90 patients with invasive cancer associated with an IPMN (median survival = 38 months) are depicted in Figure 2. In the total cohort of patients with ductal adenocarcinoma of the pancreas, 865 patients (73.7 of the total cohort) reached the endpoint of death. The 1-, 2-, 5- and 10-year survivals in the patients with ductal adenocarcinoma were 65%, 37%, 18%, and 11%, respectively. The longest survivor remains alive 27 yars after PD.

Statistically significant and favorable univariate predictors of long-term survival for patients with ductal adenocarcinoma included the absence of diabetes mellitus, the absence of COPD, age younger than 65 years, no intraoperative blood transfusions, tumor diameter less than 3 cm, negative lymph node status, negative resection margin status, well or moderately differentiated cancer, absence of postoperative pneumonia, absence of postoperative sepsis, absence of postoperative bile leak, and the administration of adjuvant therapy. In a multivaritate Cox proportional hazards regression, the most important predictors of long-term survival included tumor diameter less than 3 cm, negative lymph node status, negative resection margin status, well or moderately differentiated cancer, the absence of COPD, the absence of a postoperative bile leak, and the administration of adjuvant therapy (Table 6). Figure 3 shows the Kaplan-Meier survival curves of patients with ductal adenocarcinoma of the pancreas, according to the four pathological features highlighted in the multivariate Cox regression. There were 56 patients who underwent a PD for ductal adenocarcinoma with favorable pathological features in each of these four categories: tumors less than 3 cm, negative lymph nodes and resection margins, and well or moderately differentiated. These patients had a median survival of 44 months; their 1-, 2- and 5-year survivals were 90%, 75%, and 43%, respectively.

The Kaplan-Meier survival curve for patients undergoing PD for ductal adenocarcinoma of the pancreas by the decade of resection is plotted in Figure 4. The long-term survival of patients who underwent PD in the 2000s was superior to the survival of patients who underwent resection in the 1970s ($P = 0.01$) or 1980s ($P = 0.06$). The survival comparison between the 1980s and 2000s just missed statistical significance, due to the relatively small sample size in the earlier decade (n = 65 patients). Although the long-term survival data in the present decade are insufficiently mature to observe a difference compared to the 1990s, the 2-year survival is significantly better (35% for 1990s versus 42% for 2000s, $P = 0.0009$). The median survival in the 1970s, 1980s, 1990s, and 2000s was 8, 14, 17, and 19 months, respectively.

DISCUSSION

Until the past decade, the greatest advances in the management of pancreatic cancer have been limited to surgical therapy. A brief overview of some of the more important developments in the history of pancreatic cancer treatment serves to place this present series into its proper context.

Dubious descriptions of pancreatic cancer were reported throughout eighteenth and nineteenth

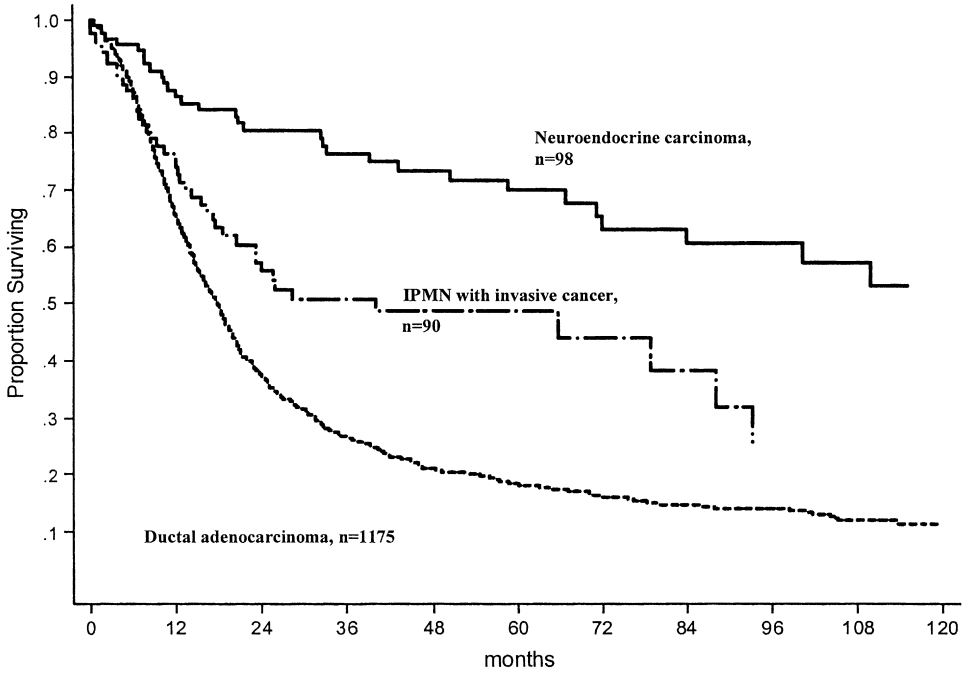

Fig. 2. Kaplan-Meier actuarial survival curves for patients undergoing PD for ductal adenocarcinoma of the pancreas (n = 1175), IPMN with invasive cancer (n = 90), or neuroendocrine carcinoma of the pancreas (n = 98). Ductal adenocarcinoma: median survival = 18 months, 1-year survival = 65%, 2-year survival = 37%, 5-year survival = 18% and 10-year survival = 11%. IPMN with invasive cancer: median survival = 38 months, 1-year survival = 74%, 2-year survival = 56%, 5-year survival = 48%, and 10-year survival = 26%. Neuroendocrine carcinoma: median survival = 139 months, 1-year survival = 86%, 2-year survival = 81%, 5-year survival = 70%, and 10-year survival = 53%.

centuries, including one series of five autopsies with hardened pancreata by the Italian anatomist Giovanni Battista Morgagni (1682–1771).[18] Perhaps the first reliable description of a series of pancreatic cancers was made by an American internist from the Jefferson Medical College in Philadelphia, Jacob Mendez Da Costa. In 1858, Da Costa reported a series of 37 autopsies with pancreatic cancer, including the first one with a microscopic diagnosis.[18]

Table 6. Multivariate Cox proportional hazards regression for patients with ductal adenocarcinoma (n = 1175)

Risk factor	Hazard ratio	*P* value
Tumor diameter (⩾3 cm)	1.6	<0.001
Positive lymph nodes	1.3	0.05
Positive resection margin	1.6	<0.001
Histological grade (poorly or undifferentiated)	1.6	<0.001
COPD	2.0	0.006
Bile leak	7.0	<0.001
Adjuvant therapy	0.5	<0.001

Surgeons resected pancreatic neoplasms in the body and tail of the pancreas prior to any attempts at removing lesions in the head. In 1882, the German surgeon Friedrich Trendelenburg removed a sarcoma originating in the left side of the pancreas, although the patient died perioperatively.[19] Periampullary cancers posed a greater challenge at the time because it was widely believed that the duodenum was essential for digestion.[20] In 1898, William Stewart Halsted performed the first local resection of a periampullary cancer for an ampullary carcinoma, preserving most of the duodenum.[21] That same year, an Italian surgeon, Alessandro Codivilla, performed the first regional resection on a patient with a pancreatic cancer. A discharge summary remains the only primary record of this procedure and indicates that the patient died 24 days after the operation.[18,22]

Survival beyond the perioperative period following PD was rarely achieved in the subsequent 25 years (notable exceptions included PDs performed by the German surgeons Walter Kausch and George Hirschel, and the Italian surgeon Ottorino Tenani[18,22]). Allen O. Whipple, the chairman of surgery

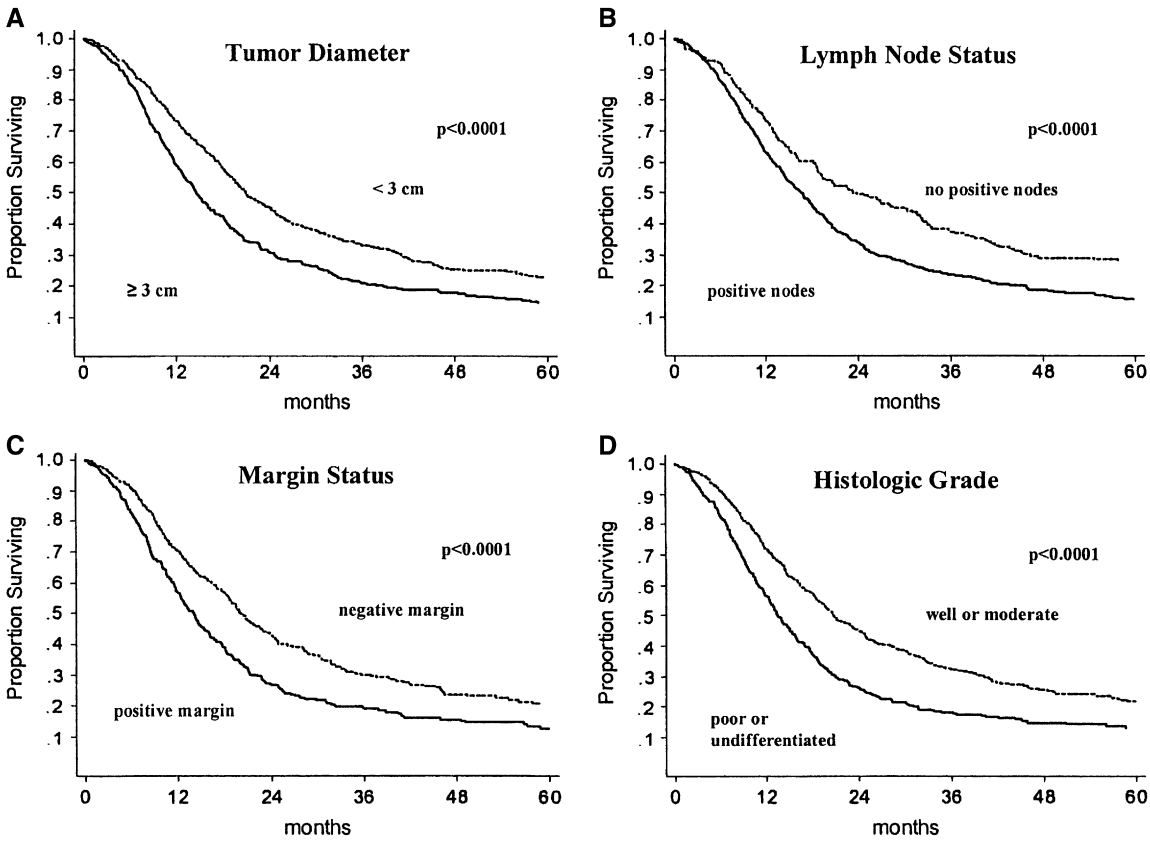

Fig. 3. Kaplan-Meier survival curves for patients undergoing PD for ductal adenocarcinoma of the pancreas, according to tumor diameter, lymph node status, resection margin status, and histologic grade. **A**, For cancers <3 cm, median survival was 21 months; 1-, 2-, and 5-year survivals were 73%, 45%, and 23%, respectively. For cancers ⩾3 cm, median survival was 15 months; 1-, 2-, and 5-year survival were 59%, 31%, and 4%, respectively. **B**, For cancers with no positive lymph nodes, the median survivals was 23 months; 1-, 2-, and 5-year survivals were 73%, 50%, and 27% respectively. For cancers with positive lymph nodes, the median survival was 17 months; 1-, 2- and 5-year survivals were 63%, 34%, and 16%, respectively. **C**, For cancers with negative resection margins, the median survival was 20 months; 1-, 2-, and 5-year survivals were 70%, 43%, and 21%, respectively. For cancers with positive margins, the median survival was 14 months; 1-, 2-, and 5-year survivals were 57%, 26%, and 12%, respectively. **D**, For well or moderately differentiated cancers, the median survival was 21 months; 1-, 2-, and 5-year survivals were 72%, 45%, and 22%, respectively. For poorly or undifferentiated cancers, the median survival was 13 months; 1-, 2- and 5-year survivals were 56%, 26%, and 13%, respectively.

at Columbia Presbyterian, became the first American surgeon to perform a PD and presented a series of three PDs at the American Surgical Association meeting in 1953.[23]

The perioperative mortality rate reported in a series of 41 PDs performed by Whipple and other surgeons in 1941 was 29%.[20] Perioperative mortality associated with PD during the 1940s through the 1980s remained high, ranging from 8% to 24%.[5,24–30] Due to subsequent progress in many aspects of surgical care, mortality rates dropped dramatically through 1980s and into the 1990s, to under 2% at high-volume centers.[15,31] Improvement in the long-term survival has been more modest. Reported 5-year survival rates for resected right-sided pancreatic cancers prior to 1980 ranged from 0% to 15%.[28,32–36] More recently, there have been large series with 5-year survival rates in excess of 15%.[9,10,37] The 5-year survival rate for pancreatic cancer in the United States, including both resected and unresected cases, increased by a marginal but statistically significant amount between 1975 and 2000 (from 3% to 5%).[1] The present series of PDs for pancreatic cancer highlights the progress made in the treatment of pancreatic cancer over the past four decades and the opportunities for further progress.

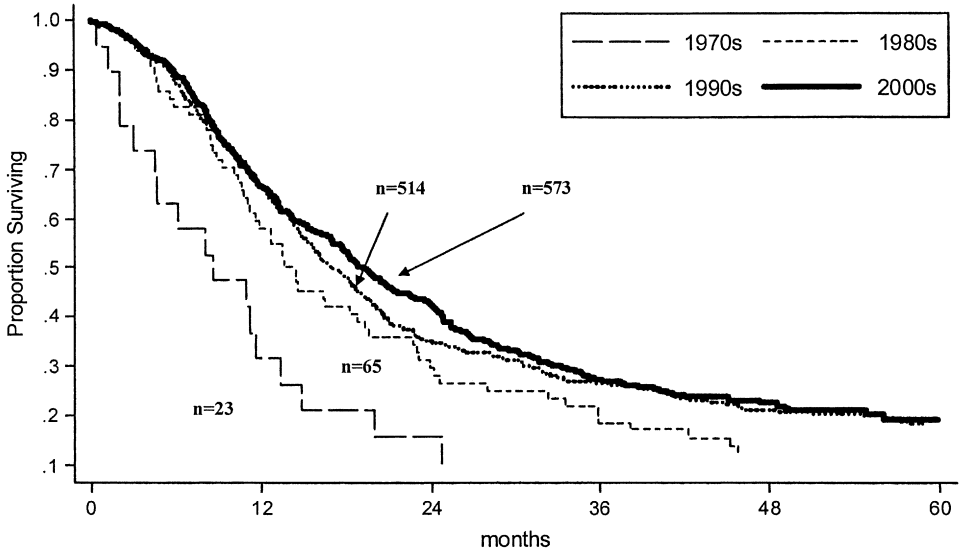

Fig. 4. Kaplan-Meier survival curves for patients undergoing PD for ductal adenocarcinoma of the pancreas, by decade. 1970s: Median survival was 8 months; the 1-, 2-, and 5-year survivals were 31%, 11%, and 5%, respectively (P = 0.01, compared to the survivals of patients in 2000s). 1980s: Median survival was 14 months; 1-, 2-, and 5-year survival were 55%, 29%, and 11%, respectively (P = 0.06, compared to the survival of patients in 2000s). 1990s: Median survival was 17 months; 1-, 2-, and 5-year survivals were 66%, 35%, and 18%, respectively (P = 0.0009 for 2-year survival in 1990s, compared to the 2-year survival for 2000s). 2000s: Median survival was 19 months; 1-, 2-, and 5-year survivals were 66%, 42%, and 20%, respectively.

The geographic referral pattern in this series of patients, with the majority coming from outside of Maryland, suggests that referring physicians and internet-savvy patients and families are seeking out high-volume centers of excellence for complex surgery. The annual number of PDs performed nationally for pancreatic cancer and the fraction of these PDs that have been performed at our institution can be estimated. Assuming a resectability rate of 20%[3] for the 18,000 cancers occurring in the head of the pancreas (60% of 30,000 pancreatic cancers originate in the head),[3] 3600 PDs are estimated to be performed annually in the United States. Since approximately 120 PDs for pancreatic cancer are performed annually at our institution, roughly 3% of the PDs performed annually in the United States for pancreatic cancer have been performed at Johns Hopkins in recent years.

Detailed statistical analyses focused on the 1175 ductal adenocarcinomas resected by PD (ductal adenocarcinoma was the most common type of pancreatic cancer, of 18 different pathological types resected in the present series). The median age of 66 years in our series is younger than the national median of 72 years for patients with pancreatic cancer. This discrepancy reflects a pattern observed in a recent population-based study that showed patients who underwent resection for pancreatic cancer were on average 5 years younger than patients with pancreatic cancer who did not undergo resection.[3] It should be noted, however, that the average age of patients undergoing PD for pancreatic cancer at our institution has gradually increased over time and this trend is likely to continue in the future as the population ages. A recent study from our institution analyzed 207 PDs in patients 80 years or older and found that perioperative morbidity and mortality of the very elderly were 3.9% and 53%, respectively.[38] Age alone was not a predictor for death or postoperative complications in multivariate analyses.

Nearly 90% of the patients in this series had a preoperative procedure. Approximately three-quarters of the patients had a preoperative procedure to manage biliary obstruction, which is consistent with the observed frequency of preoperative jaundice. The role of endoscopic and radiologic biliary decompression in the management of patients is controversial. In a recent large meta-analyssis, no benefit to preoperative biliary stenting was observed for periampullary cancers.[39] In our series, most patients had preoperative stents in place by the time that they were seen by a surgeon at our institution. There are occasional instances when preoperative decompression is clearly warranted, such as to treat cholangitis, to treat refractory pruritis, or to temporize patients who require additional medical optimization prior to surgery.

While roughly 96% of the cases of pancreatic cancer resected were stage II or less (based on AJCC cancer staging[17]), a small percentage of patients underwent a PD with locally advanced or distant disease. Three patients had stage IV disease, including two with solitary liver metastases (survival of 6 and 7 months) and one with a solitary skin lesion (survival of 11 months). In one study that compared simultaneous PD and partial hepatectomy in patients with hepatic metastases, to palliative bypass in similarly staged patients, there was no difference in long-term survival (median of 6 and 4 months, respectively). Furthermore, all of the patients undergoing PD and a liver resection died of recurrent liver metastases within 1 year.[40] The data from this series also suggest that documented hepatic metastases confers an especially poor prognosis.

The decline in the perioperative mortality following PD over time is perhaps the most striking accomplishment in pancreatic surgery. There were just six deaths in 548 patients in the present decade, resulting in a 1% mortality rate, which is comparable to mortality rates with other elective operations of the gastrointestinal tract such as colectomy for colon cancer[41] and antireflux surgery.[42] The postoperative morbidity was significantly higher in the present decade compared to the previous one (44.6% versus 30.8%, respectively; $P < 0.001$). This observed increase is most likely a function of more complete data collection in the present era of electronic records, rather than an increased complication rate. Potentially serious complications such as pancreatic fistula, delayed gastric emptying, cardiac events, and pneumonia occured with similar frequency during the 2000s and 1990s (data not shown), while less serious complications such as urinary tract infections (0.2% versus 1.6%, $P = 0.02$) and superficial wound infections (6.9% versus 9.9%, $P = 0.08$) occured more frequently in the 2000s compared to the 1990s. A more telling trend is the decrease in the median postoperative length of stay with each decade (16 days in the 1980s, 11 days in the 1990s, and 8 days in the 2000s).

The survival analyses performed in this study confirmed previous studies suggesting that pathological factors are important indicators for long-term survival.[9,43,44] Tumor diameter less than 3 cm (3 cm was arbitrarily selected as a cutoff because it is the median tumor diameter in this series), negative lymph nodes, a negative resection margin status (or R0 resection), and well or moderately differentiated tumors were all favorable prognostic factors in univariate and multivariate analyses. Roughly 5% of the patients in our experience (n = 56) had favorable pathology in all four parameters. The median survival in these patients was nearly 4 years. These data strongly suggest that early detection of pancreatic cancer would dramatically alter its clinical course and improve survival rates.

The long-term survival of patients who underwent PD for pancreatic cancer in the present decade (median 19 months) exceeds survivals from the 1970s (8 months, $P = 0.01$) and the 1980s (11 months, $P = 0.06$). There has not been sufficient follow-up in the present decade to detect a difference compared to the long-term survival of patients who underwent PD in the 1990s; however, the curves begin to diverge at 15 months and are significantly different when the first 2 years after surgery are compared (35% 2 year-survival for the 1990s versus 42% 2 year-survival for the 2000s, $P = 0.0009$). This survival benefit is apparent despite the fact that patients in the 2000s are significantly older and have significantly more positive lymph nodes (68 years and a median of three positive lymph nodes) compared to patients in the 1990s (66 years, $P = 0.03$, and a median of two positive lymph nodes, $P = 0.002$). Furthermore, the staging for ductal adenocarcinomas resected by PD during the 1990s and 2000s is comparable (6.2% stage I in 1990s versus 4.8% stage I in 2000s, P = NS). Since the pathological features of ductal adenocarcinomas in the present decade are comparable to ductal adenocarcinomas resected in prior years and only a small percentage of the PDs were performed for incidentally discovered cancers ($\sim$1%), lead-time bias was not likely a factor contributing to the improved long-term survival. Although still unproved, it is more likely that greater use of adjuvant therapy in the present decade and more effective adjuvant regimens played an important role.

There have been two large phase III trials for adjuvant therapy following resection for pancreatic cancer that have been reported within the past 10 years.[45,46] Both trials were performed in Europe and were generally interpreted by the European community as providing weak evidence that chemotherapy (but not chemoradiation therapy) may be beneficial in the treatment of pancreatic cancer. American studies reported by the Gastrointestinal Tunmor Study Group[47] and our institution[48] favor the use of chemoradiation to treat pancreatic cancer. The results of the latest North American phase III trial, comparing gemcitabine versus 5-FU adjuvant therapy before and after 5-FU–based chemoradiation, indicates a survival benefit for the gemcitabine-based therapy in patients undergoing PD.[49] In addition, alternative adjuvant strategies, such as ones based on interferon-alpha,[50] small molecule inhibitors,[51] and allogeneic GM-CSF–secreting pancreatic tumor vaccines,[52] have shown promise in phase I and phase II studies.

Earlier cancer detection also holds great promise. In a recent study of pancreatic incidentalomas from our institution, 12 ductal adenocarcinomas originating in the head of the pancreas were detected "by accident" in asymptomatic individuals. These pancreatic cancers, when resected, were lower staged and associated with improved long-term survival as compared to resected symptomatic pancreatic cancers.[53]

A new chapter in the story of pancreatic cancer is under way. The creativity and dedication of Codivilla, Kausch, Whipple, and others have been matched by oncologists from many different disciplines. It is our expectation that improvements in long-term survival will continue, perhaps with even accelerated pace, as improved cancer detection technologies and adjuvant therapies are widely integrated into the clinical arena. Future prospective randomized trials with novel therapeutic agents and studies with longer patient follow-up will be necessary to better understand the impact of these advances.

The authors wish to thank the residents and nurses of the Johns Hopkins Hospital for their role in the care of these patients.

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Discussion

Howard A.H. Reber (Los Angeles, Calif): I want to begin by thanking you for the opportunity to read the paper and to ask some questions.

It is impossible to begin any kind of a discussion of a paper like this without recognizing the enormous contributions that have come from the Hopkins group, and I could go on and talk more about those contributions and their great value to the field. But because I wouldn't have time to ask some questions, I am not going to say anything more about that. Instead, Jordan, I will ask some questions and make some comments that came to my mind as I read the manuscript.

The first thing that I want to focus on is the 42% positive margin status, which seems rather high. In our own recent experience, we had a 15% positive margin rate, obviously much lower. Would you comment on this, and tell us which margins were usually involved?

I also wonder whether this 42% positive margin status that you identified has changed over the years. You are reviewing a large number of patients over a 36-year interval. Has the frequency of margin positivity changed over the review period?

I wonder as well whether you are doing resections in patients who have more advanced disease than

many of the rest of us would normally consider operative candidates? Thus, in the manuscript you indicated that there were 13 patients who had stage III pancreatic cancer, meaning that they had either celiac or superior mesenteric artery involvement. Most of us wouldn't resect in a patient who had involvement of those vessels. There also were 47 patients who had partial resections of the superior mesenteric vein or the portal vein. You found as well that 78% of your patients had positive lymph nodes. At UCLA, the node positivity rate is about 50%. You even mention in the paper that there were a couple of patients who had resections with liver metastases.

So what I would ask you to do is to outline for us your current *contraindications* to resection? When don't you resect if you have resected tumors in the kinds of patients that I have just mentioned?

Another question comes as well from a comparison of your data and our experience. For example, we found that 14% of our patients had well-differentiated tumors. The number that Dr. Yeo gave was 3% with well-differentiated tumors. We found that 60% of the patients in our series had perineural invasion. You showed 91%. Thus, it looks as though we may be resecting patients who have earlier or less aggressive disease, and I am not sure that is true. Because the UCLA review included only patients from the last 15 years or so, I wonder whether the disease itself may be changing. In other words, is pancreatic cancer in this 21st century really the same disease in terms of its biological aggressiveness as it was 30 years ago?

I hoped that you would provide an answer to that, but I was unable to find it in the manuscript. Although you have made decade-by-decade comparisons of mortality rate, length of hospital stay, etc., I found no similar analysis in regard to the pathological characteristics of the tumor. So, for example, were patients more likely to have poorly differentiated tumors 30 years ago than they are today? Sometimes these diseases do change, and that would be very interesting.

The final question relates to an observation that you made a few years ago, that the operative blood loss was an important prognostic factor. Because of that observation, we have made a great effort to minimize blood loss in our resections, and found that in patients in whom we lose less than 400 ml of blood, the 5-year survival rate is twice as good as in the group where we lose more than 400 ml. So I was surprised to note that blood loss no longer breaks out as a prognostic factor in your most recent analysis.

Again, an incredible experience, a great presentation by the senior member of your group, and I appreciate the chance to have read the manuscript. I look forward to your comments.

Jordan Winter, M.D. (Baltimore, Md): Thanks, Dr. Reber, for challenging me with several poignant questions.

Regarding your question on resection margin, there are basically five locations on a Whipple specimen where our pathologists look for resection margin positivity: the bile duct, duodenum, the pancreatic neck, the uncinate process, and the retroperitoneum. Most of our positive margins are in the vascular groove along the superior mesenteric artery where it is very difficult to achieve negative margins in the presence of microscopic tumor invasion. It is very seldom the case that any of the other margins are positive. An exception may be if there is margin posititivity in the vascular groove on frozen section and another margin is coincidentally found to be positive. After considering patient and operative factors in these instances, the surgeon may opt to take a less aggressive approach in achieving a negative margin at the second location then he or she otherwise would in the absence of a positive margin at the vascular groove.

In addition, although we didn't mention this point in the manuscript, there has been a decrease in the proportion of specimens with positive resection margins by decade. Although the observed rate in the present decade is still not quite as low as you see at your institution, it is around 30%. Perhaps the higher rate at our institution may be the result of a referral bias that includes patients who have sought second and third opinions after being deemed unresectable at other institutions and we are seeing a disproportionate number of patients that have locally aggressive cancers.

You also pointed out that 13 patients had stage III disease and 3 patients had stage IV disease on the final pathology. I want to point out that these are absolute numbers and that in a large series such as this, 13 patients are about 1% of all patients and 4 patients are about 0.3% of all patients. I think it's also important to consider that in some of the patients with stage III disease in this series, the patients were relatively young, and if the surgeon felt that the operation could be completed safely, than an aggressive course of action in conjunction with chemoradiation therapy may indeed be the best alternative for that patient.

Pancreatic Cancer in the General Population: Improvements in Survival Over the Last Decade

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Background: It is unknown whether the improved survival seen at high-volume centers has been translated to all patients with pancreatic cancer.

Objective: To use the Surveillance, Epidemiology, and End Results (SEER) database to evaluate population-based trends in surgical resection and survival.

Methods: All patients diagnosed with pancreatic cancer from 1988–1999 were identified. The survival and proportion of patients undergoing surgical resection were compared for each of three equal time periods.

Results: There were 24,016 patients with pancreatic cancer. 19,533 had stage data available. 9% had localized, 29% had regional, and 62% had distant disease. Resection rates increased for patients with localized and regional disease over the three time periods. Survival increased for patients with regional and distant disease. For regional pancreatic cancer patients, 2-year survival increased from 9.5% to 13.5% ($p < 0.0001$) and from 21.5% to 28.9% following surgical resection ($p = 0.002$). For resected local/regional pancreatic cancer, the year of diagnosis was and independent predictor of improved survival ($p = 0.0001$).

Conclusions: SEER patients with regional and distant pancreatic cancer have improved survival over the past decade in both unadjusted and adjusted models. The improvement is most striking for patients with regional disease and reflects increased resection rates and improved resection techniques over time.
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KEY WORDS: SEER, survival, pancreatic cancer

Pancreatic adenocarcinoma remains the fourth leading cause of cancer deaths in men and women in the United States.[1] There are approximately 30,000 new cases annually for an incidence rate of 9 cases per 100,000 population. The incidence is slightly higher in males and African Americans. Sixty-five percent of pancreatic adenocarcinomas arise in the head, neck, or uncinate process, 15% in the body or tail, and 20% diffusely involve the gland. Unfortunately, pancreatic adenocarcinoma is an aggressive cancer, with a death-to-incidence ratio approaching one. Because of the vague nature of the presenting symptoms, the majority of patients present with advanced-stage disease and are not candidates for surgical resection. As a result, the overall 5-year survival is less than 4%.[1,2]

Many lay people and medical professionals view the diagnosis of pancreatic adenocarcinoma as a death sentence. However, this is not the case for those patients who present with early stage or locoregional disease amenable to surgical resection. In the 1970s, high morbidity and mortality rates in excess of 25% after pancreatic resection led many authors to suggest that such an aggressive approach was not indicated.[3,4] Since then, many centers have reported significant improvements in perioperative 30-day mortality, with rates of less than 5%.[5–9]

Concomitant with improvements in perioperative mortality rates, pancreatic cancer patients who were treated with surgical resection at high-volume centers had improved 5-year actuarial survival rates of 15%–21% after pancreaticoduodenectomy[5–11] and

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approximately 12% after distal pancreatectomy.[5,12–14] In addition, an actual 5-year survival rate of 15% has been reported.[15] Although some believe that there is no hope for long-term survival, a recent single institution study reported a 17% actual 5-year survival rate, with 96 5-year survivors of pancreatic adenocarcinoma. In addition, the authors demonstrated that the subsequent 5-year survival for those patients achieving the 5-year landmark was 55%. Long-term survival did, in fact, occur.[16]

Improvement in mortality and survival at high-volume centers has led to increased use of surgical resection for this disease. The results obtained from these centers have led many to suggest regionalization of care to such specialized hospitals. Many studies demonstrate that regionalization of care decreases lengths of stay, decreases hospital costs, and improves short- and long-term surgical outcomes after complex pancreatic surgery.[17–22] However, the majority of patients in the U.S. population with pancreatic cancer are not treated at high-volume, specialized centers. Therefore, it is unclear whether this increased resection rate and long-term survival seen at major centers has been translated to the general population.

A recent study by Cress and colleagues[23] also reports a population-based survival analysis of patients with pancreatic cancer. Their study evaluated 10,612 patients with pancreatic cancer from the California tumor registry. They report a median survival of 3.5 months in the 8,938 patients not resected compared with 13.3 months in the 1,674 patients resected. The goal of this paper is to use the Surveillance, Epidemiology, and End Results (SEER) tumor registry[24] to evaluate trends in surgical resection and overall survival over the last decade.

Our current study differs from the former in that we use the SEER data, representative of the entire U.S. population. In addition, we evaluate not only overall survival but trends in survival over time to understand whether the improvements in survival seen at high-volume centers are being translated to the general population.

METHODS

Using the publicly available SEER database, we identified all people in the registry with the diagnosis of pancreatic cancer between 1998 and 1999. The SEER program is sponsored by the National Cancer Institute, is complete for the time period 1973–2001, and contains over three million cancer cases with 170,000 new cases added annually. SEER registries exist in fourteen geographic areas which were added to the registry at different times. They are summarized in Table 1. The database contains 26% of the total U.S. population. While the database is largely representative of the U.S. population, it is designed to slightly overrepresent minority groups, with increasing representation for smaller groups. The database covers 23% of all U.S. African Americans, 40% of U.S. Hispanics, 42% of American Indians and Alaskan Natives, 53% of U.S. Asians, and 70% of Hawiian/Pacific Islanders. The SEER tumor registry collects information of demographics, primary tumor site, stage of disease, first course of treatment, and survival status. This makes it an ideal source to study population-based trends in treatment and outcomes for patients with pancreatic cancer.

Patients diagnosed before 1988 were eliminated from the analysis because there were no SEER data available on surgical resection. To ensure that we had adequate follow-up to evaluate 2-year survival, we excluded patients diagnosed after 1999. Furthermore, the SEER areas of New Jersey, Louisiana, Kentucky, and Greater California were not added until after 2002 and are not included in this study. This analysis included only patients with pancreatic adenocarcinoma and pancreatic adenocarcinoma arising in an intraductal papillary mucinous neoplasm (IPMN). Patients with mucinous cystadenocarcinomas, neuroendocrine tumors, acinar cell tumors, or unclear pathologies were excluded. Patients without microscopic confirmation of tumor, those patients identified at autopsy, or those patients identified through death certificate only were excluded.

Table 1. SEER tumor registry regions

Region	Year added*
Connecticut	1973
Iowa	1973
New Mexico	1973
Utah	1973
Hawaii	1973
Metropolitan Detroit	1973
San Francisco/Oakland	1973
Atlanta	1975
Seattle/Puget sound	1975
Georgia (10 rural counties)	1978
Alaska (natives)	1990
Los Angeles County	1992
San Jose/Monterey	1992
Kentucky	2001
New Jersey	2001
Louisiana	2001
Greater California	2001

*Only registries included in SEER before 2001 are included in the analysis.

Patients were divided into subgroups based on SEER summary stage. The SEER summary stages were: (1) localized disease, (2) regional disease, or (3) distant disease. Localized disease was defined as tumor in situ or tumor confined to the pancreas. Regional disease was defined as tumor invading adjacent structures including the duodenum, bile duct, ampulla of Vater, superior mesenteric vessels, and hepatic artery. Locoregional lymph node involvement was also categorized as regional disease. Distant disease required the presence of distant metastases (liver, lung) or metastases outside of the locoregional areas.

Localized pancreatic cancer is defined as having tumor in-situ or tumor confined to pancreas and all patients with localized disease are candidates for surgical resection from a technical viewpoint. Patients with regional disease are resectable if they have tumor extending into the peripancreatic fat (not involving major vessels or other organs), bile duct, duodenum, or ampulla of Vater, or nodal basins within the field of resection (lymph node stations 12b, 12c, 13, 14b, 14v, and 17). Patients are considered unresectable if they have disease involving the portal vein/hepatic artery/superior mesenteric vessels, tumor involving organs other than those in the primary resection field such as transverse colon, and/or tumor involving remote lymph nodes.

For each year in the time period studied, we identified the percentage of patients with localized, regional, distant, or unstaged disease. We also identified the percentage of patients with localized and regional disease that underwent potentially curative surgical resection each year. The time period was then divided into three equal intervals: 1988–1991, 1992–1995, and 1996–1999. Using log-rank tests, the Kaplan-Meier[25] actuarial survival curves for the three time periods were compared in the overall cohort. In addition, survival in each time period was compared for those with localized, regional, and distant disease. For those undergoing surgical resection, the Kaplan-Meier survival curves were compared in similar fashion. This was done for both localized pancreatic cancer with resection and regional pancreatic cancer with resection.

To determine if the year of diagnosis was an independent predictor of survival, a multivariate analysis was performed using a Cox proportional hazards model.[26] Demographic factors including age, gender, race, and marital status were included in the model. In addition, other factors known to influence survival such as conventional tumor stage, histology type (adenocarcinoma vs. adenocarcinoma arising in an IPMN), site of the primary tumor (head vs. body/tail vs. other), lymph node status, and resection status were included in the model. For all models, age and year of diagnosis were continuous variables. Cox proportional hazards models were also performed for patients with localized and regional disease. Separate models were obtained for those undergoing surgical resection and for those not resected.

All data analysis was performed using SAS statistical software, version 9.1.3 (SAS Institute, Cary, NC). Significance was accepted at the $P < 0.05$ level. All means are expressed as mean $\pm$ standard deviation, and all proportions are expressed as percentages. Chi-square analysis was used to compare proportions for all categorical data. When evaluating trends, P values from Cochran-Armitage trend test were reported. Hazard ratios (HR) and confidence intervals were given for each level of each category in the Cox proportional hazards models, with the reference group listed first (hazard ratio = 1.0). P values in all Cox proportional hazards models were reported for each category of analysis, with the number of degrees of freedom being equal to the total number of categories minus one. P values for each individual level within categories were not calculated separately. However, any level within a category that had a hazard ratio of less than or greater than one and 95% confidence intervals that did not include the null value of 1.0 were significantly different from the comparison group.

RESULTS

Using the publicly available SEER tumor registry, we identified 24,016 patients with pancreatic adenocarcinoma or pancreatic adenocarcinoma arising in an IPMN diagnosed between January 1988 and December 1999, inclusive. The mean age of the patients was 70.2 $\pm$ 12.1 years. 11,543 patients (48%) were male. 12,928 (54%) were married and 10,415 (43%) were unmarried (6355 (26%) widowed, 2182 (9%) single, 1776 (7%) divorced, 102 (1%) separated) and the marital status was unknown in the remaining 3%. 18,590 (77%) of patients were white, 2775 (12%) were African American, 1725 (7%) were Hispanic, and 926 (4%) were other races.

Cancers of the pancreatic head, neck, and uncinate process occurred in 12,602 patients (52%). 3804 (16%) had cancers in the body and/or tail of the gland. The remaining 32% did not specify the location within the gland 22,758 (95%) were pancreatic adenocarcinomas and 1258 (5%) were adenocarcinomas arising in IPMNs. Nodal status was available on 9103 patients (38%), most likely those that underwent surgical resection or lymph node

biopsy. Of these 9103 patients, 4584 (50%) had negative lymph nodes and 4519 (50%) had positive lymph nodes. For those undergoing surgical resection, only 61 had no nodal data and of the remaining 1945 patients, 53% were node positive.

Stage data was not available on 4,483 of the 24,016 patients (19%). Of the 19,533 patients with stage data available, 1,745 (9%) had localized disease, 5,745 (29%) had regional disease, and 12,043 (62%) had distant disease at the time of diagnosis. This selection process is summarized in Fig. 1.

The overall survival for the entire cohort (N = 24,016, resected and unresected) was 6.2% at 2 years, with a median survival rate of 3 months. For patients with localized disease, survival at 2 years was 15.8% (median survival = 7 months), whereas for patients with regional disease, the survival at 2 years was 11.8% (median survival = 7 months). After calculating overall survival, the time period 1988–1999 was divided into three equal length periods. There were 7,691 patients diagnosed from 1988–1991, 7,869 diagnosed from 1992–1995, and 8,456 diagnosed from 1996–1999. The overall 2-year survival was 5.2% for the first time period, 6.3% for the second time period, and 7.0% for the third time period (Table 2; $P = 0.08$).

From 1988–1999, the distribution of patients with localized, regional distant, and unstaged disease changed over time. The overall trends are summarized by the line graph in Fig. 2. When broken down into three equal time periods for easier comparison, the percentage of patients with localized disease was fairly constant, ranging from 7.4% in 1988–1991, to 7.4% in 1992–1995, to 7.0% in 1996–1999. The percentage of patients with distant disease was also relatively constant over the three time periods at

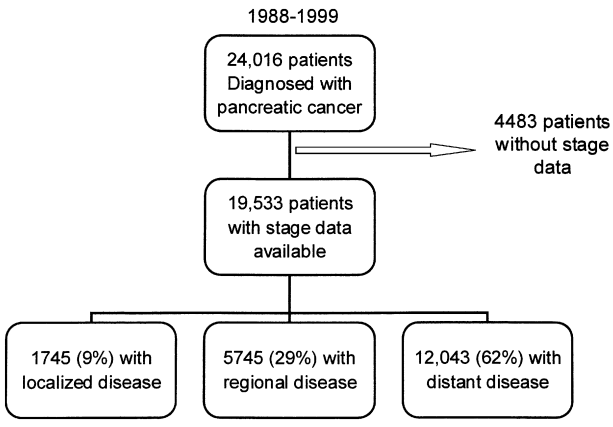

Fig. 1. Establishment of a cohort of patients diagnosed with pancreatic adenocarcinoma or adenocarcinoma arising in an IPMN by using the SEER databases. All cases were diagnosed from 1988–1999.

Table 2. Survival by historical tumor stage and time period

		2-year survival rate			
	Total No.	1988–1991, %	1992–1995, %	1996–1999, %	*P* value*
Overall	24,016	5.2	6.3	7.0	0.08
Localized	1,745	13.8	15.9	17.5	0.69
Localized with resection	376	43.0	44.9	46.5	0.93
Localized without resection	1,369	7.0	8.6	7.7	0.69
Regional	5,745	9.5	12.0	13.5	0.0008
Regional with resection	1,630	21.4	27.6	28.9	0.0015
Regional without resection	4,115	5.9	5.8	6.0	0.43
Distant	12,043	1.4	2.0	2.3	<0.0001
Unstaged	4,483	6.6	6.9	6.6	0.06

*P value is the log-rank P value for differences between the three time periods.

49.0%, 50.5%, and 50.9%, respectively. The change in distribution was mainly seen for regional and unstaged disease, which is best understood when looking at Fig. 2. To emphasize the trend, the graph is continued through 2001. The percentage of patients with regional disease increased from 23.1% in 1988–1991 to 25.7% in 1996–1999, whereas the number of unstaged patients decreased from 20.5% to 16.4%. The chi-square P value for differences between all four groups was <0.0001.

The 2-year survival was then compared over the three time periods. This survival analysis was performed by tumor stage: localized, regional, and distant and is summarized in Table 2. In the 1745 patients with localized disease, 569 were diagnosed from 1988–1991, 585 were diagnosed from 1992–1995, and 591 were diagnosed from 1996–1999. The 2-year survival was 13.8% in the first time period, 15.9% in the second, and 17.5% in the third. This observed 3% increase in survival was not statistically significant (Fig. 3, *A*; $P = 0.69$).

For the 5,745 patients with regional disease, the 2-year survival increased significantly over time. The 2-year survival rate was 9.5% in 1988–1991 (n = 1,779), 12.0% in 1992–1995 (n = 1,789), and 13.5% in 1996–1999 (Fig. 3, *B*; n = 2,177; $P = 0.0008$). For patients with distant or metastatic disease (12,043), the 2-year survival increased from 1.4% (n = 3,770) to 2.0% (n = 3,970) to 2.3% (n = 4,303) over the three time periods ($P < 0.0001$). This difference is statistically significant,

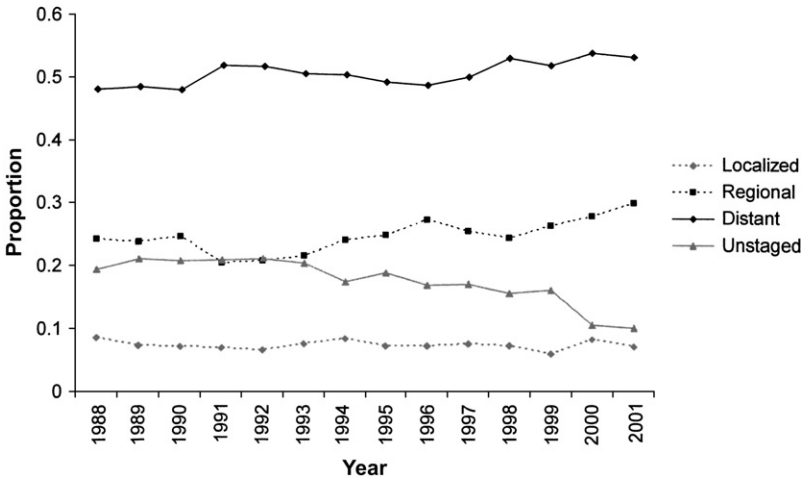

Fig. 2. Distribution of pancreatic cancer cases by stage from 1988–2001. The proportion of patients with localized and distant disease has remained constant. As the proportion of those with regional disease increases, those with unstaged disease are decreasing, suggesting improved diagnostic capability ($P < 0.0001$).

but likely not clinically significant, as the analysis is significantly overpowered to assess such a small difference in survival.

Unstaged patients (n = 4483) had 2-year survival rates of 6.6% in 1988–1991, 6.9% in 1992–1995, and 6.6% in 1996–1999. This analysis was performed to determine the approximate stage of these patients. Based on their observed survival rates, the majority were unstaged or had advanced regional disease yielding survival rates of slightly better than those with distant disease. These patients are not included in any further analyses.

Patients with localized and regional disease are potential candidates for surgical resection. For the localized and regional groups, we determined the percentage of patients resected over each time period. Note that not all patients with regional disease are technically resectable given the definition of resectability in the methods section. Three hundred sixty-six of the 1745 patients with localized disease (21%) and 1630 of the 5745 patients with regional disease (28%) underwent surgical resection. The number of patients with localized disease undergoing surgical resection increased from 18.8% in 1988–1991 to 20.3% in 1992–1995 to 25.5% in 1996–1999 ($P = 0.0025$ for trend). Likewise, the proportion of patients with regional disease undergoing surgical resection increased from 23.0% to 23.8% to 32.5% over the three time periods ($P < 0.0001$ for trend). This trend is summarized in Fig. 4.

After potentially curative surgical resection, the 2-year survival was significantly improved for those with regional disease over the three time periods (Fig. 5). The 2-year survival with regional disease after resection (n = 1630) was 21.4% in 1988–1991, 27.6% in 1992–1995, and 28.9% in 1996–1999 (Table 2; $P = 0.0015$). This improvement in survival after surgical resection was not observed for patients with localized disease. After surgical resection, patients with localized disease had 2-year survival rates of 43.0%, 44.9%, and 46.5%, respectivley, ($P = 0.93$) over the three time periods (Table 2).

To determine if the year of diagnosis was an independent predictor of survival, we performed several Cox proportional hazards model, including demographic and pathologic factors known to influence survival. The first model was performed in patients with localized disease and is shown in Table 3. Consistent with the Kaplan-Meier analysis, the year of diagnosis was not an independent predictor of survival. The strongest positive predictor of survival was surgical resection (HR = 0.388; 95% CI, 0.329–0.458). Factors that negatively influenced survival in this multivariate model were age (HR = 1.019; 95% CI, 1.014–1.025, a 2% decrement in survival per year of age), male gender (HR =1.184; 95% CI, 1.057–1.328), and African American race (HR = 1.189; 95% CI, 1.015–1.393).

The multivariate Cox model for patients with regional disease is shown in Table 4. After controlling for demographic and pathologic factors, the year of diagnosis was no longer a significant predictor of survival. Again, surgical resection was the strongest positive predictor of survival, with a hazard ratio of 0.525 and a 95% CI of 0.489–0.565. Factors that were negative independent prognostic indicators included increasing age (HR = 1.016; 95% CI,

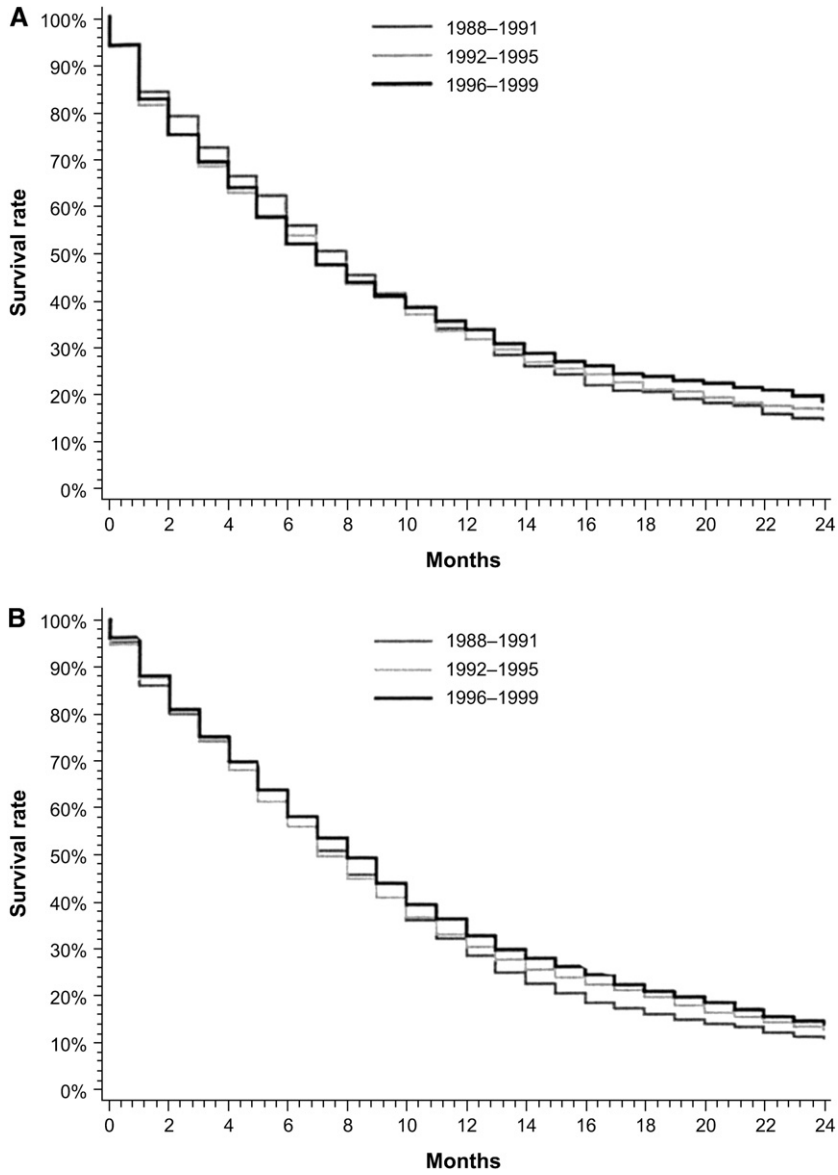

Fig. 3. (**A**) Kaplan-Meier actuarial survival curves for patients with localized disease (resected and unresected; n = 1,745) by time period. The 2-year survival was 13.8% in patients diagnosed from 1988–1991 (n = 569), 15.9% if diagnosed from 1992–1995 (n = 585), and 17.5% if diagnosed from 1996–1999 (n = 591). This observed 3% increase in survival was not statistically significant (P = 0.69). (**B**) Kaplan-Meier actuarial survival curves for patients with regional disease (resected and unresected; n = 5,745) by time period. The 2-year survival was 9.5% in for patients diagnosed from 1988–1991 (n = 1779), 12.0% if diagnosed from 1992–1995 (n = 1789), and 13.5% if diagnosed from 1996–1999 (n = 2,117). This difference was statistically significant (P = 0.0008).

1.013–1.019, a 2% decrement in survival per year of age), African American race (HR = 1.115; 95% CI, 1.018–1.221), unmarried patients (HR = 1.169; 95% CI, 1.101–1.214), adenocarcinoma compared with IPMN (HR = 1.285; 95% CI, 1.112–1.470), lesions in the body and tail of the pancreas (HR = 1.127; 95% CI, 1.021–1.244), and the presence of positive lymph nodes (HR = 1.133; 95% CI, 1.058–1.212).

Because resection was such a strong indicator of survival, a Cox model was performed for patients with regional disease with and without resection. The results are summarized in Table 5. For patients with regional disease undergoing surgical resection, a 3% increase in survival per year studied was noted. This difference was statistically significant (HR = 0.967; 95% CI, 0.951–0.984) after controlling for age, gender, marital status, pathologic diagnosis,

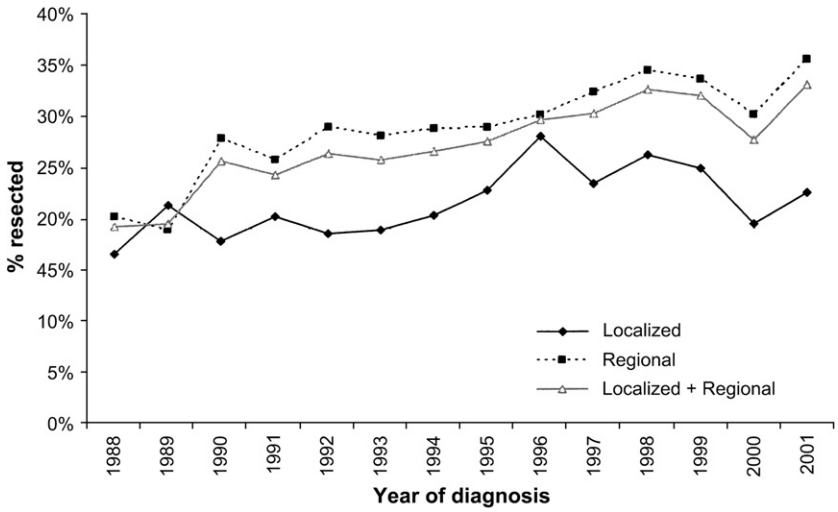

Fig. 4. The percentage of patients with localized and regional disease undergoing surgical resection from 1988–2001. This percentage has steadily increased for both groups. The *P* value for trend is 0.025 in the localized group and <0.0001 in the regional group.

and lymph node status. For regional disease without resection, the year of diagnosis was not an independent predictor of survival.

DISCUSSION

Our study evaluated the overall 2-year survival as well as changes in survival over the last decade in a population-based cohort of 24,016 patients with pancreatic adenocarcinoma identified in the SEER tumor registry. The majority of previously published literature on the treatment of patients with pancreatic adenocarcinoma is generated from high-volume centers. Few of these centers report statistics on all comers with pancreatic cancer, but when reported, outcomes are similar to those observed in the U.S. population,[1] with overall 5-year survival rates of less than 3%.[27]

Over the past two decades, high-volume centers have reported significant improvements in survival

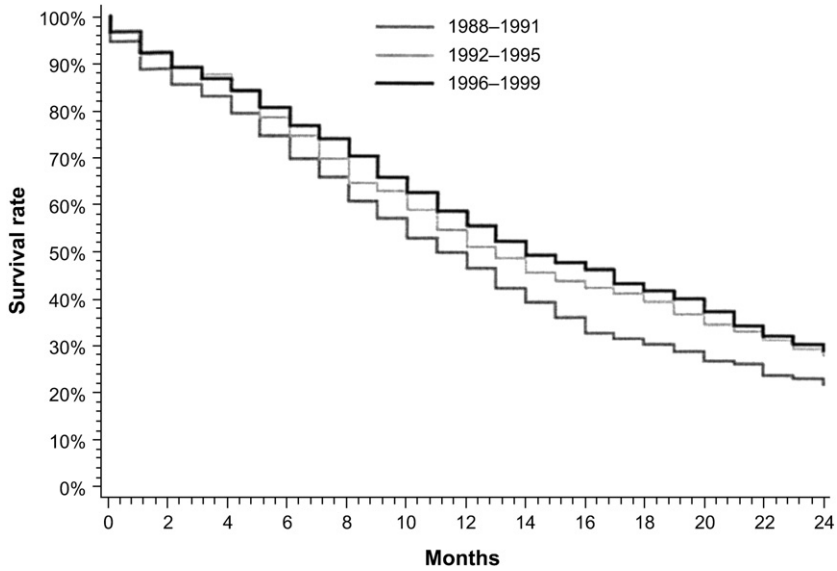

Fig. 5. Kaplan-Meier actuarial survival curves for patients with regional disease undergoing surgical resection (n = 1,630) by time period. The 2-year survival was 21.5% in patients diagnosed from 1988–1991 (n = 410), 27.6% if diagnosed from 1992–1995 (n = 512), and 28.9% if diagnosed from 1996–1999 (n = 708). This difference was statistically significant ($P = 0.0015$).

Table 3. Cox proportional hazards model: localized disease (resected and unresected)

	Final adjusted model (n = 1745)		
Variable	**Hazard ratio**	**95% CI**	***P* value***
Year of diagnosis	1.002	0.987–1.017	0.804
Age (continuous)	1.019	1.014–1.025	<0.0001
Gender			
Female	1.000	—	0.004
Male	1.184	1.057–1.328	
Ethnicity			
White	1.000	—	0.149
Black	1.189	1.015–1.393	
Hispanic	1.135	0.890–1.446	
Other/unknown	1.052	0.853–1.299	
Married			
Yes	1.000	—	0.705
No	1.023	0.911–1.148	
Histology type			
Adenocarcinoma	1.000	—	0.076
IPMN	0.778	0.590–1.026	
Site			
Head	1.000	—	0.848
Body/tail	1.002	0.891–1.234	
Others	1.036	0.870–1.150	
Positive lymph nodes			
No	1.000	—	0.074
Yes	—	—	
Unknown	1.106	0.990–1.236	
Resection			
No	1.000	—	<0.0001
Yes	0.388	0.329–0.458	

**P* value for entire category of each variable, not individual levels.

Table 4. Cox proportional hazards model: regional disease (resected and unresected)

	Final adjusted model (n = 5745)		
Variable	**Hazard ratio**	**95% CI**	***P* value***
Year of diagnosis	0.993	0.985–1.001	0.091
Age (continuous)	1.016	1.013–1.019	<0.0001
Gender			
Male	1.000	—	0.086
Female	0.951	0.898–1.007	
Ethnicity			
White	1.000	—	0.042
Black	1.115	1.018–1.221	
Hispanic	0.990	0.857–1.142	
Other/unknown	0.931	0.841–1.031	
Married			
Yes	1.000	—	<0.0001
No	1.169	1.101–1.241	
Histology type			
IPMN	1.000	—	0.0002
Adenocarcinoma	1.285	1.112–1.470	
Site			
Head	1.000	—	0.002
Body/tail	1.127	1.021–1.244	
Others	1.123	1.041–1.212	
Positive lymph nodes			
No	1.000	—	<0.0001
Yes	1.133	1.058–1.212	
Unknown	1.192	1.108–1.282	
Resection			
No	1.000	—	<0.0001
Yes	0.525	0.489–0.565	

**P* value for entire category of each variable, not individual levels.

after surgical resection. Few studies specifically report 2-year survival, but survival curves are given and 2-year survival is estimated to be 30%–40% for head lesions,[1–6] increasing to 55%–65% in node-negative, margin-negative patients,[5] and 15%–25% for body and tail lesions in these studies. The goal of our study was to determine whether the improvement in survival observed at major centers has been translated to the general population of patients with pancreatic cancer. Although 5-year survival is usually reported in the literature, we chose to report 2-year survival for the following reasons: (1) In this study and most reported studies, the median follow-up is less than 2-years, making 5-year survival estimates inaccurate. (2) The median survival for all patients with pancreatic cancer is less than 6 months and for those resected is less than 2 years, so 2-year survival has more clinical importance and is a better measure of improvements.

The overall 2-year survival rate for the 24,016 patients with pancreatic cancer identified in the SEER tumor registry was 6.2%. In analyzing the SEER data, we found that there has been no statistically significant change in overall survival over the last decade (1988–1999). However, when evaluated by stage, we found that significant improvements were achieved for those patients with regional and distant disease, but not for patients with localized disease. This improvement included patients with regional disease who underwent surgical resection. Furthermore, over the same time period, the percentage of patients undergoing surgical resection increased over time in patients with localized and regional disease. Improved staging was also noted with decreasing numbers of unstaged patients and more patients identified as having regional disease.

Our data demonstrate improvements in survival in certain subgroups of patients with pancreatic cancer

Table 5. Cox proportion hazard models: regional disease with and without resection

	Patients with resection adjusted model (n = 1630)			Patients without resection adjusted model (n = 4115)		
Variable	**HR**	**95% CI**	***P* value***	**HR**	**95% CI**	***P* value***
Year of diagnosis	0.967	0.951–0.984	<0.0001	1.000	0.991–1.009	0.935
Age (continuous)	1.012	1.007–1.018	<0.0001	1.017	1.014–1.020	<0.0001
Gender						
Male	1.000	—	0.226	1.000	—	0.167
Female	0.929	0.826–1.046		0.954	0.893–1.020	
Ethnicity						
White	1.000	—	0.659	1.000	—	0.063
Black	1.120	0.935–1.342		1.113	1.002–1.237	
Hispanic	1.051	0.787–1.404		0.960	0.814–1.133	
Other/unknown	0.993	0.790–1.249		0.917	0.818–1.027	
Married						
Yes	1.000	—	0.030	1.000	—	<0.0001
No	1.147	1.013–1.299		1.170	1.093–1.252	
Histology type						
Adenocarcinoma	1.000	—	0.001	1.000	—	0.050
IPMN	0.651	0.505–0.839		0.854	0.729–1.000	
Site						
Head	1.000	—	0.481	1.000	—	0.002
Body/tail	1.088	0.882–1.342		1.151	1.029–1.288	
Others	1.107	0.906–1.451		1.132	1.042–1.229	
Positive lymph nodes						
No	1.000	—	0.002	1.000	—	0.001
Yes	1.248	1.103–1.41		1.088	1.002–1.181	
Unknown	1.302	0.885–1.1915		1.162	1.076–1.256	

**P* value for entire category of each variable, not individual levels.

over the last decade that parallel the improvements seen at high-volume centers. Despite this improvement, the observed 2-year survival rates still lag behind those reported by major centers. In addition, the resection rates in the general population are low. The next several paragraphs will elaborate on these findings for each stage group (distant, regional, and localized), discussing the interpretation of the univariate and multivariate models and their significance. In addition, we will discuss the strengths and limitations of using the SEER dataset for this type of analysis.

Our data show that patients with distant disease had 0.9% improvement in survival from the first to the last time period. This modest observed difference is likely due to advances in chemotherapy over this same time period.[28–35] Although this difference is statistically significant, it is not clinically significant, with the statistical significance resulting from the overpowering of the study (12,043 patients) to detect the difference observed. This is consistent with data from major centers where palliative chemotherapy regimens have had little effect on long-term survival.

For those patients with regional disease, the improvement in survival is both statistically and clinically significant and parallels that seen at high-volume centers. In the multivariate model, the year of diagnosis was not a significant independent predictor of survival after controlling for surgical resection, which was a strong predictor of improved survival. This suggests that the improvements in survival for those with regional disease seen over this decade are in large part due to increased surgical resection rates. In addition to improved resection rates, the outcomes after resection have also improved, with 2-year survival increasing from 21.4% in 1998–1991 to 28.9% in 1996–1999, supporting the hypothesis that improvements in surgical technique as well as increased surgical resection rates have led to increased survival. Refinements in surgical technique may lead to lower surgical mortality and a higher proportion of R0 resections, leading to the improved survival observed. After controlling for patient demographics and tumor characteristics in the multivariate model, the year of diagnosis is a significant factor in resected patients but not in unresected patients. Because SEER does not measure

changes in mortality for surgical resection and advances in surgical technique, it is likely reflected in the year of diagnosis variable, supporting the conclusion that resection technique has improved over the years.

Similar to the group with regional disease, resection rates increased in those with localized disease, although not as dramatically. However, no statistical differences in survival were noted. The lack of improvement over time in this group is likely multifactorial. Patients with localized disease represented the minority of patients with pancreatic cancer and were probably the most aggressively treated group. Therefore, the increased surgical aggressiveness that led to improvements in survival for those with regional disease did not affect those with regional disease to the same degree. Negative margin status, or R0 resection, has been shown to be an important prognostic indicator in long-term survival after resection for pancreatic cancer.[5,6,9,10,15,16,27,36,37] Improvements in surgical technique would not increase the R0 resection rate in this group of patients given that they had disease localized to the pancreas, explaining why resected patients did not, over time, gain the same benefit seen in those patients with regional disease.

Our study suggests that surgical resection is underutilized in pancreatic cancer patients. Only 21% of patients with localized disease and 28% of patients with regional disease underwent surgical resection in this series. This is similar to findings by Krzyzanowska and colleagues[38] in a study evaluating the utilization of chemotherapy in advanced pancreatic cancer. Using the SEER data, they conclude that, despite its proven effectiveness, there is a low utilization of chemotherapy in the general population of patients with pancreatic cancer.

The SEER tumor registry is an ideal data set to study population-based outcomes. In contrast to individual state tumor registries, the SEER registries capture cases from many different regions of the country. The population covered by SEER is comparable to the general U.S. population with regard to measures of poverty and education. However, the SEER population tends to be somewhat more urban and has a higher proportion of foreign-born persons than the general U.S. population.

The SEER public use data set from 1973–2001 presented limitations in this study. Patients before 1988 were excluded from the analysis because SEER lacked coding for surgery to the primary site. Because surgical resection strongly affects survival, we felt it was important to include only the time period with this data available. In addition, 4483 patients were unstaged and could not be included in stage specific analyses. However, after exclusion, there still remained a large number of patients in each group, providing significant power to extrapolate our findings to the general population of patients with pancreatic cancer.

The data on nodal status may be inaccurate and reflect differences in pre- and post-operative staging. In unresected patients, nodal data is usually obtained from a biopsy of a lymph node and sampling error plays a significant role. The 50% figure from the SEER data, may be actually much higher. For those who are resected, this data is probably more accurate, as the complete specimen is reviewed. The lower rates of nodal positivity could be the result of varying expertise of pathologists, especially outside of high-volume centers. All nodes may not be evaluated, leading to false negative nodal status. Another explanation may be that, in the general population, surgeons are less aggressive with surgical resection and only lower stage tumors are being resected.

Unlike single institution studies, we have no way of confirming the staging information. In addition, margin status or data on R0 versus R1 resections is not available. In addition, the number of patients diagnosed with regional disease increased over time, whereas the number of unstaged patients decreased over time. It is possible that stage migration[39] could account for some of the improvement in survival seen in the group with regional disease. However, there was not a concomitant improvement in survival in the unstaged group and the unresected group showed no improvement in survival with time, suggesting that this was not the case.

Lastly, it is possible that the observed improvement in survival is driven by a subset of the patients who were treated at major centers. The SEER data does not provide individual hospital or doctor information, and this information cannot be definitively sorted out. However, many of the major pancreatic cancer surgery centers (M.D. Anderson, Memorial Sloan-Kettering, The Massachusetts General Hospital, Johns Hopkins, The Mayo Clinic, etc.) are not located in SEER regions. Nonetheless, referral to and treatment at specialized centers within this cohort may explain some of the improvement observed in our study.

In conclusion, concomitant with the improved survival seen at major centers, survival has improved in the general population of patients with pancreatic cancer. This improvement in survival can be attributed to increased surgical resection rates and improved surgical techniques over the time period studied. Surgical resection, however, seems to be underused in patients with pancreatic cancer. Further population-based studies are needed to determine

the reasons for low surgical resection rates. Are these patients too old? Are they too sick? Do they reside in an area that lacks the expertise to understand the management of this complex disease or perform the necessary operation? Strategies designed to maximize surgical resection rates may lead to further improvements in survival for this disease.

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Discussion

David B. Adams, M.D., (South Carolina): In the calendar year of 1971, Patton was named the best picture here in LA at the Oscars. Bridge over Troubled Water was album of the year. Phyllis George was crowned Miss America. Idi Amin seized power in Uganda. Mao Tse tung invited the U.S. ping pong team to visit Beijing. And President Richard Nixon declared war on cancer. A spin-off of the war on cancer and the National Cancer Act of 1971 was the Surveillance, Epidemiology and End Results program of the NCI, which began collecting data in 1973 from nine population-based cancer registries. Today's report represents the admirable and Sisyphean efforts of Dr. Riall and her colleagues to interpret an enormous amount of SEER data from 1988 to 1999 to identify pancreatic cancer treatment patterns and outcomes. As the authors note, population databases are rich and versatile tools and have the ability to identify real world practice, frequently distinct from that of clinical protocol. The large numbers of a population database allow for extraordinary statistical power. However, no large database is without problems, and there are inherent limitations in the evaluation of epidemiology and outcomes for population-based studies. Some of these well known SEER data limitations which influence the conclusions that Dr. Riall and her colleagues have drawn are as follows:

1. Miscoding and inaccuracies of hospital tumor registries are well known and may be as high as 20%.
2. Stage migration may be responsible for improvements in survival.
3. Lead time bias likewise may be responsible for apparent survival improvement when actually earlier identification of disease is what is being demonstrated.
4. Co-morbidity information and other critical data are lacking.
5. Changes in coding schemes used to classify disease stage and histology change over the study period.
6. SEER data may not be truly representative of the U.S. population with over-reporting of urban and foreign-born populations and under-reporting of southern and rural populations.
7. Reliance on secondary data lends itself to post hoc hypothesis generation.
8. Inpatient mortality is claimed not to be an ideal measure for quality and safety outcomes.
9. Deaths within 30 days of surgery are not counted as cancer deaths although frequently they are.

And finally, just to make 10. The SEER facts are the facts, but if the facts were the whole truth, then the phone book would be the *Book of Books*.

Those are my comments and these are my questions, many of which are answered in the manuscript.

1. Would you please comment on the lead time bias issue? Is widespread use of CT scanning detecting earlier disease over the study period?
2. Similarly, would you speculate on the influence of postoperative chemotherapy in improving patient outcome in the study period?
3. Would you comment on the high number of unstaged patients, 19% of the total cohort? Would this cause a significant bias in the analysis?
4. Why is surgical resection underutilized in the study group? You mentioned that but I think it bears responding to again. Does this data mean that 79% of patients within the U.S. with localized disease are inadequately treated?
5. And then finally, would you discuss the shortcomings of a two-year follow-up time period? To me this seems adequate for patients with distant disease with expectant short survival but not for those with resection for local and regional

disease. Is the 7% difference in the two-year survival of those undergoing resection for regional disease truly clinically relevant and do you expect it to be sustained in follow-up studies?

I am very grateful to Dr. Riall for providing me with a copy of this wealthy, coherent and thoughtful manuscript well in advance of this meeting and commend it enthusiastically. I look forward to further analysis of SEER data from this group and commend them for their creative and disciplined analysis they brought to us today.

Thank you very much.

Response by ***Taylor Riall, M.D.,*** (Galveston, Texas).

Dr. Riall: I would like to thank Dr. Adams for carefully reviewing our manuscript. As he details nicely, there are many limitations to using administrative data sets. Most of these are addressed in the discussion section of our paper.

You asked specifically about lead time bias. Unfortunately in pancreatic cancer we don't have a good screening test, for example a PSA and over the last decade we really haven't seen significant improvements in earlier detection for this disease. I think in this particular cohort lead time bias does not contribute significantly to the improved survival that we observed.

Postoperative chemotherapy would be related specifically to the group of patients with regional disease who underwent surgical resection. Postoperative chemotherapy probably accounts for some proportion of the improvement in survival seen in our cohort, however, it is likely a small proportion. Unfortunately, it cannot be studied using the SEER data. The data on adjuvant chemotherapy, although we recommend it, demonstrates only marginal improvement in long-term survival for patients with pancreatic cancer.

You asked about why we evaluated two-year instead of five-year survival. The median survival in patients with pancreatic cancer is approximately six months, and only eighteen months to two years for patients who undergo surgical resection. Therefore, we chose to evaluate two-year survival even though the majority of studies evaluate five-year survival. In addition, this study only has a 24-month median follow-up in our live patients, so it is inaccurate to evaluate five-year survival in this cohort with actuarial survival curves. Finally, I also think that two-year survival, given the short survival for pancreatic cancer, has clinical significance. In fact, if you project these survival curves out five years, the observed survival difference persists.

Why is surgical resection underutilized? I don't know. We have purchased the SEER Medicare data, which will provide information on patient comorbidities, as well as regional practice patterns, perhaps providing more insight into why patients did not undergo surgical resection. What I suspect is that among gastroenterologists and medicine doctors in the community there is a nihilistic attitude toward pancreatic cancer, and I suspect, that many of these patients are never referred to surgeons. I think the number is less than 79% you quoted for several reasons. First, patients with localized disease, by definition, are technically resectable, but patients with regional disease are not all technically resectable. So a proportion of those patients with regional disease probably would not be candidates for surgical resection. In addition, comorbidities may limit resectability.

Discussion by ***Russell G. Postier, M.D.,*** (Oklahoma City, OK): Most my questions have already been answered, but I think the problem we have here is we are preaching to the choir. If anything approaching 75% of patients with local or regional resectable disease were not offered resection, then that is a major problem. This doesn't need to be presented in our Journal but maybe to U.S.A. Today, or on the Oprah show or something, because this is clearly not something that we can do anything about unless we see the patients.

Response by ***Taylor Riall, M.D.,*** (Galveston, Texas).

Dr. Riall: I appreciate your comments, Dr. Postier. We have looked at the group of patients with locoregional disease and categorized the resectable and unresectable groups. We are currently analyzing the reasons for non-resection (or factors predicting surgical resection. After identifying the reasons why patients do not undergo surgical resection, we plan to present the data to nonsurgeons in an effort to maximize resection rates in appropriate candidates.

Exocrine Function Following the Whipple Operation as Assessed by Stool Elastase

Joe Matsumoto, M.D., L. William Traverso, M.D.

What impact does pancreaticoduodenectomy (PD) have on exocrine function? Does the pancreatic anastomosis remain patent? When stool elastase became available for testing in November 2001, we began preoperative assessment and then increasingly employed postoperative measurements. From December 2001 until March 2006, 182 patients underwent PD by the same surgeon. Preoperative stool elastase was measured in 138 (76%) patients and was repeated postoperatively at 3 ± 1 month, 12 ± 2 months, and 24 ± 3 months. At the same time periods, an abdominal CT scan was used to assess patency of the pancreatic anastomosis as implied by pancreatic duct dilation in the remnant (dilation = duct >3 mm or, if duct dilated preoperatively, then duct that failed to decrease in size). All cases were reconstructed with duct-to-mucosa pancreaticojejunostomy. Stool elastase was expressed as normal (>200 μg/gram stool), moderately reduced (100–200 μg/gram), or severely reduced (<100 μg/gram). Preoperative stool elastase values were "normal" in 78% (pancreatic cancer 32% normal vs. all other groups >78%; $P \leq 0.001$). As compared with preoperative values, the percent of cases with reduced elastase levels at 3 months, 1 year, and 2 years postoperatively was 48%, 73%, and 50%, respectively. The CT scans at the time of the 69 stool elastase measurements after PD showed pancreatic duct dilation in the pancreatic remnant in 9 of 69 (9%) stools but was not more frequent in the group with decreased elastase. Based on cases elastase, one third of patients about to have PD will have exocrine insufficiency, an observation most common among the patients with pancreatic cancer (68%). Stool elastase levels are further depressed in the majority of cases after PD from parenchymal loss because we could not implicate an occluded pancreatic anastomosis. These results suggest that, after PD, exocrine supplementation should be given to all patients with pancreatic cancer, especially those with impending adjuvant therapy. To further improve the long-term results after PD, each surgeon should assess the effect of their own type of pancreaticoenteric technique on exocrine function. (J GASTROINTEST SURG 2006;10:1225–1229) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Pancreaticoduodenectomy, exocrine insufficiency, pancreas

The ideal pancreatic resection preserves function of the gastrointestinal tract, the endocrine system, and exocrine secretory capacity. This is particularly important when the surgeon must deliver a healthy patient without postoperative complication for timely adjuvant therapy. Exocrine function has received the least assessment. How frequent is exocrine insufficiency present in patients who will require pancreaticoduodenectomy (PD)? What impact does subsequent PD have on exocrine function due to the reduction in pancreatic parenchyma? Beyond the effect of parenchymal reduction, how often might a stricture of the pancreatic anastomosis further compromise exocrine function? These questions have not been addressed to any degree in the literature.

When stool elastase test became commercially available to us at the end of 2001, we began preoperative assessment and then increasingly employed postoperative measurements. Testing for elastase-1 in the stool has several advantages over testing for fecal fat, trypsin, or chymotrypsin. The test does not require a timed stool collection or special diet and has a 99% negative predictive value for pancreatic insufficiency.[1] Elastase-1 is concentrated in stool yet remains enzymatically stable after intestinal transit.[2] In addition, the measurement of stool elastase-1 is not effected by exogenous pancreatic supplements,

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and the enzyme remains stable at room temperature up to 1 week after collection.[3–5]

MATERIAL AND METHODS

Over a 4.3-year period from December 2001 until March 2006, 182 patients underwent proximal PD with pancreaticojejunostomy by the same surgeon. Indications for PD were cystic tumors (36%), pancreatic adenocarcinoma (22%), periampullary tumors other than pancreatic adenocarcinoma (20%), chronic pancreatitis (20%), and other (2%). PD was pylorus-preserving in 94%.

Surgical Technique

The technique of pancreatic anastomosis was the same in all 182 cases—an end-to-side, internally stented, two-layered, duct-to-mucosa pancreaticojejunostomy. Briefly, the duct-to-mucosa anastomosis was performed with 5/0 or 6/0 interrupted absorbable sutures depending on duct size. For ducts ⩽3 mm, 4 sutures were used with one at each quadrant. With larger ducts, 5 or 6 sutures may have been required. All knots were tied down on the outside of the new lumen. The internal stent was a 4 cm long, 3 French pancreatic stent cut from a 10 cm long commercially available endoscopic pancreatic stent (Wilson-Cook Medical Inc., Winston-Salem, NC). The outer layer of the seromuscular envelope was completed with interrupted 3-0 silk Lembert sutures. Prophylactic somatostatinlike substances were not used.

Stool Elastase

Preoperative stool elastase was measured in 138 of 182 (76%) patients and was repeated postoperatively at 3 ± 1 month, 12 ± 2 months, and 24 ± 3 months. The analysis was based on a solid phase ELISA used for the quantitative determination of human elastase-1 in feces (Elastase-1 ELISA, BIOSERV Diagnostics, Rostock, Germany). The polyclonal antibodies used in this assay are specifically directed against defined sequences of the human pancreatic elastase-1 molecule. The assay is species-specific and does not cross-react with porcine elastase found in pancreatic exocrine supplements. Human elastase-1 is remarkably stable and is found in the stool in about a sixfold concentration as compared with pancreatic juice, thereby stool elastase reflects the secretory capacity of the pancreas.[2] Stool elastase was expressed as μg/gram of stool and was considered "normal" if greater than 200 μg/gram stool, "moderately reduced" if 100–200 μg/gram, or "severely reduced" if less than 100 μg/gram.

To determine if a low stool elastase value in the postoperative period was due to parenchymal reduction or stricture of the pancreatic anastomosis, we viewed an abdominal CT scan, if available, that was obtained during the same time period of each stool test. If the size of the main pancreatic duct (MPD) anywhere in the pancreatic remnant was greater than 3 mm, then the anastomosis was considered to be "strictured." If the duct in the remnant was dilated preoperatively (>3 mm), then "stricture" was assigned only if the duct failed to decrease in size.

Statistical Analysis

Continuous data are presented as mean ± standard deviation. Categorical variables were compared using the chi-square test or the Fisher exact test, where appropriate. Because some patients did not provide a stool sample at every postoperative time period, we dealt with the missing data by pairing the stool elastase values for each postresection time period to that patient's preoperative stool elastase. This comparison of continuous variables was accomplished with a paired Student's t test and the nonparametric Wilcoxon matched pairs signed rank test. The latter is more sensitive when sample sizes are small. We considered statistical significance to be achieved with a $P < 0.05$.

RESULTS

In 138 patients, 220 fecal elastase samples were measured: preoperative (n = 138), 3 months postoperative (n = 40), 1 year (n = 22), and 2 years (n = 20). Preoperative stool elastase levels were normal in 67% of 138 patients and are listed by disease in Table 1 according to the number of cases and the test results, that is, normal, moderately reduced, or severely reduced. Among the disease groups requiring PD, cases of pancreatic adenocarcinoma had the lowest rate of normal stool elastase values at 32%. When compared with all other groups, this lower rate of normal stool elastase values in pancreatic cancer cases was significantly different (Table 2). We also observed a lower incidence of normal preoperative stool elastase tests in cases with malignant versus benign disease (Table 2). Specifically, the cases considered malignant in this analysis were from the list of Table 1 and were adenocarcinoma of the pancreas, ampulla, bile duct, and duodenum. Cases also placed in this malignant group were those with islet cell cancer and those with intraductal papillary mucinous neoplasms that were invasive or carcinoma in situ.

Table 1. Results of preoperative stool elastase by disease

Disease	Cases	Normal, %	Moderate, %	Severe, %
Pancreatic adenocarcinoma	31	32	26	42
Periampullary cancer	22	77	9	14
Islet cell cancer	1	100	—	—
IPMN	41	71	10	19
Serous cystadenoma	7	100	—	—
Mucinous cystadenoma	1	100	—	—
Chronic pancreatitis	27	74	11	15
Other	8	100	—	—
Total cases	138	67	13	20

IPMN = intraductal papillary mucinous neoplasm.

Initially, we examined the change of stool elastase category in the postoperative period by tabulating if the category of preoperative elastase (normal, moderately reduced, or severely reduced) had increased, maintained, or been reduced (Table 3). Overall, the 82 postresection stool samples showed that 55% had lost their preresection level and 39% had maintained it, whereas only 6% had increased their category. At the 1-year period, the effect was most pronounced, but after the nadir at 1 year, an improvement to 50% was noted from 73% in the reduced category.

In Table 4, the actual value of stool elastase in μg/gram of stool was compared over time periods. The changes in stool elastase from preoperative to postoperative values are listed for the following subgroups: all patients, those with normal preoperative levels of elastase, and those with abnormal preoperative levels. To complete an analysis of these groups, we paired the available postoperative stool samples to the patient's preoperative level. In the "all patients" and "normal preoperative elastase" groups, a significant lowering of the stool elastase after resection was noted at 3 months, 1 year, and 2 years. In the normal preoperative elastase group, we noted a trend to increasing elastase at 2 years after a nadir

Table 2. Preoperative stool elastase by pancreatic cancer or malignant disease in 138 patients

Disease	No. samples	Normal, %	Chi-square
Pancreatic adenocarcinoma	31	32	$P < 0.001$
All other groups	107	78	
Malignant tumors	65	54	$P < 0.01$
Benign tumors	73	79	

Table 3. Change in preoperative stool elastase level at each postoperative time period

Months after PD	Increased, %	Maintained, %	Reduced, %
3 mo (n = 40)	5	48	48
1 yr (n = 22)	14	14	73
2 yr (n = 20)	0	50	50
Total (n = 82)	6	39	55

was observed at 1 year postoperatively. In the abnormal preoperative elastase group there was no difference—the values remained depressed in the abnormal range. Results for significance were similar after analysis with either Student's *t* test or the Wilcoxon matched pairs signed rank test.

A different perspective can be gained by examining the percentage of patients that began with normal stool elastase preoperatively (>200 μg/gram of stool) and how well they maintained this normal status. Only a minority of these patients still had normal values at the postresection periods: 11 of 28 (39%) at 3 months, 2 of 17 (12%) at 1 year, and 5 of 14 (35%) at 2 years.

To determine if a strictured pancreatic anastomosis might be influencing the decline in postresection stool elastase rather than just parenchymal loss or pancreatic atrophy from disease, we looked for dilation of the MPD in the postoperative period. Was there a relationship of postresection stool elastase in those with MPD dilation? A CT scan could be

Table 4. Change in preoperative stool elastase level at each postoperative time period using each patient's postoperative value paired to their preoperative value

Time period	No. of cases	Stool elastase preoperative*	Stool elastase postoperative*	Preoperative, %
All patients with normal or abnormal preoperative elastase				
3 mo	40	327 ± 140	190 ± 130†	58%
1 yr	22	304 ± 146	129 ± 59†	42%
2 yr	20	307 ± 174	175 ± 145†	57%
Patients with normal preoperative elastase				
3 mo	28	418 ± 110	223 ± 140†	53%
1 yr	17	358 ± 116	121 ± 52†	34%
2 yr	14	397 ± 116	213 ± 160†	54%
Patients with abnormal preoperative elastase				
3 mo	12	115 ± 47	112 ± 50	97%
1 yr	5	121 ± 57	154 ± 79	127%
2 yr	6	98 ± 61	87 ± 21	89%

*Elastase data in μg/gram of stool as mean ± SD.
†$P < 0.01$; paired *t* test, preoperative vs. postoperative.

matched to 69 of 82 postoperative stool samples (84%), and in 9 of 69 cases MPD dilation was observed. Cases with and without MPD dilation were then compared depending on their postresection level of stool elastase. The 69 stool samples were divided into two groups: those with elastase values that were maintained (or increased) from their preoperative levels (n = 32) and those that reduced their elastase level after surgery (n = 37). In Table 5, a relationship between MPD dilation and the level of stool elastase was not observed. For instance, at the 3-month time period in the group with stool elastase maintained or increased, 9% of 21 patients had MPD dilation observed, yet in the group with decreased stool elastase postoperatively, a similar percentage—or 6% of 17 patients—had MPD dilation. To examine if an effect on elastase levels would be better noticed in just those patients with normal preoperative stool elastase, we repeated the analysis within that subgroup but still found no significant relationship of reduced stool elastase and MPD dilation; however, few cases of MPD dilation were noted at the 1- and 2-year time periods (Table 5).

DISCUSSION

How frequent is exocrine insufficiency present in patients that will require PD? The literature does not address this question. The current study suggests that pancreatic exocrine insufficiency is common (33%) in this pre-PD group and that enzyme supplementation should be considered in many patients. Particular focus should be made on patients with pancreatic adenocarcinoma because 68% of our patients had abnormal secretory function of the pancreatic exocrine system based on stool elastase.

Table 5. MPD dilation was not associated with the ability to maintain* preoperative stool elastase levels

Period	MPD dilation if stool elastase maintained	MPD dilation if stool elastase decreased	Total samples
All postoperative samples (n = 69)			
3 mo	2/21 (9%)	1/17 (6%)	38
1 yr	0/6 (0%)	3/13 (23%)	19
2 yr	0/5 (0%)	3/7 (18%)	12
No. samples	32	37	69
Cases with normal preoperative levels (n = 50)			
3 mo	2/14 (14%)	1/14 (7%)	28
1 yr	0/2 (0%)	3/12 (25%)	14
2 yr	0/2 (0%)	3/6 (50%)	8
No. samples	18	32	50

*No significant differences–maintained versus decreased (Fisher Exact test).

Also in this study, the likelihood of finding abnormal exocrine function extended to half of the patients with malignancy and to one fifth of those with benign disease (46% vs. 21%).

What impact does the reduction in pancreatic parenchyma secondary to PD have on exocrine function? In our cases with normal exocrine secretory capacity before resection, we observed a significant reduction in stool elastase, with a nadir at 1 year and a tendency to increase at 2 years. In those with normal levels before resection, this level could be maintained at the 3-month, 1-year, and 2-year time periods in only 39%, 12%, and 35%, respectively. As in Table 4, the effect of removing about 50% of the pancreas was a 50% reduction in exocrine secretory capacity. The increased secretory capacity after the 1-year time period needs to be further investigated with more patients. Can the pancreatic secretory capacity increase over time? Or does this trend reflect only the survivors?

In Table 4, the group with abnormal preoperative elastase already had depressed secretory exocrine capacity before PD. Surprisingly, no further depression in elastase was noted after the 50% reduction of parenchyma by resection. Evidently, the sequela of MPD obstruction by the disease process had resulted in permanent exocrine atrophy, so that after the pancreatic head was removed and the MPD was decompressed, the pancreatic remnant was not able to return to predisease secretory capacity. An additional explanation might be that, if an already low elastase was detected preoperatively, perhaps the stool elastase analysis could not sense any further decline. The ELISA method has a 99% negative predictive value and an 88% positive predictive value for pancreatic insufficiency in those patients with a stool elastase of less than 100 μg/gram of stool.[1]

Very little has been written about exocrine function after PD, but all studies have found marked reductions.[6–11] Clinically, steatorrhea has been noted in 58% of 19 patients[6] and 42% of 52 patients.[7] A few reports have evaluated stool elastase after PD,[6,8–10] whereas only one reported measurements of preoperative stool elastase—and that was only in four cases.[8] Seventy-four patients in these reports were evaluated with postoperative stool elastase, from 11 days to 104 months after PD; a variety of pancreaticoenteric reconstruction techniques were used. All studies showed a marked lowering of the mean or median stool elastase below the normal level of 200 μg/gram stool (Table 6).

An ideal pancreatic anastomosis technique would be one that does not leak in the postoperative period and also does not stricture to compromise further exocrine function beyond the parenchymal

Table 6. A summary of reports measuring stool elastase after PD

Author	N	Postoperative time mean (range)	Mean or median stool elastase μg/gram stool*
Lemaire et al., 2000[6]	19	32 (12–70) mo	12 (1–34)
Lyubimova et al., 2003[8]	24	(14 d–20 yr)	29 (16–85)
Pessaux et al., 2002[9]	18	40 (3–104) mo	74 (0–32)
Mariani et al., 1999[10]	13	21 (11–44) day	~50

*Normal value is >200 μg/gram.

reduction. How often might a stricture of the pancreatic anastomosis further compromise exocrine function? First we had to assume that a strictured pancreatic anastomosis would be associated with MPD dilation. Then was MPD dilation associated with a depression in stool elastase levels? The rate of MPD dilation at the time of stool sampling was similar regardless whether the stool elastase had been maintained or decreased because the surgery. If an anastomosis was in the process of stricturing, an effect on stool elastase would be best shown in those patients with a normal preoperative stool elastase. We did not see this effect in the subgroup with normal preoperative stool elastase (Table 5) at 3 months postoperatively, but a trend was seen at 1 and 2 years. The number of cases was too small but the process of stricturing interfering with exocrine function may still be a possibility. Because of this possible change in exocrine function, we believe that stool should be periodically monitored for elastase to determine if pancreatic enzyme supplementation is required.

In summary, pancreatic exocrine insufficiency is common in those patients about to undergo PD, particularly those with pancreatic adenocarcinoma or other malignancy. In those with normal exocrine function preoperatively, the pancreatic parenchymal loss with PD will result in a reduction to abnormal secretory capacity in two thirds of cases. Pancreatic enzyme supplementation should be considered preoperatively in many patients—and in almost all cases postoperatively—because of the parenchymal reduction associated with PD. Stool for elastase should be periodically monitored to guide this therapy, realizing that exocrine function may improve if the anastomosis remains open after 1 year, or that a stricturing anastomosis might require months to interfere with exocrine function. Evaluation of any proposed pancreaticoenterostomy technique should include the ability of the technique to maintain exocrine function. Because exocrine insufficiency is so common in patients with malignant disease who undergo PD, the use of exocrine enzyme supplementation should be routine, especially for those patients about to undergo adjuvant chemoradiation, which will further compromise exocrine function due to external beam radiation.

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Pancreatic Regeneration in Chronic Pancreatitis Requires Activation of the *Notch* Signaling Pathway

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Chronic pancreatitis as an inflammatory process characterized by morphological changes, pancreatic dysfunction, and pain. During pancreatic injury and repair the *Notch* signaling pathway is reinstated. The current study analyzed this pathway in chronic pancreatitis and characterized its influence on fibrogenesis. Real-time quantitative PCR and immunohistochemistry were used for expression studies. *Notch* activation was determined by a specific luciferase-HES-1-reporter gene constructs. Cells were stimulated with alcohol, glucose, bile acids, and steroids. *Notch-2*, *-3*, and *-4* mRNA, were overexpressed in chronic pancreatitis specimens. The ligands *Jagged-1*, *-2*, and *Delta-1* were highly overexpressed. *Jagged-1* and *Notch* receptors were observed in nerves, regenerating exocrine cells, and endocrine cells. *Delta* staining was present in ductal but not in acinus cells and not in nerves. Activation of *Notch* signaling was detectable upon cell stimulation with glucose, steroids, and bile acids. High glucose levels were further associated with increased collagen-I production. The *Notch* pathway is reactivated during chronic pancreatitis. Among the stimuli activating the *Notch* pathway are steroids, high glucose levels, and bile acids. These findings suggest a possible role of the *Notch* pathway during pancreatic regeneration since *Jagged-1* inhibits inducible collagen-1 production, suggesting a new mechanism of tissue repair in this disease. (J GASTROINTEST SURG 2006;10:1230–1242) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Chronic pancreatitis, *Notch*, *Jagged*, fibrosis, pancreas

Chronic pancreatitis remains a major source of morbidity in Western countries. Most patients currently diagnosed with chronic pancreatitits have abdominal pain, maldigestion with steatorrhea or weight loss, and, with advancing disease, diabetes mellitus. However, intractable pain—often with narcotic addiction—usually dominates the clinical picture, being recalcitrant to most conservative therapies and commonly requires surgery.[1] The phrase "chronic pancreatitis" refers to a syndrome of destructive, inflammatory conditions that encompasses the many sequelae of long-standing pancreatic injury. The pathological hallmarks are inflammatory cell infiltrates, glandular atrophy with acinar cell damage, ductal changes, and irregular fibrosis with islet cell loss.[2–4] Noteworthy is that outgrowth of nerves into the inflammatory mass is a unique histological finding for this disease. Four major theories emerged to explain the pathogenesis of chronic pancreatitis: toxic-metabolic, oxidative-stress, stone and duct obstruction, and necrosis-fibrosis theories. Newer concepts of chronic pancreatitis also identified autoimmune processes with a "duct-destroying" phenotype, suggesting that chronic pancreatitits represents a primary autoimmune or inflammatory condition.[5,6] Similarly, there is growing evidence that pancreatic stellate cells and fibrogenic cytokines may play a predominant role in the pathogenesis of chronic pancreatitis.[7,8] Likely, truth is contained in each of these theories, but there is no generally accepted trigger for the development and progression of chronic pancreatitis. Futhermore, there remains

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a lack of a precise classification and stratification systems. What is known, however, is that patients with long-standing chronic pancreatitis are at a markedly increased risk of developing pancreatic cancer.[9]

On the molecular level, there is increasing evidence that transforming growth factor β1 (TGF-β1) plays a major role in fibrogenesis, because mice overexpressing TGF-β1 develop pancreatic fibrosis. In patients with chronic pancreatitis enhanced pancreatic TGF-β1 expression was observed. Similarly, interferon-γ, interleukin-10, tumor necrosis factor (TNF), inducible nitric oxide synthetase, and connective tissue growth factor may also contribute to pancreatic fibrogenesis.[8,10] The molecular correlate of nerve "sprouting" into chronic pancreatitis might be overexpression of nerve growth factors.[11–13]

Notch signaling is important in pancreatic development. Suppression of *Notch* activity leads to differentiation of pancreatic progenitor cells into endocrine cells paralleled by a depletion of exocrine progenitor cells.[14–16] Among those functions of the *Notch* signaling pathway that have been characterized most intensively are its predominant role in neuronal development and its role in T-cell specification.[17–22] Although detailed immunological characterization of chronic pancreatitis in tissue specimens remains to be shown, *Notch*-induced TCR-mediated activation of peripheral T cells with NF-κB activity and IFN-γ production might play an important role in both inflammation and nerve development of chronic pancreatitis. *Notch* and its ligands are in the center of a key link in peripheral T-cell activation and cytokine secreation.[23] Building on these findings of *Notch* as a key player in nerve development and immunological specification, we hypothesized in the present study that *Notch* genes and their ligands might play a pivotal role in the development and progression of chronic pancreatitis as well as neuronal transformation.

MATERIALS AND METHODS

Human Chronic Pancreatitis Specimens

Human chronic pancreatitis tissue samples were obtained from 22 patients undergoing pancreatic resection for chronic pancreatitis at the University Hospital of Bern, Switzerland, and the University of Heidelberg, Germany. The etiology of chronic pancreatitis was alcohol overconsumption, and surgical procedures consisted of either a partial duodenopancreatectomy or a duodenum-preserving pancreatic head resection. Histologically, chronic pancreatitis was graded as moderate to serve in all the patients. Tissue samples of 24 previously healthy organ donors served as controls and were obtained through an organ donor program. Tissue samples for RNA extraction were frozen in liquid nitrogen immediately after removal in the operating room and stored at −80°C until further analysis. The study protocol was approved by the Ethics Committees at the Universities of Bern and Heidelberg.

Immunohistochemistry

Sections of 3 μm of formalin-fixed tissues were deparaffinized and rehydrated as described previously.[24–26] Immunostaining was performed using the DAKO Envision System (DAKO, Carpinteria, Fremont, CA) according to the manufacturer's instructions. Primary antibodies were added and incubated at 4°C overnight. The following antibodies were used for immunohistochemistry: the mouse polyclonal antibody against *Notch-1* (Ab-1; Neomarkers, Fermont, CA) was used in a 1:100 dilution; the rabbit polyclonal antibody against *Notch-2* (25-255; Santa Cruz Biotechnology, Heidelberg, Germany) was used in a 1:500 dilution, and the rabbit polyclonal antibodies against *Notch-3* (M-134; Santa Cuz Biotechnology) and *Notch-4* (H-225; Santa Cruz Biotechnology) were used in 1:100 dilutions. The goat polyclonal antibody against *Jagged-1* (C-20; Santa Cruz Biotechnology) was used in a 1:500 dilution, and the goat polyclonal antibody against *Delta* (C-20, Santa Cruz Biotechnology) was used in a 1:100 dilution.

After thorough rinsing in TBS-Tween, incubation of the secondary antibody labeled with streptavidin-biotin was followed by incubation with streptavidin peroxidase and color development by DAB (3,3′-diaminobenzidine tetrahydrochloride) according to the manufacturer's instructions. Control slides were incubated either in the absence of primary antibody or with a non-specific IgG antibody. All slides were analyzed by two independent observes blinded to patient status, followed by resolution of any differences by joint review and consultation with a third observer.

Cell Culture

The fibroblast cell line (NIH-3T3) were purchased from the American Tissue Type Culture Collection (ATCC, Rockville, MD) and were cultured as previously described.[24] Normal human dermal fibroblasts (NHDFs) were purchased from PromoCell (PromoCell GmbH, Heidelberg, Germany) and cultured in fibroblast growth medium supplemented with insulin (5 μg/ml) and basic fibroblast growth factor at a final concentration of 1 ng/ml. Highly purified bile acids (>95%) chenodeoxycholic acid,

cholic acid, dehydrocholic acid, deoxycholic acid, and lithocholic acid were purchased from Sigma-Aldrich (Munich, Germany). The γ-secretase inhibitor L-685,458 was from Sigma-Aldrich and was used as in the two concentrations of 1 and 10 μM. Recombinant active human recombinant TGF-β was purchased from R&D Systems (Minneapolis, MN) and rabbit antitype I collagen from Biodesign (Saco, ME).

Preparation of Cell Lysates and Western Blot Analysis

Cells were switched to medium containing 1% FBS and were—in the case of inhibition experiments—pretreated for 1 hour with the γ-secretase inhibitor L-685,458 (10 μM). Transfected cells were incubated for 24 hours with 5 ng/ml TGF-β. Cell lysis at 4°C was done with in RIPA or lysis buffer (10 mM Tris•HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40) containing protease and phosphatase inhibitors (1 mM sodium orthovanadate, 50 mM sodium fluoride, 40 mM glycerophosphate). Lysates were centrifuged at 18,000 *g* for 10 minutes. Proteins were separated by SDS-PAGE (6% or 10% acrylamide gels), transferred onto a PVDF membrane (Millipore, Bedford, MA), and immunoblotted with anti-type I collagen antibody (0.2 μg/ml). The blots were developed with chemiluminescence. Autoradiograms were scanned and densitometric analysis was performed.

RNA Isolation and Northern Blot

Three days after cells were plated in 100-mm culture dishes, cells were switched to medium containing 1% FBS. They were preincubated with γ-secretase inhibitor L-685,458 (10 μm) for 1 hour before addition of 5 ng/ml TGF-β1 or control vehicle for 24 hours. Total RNA was harvested using TriZOL (Invitrogen, Karlsruhe, Germany) and analyzed by Northern blot as described previously. cDNAs for human 1(I) and 2(I) collagen chains were used for hybridization. Quantification of the bands on autoradiograms was performed using laser densitometric analysis. The signals obtained by hybridization with these probes were corrected for loading using the signal obtained with a human cDNA for 7S ribosomal RNA.

Transfection of NIH-3T3 Cells With *Jagged-1* and *Delta-1*

A retroviral vector containing the full-length cDNAs of human *Delta-1* or *Jagged-1* along with green fluorescent protein (eGFP) was kindly provided by Leonor Parreira (Instituto de Histologia e Embriologia, Faculdade de Medicina de Lisboa, Lisboa, Portugal). The *Notch* reporter gene construct HES-l-luc, which contained the firefly luciferase cDNA under control of the HES-1 promoter was a kind gift from Dr. Alain Israel and was described previously.[27] Cells were transfected using Lipofect-AMINE Plus Reagent according to the manufacturer's instructions (Life Technology, Rockville, MD). At 48 hours after transfection, cells were analyzed for GFP expression by fluorescence microscopy. Analysis was continued only in the case that greater than 90% of the cells expressed GFP.

Luciferase Assays

NIH-3T3 cells (1×10^5 cells) were co-transfected with 3 μg of the different luciferase reporter gene constructs along with 1 μg of pRL-CMV-Rluc, an expression vector of renilla luciferase (Promega, Madison, WI). At 24 hours posttransfection, various compounds were added and incubated for an additional 3 hours. Luciferase assays were performed using the Dual-Luciferase Reporter System (Promega), in which relative firefly luciferase activities were calculated by normalizing transfection efficiency according to the renilla luciferase activities. Fold activation of luciferase activity was calculated relative to control cells that were given the reference value of 1 as described.[28] The experiments were performed in triplicate, and similar results were obtained from independent experiments. The reported data represent the mean results from three different experiments, each performed in triplicate.

Quantification of Collagen-1 Production

Quantitative determination of collagen-1 protein levels were done by ELISA technique. After 24 hours culture media was removed and cells detached using a cell-scraper. An equal volume of the pepsin solution was added to the cells (0.1 mg/ml) and incubated on a rotator overnight at 4°C. After centrifugation (10 minutes 10,000g) the supernatant was recovered and one-third volume of neutralization solution (200 mM Tris, 150 mM NaCl) was added to the pepsin solution. After thourough mixing, 50 μl was used per well for the ELISA. The ELISA procedure was carried out according to the manufacturer's instruction (Cosmo Bio, Tokyo, Japan). All samples were measured in duplicates. Finally, optical density was determined at 450 nm, and collagen-1 concentration was calculated as μg/ml.

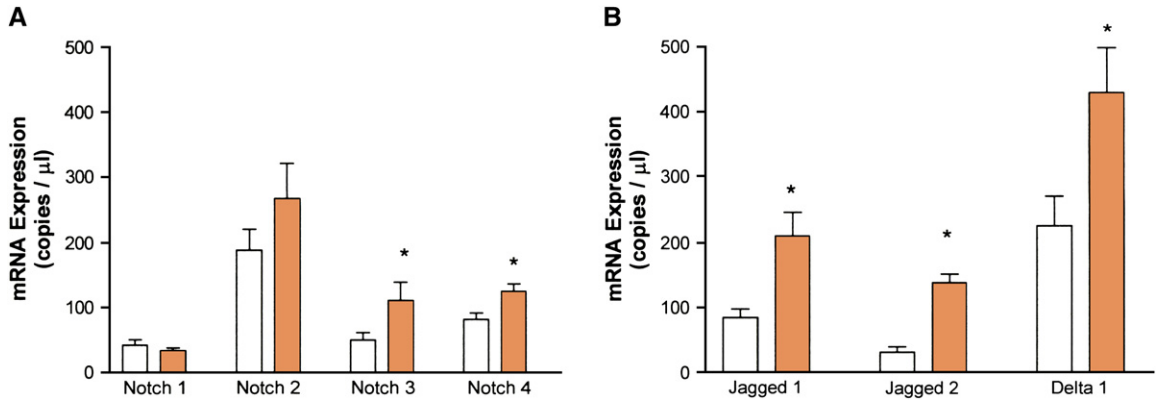


Fig. 1. Real-time quantitative RT-PCR. Expression of *Notch-1*, *Notch-2*, *Notch-3*, *Notch-4*, *Jagged-1*, *Jagged-2*, and *Delta-1* was quantified in 22 human chronic pancreatitis specimens and compared to expression of these mRNA moieties in normal pancreatic tissue specimens (n = 24). *Notch-3* and *Notch-4* were statistically significant overexpressed ($P < 0.05$), whereas differences in *Notch-2* mRNA expression did not reach statistical significance ($P = 0.06$). All ligands were significantly overexpressed ($P < .05$). Normalization of expression levels was done using cyclophilin-B as a housekeeping gene.

Real-Time Quantitative RT-PCR

Unless indicated otherwise, real-time quantitative RT-PCR was performed as previously described, and all reagents were purchased from Roche Applied Science (Mannheim, Germany).[29,30] All used primer sets had an efficiency >1.86. The data of two independent analyses for each sample and parameter were averaged. The copy number of mRNA moeties was normalized by the housekeeping gene cyclophilin-B and is presented as the number of transcripts per 10^3 copies of cyclophilin-B.

The following primers sets were used (in 5′-3′ orientation):

Statistical Analysis

Median and mean values of the respective RT-PCR results were statistically analyzed using the SAS program (Statistical Analysis System, Version 6.11; SAS Institute, Cary, NC) and the SPSS program (Version 10.0, SPSS, Munich, Germany). The *t* test procedure for unpaired samples was used to compare the overall expression in cancerous and normal pancreatic tissue samples. *P* values less than 0.05 were considered as significant.

RESULTS

Expression of *Notch* Receptors in Human Specimens

Expression of the members of the *Notch* gene family in human specimens of chronic pancreatitis was determined by real-time quantitative PCR (Fig. 1A). Included in this analysis were 22 patients with chronic pancreatitis and 24 previously healthy individuals. All four members of the *Notch* gene family were detectable in normal and in chronic pancreatitis samples. Lowest basal levels were found for the

Gene Name (GenBank Accession Number)	Forward	Reverse
Notch-1 (NM_017617)	CAATGTGGATGCCGCAGTTGTG	CAGCACCTTGGCGGTCTCGTA
Notch-2 (NM_024408)	AAAAATGGGGCCAACCGAGAC	TTCATCCAGAAGGCGCACAA
Notch-3 (NM_000435)	AGATTCTCATCCGAAACCGCTCTA	GGGGTCTCCTCCTTGCTATCCTG
Notch-4 (NM_004557)	GCGGAGGCAGGGTCTCAACGGATG	AGGAGGCGGGATCGGAATGT
Jag-1 (NM_000214)	CGGGATTTGGTTAATGGTTATC	ATAGTCACTGGCACGGTTGTAGCAC
Jag-2 (NM_002226)	ACCAGGTGGACGGCTTTG	CCGCGACAGTCGTTGA
Delta-1 (NM_005618)	CCTACTGCACAGAGCCGATCT	ACAGCCTGGATAGCGGATACAC

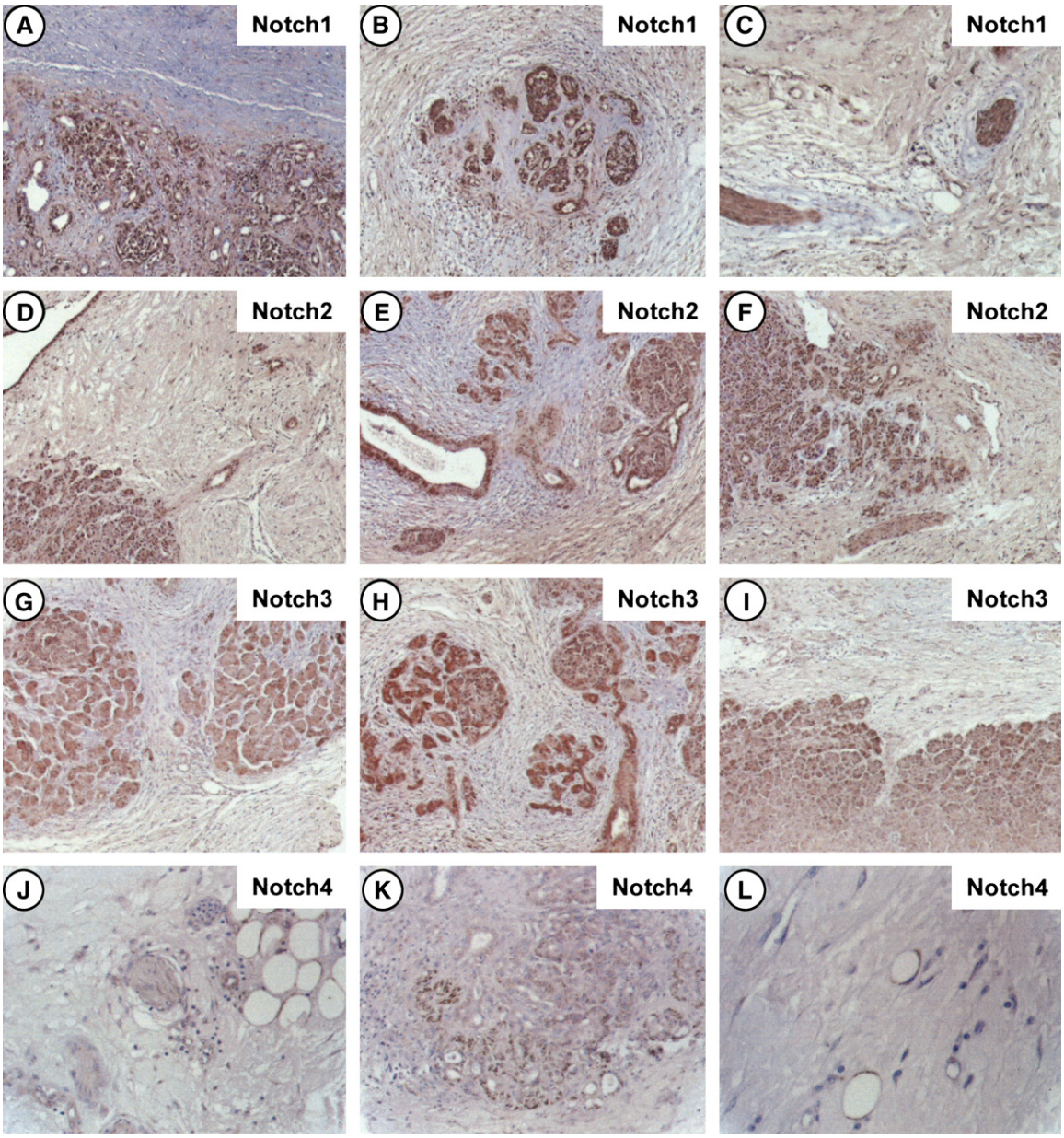
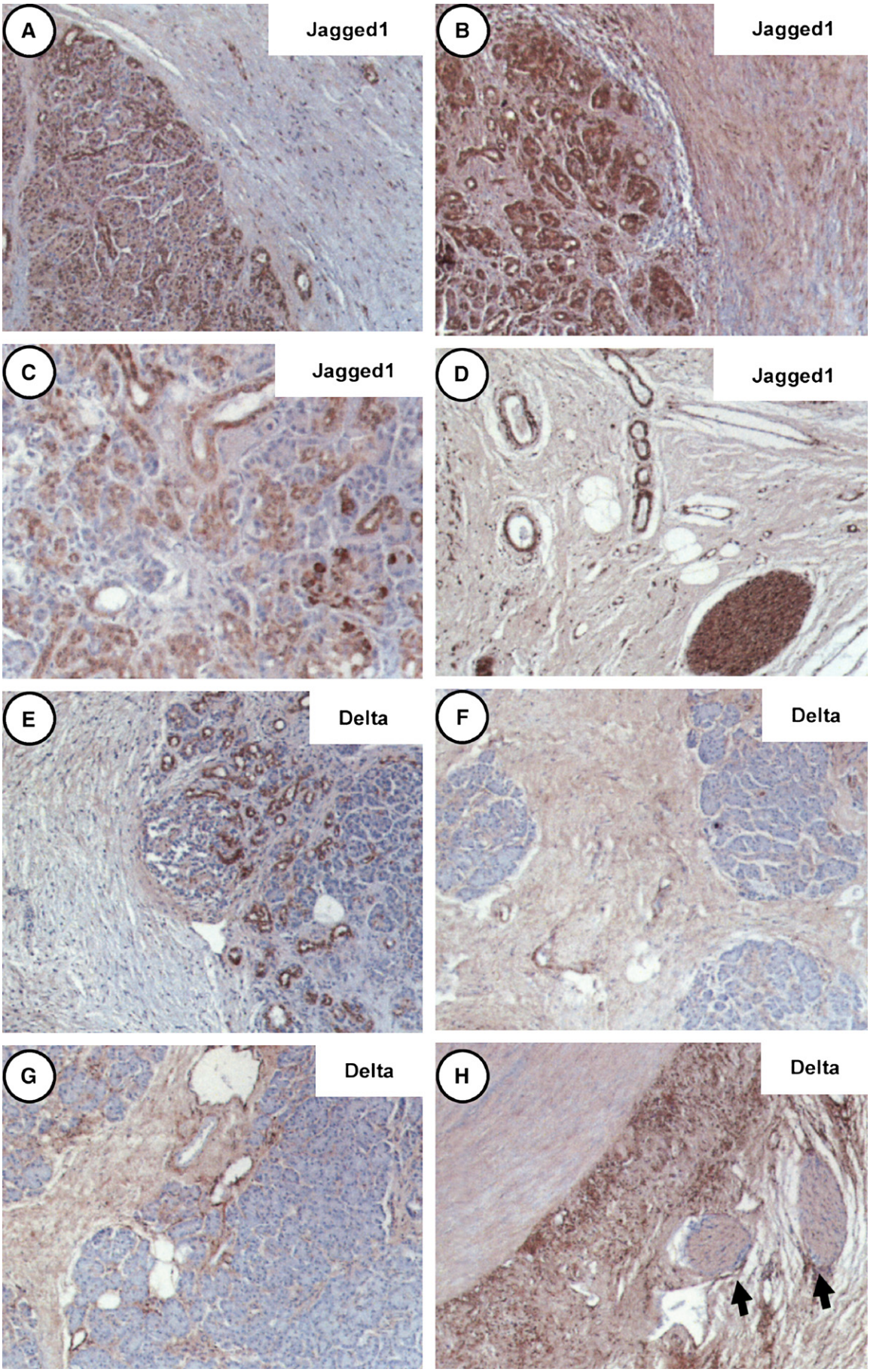


Fig. 2. Immunohistochemistry of *Notch* family members in chronic pancreatitis samples. *Notch-1* immunoreactivity was present in damaged acinar tissue, metaplastic ducts (**A, B**) and in CP associated nerves (**C**). *Notch-2* was detectable in metaplastic ducts and acinar tissue cells (**D–F**). *Notch-3* immunoreactivity was marked in acinar and ductal cells close to fibrosis (**G–I**) *Notch-4* was in acinar tissue and transforming ducts (**J–L**). Magnification ×200.

Notch-1 gene. Its expression level did not change during the course of chronic pancreatitis. In contrast, higher mRNA levels were detectable for *Notch-2* gene, which was upregulated in chronic pancreatitis tissues, but due to the small sample size this increase did not reach statistical significance. *Notch-3* and *Notch-4* gene expression was significantly upregulated in chronic pancreatitis ($P < 0.05$). *Notch-3* was 2-fold higher in chronic pancreatitis tissues than in normal pancreatic specimens, and *Notch-4* was about 1.5-fold higher in the diseased pancreas.

Expression of *Notch* Ligands in Human Specimens

mRNA expression of the ligands *Jagged-1*, *Jagged-2*, and *Delta* were measured in the same specimens as the *Notch* receptors (Fig. 1B). All ligands were


A
Jagged1
B
Jagged1
C
Jagged1
D
Jagged1
E
Delta
F
Delta
G
Delta
H
Delta

expressed in normal pancreatic tissues with marginally low levels of *Jagged-2* expression. *Jagged-1* mRNA expression was more than 2.5-fold upregulated in chronic pancreatitis specimens but was also detectable in normal pancreatic tissues ($P < 0.05$). *Jagged-2* mRNA expression was not expressed in biologically relevant levels in normal pancreatic tissue specimens but was almost 4.5-fold increased in chronic pancreatitis specimens ($P < 0.05$). *Delta-1* expression was detected at relatively high levels in normal pancreatic specimens with a significant upregulation (1.9-fold) in chronic pancreatitis samples ($P < 0.05$).

Localization of *Notch* Expression in Human Tissue Specimens

Localization of *Notch* gene members in human tissue specimens was done by immunohistochemistry *Notch-1* immunoreactivity was detectable in pancreatic acini close to fibrosis (Fig. 2, *A* and *B*) but was also detectable in nerves within the inflammatory mass (Fig. 2, *C*). Similarly, strong *Notch-2* immunoreactivity for was present in ducts within the fibrotic tissue as well as pancreatic acini, nerves and transforming ducts (Fig. 2, *D–F*). Fibrotic tissue itself did not stain positive for *Notch-2*. Similarly, *Notch-3* was strongly positive in pancreatic acini and ductal cells close to or within the fibrotic tissue (Fig. 2, *G–I*). *Notch-4* was found to be expressed by acinar tissue and tubular complexes next to pancreatic acini and fibrosis (Fig. 2, *I–L*). Furthermore, *Notch-4* expression was seen in endothelial linings of blood vessels (Fig. 2, *L*). For negative controls, we used immunization peptides or omitted either first or second antibody (data not shown).

Localization of *Notch* Ligands in Human Tissue Specimens

Localization of *Jagged* and *Delta* in human specimens revealed that *Jagged-1* was strongly present in exocrine and endocrine tissue. A strong signal for *Jagged-1* was present in areas next to fibrotic tissue, transforming acinar/ductal tissue structure, and in nerves entrapped in the fibrotic tissue (Fig. 3, *A–D*). *Jagged-2* immunostaining in human specimens did not reveal a specific positive signal. In clear contrast, *Delta* staining was present in tubular complexes and metaplastic ducts but was largely absent in acinar cells (Fig. 3, *E–H*). Furthermore, the connective tissue itself was found to stain positive for *Delta*. Nerves were devoid of *Delta* immunoreactivity (Fig. 3, *H*). For negative controls, we used immunization peptides or omitted either first or second antibody (data not shown).

Microenvironmental Factors Induce *Notch* Signaling

In order to test whether some of the proposed triggers of chronic pancreatitis induce *Notch* signaling, we transfected NIH-3T3 cells with a *Notch* reporter gene construct (*HES-1-luc*). Among the factors tested for *Notch* activation was alcohol (1 mm), D-glucose (30 mm D-glucose), dexamethasone (10 μM DEX), and bile acids (50 μM cholic acid and chenodeoxycholic acid (GCDCA). After transfection of NIH-3T3 cells with the *HES-1-luc* construct, compounds and inhibitors were added at the indicated concentrations. Three hours after compound addition, dual luciferase assays were performed and transcriptional activity of the HES-1 promoter in each condition was determined. Addition of dexamethasone sharply induced HES-1 promoter activation (Fig. 4, *A*). A similar increase was observed after the addition of D-glucose. Both dexamethasone and glucose induced activation of the HES promoter element were revertible by the addition of 10 m γ-secretase inhibitor L-685,458, which was added 1 hour ahead of the compounds (Fig. 4, *A*). Bile acids increased transcriptional activity of the HES-1 promotor but in contrast to the aforementioned compound, the transactivation could not be inhibited completely by γ-secretase inhibitor L-685,458 (Fig. 4, *B*).

Measurement of Collagen-1 in Cell Culture

In order to measure collagen production upon activation of the *Notch* signaling pathway, we performed an ELISA procedure quantifying collagen-1 production. Among the microenvironmental factors tested, only high glucose levels (30 mm D-glucose) increased collagen-1 production in NHDF and NIH-3T3 fibroblasts (Fig. 5). For positive control, recombinant TGF-β (5.0 ng/ml) was used to

Fig. 3. Immunohistochemistry of *Notch* ligands in chronic pancreatitis tissues. Strong immunoreactivity was present for *Jagged-1* in acinar cells close to fibrosis and damaged ductal cells (**A–C**) as well as in encased nerves nervs (**D**). *Delta-1* immunoreactivity was primarily found in ductal cells, whereas acinar cells did not stain positive for *Delta-1* (**E–H**). *Nerves* were devoid of *Delta-1* expression (*H*, *arrows*). Magnification ×200.

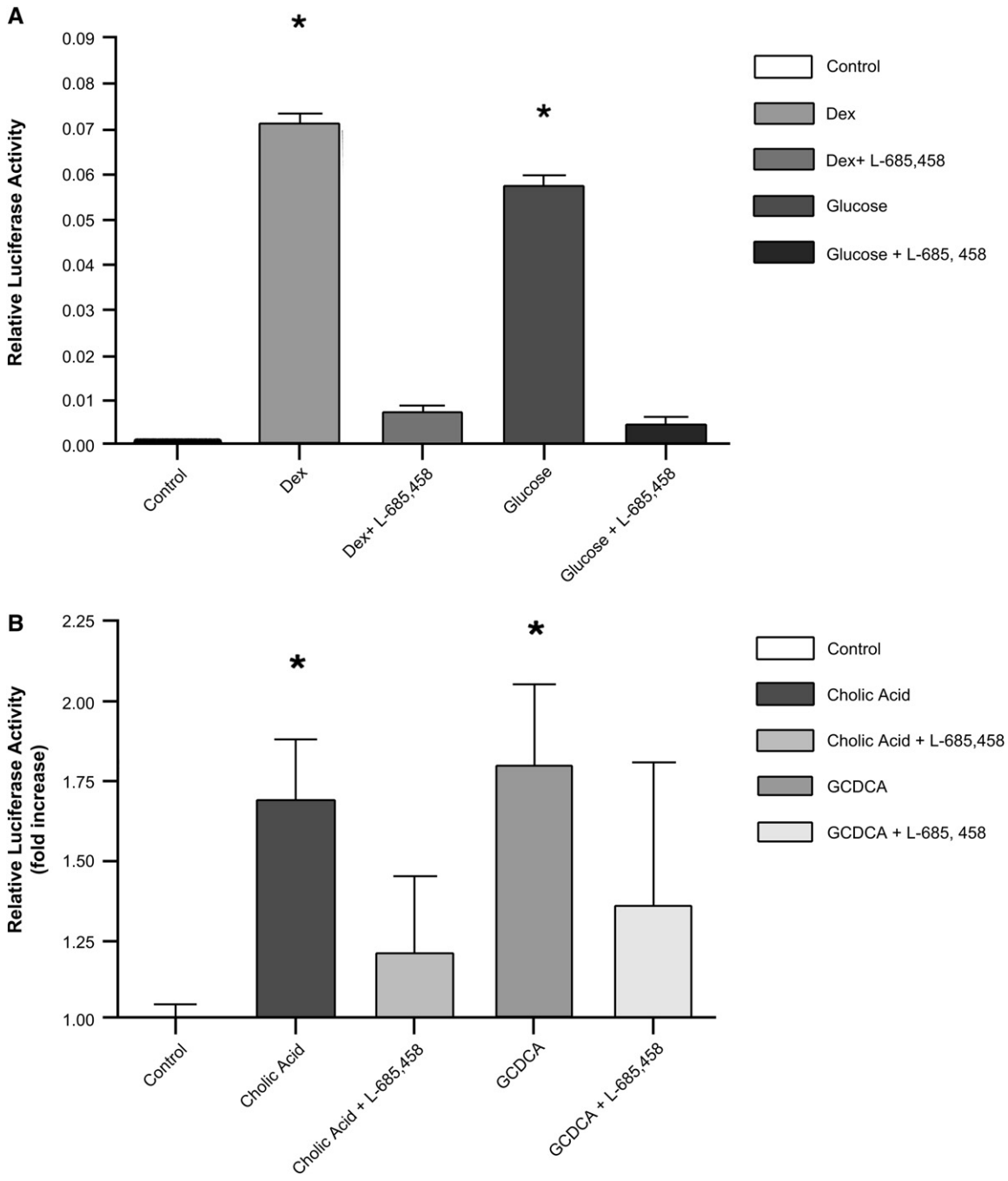


Fig. 4. Notch activation assays. NIH-3T3 cells were transfected with a *Notch* reporter gene construct (HES-1-luc). The concentrations used were dexamethasone (10 μM DEX), D-glucose (30 mm) (**A**), and bile acids (50 μM) (**B**). The γ-secretase inhibitor L-685,458 was used at 10 μM concentration and was added 1 hour prior to the addition of compounds. Three hours after compound addition, a dual luciferase reporter assay was performed. HES-1 promoter activity was measured as the relative ratio of firefly and renilla luciferase activities. All values were corrected by the background luciferase activity in endogenous *Notch*-expressing cells transfected with HES-1-luc and pRL-TK.

stimulate collagen synthesis in fibroblasts. Addition of the γ-secretase inhibitor L-685, 458 did not change collagen production after D-glucose stimulation or in TGF-β–stimulated fibroblasts suggesting a *Notch* independent collagen-1 increase (Fig. 5). Dexamethasone (10 μM DEX) and bile acids (50 μM cholic acid, 50 μM chenodeoxycholic acid) did not change collagen-1 synthesis (data not shown).

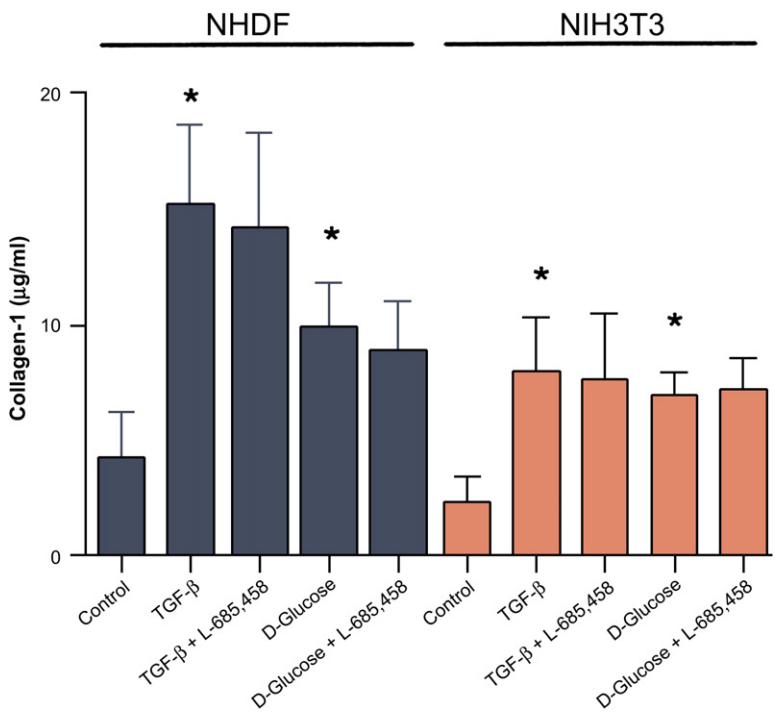


Fig. 5. Collagen-1 quantification. NIH-3T3 cells were transfected with a *Notch* reporter gene construct (HES-1-luc). D-Glucose concentration was 30 mM. The γ-secretase inhibitor L-685,458 was used at 10 μM concentration and was added 1 hour prior to the addition of compounds. For positive control, TGF-β (5.0 ng/ml) was used. Cells were grown for 48 hours in DMEM supplemented with 10% FBS, washed three times with PBS, and changed to 1 ml, of DMEM supplemented with 1% FBS and compounds. After 48 hours, cells were analyzed by ELISA, and collagen-1 concentration was measured. All experiments were repeated at least twice. $^*P < 0.05$ indicates a significant increase compared with untreated cells.

Overexpression of *Jagged-1* and *Delta-1* in Fibroblasts

NIH-3T3 cells were transfected with a retroviral pEGFP carrying cDNA expression plasmids encoding the human *Jagged-1* and *Delta-1* genes. mRNA and protein extraction was done 36 hours after cell transfection. *Jagged-1* transfection suppressed basal collagen-1 production in fibroblast cultures (Fig. 6), whereas *Delta-1* overexpression exerted only a moderate effect (data not shown). More strikingly, however, was the suppression of collagen production in fibroblasts stimulated by TGF-β. The TGF-β–mediated induction of collagen-1 production could almost be antagonized by *Jagged* overexpression (Fig. 6). In order to further address whether this effect was specific to *Jagged-1* overexpression, we added the γ-secretase inhibitor L-685,458 (10 μM). Inhibition of γ-secretase neutralized the suppressive effect in *Jagged-1* transfected cells and increased collagen-1 production. This finding suggests that TGF-β–induced activation of collagen-1 production was specific to *Jagged-1* expression.

DISCUSSION

Chronic pancreatitis is a disease that primarily affects the exocrine pancreas but also involves the endocrine tissue Chronic pancreatitis is characterized by continuous destruction of pancreatic acinar and ductal cells and deposition of fibrous tissue. Ectopic deposition of extracellular matrix can cause a mass phenomenon in the pancreatic head with subsequent compression and occlusion of adjacent structures. Overproduction of extracellular matrix often compromises the function of the remaining vital parenchyma. Several hypotheses have been postulated to explain the development of chronic pancreatitis, but so far the pathogenesis of this disease remains largely unclear. Nothing is known as to whether the *Notch* pathway is involved in the pathogenesis of chronic pancreatitis today. However, it is known that *Jagged-1*, a ligand of the *Notch* receptors, is inducible by laminin, fibronectin, and Matrigel.[31–33] Furthermore, *Notch* signaling has been associated with mesenchymal specification, suggesting possible antifibrotic effects in chronic pancreatitis.[34–36]

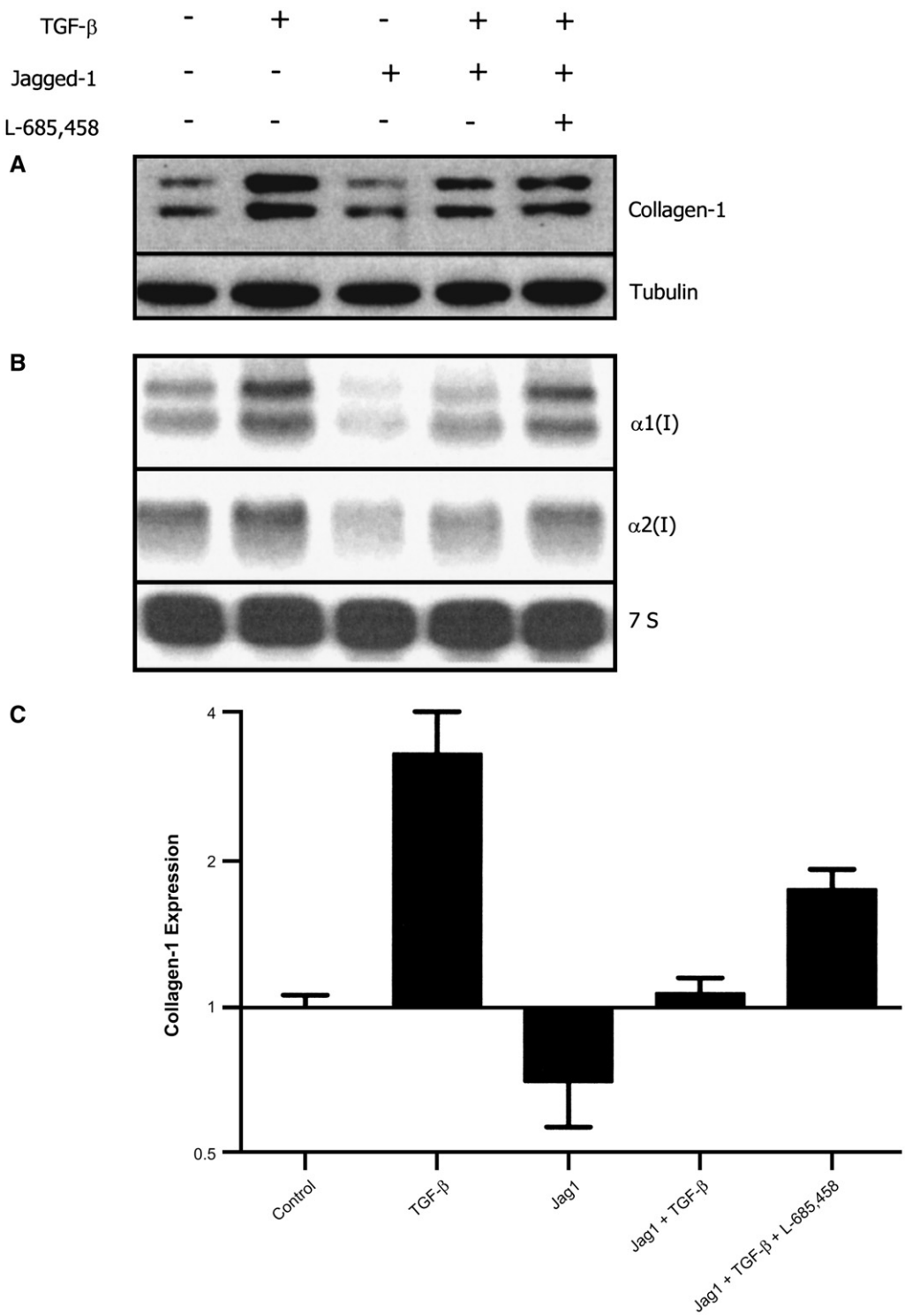


Fig. 6. Effect of *Jagged-1* on collagen-1 expression. NIH-3T3 cells were grown under the indicated conditions. As positive control, TGF-β (5.0 ng/ml) was used. *Jagged-1* indicates transfection of NIH-3T3 cells with an expression plasmid of *Jagged-1*. Western blot analysis was done (**A**) and collagen-1 protein expression was analyzed. Tubulin served as loading control. MRNA expression was quantified by Northern blot analysis for collagen-1 (**B**, both subunits) and normalized against 7S. The corresponding laser densitometry of expression studies is shown in **C**, where untreated NIH-3T3 cells were set as 1.

In the present study, we found that *Notch* family members were differentially expressed in human chronic pancreatitis tissues compared with normal pancreatic tissues. *Notch-3* and *Notch-4* were found to be expressed at higher levels in chronic inflammatory pancreatic tissue. *Notch-2* was also upregulated but, due to the small sample size, not significantly elevated. The highest relative increase in mRNA expression was found for *Notch-3*, which was more than 2-fold upregulated. It is well known from studies in zebra fish that *Notch-3* maintains cell–cell interactions and cellular communication of vascular smooth muscle cells, which may trigger fibrosis as seen, for example, in the CADASIL syndrome, a disease caused by a single point mutation of *Notch-3*.[37] Immuohistochemical analysis revealed that *Notch* receptors were expressed by damaged acinar cells and metaplastic ducts that were either close to or encased within the fibrotic tissue. *Notch* expression was not only detectable in exocrine pancreatic cells but also in nerves within the inflammatory tissue.

The *Notch* ligands *Jagged-1*, *-2*, and *Delta-1* were also significantly upregulated in chronic pancreatitis tissue. *Jagged-2* was not expressed in relevant levels in normal pancreas but was markedly upregulated in chronic pancreatitis. Spatial expression analysis in tissue samples indicated that *Jagged-1* expression was mainly present in damaged ductal cells and metaplastic ducts as well as in nerves in chronic pancreatitis. In contrast to *Jagged-1*, immunohistochemical analysis of *Delta-1* revealed that acinar cells and nerve tissue were devoid of *Delta-1*, which stained strongly positive in ductal cells and in connective fibrous tissue.

In further analyses, we tested several compounds that are associated with the pathogenesis or therapy of chronic pancreatitis. We tested ethanol, amylase, lipase, glucose, bile acids, and steroids, which are currently used for therapy of autoimmune chronic pancreatitis with regard to induction of HES promoter activity, serving as a reporter gene assay for *Notch* activation. Among the factors that increased *Notch* reporter gene activity were dexamethasone and high glucose levels, as well as bile acids. In order to further delineate the specific role of these compounds, we used a γ-secretase inhibitor, L-685, 458, to specifically block *Notch* signaling. In contrast to steroids and glucose, bile acids increased the transcriptional activity of the HES-1 promotor, but transactivation could not be inhibited completely by *Notch* inhibitors. In order to determine whether *Notch* signaling may influence fibrosis in chronic pancreatitis we measured collagen production upon activation and found that high glucose levels (30 mm D-glucose) increased collagen-1 production, as recently shown also for pancreatic stellate cells.[38] Because glucose-induced upregulation of collagen-1 synthesis could not be reverted by the addition of *Notch* inhibitors, it is likely that this effect was independent of *Notch* signaling despite glucose itself induced *Notch* reporter gene activity. Because it has previously been reported that *Notch* activation negatively regulates chondrogenic as well as osteogenic differentiation and recent reports demonstrated that *Jagged-1* was able to inhibit of pro-alpha 1(I) collagen expression, *Jagged-1* and *Delta-1* transfection studies were done to further address the role of this pathway in fibrogenesis. *Jagged-1* transfection suppressed basal collagen-1 production in fibroblast cultures, whereas *Delta-1* overexpression exerted no substantial effect. More important, suppression of collagen-1 production induced by TGF-β was detectable in *Jagged-1*–overexpressing fibroblasts, suggesting that *Jagged-1* neutralizes the profibrotic activity of TGF-β. This neutralizing effect was blocked by addition of a γ-secretase inhibitor, suggesting that TGF-β–induced activation of collagen-1 production was dependent on *Jagged-1* expression.

Taken together, our present study defines for the first time a new role of the *Notch* signaling pathway in the pathogenesis of chronic pancreatitis, especially in fibrogenesis. We have demonstrated that *Notch* receptors and ligands are overexpressed in chronic pancreatitis and it appears that *Jagged-1* exerts an antifibrotic effect and might therefore play an important role in pancreatic regeneration and repair or activates protective mechanisms in this disease.

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Discussion

Stanley W. Ashley, M.D. (Boston, Mass): Peter, that is an elegant study. We have very little understanding of why some pancreatic insults are associated with complete recovery while in others there may be some regeneration and or just fibrosis. I had a little trouble understanding your findings—was there any evidence of increased *Notch* signaling? What does the normal pancreas look like in terms of the expression of these factors? Do you think this activation is something that is occurring predominantly in the acinar cells, or is it is a response that is occurring in the fibroblasts and that is why there is fibrosis, a result of upregulation of these factors? Finally, I wonder if you would speculate on where you are going to go with this—could you direct therapeutic maneuvers at the *Notch* pathway?

Peter Buechler, M.D. (Heidelberg, Germany): The problem with this disease is currently that we have no good animal model, so we were mostly dependent upon descriptive observations. As a matter of fact, *Notch-1* and *Notch-2* also do stain positive in a normal pancreas; however, there is a clear difference toward the fibrotic area. So there must be a regulation of this genes in one or the other way. Certainly the main problem is that we don't have a good model and we cannot test these systems in an in vivo setting. So whatever these observations mean, we frankly don't know. We are dependent upon the development of a chronic pancreatitis model where we can analyze these pathways in vivo.

With regard to the *Jagged* expression and collagen regulation, there is almost nothing reported in the literature. To my best knowledge, this is the first study showing that it regulates collagen production, which certainly has something to do with fibrosis in this disease. So we think *Jagged-1* may be a protective gene, in the development of fibrosis, and from there we are certainly dependent on transgenic models to further investigate the real effect in vivo.

Herbert Chen, M.D. (Madison, Wisc): I enjoyed your talk. I had a quick question about your expression patterns in the tissues. As you know, *Notch* is a full-length receptor that needs to be cleaved to be activated, and you have shown nice data showing that both the receptor and possible ligands are present, but do you have any evidence that the pathway is activated by either upregulation of HES or perhaps downregulation of neurogeninor some other markers downstream of *Notch*?

Dr. Buechler: We do mention HES. This question we addressed in a separate study. As a matter of fact, HES is activated and also overexpressed in this disease, but at this point we do not have more data on HES-1. Neurogenin, we did not analyze so far.

Hospital Readmission After Pancreaticoduodenectomy

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Data exist on the morbidity and mortality of patients undergoing pancreaticoduodenectomy (PD), but there are few reports about hospital readmissions after this procedure. Our aim was to evaluate the number of and reasons for readmission after PD and the factors influencing readmission. We reviewed the initial hospitalization and readmissions for 1643 patients undergoing PD compared patients requiring readmission to patients that did not require readmission. Twenty-six percent of patients were readmitted a total of 678 times after PD. Patients readmitted were younger than those not readmitted (61.8 versus 64.6 years, $P < 0.0001$). Vessel resection, abscess formation, wound infection, postoperative percutaneous biliary stents, estimated blood loss >1000 ml, and age ≤ 65 years were independently associated with readmission. The length of stay for all patients decreased over time, from 10.5 days in 1996 to 7 days in 2003. The percentage of patients being readmitted also decreased from 33% in 1996 to 20% ($P = 0.004$) in 2003. The readmission rate after PD was 26%. Younger age, blood loss, postoperative complications, and vessel resection were independent risk factors for readmission. The early hospital readmission rate has not increased in association with a decreased LOS, supporting the idea that reduction in LOS did not lead to increased readmission rates. (J GASTROINTEST SURG 2006;10:1243–1253) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Readmission, pancreaticoduodenectomy, length of stay

Over the last two and a half decades, pancreaticoduodenectomy (PD) has been performed with increasing frequency for both benign and malignant diseases of the head, neck, and uncinate process of the pancreas and periampullary region. Many major centers report mortality rates of less than 5% after this procedure.[1–7] The observed decrease in postoperative mortality is multifactorial and includes improved surgical technique, improved critical care, as well as improved prevention and management of the complications of pancreaticoduodenal resection such as pancreatic fistula, biliary leak, and intra-abdominal abscess, which were often fatal in the 1970s.[8,9] Concomitant with the decreased postoperative mortality rates, postoperative lengths of stay have significantly decreased after PD.[5,7,10]

Many studies have documented the effects of regionalization of care at specialized "Centers of Excellence" on in-hospital mortality after PD.[11–16] Such regionalization of care contributes to decreased lengths of stay, decreased hospital costs and improved short- and long-term surgical outcomes after complex pancreatic surgery. The implementation of clinical pathways (critical pathways, care maps, etc.) for PD[17] and other complex gastrointestinal procedures[18] at these high-volume centers optimizes clinical outcomes by streamlining care across the multidisciplinary team of providers caring for these patients.

While the postoperative lengths of stay, postoperative mortality, and overall costs of care after PD have been decreased by the implementation of clinical pathways and regionalization of care,[7–19] the morbidity rates after PD remain high, with many centers reporting overall complication rates of 30–60%.[3,5,6,8,20] Readmission to the hospital after PD is a common occurrence and increases hospital costs. Patients readmitted with tumor recurrence have

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been shown to have a poor long-term prognosis, while patients readmitted for many postoperative complications have a better long-term prognosis, provided their problem that prompted readmission is addressed.[21]

The objectives of this report are threefold. First, we will report the number of readmissions to The Johns Hopkins Hospital after PD and determine the factors that predict readmission after this complex surgical procedure. Second, we will characterize the reasons for readmission and compare early readmissions ($\leq$1 year after surgery) to late readmissions ($>$1 year after surgery). Third, we will test the hypothesis that the readmission rate has not increased concomitantly with the decrease in postoperative lengths of stay observed over the past two decades.

PATIENTS AND METHODS

We reviewed the initial hospitalization and subsequent readmissions for 1643 patients undergoing PD between January 1996 and December 2003 at The Johns Hopkins Hospital. Patients with both benign and malignant diseases of the pancreas and periampullary region were included. Patients readmitted to any Johns Hopkins Hospital service between the time of their initial surgery and the time of data analysis (November 12, 2005) were candidates for inclusion in the analysis. Patients readmitted for reasons unrelated to their PD or underlying disease process were excluded. Readmissions to outside hospitals were also excluded, as our ability to capture outside hospital readmission data was limited. It should be noted that many of these patients were the subjects of several previous reports from this institution.[5,6,20,22–33]

The surgical approach to the patients included in this study is as follows: A standard pancreaticoduodenal resection without extended retroperitoneal lymph node dissection was performed, except during the time period of 1996–2001 when our institution performed a prospective, randomized trial comparing standard pylorus-preserving resection to extended resection with distal gastrectomy and retroperitoneal lymphadenectomy for those patients with periampullary adenocarcinoma.[6] The majority of resections were pylorus-preserving, adding a distal gastrectomy only for cancers involving the distal stomach or first portion of the duodenum or as part of the above randomized trial.[6] Partial pancreatic resection was preferred, leaving the pancreatic body and tail in place unless the tumor extended across the pancreatic neck margin into the body and tail of the gland. A pancreaticogastrostomy or pancreaticojejunostomy was used for pancreatic-enteric reconstruction. Vagotomy, tube gastrostomy, feeding jejunostomy, total parenteral nutrition, and prophylactic octreotide administration were not routinely used.

Patients were placed into two groups based on readmission status (readmitted versus not readmitted) to The Johns Hopkins Hospital. The demographic factors, presenting symptoms, comorbidities, intraoperative course, pathology, and postoperative course of those patients requiring readmission were compared to those patients who did not require readmission. To determine factors associated with readmission, a univariate analysis was performed. χ^2 Tests were used to compare all categorical variables and Wilcoxon rank sum tests were used to compare all continuous variables. A multivariate linear logistic regression analysis was used to determine the odds ratios of the variables found to independently increase the likelihood of hospital readmission after PD.

For those patients requiring readmission, the number of readmissions, reasons for readmission, and tests/procedures performed during the readmission were evaluated. Readmitted patients were then further classified into early readmissions (within 1 year of pancreaticoduodenal resection) or late readmission (more than 1 year after pancreaticoduodenal resection). The reasons for readmission in the early and late groups were compared using a univariate χ^2 analysis. Follow-up was obtained from office records, telephone contact, the Social Security Administration database, or public records. Survival information was complete on 1640 of 1643 patients undergoing PD during this time period, with only three patients lost to follow-up.

As many patients required more than one readmission, those patients admitted more than once were compared to those requiring only one readmission in a univariate analysis using the same statistical analyses mentioned previously.

All means are reported as mean $\pm$ standard deviation of the mean. Median values are provided. All categorical data were compared using a χ^2 analysis and all continuous variables were compared using nonparametric Wilcoxon rank sum tests. Statistical significance was defined at the $P \leq 0.05$ level.

RESULTS

Of the 1643 patients undergoing pancreaticoduodenal resection at The Johns Hopkins Hospital between January 1996 and December 2003, inclusive, 431 patients (26%) were readmitted a total of 678

times for a total of 4847 hospital readmission days. The mean duration of readmission was 7 days. 142 of the 431 patients (33%) were readmitted more than once. Of the patients readmitted, 308 (71%) were readmitted within 1 year of their initial PD, while 123 (29%) were readmitted more than 1 year after initial resection.

The 431 patients requiring readmission were then compared to the 1212 patients who did not require readmission. The demographic characteristics and presenting symptoms of the two groups are compared in Table 1. Patients who were readmitted were younger, more likely to be African American, and more likely to be male. The presenting symptoms were similar between the two groups. Despite a similar incidence of jaundice in the two groups, readmitted patients were less likely to have had a preoperative endoscopic biliary stent placed, but more likely to have had a preoperative percutaneous biliary stent placed when compared to those patients not requiring readmission.

The incidences of specific comorbidities were similar between the two groups with no statistical differences. For the overall cohort of 1643 patients, 38% of patients had hypertension, 17% had coronary artery disease, 7% had a myocardial infarction, 21% were smokers, 18% had diabetes mellitus, 12% abused alcohol, 5% had peripheral vascular disease, chronic obstructive pulmonary disease, chronic pancreatitis, or acute pancreatitis, 4% had peptic ulcer disease, and 1% had inflammatory bowel disease or a pancreatic pseudocyst. Overall poor health may increase the risk of readmission, not just individual comorbidities. Therefore, we evaluated patients with ⩽1 of the above listed comorbid conditions compared to patients with two or more conditions. Twenty-nine percent of readmitted patients and 30% of patient not requiring readmission had ⩽1 comorbidity ($P = 0.7$).

The pathologic diagnoses of patients in each group are shown in Table 2. In the overall cohort, 76% of patients had malignant disease and 24% had benign disease. When comparing those patients requiring readmission to those not requiring readmission, there was no difference in the distribution of benign and malignant disease. Table 2 provides a breakdown by specific diagnosis in each group.

By univariate analysis, the development of postoperative complications correlated with subsequent readmission (Table 3). When compared to patients who were not readmitted, patients requiring readmission were more likely to have a complication (46% versus 37%, $P = 0.003$) in the postoperative period. Specific complications associated with readmission by univariate analysis were pancreatic fistula, wound infection, intra-abdominal abscess formation, bile leak, and cholangitis. Other complications were

Table 1. Univariate analysis: Demographics and presenting symptoms

	Readmission (n = 431)	No readmission (n = 1212)	*P* value
Age at surgery (yr)	61.8 ± 12.7	64.6 ± 12.7	<0.0001
Gender	58% male	52% male	0.05
Race (%)			
White	85	88	0.01
Black	11	7	
Other	4	5	
Presenting symptoms (%)			
Jaundice	58	59	0.74 (NS)
Weight loss	41	40	0.75 (NS)
Abdominal pain	39	37	0.74 (NS)
Nausea/ vomiting	14	11	0.13 (NS)
Pruritis	6	8	0.1 (NS)
Gastrointestinal bleeding	3	3	0.78 (NS)
Fever/chills	6	2	0.76 (NS)

Table 2. Pathologic diagnosis

	Readmission (n = 431)	No readmission (n = 1212)	*P* value
Malignant (%)	77	75	0.32 (NS)
Periampullary cancer (%)	66	68	0.56 (NS)
Specific diagnoses (%)			
Pancreatic cancer	39	40	—
Ampullary cancer	11	11	—
Distal bile duct cancer	11	8	—
Duodenal cancer	4	4	—
IPMN	4	5	—
IPMN with invasive cancer	2	4	—
Chronic pancreatitis	7	8	—
Neuroendocrine	8	5	—
Cystadenoma/ adenocarcinoma	3	4	—
Ampullary/ duodenal adenoma	2	3	—
Other	9	8	—

IPMN = intraductal papillary mucinous neoplasm.

not significantly different between the two groups and are detailed in Table 3. Perioperative mortality rates were different between the two groups, with 0.2% of readmitted patients dying within 30 days of surgery and 2% of patients not readmitted dying within 30 days of surgery. This is to be expected, as those patients dying are obviously not readmitted. Only 0.2% of patients were readmitted early and died within 30 days. Longer initial postoperative length of stay (LOS) predicted readmission. The readmission group had a mean postoperative LOS 13.8 ± 19.7 days versus 11.6 ± 12.5 days for patients not requiring readmission (median: 10 versus 9 days, $P = 0.02$). Fifty-one percent of readmitted patients had an initial postoperative LOS ⩾10 days, while only 42% of patients not requiring readmission had a prolonged postoperative LOS ⩾10 days ($P = 0.003$).

Intraoperative factors were compared between the two groups. Readmitted patients had longer operative times (mean = 392 minutes versus 362 minutes, $P < 0.0001$), greater estimated blood loss (EBL, mean = 1175 ml versus 927 ml, $P = 0.0002$) and larger volume of transfused packed red blood cells (mean = 1.0 unit versus 0.8 unit, $P = 0.04$). Only 9% of patients not requiring readmission had a total operative time exceeding 8 hours, while 15% of readmitted patients had operative times over 8 hours ($P = 0.002$). Forty-seven percent of readmitted patients had an estimated blood loss (EBL) ⩾1000 ml, compared to only 30% of patients not readmitted ($P = 0.001$). Thirty-five percent of patients in both groups had transfusion of at least 1 unit of packed red blood cells ($P = 0.8$). Pylorus-preserving PD was performed less often in patients who get readmitted (69% versus 76%, $P = 0.004$). Finally, resection of part of any visceral vessel during surgery, although an uncommon occurrence, was performed more often in patients who eventually required readmission (4% versus 1.5%, $P = 0.005$). Vessels resected included the superior mesenteric vein (SMV), portal vein (PV), hepatic artery, and inferior vena cava (IVC).

A backward stepwise logistic regression model was then performed. The choice of factors included in the model was based on the univariate analysis. Factors analyzed included age (<65 versus ⩾65 years), gender, race (black versus white/other), pathology (benign versus malignant), endoscopic biliary stent placement, percutaneous biliary stent placement, EBL (<1000 ml versus ⩾1000 ml), operative time (< 8 hours versus ⩾8 hours), vessel resection, pylorus-preservation, intra-abdominal abscess, wound infection, delayed gastric emptying, pancreatic fistula, bile leak, cholangitis, other complications, and LOS (<10 d versus ⩾10 days). The final logistic regression model is shown in Table 4 with reported odds ratios, confidence intervals, and *P*-values. In the final model, the independent predictors of readmission were age ⩽65 years (odds ratio [OR] = 1.6), EBL ⩾1000 ml (OR = 1.4), placement of a percutaneous biliary stent prior to surgery (OR = 1.4), visceral vessel resection at surgery (OR = 2.4), intra-abdominal abscess (OR = 2.7), wound infection (OR = 1.8), and other complications (OR = 1.5). The category "other complications" included any complication not otherwise listed. Bile leak, pancreatic fistula, and cholangitis, while significant in the univariate model, were not significant in the final multivariate model.

For the 431 patients readmitted to the hospital, the most common reason for the first readmission was recurrence or metastatic disease (23%), followed by intra-abdominal abscess (15%, Table 5). We further divided patients into early (within 1 year of initial resection, 71% of patients) and late (>1 year of initial resection, 29% of patients) readmission status. Patients in the early readmission group were more likely to be readmitted for intra-abdominal abscess, delayed gastric emptying, and wound infection, while patients in the late readmission group were more likely to be readmitted with recurrence/metastatic disease, gastric outlet obstruction, obstructive jaundice, and incisional hernia than those readmitted

Table 3. Post operative complications

	Readmission (n = 431)	No readmission (n = 1212)	*P* value
Any complication (%)	46	37	0.003
Specific complications (%)			
Delayed gastric emptying	13	11	0.16 (NS)
Pancreatic fistula	13	8	0.0003
Wound infection	12	6	<0.0001
Intra-abdominal abscess	12	4	<0.0001
Cardiac complication	3	5	0.07 (NS)
Bile leak	4	2	0.04
Cholangitis	2	1	0.03
Sepsis	2	2	0.37 (NS)
Lymphatic or chylous leak	2	1	0.16 (NS)
Postoperative pancreatitis	1	1	0.69 (NS)
Pneumonia	0.4	1	0.31 (NS)
Mean length of initial hospital stay (days)	13.8 ± 19.7	11.6 ± 12.5	0.02

Table 4. Multivariate logistic regression model for readmission

Factor	Odds ratio	95% Confidence intervals	*P* value
Demographics			0.001
Age <65 years	1.6	1.2–1.7	0.3 (NS)
Female gender	1.2	0.9–1.5	0.054 (NS)
Black race	1.6	1.0–2.4	
Preoperative factors			
Malignant pathology	1.1	0.8–1.5	0.6 (NS)
Endoscopic biliary stent	0.9	0.7–1.2	0.6 (NS)
Percutaneous bile stent	1.4	1.0–1.9	0.03
Operative factors			
Estimated blood loss ⩾1000	1.4	1.0–1.8	0.03
Time ⩾ 8 hours	1.2	0.8–1.8	0.4 (NS)
Vessel resection	2.4	1.0–5.5	0.05
Classic resection	1.3	1.0–1.7	0.09 (NS)
Postoperative factors			
Intra-abdominal abscess	2.7	1.6–4.6	<0.0001
Wound infection	1.8	1.1–2.9	0.01
Pancreatic fistula	1.4	0.9–2.2	0.2 (NS)
Delayed gastric emptying	1.2	0.8–1.8	0.4 (NS)
Bile leak	0.9	0.4–2.0	0.8 (NS)
Cholangitis	1.9	0.7–4.8	0.2 (NS)
Other complication	1.5	1.1–2.1	0.01
Postoperative length of stay ⩾10 days	1.0	0.7–1.3	0.8 (NS)

in the first year. Overall, 4% of patients were readmitted for hemorrhage. Hemorrhage was due to luminal gastrointestinal bleeding in nine patients, from pseudoaneurysms and massive hemobilia in six patients, and radiation induced gastritis in one patient. The majority of readmissions related to bleeding were within the first year. There was no statistical difference in the incidence of hemorrhage in the early and late readmission groups. Other differences in readmission diagnosis were not significant between the two groups and are detailed in Table 5.

Patients readmitted to the hospital had a variety of procedures performed which are shown in Table 6. The most common procedure among all patients was an operation other than incisional hernia repair (19%). This category is broad and includes reoperations for repair of anastomotic dehiscences, lysis of adhesions, and resection of metastatic disease, but does not include repair of incisional hernias. Patients readmitted early were less likely to undergo an operation, have an incisional hernia repair, have a biopsy performed, or have chemoembolization of liver metastases and were more likely to need a nasogastric tube, have an abscess drained, or be placed on intravenous antibiotics than those admitted more than 1 year after initial resection. Procedures not shown in Table 6 included thoracentesis, paracentesis, TPN, endovascular procedures, blood transfusions, chemotherapy for recurrence, celiac nerve block, and anticoagulation. All occurred less than 3% of the time and there were no differences between the early and late readmission groups.

In addition to invasive procedures, the majority of patients readmitted had imaging studies performed during their readmission. The most frequent study performed was a CT scan in 59% of all readmitted patients. Patients readmitted early were more likely to have a CT scan at the time of readmission than those readmitted late (64% versus 47%, $P = 0.001$). Other studies included upper gastrointestinal fluoroscopy with or without small bowel follow through (23%), cholangiography (18%), EGD (13%), magnetic resonance imaging (2%), angiography (2%), ultrasound (2%), and sinography (2%). These procedures were performed equally in the early and late readmission groups.

The postoperative length of stay consistently decreased throughout the study period, decreasing from a median of 10.5 days in 1996 to 7 days in 2003. The rate of readmission during this time also decreased, from 33% in 1996 to 20% in 2003. For each year, the median postoperative lengths of stay and readmission rates are graphically summarized in Figure 1. Decreasing lengths of stay did not increase readmission rate over the time period studied. The denominator in our annual readmission rate calculation is the total number of PD procedures performed each calendar year. The numerator is the total number of those patients from each year who are readmitted at any time point between discharge and the time of this data analysis.

Using Kaplan-Meier survival curves,[34] the long-term survival of readmitted patients was compared to patients not requiring readmission. The 1-, 3-, and 5-year survival rates for readmitted patients were 82%, 55%, and 39% (median = 41 months) compared to 76%, 50%, and 39% (median = 36 months, P = NS, Fig. 2).

Among the 431 patients readmitted, 142 (33%) were readmitted to the Johns Hopkins Hospital more than one time. Patients who were initially readmitted for cholangitis or obstructive jaundice were most likely to require multiple readmissions. Forty-seven percent of patients with an initial

Table 5. Primary readmission diagnosis

Diagnosis	All readmissions (n = 431)	Early (<1 yr) (n = 308)	Late (>1 yr) (n = 123)	*P* value
Recurrence/meatastases	23%	12%	50%	<0.0001
Intra-abdominal abscess	15%	19%	5%	0.0001
Cholangitis	12%	13%	9%	0.2 (NS)
Delayed gastric emptying	12%	15%	3%	0.001
Gastric outlet obstruction	11%	9%	16%	0.04
Small bowel obstruction	8%	8%	7%	0.73 (NS)
Obstructive jaundice	7%	4%	15%	<0.0001
Incisional hernia	6%	3%	12%	0.0002
Wound infection	5%	6%	1%	0.01
Hemorrhage	4%	5%	2%	0.3 (NS)
Pleural effusion	3%	3%	3%	0.97 (NS)
Scheduled biliary stent change	2%	2%	0%	0.08 (NS)
Pancreatitis	1%	2%	1%	0.5 (NS)
Chylous ascites	1%	2%	0%	0.15 (NS)
Enterocutaneous fistula	1%	2%	0%	0.15 (NS)
Colitis	1%	1%	0%	0.2 (NS)
Metabolic derangement	1%	1%	0%	0.3 (NS)
Pneumonia	1%	1%	0%	0.3 (NS)
Other	6%	8%	2%	0.01

readmission diagnosis of cholangitis and 50% of patients with obstructive jaundice as their initial readmission diagnosis were admitted more than once. Patients who initially presented with hemorrhage, gastric outlet obstruction and wound infections were less likely to be readmitted a second time, with 24% of patients with wound infection, 23% of patients with gastric outlet obstruction, and 18% of patients with hemorrhage being admitted a second time.

DISCUSSION

While postoperative mortality and length of stay after PD have continued to decrease, postoperative complications and readmission rates remain quite high. Few studies have reported the readmission rates or reasons for readmission in post-PD patients.[21] The current report details the reasons for readmission and identifies risk factors for readmission in 431 patients, with an overall readmission rate of 26%. Readmission was more common within the first year, with 71% of 431 readmissions occurring during this time period.

It is not surprising that the reasons for readmission differed depending on the time elapsed since pancreaticoduodenal resection. Patients presenting for readmission with abdominal pain, nausea, vomiting, or fever within the first year are more likely to have an infectious or mechanical process including

Table 6. Readmission procedures and interventions

	All readmissions (n = 431)	Early (<1 yr) (n = 308)	Late (>1 yr) (n = 123)	*P* value
Procedure/intervention				
Operation	19%	13%	33%	<0.0001
Biliary stent placement	17%	16%	21%	0.2 (NS)
Nasogastric tube decompression	15%	18%	7%	0.002
Abscess drainage	12%	15%	3%	0.0004
Antibiotics	9%	11%	2%	0.000
EGD with luminal dilation	5%	6%	4%	0.5 (NS)
Incisonal hernia repair	5%	3%	12%	<0.0001
Biopsy	1%	0.3%	4%	0.003
Chemoembolization of liver metastasis	1%	0%	3%	0.002

Totals do not add up to 100% because some patients had no procedures some had more than one, and some had less than one procedure performed.

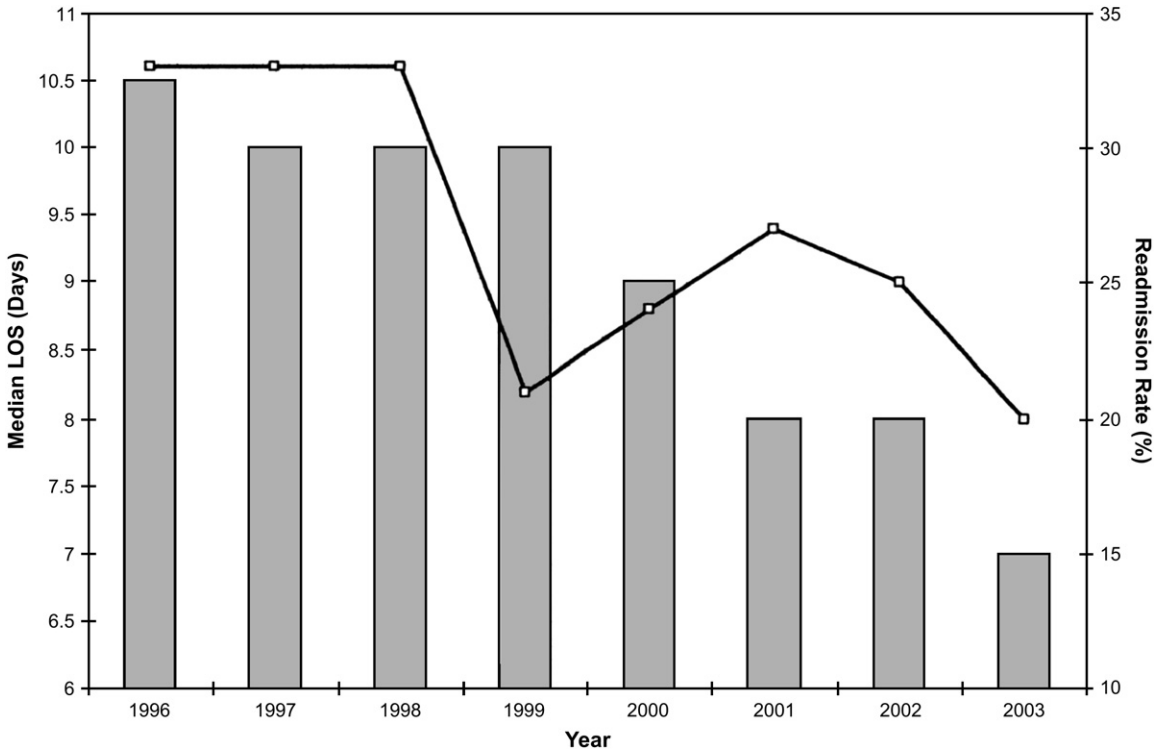

Fig. 1. The readmission rate and median postoperative length of stay per year from 1996 to 2003. The year is demonstrated on the x-axis. The vertical bars demonstrate the median length of stay (labeled on the left side of graph) and the line represents the readmission rates over time (labeled on the right side of the graph).

a wound infection, cholangitis, intra-abdominal abscess, delayed gastric emptying, or adhesive bowel obstruction rather than tumor recurrence. However, a patient presenting with similar symptoms greater than 1 year after PD for malignant disease is more likely to have tumor recurrence. While the postoperative complication rate remains high (39% overall), this study nicely demonstrates similar long-term survival in the readmission and no readmission groups implying that patients readmitted for postoperative complications have a good long-term prognosis, provided their problem which prompted readmission is adequately addressed.

After controlling for all possible risk factors for readmission identified in the univariate analysis, the factors shown to independently predict readmission in the multivariate model were age <65 years, preoperative placement of a percutaneous biliary stent, estimated blood loss of >1000 ml at initial operation, visceral vessel resection, intra-abdominal abscess formation, wound infection, and other complications. It is interesting that age <65 years and not older age was associated with readmission. It is documented that elderly patients have more complications and longer postoperative lengths of stay then younger patients. Given their increased age, it may be that surgeons are more cautious about discharging these patients quickly. As a result, they may require readmission less frequently. The tendency in the younger patient is to be extremely aggressive, perhaps performing larger operations with more extensive dissection, which may lead to increased complications.

Similar to the finding by van Geenen and colleagues,[21] postoperative complications predicted readmission in both the univariate and multivariate models. While intra-abdominal abscess and wound infection remained significant predictors of readmission in the multivariate model, pancreatic fistula and bile leak did not. Pancreatic fistula is known to increase the rates of pancreatitis, intra-abdominal abscesses, and wound infection, all common reasons for patient readmission in this current report. However, the majority of fistulas reported consist of asymptomatic leakage of amylase rich fluid into intraoperatively placed drains (fistula definition: >50 ml of amylase-rich fluid on or after postoperative day 10). These well controlled fistulas do not lead to increased readmissions. Likewise, well controlled bile leaks are not problematic and only when they are undrained

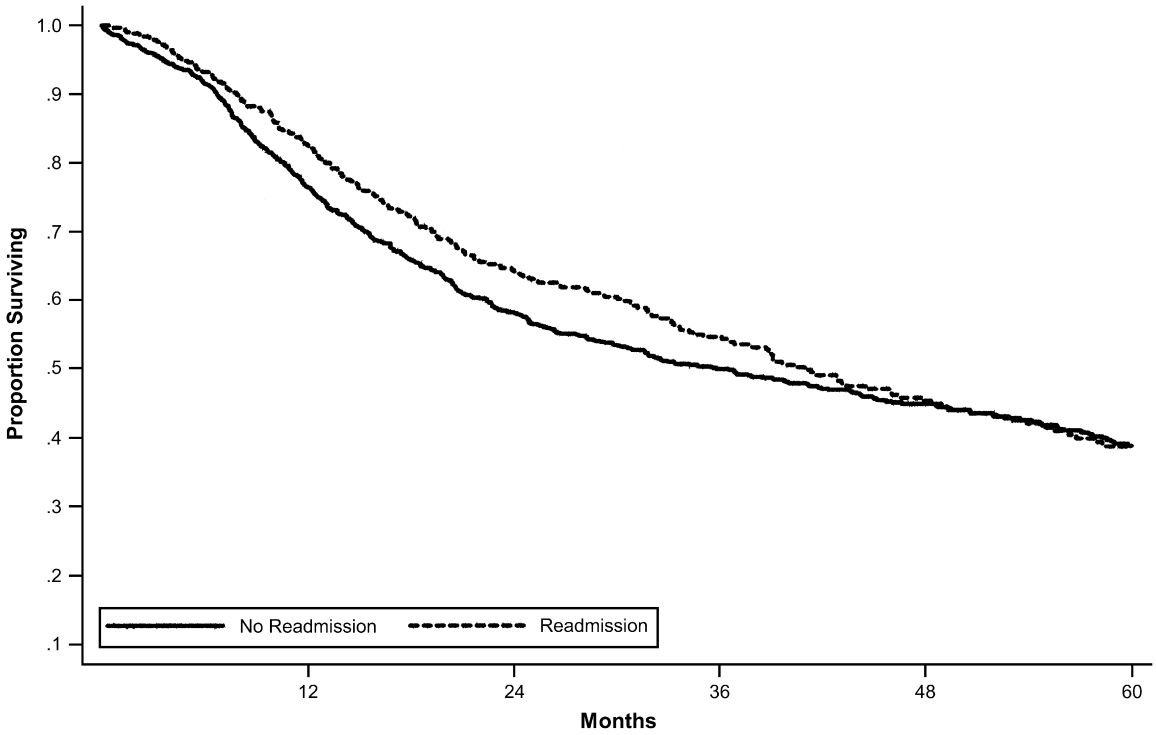

Fig. 2. The Kaplan-Meier actuarial survival curves comparing long-term survival in all pancreaticoduodenectomy patients. Patients requiring readmission (dashed line, n = 431) were compared to those not requiring readmission (solid line, n = 1212, p = 0.008).

and form an abscess do they contribute to significant morbidity. Therefore, after adjusting for the presence of these more morbid complications, pancreatic fistula and bile leak are no longer significant. This finding suggests that well-placed perioperative drains may prevent significant morbidity related to perioperative anastomotic leakage.

Cholangitis was shown to be the most common reason for multiple readmissions and was most often related to indwelling percutaneous biliary stents. Of note, cholangitis was significant in the univariate model, but not in the multivariate model after adjusting for percutaneous stenting, supporting the hypothesis that cholangitis often results from stenting, leading to multiple readmissions. It has been well demonstrated that preoperative biliary stents cause increased infectious complications including cholangitis and wound infections.[30,35,36] Preoperative percutaneous biliary stents, but not endoscopic stents were strong predictors of readmission in both the univariate and multivariate models. While both lead to increased complications following PD, only percutaneous and not endoscopic stents are maintained postoperatively. The indwelling biliary stent likely leads to more ongoing infectious complications and episodes of cholangitis postoperatively, explaining why only percutaneous stents were independent predictors of readmission.

The incidence of DGE was similar between the readmission and no readmission groups, but was the reason for readmission in 12% of patients. It is likely that with short length of stay now observed, this entity is underdiagnosed during initial admission.

The readmission rate or 26% in our study differs from the 2001 report by van Geenen and colleagues.[21] They reported a readmission rate of 38% after PD in 283 patients for both benign (n = 40) and malignant (n = 243) disease. In their study, data from readmissions outside the academic medical center were captured and included. Our study only includes patients readmitted to the Johns Hopkins Hospital, thus it necessarily underreports the actual number of readmissions. It is difficult to know the magnitude of the underreporting. Since Johns Hopkins is a tertiary referral center, many patients travel a distance to Johns Hopkins Hospital to have complex pancreatic surgery.

In the immediate postoperative period (within 1 year of surgery), readmissions are due primarily to postoperative complications. Patients who traveled a distance to have a PD at Johns Hopkins were asked to stay in the area for 48 hours to 1 week after

discharge from the hospital. In addition, it is our preference to manage any postoperative complications that occur in our PD patients, so whenever possible, patients readmitted to outside hospitals for complications related to their surgery are transferred back to Johns Hopkins. We try to maintain a good relationship with referring physicians to facilitate this process and, as a result, many immediate readmissions are to the Johns Hopkins Hospital. However, it is likely that some minor complications such as wound infections are taken care of elsewhere. The underestimation of readmission rates is likely higher for recurrence and palliative end-of-life care, as well as readmissions related to adjuvant chemoradiation delivered elsewhere. These issues are commonly taken care of by local primary care physicians or oncologists and patients may never come to our attention. These late readmissions constitute only 29% of all readmissions, but this remains a limitation of the study.

Patients undergoing PD through 2003 were included in the analysis. Therefore, patients operated on in 2003 are only followed 22–35 months (until November 2003), whereas those operated on in 1996 are followed for up to 118 months. This, too, may lead to underestimation of the 2003 readmission rates.

Over the past decade, with the push toward regionalization of care and the implementation of clinical pathways,[17–19] hospital stay after PD has decreased significantly. The above reports of clinical pathway implementation focused on decreased postoperative lengths of stay and thereby decreased costs, without examining readmission rates. It is critical to know if this decrease in length of stay is associated with higher mortality or readmission rates, which would obviously offset any benefit of decreased postoperative hospital stay. At Johns Hopkins, such pathways were implemented in 1995, just prior to the time of the start of this study. After initiation of the critical pathway for PD there was an initial drop in postoperative length of stay. Since its initiation and success the pathway is continually being refined (for example, the day that clear liquid diet is started was moved two days earlier over the time period of the study, contributing to decreased length of stay) leading to further improvements in length of stay. Over the 8-year time period of this study, readmission rates have actually decreased concomitantly with the observed decrease in postoperative lengths of stay. This nicely demonstrates that critical pathway implementation can safely target reductions in postoperative length of stay, without increasing mortality or readmission. Finally, we have reported the number, average duration, and the procedures and imaging studies associated with readmission to the hospital, but have not performed a formal cost analysis on these data. Future cost analysis for this complicated surgical procedure should take into account these late sequelae of PD.

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Discussion

Dr. Michael Sarr (Rochester, MN): First of all, I want to stress the point that Ms. Emick will be Dr. Emick on Friday. It is great to have a medical school student present such good work.

(Applause).

I have three questions. First, your study allegedly debunks the argument that earlier discharge and our concept of fast track surgery is associated with more readmissions. Many studies have shown this effect. You have shown that readmissions to outside hospitals, nursing homes, and extended care facilities were not collected by your study, and I would bet that you are probably markedly underestimating the number of readmissions. Why don't you have those data, can't you get those data, and would those data change your conclusions?

Second, again you have described the use of clinical pathways, and you show a nice decrease in duration of stay in the hospital; but your critical pathways were introduced before your study started. Are you changing those critical pathways continuously?

Third, your study shows that younger age is a risk for readmission, but when you look at the data, there is only a two- or three-year difference in age of admission and nonreadmission. Is that clinically important, and are the older patients being selected more carefully because they are in better shape?

Thank you.

Dr. Emick: Your first question was about our readmissions to outside hospitals. It is definitely a limitation of our study that we couldn't obtain that data, and we just don't have that data, so we surely

underestimate the amount of readmissions to outside hospitals. I could speculate all the reasons that people are getting readmitted to outside hospitals, but I think suffice it to say that we do underestimate. I think the conclusion of our study, though, is that the readmission rate has been decreasing over time. No matter what that absolute number is, there is no reason to suspect that the readmissions that were missing in 1996 are any different than the ones we are missing in 2003. So I think the general trend for the rate to be decreasing over time still holds, although the absolute number is probably low.

Your second question was about implementation of our clinical pathways, and it is true that started right before we started collecting data for this study. We did see an initial drop in postoperative length of stay, but in the bar graph you can see that our drop kept gradually decreasing over the study period, and that was actually revised and refined and just simply got better at executing those postoperative pathways.

And then finally, older patients being highly selected for, there was a study recently out of our institution, Dr. Makary, that showed that older patients are actually more prone to have complications and higher mortality. So you would actually expect the opposite based on that data. So we have hypothesized that surgeons tend to be more aggressive with younger patients, but I think your point about it being a significantly different number but not clinically significant is probably true.

Dr. L. Traverso (Seattle, WA): Your lecture and slides were easily followed. I was looking for some more specific conclusions at the end of your talk. In order to improve the outcomes of a complex operation such as the Whipple operation, we need to make measurements like you have done. The process of pancreaticoduodenectomy and its outcomes are measured. Each outcome allows us to see where we can improve. Other than the critical pathways, which are a very important aspect of any complex process, could you share with us any specific items that you have learned? Are you now not going to use percutaneous drains? Are you going to implement measures that will allow you to lower blood loss, et cetera, et cetera? How has the examination of all these cases helped you improve the process of pancreaticoduodenectomy?

Dr. Emick: We are now looking at percutaneous drains and how they are related to other complications after pancreaticoduodenectomy. I don't think we are going to stop using them at our institution. And, I am sorry, the second part of your question?

Dr. Traverso: Blood loss.

Dr. Emick: Blood loss. That I don't know.

Lentivirus-Mediated RNA Interference of *HMGA1* Promotes Chemosensitivity to Gemcitabine in Pancreatic Adenocarcinoma

Siong-Seng Liau, M.B.Ch.B., Stanley W. Ashley, M.D., Edward E. Whang, M.D.

The high mobility group A1 (HMGA1) proteins are overexpressed in pancreatic cancers. They are architectural nuclear proteins, which regulate expression of multiple genes implicated in the malignant phenotype. In this study, we hypothesized that HMG A1 silencing will promote chemosensitivity in pancreatic adenocarcinoma. We studied highly malignant pancreatic adenocarcinoma cell lines (MiaPaCa2 and PANC1). Lentiviral short-hairpin RNA (sh*HMGA1*) expression vectors targeting HMGA1 were used for generation of lentiviral particles. Stable transfectants were developed after lentiviral transduction. Nuclear expression of HMGA1 was assayed using Western blot analysis. Chemosensitivity to gemcitabine was determined by IC50 analysis. Caspase activity was quantitated using fluorometric caspase profiling. Apoptosis was assessed by flow cytometric analysis. Lentivirus-mediated RNA interference resulted in 90% silencing of HMGA1 expression in each of MiaPaCa2 and PANC1 cell lines. *HMGA1* silencing enhanced chemosensitivity to gemcitabine with an approximately 50% reduction in IC50 in each cell line. Lentivirus-mediated *HMGA1* silencing promoted the activation of caspases 3, 2, 9, and 8, on exposure to gemcitabine. *HMGA1* silencing resulted in reduction in Akt kinase activity. Lentivirus-mediated RNA interference of *HMGA1* promoted chemosensitivity to gemcitabine in pancreatic adenocarcinoma. *HMGA1* may represent a novel therapeutic target in pancreatic cancer. (J GASTROINTEST SURG 2006;10:1254–1263) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: *HMGA1*, gemcitabine, chemotherapy, pancreatic adenocarcinoma

Pancreatic adenocarcinoma is the fourth leading cause of cancer-related death in the United States.[1] Its biology is characterized by the propensity for early and aggressive invasion and metastasis, such that less than 10% of patients have surgically resectable disease at the time of diagnosis.[2] Gemcitabine, a nucleoside analog, is generally considered to be first-line therapy for unresectable pancreatic cancer.[3,4] However, the impact of gemcitabine on overall survival and clinical outcomes remains modest, largely because of chemoresistance. Further understanding of the molecular mechanisms underlying pancreatic adenocarcinoma chemoresistance may facilitate the identification of novel strategies for increasing chemosensitivity in this deadly cancer.

The human *HMGA1* gene, located on chromosomal locus 6p21, encodes two high mobility group A1 (HMGA1) splice variants (HMGA1a and HMGA1b).[5] These HMGA1 proteins are architectural transcription factors that play a role in both positive and negative transcriptional regulation of human gene expression in vivo.[6–8] They form stereo-specific, multiprotein complexes termed "enhanceosomes" on the promoter/enhancer regions of genes, where they are able to bind to the minor groove of AT-rich DNA sequences to induce DNA helix bending.[6,9] HMGA1 proteins are overexpressed in a range of human cancers, including pancreatic adenocarcinoma.[10–17] HMGA1 proteins have been reported to regulate signaling pathways implicated in the malignant behavior of cancer cells, including KIT ligand expression[18] and Ras/ERK signaling.[19] Moreover, *HMGA1* is a c-Myc and AP-1 target gene and has been shown to play a role in malignant cellular transformation.[20–22] Recently, it has also been reported that HMGA1

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proteins bind to *p53* in vivo and inhibit their tumor suppressor activity in thyroid cancer cells.[23]

Although there is extensive evidence for the prooncogenic roles of *HMGA1*, little is known about its roles in chemoresistance. The purpose of this study was to test the hypothesis that *HMGA1* is a determinant of pancreatic adenocarcinoma chemoresistance and that suppression of *HMGA1* expression would enhance pancreatic adenocarcinoma chemosensitivity to gemcitabine. Using lentivirus-mediated RNA interference, we assessed the effect of suppressing *HMGA1* expression on pancreatic adenocarcinoma cell gemcitabine chemoresistance and apoptotic pathways. Our observations indicate that *HMGA1* represents a rational therapeutic target in pancreatic adenocarcinoma.

MATERIALS AND METHODS

Cells and Cell Culture

MiaPaCa2 and PANC1 human pancreatic ductal adenocarcinoma cells were obtained from American Type Culture Collection (Manassas, VA). Cells were maintained in DMEM containing 10% fetal bovine serum (Gibco Life Technologies Inc, Gaithersburg, MD) and incubated in a humidified (37°C, 5% CO_2) incubator, grown in 75-cm^2 culture flasks, and passaged on reaching 80% confluence.

Lentivirus-Mediated HMGA1 RNA Interference

Lentiviral hairpin RNA interference plasmids (pLKO.1-HMGA1, TRCN0000018949), constructed as described previously [24], were obtained from The RNAi Consortium (Mission TRC Hs. 1.0; Sigma Aldrich, St Louis, MO). The sequence of short hairpin RNA targeting the human *HMGA1* gene (GenBank accession no. NM_002131) was "5′–CAACTCCAGGAAGGAAACCAA-3′, corresponding to coding region positions 446–466 of HMGA1 mRNA transcript variant 2" rather than "5′–AACTCCAGGAAGGAAACCAA-3′, corresponding to coding region positions 446–466". The control plasmid that has a scramble nontargeting short-hairpin RNA sequence was obtained from Addgene (Cambridge, MA), deposited by Dr. David Sabatini.[25] Each of these vectors had been sequence-verified. Vectors were expanded in chemically competent *Escherichia coli* (TOP10 cells; Invitrogen, Carlsbad, CA) and purified using Genelute maxiprep kit (Sigma Aldrich). To generate lentiviral particles, human embryonic kidney 293 cells (ATCC) were co-transfected with the lentiviral vector and compatible packaging plasmid mixture (Virapower lentiviral packaging system, Invitrogen) using LipofectAMINE 2000 (Invitrogen), in accordance to the manufacturer's instruction. Pancreatic adenocarcinoma cells were exposed to lentivirus-containing supernatant for 16 hours in the presence of 6 μg/ml Polybrene (Sigma). Pooled stable transfectants were established using puromycin selection. Stable transfectant cells were maintained in medium containing 3 μg/ml puromycin (Invitrogen).

Cytotoxicity Assay

Gemcitabine-induced cytotoxicity was quantified by an MTS [3-(4,5 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl-2-(4-sulfophenyl)-2*H*-tetrazolium] assay (CellTiter 96; Promega), in accordance with the manufacturer's instructions. Cells were seeded into 96-well plates at 5×10^3 cells per well and allowed to adhere overnight in medium containing 10% FBS. Cell viability was determined after 72 hours in presence or absence of 0–10 μM gemcitabine. Plates were read with the use of the SpectraMax M5 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) at a wavelength of 490 nm. Six samples were used for each experimental condition, and experiments were performed in triplicate. IC_{50} values were calculated using the SoftMax Pro software (Molecular Devices). At identical time points, cell counting was performed. Cells were trypsinized to form a single-cell suspension. Viable cells, determined by Trypan blue exclusion, were counted with the use of a Neubauer hemocytometer (Hausser Scientific, Horsham, PA). Cell counts were used to confirm MTS results.

Western Blotting

Cells were harvested and rinsed twice with PBS. Total cell extracts were prepared with lysis buffer (20 mM Tris, pH 7.5, 0.1% Triton X, 0.5% deoxycholate, 1 mM PMSF, 10 mg/ml aprotinin, 10 mg/ml leupeptin) and cleared by centrifugation at 15,000*g*, 4°C. Nuclear extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents based on the manufacturer's instruction (Pierce, Rockford, IL). Total protein concentration was measured using the BCA assay kit (Sigma) with bovine serum albumin as a standard, according to the manufacturer's instructions. Total cell lysates containing 50 μg of total protein or nuclear protein containing 10 μg of total protein was subjected to 10% SDS/PAGE, and the resolved proteins were transferred electrophoretically to PVDF membranes (Invitrogen). Equal protein loading was confirmed by Coomassie (BioRad, Hercules, CA) staining of the gel. After blocking with PBS containing 3%

bovine serum albumin for 1 hour at room temperature, membranes were incubated with 3–5 mg/ml antibody in PBS containing 0.1% Tween 20 overnight at 4°C. Anti-*HMGA1* and anti-lamin B1 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Chemoluminescent detection (Amersham Biosciences, NJ) was performed in accordance with the manufacturer's instructions. The densitometric signal was quantified using ImagePro Plus software version 4.0 (Media Cybernetics, Silver Spring, MD) and normalized to that of actin. Blots were performed in triplicate in at least three independent experiments. Mean densitometric values (±SD) are shown.

Apoptosis Assay

After gemcitabine (1 μM) treatment for 48 hours, 1×10^6 cells were washed, trypsinized, and resuspended in 0.5 ml of PBS containing 2% FBS and 0.1 μM EDTA. Apoptosis staining was performed using 1 μl/ml YO-PRO-1 and propidium iodide (Vybrant Apoptosis Assay Kit #4; Molecular Probes, Eugene, OR). Cells were incubated for 30 minutes on ice and then analyzed by flow cytometry (FACScan; Becton Dickinson, Franklin Lakes, NJ), measuring fluorescence emission at 530 and 575 nm. Cells stained with the green fluorescent dye YO-PRO-1 were counted as apoptotic; necrotic cells were stained with propidium iodide. The number of apoptotic cells was divided by the total number of cells (minimum of 10^4 cells), resulting in the apoptotic fraction. Data were analyzed using CellQuest software (Becton Dickinson). All assays were performed in triplicate.

Fluorometric Caspase Profiling

Whole cell lysates were assayed for caspase 2, 3, 8, and 9 activities using the BD ApoAlert fluorometric Caspase Assay Plate (BD Biosciences Clontech, Palo Alto, CA) according to the manufacturer's instructions. Plates were read (excitation, 360 nm; emission, 480 nm) using SpectraMax M5 microplate reader in fluorescence mode (Molecular Devices). All measurements were performed in triplicate, each with three determinations for each condition.

Akt Kinase Assay

Active Akt was immunoprecipitated from 1 mg of clarified total cell lysate using the catch and release reversible immunoprecipitation system (Upstate, Charlottesville, VA) according to the manufacturer's protocol. Four micrograms of mouse monoclonal anti-Akt (PH domain) antibody (Calbiochem, San Diego, CA) was used per 500 μg of cell lysate. Following immunoprecipitation, equivalent amounts of eluate were used for Akt kinase assay with an ELISA-based Akt activity assay that uses a biotinylated peptide substrate that is phosphorylated by Akt kinase (K-LISA Akt activity assay; Calbiochem). Akt activity was quantified by reading the absorbance at 450 nm, with a reference wavelength set at 540 nm, using SpectraMax M5 microplate reader (Molecular Devices). All measurements were performed in triplicate, each with three determinations for each condition.

Statistical Analysis

Differences between groups were analyzed using Student's *t*-test, multifactorial ANOVA of initial measurements, and Mann–Whitney *U* test, for nonparametric data, as appropriate, using Statistica 5.5 software (StatSoft, Inc, Tulsa, OK). In cases in which averages were normalized to controls, the standard deviations of each nominator and denominator were taken into account in calculating the final standard deviation. $P < 0.05$ was considered statistically significant.

RESULTS

Lentivirus-Mediated RNA Interference of *HMGA1*

Cell lines stably expressing hairpin RNA were developed following lentiviral transduction and selection with puromycin. Lentivirus-mediated RNA interference of *HMGA1* (shHMGA1) resulted in up to 90% silencing of *HMGA1*, as confirmed by Western blot analysis (Fig. 1). Infection with control lentivirus encoding scramble hairpin RNA (shControl) had no effect on *HMGA1* expression.

Suppression of *HMGA1* Expression Enhances Gemcitabine-Induced Cytotoxicity

The baseline level of HMGA1 protein expression was approximately 3-fold higher in PANC1 cells than in MiaPaCa2 cells (Fig. 1). PANC1 cells were found to be more resistant to gemcitabine-induced cytotoxicity than MiaPaCa2 cells, with the IC_{50} of PANC1 cells being 128 nM compared to 64 nM for MiaPaCa2 cells. To determine the IC_{50}, cells were exposed to 0 to 10 μM gemcitabine for 72 hours. The IC_{50} was calculated from MTS cytotoxicity assay data. Suppression of *HMGA1* expression resulted in reduction of the gemcitabine IC_{50} in both MiaPaCa2 and PANC1 cells (Fig. 2). The *HMGA1* silencing-induced increases in gemcitabine-induced cytotoxicity were accompanied

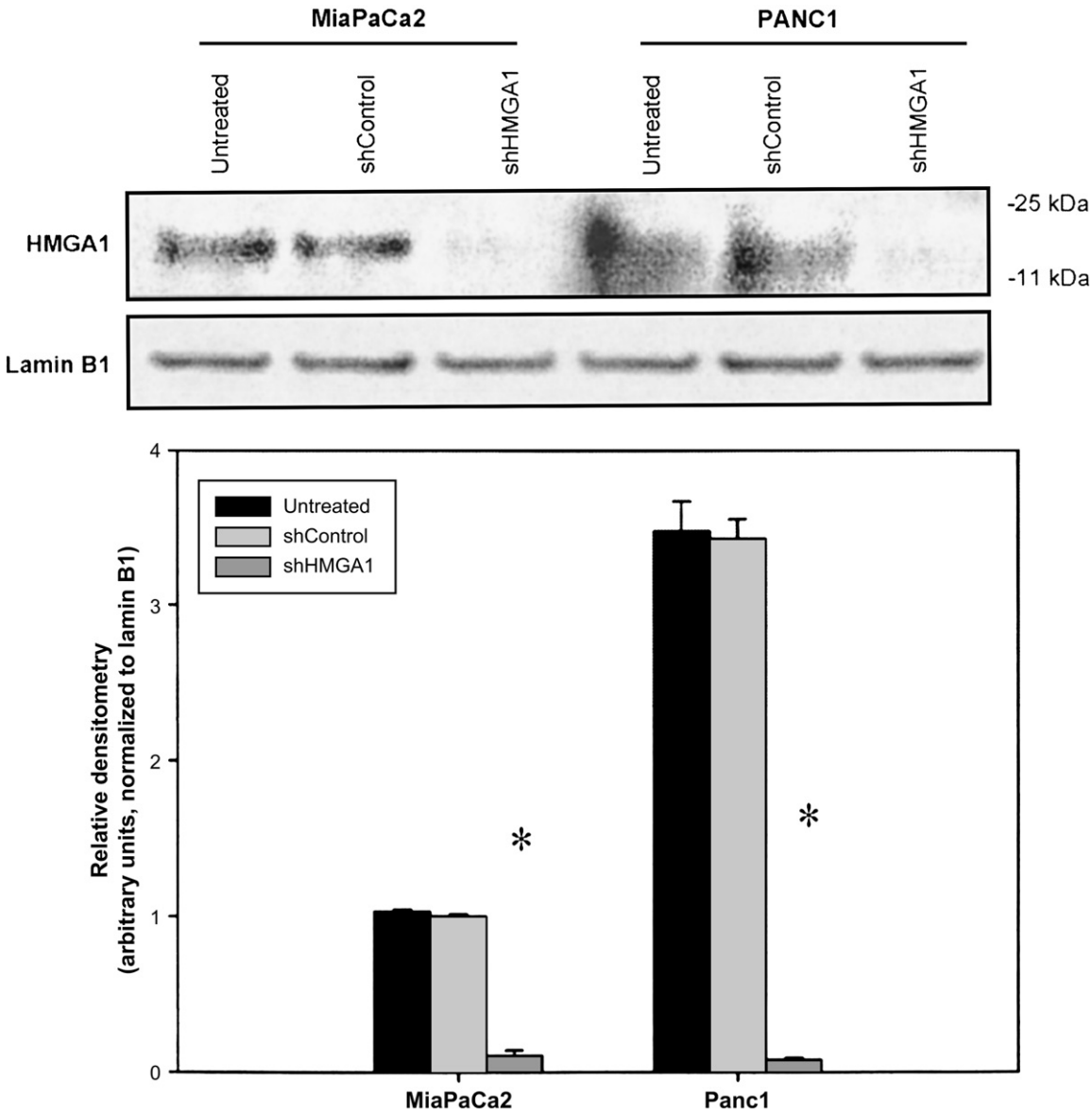

Fig. 1. Stable silencing of *HMGA1* expression using lentivirus encoding short-hairpin RNA (shRNA) was confirmed on Western blot analysis of nuclear extracts. Up to 90% silencing of *HMGA1* expression was achieved using the lentivirus-mediated shRNA approach. MiaPaCa2 and PANC1 cell lines differentially express *HMGA1*, with PANC1 cells having higher expression (up to 3-fold higher than MiaPaCa2 cells). In each experiment, controls were cells stably transfected with lentivirus encoding scramble shRNA (shControl). Densitometry values are mean (± SD). $^*P < 0.05$ versus shControl or untreated cell line.

by significant increases in cellular apoptotic fractions (Fig. 3).

HMGA1-Specific Silencing Enhances Gemcitabine-Induced Activation of Caspases 3, 8, 9, and 2

Caspase activation is required for gemcitabine-induced cytotoxicity in cancer cells.[26] As such, we sought to determine the effect of *HMGA1* silencing on caspase activities after exposure to gemcitabine for 48 hours. Gemcitabine-induced activation of caspases 3, 8, 9, and 2 was markedly increased with targeted suppression of *HMGA1* in MiaPaCa2 cells, compared to controls (Fig. 4). On exposure to 1 μM gemcitabine for a similar duration of time, PANC1 cells exhibited modest but statistically significant elevations in activities of each of the caspases profiled with suppression of *HMGA1*.

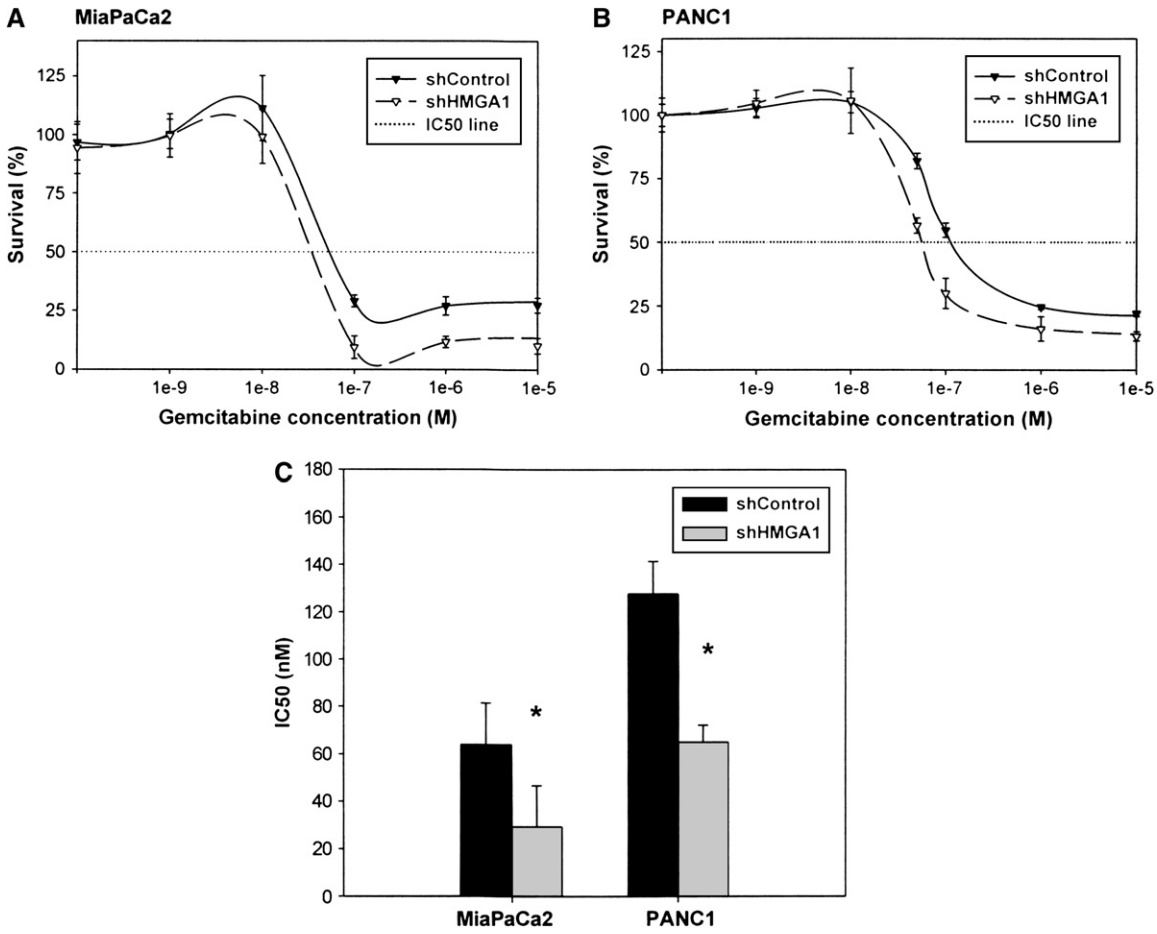

Fig. 2. Lentiviral-mediated RNA interference of *HMGA1* expression enhances gemcitabine-induced cytotoxicity in MiaPaCa2 and PANC1 cell lines. Growth curves of MiaPaCa2 (**A**) and PANC (**B**) cells show the effect of silencing *HMGA1* on chemosensitivity to gemcitabine, as determined using the 3-(4,5 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl-2-(4-sulfophenyl)-2*H*-tetrazolium) assay. Suppression of *HMGA1* expression shifts the growth curves to the left in both MiaPaCa2 and PANC1 cells, indicating an increased in chemosensitivity to gemcitabine. (**C**). The mean IC50 of gemcitabine was reduced by approximately 2-fold in both MiaPaCa2 (shHMGA1 versus shControl: 64 nM versus 29 nM) and PANC1 (128 nM versus 65 nM).

Akt Activity Is Inhibited by Suppression of HMGA1 Expression

Activation of the serine/threonine kinase Akt is common in pancreatic cancer.[27] Akt has been recognized as a determinant of pancreatic adenocarcinoma gemcitabine chemoresistance.[28–30] As such, we examined the effect of *HMGA1* silencing on Akt activity using an ELISA-based Akt kinase activity assay. Suppression of *HMGA1* expression resulted in significant reduction Akt kinase activity in MiaPaCa2 and PANC1 cell lines, with a greater effect seen in MiaPaCa2 cells (Fig. 5). The decrease in Akt activity we observed with targeted suppression of *HMGA1* expression may in part contribute to the increase in gemcitabine-induced, caspase-mediated cytotoxicity and apoptosis.

DISCUSSION

Pancreatic adenocarcinoma is among the most aggressive and chemoresistant of human malignancies. The prognosis associated with this cancer remains dismal, despite considerable advances in the medical and surgical management of this disease.[31] At the time of diagnosis, most patients will have unresectable disease. Although the nucleoside analog gemcitabine has proven efficacy against pancreatic cancer, it is associated with only modest improvement in clinical outcomes.[3,4] There is an urgent need to identify new therapeutic approaches.

Overexpression of *HMGA1* has previously been reported to be present in a range of human cancers, including pancreatic adenocarcinoma.[10–17] *HMGA1* overexpression is causally associated with both

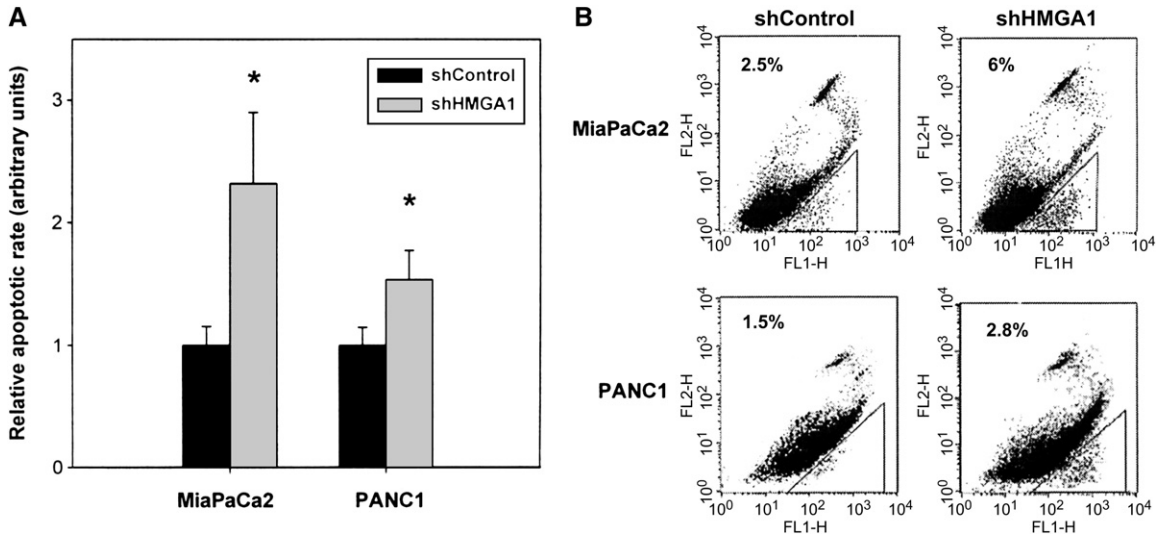

Fig. 3. Apoptotic fraction was quantitated using flow cytometry after staining of cells with Yo-Pro-1 and propidium iodide dyes. (**A**). Consistent with results of the cytotoxicity assay, suppression of *HMGA1* expression promotes apoptosis with increases in apoptotic fraction on exposure to gemcitabine 1 μM for 48 hours. Data are means of at least three determinations ±SD. $^*P < 0.05$ versus shControl transfectants. (**B**). Representative flow cytometric analyses of apoptotic fraction showing suppression of *HMGA1* expression led to increased apoptotic fractions on exposure to gemcitabine 1 μM for 48 hours. The apoptotic cell population is shown by the triangle drawn around the cell population in each analysis.

neoplastic transformation and metastatic progression in breast cancer.[32] Furthermore, *HMGA1* is a c-Myc and AP-1 target gene[20–22] and has been reported to regulate pro-oncogenic signaling pathways, including KIT ligand expression[18] and Ras/ERK signaling.[19] Recent reports also suggest that HMGA1 proteins bind to *p53* in vivo and inhibit their tumor suppressor activity in thyroid cancer cells.[23] Suppression of *HMGA1* expression by antisense oligonucleotides has been reported to inhibit pancreatic cell proliferation.[33] In addition, antisense-mediated suppression of *HMGA1* expression has been reported to inhibit the growth of experimental pancreatic cancers in vitro and in vivo.[33]

HMGA1 has received little attention in the context of chemoresistance. In our study, we have identified *HMGA1* as a potential target through which chemosensitivity to gemcitabine may be increased in pancreatic adenocarcinoma cells. From a therapeutic standpoint, targeting *HMGA1* is attractive in that although it is overexpressed in a range of human malignancies, *HMGA1* expression is absent or present at only very low levels in normal adult tissues.[34] As such, targeting *HMGA1* may have little or no effect on noncancerous tissues.[35] An important caveat is that the role of *HMGA1* in chemoresistance varies according to the chemotherapeutic agent used. For instance, overexpression rather than suppression of *HMGA1* has been shown to chemosensitize MCF-7 human breast adenocarcinoma cells to cisplatin.[36] In view of these findings, therapeutic applications of *HMGA1* silencing would need to be carefully evaluated in the context of cancer characteristics and the specific chemotherapeutic agents used.

Our observation that *HMGA1* silencing suppresses Akt activity is interesting for several reasons. Inhibition of the PI3K/Akt pathway is reported to induce chemosensitization in pancreatic cancer cells both in vitro[37] and in vivo.[38] Active Akt has been reported to protect cells from apoptotic stimuli by inhibiting activation of initiator caspase 9 and effector caspase 3 at a postmitochondrial level.[39] As such, we have identified the PI-3K/Akt kinase signaling pathway as one of the likely molecular mechanisms by which overexpression of HMGA1 proteins promotes chemoresistance to gemcitabine. However, in view of the modest effect of *HMGA1* suppression on Akt kinase activity, it is unlikely that PI-3K/Akt signaling is the sole effector of *HMGA1*-mediated chemoresistance to gemcitabine.

In this study, we have shown that lentivirus-mediated RNA interference of *HMGA1* promotes chemosensitivity to gemcitabine. As such, *HMGA1* represents a rational molecular therapeutic target. The feasibility of in vivo gene silencing using lentiviral vectors has already been demonstrated.[40] The lentivirus vector used in this study is derived from HIV-1 and is replication deficient on transducing the first cell with which it comes into contact. The ability of lentivirus to efficiently transduce cells,

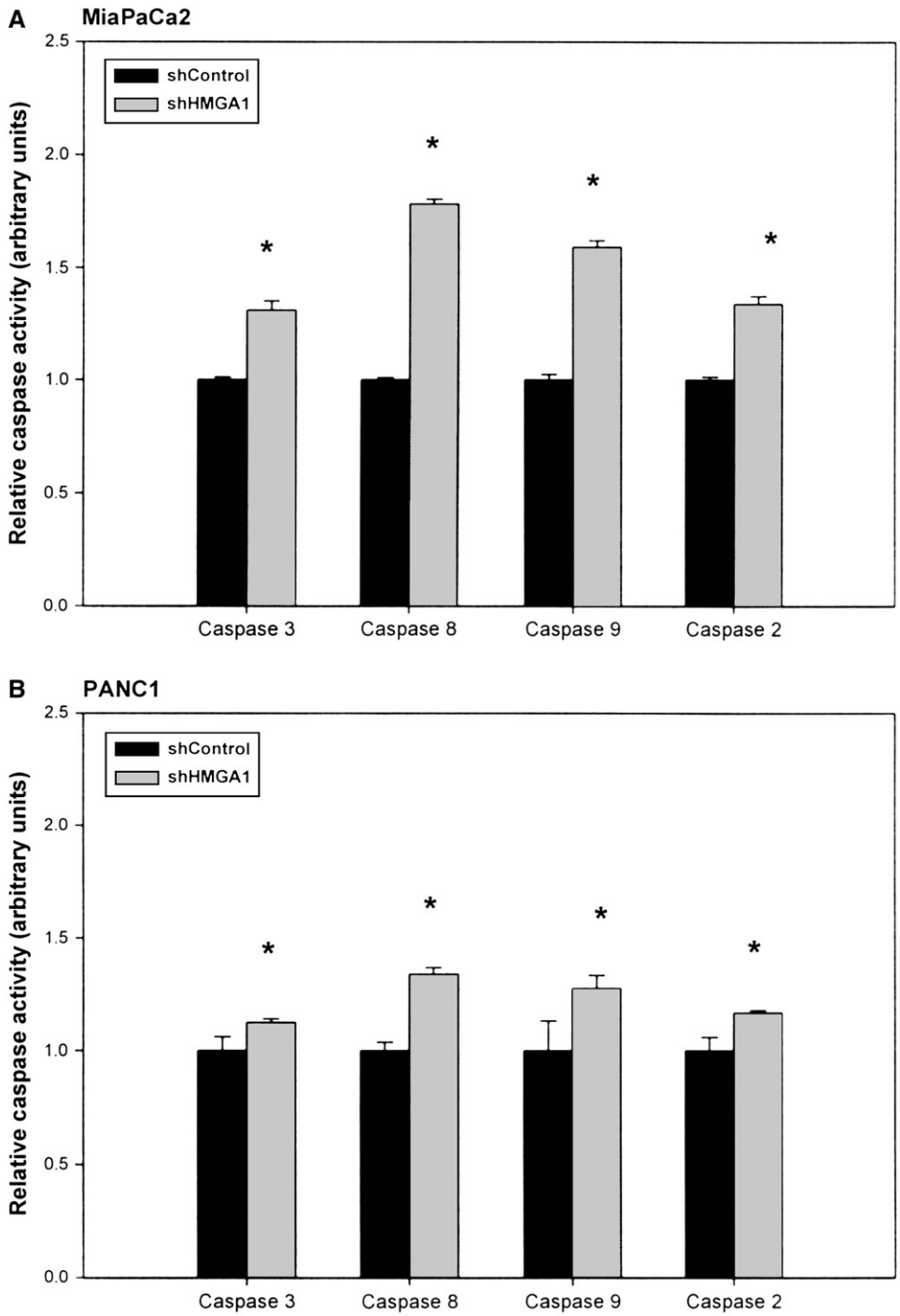

Fig. 4. The effect of targeted suppression of *HMGA1* expression resulted in increased caspase activation on exposure to gemcitabine. Activities of caspases 3, 8, 9, and 2 were quantified using a fluorometric assay after exposure to 1 μM gemcitabine for 48 hours. Activities of each of the four caspases profiled exhibited a significant increase in both MiaPaCa2 (**A**) and PANC1 (**B**) cell lines with lentiviral-mediated *HMGA1* silencing, compared to controls. Controls were cells stably transfected with lentivirus encoding scramble shRNA (shControl). Values are means (±SD) of three experiments with triplicate determinations. $^*P < 0.05$ versus shControl transfectants.

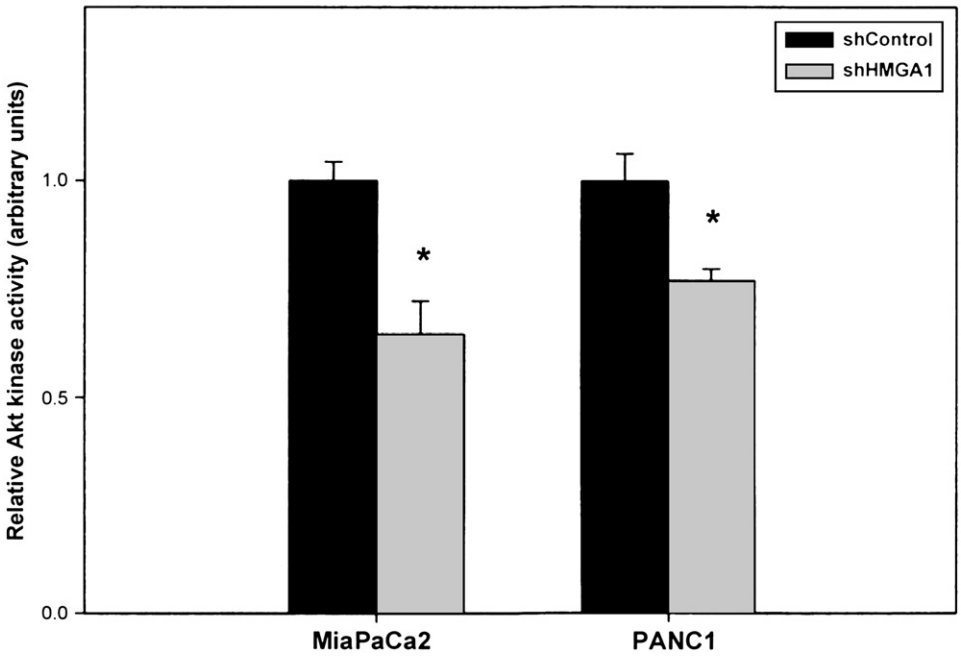

Fig. 5. Akt kinase activity was quantitated using an ELISA-based Akt activity assay following immunoprecipitation of active Akt from total cell lysates. Targeted suppression of *HMGA1* expression using lentivirus-mediated RNA interference resulted in approximately 30–40% reductions in Akt kinase activity in each of MiaPaCa2 and PANC1 cells, compared to controls. Silencing of *HMGA1* has no effect on the level of expression of total Akt, as determined on Western blot analysis (data not shown). Controls were cells stably transfected with lentivirus encoding scramble shRNA (shControl). Values are means (±SD) of three experiments with triplicate determinations. $^*P < 0.05$ versus shControl transfectants.

even nonproliferating ones, is a considerable advantage over other vectors. This feature in combination with the emerging power of RNA interference will facilitate development of viral RNA interference-based therapies in oncology. The first clinical trial involving a HIV-based lentiviral vector in AIDS patients has already been completed in the United States in 2005.[41]

In summary, our findings demonstrate for the first time that suppression of *HMGA1* expression by lentivirus-mediated RNA interference represents a novel strategy for chemosensitizing pancreatic adenocarcinoma to gemcitabine. As such, *HMGA1* warrants further investigation as a therapeutic target in pancreatic adenocarcinoma.

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Discussion

Mark P. Callery, M.D., Boston, Mass: Thank you. Dr. Liau, you have shown us that HMGA1 knockdown chemosensitizes these pancreatic cancer cell lines to gemcitabine. You achieved this both in cultured cells and in xenografts generated by these HMGA1 knockdown cells. You implicate a reduction in Akt kinase activity upon HMGA1 knockdown, and because you could defeat this anti apoptotic survival pathway, you suggest we can use this molecule as a target for future therapy. Your hypothesis was tested with a logical series of experiments and presented to us quite nicely.

Do you have actual data for HMGA1 overexpression in human pancreatic cancer specimens, and do you have any idea as to the mechanism of HMGA1 overexpression? Does HMGA1 correlate or better cause a particularly malignant phenotype, for example, cellular invasiveness or metastasis? Finally, how might you suggest targeting HMGA1 for theraply? Are there any available drugs today?

Now, I asked you this on Saturday as well, but your xenografts were all made with customized knockdown cells, something that is just not possible in the clinical setting. Can you deliver somehow your silencer to native xenografts, prove that HMGA1 knockdown occurs, and in fact sensitizes them to treatment with gemcitabine?

Congratulations to you and Stan Ashley, and particularly Ed Whang. You all can be justifiably proud of your contribution.

Siong-Seng Liau, M.D., Boston, Mass: Thank you very much, Dr. Callery. To answer these questions, we have embarked on looking at the expression of HMGA1 in pancreatic cancer tissues. We previously constructed a tissue microarray containing samples from 89 patients with pancreatic cancer. Of these 89 patients, 92% have tumoral HMGA1 overexpression on immunohistochemistry, with little or no expression in normal pancreas.

In terms of the effect of HMGA1 on the malignant phenotype of pancreatic cancers, we previously have shown that silencing of HMGA1 results in significant reductions in cellular invasiveness and in in vivo metastasis. In addition, we have shown that overexpression of HMGA1 allows these cells to grow under anchorage independent conditions, that is, in a soft agar colony formation assay. The reverse is also true; as we silence HMGA1, there is a significant reduction in the ability of these cells to grow under anchorage independent conditions. HMGA1 silencing is also associated with a reduction in tumor size in a nude mouse xenograft model of pancreatic cancer.

There is no drug that specifically targets HMGA1. There is a family of drugs, related to mitomycin C, that crosslinks HMGA1 to DNA. Howeer, these agents are by no means specific inhibitors of HMGA1 activity.

It is true that the xenografts we implanted to demonstrate in vivo chemosensitivity were derived from stably transfected cells. We are currently embarking on a gene therapy approach to pancreatic cancer in which we generate a high titre lentivirus expressing hairpin RNA targeting HMGA1. We hope to demonstrate that intratumoral injection of this lentivirus will chemosensitize pancreatic cancer xenografts.

In closing, I'd like to thank you for insightful questions and kind comments.

Postoperative Pancreatic Fistulas Are Not Equivalent After Proximal, Distal, and Central Pancreatectomy

Wande Pratt, B.A., Shishir K. Maithel, M.D., Tsafrir Vanounou, M.D., Mark P. Callery, M.D., Charles M. Vollmer, Jr., M.D.

It is uncertain whether postoperative pancreatic fistulas after distal and central pancreatectomies behave similarly to those after pancreaticoduodenectomy. To date, this concept has not been validated either clinically or economically. Overall, 256 consecutive pancreatic resections from October 2001 to February 2006 (184 pancreaticoduodenectomies, 66 distal pancreatectomies, and 6 central pancreatectomies) were evaluated according to the International Study Group of Pancreatic Fistula classification scheme. Pancreatic fistula was defined as any measurable drainage on or after postoperative day 3, with amylase content greater than three times the normal serum value. Outcomes were divided into four grades: (1) no fistula, (2) grade A: biochemical fistula without clinical sequelae, (3) grade B: fistula requiring any therapeutic intervention, or (4) grade C: fistula with severe clinical sequelae. Grades B and C are considered clinically relevant fistulas based on worsening morbidity, increased length of stay, frequent hospital readmission, and increased costs/resource utilization. Clinical and economic outcomes were compared—grade for grade—across the three resection types. Fistulas of any extent (Grades A–C) occurred in one third of all patients; two thirds had no fistula. Overall, there were 16 readmissions (6%), six reoperations (2%), and no deaths attributable to pancreatic fistula. Outcomes between no fistula and grade A patients were identical across resection types, though grade A fistula was more common in distal pancreatectomy. For each resection type, length of stay and costs progressively increased with grades B and C. However, the negative impact of these clinically relevant fistulas varied between resection types. Rates for intensive care unit admission and rehabilitation placement were higher among pancreaticoduodenectomy patients. Total parenteral nutrition and antibiotic use were similar, but percutaneous drainage was used more often for distal pancreatectomy. Grade B fistula was more severe after distal pancreatectomy, as indicated by increased length of stay, readmissions, and total cost. Although reoperation rates for grade C fistulas were equivalent, intervals to reoperation were substantially longer after distal and central pancreatectomies. When classified according to International Study Group of Pancreatic Fistula criteria, clinically relevant pancreatic fistulas behaved differently depending on type of pancreatectomy. This translates into variable severity that guides management decisions, which ultimately dictate clinical outcomes and economic impact. (J GASTROINTEST SURG 2006;10:1264–1279) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Pancreatic fistula, cost analysis, International Study Group of Pancreatic Fistula, pancreatic resection

Although distal and central pancreatectomies are now performed more frequently for treatment of benign and malignant diseases of the pancreas, less is known about the short-term outcomes after these operations as opposed to pancreaticoduodenectomy. Distal pancreatectomy, like pancreaticoduodenectomy, presents a formidable challenge for surgeons managing diseases of the pancreas. Malignant tumors arising in the body or tail often emerge at an advanced stage, and when performed for pancreatitis, inflammatory effects make this resection particularly challenging.[1,2] Furthermore, cystic diseases increasingly require pancreatic resection, often in the setting of normal glandular texture with an attendant risk for fistula development. Despite these factors, distal pancreatectomy remains a favored approach

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for curative treatment of these conditions and can be accomplished with a morbidity of 31%–47% and mortality of 0.9%–4%.[3–7]

Central pancreatectomy—also referred to as medial, segmental, or middle segment pancreatectomy—is technically alluring but no less challenging than proximal or distal pancreatectomy. First described by Guillemin and Bessot[8] in 1957, it has subsequently gained acceptance as an alternative to distal and proximal resections for select benign or preneoplastic lesions of the neck or body, [9–13] given its maintenance of pancreatic endocrine and exocrine function. It involves transection to the left and right of the lesion, with definitive closure of the proximal gland and construction of a pancreatico-enteric anastomosis to the distal remnant. Postoperative morbidity (25%–63%) and mortality (0%–2%) rates for this operation are equivalent to those after other resections.[10–15]

The major dilemma for these procedures lies in management of the remnant pancreas, particularly as it relates to the technical prevention of pancreatic fistula. Commonly used techniques for closure of the pancreatic duct after distal pancreatectomy include the hand-sewn suture technique, staple closure, or a combination of both. Suture ligation of the main pancreatic duct, application of adhesive sealants, and enteric drainage of the proximal gland are additional methods to avoid fistula formation.[1,5–7] Typically after central pancreatectomy, the proximal aspect is often closed with a hand-sewn suture or staple technique, whereas the distal remnant is managed with either Roux-en-Y pancreaticojejunostomy or pancreaticogastrostomy.[11,15,16]

Despite these various surgical approaches, pancreatic fistula remains the single most common complication after pancreatic resections. Published postoperative pancreatic fistula rates are similar for proximal[17–24] (5%–20%)and distal[3,5,7] pancreatectomy (5%–26%), but are remarkably higher for central[10,13–15] pancreatectomy (17%–63%). Although the clinical impact of pancreatic fistula after proximal pancreatectomy is well described, evidence demonstrating the clinical consequences of fistula formation after distal or central pancreatectomy is less robust and poorly described. In fact, some still believe that pancreatic fistulae after these operations behave similarly to fistulae after pancreaticoduodenectomy. To date, this has not been demonstrated either clinically or economically. Furthermore, although a novel classification scheme describing fistula severity has been conceived by the International Study Group on Pancreatic Fistula (ISGPF)[25] and validated in a large series of patients undergoing pancreaticoduodenectomy,[26] it has yet to be applied for patients undergoing distal or central pancreatectomy.

The objectives of this study are to examine a contemporary experience with fistula development after various resection modalities of the pancreas at a single, high-volume pancreato-biliary surgical specialty center; to apply the ISGPF classification scheme in patients undergoing pancreatic resections; and to directly compare the clinical and economic impacts, as well as management, of fistulae after proximal, distal, and central pancreatectomy.

MATERIAL AND METHODS

Patients

Two surgeons performed 256 consecutive pancreatic resections from October 2001 to February 2006, including 184 proximal, 66 subtotal or distal, and 6 central pancreatectomies. Preoperative diagnoses included suspected periampullary or neuroendocrine tumors, intraductal papillary mucinous neoplasms, cysts, and pancreatitis. Final pathology revealed that patients most commonly had pancreatic ductal adenocarcinoma (n = 75), cystic disease (n = 50), or pancreatitis (n = 44). Other pathologies encountered included other periampullary malignancies (n = 39), neuroendocrine tumors (n = 13), and other various benign (n = 31) or malignant conditions (n = 4). Patients undergoing pancreaticoduodenectomy most often had ductal adenocarcinoma (n = 66, 36%), whereas those undergoing distal pancreatectomy had cystic disease (n = 27, 41%). Central pancreatectomy was most frequently performed for intraductal papillary mucinous neoplasms (n = 2) or pancreatitis (n = 2).

Surgical Technique

Management of the pancreatic remnant after pancreatectomy depended on the resection performed.

Proximal pancreatectomy. After proximal resection of the pancreas, a pancreatico-jejunal anastomosis was constructed in a duct-to-mucosa, end-to-side fashion with either a single- (n = 108) or two-layer (n = 76) interrupted anastomosis. Ductal stents were seldom used (n = 24, 13%). No pancreaticogastrostomies were performed. Prophylactic octreotide was given subcutaneously (dose 150 μg every 8 hours) and continued postoperatively in 93 patients considered high risk for pancreatic fistula based on gland texture, duct size, or disease process. A single drain was routinely placed anterior to the pancreatico-jejunal anastomosis and exteriorized through the lateral abdominal wall. In five patients, who were intraoperatively felt to be at extremely high risk for

either pancreatic or biliary fistula development, multiple drains were placed.

Distal pancreatectomy. Distal pancreatectomy was overwhelmingly (89%) performed with stapled transection/closure proximal to the pancreatic lesion. After resection, the staple line along the proximal pancreatic remnant was frequently oversewn with absorbable suture (n = 42, 63%). Application of fibrin sealant was seldom used (n = 4), and ductal stents were placed antegrade through the ampulla in only two cases. Retrograde enteric drainage of the remnant was not performed, and prophylactic octreotide was rarely employed (n = 4). A single drain was routinely placed (n = 51, 71%) in the vicinity of the proximal transection margin and exteriorized through the lateral abdominal wall. Multiple drains were placed in nine patients for further control of potential intra-abdominal collections in the left upper quadrant. No drains were placed in only six cases.

Central pancreatectomy. After central pancreatectomy, the proximal pancreatic remnant was similarly controlled with either staple (n = 3) or suture closure (n = 3). In only one case was adhesive sealant applied. A single-layered pancreaticojejunostomy was constructed for management of the distal pancreatic remnant in all cases. Prophylactic octreotide was frequently administered (n = 5, 83%). A single drain was routinely placed anterior to the pancreatico-jejunal anastomosis and exteriorized through the lateral abdominal wall. In only one case did a patient receive a second drain in the resection bed.

Postoperative Management

All aspects of care were directed by the operating surgeon according to a standardized postoperative care path for pancreatic resection employed at our institution. Outputs from all operatively placed drains were recorded daily for at least 6 postoperative days. Amylase levels were obtained from drains usually after tolerance of a soft solid diet on or after postoperative day (POD) 6. In those patients with more than one operatively placed drain, amylase levels were obtained from the drain known to be adjacent to the pancreatic remnant. All patients had drains removed at the operating surgeon's discretion, most often when amylase content was below three times normal serum levels. Drains were maintained longer if patients had high drain amylase levels, generous fluid output, or sinister appearance to the effluent. Computed tomography was used to assess for fluid collections whenever indicated based on clinical suspicion (n = 50, 20%), with 28% of these cases (n = 14) resulting in CT-guided percutaneous drainage. Additional management methods for suspected fistulas included administration of antibiotics, subcutaneous octreotide, supplemental (i.e., parenteral or enteral) nutritional support, and infrequently, surgical exploration.

Data Collection

Data on preoperative, intraoperative, and postoperative care was prospectively collected for each case. Preoperative parameters include patient demographics (i.e., age, gender, and comorbidities), presenting symptoms (i.e., jaundice, weight loss, diarrhea, pain, etc.), laboratory tests, prior imaging studies, and preoperative therapies (i.e., endoscopic ductal stenting or sphincterotomy). Intraoperative parameters include total operative time, blood loss, fluid resuscitation, blood transfusions, gland characteristics, surgical technique, as well as the use of drains, stents, somatostatin analogues, and adhesive sealant. Postoperative events and clinical outcomes were recorded, including therapeutic and diagnostic strategies, requirements for nutritional support, laboratory and imaging studies, incidence and type of complications, intensive care unit (ICU) transfers and duration, length of stay (LOS), discharge disposition, hospital readmissions, reoperations, and death within 30 days postoperatively. Data was stored on a secured database and analyzed independently.

Classification of Pancreatic Fistula

A detailed analysis of the data and clinical course was individually performed for each of the 256 consecutive patients. Pancreatic fistula, according to the ISGPF classification scheme, was defined as any measurable drainage from an operatively placed drain (or a subsequently placed percutaneous drain) on or after POD 3, with an amylase content greater than three times the upper limit of normal serum amylase level (i.e., >300 IU/L).[25] All patients below this threshold were considered to have no biochemical evidence of fistula.

Those patients with fistula were then classified into three grades of severity according to ISGPF clinical criteria *only* after complete postoperative follow-up was accomplished. Table 1 summarizes these distinctions of fistula severity based on the presence or absence of 10 clinical parameters. A fistula is classified according to a particular grade if at least one criterion for that particular grade occurs.

No fistula patients. The no fistula group of patients lacks both elevated drain amylase levels and any clinical sequelae of fistula. Treatments specific for pancreatic fistula management (i.e., somatostatin

Table 1. Criteria for grading postoperative pancreatic fistula according to International Study Group of Pancreatic Fistula[25]

Criteria	No fistula	Grade A fistula	Grade B fistula	Grade C fistula
Drain amylase	<3 times normal serum amylase	>3 times normal serum amylase	>3 times normal serum amylase	>3 times normal serum amylase
Clinical conditions	Well	Well	Often	Ill appearing/bad
Specific treatment	No	No	Yes	Yes
US/CT (if obtained)	Negative	Negative	Negative	Positive
Persistent drainage (>3 weeks)	No	No	Usually	Yes
Signs of infection*	No	No	Yes	Yes
Readmission†	No	No	Yes	Yes/No
Sepsis‡	No	No	No	Yes
Reoperation	No	No	No	Yes
Death related to fistula	No	No	No	Yes

ISGPF classification scheme.
*Signs of infection include elevated body temperature >38° C, leukocytosis, and localized erythema, induration or purulent drainage.
†Readmission is any hospital admission within 30 days following hospital discharge from the initial operation.
‡Sepsis is the presence of localized infection and positive culture with evidence of bacteremia (i.e. chills, rigors elevated are requiring antibiotic treatment, or hemodynamic compromise as demonstrated by high cardiac output and low systemic vascular resistance (SVR) within 24 h even of body temperature >38° C.

analogues, percutaneous drainage of peripancreatic fluid collections) are neither required nor instituted. Patients without pancreatic fistulas may certainly suffer from other postoperative complications with varying (sometimes severe) clinical manifestations, but these are not directly attributable to pancreatic fistula.

Grade A fistulas. Grade A fistulas are transient, asymptomatic fistulas, evident only by elevated drain amylase levels. The clinical sequelae of pancreatic fistula do not manifest in these patients. Consequently, treatments are not required, nor do deviations in clinical management occur for this fistula grade. Drains are removed within 3 weeks—almost always within the first 7 days after the operation. Diagnostic imaging studies, if obtained at all, do not reveal worrisome or suspicious peripancreatic collections. Antibiotics, supplemental nutrition, somatostatin analogues, percutaneous drainage, reoperation, and readmission for fistula management are neither required nor employed for this group. These *biochemical* fistulas are clinically insignificant.

Grade B fistulas. Grade B fistulas are symptomatic, clinically apparent fistulas that require diagnostic evaluation and therapeutic management. Patients may complain of abdominal pain, fever, nausea, intolerance to oral intake, other bowel-related symptoms, or systemic malaise. Diagnostic imaging studies may show worrisome or suspicious peripancreatic fluid collections. Antibiotic therapy, supplemental nutrition, and/or percutaneous drainage are indicated to control and prevent exacerbation of grade B fistulas. Operatively placed drains may remain in situ at the time of discharge, and are frequently required for management longer than 3 weeks. Therefore, grade B is considered the first tier of clinically relevant fistulae.

Grade C fistulas. Grade C fistulas are severe, clinically significant fistulas that require major deviations in clinical management. In addition to supplemental nutrition, intravenous antibiotics, and somatostatin analogues, aggressive therapeutic interventions are unquestionably warranted. Drainage from operatively placed drains persists for several weeks, whereas diagnostic imaging studies demonstrate worrisome peripancreatic and/or other intra-abdominal fluid collections. Patients with these fistulas appear ill, present in critical and unstable condition, and are vulnerable to sepsis, organ dysfunction, even death. Surgical exploration may be indicated with one of four options: (1) wide peripancreatic drainage only, (2) attempt to primarily repair the site of leakage, (3) conversion to alternative means of pancreatic-enteric anastomosis, and (4) completion pancreatectomy. Grade C represents the second level of clinically relevant fistulae.

Late fistulas. In our previous analysis of the ISGPF classification scheme for pancreaticoduodenectomy,[25] a subclass of patients with low or normal initial amylase levels subsequently developed clinical manifestations of fistula (with biochemical proof) and was classified as "late" fistula.[26] These fistulas

all meet criteria for grade B or C fistulas, but are distinct from "early" fistulas, whose amylase levels are initially elevated. Clinical and economic outcomes for this subclass of presentation are, grade for grade, worse than for their early counterparts. Similarly, we have elucidated and compared the incidence of these latent fistulas after proximal, distal, and central pancreatectomy.

Analysis by the ISGPF Classification Scheme

Once appropriately classified, clinical and economic parameters distinct from ISGPF criteria were used to analyze and compare the three grades of fistula severity among each resection modality. The following are considered clinically relevant parameters: index LOS, total hospital stay, complications, ICU transfers and duration, blood

Table 2. Clinical parameters for analysis of pancreatic fistula

Parameters	Definition
Length of stay	Days from the initial operations to hospital discharge
Total hosptial of stay	Days from the initial operation to hospital discharge *plus* any readmissions within 30 days postoperatively
Postoperative complications	
Ileus	Absence of bowel sounds, failure to pass flatus, or bowel movement by postoperative day 5, and the need for total parenteral nutrition
Delayed gastric emptying	Failure to resume oral liquid intake by postoperative day 10, and/or emesis >500 ml on or after postoperative day 5, and/or continued nasogastric drainage >500 ml on after postoperative day 5.
Biliary leak	Bilious drainage from intra operatively placed drains, and/or radiographically-confirmed fluid collection requiring surgical, endoscopic, or radiographic intervention.
Gastrointestinal bleed	Guaiac-positive hematemesis, hematoochexia, or melena and no other source of ongoing blood loss, or the sudden appearance of frank blood either on NG lavage or per rectum, with subsequent fall in hemoglobin of 2 gm/dL and requiring blood product transfusion or reoperation
Abscess	Culture-positive purulent drainage from intra-abdominal fluid collection obtained percutaneously or operatively, and/or radiographically-confirmed fluid collection with systemic or localized signs of infection (i.e., elevated, were body temperature $>38^\circ$ C, purulent drainage).
Myocardial infarction	Increase in serum concentration of CK-MB and troponin, and/or the following EXE changes new Q-waves at least 0.04 duration, new persistent ST elevation/depression.
Acute renal failure	Serum creatinine greater than 3.0 mg/dL or doubling of baseline value, and/or need for dialysis
Pulmonary embolism	Acute onset of dyspnes or tachypnea, hypotension or increased CVP, positive V/Q scan and/or chest CTA, and requiring pharmacological therapy.
Respiratory distress	$PaCO_2 > 60$ mmHg and requiring pharmacological therapy or intubation, or the need for intubation of mechanical ventilation for more than 2 postoperatively
Pneumonia	Presence of new infiltrate on CXR, and the following: body temperature $>38^\circ$ C, abnormal elevation of WBC, or positive sputum Gram stain or culture, and requiring IV antibiotic treatment
Wound complications	Any evidence of infection (i.e., eyrthema, purulent discharge, induration) and requiring antibiotic treatment, or evidence of dehiscence
Urinary tract infection	Culture-positive urine, pyuria and bacteriuria on urinalysis, and requiring antibiotic treatment
Neurological complications	Cerebral hypoxia, cerebral vascular accidents, or intracranial hemorrhage, with the onset of hemiplegia, hemianesthesia, hemianopia, aphasia, or unconsciousness
ICU transfer	Treatment in the ICU on or after postoperative day 1, excluding admissions to the ICU directly from the operating room
Blood transfusion	Units of packed red blood cells required postoperatively, excluding blood products received during the initial operation
Patient discharge disposition	Hospital discharge to one of three options following the initial operation: to home, to home with arrangements for visiting nurse assistance, or to a rehabilitation facility.

WBC = white blood cell count; CKMB = creatine kinase MB fraction; EKG = electrocardiogram; NG = nasogastric; ST = ST segment; CVP = central or jugular venous pressure; V/Q = ventilation perfusion; CTA = computed tomography angiography; CXR = chest radiograph.

Table 3. Economic parameters for analysis of pancreatic fistula

Economic parameters	Definition
Total hospital costs	Cost per patient form the initial operation to hospital discharge plus any costs for readmissions 30 days postoperatively
Itemized costs	
Pharmacy costs	Cost per patient for all medications, fluid management, and nutritional support, including parenteral and enteral nutrition received postoperatively
Radiology costs	Cost per patient for all imaging studies (i.e, chest radiographs, computed tomography scans, ultrasound) and interventional radiology procedures(i.e, percutaneous drainage, endoscopy) obtained postoperatively
Transfusion costs	Cost per patient for all blood products (i.e, packed red blood cells, fresh frozen plasma, cryoprecipitate, platelets) received postoperatively
Laboratory costs	Cost per patient for laboratory studies, including serum chemisty panel, complete blood count, and drain amylase levels obtained postoperatively
ICU costs	Cost per patient attributable to management in the post anesthesia or intensive care units
Room costs	Cost per patient for postoperative hospital accommodations and routine nursing care
Operating costs	Cost per patient for the initial operation, and for any reoperations 30 days postoperatively

transfusions, and discharge disposition. Additionally, fiscal parameters were examined, including itemized and total hospital costs. Tables 2 and 3 define the respective clinical and economic parameters employed.

Comparison of Clinically Relevant Fistula

A detailed analysis of fistula severity across all pancreatic resection modalities ensued, with particular concentration on clinically relevant fistulas (i.e., grades B and C). Additional parameters were considered in an effort to rigorously examine fistula behavior and its persistent impact after three distinct operations. Furthermore, clinical management approaches were scrutinized. Specifically, we explored urgent treatment methods (i.e., ICU transfers, CT-guided percutaneous drainage, and reoperations), as well as prolonged therapeutic approaches (i.e., continuous intra-abdominal drainage, rehabilitation placement, and hospital readmissions) employed for treatment of clinically relevant fistulas vis-à-vis each pancreatic resection modality.

Statistical Analysis

Fistula grades and resection modalities were compared using the chi-square statistic, analysis of variance, and the Student's *t* tests. Factors associated with fistula severity were calculated based on cross-tabulations using chi-square statistic and the Pearson correlation test. Statistical significance was accepted at a P value < 0.05. All statistical computations were performed using Statistical Package for the Social Sciences 14.0 for Windows (SPSS, Inc. Chicago, IL).

RESULTS

Incidence of Pancreatic Fistula

All patients met criteria for evaluation by the ISGPF classification scheme. Table 4 lists the incidence of pancreatic fistula for each resection modality. Eighty-three patients had a fistula for an overall incidence of 32.4%. However, one half of these fistulas (n = 41) were clinically insignificant grade A, or biochemical, representing 16.0% of all patients.

Table 4. Incidence of pancreatic fistula following pancreatic resection

	Proximal	Distal	Central	*P* value*
Patients (N)	184	66	6	—
No fistula (%)	129 (70)	44 (67)	0 (0)	0.001
Fistula (%)				
Overall	55 (30)	22 (33)	6 (100)	0.001
Biochemical grade A	26 (14)	14 (21)	1 (17)	0.41
Clinical grade B	23 (13)	5 (8)	4 (66)	0.001
Clinical grade C	6 (3)	3 (5)	1 (17)	0.24

*All *P* values for comparison among resections.

Clinically relevant fistulas comprised the remainder; 32 patients (12.5% overall) developed grade B fistulas, and only 10 (3.9% overall) developed grade C fistulas. Overall, there were 16 readmissions (6.3%), six reoperations (2.3%), and no deaths attributable to pancreatic fistula. Antibiotics were administered for fistula management in 33 of 83 patients (39.8%); supplemental nutrition was initiated for 19 patients (22.9%); percutaneous drainage was required in 14 patients (16.9%); and therapeutic octreotide was seldom used (8.4%).

The overall incidence of fistula was equivalent after proximal and distal pancreatectomy (30% vs. 33%; $P = 0.61$); yet, the incidence of biochemical and clinical (grade B or C) fistulas differed. When fistulas occurred after proximal pancreatectomy, they equally were likely to be biochemical or clinical (47 /53 %). In contrast, fistulas after distal pancreatectomy were more often biochemical (64%), and thus, had no clinical consequence.

The overall incidence of fistula was highest after central pancreatectomy. All six of these patients developed pancreatic fistulas, five of which (83%) were clinically relevant fistulas.

Biochemical (Grade A) Fistula Characteristics

Overall, 41 patients (16.0%) developed grade A fistulas after pancreatectomy. Specific treatments for this fistula grade were neither indicated nor administered after any pancreatic resection, and readmissions or reoperations for fistula management did not occur for this fistula grade. No patient had persistent drainage for more than 3 weeks.

Proximal pancreatectomy. Grade A fistulas occurred in 26 of 184 patients (14%) undergoing pancreaticoduodenectomy. The median drain amylase level for this fistula grade was 900 IU/L. Only two patients with this fistula type required any diagnostic imaging, and in both patients, CT scans failed to show peripancreatic fluid collections.

Distal pancreatectomy. The incidence of grade A fistulas, however, was higher after distal pancreatectomy, occurring in 14 of 66 patients (21%). Furthermore, the median drain amylase level (4,610 IU/L), when compared to proximal pancreatectomy, was significantly higher ($P = 0.03$). Only three patients required any abdominal imaging after distal pancreatectomy, and all failed to demonstrate radiographic evidence of a fluid collection, leak, or fistula.

Central pancreatectomy. A single patient developed a grade A fistula after central pancreatectomy. Postoperatively, the patient suffered acute renal failure secondary to hypovolemia and was transferred to the ICU for monitoring. The patient's hemodynamic profile gradually improved, and total parenteral nutrition (TPN) was initiated and continued until gastrointestinal function resumed on POD 7. A drain amylase level (334 IU/L) was obtained after the patient tolerated a soft solid diet. Thus, with this marginally elevated amylase level and lack of clinical sequelae attributable to fistula, this was categorized as a biochemical fistula. Diagnostic imaging was not indicated, and the patient did not receive any antibiotics, octreotide therapy, or percutaneous drainage.

Clinically Relevant (Grade B and C) Fistula Characteristics

Clinically relevant fistulas occurred in 42 patients (16.4% overall incidence) undergoing any pancreatic resection (see Table 5). The majority of these patients (81%) had CT scans positive for suspicious fluid collections, with 41% (14/34 cases) amenable to percutaneous drainage. All but two patients received at least

Table 5. Outcomes for clinically relevant fistulas following pancreatic resection

Outcomes	Proximal	Distal	Central	*P* value*
Patients (%)	29 (16)	8 (12)	5 (83)	—
Age (median, yrs)	73	62	59	0.16
Drain amylase (median, IU/L)	1,048	3,688	831	0.90
CT positive finding (%)	22 (76)	8 (100)	4 (80)	0.32
Hyperalimentation (%)†	16 (55)	2 (25)	2 (40)	0.31
Antibiotics (%)†	25 (86)	5 (63)	3 (60)	0.21
Therapeutic octreotide (%)†	6 (21)	1 (13)	0 (0)	0.51
Percutaneous drainage (%)†	6 (21)	5 (63)	3 (60)	0.03
Persistent drainage (%)	12 (41)	4 (50)	2 (40)	0.91
Readmisssion (%)†	6 (21)	7 (88)	3 (60)	<0.001
Reoperation (%)†	3 (10)	2 (25)	1 (20)	0.55

*All *P* values for comparison among resections.
†Treatment for fistula management.

one treatment for fistula management. Antibiotics were the most commonly administered treatment (79%), followed by supplemental nutrition (45%), octreotide therapy (17%), and surgical exploration (14%). Despite the severity of these pancreatic fistulas, no fistula-related death occurred in any patient.

Proximal pancreatectomy. After proximal pancreatectomy, clinically relevant fistulas occurred in 29 patients (16% overall incidence). The majority of these fistulas (79 %) represented grade B fistulas. The median drain amylase level was 1048 IU/L. Three fourths of patients demonstrated radiographic evidence of fistula, and in twelve patients, drainage of fluid collections persisted longer than 3 weeks. Antibiotics were the most commonly administered treatment (86%), whereas percutaneous drainage and octreotide therapy were employed only 21% of the time. Surgical exploration with repair of the pancreatic-enteric anastomosis was required in only three patients after proximal pancreatectomy.

Distal pancreatectomy. These fistulas occurred in only eight patients and represented 36% of all fistulas and 12% of all patients presenting after distal pancreatectomy. The median drain amylase level for these clinically relevant fistulas was 3688 IU/L. Similar to proximal pancreatectomy, grade B fistulas represented the majority of these clinical fistulas (63%). All eight patients had CT scans positive for suspicious fluid collections. In contrast to proximal pancreatectomy, the vast majority of clinical fistulas after distal pancreatectomy (63%, $P = 0.02$) proceeded to CT-guided drainage for fistula management. Antibiotics were also employed for 63% of clinical fistulas, whereas therapeutic octreotide was seldom administered. Two patients required surgical exploration for drainage of the peripancreatic fluid collection, and a third developed sepsis, categorizing these fistulas as grade C fistulas.

Central pancreatectomy. Clinically relevant fistulas occurred in five patients (83% of fistula cases) undergoing central pancreatectomy. Grade B fistulas comprised 80% of all clinically relevant fistulas (four out of five). The median drain amylase level, at 831 IU/L was more in line with that observed after proximal pancreatectomy. All patients received at least one specific treatment for fistula management. Antibiotics and percutaneous drainage were similarly the most common treatments administered (60%). Although no patient received octreotide therapy, two received supplemental nutritional support for fistula management.

A single patient developed a grade C fistula after central pancreatectomy, requiring surgical exploration with wide peripancreatic drainage and a complicated recovery course. The initial drain amylase level, obtained on POD 10, was 177 IU/L, and the drain was subsequently removed. However, the patient returned on POD 63 with signs of infection and a 6.7 cm fluid collection anterior to the pancreatic resection bed. He underwent CT-guided percutaneous drainage, and this fluid amylase level demonstrated 1,779 IU/L. The patient rapidly improved, and was discharged home the following day, only to return 2 days later with an infection of the initial operative incision. The patient received antibiotics and was provided home nursing care for continued wound management. Interval imaging demonstrated a persistent fluid collection that was conservatively managed, but the patient returned to the operating room 260 days after the index operation for enteric drainage of a large (6.6 cm $\times$ 5.1 cm) fluid collection adjacent to the proximal remnant, and a second collection in the vicinity of the distal pancreaticojejunostomy (5.4 cm $\times$ 4.6 cm). Although not acutely ill at the time, the requisite operation categorized this fistula as a grade C.

Late Fistulas

The incidence of late fistulas was examined among all pancreatic resections. Of all fistulas (grade A–C), 15.7% presented in this fashion. These fistulas presented significantly later in the postoperative period than did early fistulas (10 days vs. 6 days, $P = 0.006$). This presentation was equivalent between proximal and distal resections, representing 31.0% of all clinically relevant proximal fistulas and 37.5% of those occurring after distal pancreatectomy ($P = 0.74$). Only one patient (20.0%) developed a latent fistula after central pancreatectomy.

Clinical Analysis of Pancreatic Fistula

Clinical parameters, distinct from those used for defining the ISGPF classification scheme, were considered for all patients and are summarized in Table 6.

Proximal pancreatectomy. Among proximal pancreatectomy, there was no statistically significant difference in LOS between no fistula and grade A fistulas ($P = 0.06$). However, postoperative LOS (index admission) progressively increased as fistula severity increased from grade A to grade C. Total hospital stay was also shortest for grade A fistulas and longest for grade C fistulas (grade A, 8 days; grade B, 13 days; and grade C, 35 days).

The incidence of complications after proximal pancreatectomy also increased as fistula severity increased (see Table 6). Overall, 76 of 184 patients (41.3%) developed complications exclusive of pancreatic fistula. Patients with grade A fistulas seldom (11.5%) developed complications. However, the

Table 6. Clinical and economic analysis of pancreatic fistula following pancreatic resection

Pancreaticoduodenectomy					
Clinical parameters	**No fistula**	**Grade A**	**Grade B**	**Grade C**	**p Value†**
Patients (%)	129 (70)	26 (14)	23 (13)	6 (3)	—
Length of stay (median, day)	8	8	10	27	< .001
Total hospital stay (median, day)	8	8	13	35	< .001
Complications (%)	50 (39)	3 (12)	17 (74)	6 (100)	< .001
ICU transfer (%)	17 (13)	0 (0)	6 (26)	5 (83)	< .001
Blood transfusions (%)	17 (13)	2 (8)	8 (35)	3 (50)	.004
Rehabilitation placement (%)	19 (15)	1 (4)	8 (35)	5 (83)	< .001
Total hospital costs (median)	$19,179	$18,075	$27,788	$135,933	< .001
Distal pancreatectomy					
Clinical parameters	**No fistula**	**Grade A**	**Grade B**	**Grade C**	**p Value†**
Patients (%)	44 (67)	14 (21)	5 (8)	3 (4)	—
Length of stay (median, day)	7	7	8	8	.12
Total hospital stay (median, day)	7	7	17	17	< .001
Complications (%)	9 (20)	1 (7)	3 (60)	3 (100)	< .001
ICU transfer (%)	5 (11)	0 (0)	0 (0)	0 (0)	.45
Blood transfusions (%)	5 (11)	0 (0)	2 (40)	1 (33)	.08
Rehabilitation placement (%)	0 (0)	0 (0)	0 (0)	0 (0)	—
Total hospital costs (median)	$15,241	$14,836	$34,555	$38,509	.02
Central pancreatectomy					
Clinical parameters	**No fistula**	**Grade A**	**Grade B**	**Grade C**	**p Value†**
Patients (%)	0 (0)	1 (17)	4 (66)	1 (17)	—
Length of stay (median, day)	—	14	8	10	.96
Total hospital stay (median, day)	—	14	11	28	.41
Complications (%)	—	1 (100)	2 (50)	1 (100)	.65
ICU transfer (%)	—	1 (100)	1 (25)	1 (100)	.35
Blood transfusions (%)	—	1 (100)	1 (25)	1 (100)	.35
Rehabilitation placement (%)	—	0 (0)	1 (25)	0 (0)	.85
Total hospital costs (median)	—	$35,639	$28,044	$85,967	.34

†All p values for comparison between fistula grades.

incidence of concomitant complications was significantly increased for patients with grade B and C fistulas (73.9% and 100%, respectively). Wound infections, postoperative ileus, intra-abdominal abscess, and respiratory complications were commonly observed.

Approximately 15% of all patients required ICU management at any time after proximal pancreatectomy. No patient who developed a grade A fistula was transferred to the ICU, compared with six patients with grade B fistulas (26.1%) and five with grade C fistulas (83.3%).

Blood transfusions were frequently required for patients who developed fistulas after proximal pancreatectomy. Overall, 13 patients with a pancreatic fistula received blood products in the postoperative period, 11 of which had grade B or C fistulas. The number of units of packed red blood cells was greatest for patients with grade C fistulas (median, 2 units).

Patient discharge disposition after proximal pancreatectomy was also compared across all fistula grades. Patients who developed grade A or B fistulas were often discharged to home (96.2% and 65.2%, respectively), whereas patients with grade C fistulas were more regularly discharged to rehabilitation facilities (83.3%).

Distal pancreatectomy. The preceding findings demonstrate that, according to ISGPF criteria, clinical outcomes after proximal resections worsen in escalating fashion as fistula severity increases from grade A to grade C. Similar trends were observed in patients undergoing distal pancreatectomy, but the clinically relevant fistula grades are marginally

differentiated (see Table 6). Although clinical outcomes after distal pancreatectomy worsened as fistula severity increased from grade A to grade C, the clinical impact of grade C fistulas was no more severe than that of grade B fistulas. Postoperative LOS was equivalent for grade B and C fistulas (median, 8 days), as was total hospital stay (median, 17 days). Postoperative complications did occur more often with grade C fistulas compared to grade B fistulas (100% vs. 60%, respectively), but this was not statistically significant ($P = 0.27$). Patients undergoing distal pancreatectomy did not require any postoperative ICU management or rehabilitation placement, but were frequently managed with postoperative blood transfusions—37.5% of clinically relevant distal fistulas (grade B, 40.0%; grade C, 33.3%; $P = 0.88$).

Central pancreatectomy. Analysis of clinical outcomes after central pancreatectomy shows fewer distinctions and was obviously limited by a smaller cohort (see Table 6). The single patient that subsequently developed a grade A fistula was transferred to the ICU for acute renal failure recognized on the night after the operation. This patient was resuscitated with intravenous fluids and blood transfusion and returned home on POD 14. Four of six patients experienced grade B fistulas, with lengths of stay for these fistulas ranging from 6 to 26 days (median, 8). Complications exclusive of pancreatic fistula occurred in two of the four (50%) patients, but only one patient with a grade B fistula was transferred to the ICU. This patient received transfusion for management of blood loss anemia and subsequently required rehabilitation placement for continued postoperative management. Only one patient had a grade C fistula after central pancreatectomy. This patient developed delayed gastric emptying during the index hospital admission, but left the hospital after 10 days only to return on POD 63 with a suspicious fluid collection amenable to CT-guided percutaneous drainage. After a second hospital readmission for a wound infection 2 days later, the patient remained clinically stable. However, on POD 260, the patient underwent enteric drainage of two persistent fluid collections and remained in the hospital for 14 days. The total hospital stay, including the index admission and three hospital readmissions, was 28 days.

Economic Analysis of Pancreatic Fistula

To further understand the impact of these fistulas, fiscal parameters for each fistula grade were analyzed. Outcomes comparing each resection type are listed in Table 6.

Proximal pancreatectomy. As fistula severity increased after proximal pancreatectomy, all cost metrics correspondingly escalated. Hospital costs for grade A fistulas did not significantly differ from those for no fistula patients ($P = 0.17$), and costs for resource utilization (i.e., radiology, pharmacy, laboratory, and transfusion costs) were equivalent between no fistula and grade A classes. However, itemized costs for grade B fistulas were significantly greater than those for grade A fistulas ($P < 0.01$). By definition, grade B fistulas frequently required diagnostic evaluations and therapeutic management, as radiology, pharmacy, and laboratory costs all increased significantly ($P < 0.01$). Hospital costs for grade C fistulas were even more expensive and were significantly greater than those for any other fistula type ($P < 0.01$). A detailed economic analysis of itemized costs revealed three distinct features of grade C fistulas. First, resource utilization in the setting of grade C fistulas was greatest, as combined radiology, pharmacy, laboratory, and transfusion costs for this fistula grade ($23,365) exceeded total hospital costs for grade A fistulas ($19,179), and almost equaled total hospital costs for grade B fistulas ($27,788). Second, grade C fistulas not only required frequent ICU management, but once transferred to the ICU, these fistulas were remarkably more costly—ICU costs alone represented 41% of overall hospital costs. Finally, whereas total operating costs for no fistula, grade A and B fistulas were equivalent and ranged from $4,511 to $4,821, operating costs for grade C fistulas were significantly increased ($7,786, $P < 0.001$), demonstrating the high rate (50%)—and additional cost—of surgical exploration for management of grade C fistulas.

Distal pancreatectomy. Although dramatic differences in economic parameters were observed among proximal pancreatic resections, these distinctions between fistula grades after distal resections were less remarkable. As with proximal resections, total hospital costs for no fistula and grade A fistulas were equivalent after distal pancreatectomy ($15,241 and $14,836, respectively; $P = 0.42$), but significant increases occurred as fistula severity worsened from A to C. However, grade B fistulas did not behave in an intermediate fashion, and more closely resembled grade C fistulas ($34,555 and $38,509, respectively; $P = 0.63$). Costs for grade B and C fistulas—across all economic parameters—were not statistically different, and therefore preclude any economic distinction between these two fistula classes.

Central pancreatectomy. The economic impact of each fistula class after central pancreatectomy was also similar. Increased resource utilization for the single patient with a grade A fistula is demonstrated

by increased pharmacy, laboratory, and ICU costs. Although pharmacy costs for grade A fistulas exceeded those costs for grade B fistulas ($4,807 vs. $1,477; $P = 0.60$), the patient did not receive any treatments specific for fistula management (i.e., antibiotics, octreotide therapy, supplemental nutritional support). Furthermore, radiology costs were not increased. Other than an early chest radiograph in the surgical recovery room and an ultrasound to assess kidney perfusion in the setting of acute renal failure, diagnostic imaging was neither a significant nor a contributing factor to total hospital costs. Increased ICU costs, however, represent 39% of total hospital cost and demonstrate the 6-day ICU stay necessary to manage the patient's renal disease.

Among central pancreatectomy cases, patients with grade B fistulas had economic outcomes that most resembled their proximal pancreatectomy counterparts. Total hospital costs for this fistula grade were equivalent between both resection types ($28,044 vs. $27,788; $P = 0.88$). Furthermore, all itemized costs for central pancreatectomy matched those for proximal pancreatectomy.

The grade C fistula after central pancreatectomy also resembled its proximal counterpart. Total hospital costs for this fistula class were substantially greater than those for any other fistula type. Furthermore, resource costs (i.e., radiology, pharmacy, laboratory, ICU) in the setting of a grade C fistula were also greatest, and alone ($24,298) almost equaled total hospital costs for grade B fistulas ($28,044). Finally, although total operating costs for a grade C fistula exceeded operating costs for grade A and B fistulas, the cost of the index operation for the grade C fistula ($4,197) was also equivalent to those for grade A and B fistulas ($4,939; $P = 0.22$).

Comparison and Management of Clinically Relevant Fistulas

The previously described analyses of fistula severity indicate that not only does the incidence of pancreatic fistula differ among proximal, distal, and central pancreatectomy, but so too does the impact of clinical (grade B and C) fistulas. Therapeutic approaches to these fistulas were examined and compared. Furthermore, intervals to each management approach were explored in an effort to further describe whether clinically relevant fistulas behave differently after the various pancreatic resections (Table 7). Grade A fistulas do not impact clinical and economic outcomes, and were not examined or compared.

Table 7. Clinically relevant fistulas after each pancreatic resection

Outcomes	Proximal	Distal	Central	*P* value*
Patients (N)	29	8	5	—
Critical condition (%)	19 (66)	2 (25)	2 (40)	.10
ICU transfer (%)	11 (38)	0 (0)	2 (40)	.11
Rehabilitation placement (%)	13 (45)	0 (0)	1 (20)	.05
Drain removal after 28 days				
No (%)*	8 (28)	4 (50)	2 (40)	.48
Postoperative interval (median, days)	16	30	14	.11
CT-guided Percutaneous drainage (%)†				
No (%)*	6 (21)	5 (63)	3 (60)	.03
Postoperative interval (median, days)	21	28	20	.37
Reoperation†				
No (%)*	3 (10)	2 (25)	1 (20)	.55
Postoperative interval (median, days)	7	92	260	.002
Hospital readmission†				
Any readmission (%)	6 (21)	7 (88)	3 (60)	.001
≥ 2 readmission (%)	3 (10)	4 (50)	2 (40)	.03
Hospital stay (median, days)				
Initial	12	8	8	.23
Total	15	17	12	.91
Hospital costs (median)				
Initial	$27,703	$14,887	$28,241	.19
Total	$29,821	$35,591	$34,644	.58

*All *P* values for comparison among resections.
†Treatment for fistula management.

Proximal pancreatectomy. Patients with clinically relevant fistulas after proximal pancreatectomy often presented in critical condition and regularly required aggressive management in intensive care settings (38%). The rate of reoperation for fistula management was not statistically different across the proximal, distal, and central pancreatectomies. However, when required after proximal pancreatectomy, urgent surgical exploration was employed within 1 week of the index operation, and consequently delayed hospital discharge by approximately 4 days. Furthermore, when these patients were approved for hospital discharge, they were eight times more likely to require rehabilitation placement than patients who did not develop clinical fistulas after proximal pancreatectomy ($P < 0.001$; odds ratio, 8.3, 95% confidence interval, 3.5–19.8). Despite their high acuity, patients with clinical fistulas infrequently required any hospital readmissions after proximal pancreatectomy and almost never required multiple readmissions. When complete management of these clinically relevant proximal fistulas was accomplished, costs per patient totaled $29,821 (median).

Distal pancreatectomy. The impact of clinically relevant fistulas after distal pancreatectomy, however, followed a much more indolent and prolonged therapeutic course. Patients with these fistulas seldom presented in critical condition, and none required any ICU management. In contrast to proximal pancreatectomy cases, cautious drain management ensued with intra-abdominal drains placed during distal pancreatectomy remaining in situ 2 weeks longer (16 days vs. 30 days, $P = 0.05$). Furthermore, CT-guided percutaneous drainage was more frequently employed, and in each case, resulted in further hospital readmission. Two patients required surgical exploration with wide peripancreatic drainage, but unlike after proximal pancreatectomy, reoperation occurred several months later after distal pancreatectomy (range, 71–112 days). However, the most striking feature of these clinically relevant fistulas is that they almost always required at least one hospital readmission (88%) and frequently required multiple readmissions (50%). In fact, among all distal pancreatectomy cases, clinical fistula was the only complication that resulted in hospital readmission. Although the median hospital cost for the index admission was $14,887, these hospital readmissions contributed to greater total hospital costs (median, $35,591). This further demonstrates that the overall management of these lingering clinically relevant distal fistulas is potentially more costly than the treatment of proximal fistulas (see Fig. 1).

Central pancreatectomy. Clinically relevant fistula after central pancreatectomy behaved in an intermediate fashion to those fistulae after either proximal or distal pancreatectomy. Two of five patients were managed aggressively in the ICU, and only one patient required rehabilitation placement. Like proximal pancreatectomy, drains were removed early in the postoperative period, usually on day 14. However, the use of invasive interventions was similar to that after distal pancreatectomy. Three of five (60%) patients underwent CT-guided percutaneous drainage, and in all three, urgent drainage evoked hospital readmission. The single patient who underwent surgical exploration and enteric drainage of two peripancreatic fluid collections did so several months after the index operation, but only subsequent to

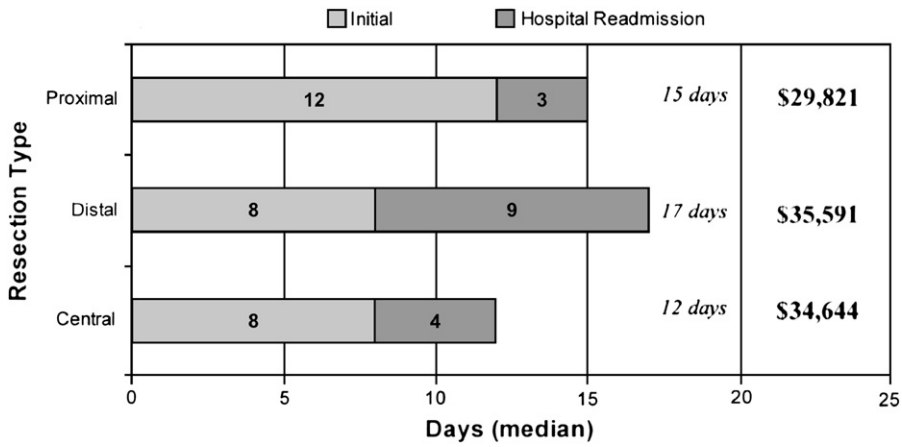

Fig. 1. Patients with proximal fistulas initially had longer hospital stays and increased hospital costs than those with distal fistulas. However, when hospital readmissions for fistula management were included, patients with distal fistulas remained in the hospital longer than those with proximal fistulas and subsequently incurred greater total hospital costs.

failed percutaneous drainage. These clinically relevant fistulas often required hospital readmission (60%) and occasionally required multiple readmissions. When the clinical course had finally ended, hospital costs totaled $34,644 (median) for these central fistulas.

DISCUSSION

Although the severity of pancreatic fistula after pancreaticoduodenectomy has been rigorously examined in prior studies,[17–19,21–24] little is known about the impact of this complication after other pancreatic resections. During the past decade, the incidence of pancreatic fistula after distal pancreatectomy has been reported to equal, but not exceed, rates of fistula after pancreaticoduodenectomy.[1,5,27,28] In the single largest series of distal pancreatectomy reported by Lillemoe et al.,[3] pancreatic fistula occurred in only 12 of 235 patients (5%), and this, therefore, represents a benchmark standard for pancreatic fistula after distal pancreatectomy. Although this complication was managed without surgery, its occurrence extended the length of postoperative stay. In that series, however, pancreatic fistula was not defined in the published report, and no distinction was made between biochemical and clinical fistulas, so it remains unclear whether pancreatic fistula had a negative clinical and/or economic impact on these 12 patients.

Even less is known about pancreatic fistula after central pancreatectomy. Reported rates of fistula range from 30%–63% in these infrequent series limited by few patients.[11–16] The largest series of central pancreatectomy cases—published by Sauvanet et al.[14] from a retrospective, multi-institutional study of 53 resections performed at twelve French and Belgian university hospitals—had an overall incidence of 30%, but there is no detailed description of the effects of this complication.

Given the findings from these previous studies, we sought to examine whether pancreatic fistula after three distinct operations occurred with similar frequency. The International Study Group on Pancreatic Fistula grading system for pancreatic fistula was applied to each resection modality, judged for its clinical and economic validity, and analyzed to determine the comparative impact of clinically significant fistulas across proximal, distal, and central pancreatectomy.

In this current analysis, the overall incidence of fistula was equivalent between proximal and distal pancreatectomy cases (30% and 33%, respectively) and significantly higher after central pancreatic resections. However, the impact of these fistulas depended on the type of resection performed and is dictated, we hypothesize, by the need for enteric reconstruction. Although fistulas occurring after proximal resections were equally likely to be either clinically silent or clinically significant, two thirds of fistulas occurring after distal resections were biochemical and had no clinical impact. In contrast to both proximal and distal resections, the majority of fistulas after central pancreatectomy had at once a measurable detrimental clinical impact. These results suggest that although the presence of a pancreatico-enteric anastomosis does not affect the overall *incidence* of pancreatic fistula, operations requiring enteric reconstruction are more susceptible to developing clinical fistulas that negatively impact clinical and economic outcomes.

These findings are comparable to results from a report by Sauvanet et al.,[14] who suggested that a pancreatic fistula originating from a pancreatico-enteric anastomosis seems to have a worse prognosis than those originating from a pancreatic remnant. Among 16 patients who developed pancreatic fistula after 53 central pancreatectomies, the source of fistula was identified in four patients, including three who required reoperation. All three had leakage of the pancreatico-enteric anastomosis, and one subsequently died from multiple organ failure. The fourth patient did not leak from the pancreaticojejunostomy, but instead developed an amylase-rich fluid collection adjacent to the proximal pancreatic remnant. However, this collection did not result in severe clinical sequelae. These findings suggest that leakage at the site of a pancreatico-enteric anastomosis is the precipitating factor for more severe clinical outcomes.

It has long been appreciated that activation of pancreatic juice by enterokinase is an early and necessary mechanism that stimulates the proteoclastic activity of various pancreatic enzymes.[29] This process may contribute to clinical fistula development and may further distinguish operations that require enteric reconstruction (i.e., proximal, central pancreatectomy) from those that do not (i.e., distal pancreatectomy). However, this remains speculation only, as this current study was not designed to determine whether varying degrees of enterokinase activation result in different levels of fistula severity, but rather to identify important clinical distinctions in pancreatic fistulas occurring after three pancreatic operations.

The International Study Group on Pancreatic Fistula classification scheme provides a universal and reproducible definition of pancreatic fistula and a grading system for fistula severity. Although

this scheme accurately characterizes three fistula classes of increasing severity in patients undergoing pancreaticoduodenectomy, this stratification does not appear to be as explicit in distal and central pancreatectomy cases. Among proximal resections, clinical and economic outcomes worsen as fistula severity increases from grade A to grade C. Yet, among distal and central resections, grade B and C fistulas are indistinguishable both clinically and economically. Lengths of stay, as well as rates of complications, ICU transfers, blood transfusions, and rehabilitation placement, are similar between these fistula grades. Hospital costs are similarly equal between grade B and C fistulas. These results demonstrate that clinical and economic outcomes worsen as fistula severity increases according to ISGPF criteria, but that the impacts of grade B and C fistulas are equivalent after distal and central pancreatectomy.

These clinically relevant grade B and C fistulas were considered for further analysis in an effort to explain the differences among proximal, distal, and central pancreatectomy. Our results suggest that a clinically relevant fistula is an acute complication after proximal pancreatectomy and will often require aggressive management approaches in intensive care settings. Surgical exploration, when indicated, is urgent and usually occurs early in the postoperative period. Removal of intra-abdominal drains, however, is seldom delayed, and patients infrequently require CT-guided percutaneous drainage or hospital readmission. Patients may benefit from rehabilitation placement for continued postoperative care, as clinical fistulae after proximal pancreatectomy are often associated with other complications such as wound and respiratory infections. Clinically relevant fistula in distal pancreatectomy neither demands aggressive management approaches nor extends the initial hospital stay. Patients can be discharged home rather than to rehabilitation facilities. However, prolonged drainage of intra-abdominal collections occurs, and multiple hospital readmissions, usually for image-guided percutaneous drainage, are almost always required. Finally, when clinically relevant fistulas develop after central pancreatectomy, they more often resemble those after proximal pancreatectomy. The creation of two pancreatic remnants in this procedure effectively increases the risk of pancreatic fistula (double jeopardy?), and higher rates of clinically relevant fistula should be expected. Acute interventions are routinely indicated, but may depend on whether the fistula involves the proximal pancreatic stump or the distal pancreatico-enteric anastomosis.

CONCLUSION

In summary, the results of this study indicate that the overall incidence of fistula is similar among proximal and distal pancreatic resections, but that the severity and behavior of these fistulae differ. Management experiences with this difficult and costly complication vary across resections and may be influenced by the presence of a pancreaticoenteric anastomosis.

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Discussion

Discussion by William H. Nealon, M.D., Texas
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Dr. W. Nealon (Galveston, TX): Well, I will first break the code by letting you know that Mr. Pratt is a third-year medical student.

I rise to congratulate the authors on a superb clinical and financial analysis that will serve as a benchmark for future work. Their paper is unique for including pancreaticoduodenectomy and distal pancreatectomy, comparing the frequency, severity, and cost of fistulas in both. It is also among the first reports to completely embrace the recently derived international system for classifying fistulas, the ISGPF.

The authors report on 256 patients, approximately one third with distal and two thirds with head resection. There were 81 fistulas, half of which were clinically irrelevant with no negative clinical or financial outcome. Of the remaining patients with clinically significant fistulas, three fourths of them were grade B, and only 10 patients had fistulas associated with sepsis and percutaneous or operative intervention and prolonged ICU stays; these are the grade C—six required reoperation and none died.

Comparing head resection to tail resection, significant differences were only noted for percutaneous drainage and readmission, which was more common after tail resection, and, as Mr. Pratt noted, these are patients who often have gone home and come back with somewhat less significant sickness; however, their costs were greater. Length of stay, ICU admissions, expensive antibiotics, octreotide, supplemental nutrition, referral to rehab facility, and hospital costs were all progressively higher in B and C fistulas.

I have three questions.

You may know that prior to the assumption of the ISGPF standards for pancreatic fistula, every report of fistula is based upon the volume of the fistula. Did you examine that variable at all?

Number two. In your patients with clinically relevant fistulas, 81% had fluid collection seen by cross-sectional imaging: 15.7% were found late. This raises the question of drain failure. Do you have any information on the timing of drain removal or of drain function when these fluid collections were identified?

Number three. It strikes me that infected fluid collection and subsequent sepsis had the most profound effect on outcome. Can you tell me about those patients, what bacteria were found, was there any early clue with leukocytosis or fever in these patients?

The overall outcomes in this study are superb and the precise evaluation of the clinical and economic impact of fistulas is a model to be emulated.

Thank you.

Mr. Pratt: Thank you, Dr. Nealon, for all your questions. I appreciate your excellent commentary and thank you for reviewing our manuscript ahead of time. I will take your questions in their original order.

With regards to drain volume, we do acknowledge that the ISGPF classification scheme refers to any measurable drainage on or after postoperative day three. In our series, we did identify daily drain volumes. However, we did not compare these volumes across the various resection types or evaluate volume as an independent risk factor for the severity of pancreatic fistula. This is indeed a variable that we should, and will, investigate.

With regards to imaging of fluid collections and whether the time to drain removal correlated with the size or severity of fluid collections, it is—to our knowledge—that patients with distal fistulas typically required prolonged drain placement and almost always went home with drains in situ. These patients would occasionally return for hospital readmissions or management of fluid collections if we suspected signs of infection, or in situations in which something was brewing at the time. The median drain removal was 30 days for the distal group and 16 days for the proximal group. This demonstrates that patients with distal fistulas tended to require continued in situ drainage and usually required drainage longer than patients in the proximal group.

Finally, in response to your last question regarding infections and the types of infectious species that might have been associated with these fistulas, I unfortunately do not have data on which to comment. I apologize for that. However, what we have discovered is that most patients with proximal or central fistulas often presented with suspicious signs of infection, including leukocytosis, fever, and purulent drainage—either from the drain itself or within the abdominal incision. In those cases, we suspected an infection had occurred, although the source of infection was not always identified.

Does Pancreatic Duct Stenting Decrease the Rate of Pancreatic Fistula Following Pancreaticoduodenectomy? Results of a Prospective Randomized Trial

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Pancreatic duct stenting remains an attractive strategy to reduce the incidence of pancreatic fistulas following pancreaticoduodenectomy (PD) with encouraging results in both retrospective and prospective studies. We performed a prospective randomized trial to test the hypothesis that internal pancreatic duct stenting reduces the development of pancreatic fistulas following PD. Two hundred thirty-eight patients were randomized to either receive a pancreatic stent (S) or no stent (NS), and stratified according to the texture of the pancreatic remnant (soft/normal versus hard). Four patients were excluded from the study; in three instances due to a pancreatic duct that was too small to cannulate and in the other instance because a total pancreatectomy was performed. Patients who randomized to the S group had a 6-cm-long segment of a plastic pediatric feeding tube used to stent the pancreaticojejunostomy anastomosis. In patients with a soft pancreas, 57 randomized to the S group and 56 randomized to the NS group. In patients with a hard pancreas, 58 randomized to the S group and 63 randomized to the NS group. The S and NS groups for the entire study population, as well as for the subgroup of high-risk patients with soft pancreata, were similar as regard to demographics, past medical history, preoperative symptoms, preoperative procedures, and intraoperative data. The pancreatic fistula rate for the entire study population was 9.4%. The fistula rates in the S and NS subgroups with hard pancreata were similar, at 1.7% and 4.8% ($P = 0.4$), respectively. The fistula rates in the S and NS subgroups with soft pancreata were also similar, at 21.1% and 10.7% ($P = 0.1$), respectively. A nonstatistically significant increase in the pancreatic fistula rate in the S group persisted after adjusting for the operating surgeon and technical details of the operation (e.g., anastomotic technique, anastomotic orientation, pancreatic duct size, and number of intra-abdominal drains placed). In patients with soft pancreata, 63% percent of the pancreatic fistulas in stented patients required adjustment to the clinical pathway (including two deaths), compared to 47% of the pancreatic fistulas in patients in the NS group ($P = 0.3$). Internal pancreatic duct stenting does not decrease the frequency or the severity of postoperative pancreatic fistulas. (J GASTROINTEST SURG 2006;10:1280–1290) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Pancreaticoduodenectomy, pancreatic cancer, stent, pancreatic fistula

Pancreaticoduodenectomy (PD) has less than a 3% mortality rate at high volume centers but continues to have a complication rate of at least 40% in most series. In our institution's experience, the most common complications following PD in descending order of frequency are delayed gastric emptying, pancreatic fistula, and wound infection.[1] These complications, particularly the first two, can have a profound impact on the patient's physical and emotional well being. They often require pharmacologic

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interventions and invasive procedures, prolong the hospital stay, add substantially to the cost of treatment, and in some cases lead to death.

Many recent series suggest only a modest improvement in the 19.5% pancreatic fistula rate following PD reported by Dr. Allen Whipple more than 60 years ago.[2] Although the current rates vary widely between different centers,[3] several large series using a similar definition for pancreatic fistula describe rates around 10%.[1,4,5] Perhaps a larger reduction in pancreatic fistula rates over time has been hindered by the increasing proportion of PDs performed for cystic lesions, which are often associated with soft pancreatic remnants (all 41 patients in Dr. Whipple's series underwent resection for a periampullary cancer, usually associated with a hard gland). Nevertheless, the pancreatic fistula rate continues to be considered quite high for a gastrointestinal anastomosis.

Surgeons have attempted to identify risk factors for pancreatic fistulas, with the hope of devising effective strategies to reduce the leak rate. While many different risk factors have been proposed, a soft pancreatic texture has been most consistently linked to high rates of fistulas at our institution and others.[6–10] Many different technical and pharmacologic approaches to deal with soft pancreatic remnants have been attempted, but none have been able to unequivocally and reproducibly lower the pancreatic fistula rate in prospective randomized trials. Failed or unproven strategies include reconstruction with a pancreaticogastrostomy (versus a pancreaticojejunostomy),[11,12] the use of octreotide,[7,13] the use of fibrin glue sealant,[14–16] the placement of intra-abdominal drains,[17] the use of an isolated jejunal (Roux-en-Y) limb,[18] and main pancreatic duct occlusion.[19,20]

The placement of a plastic stent across the pancreaticojejunostomy (PJ) anastomosis is an attractive strategy to reduce the pancreatic fistula rate following PD. The stent theoretically may help to facilitate precise placement of sutures through the pancreatic parenchyma or duct when performing the PJ anastomosis, and it may also provide some protection of the PJ anastomosis against activated pancreatic enzymes by directing the exocrine secretions directly into the jejunal lumen. Several published reports advocate this technique, but these studies are uncontrolled retrospective studies[21–24] or prospective studies that do not compare their results to an appropriate control group without a pancreatic duct stent.[25–27] This prospective randomized single-institution trial was designed to test the hypothesis that internal pancreatic duct stenting is less likely to be associated with a postoperative pancreatic fistula.

MATERIALS AND METHODS

Protocol

The study protocol was approved by the Johns Hopkins Medicine IRB, and reviewed annually by a Data Safety Monitoring Board (DSMB). Enrollment was offered to all adult patients at the Johns Hopkins Hospital who were anticipated to undergo PD. For each patient agreeing to participate, informed consent was obtained. Two hundred thirty-eight patients were accrued between March 8, 2004, and November 21, 2005.

Patients were randomized intraoperatively to either receive a pancreatic duct stent or no pancreatic duct stent. This process was based on a randomly generated number pattern, and occurred after removal of the PD specimen and prior to initiating the PJ anastomosis. Four patients were withdrawn from the study after enrollment and randomization. In one case, a total pancreatectomy was performed when the PD specimen frozen section analysis revealed tumor involvement of the pancreatic body and tail remnant. In three cases, the pancreatic duct diameter was too small to allow cannulation and stent placement. The reported results include the 234 patients who completed the study.

Surgical Technique

The majority of patients underwent a pylorus preserving PD, with a standard lymph node harvest, as previously described.[1,11] A distal gastrectomy was performed if a pylorus preserving procedure would have compromised the duodenal margin because of ischemia or tumor involvement. Vagotomy, tube gastrostomy, and feeding jejunostomy were not routinely performed. Upon completion of the PD, the proximal 2–3 cm of the pancreatic body remnant was mobilized in preparation for the PJ anastomosis. A 3.5 to 8 French plastic pediatric feeding tube (The Kendall Company, Mansfield, MA; Bard Access Systems, Salt Lake City, UT) was cut to a length of 6 cm and served as a pancreatic duct stent in patients who randomized to the stent group. The largest sized stent that could easily pass into the pancreatic duct was used. The stent was positioned with half of the stent (3 cm) in the pancreatic duct and half (3 cm) in the jejunal lumen. It was secured in place with a single absorbable suture.

The pancreatic body remnant was hand-sewn to the proximal jejunum in two layers: an inner layer with absorbable suture and an outer layer with interrupted 3-0 silk suture (Fig. 1). The PJ anastomosis was performed either by invaginating the pancreatic remnant into the jejunal lumen or by sewing the

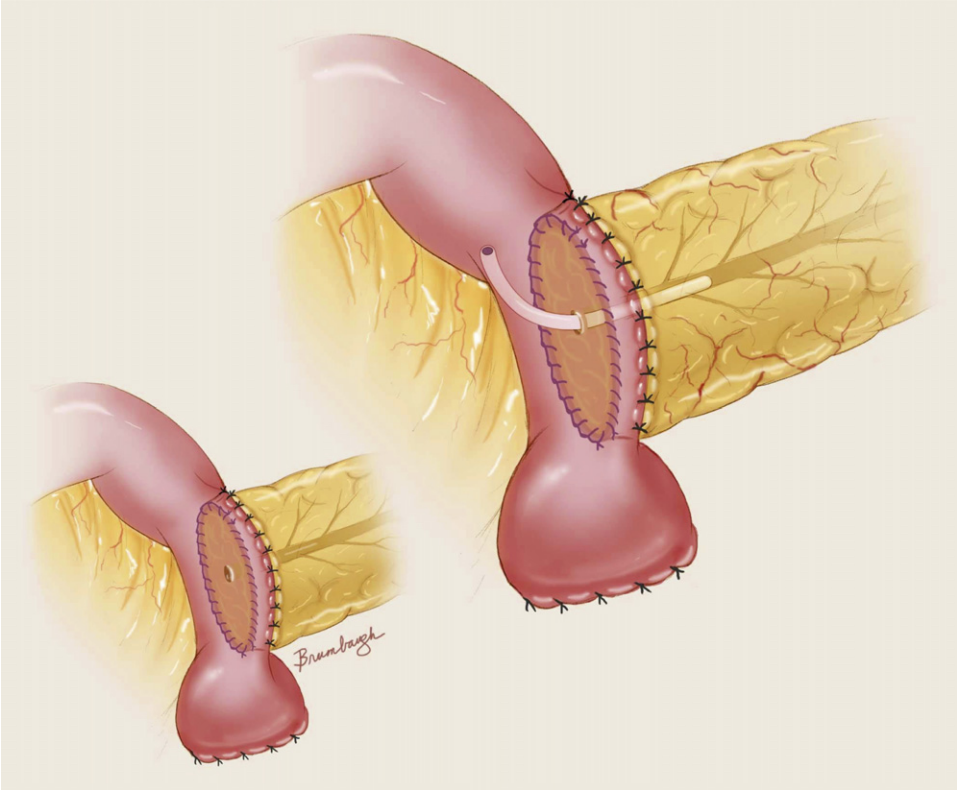

Fig. 1. Schematic of the placement of the pancreatic duct stent. In both illustrations, an end-to-side pancreaticojejunostomy is depicted. On the *left panel*, no stent is shown. On the *right panel*, a 6-cm-long stent is depicted, with 3 cm residing in the jejunal lumen, and 3 cm residing in the pancreatic duct. The stent is secured in place with one nonabsorbable suture.

pancreatic duct to the jejunal mucosa. The pancreatic remnant and the jejunum were juxtaposed in either an end-to-side (usually) or end-to-end fashion. The remaining two anastomoses were performed downstream on the jejunal limb, with both the hepaticojejunostomy and the duodenojejunostomy being handsewn and left in a retrocolic position. One or more 10-mm Jackson-Pratt silicone drains (CardinalHealth, Dublin, OH) were introduced through separate abdominal stab incisions and placed in the vicinity of the PJ and hepaticojejunostomy anastomoses. The PJ technique, orientation of the pancreatic remnant and the jejunum, and the number of surgically placed abdominal drains were left to the discretion of the operating surgeon but documented in a postoperative surgeon questionnaire.

Perioperative Management

Perioperative management was based on the Johns Hopkins Hospital critical pathway for PD. All patients underwent a bowel prep with Fleet phospha soda (Fleet, Lynchburg, VA) the day before surgery. A second-generation cephalosporin (or an appropriate substitute for patients with a penicillin allergy) was given intravenously within 2 hours of the skin incision. All patients were put on a histamine H2-receptor antagonist postoperatively. Prophylactic octreotide was not administered. Surgically placed drains near the PJ anastomosis were left in place for at least 4 postoperative days. Samples of the drain fluid were sent for fluid amylase whenever the drain output was greater than 50 ml/day or the effluent appeared abnormal. Abdominal drains were removed after the fourth postoperative day in the absence of a pancreatic fistula (see definition below). Management of a pancreatic fistula was left to the discretion of the operating surgeon.

Data Collection

Data were collected prospectively and entered into an IRB-approved PD database. Each surgeon completed a postoperative questionnaire indicating the pancreas texture (soft/normal versus hard/fibrotic), the randomization group (stent versus no

stent), pancreatic duct diameter (mm), size of the pancreatic duct stent (Fr), technique of the PJ (invagination versus duct-to-mucosa), orientation of the PJ (end-to-side versus end-to-end), and the number of surgically placed abdominal drains.

Study End Points and Definitions

Data are presented for patients with both soft and hard pancreata; however, greater analysis is presented for patients with soft pancreatic remnants, since patients in this group are at higher risk for pancreatic fistulas.[6,11] The primary study end points included pancreatic fistula rates, severity of pancreatic fistulas, postoperative complications, postoperative length of hospital stay, and death. The primary study analysis used the same local definition of pancreatic fistulas described in prior studies from this institution: a) a 24-hour drain output of 50 ml or greater, containing amylase-rich fluid (greater than three times the upper limit of normal in the serum), on or after postoperative day 10; or b) radiographic evidence of a PJ disruption.[7,11,14] A separate analysis of pancreatic fistulas was performed using a more liberal definition for pancreatic fistulas recently proposed by the International Study Group on Pancreatic Fistula (ISGPF): the presence of amylase-rich fluid (greater than three times the upper limit of normal in the serum) of any measurable volume on or after postoperative day 3.[3] The local definition for pancreatic fistulas is used in this study, unless otherwise indicated. Definitions for other postoperative complications and endpoints have been described previously.[1,11]

Two complication grading systems were used to evaluate the severity of pancreatic fistulas. The Clavien classification system of postoperative complications includes five grades that are based on the magnitude of the intervention used to treat a complication.[28] Grade 1 and 2 complications are managed with pharmacologic agents or other noninvasive measures, grade 3 complications are managed with invasive procedures, grade 4 complications involve intensive care unit management, and grade 5 complications indicate perioperative death. For the purpose of statistical analysis, the highest three grades are considered to be "severe" complications in this study. The ISGPF working group proposed a three-tiered grading system (A or low grade, B or medium grade, and C or high grade) restricted to the stratification of pancreatic fistulas.[3] In general, grade A fistulas are transient and do not require any intervention, grade B fistulas required adjustment to the clinical pathway but the patients are clinically well, and grade C fistulas often require operative intervention and are associated with sepsis or death.

Statistical Analyses

The study was designed to demonstrate a significant benefit of pancreatic duct stenting for the prevention of pancreatic fistulas (one-sided). For patients stratified to the soft pancreas group, the calculation was based on the goal of improving the pancreatic fistula rate from 25% to 10%, with α set at .05 and β set at .2, yielding a power of 80%. Ninety-two patients were calculated to be required in each arm of the soft pancreas group. For patients stratified to the hard pancreas group, the calculation was based on the goal of improving the pancreatic fistula rate from 15% to 5%, with α set at .05 and β set at .2, yielding a power of 80%. One hundred thirty patients were calculated to be required in each arm of the hard pancreas group. The total number of patients needed to realize a significant benefit of pancreatic duct stenting in soft and hard pancreas glands was 444 patients. However, at the time of the second annual review by the DSMB, a negative trend toward increased pancreatic fistulas was observed in the stent group. After careful consideration and discussions with the IRB and DSMB, the decision was made to stop patient accrual and report the results of the trial.

Comparison of continuous variables was performed using the Mann-Whitney rank sum test and comparison of categorical variables was performed using a χ^2 test. Multivariate analyses were performed with logistic regression. Results are reported as median values, unless indicated otherwise. Statistical significance was accepted for $P < .05$. Data analyses were performed using Intercooled Stata Version 8.0 (Chicago, IL).

RESULTS

Patient Population

The study included 234 patients who underwent a PD by nine surgeons. Two surgeons (J.L.C. and C.J.Y.) performed 90% of the operations. Figure 2 gives the distribution of patients in each study arm. One hundred fifteen patients randomized to the stent group and 119 patients to the no-stent group. One hundred thirteen (48%) patients had a soft pancreatic remnant and 121 (52%) patients had a hard pancreatic remnant. Of the patients with a soft pancreatic remnant, 57 were randomized to the stent group and 56 to the no-stent group. Of the patients with a hard pancreatic remnant, 58 were randomized to the stent group and 63 to the no-stent group.

For both the total study population and the subgroup of patients with soft pancreatic remnants, there were no significant differences in patient demographics, past medical history, preoperative symptoms, or

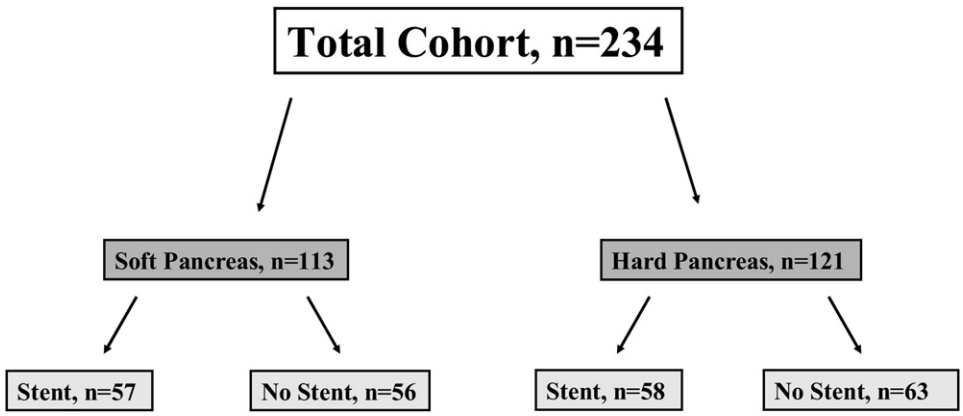

Fig. 2. The distribution of patients in each study arm, stratified according to the texture of the pancreatic remnant. In total, there were 115 patients who received a pancreatic stent and 119 patients who did not receive a pancreatic stent.

preoperative procedures (Table 1). Of note, a small and nonsignificant difference existed between the stent and nonstent groups in the incidence of COPD ($P = 0.2$ when analyzing the entire study population or the subgroup of patients with soft pancreatic remnants), acute pancreatitis ($P = 0.08$ when analyzing the subgroup of patients with soft pancreatic remnants), and weight loss ($P = 0.1$ when analyzing the entire study population and $P = 0.06$ in the subgroup of patients with soft pancreatic remnants). None of these preoperative factors contributed to pancreatic fistula development in univariate or multivariate analyses.

There were no differences in the proportions of specimens harboring malignant disease or periampullary cancers between the stent group and the no-stent group (Table 2). Specific pathologic diagnoses were comparable between the two groups with two exceptions. Chronic pancreatitis was significantly more common in the stent group (7.0%, $P = 0.05$) compared to the no-stent group (1.7%) in the total study population. IPMNs were less common in the stent group (5.2%, $P = 0.03$) compared to the no-stent group (13.5%) for the total study population, as well as in the subgroup of patients with soft pancreatic remnants (8.8% stent-group and 23.2% no-stent group, $P = 0.04$). Neither of these diagnoses was associated with pancreatic fistulas in univariate or multivariate analyses.

Intraoperative parameters were not statistically different between the stent group and the no-stent group (Table 3). The difference in blood loss in patients with soft pancreatic remnants approached statistical significance ($P = 0.1$); however, the difference was due to a disproportionate number of patients in the stent group with blood loss between 750 and 1000 mL. Blood loss in this range was not associated with pancreatic fistulas rates in univariate or multivariate analyses.

Complications

Complications occurred in 58% of patients in the study and there were six deaths (2.7%). Four deaths were due to sepsis, one death from intraoperative cardiac arrest, and one death from aspiration pneumonia and respiratory failure. There were no statistical differences in mortality and morbidity between the stent group and the no-stent group in the entire study population or the subgroup of patients with soft pancreatic remnants (Table 4). The median postoperative length of stay for the entire study population was 7 days (range 5–85 days). The length of stay was also similar between the stent and no-stent groups. None of the specific complications tabulated in Table 4 were statistically different between the two groups.

Fistulas

The pancreatic fistula rates for the different study arms were calculated using the local definition (⩾50 mL amylase-rich fluid on or after postoperative day 10 or radiographic evidence of a fistula), as well as the recently proposed definition by the ISGPF (amylase rich fluid on or after postoperative day 3). The results are presented in Table 5. The fistula rate for the entire study population using the local definition was 9.4%. The rates in the stent and the no-stent groups were 11.3% and 7.6%, respectively ($P = 0.3$). The rates in patients with hard and soft pancreatic remnants were significantly different, at 3.3% and 15.9%, respectively ($P = 0.001$). In the hard pancreatic remnant group, the pancreatic fistula rates were 1.7% in the stent group and 4.8% in the no-stent

Table 1. Patient characteristics and preoperative data*

	All patients		Soft pancreas only	
	No stent (n = 119)	Stent (n = 115)	No stent (n = 56)	Stent (n = 57)
Age (yr), median (range)	67 (33–88)	63 (27–89)	67 (33–88)	68 (39–89)
Male, n (%)	65 (54.6)	67 (58.3)	27 (48.2)	32 (56.1)
White, n (%)	102 (90.3)	99 (92.5)	43 (86.0)	51 (94.4)
PMH, n (%)				
Hypertension	57 (48.7)	42 (38.5)	25 (44.6)	24 (44.4)
Coronary artery disease	22 (18.8)	18 (16.5)	8 (14.3)	6 (11.3)
Diabetes mellitus	17 (14.5)	20 (18.2)	4 (7.1)	8 (14.8)
Peripheral vascular disease (includes CVA)	14 (12.0)	12 (10.9)	6 (10.7)	7 (13.0)
Tobacco use	9 (7.8)	9 (8.3)	3 (5.5)	4 (7.6)
Acute pancreatitis	9 (7.7)	9 (8.2)	1 (1.8)	5 (9.3)
COPD	8 (6.8)	3 (2.7)	4 (7.1)	1 (1.9)
Myocardial infarction	6 (5.1)	7 (6.4)	3 (3.6)	3 (5.6)
Chronic pancreatitis	3 (2.6)	7 (6.4)	1 (1.8)	3 (5.6)
PUD	3 (2.6)	2 (1.8)	2 (3.6)	1 (1.9)
EtOH abuse	2 (1.7)	5 (4.6)	0 (0)	2 (3.8)
Preoperative symptoms, n (%)				
Jaundice	68 (58.1)	60 (54.6)	25 (44.6)	22 (40.7)
Abdominal pain	48 (41.7)	49 (45.8)	24 (42.9)	21 (40.4)
Weight loss	40 (34.8)	49 (45.4)	13 (23.2)	21 (40.4)
Nausea or vomiting	15 (13.0)	7 (6.5)	5 (8.9)	3 (5.8)
Pruritits	12 (10.3)	6 (5.6)	5 (8.9)	2 (3.9)
GI bleed	5 (4.3)	4 (4.7)	4 (7.1)	4 (5.8)
Fevers or chills	9 (7.8)	5 (4.6)	5 (8.9)	2 (3.9)
Preoperative invasive procedure n (%)	106 (9.6)	98 (88.3)	47 (85.5)	44 (81.5)
ERCP	75 (64.7)	68 (61.3)	32 (52.2)	26 (48.1)
Biopsy	59 (53.2)	58 (53.2)	29 (54.7)	34 (63.0)
Ednostent	58 (50.0)	48 (44.0)	26 (47.3)	19 (35.9)
EUS	29 (25.4)	26 (23.4)	16 (29.1)	16 (29.6)
PTC/PBD	22 (19.0)	15 (13.5)	6 (10.9)	3 (5.6)

*No significant differences were noted comparing the no-stent with the stent group for any measured parameter in this table.
COPD = chronic obstructive pulmonary disease; PUD = peptic ulcer disease; CVA = cerebrovascular accident; EtOH = alcohol; ERCP = endoscopic retrograde cholangiopancreatography; EUS = endoscopic ultrasound; PTC/PBD = percutaneous transhepatic cholangiogram/percutaneous biliary drain.

group ($P = 0.4$). In the soft pancreatic remnant group, the fistula rates were 21.1% in the stent group and 10.7% in the no-stent group ($P = 0.1$, power = 0.32)

The technical and surgeon factors that were tracked using a postoperative questionnaire are provided in Table 6, along with their associated odds ratios for pancreatic fistula development. Both a soft pancreatic remnant and an end-to-end PJ were statistically significant risk factors in the univariate analysis, using the local Johns Hopkins definition of pancreatic fistulas; only a soft pancreatic remnant was statistically significant in the multivariate model adjusting for the operating surgeon. When the ISGPF definition was used for pancreatic fistulas, both a soft pancreatic remnant and a small pancreatic duct were significant risk factors for a pancreatic fistula after adjustment for the operating surgeon. The results of the multivariate analysis were consistent when preoperative and additional intraoperative variables were included in the model (data not shown).

Two different complication grading systems were utilized to compare the severity of the complications and fistulas in the stent and no-stent groups in patients with soft pancreatic remnants. The Clavien classification system grades complications according to the invasiveness of the intervention used to treat the complication. The proportion of patients with severe complications (grades 3–5) were similar in the stent (24.6%) and no-stent groups (26.8%, $P = 0.8$). The ISGPF classification system stratifies pancreatic fistulas according to severity. According to ISGPF criteria, the proportion of fistulas requiring adjustment to the clinical pathway in the stent and no-stent groups were 63% and 43%, respectively ($P = 0.3$).

The mortality rate for patients who had pancreatic fistulas was significantly higher than the rate

Table 2. Pathologic diagnoses

	All patients		Soft pancreas only	
	No stent (n = 119)	Stent (n = 115)	No stent (n = 56)	Stent (n = 57)
Malignant, n (%)	90 (75.6)	85 (73.9)	36 (64.3)	36 (63.2)
Periampullary cancer, n (%)	79 (66.4)	78 (67.8)	29 (51.8)	31 (51.4)
Specific diagnosis				
Pancreatic ductal adenocarcinoma	49 (41.2)	51 (44.4)	12 (21.4)	13 (22.8)
IPMN	16 (13.5)	6 (5.2)*	13 (23.2)	2 (8.8)*
Distal bile duct cancer	12 (10.1)	9 (7.8)	13 (23.2)	2 (10.5)
Ampullary adenocarcinoma	9 (7.6)	11 (9.6)	6 (10.7)	8 (14.0)
Duodenal adenocarcinoma	5 (4.2)	5 (4.4)	3 (5.4)	4 (7.0)
IPMN with invasive cancer	4 (3.4)	2 (1.7)	0 (0)	0 (0)
Cystadenoma	4 (3.4)	5 (4.4)	4 (7.1)	4 (7.0)
Malignant neuroendocrine tumor	3 (2.5)	1 (0.9)	1 (1.8)	1 (1.8)
Chronic pancreatitis	2 (1.7)	8 (7.0)*	1 (1.8)	4 (7.0)
Periampullary adenoma	1 (0.8)	3 (2.6)	1 (1.8)	3 (5.3)
Gastrointestinal stromal tumor	0 (0)	1 (0.87)	0 (0)	1 (1.75)
Metastatic disease	0 (0)	1 (0.9)	0 (0)	
Cystadenocarcinoma	0 (0)	0 (0)	0 (0)	0 (0)
Bengin neuroendocrine tumor	0 (0)	0 (0)	0 (0)	0 (0)
Other	14 (11.8)	12 (10.4)	7 (12.5)	7 (12.3)

IPMN = intraductal papillary mucinous neoplasm.
*$P < 0.05$.

for patients without pancreatic fistulas (9.1% versus 1.9%, respectively, $P = 0.04$). The postoperative length of stay was also significantly longer for patients with pancreatic fistulas (median 19 days versus median 7 days for patients without fistulas, respectively, $P < 0.001$). Clinically significant pancreatic fistulas (ISGPF grades B or C) occurred in two of the patients who died perioperatively. Both patients had a stent placed in a soft pancreatic remnant.

DISCUSSION

In this prospective randomized trial of 234 patients undergoing PD with or without an internalized pancreatic duct stent, no benefit was observed for pancreatic duct stenting. A trend was observed toward increased pancreatic fistulas in patients with soft pancreatic remnants and stents (21.1%, $P = 0.13$), as compared to those with soft pancreatic remnants and no stents (10.7%). This negative trend was also observed using a less restrictive definition of pancreatic fistulas (the ISGPF definition) which includes transient pancreatic fistulas in addition to the clinically significant fistulas accounted for in the local definition (47.4% stent group versus 33.9% no-stent group, $P = 0.15$).

Although the ad hoc power analysis suggests that there is only a 32% chance that a difference exists between the two groups, the trial was stopped early because of the real possibility that pancreatic stents

Table 3. Intraoperative data*

	All patients		Soft pancreas only	
	No stent (n = 119)	Stent (n = 115)	No stent (n = 56)	Stent (n = 57)
Pylorus, preserving (vs. hemigastrectomy), n (%)	104 (89.7)	99 (86.1)	51 (91.1)	50 (80.7)
Resected major visceral vessel,† n (%)	4 (3.6)	6 (5.5)	2 (3.7)	0 (0)
Estimated blood loss, median (range)	675 (150–2400)	750 (100–3700)	500 (150–2400)	750 (100–1600)
Transfused units, median (range)	0 (0–5)	0 (0–6)	0 (0–4)	0 (0–4)
Operative time (min), median (range)	350 (234–600)	345 (230–680)	350 (235–480)	340 (230–560)

*No significant differences were noted comparing the no stent with the stent group for any measured parameter in this table.
DGE = delayed gastric emptying; UTI = urinary tract infection.
†Major visceral vessels include the superior mesenteric vein and portal vein.

Table 4. Postoperative data*

	All patients		Soft pancreas only	
	No stent (n = 119)	Stent (n = 115)	No stent (n = 56)	Stent (n = 57)
Mortality, n (%)	4 (3.5)	2 (1.8)	2 (3.6)	2 (3.7)
Complications, n (%)	69 (58.0)	66 (57.4)	35 (62.5)	40 (70.2)
Reoperation, n (%)	10 (8.4)	5 (4.4)	5 (8.9)	4 (7.0)
Specific complications, n (%)				
Wound infection	22 (18.5)	15 (13.0)	12 (21.4)	8 (14.0)
DGE	15 (12.6)	16 (13.9)	5 (8.9)	7 (12.3)
Pancreatic fistula†	9 (7.6)	13 (11.3)	6 (10.7)	12 (21.0)
Cardiac	8 (6.7)	3 (2.6)	7 (12.5)	2 (3.5)
Intra-abdominal abscess	6 (5.1)	8 (7.0)	2 (3.6)	6 (10.5)
Bile leak	3 (2.5)	4 (3.5)	1 (1.8)	4 (7.0)
Lymph leak	3 (2.5)	2 (1.7)	2 (3.6)	1 (1.8)
Pneumonia	2 (1.7)	1 (0.9)	2 (3.6)	1 (1.8)
Sepsis	2 (1.7)	2 (1.7)	2 (3.6)	2 (3.5)
Ulcer	2 (1.7)	0 (0)	0 (0)	0 (0)
UTI	2 (1.7)	3 (2.6)	1 (1.8)	3 (5.3)
Small bowel obstruction	1 (0.8)	0 (0)	0 (0)	0 (0)
Cholangitis	1 (0.8)	1 (0.8)	1 (1.8)	1 (1.8)
Pancreatitis	0 (0)	0 (0)	0 (0)	0 (0)
Postoperative length of stay (days), median (range)	7 (5–9)	8 (5–85)	8 (5–59)	8 (6–85)

*No significant differences were noted comparing the no stent with the stent group for any measured parameter in this table.
DGE = delayed gastric emptying; UTI = urinary tract infection.
†Johns Hopkins definition.

were causing harm, and the remote possibility that the stents would in fact prove to be beneficial if the planned study size was achieved. If the study had continued, the minimum fistula rate that would have been necessary to reject the null hypothesis, in the soft pancreatic remnant/no-stent arm, is easily calculated. If there were no fistulas in the remaining 35 patients required to achieve the predetermined sample size in the soft pancreatic remnant/stent arm, then a remarkably high 57% fistula rate (defined according to the local definition of pancreatic fistulas, see above) would be needed in the soft pancreatic/no-stent arm. The chances are extremely unlikely of observing a near-zero percent fistula rate in one group and a concurrent fistula rate in the other group that exceeds 2.5 times the expected rate[6] for soft pancreatic glands.

Table 5. Pancreatic fistula rates according to two definitions

Group	JHH, n (%)	ISGPF, n (%)
Pancreatic fistulas in entire cohort (n = 234)	22 (9.4)	57 (24.4)
Stent (n = 115)	13 (11.3)	31 (27.0)
No stent (n = 119)	9 (7.6)	26 (21.9)
Hard pancreatic remnant (n = 121)	4 (3.3)	11 (9.1)
Stent (n = 58)	1 (1.7)	4 (6.9)
No stent (n = 63)	3 (4.8)	7 (11.1)
Soft pancreatic remnant (n = 113)	18 (15.9)	46 (40.7)
Stent (n = 56)	12 (21.1)†	27 (47.4)*
No stent (n = 57)	6 (10.7)	19 (33.9)

JHH = Johns Hopkins Hospital local definition; ISGPF = International Study Group for Pancreatic Fistula definition.
†Estimated power = 0.33.
*Estimated power = 0.32.

No confounding variables were identified in this study that could have accounted for the lack of a benefit for pancreatic duct stenting. Patient demographics, past medical history, preoperative symptoms, preoperative procedures, and intraoperative factors were all similar between the stent and no-stent groups. There were statistical differences noted in two pathological diagnoses (chronic pancreatitis being more common in the stent group and IPMN being less common in the stent group) between the two experimental groups, but neither of these pathologies were associated with pancreatic fistula development.

The severity of the postoperative complications and pancreatic fistulas were comparable between high-risk patients (patients with a soft pancreatic remnant) in the stent group and the no-stent group,

Table 6. Associated risk for pancreatic fistula development for various technical and operative factors: univariate and multivariate analyses

	JHH definition of fistula		ISGPF definition of fistula	
Variable	**Univariate**	**Multivariate**	**Univariate**	**Multivariate**
P-J (end-to-end vs. end-to-side)	10.5*	14.7	9.8	10.5
Soft gland	5.5*	7.5*	6.9*	8.1*
Anastamosis (duct-to-mucosa vs. invagination)	1.9	4.2	2.4*	2.8
Stent	1.6	1.7	1.3	1.0
Size of duct (⩾5 mm)	0.4	0.1	0.5	0.2*
Drains (⩾3)	2.2	1	3.7*	3.2

JHH = Johns Hopkins Hospital; ISGPF = International Study Group on Pancreatic Fistula.
The multivariate analysis included the six technical and operative variables included in the table.
*$P < 0.05$ (in the multivariate analyses, significance was determined after adjusting for the operating surgeon).

according to two different complication grading systems. The ISGPF grading system, which is only concerned with pancreatic fistula (as opposed to the Clavien classification of complications which considers all complications), revealed a trend toward more severe fistulas in the stent group (63% required adjustment to the clinical pathway versus 47% in the no-stent group, $P = 0.3$).

It is worth noting that the overall complication rate in this study (58%) is high compared to previous studies form this institution (30–40%),[1,7] and is most likely related to improved data collection based on electronic records, resulting in the inclusion of minor complications into the PD database. The relatively short length of postoperative hospital stay in the present series (median length of stay = 7 days), as compared to those prior series (median length of stay, 9–13 days), supports this argument.

A potential criticism of this study may be that certain technical factors were not standardized and unknown surgeon factors could have confounded the results. However, a questionnaire was administered and completed by the operating surgeon at the conclusion of each case so that potential confounders could be considered in the analysis. Multivariate analysis using the local Johns Hopkins and the ISGPF definitions for fistulas did not show pancreatic stenting to be protective against pancreatic fistulas, after adjusting for texture, anastomotic technique, the orientation of the pancreatic remnant and jejunum, the size of the pancreatic duct, the number of drains placed, or the operating surgeon. It is therefore quite unlikely that technical factors or surgeon factors contributed to the lack of protection afforded by pancreatic duct stenting in this study.

Much of the literature on the subject of pancreatic duct stenting describes the placement of external pancreatic stents (i.e., long tubes that commence in the pancreatic duct, traverse the jejunum and the abdominal wall, and drain to a reservoir).[21–27] Proponents of this technique cite three theoretical reasons why external stents may improve outcomes following a pancreaticoenterostomy. First, external stents create a controlled pancreaticocutaneous fistula by diverting a substantial amount of pancreatic juice away from the anastomosis, which may encourage anastomotic healing and improve long-term pancreatic duct patency. Second, the stent may decompress the afferent limb and provide improved local control of secretions in the instance of an anastomotic leak. Third, the stent can facilitate precise suture placement.[26]

The technique, as described by Manabe et al.,[24] involves the placement of a long tube into the main pancreatic duct and across the PJ anastomosis. The tube exits the gastrointestinal tract through a separate enterotomy (externalization through the biliary system via a transhepatic route has also been described[26]) and continues extracorporeally through a stab incision in the abdominal wall. The tube is secured to the skin and drains to gravity for several weeks until the stent is removed. The results with externalized pancreatic duct stents have been equivocal. Matsumoto et al. reported a 16% pancreatic fistula rate using this technique,[25] Hamanaka et al. reported a 4% fistula rate,[22] and Howard reported zero pancreatic fistulas in 56 consecutive cases.[21] All of these series lacked a control group without pancreatic duct stents. Roder et al. reported a 7% pancreatic fistula rate in patients with stented external drainage of the pancreatic duct, as compared to a 29% fistula rate in patients without a pancreatic duct stent ($P = 0.007$). The experimental groups were similar with regard to certain preoperative and intraoperative factors, but the patients were not randomized.[27]

Critics of external pancreatic duct stenting make the point that neither pancreatic juice diversion,

nor decompression of the jejunal limb, has ever been proved with external stenting. Furthermore, there has not been a well-controlled prospective randomized trial to evaluate the technique, and external pancreatic stents have been associated with peritonitis following drain removal.[26] Internal pancreatic stents may be a safer alternative and have been associated with a comparable pancreatic fistula rate to externalized pancreatic stents.[26] Like external stents, internal stents may provide limited protection of the PJ anastomosis against activated pancreatic enzymes and facilitate precise suture placement. In a direct comparison of in situ and ex situ pancreatic duct stents, Ohwada et al. randomized 74 patients to one of the two techniques (a no-stent control group was not included in the study). The authors observed a 5% pancreatic fistula rate in both groups.

Biehl and Traverso[29] evaluated internal pancreatic duct stenting after PJ in a small, canine model. The authors performed the procedure in three experimental groups: a stent group, a group with a stent placed followed by stent removal after the PJ anastomosis was complete, and a no-stent group. End points included the development of pancreatic fistulas, duct occlusion, and duct stenosis. The authors observed a trend towards increased anastomotic integrity and patency in both stent groups.

To our knowledge, this study represents the largest prospective randomized controlled trial evaluating pancreatic duct stenting post-PD and provides strong evidence that there is no benefit to this technique. Despite efforts to find effective strategies to reduce the pancreatic fistula rate, this complication continues to comprise roughly one-quarter of the complications sustained following PD. In the Johns Hopkins series of over 2900 PDs, the fistulas rates during the past three decades were 7% in the 1980s, 11% in the 1990s, and 10% in the 2000s (J. Winter, personal communication). Furthermore, pancreatic fistulas following PD are associated with increased mortality and length of postoperative hospital stay. This trial is the fourth randomized prospective study from this institution in the past 11 years that fails to demonstrate reduced rates of pancreatic fistulas using a PJ after a specific intervention; the other interventions being pancreaticogastrostomy,[11] prophylactic octreotide,[7] and fibrin glue sealant at the PJ anastomosis.[14] Future prospective randomized trials to study innovative approaches to prevent pancreatic fistulas remain a high priority for pancreatic surgeons.

The authors wish to thank Jennifer Brumbaugh for her skillful illustration (Fig. 1) and the residents and nurses of the Johns Hopkins Hospital for their role in the care of these patients.

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Discussion

Andrew L. Warshaw, M.D., Boston, Mass: Dr. Winter, congratulations on another really excellent randomized study of pancreaticoduodenectomy from Hopkins and now a dispersion of other institutions. Nobody does it as often or better.

This study demonstrates whether a stent across the pancreaticojejunal anastomosis protects against a leak and consequent fistula. These fistulas do remain a common problem, and as you have outlined, various attempts to reduce their frequency, whether using octreotide, fibrin glue, duct occlusion, or other techniques, have all failed, although closed suction drains do appear to have converted a once-lethal event to one that usually heals without further complication or need for reoperation.

Your findings are partly predictable in that the fistula rate was 3.3% when the pancreas was fibrotic and 15.9% when it was soft. Whether or not a stent was used made no significant difference in either group, however. These fistula rates are almost identical to our experience, in which it was 4.4% in 158 patients with pancreatic cancer and 14.9% among 321 patients with other diseases and a soft pancreas in the years 2001 to 2005. My questions are as follows.

You used to 6-cm internal stent which passed spontaneously at some indeterminate time. Since fistulas typically appear 5 to 6 days postoperatively, how do you know the stent had not passed previously, leaving the anastomosis unstented at the critical time? In our practice, as with many others, we prefer to bring the stent out through the abdominal wall in order to retain total control over it and assure its continued presence, as well as to provide access for pancreatography to interrogate the anastomosis when needed.

Second, you did not detail how often you pancreaticojejunal anastomosis was mucosa-to-mucosa as compared to an invagination technique or the relative effectiveness of each technique in the hands of the surgeons in this series. You mentioned that both techniques were used but do not enumerate. If invagination was mostly likely to be chosen for a small pancreatic duct, which is probable, which generally correlates with a soft gland, your outcomes may be skewed or have missed a possible benefit of stenting a small duct. On the other hand, you allude to the benefit of a stent in facilitating the mucosa-to mucosa anastomosis of a small duct in your manuscript. Is it likely that the randomization to a no-stent group may have biased the choice of the anastomotic technique, mucosa-to mucosa versus invagination, in your series?

And finally, a very recent report, actually just this month, from Japan found on multivariate analysis that poorly controlled diabetes as manifested by an elevated hemoglobin A_{1c} significantly increased the likelihood of a pancreatic anastomotic leak in that patient population. Do you have any experience and can you comment?

I do congratulate you on a very well conducted trial and an excellent presentation.

Treatment With Gemcitabine and TRA-8 Anti-Death Receptor-5 mAb Reduces Pancreatic Adenocarcinoma Cell Viability In Vitro and Growth In Vivo

Leo Christopher DeRosier, M.D., Zhi-Qiang Huang, M.D., Jeffrey C. Sellers, M.S., Donald J. Buchsbaum, Ph.D., Selwyn M. Vickers, M.D.

Gemcitabine is a first line agent for pancreatic cancer, but yields minimal survival benefit. This study evaluated in vitro and in vivo effects of a monoclonal antibody (TRA-8) to human death receptor 5, combined with gemcitabine, using two human pancreatic cancer cell lines, S2VP10 and MIA PaCa-2. A subcutaneous model of pancreatic cancer was employed to test in vivo efficacy. S2VP10 and MIA PaCa-2 cells were treated with varying doses of gemcitabine and TRA-8. Cell viability and apoptosis were determined with an adenosine triphosphate assay and annexin V staining, respectively. Mitochondrial membrane destabilization was evaluated with fluorescence-activated cell sorting analysis of JC-1 stained cells. Caspase activation was evaluated by Western blot analysis. MIA PaCa-2 subcutaneous xenografts in athymic nude mice were evaluated for response to treatment with 200 μg of TRA-8 (intraperitoneal on days 9, 13, 16, 20, 23, and 27 postimplant) and 120 mg/kg gemcitabine (I.P. on days 10, 17, and 24). Tumor growth was measured with calipers. MIA PaCa-2 and S2VP10 cells receiving combination treatment with TRA-8 and gemcitabine demonstrated enhanced cytotoxicity, annexin V staining, and mitochondrial destabilization compared to either agent alone. Combination treatment produced enhanced caspase-3 and -8 activation in both cell lines compared with either agent alone. In vivo studies demonstrated mean subcutaneous tumor surface area (produce of two largest diameters) doubling times of 38 days untreated, 32 days gemcitabine, 49 days TRA-8, and 64 days combination treatment. TRA-8 is an apoptosis-inducing agonistic monoclonal antibody that produced synergistic cytotoxicity in combination with gemcitabine in vitro through enhanced caspase activation. These findings, with substantial inhibition of tumor growth in a mouse pancreatic cancer xenograft model receiving combination therapy, are encouraging for anti-death receptor therapy in the treatment of pancreatic cancer. (J GASTROINTEST SURG 2006;10:1291–1300) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Pancreatic cancer, gemcitabine, monoclonal antibody, TRAIL death receptor antibody

Pancreatic adenocarcinoma remains one of the most deadly and chemoresistant cancers. There have been multiple clinical and preclinical studies evaluating various chemotherapeutic agents, but few have produced significant improvement in survival. This study describes the use of TRA-8, a monoclonal antibody directed to human death receptor-5 (TRAIL-R2) in the treatment of pancreatic cancer in both in vitro and in vitro systems.

Gemcitabine is currently the first line agent in the treatment of pancreatic cancer. An early study of gemcitabine in advanced pancreatic cancer demonstrated measurable response in 23.8% of patients, with median survival of 5.7 months and only 18% survival at 12 months.[1] The testing of other traditional chemotherapeutic compounds alone or in combination with gemcitabine has failed to improve survival results in clinical trials.[2–8] Given these

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outcomes with current adjuvant therapy and as the biology of pancreatic cancer is better understood, interest has shifted to novel biologic agents that are targeted toward inhibition of aberrant cell proliferation. Clinical studies evaluating bevacizumab, an antivascular endothelial growth factor monoclonal antibody, or the epidural growth factor receptor tyrosine kinase inhibitor erlotinib have shown promising results.[9,10]

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) is another agent that has generated great interest as a potential antineoplastic agent. TRAIL is a 281aa protein that shares sequence homology with other members of the tumor necrosis factor family. The gene encoding TRAIL lies on chromosome 3 at position 3q26.[11,12] TRAIL has been found to induce apoptosis in a variety of cancer cell lines through binding of the ligand to cell-surface TRAIL death receptors.[13–15] There have been five TRAIL receptors identified. Two of these receptors are decoy receptors, decoy receptor-1 (DcR1) and decoy receptor-2 (DcR2), neither of which induce apoptosis when bound by TRAIL.[16–19] The two functional death receptors, DR4 (TRAIL-R1) and DR5 (TRAIL-R2), trigger apoptosis when bound by TRAIL.[16,20,21] Osteoprotegerin is a fifth soluble receptor for TRAIL found to block TRAIL-mediated apoptosis in vivo.[22] The initial optimism for TRAIL was tempered somewhat because studies with early recombinant forms of TRAIL induced apoptosis in normal human hepatocytes.[23] Although newer zinc-optimized recombinant forms of TRAIL have avoided this hepatotoxicity,[13,24] researchers worked to develop agonistic monoclonal antibodies to specific death receptors in an effort to circumvent this observed toxicity.[25,26] Ichikawa et al.[25] developed TRA-8, a mouse monoclonal antibody that specifically binds to human DR5, without binding to DR4 or decoy receptors, which induced apoptosis of tumor cell lines in vitro, with tumoricidal effects in vivo. As most studies have demonstrated DR5 expression in established human cancer cell lines, and TRA-8 has been shown to induce apoptosis, TRA-8 could be a useful adjunct to current chemotherapy regimens. This study sought to examine the effects of TRA-8 on two human pancreatic cancer cell lines (MIA PaCa-2 and S2VP10) when combined with gemcitabine in both in vitro and in vivo systems.

METHODS

Human Pancreatic Cell Lines and Reagents

The human pancreatic cancer cell line MIA PaCa-2, was obtained from the American Type Culture Collection (Manassas, VA). Human pancreatic cell line S2VP10 was a gift from Dr. M. Hollingsworth (University of Nebraska).[27] Both MIA PaCa-2 and S2VP10 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Mediatech Inc., Herndon, VA) with 10% fetal bovine serum (Hyclone, Logan, UT). Murine monoclonal TRA-8 IgG1 antibody was prepared by Dr. Tong Zhou (University of Alabama) or by Sankyo Co. Ltd. (Tokyo, Japan). Gemcitabine (Eli Lilly and Company, Indianapolis, IN) was purchased from the University of Alabama at Birmingham Hospital Pharmacy.

Fluorescence-activated Cell Sorting (FACS) Analysis of DR5 Expression on Human Pancreatic Cancer Cell Lines

MIA PaCa-2 and S2VP10 cells were plated in DMEM containing 10% fetal bovine serum in 6-well culture plates. Twenty-four hours after plating, cells were treated with 300 nmol/L gemcitabine for 24 hours. Cells were harvested and then centrifuged at 1000 rpm for 5 minutes followed by resuspension in fresh DMEM. Cells were counted and aliquots of 5×10^5 cells were added to FACS tubes. Cells were recentrifuged and the pellet was resuspended in FACS buffer. Cells were incubated with TRA-8 (2 μg/tube) for 30 minutes on ice followed by Alexa conjugated goat anti-mouse IgG antibody (Molecular Probes, Eugene, OR). Cells incubated with an equal amount of isotype-specific IgG1 were used as a control. Cells were washed again with FACS buffer and cells were fixed with 1% paraformaldehyde. Cells were examined using flow cytometry (FACScan, Becton Dickinson, San Jose, CA) for DR5 expression. Data was analyzed using Student's *t* test (2-tailed, paired).

In Vitro Cell Viability Assay

To examine the cytotoxic effects of TRA-8 alone and in combination with gemcitabine, MIA PaCa-2 and S2VP10 cells were trypsinized and plated at 1000 cells per well in Costar (Corning, NY) 96-well culture plates, in DMEM with 10% fetal bovine serum, 100 IU penicillin and 100 μg/ml streptomycin (Mediatech Inc.). Cells were incubated overnight at 37° C and treated with TRA-8 (0–1000 ng/ml). Twenty-four hours after TRA-8 treatment, cells were processed using the ATPLite Luminescence ATP Detection Assay System (Perkin Elmer, Boston, MA), and luminescence was read on a TopCount Luminescence Reader (Packard Instruments, Meriden, CT). Adenosine triphosphate (ATP)-based bioluminescence assays have been validated as a tool to evaluate cell viability after drug administration.[28,29]

Manufacturer's protocol was followed, except one half of the recommended solution volumes were added to each well. Viability data is expressed as a percentage of untreated control cells ± standard error. All data points are the mean of two independent experiments, and each experiment included 12 replicates at each TRA-8 dose, and each untreated control included 24 replicates. MIA PaCa-2 and S2VP10 cell lines were also evaluated for combination cytotoxicity with gemcitabine and TRA-8. MIA PaCa-2 and S2VP10 cells were plated in the same manner as described above. After overnight incubation, cells were treated with gemcitabine (300 nmol/L). Twenty-four hours after gemcitabine addition, cells were treated with TRA-8 (0–1000 ng/ml) for 24 hours. The ATPLite assay was performed as described above. Data points are the mean of two independent experiments, and each experiment included quadruplicate wells at each combination.

Annexin V Analysis of Human Pancreatic Cancer Cell Lines

MIA PaCa-2 and S2VP10 human pancreatic cancer cell lines were selected for apoptosis analysis based on their sensitivity and resistance to TRA-8–induced cytotoxicity, respectively. MIA PaCa-2 and S2VP10 cells were plated in 6-well culture plates at 3×10^5 cells/well in DMEM with 10% fetal bovine serum and cultured overnight. Cells were treated with 300 nmol/L gemcitabine, or an equal volume of media alone to serve as a negative control, overnight at 37° C. TRA-8 (300 ng/ml), or an equal volume of media to serve as a negative control, was added 24 hours after gemcitabine addition. Twenty-four hours after TRA-8 addition, cells were stained with annexin V and propidium iodide (PI) using an Annexin V-FITC Apoptosis Detection kit (BioVision Inc, Mountain View, CA) according to the manufacturer's protocol. Cells were then analyzed using flow cytometry (FACScan, Becton Dickinson, San Jose, CA). Data (mean of three measurements) were analyzed with CellQuest software (Becton Dickinson).

Detection of Mitochondrial Membrane Destabilization

Intact mitochondrial membranes allow accumulation of JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide) dye in the mitochondria.[30,31] When a critical concentration is reached, the dye will fluoresce red. If there is a loss of mitochondrial membrane potential ($\Delta\Psi_m$), the JC-1 dye cannot accumulate in the mitochondria and will remain as a monomer in the cytosol that fluoresces green. MIA PaCa-2 and S2VP10 cells were plated at 3×10^5 cells/well in 10% DMEM. Cells were incubated overnight at 37° C and then incubated with 300 nmol/L gemcitabine for 24 hours, or an equal volume of media to serve as a negative control. TRA-8 was added (300 ng/ml) to the media containing cells and gemcitabine, or an equal volume of media to serve as a negative control. Twenty-four hours after TRA-8 addition, cells were scraped from the bottom of the wells and aliquots of 5×10^5 cells were placed into FACS tubes. Cells were then stained using a JC-1 Mitochondrial Membrane Potential Detection Kit (Cell Technology, Mountain View, CA) according to the manufacturer's protocol. Cells were then analyzed using flow cytometry as described above.

Western Blot Analysis of Caspase-3 and -8 Activation

MIA PaCa-2 and S2VP10 cells were plated at 2×10^6 cells/well in 10% DMEM. Cells were incubated overnight and then treated with gemcitabine (300 nmol/L) for 24 hours. TRA-8 was then added (300 ng/ml) and cells were incubated for an additional 3 hours. Cells were scraped in wells, collected, and centrifuged at 1000 rpm for 5 minutes at 4° C. Cells were resuspended in phosphate buffered saline with 10 mM sodium orthovanadate and centrifuged again at 1000 rpm for 5 minutes at 4° C. Cell pellets were resuspended and lysed in radio immuno precipitation buffer with 10 mM sodium orthovanadate and 1:100 protease inhibitor cocktail (Sigma, St. Louis, MO). The lysates were incubated on ice for 20 minutes, then centrifuged at 14,000 rpm for 10 minutes at 23° C. A protein assay was then performed using DC protein assay (Bio-Rad, Hercules, CA) according to the manufacturer's protocol. Forty μg of sample in 30 μL was then loaded and run on a standard polyacrylamide electrophoresis gel. Protein was then transferred from the gel to a 0.45 μM nitrocellulose membrane (Bio-Rad). Membranes were then probed with antibodies to caspase-3 (Cell Signaling, Beverly, CA) and caspase-8 (BD Pharmingen, Chicago, IL).

Subcutaneous Pancreatic Cancer Treatment Model

Female nude mice were obtained from the NCI-Frederick Animal Production Program (Frederick, MD) and acclimated for 3 weeks in a University of Alabama at Birmingham Animal Facility before use. All handling and experiments were performed with strict adherence to the University of Alabama at Birmingham's IACUC guidelines. MIA PaCa-2

cells (2×10^7) were injected subcutaneously into athymic nude mice on day 0. Tumors were measured using calipers, and when tumors reached 6–8 mm in diameter, mice were assigned to treatment groups. The treatment groups (n = 8) were untreated controls, gemcitabine alone (120 mg/kg I.P. on days 10, 17, and 24 postimplant), TRA-8 alone (200 μg I.P. on days 9, 13, 16, 20, 23, and 27 postimplant) and combination treatment with gemcitabine and TRA-8. Tumor size (surface area equal to product of two largest diameters) was measured three times/week and results are expressed as the average change in tumor size in each group relative to size on day 9, when treatment was initiated.

RESULTS

FACS Analysis of DR5 Cell Surface Expression on Human Pancreatic Cancer Cell Lines

The level of DR5 cell surface expression was evaluated by FACS analysis on human pancreatic cancer cell lines (MIA PaCa-2 and S2VP10). As shown in Fig. 1, both human pancreatic cancer cell lines tested expressed DR5. Baseline cell surface expression of DR5 was higher in MIA PaCa-2 cells compared with S2VP10 cells.

In Vitro Cell Viability

MIA PaCa-2 cells demonstrated high sensitivity to TRA-8 ($IC_{50} < 20$ ng/ml to produce 50% cytotoxicity). S2VP10 was the more TRA-8 resistant cell line ($IC_{50} > 1000$ ng/ml). MIA PaCa-2 and S2VP10 cells demonstrated increased cytotoxicity with TRA-8 and gemcitabine combination therapy as compared with either agent alone (Fig. 2).

Annexin V Analysis of Apoptosis in Human Pancreatic Cancer Cell Lines

MIA PaCa-2 cells demonstrated positive annexin V staining after treatment with TRA-8 or gemcitabine, with the highest value in the combination treatment group (Fig. 3). In the group treated with TRA-8, annexin V positive cells were 23.97 ± 1.14% of gated events. In the group treated with gemcitabine alone, annexin V positive cells were 35.34 ± 20.77% of fluorescein isothiocyanate (FITC) gated events. This value increased to 83.43 ± 0.37% of gated events in the group receiving combination treatment, a significant increase when compared with TRA-8 alone ($P = 0.0002$) but not gemcitabine alone ($P = 0.0820$). S2VP10 cells also demonstrated enhanced annexin V

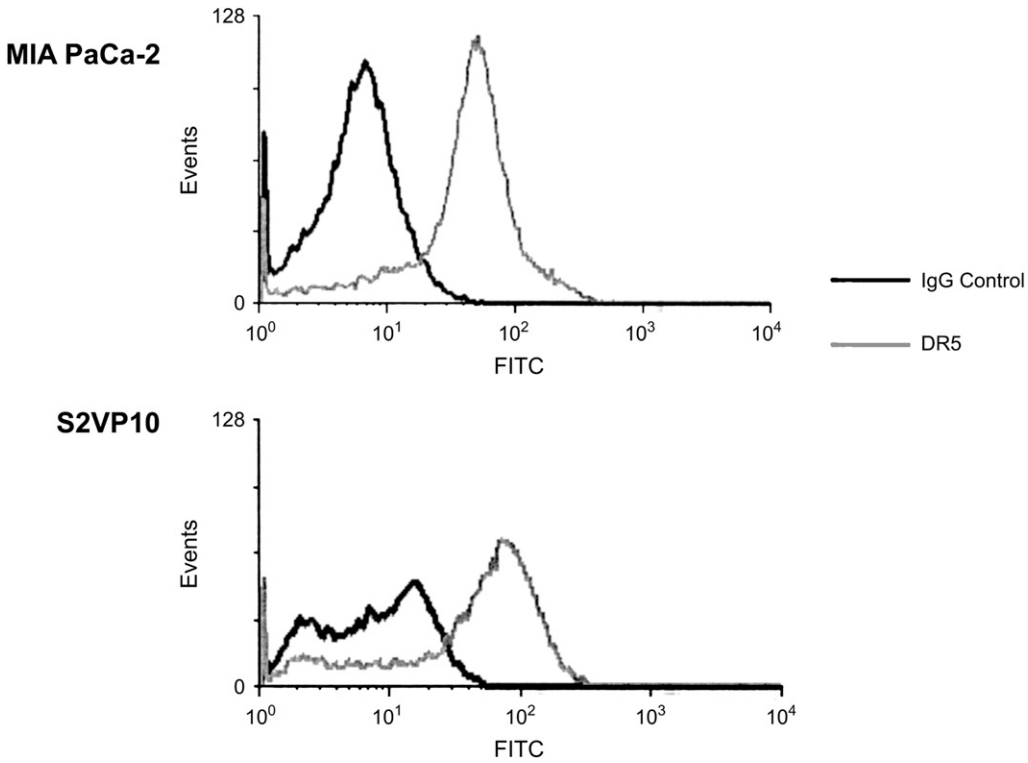

Fig. 1. Flow cytometry analysis of DR5 cell surface expression in MIA PaCa-2 and S2VP10 human pancreatic cancer cell lines. Cells were probed with 2 μg of TRA-8, then goat anti-mouse IgG conjugated with Alexa fluorochrome. Cells were analyzed using FACScan and analyzed with CellQuest software.

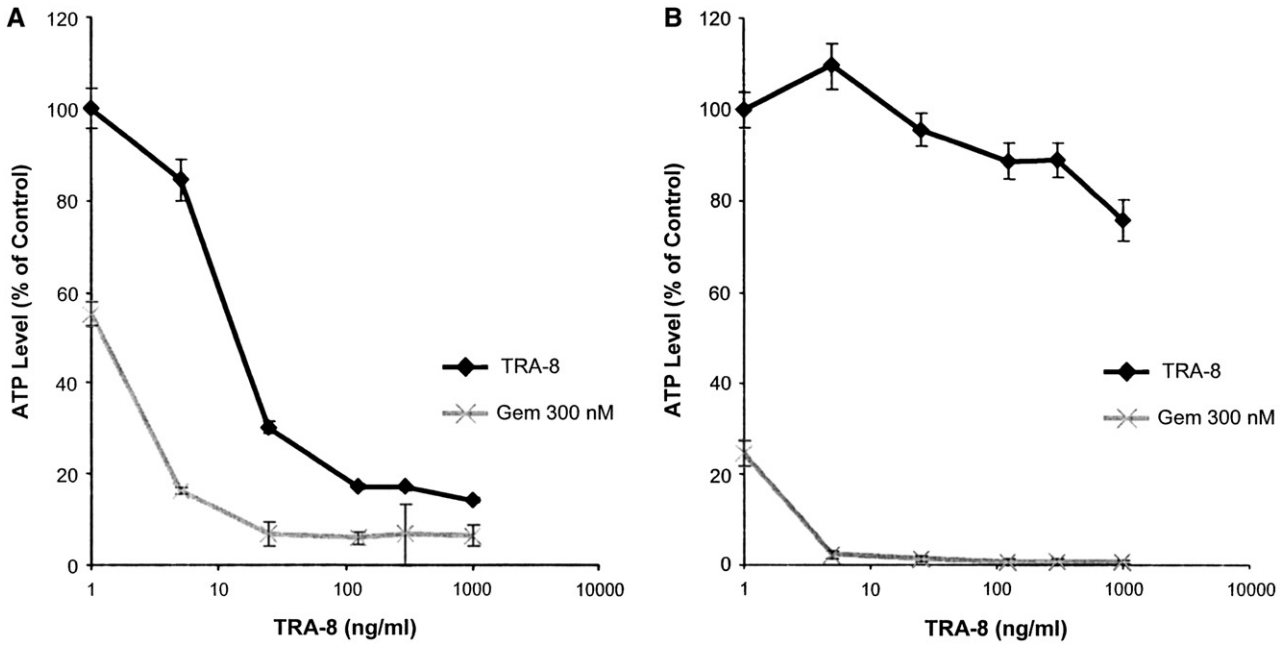

Fig. 2. TRA-8 and gemcitabine cytotoxicity against pancreatic cancer cell lines. MIA PaCa-2 (**A**) and S2VP10 (**B**) pancreatic cancer cells were treated with varying doses of TRA-8 and 300 nmol/L gemcitabine, and cell viability was determined using a luminescence-based ATP assay.

staining with combination treatment, which was greater than either agent alone. The annexin V positive cells in the TRA-8 alone group were 28.44 ± 4.71% of gated events. In the group treated with gemcitabine alone, the annexin V positive cells were 76.66 ± 3.32% of gated events. This value increased to 97.49 ± 0.18% in the group receiving combination treatment, a significant change compared with either treatment alone (TRA-8 vs. combination, $P = 0.0002$; gemcitabine vs. combination,

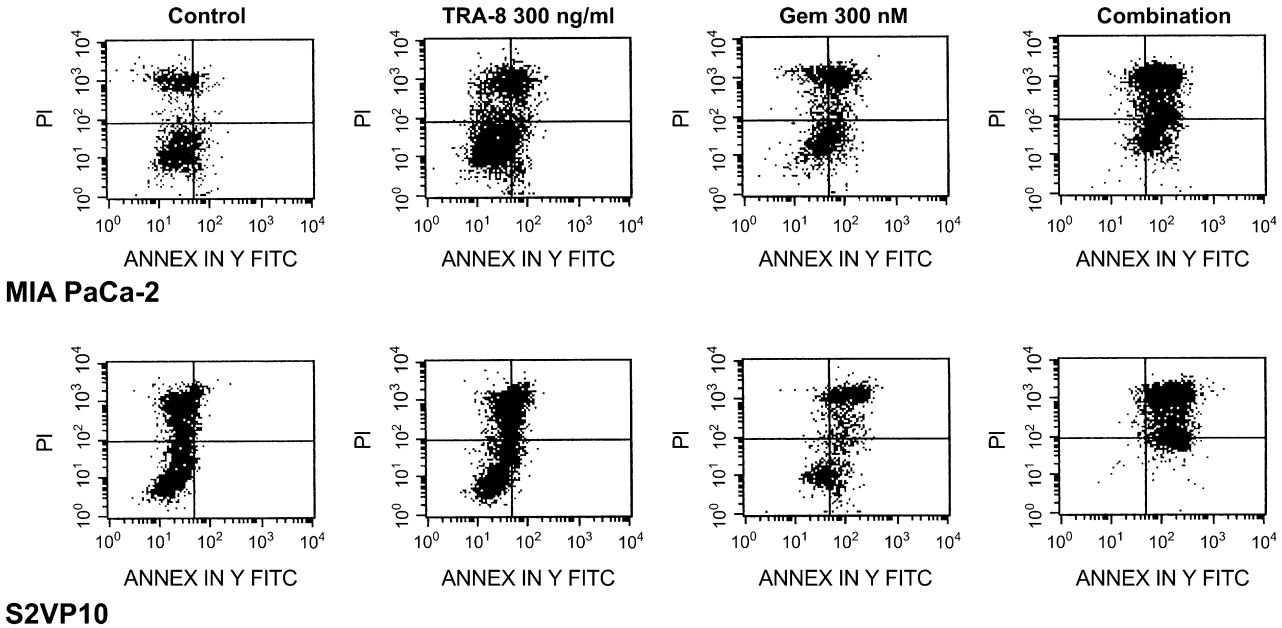

Fig. 3. Annexin V FITC/PI staining of human pancreatic cancer cell lines MIA PaCa-2 and S2VP10. Twenty-four hours after gemcitabine addition, cells were treated with TRA-8 for 24 hours. Cells were then stained with annexin V/FITC and PI. Both cell lines demonstrated enhanced annexin V/FITC and PI staining with combination therapy, indicating an apoptotic cytotoxic response.

$P = 0.01$). In untreated cells, 97.52 ± 3.44% of gated events were FITC-negative.

Detection of Mitochondrial Membrane Destabilization

MIA PaCa-2 cells demonstrated a loss of $\Delta\Psi_m$, as indicated by a reduction in red:green fluorescence ratio when exposed to combination treatment with gemcitabine and TRA-8, which was greater than with either agent alone (Fig. 4). The ratio in untreated cells was 10.0, 4.2 in TRA-8 treated cells, 2.4 in gemcitabine treated cells, and 0.3 in cells treated with combination therapy. S2VP10 cells also demonstrated a loss of $\Delta\Psi_m$, when exposed to combination treatment that was greater than with either agent alone. The results suggest that cytochrome *c* was released with combination treatment, providing evidence for activation of the intrinsic apoptotic pathway.

Western Blot Analysis of Caspase Activation

MIA PaCa-2 and S2VP10 cells treated with gemcitabine and TRA-8 demonstrated enhanced upstream caspase-8 cleavage compared with either treatment alone (Fig. 5). The inactive (pro) form of caspase-8 is a 55 and 50 kDa doublet protein, whereas the cleavage products (active caspase-8) are 40, 36, and 23 kDa. The inactive (pro) form of caspase-3 is a 32 kDa protein. Combination treatment with gemcitabine and TRA-8 enhanced cleavage of caspase-3 (activation results in a 17 and 11 kDa fragment) in both cell lines to a greater extent than did either agent alone.

Subcutaneous Pancreatic Cancer Treatment Model

A subcutaneous flank MIA PaCa-2 xenograft model was used to test the efficacy of TRA-8 in combination with gemcitabine to inhibit tumor growth. Untreated tumors doubled in size in 38 days (Fig. 6). Tumors treated with gemcitabine doubled in 32 days, and those treated with TRA-8 doubled in 49 days. The delay in tumor doubling was greatest in tumors treated with combination therapy, which produced a doubling time of 64 days. The difference in tumor doubling times was significant when the combination therapy group was compared with either treatment alone or no treatment ($P < 0.001$). TRA-8 toxicity to mice could not be evaluated because it is specific for human DR5 and does not bind to mouse DR5.

DISCUSSION

To evaluate the efficacy of TRA-8 in the treatment of pancreatic cancer xenografts, MIA PaCa-2 and S2VP10 cells were evaluated initially in vitro.

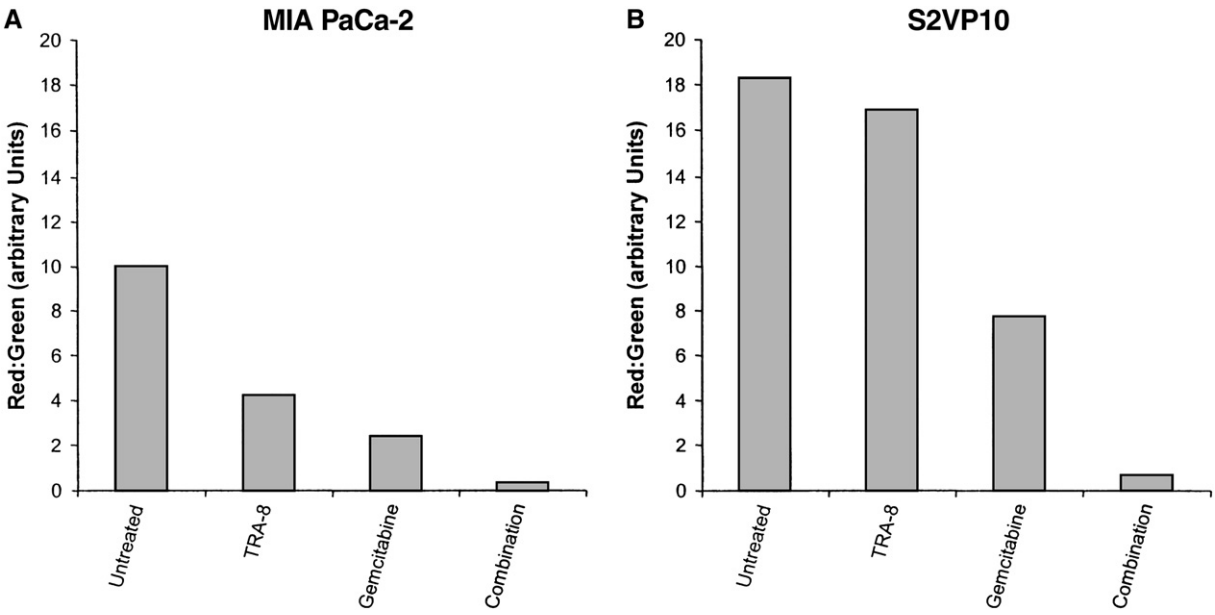

Fig. 4. Flow cytometry analysis of JC-1 dye staining in MIA PaCa-2 (**A**) and S2VP10 (**B**) human pancreatic cancer cell lines. Twenty-four hours after gemcitabine addition, cells were treated with TRA-8 for 24 hours. Cells were then stained with JC-1 dye. Both cell lines demonstrated a reduction in red fluorescence and an increase in green fluorescence (represented by reduction in red:green fluorescence ratio) with combination treatment, indicating reduction in mitochondrial membrane potential.

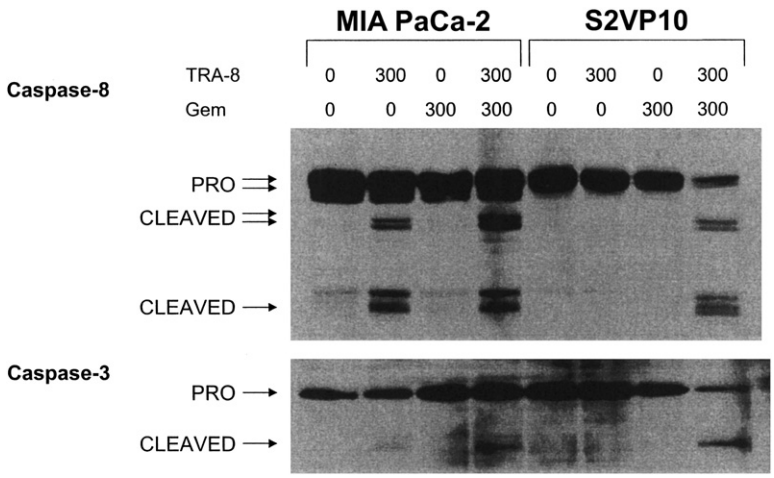

Fig. 5. Western blot analysis of caspase-8 and -3 activation in MIA PaCa-2 and S2VP10 cells. Cells treated with TRA-8 and gemcitabine showed greater caspase-8 (initiator caspase) activation, as well as caspase-3 (executioner caspase) activation.

Both cell lines were found to express DR5, with S2VP10 cells having a greater level of expression compared with MIA PaCa-2. The level of DR5 expression in these two cell lines did not correlate with sensitivity to TRA-8, as indicated by greater annexin V FITC staining in MIA-PaCa-2 cells with TRA-8 treatment alone, as compared with S2VP10 cells. This data is consistent with data reported for TRAIL treatment of pancreatic cancer cells.[32–34]

There have been many studies reporting the induction of apoptosis by TRAIL in a variety of human cancer cell lines. The potential hepatotoxicity of early versions of recombinant TRAIL led to the development of agonistic TRAIL death receptor monoclonal antibodies. TRA-8 is one such antibody, and its specificity for DR5 (vs. other TRAIL receptors) and its ability to induce apoptosis in human cancer cells was reported by Ichikawa et al.[25] There have been studies of other agonistic monoclonal TRAIL death receptor antibodies that produced apoptosis in tumor models of ovarian, glioma, colon, breast, uterine, lung, and hematogenous cancers.[35] As pancreatic adenocarcinoma is resistant to traditional chemotherapeutic agents as well as combinations of these agents, it is a suitable disease for study with TRAIL death receptor therapy.

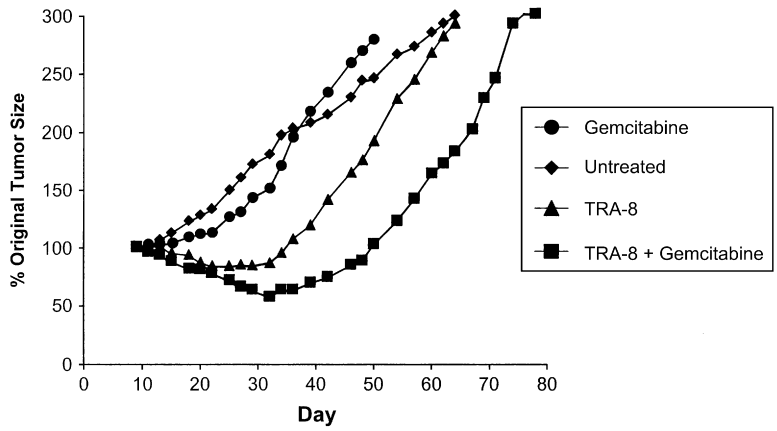

Fig. 6. MIA PaCa-2 xenograft tumors established subcutaneously in athymic nude mice were randomized to treatment groups (n = 8 mice/group). The treatment groups were untreated controls, gemcitabine alone (120 mg/kg I.P. on days 10, 17, and 24 postimplant), TRA-8 alone (200 μg on days 9, 13, 16, 20, 23, and 27 postimplant), and combination treatment with gemcitabine and TRA-8. Data is expressed as the average change in tumor size relative to size on day 9, when treatment was initiated. Combination therapy produced an increase in time to tumor doubling compared with untreated controls and to those groups receiving gemcitabine or TRA-8 alone.

To evaluate the cytotoxic effects of TRA-8 and gemcitabine on pancreatic cancer cells, MIA PaCa-2 and S2VP10 cells were treated with 300 nmol/L gemcitabine for 24 hours, followed by gemcitabine and TRA-8 for 24 hours. There was increased cytotoxicity with the combination treatment as compared with either agent alone (Fig. 2). Annexin V staining was performed to verify apoptosis and demonstrate reduction of cell viability with TRA-8 and gemcitabine treatment. As shown in Fig. 3, MIA PaCa-2 cells showed an increase in annexin V FITC staining after treatment with TRA-8 and gemcitabine as compared with either agent alone. S2VP10 cells demonstrated relative resistance to TRA-8 treatment alone but had a significant reduction in cell viability with combination treatment. This is consistent with other published data describing reduction in tumor cell viability after treatment with an anti-death receptor agonistic antibody, but enhanced reduction with combination therapy with chemotherapeutic agents.[26,35] This has also been demonstrated in cervical cancer models with TRA-8 when combined with cisplatin or topotecan in both in vitro and in vivo studies.[36] Although there have not been studies published regarding combination treatment with a TRAIL death receptor monoclonal antibody in combination with gemcitabine against pancreatic cancer cell lines, there have been several studies documenting enhanced reduction in pancreatic cancer cell viability when TRAIL was combined with gemcitabine. Xu et al. evaluated six human pancreatic cancer cell lines and found enhanced cytotoxicity when TRAIL was combined with gemcitabine.[37] Furthermore, other data has shown that TRA-8–induced cytotoxicity was enhanced when antibody treatment was combined with an adenoviral vector encoding a cytosine deaminase:uracil phosphoribosyltransferase fusion gene (Ad-CD:UPRT) and subsequent administration of the prodrug 5-FC, which was converted by CD to 5-FU.[38]

Apoptosis was verified as the mechanism of cytotoxicity through Western blot analysis of caspase-8, an initiator of apoptosis through the transduction of a signal from the activated death receptor to downstream executioner caspases.[13] As shown in Fig. 5, MIA PaCa-2 cells, when treated with TRA-8, showed activation of caspase-8, with only minimal activation when treated with gemcitabine alone. Combination therapy produced enhanced activation of caspase-8. Combination therapy also produced enhanced caspase-8 activation in S2VP10 cells compared with either agent alone. Caspase-3, an executioner caspase, has been shown to be activated after binding of TRAIL to death receptors, through the upstream proteolytic activities of initiator caspases (such as caspase-8).[39] Both MIA PaCa-2 and S2VP10 cells showed enhanced caspase-3 activation when treated with TRA-8 and gemcitabine, compared with either agent alone. This interaction between the two agents is encouraging for the potential use of TRA-8 or TRAIL as an adjuvant treatment for pancreatic cancer.

To further demonstrate apoptosis was responsible for the observed cytotoxicity, a JC-1 assay was used. Both cell lines demonstrated loss of mitochondrial membrane potential, an early step in apoptosis, with combination TRA-8 and gemcitabine treatment. In MIA PaCa-2 cells, the mitochondrial membrane potential decreased with both gemcitabine and TRA-8 treatment, although combination therapy produced a greater reduction. S2VP10 cells also had a marked reduction in mitochondrial membrane potential with combination therapy, illustrating the sensitizing effects of gemcitabine to TRA-8 treatment (Fig. 4).

After showing enhanced in vitro effects with combination TRA-8 treatment with gemcitabine, a pancreatic cancer xenograft study was undertaken to examine in vivo effects. As shown in Fig. 6, combination therapy slowed the tumor growth rate to a greater extent than either agent alone, after 3 weeks of combination treatment. Improvement in tumor growth inhibition with combination therapy is consistent with other reports from the literature. Hylander et al.[40] established human pancreatic cancer xenografts in SCID mice and treated these mice with TRAIL. The tumors that were relatively resistant to TRAIL showed increased reduction in tumor growth when gemcitabine was added. Jacob et al.[41] also demonstrated a greater reduction in pancreatic tumor growth in the livers of nude mice when gemcitabine was used in combination with TRAIL gene therapy.

Pancreatic cancer remains a deadly disease with minimal survival. These studies have shown in both in vitro and in vivo models that TRA-8 has the potential to be an adjuvant to gemcitabine in the treatment of pancreatic cancer. These results are consistent with those in the literature reporting enhanced antitumor effects with combination therapy with TRAIL and gemcitabine. Based on these results, we believe that further evaluation with other chemotherapy agents and additional studies in an orthotopic model of pancreatic cancer are warranted.

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Discussion

Dr. K. Kirkwood (San Francisco, CA): Thank you, Dr. Tanabe. I would like to congratulate you on a beautiful study, Dr. DeRosier, and to congratulate you and Dr. Vickers on the manuscript. I learned a lot about TRAIL and TRA-8 in reading this.

UAB has really led many of the investigations of the importance of TRAIL, not only in pancreatic cancer but in many other tumors, and I think line of investigation is showing great promise. I have three questions for you.

The first is about mechanism, which I couldn't quite discern from your paper. It looked as if TRA–8 alone, if I understand, had no effect on mitochondria membrane destabilization in the S2VP10 cells but was effective in combination with gemcitabine in changing membrane destabilization or mitochondrial destabilization and in an reducing cell viability. So it seems as if that may not be via cytochrome *c*, as you speculated, and I am wondering if you can expand on the possible mechanism of that sensitizing effect.

Second, I guess if somebody wants to take this into the clinicial arena as quickly as possible, the question would be, have you examined DR–5 immunostainining and TRAIL protein expression in human pancreatic tissues?

And the last question pertains to a paper by Horvak and colleagues I found in 2005, that you are probably aware of, looking at TRAIL and TRA-8 in human ovarian cancer. They found, interestingly, that there was no correlation between epithelial ovarian cell TRAIL expression and survival. So in the ovarian cells themselves, the epithelical cells, TRAIL expression was not correlated with patient survival. However, the stromal expression of TRAIL did confer a survival advantage, a survival benefit, in ovarian cancer patients. Given the increasing importance and recognition of the stroma in pancreatic cancer, I wonder if you can comment on the potential importance of TRAIL in pancreatic stroma?

Thank you.

Dr. DeRosier: Great questions. The first question, the effects of TRA-8 in the S2VP10 celll line. The S2VP10 cell line is a very aggressive cell line. It was derived from the SUIT2 line by Dr. Hollingsworth at the University of Nebraska. We didn't show the data here for it, but the inhibitors of apoptosis, so XIAP, cFLIP, and some of those molecules, you can downregulate these with combination therapy. And as in other cancer systems, and it looks like in S2VP10 as well, part of the synergistic mechanism, the interaction between the two agents, is downregulation of inhibitors of apoptosis. That is not to say that is the complete mechanism, but that probably plays a role as well.

Staining of DR-5 and TRAIL in human samples. Of the samples we have stained at UAB using TRA-8 for the DR-5 staining, and also another antibody for DR-4, about 90% to 95% are staining positive for DR-4 and DR-5. That is consistent with the small amount that is published in the literature on pancreatic cancer, where they are finding mRNA levels or cell surface expression with the majority of pancreatic tissue, but the vast majority of cases have an increased expression in the cancer cells versus the normal tissue.

The question of epithelial expression versus stromal expression of the death receptors in TRAIL, we have found across all of our cell lines that our lab has studied—lung, ovarian, breast, colon, pancreatic—that the level of DR-5 expression or DR-4 expression does not correlate with sensitivity to the antibody or correlated with the sensitivity to TRAIL, to the point that one of our initial investigations was, well, will gemcitabine alter DR-5 expression, and is that the source of this this enhanced response? We haven't been able to find that, and that has been pretty consistent with what others have published.

In terms of looking directly at tumor stroma versus tumor cell expression of the DR-5, we have not looked at that yet, and so I don't know, but that is a good idea.

The Effects of Neoadjuvant Chemoradiation on pTNM Staging and Its Prognostic Significance in Esophageal Cancer

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For esophageal cancer, it is not clear if pathologic TNM staging after chemoradiation and resection will have the same prognostic significance compared with patients who undergo resection only. From 1995 to 2004, prospectively collected data from 279 patients with intrathoracic squamous cell cancers were analyzed. Patients were given chemoradiation either as part of a randomized trial comparing neoadjuvant chemoradiation with surgical resection alone, or because of advanced disease at presentation. One hundred seventy patients had surgical resection only (surgery), and 109 had neoadjuvant chemoradiation (CRT plus surgery). In the surgery group, pT1, 2, 3, and 4 disease was found in 15, 17, 104, and 34 patients, respectively; their respective pN1 rates were 13.3%, 29.4%, 57.7%, and 64.7%, $P < 0.01$. In CRT plus surgery, pT0, T1, 2, 3, and 4 were found in 48, 12, 23, 21, and 5 patients, respectively; their respective pN1 rates were 31.3%, 16.7%, 21.7%, 52.4%, and 20%, $P = 0.44$. Logistic regression analysis of factors predictive of pN1 showed that pT stage correlated with pN1 status ($P = 0.005$) in the surgery group, but not for the CRT plus surgery group. Cox regression analysis demonstrated that in the surgery group, pT, pN, and R category, and overall pTNM stage, were independent prognostic factors, whereas pN, R category, and gender were identified as relevant for CRT plus surgery. After chemoradiation, pT and overall pTNM stage groupings were not as clearly prognostic as in patients without prior therapy. Nodal status remains an important prognostic factor. (J GASTROINTEST SURG 2006;10:1301–1311) © 2006 The Society for Surgery of the Alimentary Tract

KEY WORDS: Esophageal neoplasm, chemoradiation, multimodality treatment, staging, prognosis

Immediate surgical results of esophagectomy for cancer have improved. In dedicated centers, a mortality rate of below 5% can be achieved.[1–4] Prolonging long-term survival is a goal more difficult to attain. Prognosis for esophageal cancer remains poor throughout the world. In selected centers and in subgroups of patients who undergo radical esophagectomy, 5-year survival rates of 40% or above could be achieved.[5–7] Selection bias is difficult to disprove, and such encouraging results are infrequently seen. In most reports, a 20% 5-year survival rate is recorded.[8,9]

In recent years, neoadjuvant therapy involving chemotherapy and/or radiotherapy is commonly used as an adjunct to surgical resection.[10,11] Despite the equivocal data from randomized controlled trials that these treatments can result in better prognosis compared with surgery alone, they are frequently applied with an aim to downstage tumor—increasing the resection rate (especially R0 resection)—and to improve survival.[12–17] After neoadjuvant therapy, however, clinical restaging is difficult with conventional techniques such as CT scan or endoscopic ultrasound. Positron emission tomography scan shows some promise, but how it should be integrated into clinical practice, and whether it can be used to predict long-term survival or not requires further evaluation.[18]

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Among other factors investigated, pathological TNM stage after resection has been the gold standard in prognosis stratification, and the relationship between advancing pTNM stage and poor survival is well established. It has been our observation however, that after chemoradiation, the primary tumor is often sterilized, but persistence of nodal disease exists. It is therefore hypothesized that neoadjuvant therapy may alter the relationship between the different components of the pTNM system, such as the intercorrelation of pT and pN status, and that the postchemoradiation pTNM stages may have different prognostic implications compared with patients without prior therapy. These factors are investigated in a large group of patients undergoing surgical resection, with or without neoadjuvant chemoradiation.

METHODS

From 1995–2004, 471 patients with intrathoracic squamous cell carcinomas without prior treatments were managed at the Department of Surgery, The University of Hong Kong at Queen Mary Hospital. Patients who had cancers located in the cervical esophagus, tumors that involved the gastroesophageal junction, and cancers of other cell types were excluded from this study. Patients with synchronous or history of nonesophageal malignancies were also excluded, so that the influence of other unrelated tumors on survival was prevented. Surgical resection was carried out in 279 patients (59.2%), of whom 170 had surgical resection only and 109 received preoperative chemoradiation therapy. Data were captured in a prospectively collected database. These patients were the subjects of the present study.

The management rationale and protocols at the authors' institution have been described previously.[19] Patients were managed in an individualized manner determined by both patient (performance status, comorbidities) and tumor (stage, location) characteristics. Surgical treatment was the preferred treatment option. Patients were selected for nonsurgical treatment if they had locally advanced unresectable disease, or nonlocal-regional metastases, when medical-surgical risks were prohibitive, or in those who declined surgery.

For tumor imaging and staging purposes, all patients had a barium contrast study, an endoscopy, bronchoscopy, and since May 1996, endoscopic ultrasound examination. An ultrasound of the neck and CT scan of the thorax and abdomen were carried out. Positron emission tomography scans were available for most patients since July 2002.

The surgical techniques are described in brief: for most tumors in the middle and lower third of the esophagus, a Lewis-Tanner esophagectomy via an abdominal-right thoracotomy approach was preferred. For patients who had a tumor of the superior mediastinal segment, a three-phase esophagectomy was carried out. In this operation, usually a right-sided thoracotomy was performed first for esophageal mobilization; a synchronous laparotomy and left cervical incision then provided access for gastric and cervical esophageal mobilization, followed by a gastric pull-up to the neck, either by the posterior mediastinal or by the retrosternal route for cervical esophagogastrostomy. In patients who had limited cardiopulmonary reserve for whom a thoracotomy was judged to be of high risk, a transhiatal esophagectomy was performed. This method was mainly used for tumors of the lower esophagus. This method was uncommonly performed in the study period because the preferred approach was transthoracic and thoracoscopic esophagectomy has also largely replaced the need for transhiatal esophagectomy.[20] Altogether, 16 patients underwent thoracoscopic esophagectomy.

Lymphadenectomy usually involved a two-field lymphadenectomy with dissection of lymph nodes around the celiac trifurcation, and also an infracarinal mediastinal lymph node dissection. Lymph nodes of the superior mediastinum were sampled, but complete clearance of nodal tissues around the paratracheal area along the recurrent laryngeal nerves was not usually performed unless suspicious lymph nodes were encountered. Similarly, cervical lymphadenectomy was not carried out routinely unless there was evidence of disease because our study of recurrence patterns suggested limited value of neck dissection,[21] and that survival advantage of cervical lymphadenectomy was not proven.[22,23] In patients with obviously palliative resection, a more limited lymphadenectomy was carried out.

Reconstruction of intestinal continuity was usually restored with a gastric conduit placed in the right thoracic cavity (after Lewis-Tanner esophagectomy) or via the orthotopic route when the anastomosis was carried out in the neck. In the obviously palliative cases where residual mediastinal disease was evident, the retrosternal route was chosen. The colon was used in patients with a prior gastrectomy, the right ileocolon being the preferred conduit.[24] All these surgical techniques have been described.[25,26]

Processing of the resected surgical specimens started in the operating room. The operating surgeons dissected the different nodal stations separately and labeled them for further histological examination. Individual nodes were not dissected,

but only the fat and connective tissues containing the nodes at various anatomical locations were isolated. The tissue adjacent to the primary tumor was not disturbed so that histological examination of the circumferential margin would not be hampered. The primary tumor was serially sectioned for histology by the pathologist.

During the study period, patients were given neoadjuvant chemoradiation therapy as either part of a randomized controlled trial comparing neoadjuvant chemoradiation and surgical resection alone, or when locally advanced tumor or nonregional metastastic spread, such as cervical lymphadenopathy, was encountered, whereby an R0 resection was judged improbable. The chemotherapy regimen consisted of cisplatin at 100 mg/m^2, by intravenous infusion given on the first day and day 24, together with 5-fluorouracil (5-FU), 500 mg/m^2 per day by continuous infusion given from day 1–5, and day 24–28. Radiotherapy was given as external beam irradiation at 40 Gy in 20 daily fractions of 2 Gy each, delivered through anterior and posterior opposing fields to the primary tumor as defined by CT scan, endoscopy, and barium contrast study. Fields included the primary tumor with at least 1 cm lateral margin on each side and proximal and distal margins of at least 3 cm. Regional lymph nodes were not prophylactically irradiated. Enlarged lymph nodes were irradiated in the fields for the primary if they were close to the primary, or separate radiation fields were used for palliation of symptoms. In those who demonstrated significant response, surgical resection was carried out.

For the purpose of this study, 30-day mortality rate was defined as any death after esophagectomy within 30 days, and hospital mortality rate included any deaths within the same hospital stay. Patients were staged according to American Joint Committee on Cancer (AJCC) classification,[27] and the R category of resection was based on the International Union Against Cancer system.[28]

Statistical Analysis

Continuous variables are expressed as median (range). Chi-square and Fisher exact tests were used to compare categorical data. Survival analyses were performed using the Kaplan-Meier method from the date of operation to the time of death of any cause or to the time of last follow-up, at which point the data were censored. Comparisons between groups were assessed by the log-rank test.

To evaluate the impact of various clinicopathological parameters for long-term survival, potential prognostic factors were analyzed with univariate Cox regression analysis. The same factors were also used in Cox proportional hazard models fitted for multivariate analysis.

Statistical significance was accepted at the 5% level. All statistical analyses were performed with the SPSS statistical package, version 11.5 (SPSS Inc., Chicago, IL).

RESULTS

A total of 279 patients satisfied the inclusion criteria and underwent surgical resection. There were 228 men and 51 women, the median age was 66 years (range, 38–86). Of these patients, 170 had surgical resection only and 109 received neoadjuvant chemoradiation therapy. Their demographics are shown in Table 1. The majority of patients underwent

Table 1. Patient demographics in patients who underwent surgery only or in patients with neoadjuvant chemoradiation

	Surgery	CRT + Surgery*	*P* value
No. of patients	170	109	—
Median age, yr (range)	66 (40–86)	66 (38–82)	0.427
Gender (M:F)	132:38	96:13	0.038
Level of tumor			
Upper	23	23	
Middle	102	66	0.124
Lower	45	20	
R category			
R_0	120	97	
$R_{1/2}$	50	12	<0.001
pT stage			
pT0	0	48	
pT1	15	12	
pT2	17	23	<0.001
pT3	104	21	
pT4	34	5	
pN stage			
pN0	81	75	
pN1	89	34	0.001
pM stage			
pM0	152	100	
pM1a/b	18	9	0.68
pTNM stage			
pCR	0	31	
pT0N1	0	14	
Stage I	13	9	
Stage II	58	32	<0.001
Stage III	81	14	
Stage IV	18	9	

Numbers represent number of patients unless stated otherwise.
*Neoadjuvant chemoradiation and surgery.

a transthoracic esophagectomy (98.9%), with only three patients in the surgery group having a transhiatal approach. Thirty-day mortality rate was 0.6% (one patient) in the surgery group, and 0% in patients who received neoadjuvant therapy ($P = 1.0$). In-hospital death rates were 2.4% (four patients) and 0%, $P = 0.16$. R0 resections were possible in 89% of the neoadjuvant therapy group and 70.6% of the surgery group, indicating significant tumor downstaging. This is also reflected in the pTNM stage groupings, with significantly lower disease stage distributions in the neoadjuvant therapy group.

The relationship between pT and pN status is shown in Fig. 1. Advancing pT stage showed a clearly progressive increase in incidence of pN1 stage in the surgery group ($P < 0.01$). This correlation, however, was not significant in the neoadjuvant group, $P = 0.44$. Logistic regression analysis was used to identify factors that were predictive of pN1 status. In the surgery group, pT status was shown to be an independent factor predictive of positive nodal status (Table 2). R category, level of tumor, age, and gender of patient were not significant predictive factors. In the neoadjuvant therapy group, none of the factors above tested showed predictive value in identifying pN1 status.

Stage-specific survival curves for patients with and without neoadjuvant chemoradiation therapy are shown in Fig. 2, *a*, *b*. In the surgery group, clear separations of survival among different stages were seen ($P < 0.01$). In the chemoradiation group, although a trend could still be seen, it was not as clear, and statistically it was not significant by the log-rank test, P value = 0.09. Comparisons of stage-specific survival in patients with and without chemoradiation therapy are shown in Table 3. The survival rates for each pTNM stage between the two groups of patients were comparable, except for stage I disease ($P = 0.0485$). Patients with pathological complete response and pT0N1 had no equivalent groupings in the surgery group and could not be directly compared.

Univariate analyses of prognosis with respect to different clinicopathological factors are shown in Tables 4 and 5. In the surgery group, pT, pN, pM stage, overall pTNM stage, and R category were significant factors, whereas in the neoadjuvant therapy group, pT, pN, and R category and gender were predictive factors. Multivariate analyses using Cox regression analysis for survival are shown in Tables 6 and 7. Advancing pT stage, pN stage, and R1/2 resections were predictive of worse survival in the surgery group, whereas pN1 stage, male gender, and R1/2 resections were predictors of poor prognosis in the neoadjuvant therapy group. If overall pTNM stage groupings were analyzed as a separate factor in the Cox regression model instead of using separate pT, pN, and pM, then for the surgery group, overall pTNM stage and R category were predictive of survival replacing pT and pN status (Table 6), whereas in those patients with neoadjuvant therapy, the significant predictive factors were not changed.

DISCUSSION

Chemoradiation therapy is increasingly used up front to treat esophageal cancer, often in

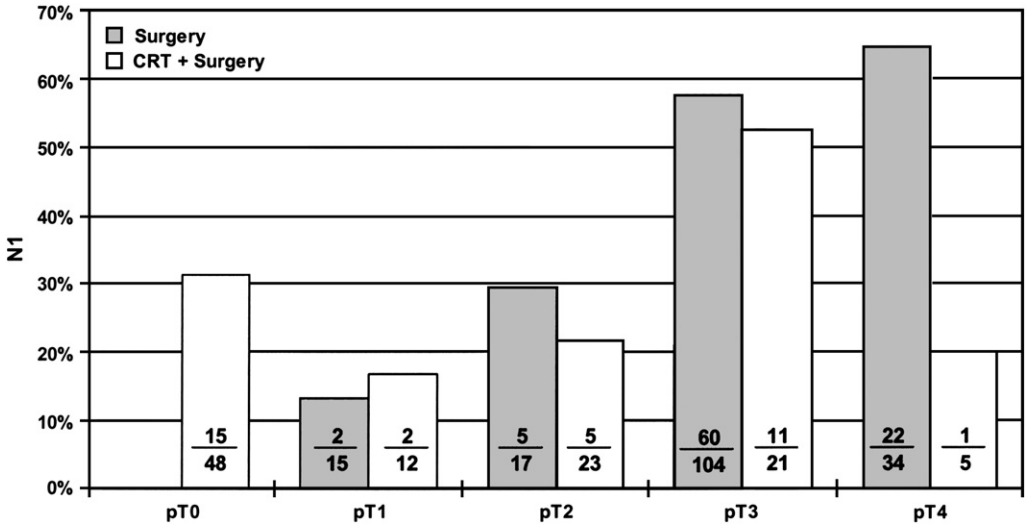

Fig. 1. Relationship between pT status and pN status for the two groups of patients (surgery only and neoadjuvant chemoradiation [CRT] with surgery). Correlation between pT vs. pN in surgery group: $P < 0.01$; in CRT + surgery group: $P = 0.44$.

Table 2. Logistic regression analysis of factors predictive of pN1 status in patients who underwent surgery without chemoradiation

Factor	No. of patients	OR	95% CI	*P* value
pT stage				0.005
pT1 (reference)	15	1		—
pT2	17	2.708	0.440–16.68	0.283
pT3	104	8.864	1.903–41.29	0.005
pT4	34	11.92	2.297–61.83	0.003

OR = odds ratio; CI = confidence interval.

multimodality programs, despite the lack of proof of benefits in randomized trials.[12–16] In our patients, significant downstaging by chemoradiation seemed possible since a substantial proportion of patients were selected for neoadjuvant chemoradiation because of their more locally advanced tumors and metastatic disease at presentation, but the postresection pathological stage distribution as well as R0 resections were more favorable in this group. Whether neoadjuvant chemoradiation in resectable tumors could truly confer a survival benefit awaits more randomized trials and their meta-analyses.[29,30]

pTNM stages are the most commonly used parameters to stratify patients for prognosis after surgical resection. However, it is unclear whether the same staging system can be used for patients after neoadjuvant chemoradiation. It has been suggested by some investigators that the prognostic implications of the current pTNM staging system is not invalidated by chemoradiation therapy.[31,32] Swisher and colleagues[32] reported that, stage-for-stage, survival rates comparisons were equivalent between patients with or without prior therapy except in stage I patients. Interestingly, our results are similar; no statistical difference was found between the two groups when stage-for-stage comparisons were made for stage II to IV disease, except for stage I disease. In the data from M. D. Anderson Cancer Center, the median survival and 5-year survival rate were 163 months and 82% for stage I disease in patients who had surgical resection only. The respective survival rates were 53 months and 47% in those who had chemoradiation. In our data, the 5-year survival rates were very similar at 80.8% and 41.7% respectively when the two groups were compared. The median survival for the surgery alone has not been reached, and it was 47.3 months for patients with chemoradiation. We hypothesize the following to explain the better survival in the surgery group for patients with stage I disease: In patients who had resection alone and a pT1 lesion, there was a certain proportion of patients who had no nodal metastases (pN0) and were classified as stage I (pT1N0). Patients in the chemoradiation group who had a pT1 stage after neoadjuvant therapy obviously had had more nodal disease burden to begin with, because this group included many patients who had had a higher T stage before treatment. After chemoradiation, many patients were downstaged to pT1, but if chemoradiation was not as effective in downstaging nodal disease, then many of these pT1 patients would also have positive nodes, and this is expected to be more frequent than in patients who had de novo T1 lesions. Because surgical resection may not be able to remove all positive nodes (equally true for both groups of patients), those patients with an apparent pT1N0 disease after chemoradiation are likely to have more "residual true-positive nodes" left in situ compared with patients with de novo pT1 lesions. This helps explain the worse prognosis of stage I patients after chemoradiation. In patients with stage II–IV disease, this effect was expected to be less because the incidence of nodal metastases was much higher, and the chance of undersampling and falsely assigning a patient to pN0 disease will be much less. The observed pN status will more likely reflect the "true" nodal status.

Swisher's data also suggested that pTNM stage was prognostic on multivariate analysis even after chemoradiation therapy. Our data do not lend full support to their findings. Although the surgery group showed clearly worsening median and 5-year survival rates with more advanced pTNM stage (Fig. 2), and pTNM was found to be significant on Cox regression analysis for the chemoradiation group, this trend seemed less evident. Statistically, overall pTNM stage was not prognostic in univariate as well as multivariate analysis, showing that the pTNM stage groupings are much less satisfactory in the postchemoradiation setting.

The reason postchemoradiation pTNM stage was less predictive of survival may be in part due to the altered relationship between pT and pN stage. In patients without prior therapy, the increasing incidence of pN1 status with advancing pT stage was expected and confirmed, as shown in Fig. 1 and the results of logistic regression analysis that demonstrated the predictive value of increasing pT stage on nodal metastases. In the chemoradiation group, however, this clear relationship was no longer evident. In patients with pT4 disease, only 20% had pN1 disease; this was lower than expected. This may be related to the small number of patients in this group (only 5). A potential selection bias may also help explain this phenomenon; patients with pT4 disease were likely to have locally advanced tumors with or without

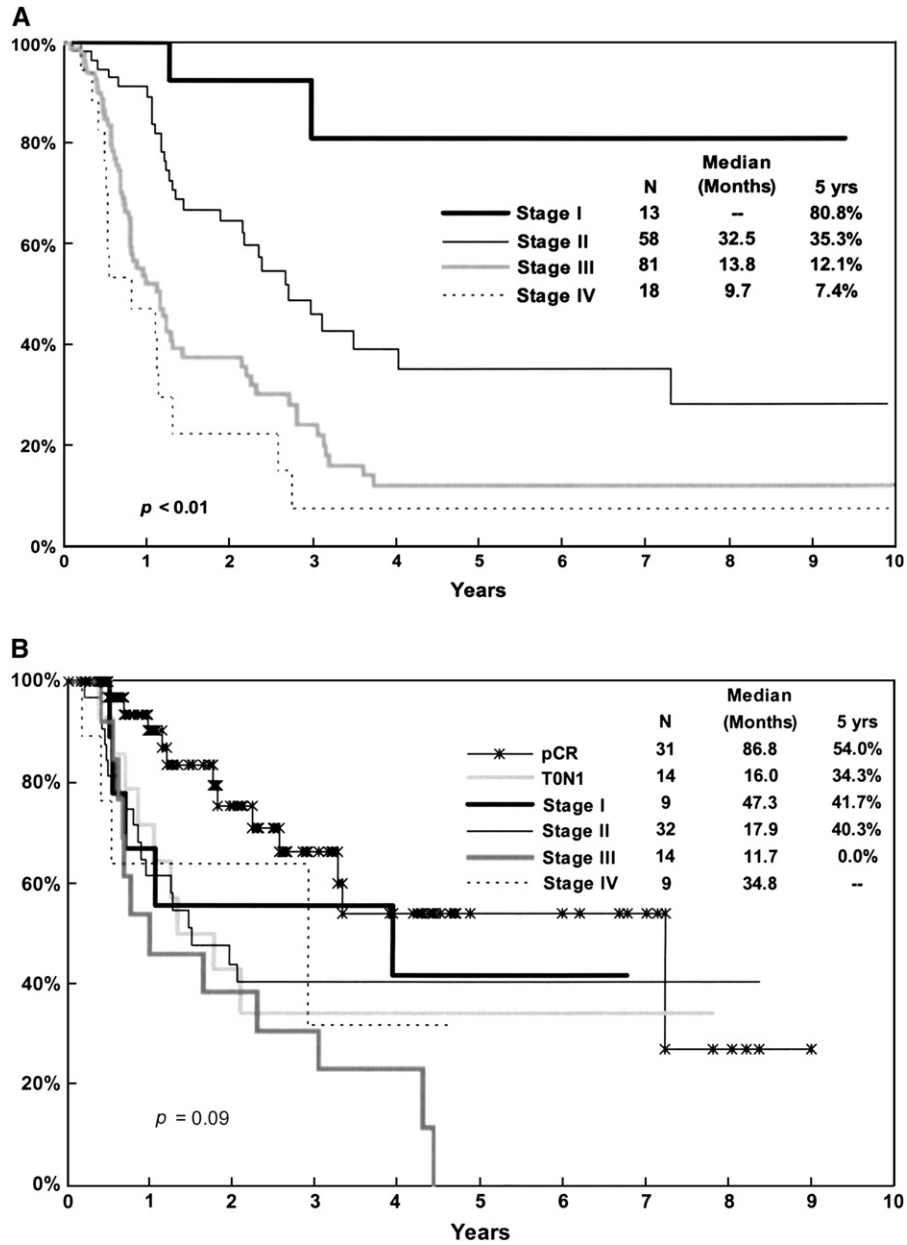

Fig. 2. Survival curves of patients stratified for different pTNM stages for surgery group (**A**) and neoadjuvant chemoradiation plus surgery group (**B**).

locoregional (or even nonlocoregional) metastases before chemoradiation therapy. When locally advanced disease was still found after treatment, patients with obvious nodal disease may not be operated upon because the chance of an R0 resection would be low. Only those with no or little nodal disease burden deemed to have a chance of cure would be resected. Thus, a group of patients with pT4N1 disease were excluded, resulting in an apparent lower than expected incidence of pN1 stage.

Another, perhaps more important, reason for the altered correlation between pT and pN status was the presence of nodal metastases in patients whose primary tumor was sterilized by chemoradiation (pT0N1). This stage grouping had no equivalence in patients who underwent surgical resection alone. Histologically, absence of tumor in the primary tumor itself occurred in 41% of patients after chemoradiation, but one third of these patients had pN1 disease.

Persistence of nodal disease despite complete response at the primary site may reflect a biological difference in responsiveness to chemoradiation between the primary tumor and metastatic cells, because the latter may behave differently. An alternative explanation is that the radiation field did not

Table 3. Comparisons of stage-specific survival in patients who underwent surgery alone or with neoadjuvant chemoradiation

	Surgery			CRT + Surgery			
Stage	**No. of patients**	**Median (mo)**	**5-yr, %**	**No. of patients**	**Median (mo)**	**5-yr, %**	***P* value***
pCR	0	—	—	31	86.8	54.0	—
pT0N1	0	—	—	14	16	34.3	—
I	13	Not reached	80.8	9	47.3	41.7	0.049
II a/b	58	32.5	35.3	32	17.9	40.3	0.43
III	81	13.8	12.1	14	11.7	0	0.97
IV	18	9.7	7.4	9	34.8	—	0.13

*By log-rank test comparing the two groups of patients stage by stage.

extend to encompass all areas with possible nodal spread, whereas the extent of surgical resection was much wider; this also implies that systemic chemotherapy had suboptimal effects on metastatic cells. It is a well-known phenomenon that esophageal cancer can have wide longitudinal spread.[33] The focus of the radiation field, however, is usually planned on the primary tumor with a limited longitudinal and lateral margin, thus nodal metastases outside the radiation field may not be treated adequately. Treating an extended area from the neck through the mediastinum to the celiac axis is regarded as too extensive and would incur too much morbidity. In assessing pN1 status, the distribution of involved nodes could

Table 4. Univariate analysis of survival with respect to different clinicopatholgoical factors in patients who underwent surgical resection without neoadjuvant therapy

Factor	**No. of patients**	**HR**	**95% CI**	***P* value**
Age	170	1.007	0.986–1.028	0.53
Gender				
Male (reference*)	132	1	—	
Female	38	0.800	0.496–1.291	0.36
Level of tumor				
Upper (reference)	23	1	—	
Mid/lower	147	0.717	0.413–1.245	0.24
pT stage				<0.01
pT0	—	—	—	—
pT1 (reference)	15	1	—	—
pT2	17	7.261	1.588–33.194	0.01
pT3	104	8.763	2.141–35.870	<0.01
pT4	34	18.499	4.382–78.101	<0.01
pN stage				
pN0 (reference)	81	1	—	
pN1	89	2.288	1.538–3.403	<0.01
pM stage				
pM0 (reference)	152	1	—	
pM1a/b	18	2.379	1.372–4.126	0.02
Overall pTNM stage				<0.01
Stage I (ref)	13	1	—	
Stage II	58	5.159	1.231–21.622	0.03
Stage III	81	11.305	2.751–46.455	<0.01
Stage IV	18	16.956	3.855–74.580	<0.01
R category				
R0 (reference)	120	1	—	
R1/2	50	2.606	1.754–3.872	<0.01

HR = hazard ratio; CI = confidence interval.
*Reference against which hazard ratios are calculated.

Table 5. Univariate analysis of survival with respect to different clinicopathological factors in patients who had neoadjuvant chemoradiation and surgical resection

Factor	No. of patients	HR	95% CI	*P* value
Age	109	0.996	0.972–1.022	0.78
Gender				
Male (reference*)	96	1	—	
Female	13	0.267	0.083–0.854	0.03
Level of tumor				
Upper (reference)	23	1	—	
Mid/lower	86	1.302	0.677–2.507	0.43
pT stage				<0.01
pT0	48	0.868	0.352–2.140	0.76
pT1 (reference)	12	1	—	—
pT2	23	0.746	0.270–2.063	0.57
pT3	21	2.284	0.902–5.783	0.08
pT4	5	8.849	2.058–38.055	<0.01
pN stage				
pN0 (reference)	75	1	—	
pN1	34	2.151	1.283–3.604	<0.01
pM stage				
pM0 (reference)	100	1	—	
pM1a/b	9	1.170	0.424–3.233	0.76
Stage				0.11
Stage I (reference)	9	1	—	
Stage II	32	1.149	0.425–3.106	0.78
Stage III	14	2.056	0.723–5.844	0.18
Stage IV	9	1.238	0.332–4.622	0.75
pCR	31	0.614	0.216–1.746	0.36
PT0N1	14	1.291	0.431–3.869	0.65
R category				
R0 (reference)	97	1	—	
R1/2	12	6.684	2.869–15.574	<0.01

HR = hazard ratio; CI = confidence interval.
*Reference against which hazard ratios are calculated.

still extend from the superior mediastinum to the left gastric artery area. Unfortunately, our data was not detailed enough to isolate individual locations of lymph node metastases to make this analysis.

In our multivariate analysis, R category of resection was important as a prognostic factor in both groups of patients. This has been a consistent finding and is not debated. Our data suggest that pT status becomes less important after chemoradiation. In patients who underwent surgical resection alone, both pT and pN (and overall pTNM stage when added to the Cox regression analysis) were independent predictive factors for survival. In patients with prior chemoradiation, pT lost its significance, whereas pN status retains its importance. One reason why pT stage's predictive value was lost may be related in part to the difficulty in assigning a pT stage according to conventional definitions of depth of esophageal infiltration. Often clusters of "viable cells" may persist in the different layers of the esophageal wall and assigning a pT stage may be arbitrary, and classifying tumors in such manner may no longer reflect its prognostic significance. A new way of assessing the primary tumor after chemoradiation may be beneficial. One suggested classification was proposed by the group at the M. D. Anderson Cancer Center. The degree of response was defined as P0 (0% residual tumor), P1 (1%–50% residual), and P2 (>50% residual).[31] It was demonstrated that this assigned degree of response in the primary tumor histologically can be integrated into the current AJCC classification to enhance its prognostic value.

Nodal status appeared to be an important prognostic factor in both groups of patients. A recent study showed that in a group of 101 patients who had chemoradiation therapy, only pN status was predictive of disease-free survival regardless of pT status; 57% of node-negative patients were alive at 3 years compared with 0% for node-positive patients. The degree of primary response was not predictive

Table 6. Multivariate analysis on factors predictive of survival for patients who underwent surgical resection only, with or without pTNM overall stage groupings entered into Cox regression model

	HR	95% CI	*P* value
Surgery (pTNM not entered)			
pT stage			<0.01
pT1 (reference*)	1	—	—
pT2	6.560	1.432–30.060	0.02
pT3	6.338	1.524–26.362	0.01
pT4	8.537	1.840–39.599	<0.01
pN stage			
pN0 (reference)	1	—	
pN1	1.731	1.152–2.601	<0.01
R category			
R0 (reference)	1	—	
R1/R2	1.738	1.025–2.945	0.04
Surgery (pTNM stage entered)			
Overall stage			<0.01
Stage I (reference)	1	—	
Stage II	4.743	1.129–19.916	0.03
Stage III	9.191	2.211–38.202	<0.01
Stage IV	12.230	2.714–55.123	<0.01
R category			
R0 (reference)	1	—	
R1/R2	1.790	1.179–2.718	<0.01

HR = hazard ratio; CI = confidence interval.
*Reference against which hazard ratios are calculated.

of survival.[34] Another study showed that the number of lymph nodes with metastasis was also important after chemoradiation in patients who had residual primary tumor. In these patients, pN0 disease had better survival compared with pN1 disease, the overall survival and disease-free survival rates among those who had one positive node were similar to the rates among pN0 patients. These patients also had significantly better prognosis than patients who had more than two involved nodes.[35] Our data also enhance the importance of nodal status.

Table 7. Multivariate analysis on factors predictive of survival for patients who underwent neoadjuvant chemoradiation and surgical resection

CRT + Surgery group*	HR	95% CI	*P* value
pN stage			
pN0 (reference†)	1	—	
pN1	2.257	1.341–3.800	<0.01
Gender			
Male (reference)	1	—	
Female	0.252	0.078–0.809	0.02
R category			
R0 (reference)	1	—	
R1/2	8.984	3.780–1.357	<0.01

HR = hazard ratio; CI = confidence interval.
*Neoadjuvant chemoradiation and surgery.
†Reference against which hazard ratios are calculated.

The rate of pathological complete response after chemoradiation has been consistently shown to be around 25%–30% in the literature.[12–15] In keeping with other reports, pathological complete response rate was 28% in our patients. These patients also had the best prognosis.[34,36,37] Specifically to address the problem of pT0N1 disease, it has been proposed that these patients should be classified as stage IIA.[32] This seems also consistent with our data with the median and 5-year survival similar to patients with stage II disease (Table 3), although more patients are required to confirm this finding.

The current AJCC pTNM staging classification is one of the best systems for prognostication after surgical resection, but it has its drawbacks,[38] and different classifications have been proposed to refine it.[39,40] With the widespread use of neoadjuvant therapy, clearly it creates an extra demand on its revision to incorporate these new therapies. Certainly our data suggest that modifications are necessary. There are other histological residual tumor grading systems in addition to what is discussed above, such as the Japanese system.[41] There are also other histological factors such as those suggested by the AJCC manual.[27] In the foreseeable future, staging may also incorporate molecular markers or data on micrometastases. Such new systems should be made simple and techniques for assessment readily accessible—before any new classifications can be widely adopted. Before then, studies like the present one will help generate valuable data for future incorporation into new staging systems.

CONCLUSIONS

In summary, we have shown that the current pTNM staging system may be inadequate in the postchemoradiation patient. This is in part related to the altered relationship between pT and pN status after treatment. Nodal status remains one of the most important prognostic factors. Further work should be done to refine staging after chemoradiation and esophagectomy.

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Prospective Trial of Laparoscopic Nissen Fundoplication Versus Proton Pump Inhibitor Therapy for Gastroesophageal Reflux Disease: Seven-Year Follow-up

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Laparoscopic Nissen fundoplication and proton pump inhibitor (PPI) therapy are both established treatments for gastroesophageal reflux disease (GERD). We have performed a prospective randomized study comparing these two treatments and now have long-term follow-up data. Between July 1997 and August 2001, 183 patients in Norwich took part in a randomized controlled trial comparing laparoscopic Nissen fundoplication and PPI therapy for the treatment of GERD. In October 2005, patients were followed up and asked to complete a reflux symptom questionnaire. Ninety-one patients were randomized to have surgery and 92 to have optimized PPI therapy. After 12 months, those who had been randomized to PPI were offered the opportunity to have surgery. Fifty-four patients went on to have antireflux surgery; the remaining 38 did not. In all three groups, there was a significant improvement in symptom score after the initial 12 months ($P < 0.01$; Mann-Whitney U test). However, those who later had surgery despite having had optimal PPI treatment beforehand experienced further symptomatic improvement ($P < 0.01$) at long-term follow-up (median 6.9 years, range, 4.3–8.3). Both optimal PPI therapy and laparoscopic Nissen fundoplication are effective treatments for GERD. However, surgery offers additional benefit for those who have only partial symptomatic relief whilst on PPIs. (J GASTROINTEST SURG 2006;10:1312–1317)
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KEY WORDS: Gastroesophageal reflux, Nissen fundoplication, antiulcer agents

Gastroesophageal reflux disease (GERD) has a prevalence of 10%–20% in the West.[1] Within GERD, there is a spectrum of severity. Lifestyle modifications together with antacids may be suitable for those with mild infrequent symptoms. Patients with severe and frequent GERD symptoms require more effective acid suppression therapy, and proton pump inhibitors (PPIs) are now the mainstay of pharmacological treatment for GERD.

PPIs are effective in the majority of patients,[2,3] but up to 30% of patients may not receive complete symptomatic relief.[4] Furthermore, approximately 80% experience recurrence of symptoms following discontinuation of therapy.[5] Antireflux surgery offers an alternative to these patients, and laparoscopic Nissen fundoplication is now the standard operation for GERD in the West. The procedure is associated with a short hospital stay, moderate postoperative pain, and complete resolution of most reflux symptoms in about 90% of patients.[6,7] Although studies have shown that both PPIs and laparoscopic Nissen fundoplication are effective in the short-term, the two have never been compared in the long-term. Between 1997 and 2001 we performed a randomized controlled trial comparing these two treatments over a period of 12 months,[8,9] and have now followed up these patients in the long-term.

MATERIAL AND METHODS

Between July 1997 and August 2001, 340 patients with symptoms of GERD for at least 6 months were

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considered for participation in a randomized controlled trial at the Norfolk and Norwich University Hospital and at the Queen's Medical Centre in Nottingham. The study was approved by the ethics committees of Norfolk and Norwich University Hospital and the University of Nottingham. All invitees underwent baseline investigations—endoscopy, 24 hour pH monitoring, and esophageal manometry. Two hundred seventeen patients met the inclusion criteria (see Table 1) and participated in the trial.

Patients were randomized to either PPI treatment with daily dose adjusted to abolish all reflux symptoms, or laparoscopic Nissen fundoplication. All patients gave informed consent prior to randomization. Surgery was performed using a five-port technique. Crural repair was performed in all patients, with division of the short gastric vessels performed as necessary. After 3 months, patients were invited to undergo repeat pH and manometry studies.

Symptom Questionnaire

A self-completion DeMeester symptom score questionnaire[10] was used to evaluate GERD symptoms at baseline and after 12 months. Heartburn, regurgitation, and dysphagia are all assessed using this scoring system, the total range varying from 0 (no GERD symptoms) to 9 (maximal symptoms).

After 12 months when the short-term study had completed, patients who had undergone surgery were kept under long-term review. Those in the medical arm were offered the chance to undergo surgery or to remain on optimal PPI medication.

Follow-Up Questionnaire

In December 2005, all patients who participated in the trial in Norwich were contacted by mail. They were asked to complete a further DeMeester symptom score questionnaire and to grade satisfaction with the treatment they had received (either PPI or surgery) from 1 (not at all) to 3 (very much). Patients who had undergone laparoscopic Nissen fundoplication were also asked whether they would have surgery again given the outcome.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Symptoms of GERD for at least 6 mo	Significant esophageal dysmotility
3 mo minimum of PPI maintenance therapy	Morbid obesity (BMI = 35)
Proven reflux (as measured by 24 h pH/manometry)	Refused pH testing and manometry
No preference for either surgical or medical treatment	
Between 16 and 70 yr old	
Fit for surgery	

RESULTS

There were 183 patients based in Norwich out of a total of 217 participating in the trial. Ninety-one patients were randomized to laparoscopic Nissen fundoplication (group 1) and 92 to PPI treatment (group 2). A profile of these patients is shown in Table 2.

Surgery

All surgical procedures were completed laparoscopically. Two patients suffered splenic bleeding and two had inadvertent esophageal injury. These were dealt with laparoscopically and there were no postoperative sequelae. In the early postoperative period, two patients suffered wrap migration requiring laparoscopic correction and four patients had dysphagia within the first 3 months, requiring endoscopic esophageal dilation. Average duration of surgery was 70 minutes (range, 37–180) and median hospital stay was 2 days (range, 1–10).

PPI Treatment

The majority of patients were on regular omeprazole (53%) at a mean dosage of 20 mgs/day. Twenty-seven percent were on lansoprazole at a mean dosage of 28 mgs/day, 10% were on another type of PPI, and the other 10% were on a combination of two or more PPIs. A proportion of patients (10%) suffered side effects of headache, diarrhea, vomiting,

Table 2. Profile of patients in trial

	Randomization	
	Laparoscopic Nissen fundoplication	Proton pump inhibitor treatment
Number of patients	91	92
Sex ratio (F:M)	1:2.0	1:2.5
Age (yr)	47 (26–69)	47 (24–69)
Weight (kg)	80 (51–126)	79 (53–116)
Duration of symptoms (mo)	72 (6–480)	84 (6–516)
Duration of PPI usage (mo)	36 (6–144)	22 (3–123)
Hiatus hernia	94%	93%
Esophagitis (grade <2)	75%	83%
Esophagitis (grade >2)	25%	17%

Figures given as median (range) unless stated otherwise.

or abdominal pain, requiring alteration of the PPI type. A further 18% required dosage escalation during the trial period to relieve GERD symptoms.

Symptom Questionnaire

After 12 months, 92% of the initial cohort completed the symptom questionnaire. The postal questionnaire was sent out at a median of 6.9 years (range, 4.3–8.3), and the response rate was 79%. Of the original 92 patients randomized to PPI, 54 had undergone laparoscopic Nissen fundoplication in the intervening period (group 2a), but 38 remained on PPI treatment alone right up to the postal questionnaire (group 2b; see Fig. 1).

The results of the symptom questionnaires are shown in Table 3. In all three groups, there was a drop in symptom score at 12 months from baseline ($P < 0.01$; Mann-Whitney U test). However, only in group 2a (i.e., those patients randomized to PPI who then chose to have surgery afterwards) was there a further significant drop in symptom score at the time of the postal questionnaire ($P < 0.01$).

Satisfaction Scores

Satisfaction scores are shown in Fig. 2. Patients who had undergone surgery at some point since the trial began had similar scores, with over 80% very satisfied with symptom control. Of those remaining on PPIs, 59% were very satisfied and 41% moderately so. There was a significant association between treatment group and satisfaction scores ($\chi^2 = 15.7$; $P < 0.01$). Finally, 88% who had undergone surgery reported that they would undergo surgery again if they had it to do over again.

DISCUSSION

Both optimal PPI therapy and laparoscopic Nissen fundoplication are effective and durable treatments for GERD. Patients who had laparoscopic Nissen fundoplication at the beginning of the trial (group 1) and those who remained on PPI for the entire course of this study (group 2b) had significantly reduced symptom scores at the time of the postal questionnaire. However, there was a substantial

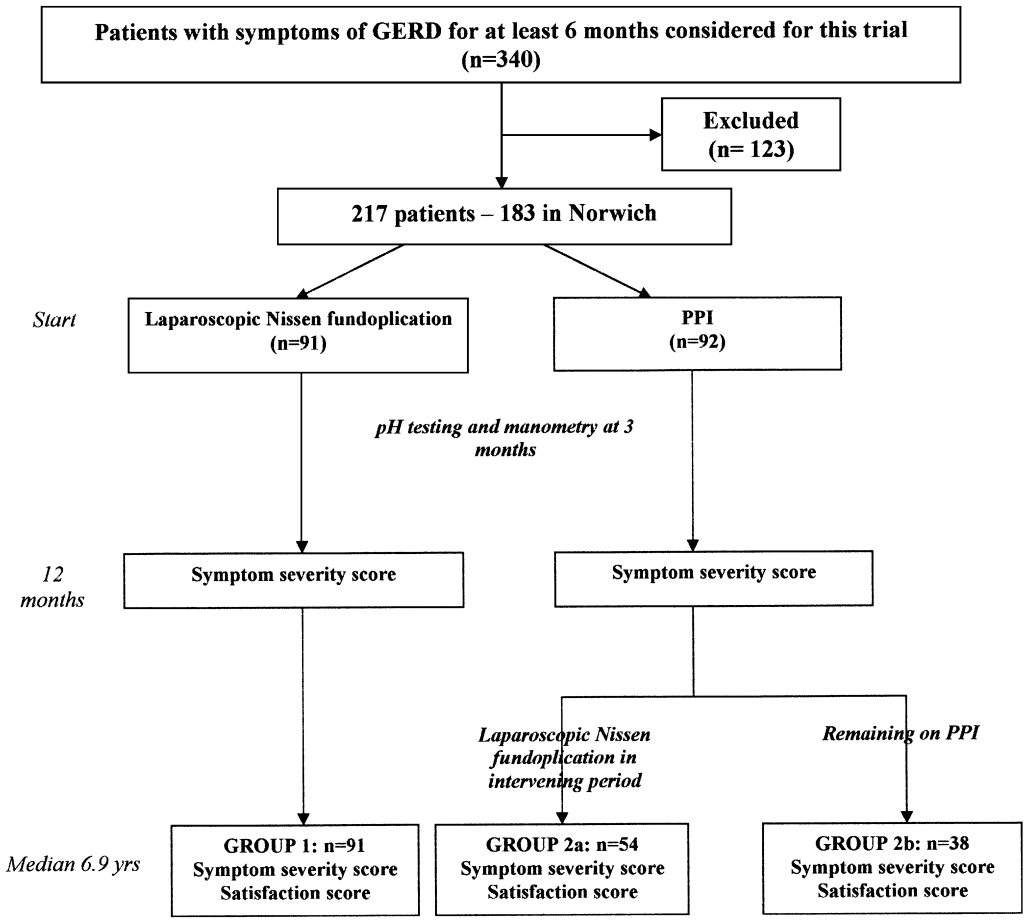

Fig. 1. Trial flow diagram.

Table 3. Mean DeMeester symptom scores at baseline

	Description	n	Baseline	12 mo	Median 6.9 yr
Group 1	Surgery	91	3.5 (1.9)	0.9 (1.4)*	1.1 (1.9)
Group 2a	PPI then surgery	54	3.3 (1.8)	2.3 (2.3)*	0.8 (1.4)†
Group 2b	PPI alone	38	2.4 (1.5)	1.1 (1.5)*	0.9 (1.0)

12 mo and median 6.9 yrs SD in parantheses.
*Significant change in score from baseline (Mann-Whitney test, $P < 0.01$).
†Significant change in score from 12 mo (Mann-Whitney test, $P < 0.01$).

cohort of patients (group 2a) who obtained only moderate benefit from 12 months of PPI treatment. These patients experienced a further significant reduction in symptom score by later undergoing laparoscopic Nissen fundoplication. Furthermore, of those who remained on PPIs for the entire duration of the study 59% were very satisfied with symptom control compared with 80% of those who had undergone laparoscopic Nissen fundoplication.

Our results are consistent with other published reports demonstrating the efficacy of laparoscopic Nissen fundoplication in eradicating GERD symptoms completely. In a study by Peters et al.[11] describing the results of 100 patients undergoing the procedure, 96% were relieved of their symptoms after a mean follow-up of 21 months, with only 5% requiring medical therapy after surgery. Our results for the 91 patients having surgery in the first 12 months of the trial (group 1) were that 93% were either moderately or very satisfied with symptom control after a median of 6.9 years. We also found that only 19% of all the patients having surgery were still on PPIs at the time of the postal questionnaire. Our results contrast with those of Spechler et al.,[12] who followed up 37 patients for a median of 6.3 years after Nissen fundoplication and found that 62% were on antireflux medication at follow-up.

Surgery also appears to be a relatively safe option if performed by an experienced operator. In the initial cohort who had surgery (group 1), there were 4 cases of intraoperative morbidity, and in all of these the problem was dealt with at the time of surgery with no postoperative sequelae. Furthermore, they were discharged after a median of only 2 days. A number of patients developed dysphagia as an early complication, but these were successfully treated with endoscopic dilation.

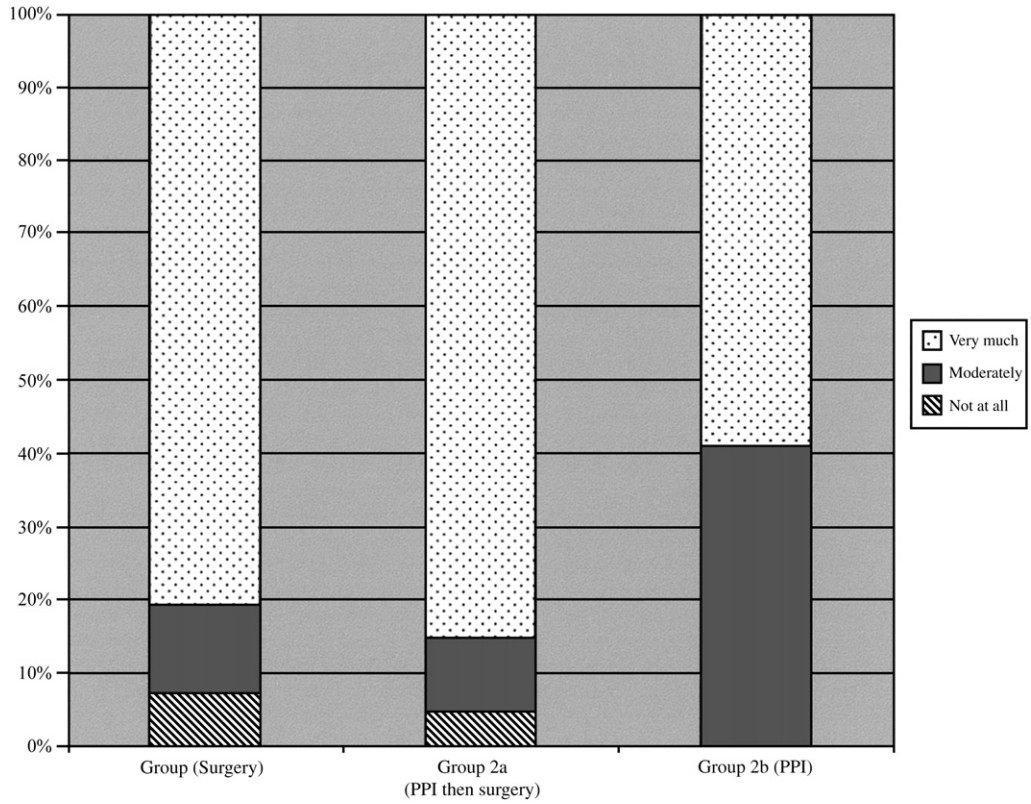

Fig. 2. Satisfaction results for each of the three groups at median 6.9 years.

Many patients with GERD will be treated successfully with lifestyle and pharmacologic management. For those with severe reflux symptoms, PPI therapy remains the first-line treatment. However, those commenced on PPIs should have their symptoms reevaluated soon afterward. Our original trial results[9] showed that randomizing patients to antireflux surgery resulted in significantly better reflux and symptom control after 12 months than if they were on optimal doses of PPI. This long-term study demonstrates that there is an additional symptomatic benefit of surgery in patients who have poor or moderate symptom control on optimized PPI therapy.

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Discussion

Dr. M. Lewis (Norwich, United Kingdom): Thank you, Sam. That was a nice presentation, and thank you for asking me to discuss it. It is important to consider the long-term consequences when considering this operation in patients, and it is good to see the long-term outcomes for your study, at last. There seems to be some variation in published results for long-term outcome of lap Nissen to date, and I know that in your manuscript that you sent me you reported a 20% relapse to PPI therapy in the patients that have had surgery. That seems to concur with a talk from the Emory group given on Monday where they had a 20% to 30% relapse to PPIs, but it differs quite considerably to Spechler's study published in 2001 looking at the long-term consequences of open Nissen fundoplication. I wonder if you have any comment on that?

Although you have shown some equivalence for the two groups, your penultimate slide hinted at a significant difference, and that was the dissatisfaction scores for the patients having surgery. It seemed to be around 5% to 10% in both groups that had had laparoscopic Nissen fundoplication compared to the PPI groups, where there was no dissatisfaction. I presume that these are patients who have severe functional symptoms such as gas bloat in the postoperative long-term. What is your approach to this small cohort of patients, because they can often be very problematic? How are you assessing them and how are you following them up?

Your use of the symptom severity score seems to be a very blunt tool for assessing long-term outcomes, and I wonder if you had considered using anything like quality of life score, SF36, perhaps?

If these two groups are broadly equivalent, both the surgery and the PPI group, we need to look at the economic outcomes for these treatments. I know your group published an economic evaluation last year in the BJS that suggested that a laparoscopic fundoplication has an equivalence to about 7 years of proton pump inhibitors, but does that include the 20% that you have shown relapse to PPIs in the long-term?

Finally, do you intend to show the long-term results to the patients who are considering surgery,

and what impact do you think it will have on whether they agree to go down route or not?

Thank you.

Dr. Mehta: I would like to address your first point. We had approximately 19% go back onto PPIs at long-term follow-up. This is a lower proportion compared to previous studies. I believe there are a number of reasons for this. Firstly, the efficacy of the operation may be significantly different between studies. In our study, we have proven the efficacy of the procedure because we have physiological data postoperatively. Other previous studies have not convincingly demonstrated this. Secondly, I think it is important to counsel these patients correctly. We inform them that they shouldn't expect to continue with PPI treatment after surgery. Finally, prescribing habits are very different in England compared to the United States. In Norwich, we have kept a very careful eye on our patients and have only allowed them to go back onto PPI treatment if there was evidence of proven reflux from physiological data.

With regard to the dissatisfaction after surgery, and you are absolutely right, there is a small cohort of patients who are dissatisfied with the outcome; I think these patients are very difficult to mange. I think they are patients who typically have atypical symptoms, a high BMI, reduced esophageal motility, and were experiencing side effects when they were on PPIs.

With regard to the cost evaluation, we have taken into account relapses in the cost evaluation published in the British Journal of Surgery last year.

My final point is that over the first 12 months, I think that patients who are initially treated on PPIs should be carefully followed up. Patients should be offered surgery if they don't appear to have complete symptom relief.

Dr. M. Patti (San Francisco, CA): Congratulations on your study. I have two questions. You treated patients with PPIs until you obtained symptom control, and you told us that you increased the dose of PPIs during this study in 16% of your patients only.

Dr. Mehta: That's right.

Dr. Patti: So, why did 60% of the patients on PPIs decide to have surgery if they were having complete symptom control?

You excluded patients who had a severe mortility problem from your study. Can you tell us how severe was the motility problem? Thanks.

Dr. Mehta: To answer your second question first, these are patients who had atypical symptoms with abnormal pH/manometry. When we talk about dysmotility, we are talking about patients who have diffuse esophageal spasm, for example, or significantly reduced primary peristalsis.

With regard to your first point, it is true, a small proportion did have to increase their PPI dosage. Many patients in our study did not want to continue on indefinite regular medication and therefore went on to have surgery, despite having good symptom control on the dosage that they were on.

Thank you.