The Bandwagon Effect

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I have been blessed with a thoroughly enjoyable and gratifying academic career in gastrointestinal surgery, but the opportunity to serve as president of The Society for Surgery of the Alimentary Tract (SSAT), the foremost association in the world devoted to surgery of the digestive tract, represents its pinnacle. For bestowing this honor on me, I am grateful to all of you, and I hope that I have served you well during the past 12 months.

Recent presidential addresses have highlighted the modern progress our society has made and its transformation from a relatively small enclave of academicians to an inclusive and proactive organization that welcomes all with an interest in gastrointestinal surgery. I will not repeat the details of this transition today. However, these changes have resulted in a stronger and more vibrant association that has effectively worked on behalf of present and future gastrointestinal surgeons.

The past year has seen two major accomplishments: finalization of the SSAT Foundation and, in partnership with the Society of American Gastrointestinal Endoscopic Surgeons (SAGES) and the Ameri-

can Hepato-Pancreato-Biliary Association (AHPBA), development of guidelines for advanced training in gastrointestinal surgery. The SSAT Foundation, which we now have the challenge of funding, has the expressed purpose of endowing key educational and research initiatives such as the Career Development Awards and the Residents' Research Conference, both of which have been central to our Society's mission. Gastrointestinal surgery fellowships have long been controversial. However, as the knowledge base that undergirds our specialty continues to rapidly expand and more general surgery chief residents seek advanced training in one or more of the interest areas that comprise the broad field of gastrointestinal surgery, their time has come. It is incumbent upon us, along with our sister societies, to ensure that this subspecialization does not cause erosion of general surgery residency training or further fragment the specialty of general surgery.

No one ascends to this podium without the help and support of many others. I am grateful to my colleagues in three separate surgery departments and to the numerous residents in whose training I have had the honor and pleasure of participating. I am particularly indebted to my three mentors, two of them former SSAT presidents, who have played seminal roles in my development as a gastrointestinal surgeon. The late Dame Sheila Sherlock, mother of the specialty of hepatology, stimulated my interest in diseases of the liver. She also taught me that good people with ideas matter more than facilities and material resources in building world-class research and clinical programs. As I will allude to later in this presentation, Dr. W. Dean Warren was the epitome of the clinical scientist. Although I spent only one year with him, it served as the foundation for my academic career that has been mainly based in clinical research.

Dr. Frank Moody was my residency program director, research supervisor, academic surgery role model, chairman, and father confessor all rolled into

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one. I have never known another person with the energy, enthusiasm, and capacity to inspire others that Frank possesses. I would not have pursued a career in academic surgery without his counsel, support, and guidance. I should add that he is also a prophet, being the first to suggest formalized post-residency fellowships in gastrointestinal surgery, which was the subject of his 1982 SSAT Presidential Address.[1]

Finally, I would like to acknowledge the unwavering support and love of my wife, DeeDee, and children, Steven and Kristin, that has allowed me to combine a gratifying and fulfilling family life with a busy and demanding career in academic gastrointestinal surgery.

While searching for a subject for this presidential address, I was advised by many that the only suitable topic is one for which you have great passion. After a great deal of thought, deliberation, and soul searching, I have settled on the "bandwagon" effect.

THE BANDWAGON EFFECT

The most famous game in the history of professional football is the 1967 NFL championship game—what has come to be known as the Ice Bowl. This victory over the Dallas Cowboys represented the third consecutive and eleventh overall National Football League championship for the Green Bay Packers, both unprecedented feats even to the present time. Although at that time the population of Green Bay, Wisconsin, was less than 100,000, this football team and their charismatic coach, Vince Lombardi, attracted millions of devoted followers from throughout the country as many jumped onto this bandwagon of success, establishing the Green Bay Packers as America's team. Unfortunately, victories were rare during the subsequent two decades and many fickle fans deserted the Packers for more popular and more successful bandwagons, leaving only those inhabitants of the frozen tundra and scattered others as faithful Packer backers.

Although it is most frequently alluded to in the world of sports, the bandwagon effect pervades nearly all aspects of our daily lives. *Webster's Third International Unabridged Dictionary*[2] defines bandwagon as: (1) something that attracts adherents by its timeliness, showmanship, vigor, or novelty; (2) a current or fashionable taste or trend; and (3) a movement that amasses power or influence by sheer size, momentum, or internal unity of a group. A bandwagon phenomenon may eventually prove to have validity, but its defining characteristic is that people initially jump on it for superficial reasons rather than for any proof of its worth.

NONMEDICAL BANDWAGONS

Bandwagons can markedly affect consumer behavior and social customs. In fact, they are the essence of advertising and marketing. A bandwagon is created, for example, when the buying public can be convinced that a product or service is better or more fashionable than its competitors without objective evidence of the product's superiority.

The bandwagon effect may also play an important role in determining who is elected to public office. Studies have shown that a significant fraction of the electorate, 6% in one analysis, will change their votes from their preferred candidate to the likely winner based on the results of pre-election polls.[3,4] This substantial desire of some voters to be associated with the predicted winner can be decisive in close elections. Because of the bandwagon effect, some countries have banned polls near election time.

There are a number of explanations for why we humans can get caught up by a trend. First, people like being identified with a winner. Being on the winning team, as in politics or sports, reinforces one's sense of belonging and social safety. Second, jumping on a bandwagon may serve self-interest. For example, backing the winning political party may fulfill a personal agenda or wearing the latest fashions may improve one's social standing.

Finally, there is simple expedience. Going along with a trend may just be easier than bucking it. According to a theory called the "Spiral of Silence," it is a fundamental human trait to want to be part of surrounding society.[5] Without this instinct, civilization as we know it would not be possible. However, this same trait can work to stifle dissent. Simply put, the Spiral of Silence theory predicts that people tend to remain silent if they feel—rightly or otherwise—that they are in the minority. The result is that the most vocalized opinion gains even more ground and alternatives retreat even further.[6]

The social psychology underlying the bandwagon effect is not limited to consumers or voters or public opinion. It is part of who we are as human beings. We physicians—however idealized our professional image—are no more immune than others to the foibles of trends and bandwagons. The difference is that our bandwagons can be far more dangerous.

MEDICAL BANDWAGONS

Medical bandwagons have been defined as "the overwhelming acceptance of unproved but popular ideas" and our history is littered with them.[7] They have led to inappropriate therapies for innumerable

patients, and they have impeded the development of more appropriate treatment.

In their treatise entitled "The Bandwagons of Medicine," Cohen and Rothschild[7] review several of these therapeutic misadventures, some of which persisted for centuries before they were abandoned, substituted by another bandwagon, or replaced by a scientifically valid alternative.

The ancient serpent cult of Aesculapius, in which sacred snakes licked the afflicted as treatment of their diseases, is an example of a bandwagon gathering momentum based on a strong personality, in this case a Roman god. The popularity of this bandwagon did not wane until the Roman gods themselves fell out of favor. We can all identify other examples of medical or surgical bandwagons that were perpetuated, in part, because of the personalities that proposed or supported them. In advertising, this phenomenon is called endorsement.

One of the more lasting and universal bandwagons of medicine that held sway for several centuries is phlebotomy. Based on balancing the four humors of the body, as proposed by Galen, this practice was applied to numerous diseases and almost certainly had a detrimental and sometimes fatal effect in patients who were already in a weakened state. Bloodletting by means of phlebotomy was eventually replaced by the use of leeches. As with many bandwagons that have adverse effects when applied indiscriminately, this one has found its modern niche in effectively relieving venous congestion in tissue flaps.

A more recent example of the inappropriate widespread use of a therapy that can be beneficial when applied more narrowly is tonsillectomy. Although generally recommended until recently as treatment for recurring pharyngitis in children, the scientific basis for such universal use of this operation was never established. Another surgical example was the unconditional acceptance for more than 75 years of the Halsted radical mastectomy before less mutilating options were even considered, and these options now have become the standard for the treatment of breast cancer.

The junk heap of medical and surgical bandwagons is impressive and represents billions of dollars of unnecessary expenditures, ineffective or harmful treatment of innumerable patients, and an impediment to development of more effective alternatives because of the overwhelming and uncritical acceptance of these unproved ideas.

WHY BANDWAGONS IN MEDICINE?

There are many potential contributors to the generation of any given medical or surgical bandwagon.

Creation, development, and testing of new ideas are core missions of academicians and have been responsible for the impressive progress both medicine and surgery have enjoyed during the past century. Without innovation there can be no progress. The innovator is a key stakeholder in the complex continuum that begins with a novel concept and ends with eventual application of the new knowledge to the therapy of a disease. Although we would hope that the research scientist, basic or clinical, would be solely focused on seeking the truth, there are numerous influences that may compromise his or her objectivity. The potential of economic gain, the need to feed the ego, and the desire for academic advancement and prominence in one's field may all cloud the impartial assessment of a new idea by its innovator and provide the framework for an eventual bandwagon. Additionally, there is a strong human tendency to take possession of, support, and promote that which we create—sometimes well past its defensibility. In the worst extreme, there may be such a strong desire to convince a variety of constituencies, including funding agencies, peer reviewers of manuscripts, and even oneself, of the worth of one's idea that research fraud is committed to make the data more compelling.

The responsibility for setting the medical agenda rests not only with the intrinsic scientific merit of a particular grant proposal, but also with those who decide which proposals get funding. Study sections are not immune to the bandwagon effect and may fund or not fund a research project for reasons secondary or peripheral to the medical issue at hand. These include pressure brought to bear on policy makers by highly motivated, single-interest groups; by prevailing political policy; or by the general state of the economy. Even without external pressures, funding agencies may tend to support those fields of research that are presently in vogue rather than potentially productive but less popular areas.

The case of Sudden Infant Death Syndrome (SIDS) is an example of a medical bandwagon prompted by special interest groups. In the early 1970s, a paper in the journal *Pediatrics* concluded that SIDS victims had an abnormality in the respiratory center of the brain, which triggered unpredictable apnea.[8] Until the publication of that article, it was assumed that SIDS victims were basically normal, healthy babies. This article, widely reported in the mass media, gave the syndrome the status of a bona fide disease, prompting intense lobbying of Congress by parents' groups and a consequent large infusion of funds for research.[9] The newly available funds invited a further influx of scientists into SIDS research, reinforcing even more the importance of the issue. This well-funded wave of SIDS research

lasted for about a decade and then petered out for lack of any meaningful results.[9] The bandwagon had run its course.

Financial pressures to order the medical agenda are also brought to bear by for-profit sponsorship of medical research. After the federal government, pharmaceutical and medical device companies are the largest supporters of medical research—research that, of course, involves drugs and devices that stand to turn a profit for the company. Although not intrinsically bad, such vested, well-funded sponsorship can skew the relative importance and value of a therapy—sometimes to the detriment of competing but less well-funded research.

In the past few years, pharmaceutical companies have been expanding their efforts to influence the choice of therapies by going directly to consumers via mass-media advertising. These represent overt efforts at initiating medical bandwagons by influencing consumers themselves. A more traditional approach of industry has been to convince physicians of the merits of a drug or appliance by the overtures of company representatives using everything from free pizzas to extravagant trips.

Of course, patients themselves can be drivers of bandwagons. Armed with the latest information from television ads, daily newspapers, the World Wide Web, or *Time* magazine, they can exert considerable pressure on their physicians to influence the management of their diseases.

Because medical innovation cannot survive without publication, academic journals are one of the primary routes down which bandwagons are driven. As gatekeepers for new information, peer reviewers and journal editors have the ultimate say about what gets published and thereby legitimized. Ideally the peer review process would be purely objective, but in the real world peer reviewers and editors are subject to a number of conscious and subconscious biases. For example, as experts in the field in which they review, peer reviewers may tend to favor manuscripts that confirm their own work and reject those that propose alternative hypotheses. In this manner, new and better ideas may be suppressed while the bandwagons of old are allowed to flourish.

These tendencies do not go unnoticed by young, aspiring academic surgeons who may then submit work that conforms to the standards of the present rather than exploring what they perceive are the unpublishable fringes where the truth may actually lie.

Even if their own favorite concept is not at risk, reviewers and editors may be susceptible to the normative pressures of the academic community. It may require courage for an editor to allow a new and provocative idea, which may lack support from the peer review process, to see the light of day. There is no doubt that scientific journals have not only provided the pathway for the impressive progress we have experienced but also at times the barriers which, in supporting the status quo, have delayed the future. In fact, there are several examples of Nobel prize–winning ideas that were initially rejected by one or more journals.[10]

However much we may want to place the blame on external agencies not under our control, most of our bandwagons are created and sustained by ourselves, academic and practicing physicians and surgeons. As a professional community, medicine in general—and each specialty group in its own right—is characterized by group processes. We surgeons, like every other group, are subject to our own group's norms. We recruit new members, our residents, for example, based on our own criteria of professional, intellectual, and social acceptability. We promote group cohesiveness with professional societies and meetings such as the one we are now attending.

These are all helpful and constructive characteristics, but they also have a downside. It has been shown that the more esprit de corps among members of a group, the greater the danger that independent, critical thinking can be replaced by "group-think."[11] Such conditions may promote group cohesiveness, but as the Spiral of Silence theory predicts, they can also discourage dissent and change.[5] In other words, the very qualities that benefit us as a group also create the environment in which bandwagons can flourish.

Laparoscopic cholecystectomy is a recent surgical example of the multiple interacting influences that lead to creation of a bandwagon—in this case one that has subsequently proved its worth. Soon after its introduction and before most surgeons were adequately trained to perform the procedure, laparoscopic cholecystectomy became the preferred method of gallbladder removal. Patients demanded this new high-tech "laser" surgery that caused minimal discomfort, industry saw a whole new market from which to profit, and general surgeons were forced to jump on the laparoscopic bandwagon or lose their patients to those who did. However, the premature application of this operation by many surgeons resulted in steep learning curves and numerous unnecessary bile duct injuries.

AVOIDING BANDWAGONS

What can be done to avoid future bandwagons and derail existing ones? In other words, what can we as individuals and as a community of surgeons do to

ensure an objective, unbiased analysis of information so that only reasonably proven therapies are applied to our patients' diseases? Considering the complex individual and social psychology that underlies the bandwagon effect, these are not easy questions to answer. However, two key components that may help us reach toward this goal are (1) basing clinical decisions on the best available evidence and (2) developing and encouraging independent, critical thinking in ourselves and in those who we educate.

Evidence-Based Surgery

Evidence-based surgery refers to a process that includes a systematic search for the best available evidence regarding a specific disorder and then integrating that knowledge with one's clinical acumen and an individual patient's preferences to optimally manage it.[12] In other words, clinical decision-making is based on an objective assessment of the medical and surgical literature rather than on anecdotal experience or opinion.

What then is the best available evidence? The quality of literature-based evidence used in solving clinical problems varies widely. When placed in a hierarchy of ascending value, the case report is generally considered the weakest evidence, whereas a meta-analysis of high-quality randomized controlled trials usually represents the most reliable evidence (Table 1). All of the clinical research designs listed beneath "randomized controlled trial" in Table 1 are considered observational studies because the patients included receive their treatment based on their own or the clinician's preferences. This leaves those analyses susceptible to bias that may artificially inflate or reduce the observed treatment effect. Nonetheless, all of these investigational methods can be helpful to the clinician in certain circumstances. For example, a case report or case series may be the predecessor to a later more rigorous study or it may present valuable information regarding an unusual disorder that, because of its rarity, may never be analyzed in a ran-

domized format. As another example, because of patients' unwillingness to be included in a controlled trial for whatever reason, the best available evidence may by necessity come from a case-control or cohort study. Finally, none of these trial designs are totally immune from bias nor do any represent the exclusive pathway to the truth.

Much of the progress achieved by surgical sciences over the past 150 years has been dependent on observational studies. This method of clinical investigation is sufficient when differences in outcome are great in magnitude. Examples include appendectomy for acute appendicitis compared to historical control subjects who were treated nonoperatively and, at the dawn of antibiotic therapy, the impact that these new drugs had on a number of diseases. Such large differences in outcome are infrequent in modern clinical research, usually necessitating a more rigorous study design. In fact, whereas in the past the main outcome measure was often survival, at present, investigations are often designed to detect subtle differences in quality of life or cost of treatment.

The randomized controlled trial in the medical sciences is a phenomenon of the latter part of the twentieth century. Although this study design was first used in horticultural research in 1926, allocating patients to experimental and control groups randomly was first accomplished in a multicenter trial of streptomycin for tuberculosis in 1948.[13,14] To my knowledge, Goligher et al.[15] were the first to compare different operative procedures by random assignment of patients in their seminal investigations into the surgical management of peptic ulcer disease in the early 1960s.

In his Presidential Address presented at the 1973 meeting of the SSAT, which coincidentally was the first Digestive Disease Week I attended, my mentor, Dr. W. Dean Warren, exhorted gastrointestinal surgeons to make greater use of the randomized controlled trial.[16] He, in part, based his argument on a cogent example of failure to do so. For nearly 25 years, the portacaval shunt had been the standard of care for variceal bleeding, but it had not been tested in a controlled trial. The prophylactic shunt (used in patients with varices that had not bled) was the first to be tested because it was considered unethical to submit patients who had previously bled to randomization. Because no other therapy appeared to be effective, it was assumed that the only way to salvage such patients was to give them shunts. After the prophylactic shunt trials showed no benefit to the surgical arm, the therapeutic shunt (used in patients who had bled) was then investigated with similar results, but not until thousands of these procedures had been done with marginal or no advantage to the patients.

Table 1. Hierarchy of clinical trial designs (in order of decreasing value)

Meta-analysis of controlled trials
Randomized controlled trial
Cohort study
Case-control study
Case series
Case report

These important but belated studies opened the way for development of better means of managing this complex clinical problem. How have we responded to Dr. Warren's challenge that "surgeons must become increasingly aware of the need to document scientifically [by means of randomized controlled trials] the value of our surgical endeavors"?[16]

A number of reviews have shown that controlled trials comprise only 3% to 7% of articles in surgical journals.[17,18] In a search of the entire 1990 medical literature, only 202 general surgical randomized controlled trials could be retrieved.[19] It was discouraging to find that a minority of these studies were published in surgical journals and that a surgeon was the principal author in only one third of them. Additionally, less than 25% of the trials compared two operative procedures, and studies published in surgical journals were generally of lesser quality than those published elsewhere. I reviewed the contents of the *Annals of Surgery* for the years 1973, when Dr. Warren delivered his address, and the most recent complete year, 2001. In 1973 less than 2% of all manuscripts represented randomized controlled trials, compared to 12% in 2001. Thus if the *Annals of Surgery* is reflective of the rest of the general surgical literature, we have improved but we still lag behind some of the other clinical sciences with respect to the rigor of our investigation. Why should this be so?

Foremost, randomized controlled trials, in general, are time intensive and expensive. By the time one is completed, advancements in technology may make its findings obsolete or a bandwagon may have gained such momentum that new evidence, even that gained from a controlled study, cannot slow it down.

There are a number of additional issues that make surgical trials particularly difficult to carry out. Whereas a comparison of two drugs is relatively straightforward, standardization of a surgical procedure is considerably more complicated. The surgeons involved may have varying technical abilities, perioperative management may differ, and the technical aspects of the operation may evolve during the investigation, all of which can compromise the final results. With complex operations, there is the question of widespread applicability to less expert surgeons. The issue of timing is also important. Initiation of a trial while the surgeons involved are still in the process of learning a new operation may produce biased results. On the other hand, undue delay may pave the way for a new bandwagon.

Possibly the most challenging aspect of surgical randomized controlled trials, and one that I have personally encountered on a number of occasions, is convincing patients that they should be allocated between operative and nonoperative therapies or between different operations based on the simple flip of a coin. Although being randomized to one drug or another may appear quite innocuous to most patients, the same individuals may have strong preferences when the choice is between medical management and surgery or between two very different operative approaches. Not only is an operation usually more painful and inconvenient, it is generally irreversible whether or not the trial eventually demonstrates its superiority.

Finally, in surgery, randomized controlled trials are not applicable in a number of situations. Solomon and McLeod[20] analyzed the 1990 gastrointestinal surgical literature to determine what proportion of therapeutic questions could be answered by a controlled trial if the clinical research setting were ideal. They judged that less than half of the treatment questions identified could have been reasonably answered by this study design. The most common problems that precluded such an approach were strong patient preferences for one treatment over another; infrequency of the condition under consideration; and a preponderance of expert opinion already favoring one of the alternatives. Although the analysis has not been done, one would expect that a greater proportion of medical than surgical questions could be resolved by randomized controlled trials.

Considering that observational studies have a number of advantages over randomized controlled trials, including lower cost, greater timeliness, and relative ease of patient enrollment, but a greater propensity for bias, do they still have a role in modern clinical research? In other words, when randomized controlled trials are not applicable, can well-designed observational studies provide reasonable guidance in treatment decisions? Although observational studies have generally been considered to exaggerate treatment effects because of the potential for bias, two recent investigations found that well-designed studies with nonrandomized comparison or control groups provided similar results as randomized controlled trials addressing the same questions.[21,22] Thus, in situations where a randomized trial is difficult or impossible to carry out, because of inadequate patient accrual or other reasons, an observational study can provide meaningful results. Ideally such an investigation should be prospective, rigorously designed, and of the cohort type, with an evenly matched, albeit not randomized, control group.

Because of the heterogeneity of the diseases and populations with which surgeons generally deal, basing one's clinical practice on the results of a single investigation, randomized or not, is unwise. The medical and surgical literature has become "balkanized" among so many subspecialties that it may be difficult

to find the prime evidence among the almost infinite number of publications regarding any given subject. When available, a meta-analysis of high-quality randomized trials usually provides the best guidance. When none exist, as is often the case in the surgical sciences, individual controlled trials or well-designed prospective studies should be sought and evaluated as to their quality. The Cochrane collaboration,[23] which is organized by special areas of interest including topics pertinent to surgeons, maintains a database of systematic reviews and is an excellent place to start in one's search for the best evidence.

An interesting conundrum that I have observed during my years as a clinical surgeon is that even when good evidence exists, we do not always incorporate it into decision-making. There may be good reasons for this, such as when a well-done trial demonstrates a statistically significant outcome that is not clinically meaningful. More often, however, the comfort we enjoy with our bandwagons of old is preferred to the cognitive dissonance that occurs when we consider a change. Cognitive dissonance refers to the stress and discomfort that arises when one of our sacred and long-standing beliefs is challenged.[24] All too often, the discomfort is reduced by maintaining our beliefs and by rejecting the new information no matter how valid it may be. An example of this is the continuing use of prophylactic nasogastric tubes after major abdominal surgery by many surgeons despite numerous randomized and observational studies that have consistently demonstrated their lack of efficacy in the majority of patients.[25]

Critical Thinking

Clearly, simply having access to the best evidence is not enough. This brings me to my final point—the need for critical, independent thinking in the practice of surgery. "Facts" in medicine or science should never be regarded as absolutes, but rather as tentatively held beliefs. The strength of any given belief is conditioned by the evidence for or against it. The critical thinker constantly seeks to update his or her fund of knowledge based on the evidence available. Because medical evidence usually arrives in numerical form as a statistical summary, one of the building blocks for practicing evidence-based surgery is the ability to properly interpret such information.

Although our surgical education system in the United States is second to none, it has not always encouraged independent and critical thinking. In fact, a common caricature of surgical training is that of the professor walking down the hall with his trainees, anxious to replicate his every move, following him like a row of imprinting ducklings would follow their

mother. Woe to the occasional duckling that gets out of line. Even though this caricature is a bit extreme, such an educational approach is a prominent part of our tradition and, if allowed to persist, almost guarantees that the bandwagons of today will become the bandwagons of tomorrow.

We are in an era of rapid expansion of medical knowledge, making much of what is learned during medical school and residency obsolescent within a few years. Therefore the greatest gift we can give our students is not merely the knowledge and experience that we possess, but rather the ability to educate themselves throughout their professional careers, and to critically and objectively assess new information as they incorporate it into their practices. The old paradigm of basing most decisions throughout one's clinical career on the knowledge gained during medical school and residency will no longer suffice in the surgery of the twenty-first century. As uncomfortable as it may be at times, this will require tolerance—and yes, even encouragement—by us as educators when our ducklings boldly propose new ways of doing things. If our egos and sense of tradition can accommodate such a shift, future surgeons should be less likely than we are to march in lock step behind prevailing bandwagons.

CONCLUSION

Although surgery has a glorious past that includes numerous seminal contributions to the medical sciences, at times progress has been and continues to be impeded by the bandwagon effect. Although many factors contribute to this phenomenon, the major obstacle that is sometimes in the pathway to truth is ourselves. The rigor of our clinical investigation has been less than it should be and our educational templates have overemphasized memorization of facts and creating clones of ourselves rather than fostering independent critical thinking by our students.

Just as good basic science research is preceded by years of training in the laboratory, good clinical research will depend on well-prepared, educated, and meticulous clinical scientists. Recent initiatives, such as the "Clinical Trials" course sponsored by the American College of Surgeons and the Clinical Investigators Preparatory Program (CIPP) funded by the National Institutes of Health, are promising aids for the development of effective clinical scientists.

Finally, we need to continually remind ourselves of the observation that "knowledge is always tentative and can only be made incrementally more certain with increasing data or evidence."[26] We must resist the easy comforts of riding bandwagons and

direct our energies toward forming the basis of a more complete knowledge. It is our challenge to make certain that the evidence generated in our clinical laboratories and published in our journals is reliable and that we and surgeons of the future can temper it with critical judgment as it is applied in the optimal care of our patients.

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The Bio-Intelligence Age: Surgery After the Information Age

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The history of surgery has many notable landmarks that have dramatically influenced the course of the practice of surgery, always in the direction of more scientific accomplishments. The Industrial Age produced the rise of the "Golden Age of Surgery" through the technologies of anesthesia, antisepsis, x-ray imaging, dedicated surgical operating rooms, formalized training programs, and countless others. The Information Age has contributed digital imaging, invasive monitoring, electronic medical records, and other procedures, with laparoscopic surgery as the latest of these examples. However, as significant as the minimally invasive surgery revolution is, laparoscopic surgery is a transition—that is, a footnote in the history of surgery. It is a halfway technology, half in the Industrial Age, in which hand-held mechanical instruments are used, and half in the Information Age, in which digital imaging (video monitoring) is used to allow surgeons to view the inner body cavities and thereby perform surgery through tiny incisions. The end of the transition is to an entirely Information Age surgical system—that is, robotics or computer-aided surgery, where both the image of the patient's organs and the manipulations of the surgeon's hands are conducted through electronic information interfaces and not directly on the patient.

The essence of understanding the Information Age is characterized by the quote from *Being Digital* by Nicholas Negroponte[1] "... it's bits instead of atoms..." The Information Age is about representing objects in the real world (atoms) by a computer representation in the information world (bits). The example is the facsimile machine. For thousands of years, information was sent by atoms—that is, clay tablets, papyrus, paper letters, and so forth. Now a fax machine scans the information on the paper (atoms) and electronically sends the signal (bits) to be reconstructed in the real world at the other end of the electronic connection; this is more efficient and less expensive. In surgery, we are approaching information science as well. Rather than viewing the patient's organs (atoms) directly, laparoscopic surgery uses a video monitor (bits). In the recovery room the surgeon views the blood pressure and pulse on the monitor—the information equivalent of the sense of touch. The medical record is going from paper to electronic, and imaging studies are now digital (bits) instead of film (atoms). Surgery is no longer blood and guts; it is bits and bytes.

To interact with information that cannot be directly touched or manipulated, there is the digital interface—where humans, machines, and information interact. For viewing information, there are displays such as a computer monitor. However, there are many other innovative display techniques that allow interacting with information (as if it were real), such as head-mounted displays, three-dimensional images in a room called a cave automatic virtual environment (CAVE), and a true three-dimensional, suspended-image hologram. Clinical examples of interacting with information include preoperative planning of patient-specific images, image-guided surgery, and virtual endoscopy. Two significant projects are currently under investigation in an attempt to realize a total information environment for surgery: (1) the holographic medical electronic representation (holomer) of any individual person and (2) the operating room of the future.

Total body scanning is making it possible to represent any patient (anatomically) as an information equivalent in a computer. The holomer permits the integration of surgery on a comprehensive scale, including not only the anatomy but also the physiol-

ogy, biochemistry, genetics, and all components of medical information into the image, which can be directly queried to provide the information to the surgeon. A scenario, entitled "Doorway to the Future," provides a framework as to how such a holomer would integrate surgery. The patient comes to the physician's office after undergoing total body scanning by means of computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography, and so forth. Just as with a pulse oximeter, the patient places a hand on a noninvasive scanner and acquires biochemical and physiologic data, for example. All data are stored in a computer, and when the patient sits at the desk next to the physician, the holomer appears between them—this image is the medical record. If the patient complains of abdominal pain, the surgeon rotates the image, uses a transparency, and "touches" the liver to get the serum glutamic-oxalate transferase (SGOT), lactate dehydrogenase (LDH), bilirubin values, or the pancreas to get the amylase or insulin levels. The data are contained within the image. If a disease is suspected, the surgeon can "fly through" the holomer, as is done in virtual colonoscopy. If something is found, the holomer becomes a patient education tool, explaining precisely and visually, exactly what is wrong with the patient. If complicated surgery is needed, the image can be used for preoperative planning or surgical rehearsal, as Jacques Marescaux of Strasbourg, France, is doing for complicated liver resections. Challenging cases can be archived and used for surgical training with virtual reality surgical simulators. The image can be used for intraoperative navigation in image-guided surgery. When the surgery is complete and the patient returns a week later for a postoperative visit, a total scan is done again and the preoperative and postoperative images are combined using data fusion and digital subtraction, and the difference is automatic outcomes analysis and quality assurance. The holomer can be stored on a personal health card (similar to a credit card) or on a secure website for telemedicine consultation. Thus the entire spectrum of surgical patient care is integrated.

The operating room of the future has had a number of descriptions; however, none have considered the operating room of the future as an information system. If the new-generation surgical robots are considered "information systems with arms" (just as CT or MRI scanners are information systems with eyes—seeing where even our own eyes cannot see), then surgical procedures can be totally integrated. Observing robots in automobile assembly lines or chip-manufacturing facilities demonstrates that there are no workers handing the robot a new tool; there are automatic tool changers. For assembling parts, there are no workers handing the robot the parts; there are automatic inventory dispensers. However, in the operating room of today, tools are passed by a scrub nurse and supplies are passed by a circulating nurse. In the operating room of the future, scrub and circulating nurses will be replaced by similar devices, and because these devices are also information systems with arms, they will communicate directly with the surgical robot (being controlled by the surgeon). Every time a tool is used or a new supply (suture, gauze, etc.) is used, the integrated system will instantly bill for the equipment, place an order to restock the operating room, and requisition the supply room to order a new supply for inventory. In addition, today's operating room requires two nurses (scrub and circulating) and one nurse for coffee breaks. A pair of operating rooms requires six nurses and a supervisor for the "cluster." Using a computer-integrated system with robots, automatic billing support, and inventory systems, the number of nurses can be reduced to a single nurse (robots do not take coffee breaks), who would supervise the two rooms—a reduction in personnel of approximately 85%. Because the cost of the personnel in staffing the operating theaters represents approximately 60% of the total cost, substantial cost savings can be realized.

The preceding two scenarios will not occur instantly; nor will they necessarily happen in the way described. The purpose of the scenarios is to describe a framework upon which the future efficiencies can be improved and costs reduced through integration with Information Age technologies. No new science is required to realize the above-mentioned potentials.

More important, however, is the fact that the Information Age is not the future—the Information Age is the present. If the Information Age is not the future, then what is? Current observations are empirically pointing to multidisciplinary technology as the future, a concept that is temporarily being called the Bio-Intelligence Age. The past few decades have shown unprecedented technologic growth based upon the revolution in Information Technology. New devices such as hand-held computers and mobile communications, as well as the explosion of the World Wide Web, have totally changed the environment and the pace of everyday life, business, and research. However, this paradigm shift is more than a century old and rightfully traces it roots back to Marconi and the first radio transmission in 1857, or Babbage's "difference engine" in 1853—the first "computer." But the real power of the Information Age began in 1955 with the introduction of the first mainframe computer, surged forward in 1976 with the first personal computer, and then reached a pinnacle in 1995 with the emergence of the software program Mosaic
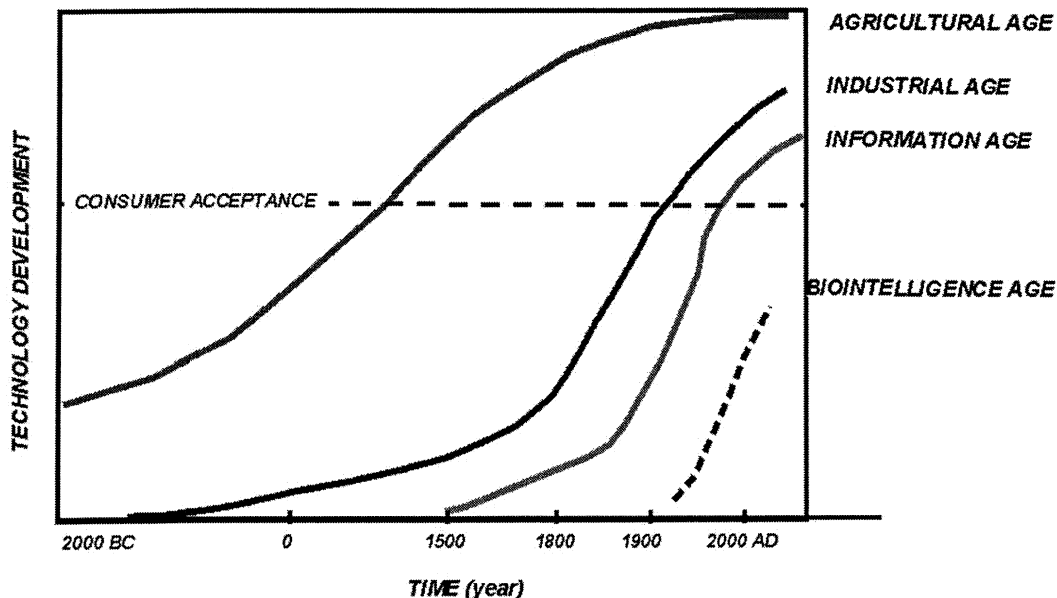
Fig. 1. A new representation of the Ages of Man.

(later to become Netscape) to popularize the World Wide Web. However, since that time there has been precious little to consider revolutionary; all the new ideas and products are simply evolutionary—that is, small incremental changes. The Information Age is moving from highly innovative to a conservative pillar of society, accepted by all areas of endeavor. If that is indeed the case, where must we turn to find the disruptive technologies that will create the next revolution?

The diagram in Fig. 1 is a conceptualization of the ages initially described by Alvin Toffler[2] in his book *The Third Wave*—including the Agricultural Age, the Industrial Age, and the Information Age. Two critical parts of the analysis of this graph are the horizontal line, which is labeled "consumer acceptance" and indicates when the revolutionary changes have been incorporated into all of society. This is when the revolution (disruptive technologies) turns into evolution (iterative technologies). With the integration of ubiquitous computing, mobile communications, and the Internet, the Information Age has reached that inflection point and has become evolutionary. That is not to say that remarkable new discoveries will not continue with Information Age technologies; what it implies is that the changes will build on established methodologies and simply add to the current direction rather than disrupt the progress in one direction and create a whole new direction. This method of change, "disruptive technologies," is best described in Clayton Christensen's[3] book *The Innovator's Dilemma*. The second critical point, according to the

diagram, is there must be another "age" that is developing; when one age turns to evolutionary change, a new age is being developed in the research laboratories. This new age, the Bio-Intelligence Age, has been developing in advanced research laboratories around the world over the last half of the twentieth century. The Venn diagram in Fig. 2 attempts to illustrate the basis for the new research direction in science and technology.

Until recently, most research has been performed within a single discipline—the biological sciences, the physical (and engineering) sciences, or the information sciences. Each "world" has unique properties. The biological world is dynamic, ever changing, measured in probabilities, flexible, adaptable, and continuously evolving. In the physical world, things are static, reliable, constant once a precise measurement is taken, rigid and nonadapting, and robust once created. In the information world, bits replace atoms, and intelligence is added to an otherwise "dumb" biological and physical world. In the past, new technologies have arisen from one of the three disciplines. The revolution in science and technology is interdisciplinary research. Throughout academia, government, and industry, there are new departments and divisions that are interdisciplinary. Hence between biological and information sciences, we see intelligent drug design, molecular engineering, the Human Genome, biocomputation, and bioinformatics departments. Between biological and physical sciences there are deoxyribonucleic acid (DNA) on a chip, biosensors, biomaterials, and biomimetic systems. And between physical and
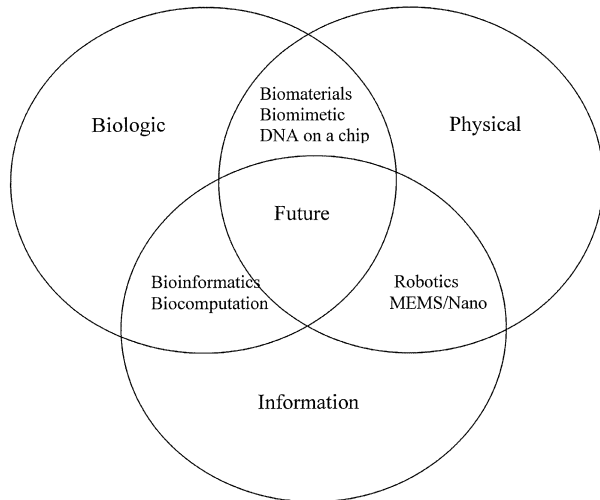
Fig. 2. Venn diagram of the Bio-Intelligence Age.

information sciences there are intelligent and cooperative robotics, next-generation Internet, microelectromechanical systems (MEMS), and nanotechnology. The real power of interdisciplinary research will be when all three disciplines are combined. One example is the bioagent surveillance bumble bee from the Defense Advanced Research Projects Agency (DARPA) program of Alan Rudolph. To a bumble bee, an MEMS biosensor that is sensitive to anthrax is attached to the back. This is integrated into a microchip with artificial intelligence to interpret the signals and then coupled to a microtransmitter. When the bumble bee flies into an area where there is anthrax, the sensor relays the signal to the transmitter, which then sends the data to the commanders and soldiers in the rear echelon. Thus it will be possible to avoid sending soldiers into environments that are contaminated with biologic agents. Another example is tissue engineering. Professor J Vacanti leads a team at Massachusetts General Hospital and Massachusetts Institute of Technology (MGH-MIT) that is developing artificial organs. There are computational mathematicians to virtually prototype the intricate, n-dimensional fractal branching pattern of the vascular system. This computer-aided design/computer-aided manufacturing (CAD/CAM) design is exported to the engineers' stereolithography machine. Using bioresorbable polymers from the biochemists, a scaffolding of this vascular tree is "printed" in full three dimensions. Molecular biologists and immunologists add to this scaffold various growth promoters, such as vascular endothelial growth factor and angiogenesis factor. Cell biologists provide vascular endothelial stem cells, which are seeded on the scaffold to create a living vascular sys-

tem. Currently such living scaffolds are being evaluated and optimized for the perfusion of the microcirculation with blood. The next step will be to test this microvascular system to determine if it truly is able to provide the nutrients to the individual cells. Then the scaffold will be seeded with organ-specific stem cells such as hepatocytes, nephrocytes, and so forth to finally create an artificial organ. Although there is still research to be completed, this example is used to illustrate how interdisciplinary research is the new foundation for change.

The implications for artificial organs is elemental, not only within medicine and science but also in society as a whole. For example, today when a person takes an automobile to be repaired, a broken part is removed and a new one is used to replace it; however in medicine, a diseased organ is usually repaired. If a person has a "stomach problem," the surgeon may perform any number of surgical procedures, depending on the disease, and may replace part of the stomach, remove most of it, or just place sutures to stop the bleeding. In the future, with artificial organs the surgeon will need only one gastric operation—that is, remove the stomach and replace it with a new one. Because the organs are engineered with the patient's own stem cells, this approach will also address the rejection problem in transplantation.

The technologies mentioned earlier raise profound moral and ethical questions. We are in the midst of the crisis over human cloning. There are numerous nontechnical issues, such as does a clone have rights, who decides who will be cloned, and what is the role of government? These and other issues have yet to be properly addressed, and yet a woman is pregnant with the first human clone. Many other similar issues must now be addressed, in as much as the current generation of surgical residents will be faced with these conundrums during their surgical careers of 20 or 30 years because the technology will not wait. The following are just a few of the more complex dilemmas for the immediate future. Computer power will exceed the computation of the human brain in 20 to 30 years. Will the machines be intelligent, will they have "rights," and can we unplug something that is intelligent? With genetic engineering, do we have the moral right to "design" our children, especially with enhanced physical or intellectual capabilities, and who decides? Will we develop a new "class" of humans? As tissue engineering and intelligent prostheses replace more and more of our bodies, what will it mean to be "human" if we no longer inhabit the body we were born with? With neural prostheses, will we connect directly with machines, another person, or the Internet; if so, what happens to our own identity?

In the context of speculation, consider the following rhetorical statement compared to the hypothetical implication: "The human species may be on the brink of a revolution that is even greater than fire or the wheel: Humans are the only species with the capacity to direct their own evolution (genetic engineering, cloning, etc.) at human's own accelerated pace—no longer by the will of Nature nor the excruciating slow pace of evolution." This may be the ultimate challenge for the next generation of surgeons (and humans). It is also critical to realize that technology has no moral value; it is neither good nor evil. Rather it is the application of the technologies that raises the moral and ethical issues. These issues must begin to be addressed at annual scientific conferences, and a sense of vital principles must be integrated into our residency training programs. Surgeons and physicians, as pillars of moral conscience, have recently been forgetting the Hippocratic oath and their stewardship to society, and have been languishing in the short-term gain of financial security and self-centered comfort and leisure. As scientists and humanists, surgeons must be aware of their responsibility in this awesome challenge and accept a leadership role throughout the coming decades of nearly impossible decisions.

In the past, these questions were merely pure speculation because the technology to make these things possible did not exist. However, the acceleration in science and technology discoveries is forcing us to look at these issues from a pragmatic standpoint, for technology waits for no one. We must address the social, political, regulatory, ethical, and moral issues now, at a time when we can slowly deliberate the consequences in the cool light of day, before the technology is upon us, and hasty decisions are made simply to accommodate a solution. The sooner we address these issues, the higher the quality of health care with which we will be able to provide our patients.

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Reasons for Conversion From Laparoscopic to Open Cholecystectomy: A 10-Year Review

Juliane Bingener-Casey, M.D., Melanie L. Richards, M.D., William E. Strodel, M.D., Wayne H. Schwesinger, Kenneth R. Sirinek, M.D., Ph.D.

Laparoscopic cholecystectomy is now considered the "gold standard" operation for patients with gall-stone disease. A number of patients require conversion to an open cholecystectomy for the safe completion of the procedure. This study investigates how the etiology and incidence of conversion from laparoscopic to open cholecystectomy has changed over time. All 5884 patients undergoing laparoscopic cholecystectomy between March 1991 and June 2001 were prospectively collected in a database. A total of 310 patients (5.2%) had had their cholecystectomies converted to an open procedure. The mortality rate for these patients was 0.7%. Causes for conversion were inability to correctly identify anatomy (50%), "other" indications (16%), bleeding (14%), suspected choledocholithiasis (11%), and suspected bile duct injury (8%). After an initial learning curve in thin patients with symptomatic cholelithiasis, inclusion of patients with acute cholecystitis, morbid obesity, or a prior celiotomy resulted in a peak conversion rate of 11% by 1994. From 1994 to the first half of 2001, the conversion rate has declined significantly for all patients (10% to 1%), as well as for patients with acute cholecystitis (26% to 1%). Although unclear anatomy secondary to inflammation remains the most common reason for conversion, the impact of acute cholecystitis on the operative outcome has decreased with time. (J GASTROINTEST SURG 2002;6:800–805.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Laparoscopic cholecystectomy, conversion, acute cholecystitis

Laparoscopic cholecystectomy has developed from an elective surgical procedure for selected patients to the "gold standard" operation for all patients with gall-stone disease. Increased surgical experience and technical innovations have extended the indications for the laparoscopic approach to patients with complicated disease processes. Still, there are a number of patients who will require conversion to an open cholecystectomy for the safe completion of the surgical procedure. To assess the factors that contribute to the need for conversion from a laparoscopic to an open approach, we investigated how the etiology and incidence of conversion from laparoscopic to open cholecystectomy has changed over time in a university teaching program.

PATIENTS AND METHODS

All patients undergoing a cholecystectomy between March 19, 1991 and June 30, 2001, at the University Hospital of the Bexar County Hospital District and the Audie L. Murphy Veterans Administration Hospital, were prospectively collected in a database. Routine intraoperative cholangiography or preoperative endoscopic retrograde cholangio-pancreatography (ERCP) was used by some but not all surgeons. The reasons for conversion, as stated in the operative report and database entry, were compiled. In four patients the reason for conversion could not be discerned because of an incomplete database entry and unavailable medical records. Causes for conversion were stratified into the following categories: inability to correctly identify anatomy, intraperitoneal bleeding, suspected choledocholithiasis, suspected common bile duct injury, and "other" indications. "Other" indications included unsuspected intraoperative findings, misdiagnosis, equipment failure, inability to establish a pneumoperitoneum, and injury to other organs.
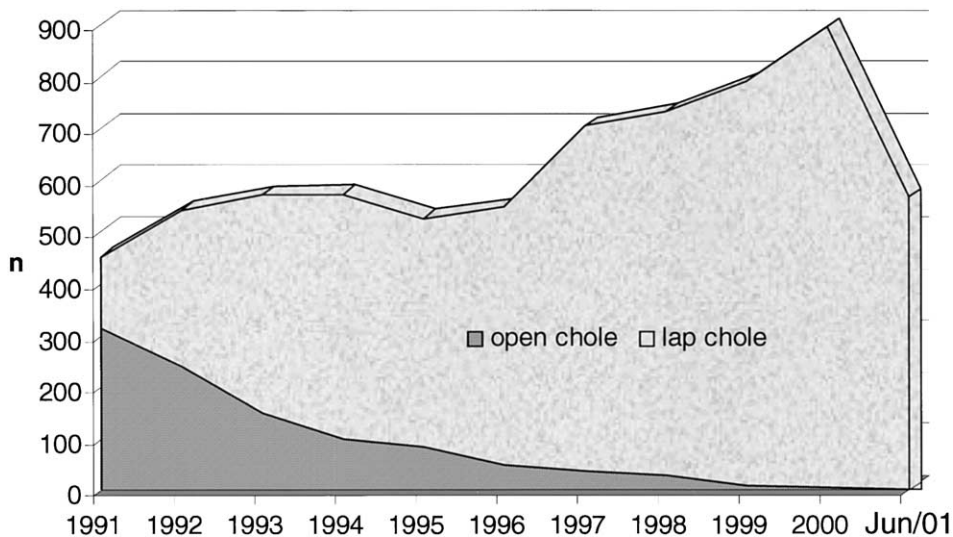
Fig. 1. Performance of primary open and laparoscopic cholecystectomy at a teaching institution from March 1991 until June 2001.

RESULTS

During a 10-year period, 6896 patients whose mean age was 40 years underwent cholecystectomy at our two teaching hospitals by either laparotomy or laparoscopy. Over the study period, the total annual number of patients undergoing cholecystectomy has steadily increased from 450 to 875 patients per year (Fig. 1). The number of patients undergoing an initial open cholecystectomy has decreased from 312 patients (69%) in 1991 to seven patients (0.78%) for the year 2000 and to two patients (0.35%) for the first half of 2001. During the same time interval, the number of patients undergoing an attempted laparoscopic cholecystectomy has increased from 140 pa-

tients (31%) in 1991 to 889 patients (99.2%) in 2000 and to 564 patients (99.6%) for the first half of 2001.

Among the 5884 patients who had undergone an attempted laparoscopic cholecystectomy, 4525 (76%) were women and 1359 (24%) were men. The indications for cholecystectomy included biliary colic (60%), acute cholecystitis (29%), and biliary pancreatitis (11%). Of the 1681 patients with a postoperative diagnosis of acute cholecystitis, 1316 (78%) were women and 365 (22%) were men. The incidence of acute cholecystitis as an indication for operation has increased from 13% in 1991 to 34% for the first half of 2001. Eighty-eight (5%) of the 1681 patients with cholecystitis had gangrenous cholecystitis at the time of operation.
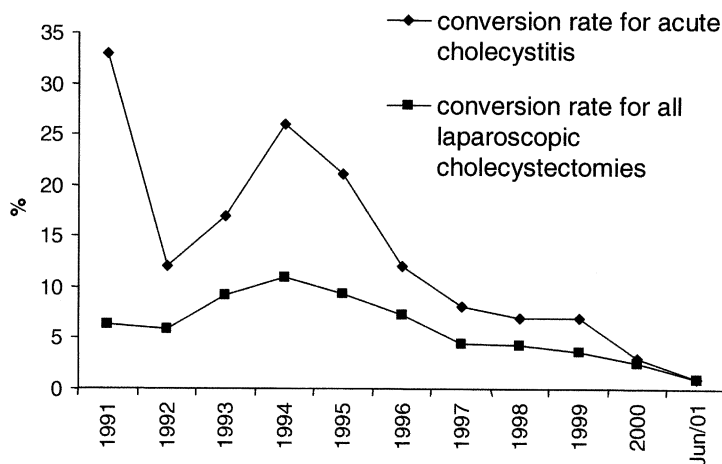

Fig. 2. Conversion rates for laparoscopic cholecystectomy for all patients with gallstone disease and for patients with acute cholecystitis from March 1991 until June 2001 at a teaching institution.

**Table 1.** Number of patients with conversion from laparoscopic to open cholecystectomy by reason and year

Year	Bleeding	Unclear anatomy	Suspected CBD injury	CBDE	"Other"	Unknown	Total
1991	1	6			1	1	9
1992	5	9			3	1	18
1993	6	16	3	5	8	1	39
1994	5	27	5	10	4	1	52
1995	8	21	2	5	6		42
1996	6	14	3	6	7		36
1997	4	15	4	1	6		30
1998	4	16	2	3	5		30
1999	1	18	3	2	5		29
2000	2	10	2	1	5		20
2001	2	2			1		5
Total	44	154	24	33	51	4	310

Of 5884 patients who had undergone an attempted laparoscopic cholecystectomy, 310 patients (5.2%) whose mean age was 42 years had their cholecystectomies converted to an open procedure. Two of the patients who underwent conversion died for a mortality rate of 0.7%. Of the 310 patients converted to an open procedure, 180 (58%) were women and 130 (42%) were men. The gradual inclusion of patients with acute or gangrenous cholecystitis, morbid obesity, or prior abdominal operations during the first 3 years of the study resulted in a peak conversion rate of 11% by 1994 (Fig. 2). From 1994 to the first half of 2001, the conversion rate to open cholecystectomy has declined for all patients (10% to 1%) and for patients with acute cholecystitis (26% to 1%). The overall conversion rate for patients with acute chole-cystitis was 8%, 20% for the first 5 years, and 4% for the most recent 5 years.

The number of patients undergoing conversion to an open cholecystectomy by year and by indication is presented in Table 1. Ranking by incidence, the reasons of conversion to the open procedure were unclear anatomy (50%), "other" indications (16%), intraperitoneal bleeding (14%), common bile duct exploration (11%), suspected bile duct injury (8%), and unknown (1%). The peak conversion rate of 11% occurred in 1994 with 52 (17%) of the 310 conversions to an open procedure occurring that year. The peak conversion rate for each of the main five categories, excluding the unknown category, also occurred between 1993 and 1995 (Fig. 3).

Inability to correctly identify the anatomy at the
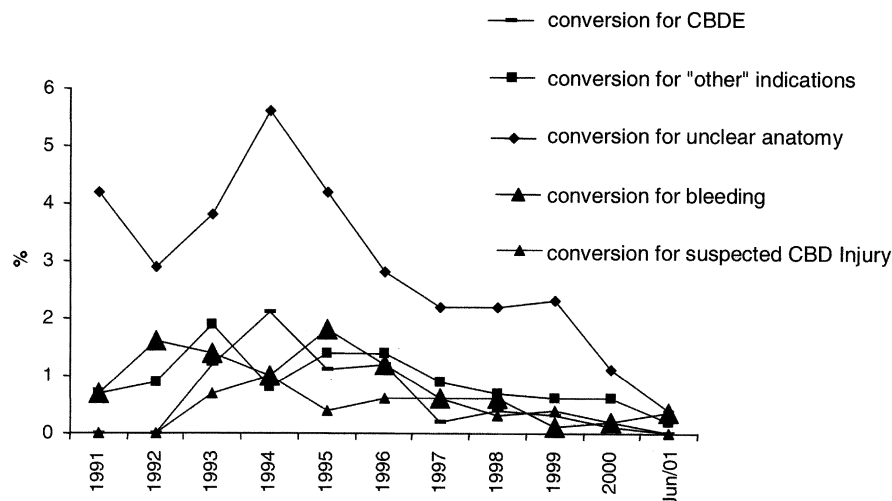
Fig. 3. Risk of conversion from laparoscopic to open cholecystectomy for unclear anatomy, bleeding, suspected common bile duct (*CBD*) injury, common bile duct exploration (*CBDE*), and "other" indications for all laparoscopic attempted cholecystectomies from March 1991 until June 2001.

porta hepatis accounted for one half of the 310 patients undergoing conversion to the open procedure (see Table 1). Individual anatomy was obscured primarily by acute inflammation (82%), but dense adhesions from prior abdominal procedures or chronic cholecystitis (12%) and aberrant anatomy (6%), including two ectopic gallbladders, were also noted. Forty-four patients underwent urgent laparotomy for bleeding. There were five major and six minor vascular injuries. Major vascular injuries occurred at the root of the mesentery with insertion of the Veress needle (n = 1) and at the right hepatic artery with dissection at the porta hepatis (n = 4). Four of the five major vascular injuries were noted between 1991 and 1993. Minor vascular injuries at the porta hepatis were cystic artery bleeding that could not be controlled laparoscopically. Other bleeding sites included the gallbladder fossa (n = 28), the liver (n = 1), the greater omentum, (n = 1) and trocar sites (n = 3). In 33 patients (11%) the attending surgeon converted to the open procedure to perform a common bile duct exploration for suspected choledocholithiasis based on intraoperative cholangiography (n = 17), failed attempts at preoperative ERCP for known choledocholithiasis (n = 4), or failed laparoscopic common bile duct exploration (12; transcystic and primary choledochotomy).

The "other" category, which included 51 patients, was the second most common reason (16%) for conversion to an open cholecystectomy; in these patients conversion was primarily for equipment failure and inability to establish a sufficient pneumoperitoneum during the early phase of laparoscopy at our institution. Additional intraperitoneal pathology and misdiagnosis are listed in Table 2. Morbid obesity was a factor in four patients. Intrahepatic position of the gallbladder prevented adequate exposure to complete the cholecystectomy laparoscopically in seven patients. The "other" category also included those patients with an inadvertent enterotomy (n = 6) at the abdominal wall trocar site or in the abdomen during lysis of adhesions from a prior celiotomy or from acute or chronic inflammation of the gallbladder itself. One patient required conversion because of concern about his cardiac status due to changes in his ECG tracing.

Suspected common bile duct injury was the reason for conversion from laparoscopic to open cholecystectomy in 24 patients. Seven patients had confirmed major bile duct injuries that required bilioenteric anastomosis (0.1%). Minor bile duct injuries were confirmed in eight patients (0.13%) and repaired with T-tube placement. Incomplete entries into the database do not allow for accurate determination of postoperative morbidity and mortality with the exception of the deaths of the patients who underwent conversion of their laparoscopic cholecystectomies.

Table 2. Additional intraperitoneal pathology and misdiagnosis

Cholecystoenteric fistula	2
Liver laceration from previous trauma	1
Suspected gallbladder cancer	1
Liver mass	3
Diaphragmatic mass	1
Ovarian cancer	1
Choledochocele	2
Perforated duodenal ulcer	1
Bowel ischemia	1

DISCUSSION

Conversion from laparoscopic to open cholecystectomy results in a significant change in outcome for the patient, who usually requires hospital admission and a longer recovery period. The condition of the patient, the level of experience of the surgeon, and technical factors all can play a role in the decision for conversion. Previous studies have examined the reasons for conversion at a point in time when experience with acute cholecystitis was still growing.[1,2] Difficult dissection and the inability to define the anatomy were the leading reasons for conversion followed by common bile duct exploration, unsuspected findings, and bleeding.

We present our findings in a large prospective study, which took place over a significant period of time, with inclusion of all initial cases of laparoscopic cholecystectomy. Similar to previous studies in the southwestern United States,[3,1] our study population was younger (mean age 40 years) than in studies in other regions where the mean age frequently ranged from 51 to 60 years.[4–8] The relatively high percentage of patients with acute cholecystitis (29%) in comparison to other studies[4,8,9] may also be based on regional differences with a large number of patients seeking late access to care because of restrictions in health care funding.

Increasing experience with the laparoscopic environment, as well as technical advances, have decreased the overall conversion rate. The overall conversion rate in our study (5.2%) compares favorably with other reports.[1,2,3,7,10,11] Increasing experience with the laparoscopic environment, as well as technical advances, have decreased the overall conversion rate. The morbidity and mortality rates for patients in our study who did not undergo conversion to the open procedure, including postoperative bile leaks and trocar site hernias, have been stable at 1.6% and 0.2%, respectively, for 7 of the past 10 years. The data are unfortunately incomplete for 3 years. The more recent years have seen a conversion rate closer to 1%, which has been

noted only in one large prospective study with a much lower percentage of patients with acute cholecystitis.[8]

Although it has been proposed that conversion rates for acute cholecystitis in a teaching institution would not improve over time because of the inexperience of the trainees,[1,12] our data suggest otherwise. Active participation of faculty members in the operating theater may have enhanced the learning experience. The contribution of two senior attending surgeons accounted for 60% of all cases since 1991. Prospective and retrospective studies involving more than 100 patients, the results of which have been published since 1994, show conversion rates for acute cholecystitis ranging from 12% to 30% (Table 3). With increasing size of the patient groups, an inverse trend in conversion rates is seen. Our results support this as 8% of the patients with acute cholecystitis underwent conversion in this large group of study patients.

A decreased conversion rate for intraoperative complications such as bleeding and suspected injury to the biliary tree reflects an overall learning curve. The relative risk for all patients who had their gallbladders operated on laparoscopically to undergo conversion for bleeding has decreased by 80% since the peak in 1995. Careful lateral retraction, dissection limited to the gallbladder–cystic duct junction, and the use of the Hasson technique for abdominal access all contribute to the reduction in vascular injuries.

The same trend is present in our data for suspected injury to the biliary tree. In a large meta-analysis published in 1996, the rate for common bile duct injury in open cholecystectomy was 0.29%. The rate for common bile duct injury in laparoscopic cholecystectomy was reported to be 0.47%.[11] A population-based study from Australia[12] estimated the increase from 0.15% in the prelaparoscopic cholecystectomy era to 0.29% in 1994 and did not yet observe a decrease in the injury frequency, as had been reported from a statewide study in Connecticut published in 1996.[17] The rate of major common bile duct injury over the 10-year period discovered immediately in our patients is 0.1%. Database entries for postoperatively discovered injuries are unfortunately incomplete starting in 1999. The change in the conversion rate for common bile duct exploration likely reflects the increased use of preoperative ERCP, as most of the recently surveyed surgeons in our group prefer to use selective preoperative ERCP to manage suspected choledocholithiasis.

"Other" indications for conversion from laparoscopic to open cholecystectomy have declined to 0.2%. Only rarely will equipment failure or the inability to establish a pneumoperitoneum now be noted as a reason for conversion to open cholecystectomy.

In accordance with other data,[2,8] the most frequent reason for conversion in our patient population was the inability to identify the anatomy correctly. The risk to undergo conversion for that reason, however, decreased by 90% from 5.5% in 1994 to 0.5% in 2001, despite an increase in the patient subgroup with acute cholecystitis. As has been indicated in previous studies, the timing of the operation likely has an impact on the outcome.[1,4,6] In recent years a clinical pathway for patients seen in the emergency department had been established in our institution, leading to operation within 24 hours of admission. This contributed to a reduction in the preoperative hospital stay for patients with acute cholecystitis and easier dissection secondary to edematous surgical planes. It coincided with a further decrease in the conversion rate and postoperative hospital stay.

CONCLUSION

Although unclear anatomy and inflammation remain the most common reasons for conversion, the impact of acute cholecystitis on the operative outcome

Table 3. Conversion rates for acute cholecystitis

Reference	Year published	Location	Study design	No. of patients	Conversion rate for acute cholecystitis (%)
Navez et al.[6]	2001	Belgium/France	retrospective	609	19
Brodsky et al.[13]	2000	Israel	prospective	215	20.5
Teixeira et al.[14]	2000	Portugal	ns	100	24
Rutledge et al.[3]	2000	United States	retrospective	251	26
Chahin et al.[15]	1999	United States	retrospective	469	22
Koperna et al.[16]	1999	Austria	retrospective	295	29
Bittner et al.[8]	1997	Germany	prospective	3,010	12
Fried et al.[2]	1994	Canada	prospective	1,676	16.9
Total		8 countries	prospective	6,708	16

in a teaching institution seems to decrease with experience, earlier operation, and improved technology.

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Gallbladder Function Before and After Fundoplication

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No study has reported an association between gastroesophageal reflux disease (GERD) or its therapies and gallbladder function. We compared pre- and postoperative gallbladder function in patients undergoing fundoplication to determine the following: (1) whether patients with chronic GERD have preexisting gallbladder motor dysfunction; (2) whether medical or surgical therapy alters gallbladder function; and (3) whether division of the hepatic branch of the anterior vagus nerve is detrimental to gallbladder motility. Nineteen patients with documented GERD consented to a preoperative cholecystokinin-stimulated technetium hepatobiliary (CCK-HIDA) scan to quantify the gallbladder ejection fraction (GBEF). All patients underwent laparoscopic Nissen fundoplication. One month after fundoplication, 12 patients completed a repeat CCK-HIDA scan for determination of GBEF, with comparison to the preoperative GBEF. Among patients with preoperative GERD, 11 (58%) of 19 met the scintigraphic criteria for gallbladder dysfunction (GBEF <35%), which is a ratio comparable to that in patients undergoing a CCK-HIDA scan for presumed biliary dyskinesia during the same time period (31 [60%] of 53; P = NS, chi-square test) and exceeds the rate of abnormal GBEF reported in healthy volunteers (3%). Six of seven patients with a low preoperative GBEF who underwent repeat evaluation postoperatively had normalization of the GBEF ($P < 0.05$, paired t-test). In the 12 patients who underwent postoperative CCK-HIDA scanning, there was no association between preservation or division of the hepatic branch of the anterior vagus nerve and postoperative gallbladder dysfunction (P = NS, chi-square test). Unexpectedly, 58% of patients with GERD demonstrated gallbladder motor dysfunction prior to fundoplication, with improvement to normal occurring in most of those studied postoperatively. These data support controlled trials to determine the effect of chronic GERD and antisecretory therapy on gallbladder and global gastrointestinal smooth muscle function. Preservation of the hepatic branch of the anterior vagus nerve during fundoplication offered no clear benefit with regard to early postoperative gallbladder function. (J GASTROINTEST SURG 2002;6:806–811.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Gallbladder, reflux, GERD, proton pump inhibitor (PPI), fundoplication

The clinical presentations of gastroesophageal reflux disease (GERD) and biliary dyskinesia are similar. In uncertain clinical situations, some physicians initiate empiric medical therapy for GERD and reserve diagnostic studies for those patients whose disease is refractory or incompletely controlled. Others may initiate biliary evaluation by ultrasound imaging, with cholecystectomy typically recommended if gallstones are identified. In both cases, the possibility that these diseases can be present concurrently is neglected, and the potential for iatrogenic morbidity from unnecessary therapy is increased. Surgeons often encounter patients who have undergone cholecystectomy prior to antireflux surgery, or who have been treated medically for presumed GERD before they present with biliary colic. No study has reported the rate of concurrence between GERD and gallbladder motor dysfunction.

In addition, despite the relatively common occurrence of both GERD and biliary disease in individual patients, no study has examined the possibility that treatment of GERD by means of antisecretory medi-

cation or antireflux surgery may result in gallbladder motor dysfunction. Despite the chemical similarity of the biologically active C-terminal regions of gastrin and cholecystokinin (CCK),[1] and the overlap in functionality of these peptides in experimental models,[2–5] medical practice patterns recognize no association between chronic proton pump inhibitor–induced hypergastrinemia and biliary dysfunction. Alternatively, even though vagal innervation of the gallbladder is thought to be important in its tone and motor function,[6,7] many surgeons routinely divide the hepatic branch of the anterior vagus nerve during antireflux surgery with no concern about inducing biliary dyskinesia.

We compared pre- and postoperative gallbladder function in patients undergoing fundoplication to determine the following: (1) whether patients with chronic GERD have preexisting gallbladder motor dysfunction; (2) whether gallbladder function changes after fundoplication and cessation of medical therapy for GERD; and (3) whether division of the hepatic branch of the anterior vagus nerve alters gallbladder motility.

METHODS
Patient Selection

All patients were evaluated in the Center for Esophageal Diseases and Swallowing at the University of North Carolina at Chapel Hill, or the Department of Surgery at Emory University School of Medicine, by one of four surgeons, between March 2000 and December 2001. GERD was diagnosed on the basis of endoscopic and/or pH-metric evidence of pathologic esophageal acid exposure, as previously described.[8] All patients underwent esophageal manometry prior to surgery. Patients who were scheduled for laparoscopic Nissen fundoplication were offered the opportunity to participate in the present study.

Patients with a history of biliary symptoms, or prior cholecystectomy, fundoplication, or vagotomy, were excluded from the study. No effort was made to determine whether asymptomatic gallstones were present.

Assessment of Preoperative Gallbladder Function

Nineteen patients (mean age 49.5 years, 62% female) consented to preoperative and postoperative cholecystokinin-stimulated technetium hepatobiliary (CCK-HIDA) scanning to quantify the gallbladder ejection fraction (GBEF) before and after fundoplication. This test involved intravenous injection of technetium (5 mCi) and scintigraphic measurement of its biliary excretion over a period of 1 to 2 hours. The change in gallbladder activity was measured af-

ter an intravenous dose of CCK (0.04 µg/kg administered over 19 minutes). The likelihood of preoperative gallbladder motor dysfunction, defined as a GBEF of less than 35%, was compared to a control group of 53 patients who underwent CCK-HIDA scanning for evaluation of presumed biliary dyskinesia, using the chi-square test.

Comparison of Preoperative and Postoperative Gallbladder Function

All patients underwent laparoscopic Nissen fundoplication, which was performed by one of four surgeons. All surgeons perform a "short, floppy" Nissen fundoplication, with complete crural dissection, mediastinal esophageal lengthening, wide fundic mobilization by division of the short gastric vessels and posterior attachments, closure of the esophageal hiatus with sutures, and creation of a 360-degree fundoplication of less than 2 cm over a 56 to 60 F bougie. At the University of North Carolina, where typical treatment of vagal fibers varies among surgeons, intraoperative decision-making dictated the treatment of the hepatic branch of the anterior vagus nerve. At Emory University, where surgeons routinely make efforts to preserve the vagal fibers, patients were randomized to either preservation or division of the hepatic branch. Patients were categorized on the basis of the final status of the hepatic branch of the anterior vagus nerve, not the intention to preserve or divide.

One month after fundoplication, patients were asked to undergo repeat CCK-HIDA scans for determination of GBEF. All patients had been off proton pump inhibitor therapy since undergoing fundoplication. Postoperative gallbladder function was compared to preoperative gallbladder function by means of a paired *t* test.

Impact of Vagal Denervation on Gallbladder Function

The association between intraoperative treatment of the hepatic branch of the anterior vagus and postoperative gallbladder dysfunction (GBEF <35%) was compared by chi-square test. The preoperative GBEF was not considered for this analysis.

RESULTS
Patient Accrual

The ability to accrue patients during the course of this study was affected by a national shortage of CCK,

Table 1. Gallbladder function in patients with GERD before and after fundoplication

Patient	Preop GBEF (%)	Institution	Nerve status	Postop GBEF (%)
1	92.4	UNC	Preserved	N/A
2	90.3	UNC	Divided	N/A
3	22.0	UNC	Preserved	63.5
4	63.3	UNC	Divided	91.5
5	61.0	UNC	Preserved	N/A
6	96.0	UNC	Divided	59
7	9.2	UNC	Divided	37.8
8	3.1	UNC	Divided	10.1
9	50.0	Emory	Preserved	19.6
10	13.0	Emory	Divided	N/A
11	33.0	Emory	Divided	41
12	69.0	Emory	Preserved	87.8
13	34.0	Emory	Divided	40
14	0.0	Emory	Divided	88
15	64.0	Emory	Preserved	86
16	33.4	Emory	Divided	N/A
17	18.8	Emory	Divided	55
18	10.0	Emory	N/A	N/A
19	21.0	Emory	Divided	N/A

and therefore, in some cases, qualified candidates were not entered or postoperative studies were not completed. Also, several patients who consented to the study refused to participate in the postoperative assessment. Overall, 19 patients underwent preoperative determination of GBEF, and 12 of them completed postoperative GBEF assessment (Table 1).

Assessment of Preoperative Gallbladder Function

Among preoperative patients with GERD, 11 (58%) of 19 had a GBEF of less than 35% (Table 2). This rate is similar to that in the control group undergoing evaluation for biliary dyskinesia (31 [60%], of

Table 2. Preoperative gallbladder function in patients with GERD vs. control subjects

Group	% With GBEF <35%	P value*
GERD-PPI patients (N = 19)	58	
Non-GERD patients (N = 53) (with suspected biliary dyskinesia)	60	NS

PPI = proton pump inhibitor.
*χ^2- test.

53; P = NS, Fisher's exact test) and exceeds the rate of abnormal GBEF reported in healthy volunteers (3%).

### Comparison of Preoperative and Postoperative Gallbladder Function

Of 19 patients who underwent preoperative CCK-HIDA scanning, 11 (57.9%) had a GBEF of less than 35%. Of the 12 patients who completed postoperative CCK-HIDA evaluation, two (16.7%) had a GBEF of less than 35%. Among all patients completing both CCK-HIDA tests (N = 12), mean GBEF improved from 38.5 ± 30.1% to 56.6 ± 27.8% after fundoplication and cessation of medical therapy (Table 3 and Fig. 1); however, this correction failed to achieve statistical significance (P = 0.082, paired t test).

Of the seven patients with an abnormally low preoperative GBEF who completed postoperative assessment, six demonstrated a normal GBEF postoperatively (P = 0.032, paired t test) (Table 4).

Table 3. Change in GBEF in all patients completing study (N = 12)

	GBEF (mean ± SD)	P value*
Preoperative	38.5 ± 30.1	0.082
Postoperative	56.6 ± 27.8	

*Paired t test.
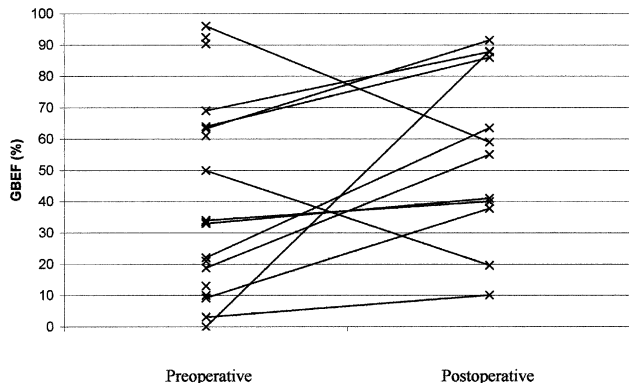
Fig. 1. Gallbladder ejection fraction for individual patients before and after fundoplication.

Impact of Vagal Denervation on Gallbladder Function

In the 12 patients who underwent postoperative CCK-HIDA scanning, there was no association between preservation or division of the hepatic branch of the anterior vagus nerve and the postoperative GBEF (P = NS; chi-square test) (Table 5 and Fig. 2).

DISCUSSION

Unexpectedly, 58% of patients studied demonstrated scintigraphic evidence of gallbladder motor dysfunction prior to fundoplication, with improvement to normal occurring in most of those studied postoperatively. Preservation or sacrifice of the hepatic branch of the anterior vagus nerve did not have an obvious impact on postoperative gallbladder function.

Regarding Improvement in Gallbladder Motor Function After Fundoplication

Iatrogenic hypergastrinemia from chronic antisecretory therapy may contribute to gallbladder motor dysfunction in patients with GERD. In the mid-1970s, Chowdhury et al.[2] presented evidence for competition between gastrin and CCK for a common receptor on the cat gallbladder, and Ryan and

Table 4. Change in GBEF in patients with preoperative GBEF <35% (N = 7)

	GBEF (mean $\pm$ SD)	P value*
Preoperative	17.2 $\pm$ 13.6	0.032
Postoperative	47.9 $\pm$ 24.3	

*Paired t test.

Table 5. Impact of vagal denervation on postfundoplication GBEF

Group	% With GBEF <35%	P value*
Nerve preserved (N = 4)	25.0	NS
Nerve divided (N = 8)	12.5	

*χ^2 - test.

Cohen[3] found that gastrin reduced the ability of CCK to increase intraluminal pressure in the opossum gallbladder. Unsulfated synthetic human gastrin was shown to have an independent stimulatory effect on gallbladder function in humans,[4] raising the potential for receptor downregulation in the setting of hypergastrinemia. In 1989, Cattey and Wilson[9] reported that 71% of patients with normal gallbladders who underwent gastrectomy for Zollinger-Ellison syndrome developed gallstones (mean 6.3 years). In
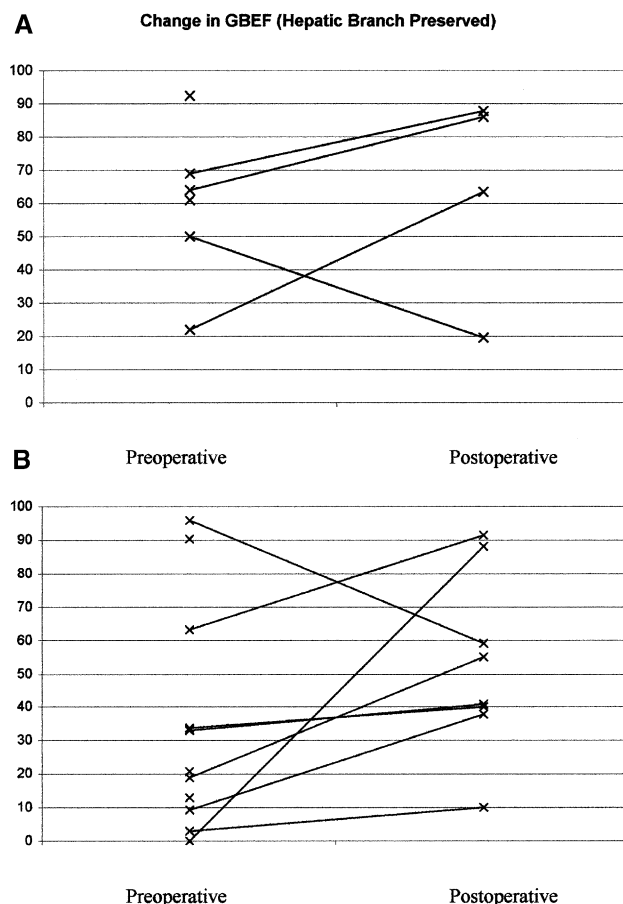
Fig. 2. Gallbladder ejection fraction before and after fundoplication for patients in whom hepatic branch vagal fibers were preserved (**A**) or sacrificed (**B**).

1997, Rasmussen[10] found that omeprazole decreased CCK secretion and postulated a possible clinical effect through the impact on biliary motility and cholesterol solubilization. These data support controlled trials to determine the effect of chronic GERD and antisecretory therapy on gallbladder motor function.

Regarding Other Smooth Muscle Function

Fundoplication has been reported to improve gastric emptying.[11–13] However, the impact of iatrogenic hypergastrinemia in preoperative patients receiving antisecretory medications, and the resultant cessation of such medications after fundoplication, has not been considered in these reports. There are conflicting data regarding the impact of chronic acid suppression on gastric emptying.[10,14] The effect of hypergastrinemia on other gastrointestinal smooth muscle function also remains to be defined.

Regarding the Importance of Preserving Vagal Gallbladder Innervation

In 1978, Csendes et al.[7] reported that 41% of patients who underwent selective hepatic vagotomy during antireflux surgery developed gallstones compared to 7.7% of control subjects (3- to 5-year follow-up).[7] In 1981, Ihasz and Griffith[6] reported that 46 of 91 patients undergoing truncal vagotomy developed distended gallbladders and 30 of these were noncontractile by cholecystography. Ten (11%) of 91 patients developed gallstones after truncal vagotomy compared to 2 (3.8%) of 53 patients undergoing selective gastric vagotomy with preservation of the hepatic vagal fibers.

Although these historical data make a compelling case for the importance of the hepatic branch vagal fibers to the gallbladder, the current study failed to identify any benefit for preservation of the nerve during fundoplication. The investigators suggest that the relatively small numbers of patients in each group, and the unexpected improvement in postoperative gallbladder motor function after fundoplication and cessation of proton pump inhibitors, may have obscured a true difference between the groups. It appears that the benefit to gallbladder motor func-

tion afforded by transition from medical to surgical therapy for GERD outweighs the impact of intraoperative treatment of the hepatic branch of the anterior vagus nerve.

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Discussion

Dr. H.A. Pitt (Milwaukee, WI): I have another possible explanation for your findings. About 25 years ago, Dr. Haile Debas described a neural reflex between the an-

trum of the stomach and the gallbladder, and about 20 years ago we described a neural reflex between the antrum of the stomach and the sphincter of Oddi, which also obvi-

ously has neural connections with the gallbladder. Antral distention causes gallbladder contraction and affects sphincter of Oddi function. I think it would be very interesting to study stomach mechanics, emptying to solid if you will, and after fundoplication in the same patients. My guess is that differences will be seen, and that effect of antral distention may be a factor in what you observed in your patients.

Dr. J. Morton: I think there was a group in Sweden who actually asked that same question in the *Scandinavian Journal of Gastroenterology*, and they reached a similar conclusion.

Dr. L.W. Way (San Francisco, CA): I want to ask the same question a different way. We know that the mean value of gastric emptying is slow in patients with reflux esophagitis. We also know that a fundoplication accelerates the rate of gastric emptying in these same patients. We know that the rate of gastric emptying is related to the release of hormones. I am wondering whether you might not have thought to measure gastric emptying in your patients.

Dr. Morton: That is certainly a valid point, and we will look at gastric emptying at some point. However, none of these patients had any symptoms that might have indicated they had problems with gastric transit time. I think the question is, does hypergastrinemia perhaps affect gastric emptying as well? Does it cause some type of global gastrointestinal smooth muscle dysfunction? However, these issues can only be answered by looking at that in particular all of these variables, including PPI use.

Dr. Way: These abnormalities that are measurable are subclinical.

Dr. Nathaniel J. Soper (St. Louis, MO): I have several questions. First, did you look at the presence or absence of gallstones in any of these patients? Second, you did show that twice as many persons, after the hepatic nerve is cut, have an abnormal gallbladder ejection fraction. I suspect that there may be an effect of just too small a number of patients and a type II error. Are you planning to conduct any more research on this with a larger group? Third, what is your recommendation for the many surgeons performing laparoscopic Nissen fundoplications? Does it make any difference whether or not they divide the hepatic branch?

Dr. Morton: I will take that last question first because that was one of the original aims of this study—that is, examining the value of dividing the nerve and its impact on gallbladder motor function. I do not think, in any way, shape, or form, that we can draw any kind of definitive conclusion about the value of preserving the hepatic nerve based on our study. To answer the second question concerning the presence of gallstones, we did not evaluate that by ultrasound. We do plan to look at some of the other questions raised by this study and other venues, in relation to healthy volunteers, to see if there is a dose-dependent correlation between proton pump inhibitors and hypergastrinemia and gallbladder function.

Invited Discussion—Expert Commentator

Dr. David W. Rattner: Dr. Morton and his colleagues present a small but intriguing series of patients in whom they studied gallbladder motility measured by CCK-HIDA scans before and after fundoplication. The concern, based perhaps more on anecdote than science, has been that disruption of the hepatic branches of the vagus during fundoplication leads to diminished gallbladder contractility and ultimately to cholelithiasis. The findings of this study certainly do not support this hypothesis. The hypothesis that hypergastrinemia contributes to gallbladder dysfunction is indeed intriguing if unproven. The number of patients is quite small making it dangerous to draw too many conclusions. The potential for selection bias is a real problem as only 19 patients from a pool of what must have been several hundred patients agreed to participate in the study,

and only 12 of those were studied postoperatively. There is no matched control group, and we are not given information about the presence or absence of gallstones. Perhaps another way of looking at the data presented in this study is to view patients with GERD as manifesting a diffuse gastrointestinal motility disorder, which also involves the biliary system and often the colon. The relationship between GERD and irritable bowel syndrome is well established, and the authors' real contribution today may be to shed light on the association between GERD and biliary dyskinesia. Certainly this work should be an impetus to expand the study to a larger group of patients. Until such time as new data are available, I will have no qualms about sacrificing the hepatic branch of the vagus while performing a laparoscopic Nissen fundoplication.

Long-Term Follow-Up After Laparoscopic Refundoplication for Failed Antireflux Surgery: Quality of Life, Symptomatic Outcome, and Patient Satisfaction

Frank A. Granderath, M.D., Thomas Kamolz, Ph.D., Ursula M. Schweiger, M.D., Rudolph Pointner, M.D.

Quality of life and patient satisfaction have been shown to be important factors in evaluating outcome of laparoscopic antireflux surgery (LARS). The aim of this study was to evaluate data pertaining to quality of life, patient satisfaction, and changes in symptoms in patients who underwent laparoscopic redo surgery after primary failed open or laparoscopic antireflux surgery 3 to 5 years postoperatively. Between March 1995 and June 1998, a total of 27 patients whose mean age was 57 years (range 35 to 78 years) underwent laparoscopic refundoplication for primary failed open or laparoscopic antireflux surgery. Quality of life was evaluated by means of the Gastrointestinal Quality of Life Index (GIQLI). Additionally, patient satisfaction and symptomatic outcome were evelated using a standardized questionnaire. Three to 5 years after laparoscopic refundoplication, patients rated their quality of life (GIQLI) in an overall score of 113.4 points. Twenty-five patients (92.6%) rated their satisfaction with the redo procedure as very good and would undergo surgery again, if necessary. These patients were no longer taking any antireflux medication at follow-up. Two patients (7.4%) reported rare episodes of heartburn, which were managed successfully with proton pump inhibitors on demand, and four patients (14.8%) reported some episodes of regurgitation but with no decrease in quality of life. Seven patients (25.9%) suffer from mild-to-moderate dysphagia 5 years postoperatively, and 12 patients (44.4%) report having occasional chest pain but no other symptoms of gastroesophageal reflux disease. Nine of these patients suffer from concomitant cardiopulmonary disease. Laparoscopic refundoplication after primary failed antireflux surgery results in a high degree of patient satisfaction and significant improvement in quality of life with a good symptomatic outcome for a follow-up period of 3 to 5 years after surgery. (J GASTROINTEST SURG 2002;6:812–818.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Gastroesophageal reflux disease, failed antireflux surgery, laparoscopic redo surgery, quality of life, patient satisfaction

Laparoscopic antireflux surgery (LARS) is an established procedure with proven success in the surgical treatment of gastroesophageal reflux disease (GERD). Long-term follow-up has demonstrated a good to excellent outcome in most patients.[1–3] However, in some patients, fundoplication fails and these patients require revision surgery.[4] Failure rates for both open and laparoscopic fundoplication are reported to range from 3% to 30%.[5] Usually, redo surgery for failed primary LARS is performed by an open technique, but since the advent of minimally invasive access, some studies have shown good functional results for laparoscopic revision surgery after primary failed open or laparoscopic antireflux procedures, at short- or midterm follow-up.[6–8] Until now, there have been few data concerning quality of life and patient satisfaction after laparoscopic revision surgery with long-term follow-up.[9,10] The aim of this study was to evaluate quality of life, symptomatic outcome, and patient satisfaction 3 to 5 years after

laparoscopic refundoplication for prior failed open or laparoscopic fundoplication.

MATERIAL AND METHODS

Between March 1995 and June 1998, a total of 27 consecutive patients (nine women and 18 men; mean age 57 years [range 35 to 78 years]) underwent laparoscopic refundoplication for primary failed open or laparoscopic antireflux surgery in the department of surgery at our institution. Patients had previously had the following antireflux procedures: open Nissen fundoplication (n = 10), open Toupet fundoplication (n = 1), laparoscopic Nissen fundoplication (n = 13), and laparoscopic Toupet fundoplication (n = 3). The corresponding symptoms were as follows: recurrent reflux in 14 patients (previous open surgery in five; previous laparoscopic surgery in nine); a combination of recurrent reflux and dysphagia in 10 patients (previous open surgery in six; previous laparoscopic surgery in four) and dysphagia alone in three patients (previous laparoscopic surgery in all).

In those patients undergoing primary open surgery, the underlying morphologic findings included telescope phenomenon in three cases, hiatal disruption in five cases, and wrap breakdown in three cases. Among those patients who had undergone primary laparoscopic fundoplication, the reasons for failed primary intervention included telescope phenomenon in one, hiatal disruption in 12, and a wrap that was too tight in three.

In every patient who was referred to our department with recurrent or persistent symptoms of GERD after primary antireflux surgery, a standardized preoperative workup was performed, which included esophagogastroduodenoscopy (EGD), barium swallow for visualization of anatomic or morphologic complications, routine esophageal manometry, and 24-hour pH monitoring. Indications for redo surgery and the technique of laparoscopic refundoplication have been described previously.[7]

In 25 patients with normal esophageal motility, a laparoscopic 360-degree "floppy" Nissen fundoplication was performed. In the two patients with poor esophageal motility and/or severely disordered peristalsis, a laparoscopic 270-degree Toupet partial fundoplication was used.

Among the complete sample, a total of 17 patients (prior open procedure in 11; prior laparoscopic procedure in six) underwent primary intervention at another institution, whereas a group of 10 patients underwent primary surgery at our hospital (all with previous laparoscopic surgery).

Follow-Up

A total of 27 patients who had undergone a laparoscopic redo procedure in our surgical unit before July 1998 were included in this study. Therefore a minimum follow-up of 3 years and a maximum follow-up of 5 years were possible. Follow-up included quality of life assessment, patient satisfaction, and symptomatic outcome at 3 to 5 years after surgery. Follow-up was completed in all 27 patients.

Quality of Life

Quality of life was evaluated by means of the German Gastrointestinal Quality of Life Index (GIQLI).[11] This questionnaire is well established,[12–15] and has been validated and recommended by the European Study Group for Antireflux Surgery.[16] The survey includes 36 items, and the general responses are assigned values ranging from 0 to 144 points. The GIQLI is also divided into five subcategories as follows: gastrointestinal symptoms (0 to 76 points), emotional status (0 to 20 points), physical function (0 to 28 points), social function (0 to 16 points), and a single item for the stress of medical treatment (0 to 4 points).

Symptom Evaluation and Patient Satisfaction

Typical symptoms of GERD, such as heartburn, dysphagia, regurgitation, and chest pain, were subjectively evaluated using a verbal rating scale in which patients described their symptoms as follows: none, mild, moderate, or severe. In addition, patients were asked the following three questions: (1) Are you satisfied with the procedure; (2) would you undergo laparoscopic redo surgery again, if necessary; and (3) do you require any antireflux medication (i.e., proton pump inhibitors, H_2 receptor antagonists)?

Statistics

The SPSS program was used for statistical analysis. All data are presented as mean ± standard deviation or median and range. Comparison of data was done using methods for repeated measurement as appropriate. $P < 0.05$ was regarded as significant.

RESULTS
Quality of Life

Preoperatively, the mean general score for the GIQLI showed a significant impairment ($P < 0.01$) in comparison to data from healthy individuals. Before laparoscopic refundoplication, the general score for

the GIQLI was 85.8 ± 9.2 points in comparison to 122.6 ± 8.5 points in healthy individuals. Three to 5 years postoperatively, the GIQLI did not reach the scores of healthy individuals but improved significantly ($P < 0.05$) in comparison to preoperative values (113.4 ± 8.3 points). The complete data for the GIQLI, including all subcategories, are shown in Fig. 1.

Symptomatic Outcome

None of the 27 patients reported having severe heartburn, dysphagia, regurgitation, or chest pain at follow-up. A total of 12 patients (44.4%) reported having one or more symptoms rated as mild or moderate.

Heartburn. Before redo surgery, heartburn was present, alone or in combination with dysphagia, in 24 of our patients (88.9%). Twenty-five patients (92.6%) were reported to be free of heartburn at 3 to 5 years after laparoscopic refundoplication. Two patients (7.4%) reported having rare episodes of heartburn, which were controlled with low doses of proton pump inhibitors (20 mg omeprazole/day) on demand. In these two patients, postoperative DeMeester scores were significantly abnormal.

Dysphagia. Preoperatively, 10 patients (37%) suffered from moderate-to-severe dysphagia in combination with recurrent reflux. Additionally, three patients (11%) had severe dysphagia as their only symptom. Therefore the total incidence of preoperative dysphagia was 48%.

Of these 13 patients, seven (25.9%) still reported suffering from mild-to-moderate dysphagia at 3 to 5 years after redo surgery, but the degree of dysphagia improved as a result of the reoperation. Within the first postoperative year, five of these seven patients suffered from persistent and severe dysphagia and

required pneumatic dilatation. After pneumatic dilatation (three patients required two postoperative dilatations), these patients were free of symptoms for approximately 1 year postoperatively but still reported mild-to-moderate dysphagia 3 to 5 years after redo surgery. In all of these patients, a laparoscopic Nissen refundoplication had been performed. The remaining 20 patients (74.1%) are free of dysphagia at 3 to 5 years after refundoplication.

Regurgitation. Preoperatively, 18 patients (66.7%) reported suffering from regurgitation. After reoperation, four patients (14.8%) were reported to have episodes of regurgitation but with no significant impairment of quality of life. These patients were not given any medication during follow-up.

Chest Pain. All patients in the present sample suffered from moderate-to-severe chest pain before laparoscopic refundoplication. Fifteen patients (55.6%) are free of chest pain 3 to 5 years postoperatively. However, 12 patients (44.4%) reported having episodes of mild-to-moderate chest pain at follow-up. Nine of these patients suffer from concomitant cardiopulmonary disease; the remaining three patients have no other symptoms of GERD.

The complete data on symptomatic outcome 3 to 5 years after laparoscopic refundoplication are presented in Figs. 2 to 5.

Patient Satisfaction. Twenty-five patients (93%) rated their satisfaction with laparoscopic redo surgery as very good at 3 to 5 years after the procedure. These patients would be willing to undergo surgery again, if necessary. None of these patients required antireflux medication during follow-up. The two remaining patients (7%) suffer from episodes of recurrent heartburn (3 to 4 times/month), as described previously. Clinical reevaluation (EGD and barium swallow) of these patients showed no anatomic or
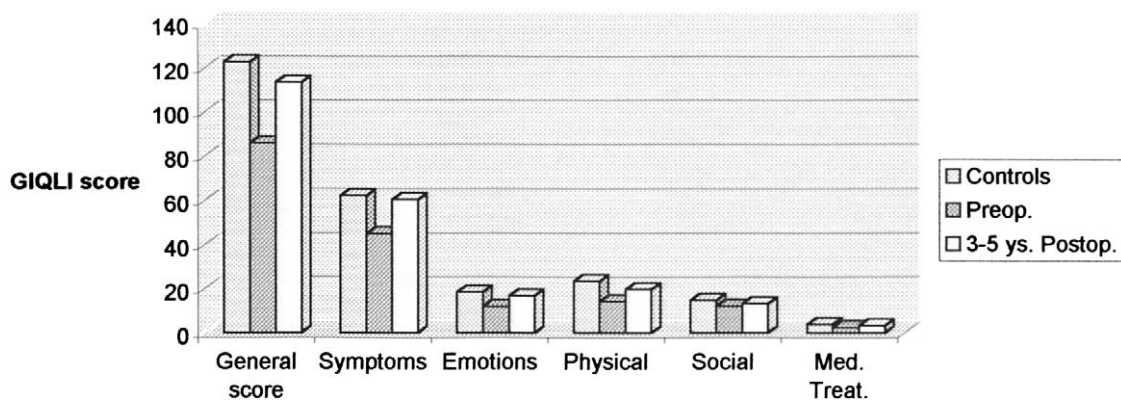

Fig. 1. Gastrointestinal Quality of Life Index (GIQLI) 3 to 5 years after laparoscopic refundoplication (n = 27).
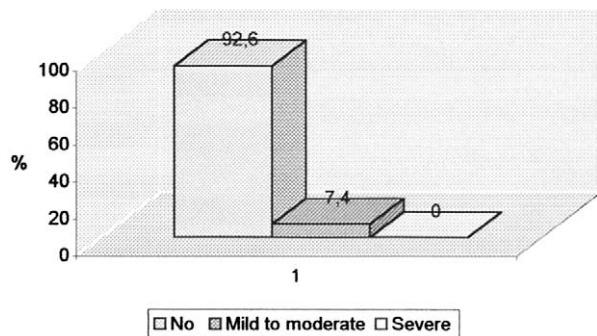
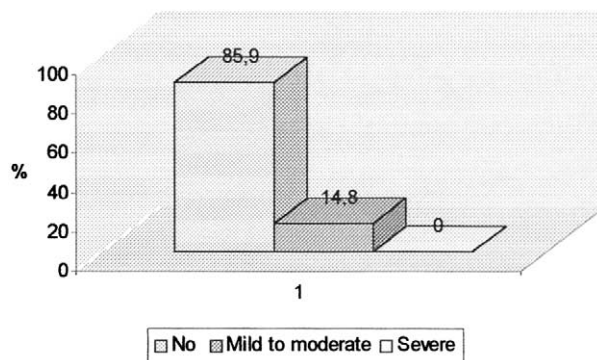**Fig. 2.** Heartburn 3 to 5 years after refundoplication.

Fig. 4. Regurgitation 3 to 5 years after refundoplication.

morphologic evidence of failure, and these patients are being managed with low doses of proton pump inhibitors on demand. Complete data are presented in Figs. 6 and 7.

DISCUSSION

During the past decade, laparoscopic fundoplication has replaced the open approach worldwide and has become the standard surgical procedure for treatment of GERD. Large studies have shown that LARS is a safe and effective surgical procedure that provides good symptomatic relief in most patients and good to excellent functional outcome at long-term follow-up.[1–3,10] However, most series include some patients with adverse outcomes after LARS necessitating redo surgery. Failure rates after laparoscopic fundoplication have been well documented and range from 3% to 17%.[5] In recent years, some reports have questioned the feasibility of laparoscopic revision surgery after failed open and/or laparoscopic antireflux surgery.[6–8,17–19] These reports have shown that laparoscopic redo surgery after failed primary intervention can be performed safely

by experienced laparoscopic surgeons with good functional and symptomatic outcome at short- or mid-term follow-up. However, there are few data regarding quality of life and patient satisfaction over a longer follow-up period.[10]

It has been shown that quality of life in patients with GERD can be impaired significantly and is comparable to that in patients with diabetes, myocardial infarction, or polyarthritis.[20] In recent years, some reports have demonstrated the efficacy of medical or surgical treatment concepts in patients with GERD by documenting quality of life data.[10,14,15]

In the present study, we present quality of life data and patient satisfaction ratings from 27 patients who had undergone laparoscopic refundoplication for primary failed open or laparoscopic fundoplication 3 to 5 years earlier.

Since the results of patient-related assessments such as quality-of-life evaluations have been reported, there has been an increasing interest in more than just objective outcome parameters, but those that are from the patient's point of view, in particular.[21] Several instruments for quality of life evaluation in gastrointestinal disorders are now available. Trus et al.[22] used the SF-36 (generic questionnaire)
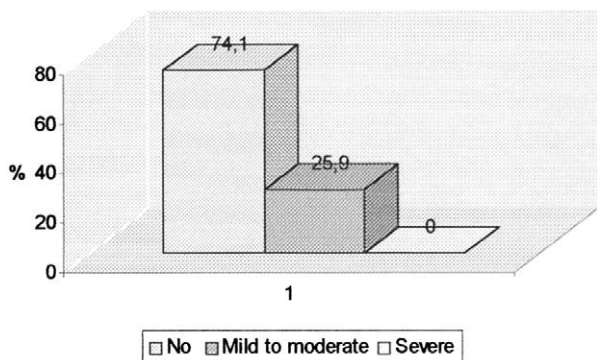

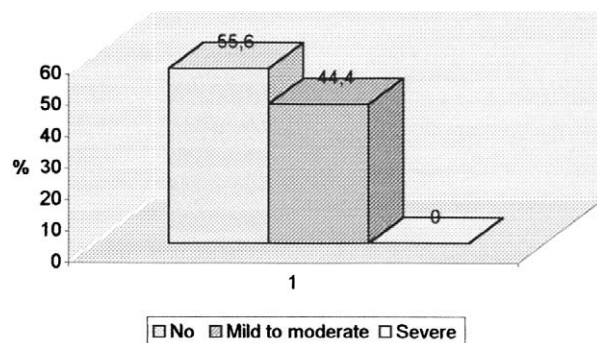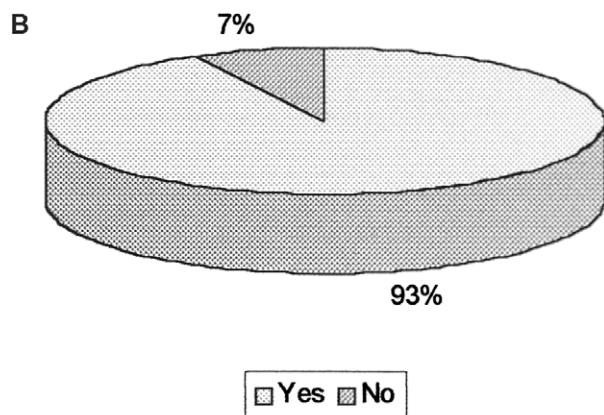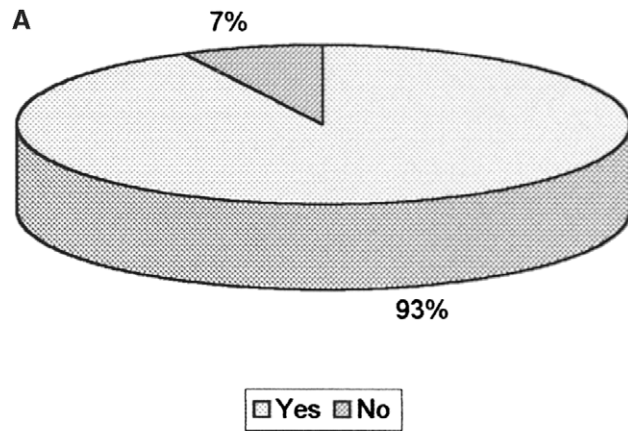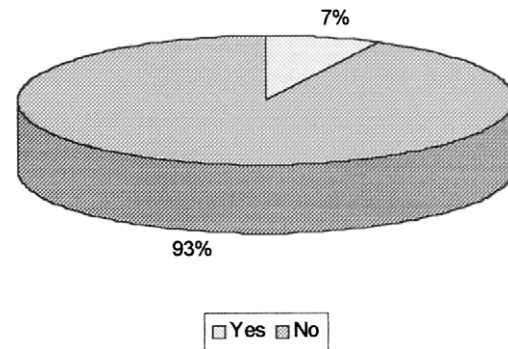**Fig. 3.** Dysphagia 3 to 5 years after refundoplication.

Fig. 5. Chest pain 3 to 5 years after refundoplication.

A   7%

93%

☐ Yes ☑ No

B   7%

93%

☐ Yes ☑ No

Fig. 6. Patient satisfaction 3 to 5 years after laparoscopic refundoplication (n = 27). Questions: **A,** Are your satisfied with procedure? **B,** Would you undergo Redo-LARS again if necessary?

7%

93%

☐ Yes ☑ No

Fig. 7. Medication (n = 27). Question: Do you need antireflux medication yet?

for quality-of-life evaluation of primary laparoscopic antireflux surgery. In a prospective study by Velanovich,[23] symptomatic and quality-of-life data from patients undergoing laparoscopic versus open antireflux surgery were compared using both the SF-36 and a disease-specific symptom score, the GERD-HRQL. As we did in our present study, Slim et al.[24] used the GIQLI to assess quality-of-life outcome in a prospective study.

In recent years, large series have shown that patient quality of life improves significantly after primary laparoscopic antireflux surgery, and a high de-

gree of patient satisfaction is demonstrated at midterm and long-term follow-up.[10,12,24]

Relatively few data are available concerning quality of life data after laparoscopic redo surgery. In a prospective nonrandomized trial by Pointner et al.,[7] the GIQLI was used to assess quality-of-life outcomes in 30 patients who underwent laparoscopic refundoplication for primary failed open or laparoscopic fundoplication over a complete follow-up period of 1 year. The general scores for the GIQLI increased from a preoperative value of 86.7 points to 120.7 points at 3 months postoperatively and 123.1 points at 1 year after redo surgery, which were comparable to scores in a healthy population (122.6 points). Floch et al.[18] reported, in a study of 46 consecutive patients, a significant improvement in patient well-being scores after laparoscopic refundoplication with a patient satisfaction rate of 89%. In a recently published prospective nonrandomized trial, the GIQLI was assessed in 20 patients who underwent laparoscopic redo surgery after primary failed open antireflux surgery for a follow-up period of 1 year.[6] At 1 year after surgery, the general score for the GIQLI did not match the scores in healthy individuals, as described previously, but showed significant improvement in comparison to preoperative values (preoperatively = 84.9 points; 1 year postoperatively = 120.1; healthy individuals = 122.6).

In the present study, quality of life was evaluated by means of the GIQLI 3 to 5 years after laparoscopic refundoplication. The mean general score for the GIQLI showed significant improvement when the preoperative data were compared with outcomes 3 to 5 years after the redo procedure. In contrast to our experience, a 2-year follow-up in another group of patients[10] did not reach a mean value comparable to that in healthy individuals. First, as shown in Figure 1, especially the subscore "physical function," showed a lower score and therefore less improvement in com-

parison to the other subscores, also in relation to healthy individuals and our earlier findings. This might be because two of the patients in our present sample suffered from a comorbid condition at the time of follow-up, which also affected their quality of life and their physical functioning in general. Second, in the present sample, a larger group of so-called "elderly" (>65 years) patients with GERD is included. It has been shown previously that quality-of-life scores in these patients generally can be lower when compared to quality of life of younger patients.[15]

Similar to what has been reported by other investigators,[18] we achieved high patient satisfaction during our long-term follow-up: 93% of our patients were satisfied with the procedure and would undergo a laparoscopic redo-procedure again, if necessary. These data correlate well with the symptomatic outcome at follow-up. None of the patients rated the subjective extent of typical GERD symptoms such as heartburn, dysphagia, regurgitation, or chest pain as severe. Only two patients (7%) reported having rare episodes of mild heartburn, which were successfully managed with low doses of proton pump inhibitors (20 mg omeprazole on demand). Despite the fact that a relatively large percentage of our patients suffered from so-called surgical side effects or recurrent problems, improvement in quality of life and patient satisfaction can be regarded as good. Even if our findings clearly show that laparoscopic refundoplication was not truly curative or resulted in mild-to-moderate symptoms in some of our patients, we think that compared to the severity of presurgical symptoms, laparoscopic redo surgery had a significant impact resulting in those high levels of improvement and satisfaction.

On the basis of our present findings, we conclude that laparoscopic refundoplication after previously failed antireflux surgery results in a high degree of patient satisfaction with good symptomatic outcome over a follow-up period of up to 5 years after surgery. However, quality of life does not reach that of healthy individuals but shows significant improvement in comparison to preoperative data. Further long-term trials concerning quality of life data after laparoscopic redo surgery are needed.

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Patients With Upright Reflux Have Less Favorable Postoperative Outcomes After Laparoscopic Antireflux Surgery Than Those With Supine Reflux

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The aim of this study was to compare symptomatic outcomes after laparoscopic antireflux surgery in patients with upright vs. supine reflux. A prospective database was used to assess postoperative clinical outcomes in relation to positional patterns of reflux in 117 patients. Supine reflux was present in 31%, upright in 24%, and the remaining 44% had bipositional reflux. Preoperatively there were no differences in the frequency of typical or atypical symptoms between groups. At a mean follow-up of 18 ± 11 months postoperatively, there were marked differences in symptoms between groups. Patients with upright reflux noted significantly more heartburn, chest pain, odynophagia, and bloating postoperatively when compared to patients with supine and bipositional reflux ($P < 0.05$). According to visual analog scales, patients with upright reflux expressed less satisfaction with operative results, ascribing more symptoms to the esophagus and stomach, when compared to those with supine reflux ($P < 0.05$). Although all patients reported improvement, the extent of the relief from preoperative symptoms was less in patients with upright reflux ($P < 0.05$). When asked if, in retrospect, they favored operative therapy, the patients with upright reflux were less enthusiastic ($P < 0.05$). Although antireflux surgery eliminates reflux in nearly all patients, postoperative symptomatic outcome is related to the preoperative pattern of reflux. Although all patients showed symptomatic improvement, the extent of that improvement was significantly less in patients with upright reflux. These patients should be carefully counseled preoperatively regarding expected symptomatic outcomes. (J GASTROINTEST SURG 2002;6:819–830.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Gastroesophageal reflux, fundoplication, ambulatory pH testing, patient outcome assessment

The influence of body position on the occurrence of gastroesophageal reflux is widely recognized by both patients and physicians. With the advent of clinical 24-hour pH monitoring of the distal esophagus in 1973,[1] it became clear that there were distinct patterns of reflux related to body position. Whereas normal volunteers had episodes of reflux only when in the upright position, those patients with gastroesophageal reflux disease (GERD) could be divided into three groups: upright reflux, supine reflux, and combined reflux.[2] The specific type of reflux pattern has been consistently shown to correlate with disease severity, with supine and combined reflux being predictors of both more severe symptoms and more advanced mucosal injury.[3,4] The calculation of the composite pH score (i.e., DeMeester score), in part, reflects the pattern of acid exposure, with supine reflux leading to higher scores than upright reflux.[1]

When the outcomes of open antireflux surgery were first examined in relation to the preoperative positional pattern of reflux, poor outcomes were seen in patients with upright reflux. Specifically, these patients seemed to have a high incidence of postoperative bloating and flatus.[2] Because of these problems, fundoplication was

not recommended in this subgroup of patients.[5] However, more recently, operative outcomes in patients with upright reflux have been reexamined and are reported to be equivalent to those of patients with supine and bipositional reflux.[6] Yet, others have continued to report an increase in the gas-bloat syndrome in this subgroup postoperatively.[7]

Because of these contradictory reports and the relatively small number of patients whose operative outcomes have been reported specifically in relation to reflux patterns, we reviewed our experience from a prospectively obtained database. The aim of the present study was to determine the influence of the preoperative positional pattern of reflux on symptomatic outcomes and overall patient satisfaction after laparoscopic antireflux surgery (LARS).

MATERIAL AND METHODS
Patients

The study population consisted of patients who participated in a comprehensive prospective evaluation of outcome predictors and who underwent LARS after 24-hour pH testing, over the 5-year period 1997 to 2001. To ensure uniformity of technique and data analysis, only patients with pH testing performed at our institution were included. All patients were referred for surgical management of GERD and underwent upper endoscopy and esophageal manometry preoperatively. Patients with an antireflux procedure performed in combination with a Heller myotomy or paraesophageal hernia repair were excluded from this analysis. In addition, patients with less than 3 months of follow-up were excluded. This study was approved by the Human Studies Committee of the Washington University School of Medicine.

24-Hour pH Testing

Patients were instructed to stop taking antacids for 24 hours, H_2-receptor antagonists for 2 days, and proton pump inhibitors for 7 days before the study and to fast the night before pH probe placement. After the lower esophageal sphincter (LES) was located manometrically, a dual-channel ComforTEC pH probe (Sandhill Scientific Inc., Highlands Ranch, CO) was inserted transnasally. The probe was positioned with its distal sensor 5 cm above the upper margin of the LES, making the proximal sensor 15 cm above the LES. Intraesophageal pH was then sampled from both the proximal and distal sensors every 6 or 8 seconds over the 24-hour period. Patients activated the event marker to indicate the onset of symptoms, meals, and recumbence, and also recorded the information in a diary for confirmation. All information

from the pH monitor was stored in a data logger. Patients were instructed not to restrict their diets or activities during the monitoring period, but diet-related pH events were edited from the tracings. The catheter was removed approximately 24 hours after placement, and the data were collected and analyzed with the Orion and MMS systems (Enschede, Holland). Thresholds for determining pathologic reflux were established for each sensor site. Because no consensus exists for normal values of distal esophageal exposure time with pH < 4, thresholds that have a high sensitivity for reflux disease have been used in our laboratory, rounded to the nearest whole percentage (>4% total, >6% upright, and >2% supine).[8,9] Thresholds for the proximal channel were extracted from the range of normal values reported in healthy volunteers (>1.1% total, >1.7% upright, and >0.6% supine).[10]

Patients were classified into one of three groups on the basis of the position in which the reflux occurred: upright reflux (UR), supine reflux (SR), or bipositional reflux (BR). Because positional data were available from both the distal and proximal sensors, patients were classified according to the predominant position in which the reflux occurred. Patients were classified as having supine reflux if (1) only supine reflux occurred in both channels, (2) only supine reflux occurred in one channel with no reflux in the other channel, or (3) only supine reflux occurred in one channel with reflux in both positions in the other channel. Patients with upright reflux were classified according to these same principles. Patients were classified as having bipositional reflux if (1) reflux occurred in both positions in both channels or (2) reflux occurred in both positions in one channel and neither position in the other channel. No patient demonstrated reflux in one position in one channel and the alternate position in the other channel.

Esophageal Manometry

Subjects fasted overnight, and medications were held on the day of the study. A 21-lumen extruded silicone catheter with recording side holes spaced at 1 cm intervals (Dentsleeve Pty, Bowden, South Australia) was used. Each lumen was attached to a pressure transducer perfused by a pneumohydraulic device (Mui Systems, Mississauga, Ontario, Canada). Pressure data were acquired at a rate of 10 Hz using a computerized manometric system (MMS; Enschede). LES pressure was determined by the station pull-through method at end expiration. The catheter was then positioned so that several distal recording sites were in the stomach and the remainder were in the LES and esophageal body. The patient took 10 swallows of water, spaced more than 20 seconds from each other; the catheter was repositioned to sample the proximal esophagus, and the

swallows were repeated. Peristaltic performance was determined from recording locations 3 and 13 cm above the LES, and contraction wave parameters were calculated using the tracings 3 and 8 cm above the LES.[11]

Operative Technique

All patients underwent a laparoscopic antireflux procedure after an overnight fast. In most cases, a short, floppy Nissen fundoplication was performed over a 60 F bougie after complete mobilization of the fundus. In a small fraction of cases, patients underwent Toupet fundoplication. The crura were approximated posterior to the esophagus in all cases. Patients were admitted overnight and offered clear liquids followed by soft solids the following morning. Patients doing well were discharged home on postoperative day 1. Postoperative physiological testing was not performed routinely.

Data Collection

Patients were questioned preoperatively with regard to typical and atypical symptoms of GERD using a comprehensive data collection form designed for this purpose. Specifically, the presence or absence of the following was elicited: heartburn, regurgitation, water brash, asthma, odynophagia, dysphagia, cough, chest pain, and nocturnal aspiration. The use of acid-buffering or acid-reducing medications and the presence of vomiting or bloating were also recorded. Responses were elicited in a yes/no fashion, with "yes" indicating any degree of the given symptom, no matter how mild or infrequent. Patients were also asked to rate global symptoms and effects of symptoms on daily living using a visual analog scale. Intraoperative details and important aspects of the perioperative course were charted. Patients were seen routinely within the first 2 to 4 weeks after surgery, at 6 months, and then annually. Patients who elected not to continue yearly office visits were contacted annually by telephone. At each postoperative contact point, patients were again surveyed for the same symptoms and were asked to complete the identical data collection form. All data were collected and compiled in a prospective fashion by two research nurses who were unaware of the patient's positional pattern of reflux. The collected data were entered prospectively into an ongoing computerized database.

Statistical Analysis

All proportional data were analyzed using the chi-square test (using the test for trend where appropriate). Three sets of continuous data were compared using one-way analysis of variance (if the data sets were shown to have equal variances with Bartlett's test) with a post-test for trend. A two-tailed unpaired Student's t test was used to compare two sets of continuous data. Continuous data are presented as mean ± standard deviation; proportional data are presented as percentages. All statistics were calculated using Graph-Pad Prism 3.0 software (San Diego, CA). $P \leq 0.05$ was considered significant.

RESULTS
Patient Population

Over the 5-year period from 1997–2001, a total of 127 patients underwent preoperative pH testing followed by LARS (both at our institution) and participated in the study. Of these, five patients (4.2%) did not have at least 3 months of follow-up data available and were excluded. An additional three patients (2.6%) who had incomplete pH data available were excluded because their reflux patterns could not be properly classified. Finally, two patients (1.7%) with normal pH studies were also excluded. One of these patients was intolerant of the pH probe and removed it after only 8 hours; the other patient had endoscopic evidence of esophagitis and typical symptoms. Thus 117 patients were included in the present analysis. Of these, 28 (24%) were classified as having upright reflux, 37 (32%) as having supine reflux, and the remaining 52 (44%) had bipositional reflux.

The demographic profiles of the three groups were similar (Table 1). The mean age, sex distribution, and body mass index were not significantly different between groups. The duration of reflux symptoms was longest in the bipositional group and shortest in the upright group. A similar proportion of smokers was found in each group.

Preoperative Esophagogastroduodenoscopy and Motility Testing

All patients underwent upper endoscopy preoperatively, but complete data were unavailable for three. Although the incidence of esophagitis was nearly half in each group, it was of much higher grade in the patients with supine and bipositional reflux (Table 2). Of the patients with esophagitis, 27% of patients with upright reflux had grade III or IV disease, compared to a full 73% of patients with supine reflux ($P = 0.02$). The incidence of stricture was also higher in the supine group, although this did not reach statistical significance ($P = 0.06$).

All 117 patients underwent preoperative manometry before surgery. When the three groups were compared with respect to esophageal peristaltic performance, pa-

Table 1. Patient demographics

	Upright reflux	Bipositional reflux	Supine reflux	*P* value*
Number	28 (24%)	52 (44%)	37 (32%)	
Age (yr)	46 ± 13	49 ± 12	46 ± 11	NS
Male sex (%)	32	48	51	NS
Body mass index	29 ± 5	31 ± 6	30 ± 5	NS
Tobacco use (%)	32	34	46	NS
Symptom duration (mo)	61 ± 47	88 ± 45	65 ± 41	0.01

*P value refers to one-way analysis of variance with post-test for trend or chi-square test for trend; NS = not significant.

tients with supine or bipositional reflux had less effective motor performance (Table 3). All patients with upright reflux had ordered peristalsis (following >70% of swallows) with no patient having failure of peristaltic propagation; in contrast, only 77% of patients with supine reflux had normal peristaltic activity (following >70% of swallows). Patients with supine reflux also had a higher incidence of esophageal hypomotility, defined as having ≥30% swallows with failure of peristalsis or a distal mean contraction wave amplitude of less than 35 mm Hg. Although only one patient with upright reflux had evidence of hypomotility, 23% of patients with supine reflux had evidence of this (P = 0.04). The mean amplitude of the distal contraction wave, the LES pressure, and the LES length were not significantly different between groups.

Preoperative 24-Hour pH Testing

All 117 patients underwent preoperative outpatient ambulatory 24-hour pH testing at our institution. As suggested by the trends in severity of endoscopic esophagitis, the modified DeMeester score was highest for the bipositional group (55 ± 25), intermediate for the supine group (41 ± 23), and lowest for the upright group (35 ± 29). This was a statistically significant trend (P = 0.003). All patients had abnormal total acid exposure times distally (>4%), but the av-

erage duration of that exposure was longest in the bipositional group (Table 4). It is important to note that although the mean distal acid exposure time in the upright position was greatest for the upright and bipositional groups, it was still higher than normal in the supine group. This is because 49% of patients classified as patients with supine reflux had reflux in both positions in the distal channel. The same point should be made for the patients with upright reflux, with 25% of them having abnormal distal acid exposure times in the supine position. In contrast, in the proximal channel, no patient with upright reflux had abnormal supine exposure; similarly no patient with supine reflux had abnormal proximal upright exposure. Thirty-eight percent of patients with bipositional reflux had abnormal acid exposure times in the proximal channel.

Preoperative Symptoms and Medication Use

Although the degree of esophagitis and the duration of acid exposure were different between groups, the frequency of typical and atypical symptoms preoperatively was quite similar (Fig. 1). These numbers represent the fraction of patients who endorsed the symptom, no matter how infrequent or mild. These numbers are similar to the remainder of patients in our database who have undergone LARS over a more extended time period (data not shown).

Table 2. Preoperative esophagogastroduodenscopy

	Upright reflux (%)	Bipositional reflux (%)	Supine reflux (%)	*P* value*
Any esophagitis	48	46	50	NS
High-grade esophagitis[†]	27	65	73	0.02
Barrett's esophagus	4	6	12	NS
Stricture	0	14	11	0.06
Hiatal hernia	39	60	54	NS

*P value refers to chi-square test for trend.
[†]Includes grade III or IV esophagitis (Savary-Miller classification).

Table 3. Preoperative esophageal manometry

	Upright reflux	Bipositional reflux	Supine reflux	*P* value*
Normal peristalsis[†]	100%	81%	77%	0.02
Hypomotility[‡]	4%	17%	23%	0.04
LES pressure (mm Hg)	6.9 ± 4.7	6.4 ± 5.0	7.7 ± 6.1	NS
LES length	2.4 ± 0.7	2.1 ± 0.7	2.4 ± 0.8	NS

**P* value refers to one-way analysis of variance with post-test for trend or chi-square test for trend.
[†]Defined by >70% swallows with normal peristaltic waves.
[‡]Defined by ≥30% swallows with aperistalsis or contraction amplitude <35 mm Hg.

Clinical Course

The operative and perioperative clinical courses of the patients in all three groups were quite similar (Table 5). The operative time was just under 2 hours in all groups, and almost 90% of all patients underwent Nissen fundoplication with the remainder undergoing Toupet fundoplication. There was one anatomic failure in the supine group and one in the bipositional group, both of which were due to intrathoracic migration of the wrap. Only one of the patients has required reoperation. Patients were discharged at a mean of 1.3 ± 1.1 days postoperatively.

Postoperative Symptoms and Medication Use

Although there were no differences in the prevalence of preoperative symptoms, at a mean follow-up of 17.6 ± 11 months, there were significant differences in symptoms between groups (Fig. 2). For most symptoms examined, the same trend was observed: the upright group had the highest percentage of patients with persistent symptoms, whereas the supine group had the lowest percentage, with the bipositional group being intermediate. This trend was statistically significant for heartburn, chest pain, asthma, odynophagia, and postoperative bloating. When the postoperative symptoms of patients were examined by type, an inter-

esting trend was seen (Fig. 3). Patients with upright reflux had a similar but less marked relief from typical symptoms (heartburn, water brash, and regurgitation) postoperatively. However, when the atypical symptoms (chest pain, nocturnal aspiration, asthma, and cough) were examined, there was a more marked difference. Although similar percentages of patients with upright (82%) and supine (79%) reflux had one of these atypical symptoms preoperatively, only 43% of patients with upright reflux had resolution of the symptom postoperatively, compared to 84% of patients with supine reflux ($P < 0.05$). When the symptoms often considered to be side effects of antireflux surgery (dysphagia, odynophagia, and bloating) were considered together, a similar trend was seen ($P < 0.05$) (see Fig. 3). Twenty-seven percent of patients with supine reflux reported postoperative bloating (of any frequency or severity) compared to 50% of patients with upright reflux ($P < 0.05$).

Comparison of Postoperative Global Satisfaction

In addition to the symptom-specific differences between groups, there were also significant differences in the subjective sense of overall postoperative improvement. In all categories, the patients with upright reflux reported a subjectively lower sense of postoperative well-being (Fig. 4). When asked to rate their overall

Table 4. Acid exposure times by channel and position

% of time pH <4	Upright reflux	Bipositional reflux	Supine reflux	*P* value*
Distal total (>4)	10.8 ± 5	15.8 ± 7	10.7 ± 7	NS
Distal upright (>6)	14.2 ± 7	15.3 ± 8	8.4 ± 8	0.0036
Distal supine (>2)	2.7 ± 5	17.4 ± 11	13.2 ± 9	0.000001
Proximal total (>1.0)	1.6 ± 1.4	2.4 ± 5	1.5 ± 2	NS
Proximal upright (>1.6)	2.5 ± 2.4	2.3 ± 4.2	0.6 ± 0.6	0.00002
Proximal supine (>0.5)	0.1 ± 0.1	2.8 ± 7.1	2.7 ± 4.9	0.0054

**P* value compares upright reflux with supine reflux by two-tailed Student's *t* test.
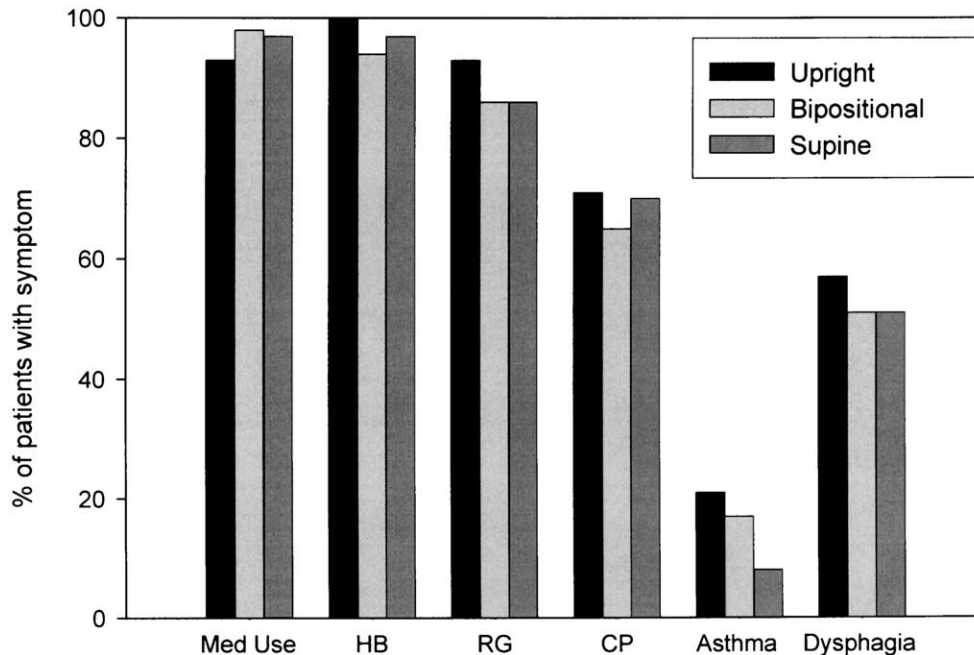
Fig. 1. Prevalence of symptoms in relation to positional pattern of reflux at preoperative evaluation. There were no differences in the proportion of patients reporting any symptom at this evaluation point. Med Use = use of acid-reducing or acid-buffering medications; HB = heartburn; RG = regurgitation; CP = chest pain.

esophageal symptoms on a 100 mm visual analog scale, with 0 mm being "as poorly as possible" and 100 mm being "completely well," patients with supine reflux averaged 96 ± 6 mm compared to those with upright reflux who averaged only 76 ± 31 mm ($P < 0.05$). Perhaps more important, when asked how their postoperative symptoms compared to their preoperative symptoms (with 100 mm indicating "100% better"), patients with supine reflux were significantly more improved compared to patients with upright reflux (98 ± 6 mm vs. 88 ± 12 mm, $P < 0.05$). It is important to emphasize that when patients were asked simply if they were "better," "unchanged," or "worse" postoperatively, all patients except two (in the bipositional group) reported that they were "better."

DISCUSSION

Since the initial description of the DeMeester scoring system, it has been clear that supine reflux is the more pathologic form of GERD compared to upright reflux, which is milder in severity.[1] In studies of normal volunteers, it has been shown that physiologic reflux occurs most often when the person is upright, specifically postprandially, and occurs very rarely in the supine position.[12,13] Many studies have documented the more severe nature of supine reflux by showing that these patients have more severe symptoms,[3] more advanced esophagitis,[2,14,15] and lower quality-of-life scores.[16] The data we have presented are consistent with these previous reports, with significantly more patients with supine reflux

Table 5. Clinical course

	Upright reflux	Bipositional reflux	Supine reflux	P values*
Time in operating room (min)	109 ± 34	113 ± 26	110 ± 35	NS
Nissen fundoplication (%)	96%	89%	94%	NS
Anatomic failure	0 (0%)	1 (2%)	1 (3%)	NS
Day of discharge	1.1 ± 0.3	1.2 ± 0.7	1.6 ± 1.8	NS
Length of follow-up (mo)	18.4 ± 10	17.5 ± 12	17.0 ± 12	NS

*P value refers to one-way analysis of variance with post-test for trend or chi-square test for trend.
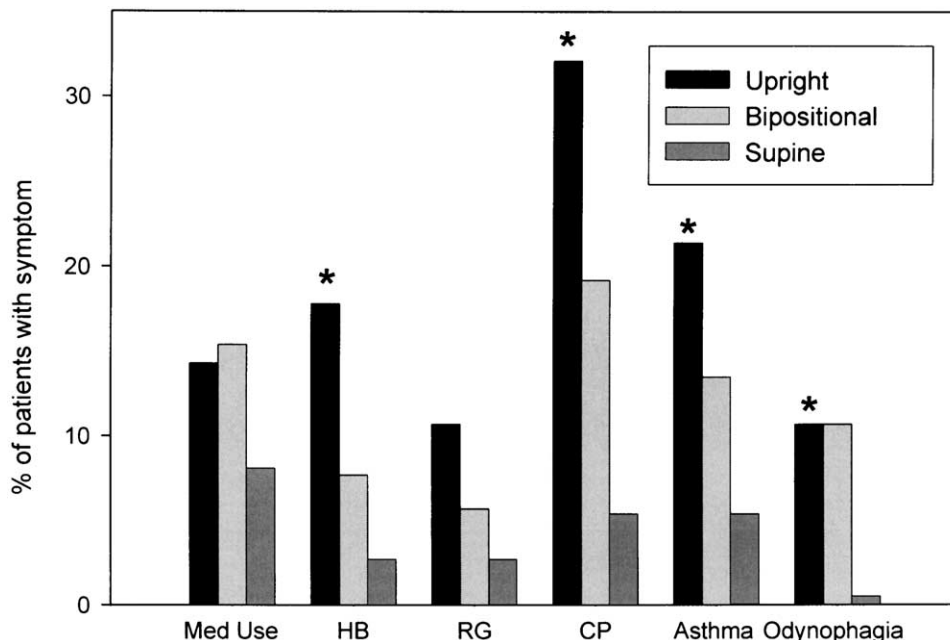
Fig. 2. Prevalence of postoperative symptoms in relation to positional pattern of reflux at most recent evaluation. There were significant differences between the patients with upright reflux and patients with supine reflux in the prevalence of heartburn (HB; $P = 0.03$), chest pain (CP; $P = 0.005$), asthma ($P = 0.05$), and odynophagia ($P = 0.04$). Med Use = use of acid-reducing or acid-buffering medications. * $P \leq 0.05$ using the chi-square test for trend.

having severe esophagitis compared to patients with upright reflux. Additionally, a trend toward a higher incidence of esophageal stricture in the patients with supine reflux was seen in this study.

In attempting to understand the difference in pathophysiology between upright and supine reflux, it has been suggested that upright reflux results from more frequent transient lower esophageal sphincter relaxations (TLESRs), whereas supine reflux is the result of an anatomically incompetent sphincter.[17] In fact, in two separate studies, one group has shown a higher incidence of a defective LES in patients with supine reflux compared to patients with upright reflux.[4,6] However, the largest and most recent study from that same group[18] did not find a difference in LES characteristics (LES length, intra-abdominal length, and LES pressure) when comparing patients with upright reflux to patients with supine reflux. We found no significant differences in LES pressure or LES length among the three groups.

The method of classifying patients as having supine, upright, or bipositional reflux in this study was somewhat different from methods used in previous studies. Whereas many other studies use a single-channel probe with information only from the distal channel, we used a dual-channel pH probe with one sensor distally (5 cm above the LES) and one proxi-

mally (15 cm above the LES). To classify patients most accurately, information from both channels was considered, and the dominant pattern was used to classify each patient. In using this type of scheme, we found that only 25% of patients classified as "upright refluxes" had abnormal supine acid exposure time in the distal channel, compared to nearly 50% of "supine refluxes" with abnormal upright exposure times in the distal channel. This could be consistent with the theory that others have proposed, namely, that there is a progression of reflux disease from mild upright reflux to the more severe supine reflux.[6] An alternate possibility, however, is that there are two separate mechanisms for reflux that can occur simultaneously. It is important to emphasize that when patients were classified according to information from only the distal channel, the same trends in terms of prevalence of postoperative symptoms and patient satisfaction were seen (data not shown).

In terms of prevalence of typical and atypical symptoms preoperatively, we did not find a difference between the three groups. Essentially all of our patients, irrespective of pattern of reflux, were taking acid-buffering or acid-suppressing medications and had typical symptoms. Although other studies have demonstrated a difference in the frequency and severity of reflux symptoms between patients with up-
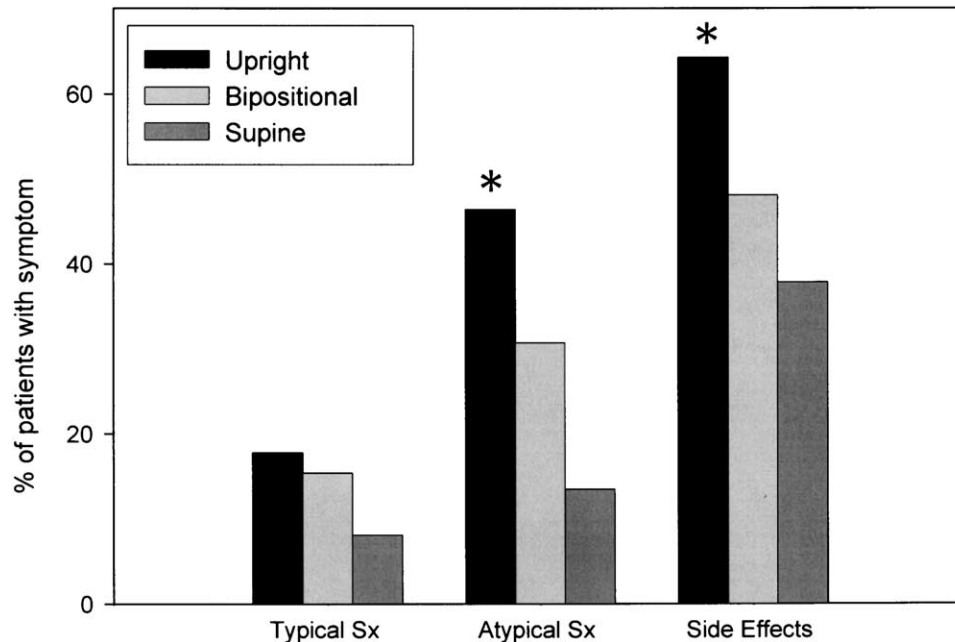
Fig. 3. Prevalence of symptoms postoperatively as stratified by type. Typical symptoms include any one of the following: heartburn, water brash, or regurgitation. Atypical symptoms include chest pain, nocturnal aspiration, asthma, and cough. Symptoms classified as side effects include dysphagia, odynophagia, and bloating. When the upright group was compared to the supine group, there was no difference in the frequency of typical symptoms postoperatively; however, patients with upright reflux had significantly more atypical symptoms ($P = 0.004$) and side effect–type symptoms ($P = 0.036$) when compared to patients with supine reflux. * $P \leq 0.05$ using the chi-square test for trend.

right and those with supine reflux, these have included all patients undergoing pH testing regardless of whether they were subsequently referred for surgery. Because all patients in our study group underwent operative management, this group would be expected to include the patients with the most severe disease. Those patients with mild or infrequent reflux symptoms are not likely to be referred for surgical therapy, and thus our patient population is biased toward those with more severe disease.

Although all groups had a similar prevalence of symptoms preoperatively, at a mean follow-up of 18 months postoperatively, there were significant differences in symptom relief. Across all categories examined, there was a clear trend: patients with upright reflux had the highest incidence of postoperative symptoms and patients with supine reflux had the lowest. The bipositional group achieved intermediate results. Although this pattern held true for typical symptoms of reflux, it was most significant for the atypical symptoms and side-effects of fundoplication. These differences seen in symptom-specific reporting were corroborated by the differences seen in terms of global patient satisfaction. When patients were asked about their overall sense of improvement in gastric

and esophageal symptoms, the patients with upright reflux reported significantly less improvement. More important, when asked if, in retrospect, they favored having had surgery for their symptoms, patients with upright reflux were less enthusiastic than patients who had supine or bipositional reflux.

Although our data support the traditional notion that patients with upright reflux do not do as well after antireflux surgery, several other studies have reported equivalent outcomes.[6,19,20] Mughal et al.[19] compared postoperative symptoms by Visick grade in 43 patients with upright reflux to the entire group of 126 patients and found no significant differences. Although they found a full 10% of patients with upright reflux had absolutely no relief of symptoms, this was not significantly different from the results of the entire group. Because they did not directly compare the results of those with upright reflux to those with supine/bipositional reflux, it is problematic to conclude that these groups have equivalent results. Gillen et al.[20] reported the results in six patients with upright reflux who underwent Nissen fundoplication and found that one patient (17%) had no relief of symptoms. Because of the small sample size in this study, no clear conclusions about the clinical outcomes can be drawn. The largest
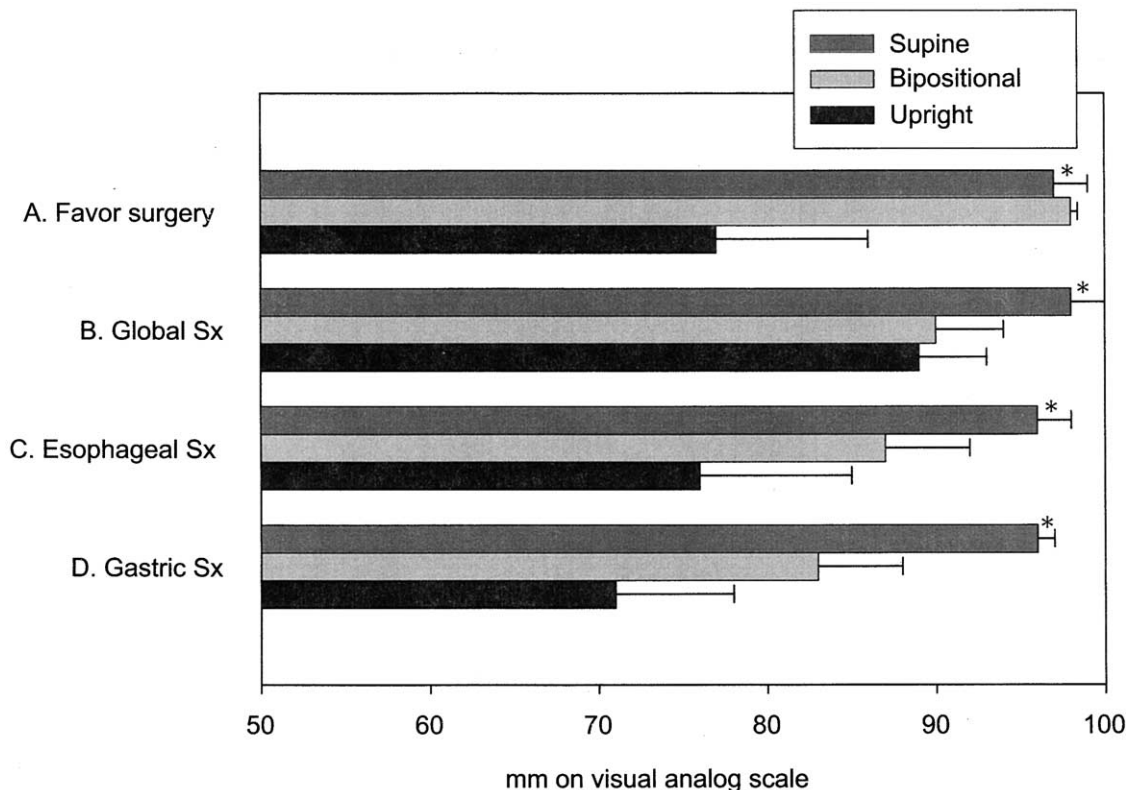
Fig. 4. Global satisfaction after surgery in relation to positional pattern of reflux. Using a 100 mm visual analog scale, patients were asked the following questions at postoperative evaluations: (*A*) "Knowing what you know now about the treatment options for your esophageal problem, indicate how strongly you favor surgical therapy?" (*B*) "Where do you see yourself from the standpoint of your esophageal symptoms now compared to prior to your surgery?" (*C*) "How have you felt overall from the standpoint of your esophagus?" and (*D*) "How have you felt overall from the standpoint of your stomach?" In each case, 100 mm indicated strongly favor (*A*), 100% better (*B*), or completely well (*C, D*). Error bars represent mean ± SEM. * $P \leq 0.05$ for Student's t test for upright reflux vs. supine reflux.

study, to date, to examine this issue compared 21 patients with upright reflux to 31 patients with supine and 45 with bipositional reflux.[6] When the prevalence of postoperative symptoms, including heartburn, regurgitation, dysphagia and bloating, was examined, no significant differences were found between groups. There are several potentially important differences between that study and the present one. First, follow-up information was only obtained in 85% of patients at a mean of 12 months. In addition, the criteria used for classifying patients into reflux groups used more extreme acid exposure times (>8.4% in the upright position and <3.4% in the supine position). As a result, some patients classified in the present study as patients with upright reflux would have been classified as patients with either supine or bipositional reflux in that study, making any differences between groups less marked.

An important question to consider is why patients with upright reflux might have less favorable out-

comes after antireflux surgery. One potential reason is that the fundoplication failed to control reflux effectively. Although we did not conduct routine postoperative pH testing as part of this study, a subgroup of patients early in our experience was routinely tested, with 97% of all patients having normalization of acid exposure times. In addition, other groups who have routinely tested patients after antireflux procedures have not found a difference in reflux control in patients with upright reflux.[2,20] Finally, 40% of our patients with upright reflux had some form of postoperative testing (26% pH testing and 14% upper gastrointestinal series) secondary to persistent symptoms, with abnormal findings in only one. This patient, who had had a partial fundoplication, had a positive pH test with a DeMeester score of 22.

A more likely explanation for the differences in postoperative outcomes relates to the difference in the underlying mechanism of reflux in these patient populations. It has been demonstrated that patients

with upright reflux have significantly lower crural pressures than patients with supine reflux.[18] Thus the patients with upright reflux are unable to prevent the natural shortening of the LES that occurs with gastric distention after meals. In addition, patients with upright reflux are thought to be more aerophagic at baseline and thus more prone to gastric distention.[21] This distention has been shown to be associated with an increase in the number of TLESRs[22] and is another important contributor to upright reflux.[13] When a fundoplication is performed, the ability of the stomach to relieve distention is significantly impaired. This is because of the wrap's ability to prevent the unfolding of the LES[23] and to decrease the frequency of TLESRs[24] when the stomach becomes distended. Although this effectively prevents reflux of acidic gastric fluid, it also inhibits the ability of the stomach to vent air, which may be especially problematic for the patient with aerophagic upright reflux. The result may be that after fundoplication, patients with upright reflux who are prone to gastric distention now have an impaired ability to relieve it. This likely explains the higher incidence of symptoms and lesser extent of overall improvement postoperatively that we have demonstrated.

Although we have shown significant postoperative differences in the outcomes between patients with upright reflux and patients with supine reflux, we do not mean to suggest that patients with upright reflux have unacceptable operative outcomes. In fact, when asked simply if they were symptomatically "better," "unchanged," or "worse" postoperatively, 100% of patients with upright reflux reported that they were "better." We have simply detected a difference in only the extent of that improvement. We conclude, therefore, that a pattern of upright reflux does not contraindicate LARS but does mandate appropriate preoperative counseling. Both the patient and the surgeon should have realistic expectations regarding the likelihood of complete relief of symptoms and the probability of associated side-effects before deciding to proceed with operative therapy. In addition, this type of information may be helpful in the development of a model, which considers all preoperative predictors, to help estimate the likely symptomatic outcome after surgical therapy.

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Discussion

Dr. D.W. Rattner (Boston, MA): In light of your group's presentation yesterday, which showed that patients with no evidence of reflux disease (NERD) did equally well as patients with GERD, I wonder how you justify with your findings that patients who have upright reflux were essentially NERD patients and the ones with supine reflux were GERD patients. I wonder if you can explain that dichotomy to us.

Dr. E.R. Winslow: There are two issues involved. The first is that, if you just look at erosive esophagitis, yes or no, there was a similar percentage in both the upright and the supine reflux groups. So, we did not actually find a difference between groups in terms of simply "esophagitis." What we did find was that there was a higher percentage of patients in the supine reflux group with high-grade esophagitis, which we did not address in our other study. The other issue is that most studies that have been able to show a higher percentage of esophagitis in patients with supine reflux have looked at all patients who undergo pH testing, not just those who are eventually operated on. So, I think that is why we were not able to show in either study that supine reflux is highly associated with esophagitis of any grade, as many others have been able to demonstrate. We were obviously looking at a different group of patients who had been selected for surgery and, therefore, were quite similar in terms of disease severity and operative outcome.

Dr. L.W. Way (San Francisco, CA): My question is how did the postoperative status of the patient correlate with pH monitoring and studies to assess the integrity of the wrap? Do you conclude, for example, that the symptoms that were not improved were not due to reflux or that there was some failure of the operation?

Dr. Winslow: We do not routinely perform postoperative pH testing unless there is a reason to do so, but in the group of patients with upright reflux who continue to have symptoms, nearly 40% underwent some postoperative study to evaluate symptoms, most of which were pH tests. Only one patient in that group had a positive test—a pH test with a DeMeester score of approximately 20—and this was a patient who had a Toupet fundoplication. No patient in the group with upright reflux had an anatomic failure. So we think most patients who have symptoms do not actually have reflux, and plenty of other groups, particularly the group from the University of Southern California, have shown that patients with upright reflux are not more likely to have reflux with an intact wrap. We think that this is more likely due to a postoperative phenomenon—gastric distention—which patients with upright reflux are more prone to, and they cannot get adequate relief postoperatively. So they may have symptoms such as heartburn, chest pain, and bloating with gastric distention, although they probably do not have acid exposure in the distal esophagus.

Dr. V. Velanovich (Detroit, MI): What were the results of esophageal manometry in patients with upright compared to those with supine reflux? I am wondering specifically whether those with upright reflux have a component of a functional esophageal disorder rather than pain coming from the acid reflux, per se?

Dr. Winslow: We have actually been interested previously in some of the hypomotility disorders of the esophagus and consider patients with this disorder to be in a group with a sensitive esophagus. However, there really was no difference in terms of that feature between groups, which I did not show you. What we did find in terms of the manometric findings was that patients with upright reflux seemed to have better peristaltic performance and less hypomotility. But, in terms of the sensitive esophagus, we do not think there is a difference.

Invited Discussion—Expert Commentator

Dr. David W. Rattner: Dr. Winslow and colleagues have shed light on the vexing question of why some of our patients with GERD do better than others after a technically successful operation. Some excellent studies have already been published on the results of antireflux surgery for atypical symptoms, patients with associated conditions

such as irritable bowel syndrome and personality disorders, and other wrinkles encountered in our practices. However, I believe every one of us has had the experience of performing a straightforward laparascopic Nissen fundoplication on a seemingly ideal surgical candidate, yet the outcome is less than perfect despite proof that the operation was technically successful, as shown by a postoperative 24-hour pH study.

This study is a model for a careful and systematic dissection of an important clinical problem. Many times we receive the results of a 24-hour pH probe study only as a DeMeester score, or "positive test" without paying attention to the pattern of reflux relative to recumbency. After hearing this presentation and reading the manuscript, I am certain that all of us will focus on identifying those patients who have upright reflux. One must wonder if there is a difference in the psychological makeup, as well as the physiology, of patients who have primarily upright reflux. Clearly these patients showed physiological improvement, yet they were much less satisfied with the results of surgery. I believe this will be a landmark paper and urge all of you not just to read it, but to study it when it is published.

Slow Progression of Periampullary Neoplasia in Familial Adenomatous Polyposis

Kouros L. Moozar, M.D., Lisa Madlensky, M.Sc., Terri Berk, M.S.S.A., Steven Gallinger, M.D.

Variable endoscopic surveillance protocols and treatment strategies have been proposed for periampullary neoplasia in familial adenomatous polyposis (FAP), primarily because of the lack of long-term, prospective natural history data. A total of 115 patients with FAP were followed prospectively for 10 years with periodic side-viewing upper gastrointestinal endoscopy by a single surgeon. The appearance of the duodenum was classified as stages 1 to 5. Statistical analysis included one-way analysis of variance for age comparisons between stage groupings and Kaplan-Meier analysis for the lifetime risks of having a particular stage of duodenal polyposis. Eighty-seven patients had multiple endoscopies over an average of 6.6 years. Thirty-three subjects had a change in stage, within an average time of 3.9 years at an average age of 41 years. The risk of having stage 3 or 4 duodenal neoplasia increased exponentially after the age of 40. The degree of dysplasia did not correlate with stage at initial classification. Progression of neoplasia in the duodenum of patients with FAP is slow. The severity of duodenal polyposis increases with age and is not influenced by the initial stage. The average time for progression of adenoma to carcinoma is likely long. (J Gastrointest Surg 2002;6:831–837.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

Key words: Familial adenomatous polyposis, periampullary polyps, duodenal polyps

There is a spectrum of extracolonic disease seen in patients with familial adenomatous polyposis (FAP) that includes clinically innocuous lesions such as benign retinal pigmentation to more serious conditions such as locally invasive desmoid tumors and periampullary malignancies.[1–3] Periampullary adenocarcinoma is a leading cause of death among patients with FAP.[4,5] Duodenal adenomas are predominantly concentrated on or around the major papilla,[6,7] and it is estimated that approximately 3% to 5% of patients with duodenal adenomas will develop periampullary adenocarcinoma.[8–10] As in colorectal cancer, it is generally believed that ampullary and periampullary adenocarcinoma in FAP arises from benign adenomatous precursor lesions. Because many patients with FAP develop duodenal polyps, but only a small fraction will develop invasive cancer, the clinical management of the duodenum in patients with FAP remains problematic.

For these reasons, regular endoscopic surveillance of the upper gastrointestinal tract in patients with FAP is recommended. However, there is no consensus on either the management of duodenal disease or the surveillance intervals. In this study, a prospectively designed upper gastrointestinal surveillance program was used to assess the natural history of duodenal neoplasia in patients with FAP.

PATIENTS AND METHODS

A total of 115 patients with FAP who had undergone colectomy were identified from the records of the Familial Gastrointestinal Cancer Registry at Mount Sinai Hospital in Toronto. These patients were followed prospectively, over a 10-year period, using a previously described upper gastrointestinal surveillance protocol[8] (Table 1). In general, we advise beginning upper gastrointestinal surveillance by age 25 years, although patient compliance is variable.

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Table 1. Staging and management of duodenal polyposis in FAP[8]

Stage	Size (mm)	Histologic findings	Management
1	0	Normal	EGD every 5 years
2	1–2	Adenoma	EGD every 3 years
3	2–10	Adenoma	EGD every 6 months
4	>10	Adenoma	Resection*
5	Any polyp/mass	Adenocarcinoma	Radical surgery†

EGD = esophagogastroduodenoscopy.

*For subjects with stage 4 disease, polyps are removed endoscopically by an interventional endoscopist. If polyps are not amenable to endoscopic treatment, they are removed through an operative transduodenal approach.

†Pancreaticoduodenectomy is usually offered to patients with stage 5 duodenal disease.

All endoscopic examinations were performed with a side-viewing video endoscope. Multiple biopsy specimens were routinely taken from grossly normal duodenal mucosa, occasionally from the ampulla of Vater, and from visible lesions. Still photographs and videotapes were used routinely to facilitate comparisons of the duodenal mucosa between endoscopic sessions.

Statistical analyses included one-way analysis of variance for the age comparisons between staged groups and Kaplan-Meier analysis to determine the lifetime risks for a particular stage.

RESULTS

All patients who had undergone at least one upper gastrointestinal endoscopy were included in this analysis. There were 54 men (47%) and 61 women (53%). The average age at first side-viewing upper endoscopy was 39.1 years (range 16 to 69 years). The duodenal polyposis stages were significantly different, depending on the patient's age at first endoscopy for patients with stage 1 (37.3 years) and stage 4 (54.0 years) disease ($P = 0.02$), as well as for those with

Table 2. Average age and stage of duodenal disease in patients with FAP at first endoscopy

Stage at first endoscopy	No. of patients	% Total (n = 115)	Average age (yr)
1	72	63	37.3
2	26	23	37.3
3	10	9	46.5
4	7	6	54.0
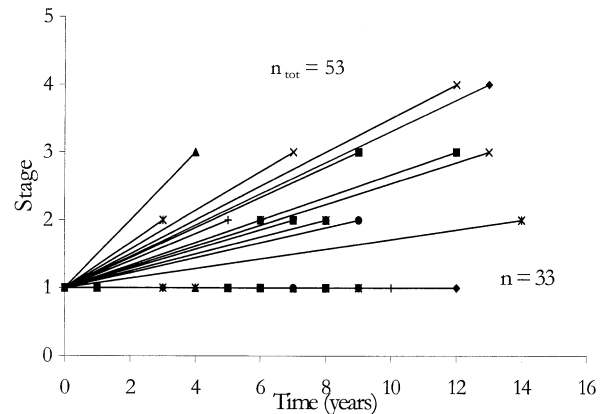
Fig. 1. Stage progression of periampullary polyps in patients with FAP who had stage 1 disease at initial side-viewing upper gastrointestinal endoscopy (n = 53). The duodenal disease in most of these patients (n = 33) remained unchanged, highlighting a very slow trend of progression. Each line and symbol represents a single patient, although some lines overlap.

stage 2 (37.3 years) and stage 4 (54.0 years) disease ($P = 0.03$) (Table 2).

Of the 115 patients, 87 (76%) had at least two endoscopies and were followed for an average of 6.6 years (range 1 to 10 years). Of these patients, 33 (38%) had a change in stage, in an average time of 3.94 years (range 0 to 13 years) and at an average age of 40.9 years (range 22 to 74 years). There were no significant differences when comparing average time to first stage change across groups categorized by stage at first endoscopy. The stage at first endoscopy did not correlate with the degree of dysplasia in the periampullary or duodenal adenomas.

One patient, a 49-year-old man with stage 4 duodenal disease, had repeated biopsies that showed severe dysplasia. A pancreaticoduodenectomy was performed with no evidence of malignancy in the resected specimen. The patient was well for 7 years after surgery, but his health declined quite rapidly after that, and he died. Widespread hepatic metastases were noted in the small bowel at autopsy from a primary lesion in the proximal jejunum.

When stages of duodenal polyposis are compared among patients undergoing two or more upper endoscopies, there is a trend toward a slow progression from stage 1 or 2 to more advanced stages. Most significantly, most patients did not progress to more advanced stages (Figs. 1 and 2). Germline adenomatous polyposis coli gene mutations have been identified for most of these subjects, and we have not noted a genotype/phenotype correlation associated with stage of duodenal polyposis or progression over time.

The Kaplan-Meier curves indicate an approximately linear progression of lifetime risk of having stage 2 dis-
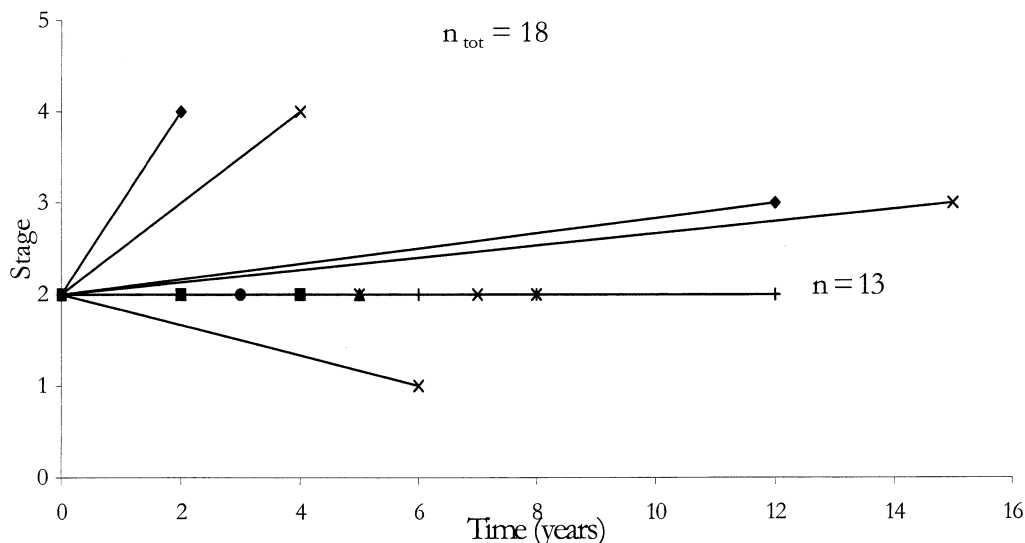
Fig. 2. Stage progression of periampullary polyps in patients with FAP who had stage 2 disease at initial side-viewing upper gastrointestinal endoscopy (n = 18). The duodenal disease in most of these patients (n = 13) remained unchanged.

ease (Fig. 3). The lifetime risk of having stage 3 or stage 4 disease gradually increases in the first three decades, followed by a sharp increase after the age of 40 (Figs. 4 and 5). This risk approaches 90% by the eighth decade, although none of these patients developed periampullary cancer during the 10-year surveillance interval of this study. The ages at 50% risk are 48 years for stage 2 disease and 56 years for stage 3 (see Figs. 3 and 4). The risk of having stage 4 duodenal polyposis does not reach 50% (see Fig. 5).
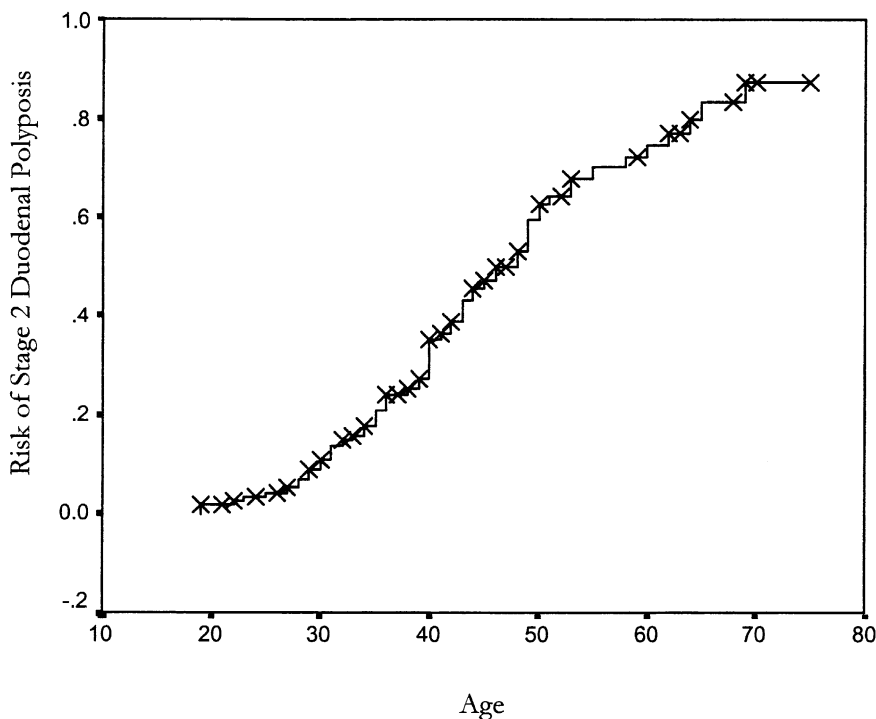
Fig. 3. Risk of having stage 2 disease. A linear trend is observed for the risk of having stage 2 disease in patients with FAP. By the age of 75, this risk is close to 90%.
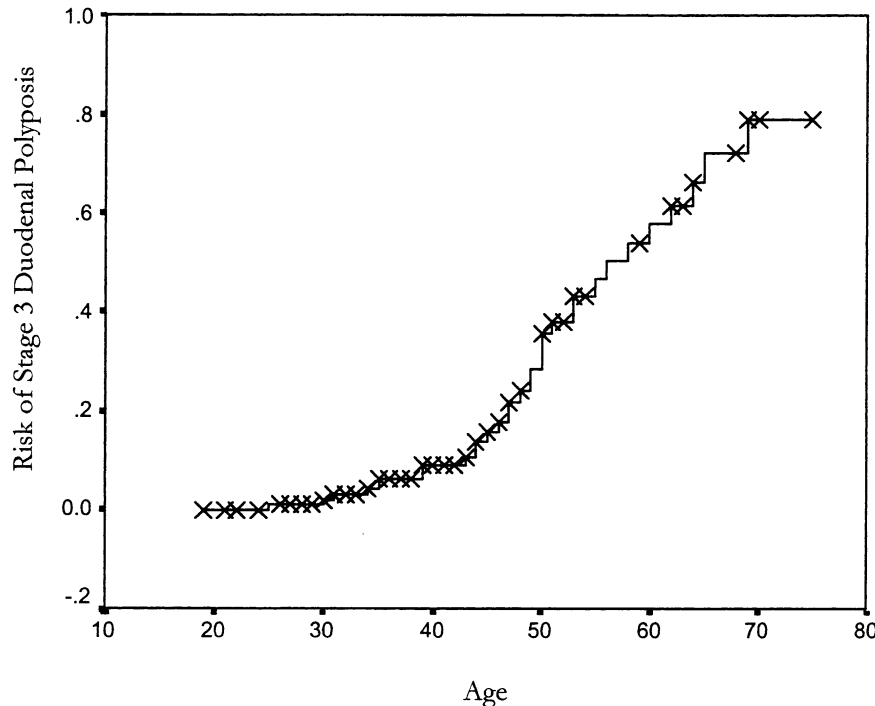
Fig. 4. Risk of having stage 3 disease. There is a slow increase in the risk of having stage 3 disease in the first three decades. This risk rises after the age of 40.

Because only 18 of these 115 patients are currently over the age of 65 years, an accurate prediction of risk for progression to frank malignancy in older patients with FAP is not possible at this time.

DISCUSSION

Clinic-based FAP registries have been instrumental in highlighting the value of secondary cancer prevention by implementing regular colonoscopic surveillance and utilizing new research findings in this rare disease. Although endoscopic surveillance protocols for FAP have been successful in reducing the mortality rate for colorectal cancer, the same is not true for upper gastrointestinal cancer, as shown by Jagleman et al.[10] who reported 39 cases of duodenal malignancy among 10 registries in 1988.

More than a decade later, there is no consensus on the frequency of upper endoscopy, which varies from 3 years for a negative result to annually, depending on morphologic and histologic findings.[1,11] The main reason for the differences in the protocols used among registries remains our poor understanding of the natural history of upper gastrointestinal neoplasia in FAP. Even though the adenoma-to-carcinoma sequence has been quite clearly defined for colorectal neoplasia, the corresponding sequence has not been as well charac-

terized in the periampullary region of patients with FAP.[12,13]

The relationship between worsening upper gastrointestinal polyposis and advancing age is not unique to the current study. Goedde et al.[14] reviewed 20 (67%) of 30 cases of upper gastrointestinal adenomas in the Roswell Park registry, two of which were diagnosed in patients aged 49 and 53 years, respectively. Debinski et al.[15] found that 35% of 200 cases of FAP in a British Registry manifested advanced duodenal disease and that age greater than 40 years was a risk factor. Bulow et al.[16] reported on FAP patients with duodenal adenocarcinoma in five Scandinavian registries and found eight cases that were diagnosed at a mean age of 47 years. In our series, the risk of having at least stage 2 disease approached 90% by the age of 75 years (see Fig. 3). Older patients showed an increase in the number and size of their adenomas. However, the relatively unimportant clinical consequence of mild (stage 2) duodenal polyposis is worth pointing out because the majority of FAP patients with stage 2 disease will not have symptoms or progression to malignancy. Interestingly, the relationship between stage 3 or higher duodenal disease and age did not follow a linear trend. As shown in Figs. 4 and 5, the cumulative risk for having stage 3 or higher disease increases slowly in the first three decades followed by a more rapid rise after the age of 40. By the age of 75 years,
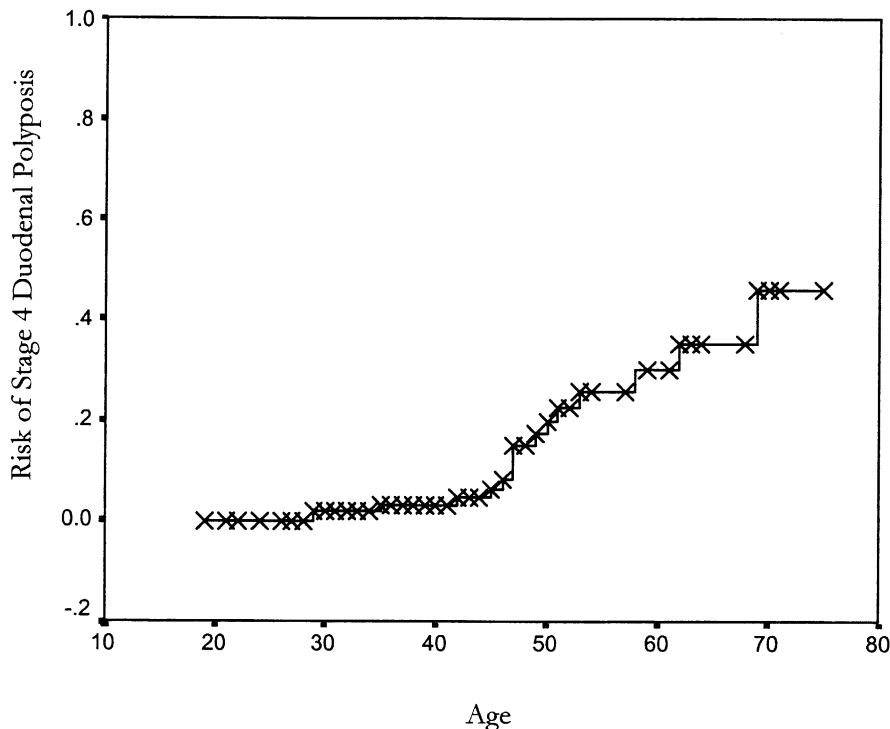
Fig. 5. Risk of having stage 4 disease. Similar to Fig. 4, there is a slow increase followed by a rapid increase in risk after the age of 40.

the risk for stage 4 disease was 45%. Furthermore, the average age at which a change in stage was noted was 41 years, regardless of the stage at the initial endoscopy. The average time for this change was 4 years, a pattern not reported previously.

In an analysis of the trend of stage progression in patients who had at least two upper endoscopic examinations, 53 patients were initially classified as having stage 1 findings (corresponding to time zero) (see Fig. 1). It is important to note that 33 of these patients (62.3%) did not show any progression of periampullary disease during a 10-year follow-up. Thirteen (72%) of 18 patients initially classified as having stage 2 disease did not change stage over the same interval (see Fig. 2). Sawada et al.[17] reported similar findings in patients with small duodenal adenomas over a 5-year observation period (age range 16 to 46 years). However, those with progression to malignancy evolved over the same period (age range 28 to 44 years). In a recent retrospective study of 180 patients with FAP, Björk et al.[18] proposed that severe polyposis was associated with a malignant course of periampullary adenomatosis. None of the patients in our series developed duodenal adenocarcinoma. However, one patient died of metastatic jejunal cancer 7 years after a pancreaticoduodenectomy for duodenal carpeting with dysplastic adenomas. Postoperative pathologic examination failed to identify any malignancy in the pan-

creaticoduodenectomy specimen. In agreement with our findings, Matsumoto et al.[19] monitored ampullary changes in 14 patients with FAP over an 8-year period and found little evidence of progression and none of malignancy. Two earlier studies by Burke et al.,[20] from the Cleveland Clinic Registry, and Bertoni et al.[21] agreed with these findings in reports of 130 cases. However, a recent report of a 10-year prospective follow up of 114 patients with FAP[22] found six cases of duodenal adenocarcinoma. Most of these patients had high-stage polyposis, and the investigators determined a 36% risk for developing cancer within this group.

With an estimated cumulative risk of 5% or less for developing duodenal cancer, the cost-effectiveness of performing frequent upper endoscopies has been questioned.[13] A lack of correlation has been suggested between endoscopic features and histologic findings and between progressive morphology and histologic findings.[20] This was reflected in our series where the degree of dysplasia did not correspond to the clinical stage, which is primarily based on the size of the adenoma.

According to the evidence presented in this study, we propose that the interval between upper gastrointestinal endoscopies should be increased, particularly in patients who are less than 40 years of age and have a less advanced stage of disease. Taken together, this natural history study demonstrates the very slow progression of periampullary adenomas in FAP. Although

we tend to treat FAP patients with advanced stage 4 adenomas by endoscopic polypectomy or surgical resection, our data raise questions concerning the value of aggressive surveillance in patients with minimal duodenal disease.[21]

The challenge for the future will be to determine appropriate management strategies for those patients with aggressive duodenal disease because local resection does not prevent recurrence, as illustrated by Penna et al.[23] and Heiskanen et al.[24] in 64 patients. Kadmon et al.[25] and Soravia et al.[26] demonstrated that malignancy may develop despite intensive surveillance since four cases of duodenal cancer were missed during follow-up. Groves et al.[22] proposed that patients with more advanced stages of duodenal polyposis should be considered for prophylactic pancreaticoduodenectomy. Despite the early disappointing results with the use of sulindac as a chemopreventive or therapeutic agent for treating duodenal neoplasia in FAP, other novel pharmacologic approaches such as cyclooxygenase-2 (COX-2) inhibitors may be useful,[27–30] particularly in the early stages of duodenal polyposis and in younger members of families with a higher risk of developing duodenal malignancy.

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Discussion

Dr. G.V. Aranha (Maywood, IL): I enjoyed your presentation. I realize that none of these polyps turned out to be carcinoma, but I would like to know whether endoscopic ultrasound would play any role in the follow-up of these patients?

Dr. K. Moozar: That is a good question, and I think it will. We have not yet begun to use endoscopic ultrasound at our institution for patients with FAP. But I think it is something that we should look into.

Dr. H.A. Pitt (Milwaukee, WI): As you know, these patients develop colon cancer first, and then they develop duodenal cancer. If they survive all that, they develop proximal jejunal polyps and jejunal cancer. Do you have any data on the jejunal polyps in your patients?

Dr. Moozar: We actually do, and since the time I submitted the abstract, we have had one patient who died of metastic jejunal disease in the afferent loop approximately 8 years after a Whipple resection for diffuse severe dysplasia in the duodenum.

Dr. J.B. Matthews (Cincinnati, OH): I share your conservatism in surveillance strategy. What happened to histology in all of this? The current standard has it that the histologic results of the endoscopic biopsy are critical to decision-making—if there is severe dysplasia, we would recommend resectional therapy. What were the histologic findings in your patients in these groups, and how did they match up with the rest of our analysis?

Dr. Moozar: When we looked at the histologic findings in our cohort, the degree of dysplasia did not seem to correspond to the size of the polyp or the progression of the polyp. So at least in our cohort, histologic findings did not play that big of a role. Occasional patients have severe dysplasia in large duodenal adenomas, but most have low-grade dysplasia. We did have one patient with severe dysplasia for whom we recommended a prophylactic Whipple procedure, and he agreed to it; that was the patient who actually died of the jejunal disease many years later. But otherwise we do not see much correlation between histologic findings and polyp size in our patients.

Dr. H.J. Sugerman (Richmond, VA): Some studies have shown that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) will prevent or retard adenomatous polyp formation. Now that you have reviewed these data, have you developed a protocol for the use of NSAIDs to prevent the development of duodenal polyps? We had one patient who required a combined Whipple–ileoanal pouch procedure because of extensive colonic and duodenal polyposis with a duodenal adenocarcinoma. Prevention of duodenal polyps might obviate the subsequent need for a Whipple procedure in a patient who has had a prior colectomy/proctectomy with an ileoanal pouch.

Dr. Moozar: We do participate in the NSAID trials. I am aware of the recent publications, as well as the one that was just published in *Gut*. We published a report showing the lack of effect of sulindac in severe duodenal polyposis. Our current strategy is to try high-dose Celebrex in patients with severe disease. It is too early to tell whether we are seeing a beneficial effect.

Selective Cyclooxygenase-2 Inhibitor Rofecoxib (Vioxx) Induces Expression of Cell Cycle Arrest Genes and Slows Tumor Growth in Human Pancreatic Cancer

William W. Tseng, B.A., Adriana Deganutti, M.S., May N. Chen, B.S., Romaine E. Saxton, Ph.D., Carson D. Liu, M.D.

Recent studies indicate that cyclooxygenase-2 (COX-2) is overexpressed in pancreatic adenocarcinoma and may play a critical role in this rapidly progressing form of cancer. A human pancreatic adenocarcinoma cell line, Mia PaCa-2, was incubated for 18 hours with 5 μmol/L of rofecoxib (Vioxx), a selective COX-2 inhibitor. Total RNA was isolated and gene expression analyzed by DNA microarray chips. In a separate experiment, athymic mice were orthotopically injected with 7.5×10^5 Mia PaCa-2 cells through a minilaparotomy. After 1 month, laparotomy was repeated to measure tumor size, and mice were randomized to receive reformulated rodent chow containing either 12.5 mg/kg/day of rofecoxib or no drug for 21 days. Tumor growth was assessed by comparing volume before and after treatment. In vitro, rofecoxib decreased gene expression of cyclin D1/PRAD1, a key component of cell cycle progression, while increasing expression of several cell cycle arrest genes, including p21/WAF1, p33/ING, GADD34, and GADD45 ($P < 0.05$). In vivo, tumor growth was significantly reduced in treated vs. control mice ($P < 0.05$). No systemic toxicity was observed in mice receiving rofecoxib. These data suggest that rofecoxib slows the growth of human pancreatic cancer through changes in gene expression that favor cell cycle arrest.   (J GASTROINTEST SURG 2002;6:838–844.)   © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Pancreas cancer, cyclooxygenase-2, rofecoxib, microarray, cell cycle

Ductal adenocarcinoma of the pancreas is associated with an overall 5-year survival rate of less than 5%, the lowest of any solid malignancy. Despite advances in treatment, overall survival has not improved for the past three decades.[1,2] Pancreaticoduodenectomy (Whipple procedure) offers the only chance for cure; however, median survival after resection is only 18 to 20 months. In addition, up to 90% of patients present with an advanced, inoperable disease state at the time of diagnosis.[1] Palliative options involve surgery, chemotherapy, and radiation but often lead to increased morbidity and do little to prolong survival.[1] Clearly, the development of more effective treatment is needed to improve the outlook for this devastating form of cancer.

Cyclooxygenases are enzymes that catalyze the conversion of arachidonic acid to various prostaglandins and thromboxanes and therefore play a key role in inflammation.[3,4] Two isoforms of the enzyme have been identified, and studies within the past decade provide evidence that overexpression of cyclooxygenase-2 (COX-2), but not COX-1, is induced in several types of human cancers. Although most extensively studied in colorectal cancer, selective overexpression of COX-2 has also been reported in primary cancers of the breast, prostate, lung, liver, and upper alimentary tract.[4] COX-2 may promote tumor growth by generating prostaglandins that inhibit apoptosis and stimulate angiogenesis; however, the precise mechanisms have not yet been clearly elucidated.[4]

Recent studies indicate COX-2 is also overexpressed in ductal adenocarcinoma of the pancreas compared to normal pancreatic tissue, and it may play a critical role in this rapidly progressing form of cancer. With the use of immunohistochemical analysis, Western blotting, and reverse transcription–polymerase chain re-

action, COX-2 expression has been detected in 56% to 100% of pancreatic adenocarcinomas, with 44% to 100% of samples characterized as having high expression relative to adjacent normal tissue.[5–11] COX-2 mRNA and protein have also been demonstrated in a number of human pancreatic cancer cell lines, and preliminary in vitro studies have achieved growth inhibition with the use of various nonselective COX and selective COX-2 inhibitors.[8,12–14] To the best of our knowledge, no previous studies have reported therapeutic effects of a selective COX-2 inhibitor on human pancreatic cancer growth in vivo.

Rofecoxib is an orally administered, selective COX-2 inhibitor that has been recently approved by the U.S. Food and Drug Administration for symptomatic relief in osteoarthritis. Because it does not inhibit the COX-1 enzyme at therapeutic dosages (25 to 50 mg/day), rofecoxib is associated with a markedly lower incidence of platelet dysfunction, gastrointestinal ulceration, and bleeding, as well as an overall reduced toxicity profile, compared to traditional nonsteroidal anti-inflammatory drugs.[3] Our laboratory recently reported that rofecoxib induced a dose-dependent growth inhibition in human pancreatic cancer cells in vitro and inhibited production of secreted vascular endothelial growth factor, a key component of tumor angiogenesis.[13] With the use of an orthotopic model of human pancreatic cancer grown in athymic mice established by our laboratory,[15] we sought to evaluate the in vivo efficacy of rofecoxib and to correlate these findings with changes in gene expression on the cellular level.

MATERIAL AND METHODS
Cell Culture

Mia PaCa2, a human pancreatic ductal adenocarcinoma cell line (American Type Culture Collection, Rockville, MD), was cultured in monolayers at 37° C in a humidified, 5% CO_2 incubator using Dulbecco's modified Eagle medium (Life Technologies, Rockville, MD) supplemented with 10% fetal calf serum (Omega Scientific, Tarzana, CA), 0.29 mg/ml L-glutamine, 0.15% sodium bicarbonate, 1 mmol/L sodium pyruvate, 50 μg/ml gentamicin, 100 U/ml penicillin, 100 U/ml streptomycin, and 0.25 mg/ml fungizone solution (Omega Scientific). Cells were detached with 0.25% trypsin-EDTA and subcultured approximately two times per week when a confluent monolayer was obtained.

Gene Expression

Mia PaCa-2 cells were incubated for 18 hours with 5 μmol/L of rofecoxib (Vioxx; Merck, Whitehouse Station, NJ), while control cells received no drug. Total RNA was isolated from both groups (RNeasy Kit; Qiagen, Valencia, CA) and dsDNA was synthesized (Superscript Kit; Gibco-BRL, Rockville, MD) before transcription as biotin-labeled cRNA (Enzo Bio-Array Kit; Affimetrix, Santa Clara, CA). cRNA was fragmented and hybridized with a large panel of human gene oligonucleotide DNA sequences arranged on microarray chips (AU-95 GeneChip Kit; Affimetrix). Chips were stained by means of streptavidin-phycoerythrin, and fluorescence intensity was quantified via GeneChip software. Gene expression was recorded as individual values, and then calculated as a ratio of treated to control cells and normalized vs. two constitutive "housekeeping" genes.

Mouse Study

Protocols involving athymic mice and human cancer cells were approved by the Animal Research Committee at the UCLA Medical Center. Six- to 7-week-old female athymic nu/nu BALB/c mice (Simonsen Laboratories, Santa Clara, CA), weighing 23 to 25 g, were quarantined for 7 days before being transferred to a separately ventilated, germ-free vivarium room.

Orthotopic Model of Human Pancreatic Cancer

Mia PaCa-2 cells were grown to near-confluence before harvesting. Cell viability greater than 90% and cell count per milliliter were determined by trypan blue exclusion on a microscope hemocytometer slide. Athymic mice were anesthetized by means of intraperitoneal injection of 0.2 ml/20 g sodium pentobarbital (7 mg/ml in 10% ethanol) and placed on a warming pad to prevent hypothermia. The level of anesthesia was assessed by toe pinch and respiratory rate. A midline minilaparotomy was performed, and the stomach, spleen, and pancreas were mobilized. The splenic pancreas was exteriorized and isolated from the abdominal viscera using a cut 2 × 2 inch sterile gauze. With the use of a 0.3 ml syringe (Becton Dickinson, Franklin Lakes, NJ), 7.5×10^5 cancer cells were injected as a stable 100 μl bolus into the pancreatic parenchyma, with special care taken to ensure no leakage into the peritoneal cavity. The abdominal wall and skin incisions were closed separately using a 4-0 monofilament suture, and the mice were allowed to recover. Separate experiments in our laboratory have determined that the rate of tumor formation and distant metastases is directly dependent on the number of cells injected (unpublished data).

Rofecoxib Treatment Regimen

A previous study reported that in mice with colorectal polyps, oral administration of 14.7 mg/kg/day of rofecoxib produced plasma concentrations that approximate those seen in humans taking 25 mg/day of rofecoxib therapeutically for osteoarthritis.[16] At this dosage, end-point efficacy was reached (significant decrease in polyps formed) without any evidence of systemic toxicity. For our in vivo study, mice were fed rodent chow reformulated to contain either rofecoxib or no drug. Rodent chow was replaced on a daily basis, and the amount of chow remaining each day, if any, was recorded for the duration of the study. Final dosages of rofecoxib in the treatment group were approximately 12.5 mg/kg/day.

Assessment of In Vivo Tumor Growth With Rofecoxib Treatment

The in vivo study protocol is outlined in Fig. 1. In mice previously inoculated with Mia PaCa-2 cells that had palpable masses in the left upper quadrant 1 month later, a repeat laparotomy was performed. Tumors were measured via caliper and volumes were calculated ($V = 0.52 \times L \times W \times H$). Mice were identified by intraoperative ear punch and allowed to recover; those with similar tumor volumes were then randomized into treatment (rofecoxib, 12.5 mg/kg/day; $n = 7$) and control (no drug; $n = 6$) groups. Twenty-one days after treatment, all mice underwent laparotomy and changes in tumor volume were

determined. During the study, systemic toxicity in both treated and control mice was monitored using weekly body weight, and general health/behavior assessment was performed frequently.

Statistical Analysis

Changes in gene expression, as determined by DNA microarray analysis, were considered significant if treatment to control ratios exceeded ±2.0. Changes in tumor volume were expressed as ratio of volume at $t = 21$ days to $t = 0$ days for each mouse, and mean values were obtained for each group. Data were expressed as mean ± SEM, and statistical significance ($P < 0.05$) was determined using Student's t test.

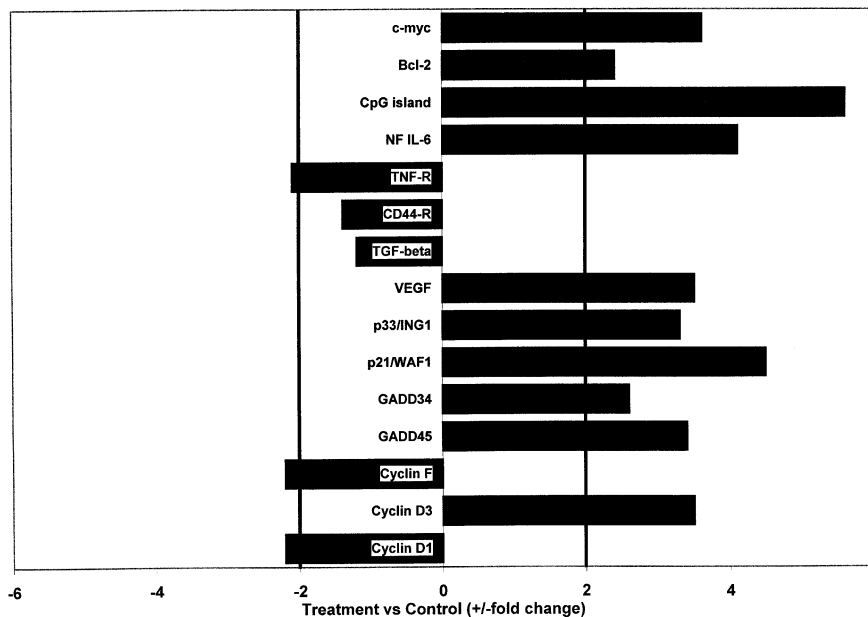## RESULTS

In Vitro Changes in Gene Expression With Rofecoxib Treatment

Mia PaCa-2 cells incubated with 5 μmol/L rofecoxib for 18 hours had a statistically significant change in expression of key genes involved in cell proliferation, apoptosis, cell adhesion, angiogenesis, and cell cycling (Fig. 2). Statistically significant changes were observed in c-myc (+3.6-fold), bcl-2 (+2.4), CpG island (+5.6), NF IL-6 (+4.1), TNF-R (−2.1), and vascular endothelial growth factor (VEGF; +3.5). Expression of other genes (CD44, TGF-beta) important in pancreatic cancer were also altered, but not to sta-
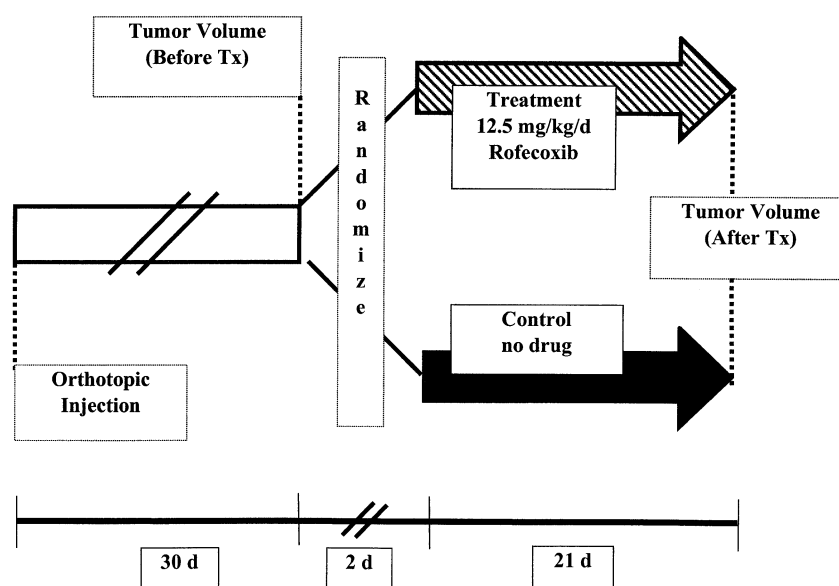
Fig. 1. In vivo study protocol. Tumor growth was assessed by comparing volume before and after 21 days of treatment with rofecoxib or no drug.

Fig. 2. In vitro changes in gene expression with rofecoxib treatment. Mia PaCa-2 cells were incubated with 5 μmol/L rofecoxib for 18 hours. Changes in gene expression were considered significant if treatment to control ratios exceeded ±2.0.

tistical significance. Among the human genes represented on the microarray chip (>8000 genes), the most consistent pattern of expression changes was noted with the cell cycle genes. Rofecoxib induced changes in gene expression in Mia PaCa-2 cells favoring cell cycle arrest. Compared to cells receiving no drug, cells incubated with the selective COX-2 inhibitor showed decreased expression of cyclin D1/PRAD1 (−2.2), a critical component enabling cell cycle progression. Consistent with this finding, several cell cycle arrest genes were upregulated with treatment including tumor suppressors p21/WAF1 (+4.5) and p33/ING1 (+3.3), as well as growth arrest genes GADD34 (+2.6) and GADD45 (+3.4).

Systemic Toxicity With Rofecoxib Treatment

No statistically significant difference in body weights between mice in the treatment and control groups was observed during the duration of the study. At 1 week (t = 7 days), average body weight in the treated mice was 22.8 ± 2.7 g vs. 23.3 ± 3.1 g in the control mice. At the conclusion of the study (t = 21 days), average body weight in the treated mice was 27.9 ± 3.7 g vs. 26.6 ± 2.5 g in the control mice. Increased tumor burden most likely accounted for the increase in average body weight as the study progressed. No mice in either the treatment or control group died prematurely or required euthanasia, defined by a loss of body

weight greater than 20%, presence of ascites, or observed reduction in mobility.

In Vivo Tumor Growth With Rofecoxib Treatment

Overall, tumor growth was significantly reduced in pancreatic cancer–bearing mice receiving rofecoxib at a dosage of 12.5 mg/kg/day (n = 7) vs. tumor-bearing mice receiving no drug (n = 6) (Fig. 3). When tumor volumes in each mouse were compared individually before and after treatment, mice receiving rofecoxib had an average 77.8% ± 20.8% increase in tumor size vs. 202.6% ± 39.6% increase in mice receiving no drug ($P < 0.05$). Average tumor volumes in treated mice increased from 484.1 ± 84.3 mm^3 to 936.5 ± 241.7 mm^3 vs. 408.3 ± 146.4 mm^3 to 1038.0 ± 296.5 mm^3 in control mice. On gross examination, tumors in rofecoxib-treated mice were not as well vascularized or locally invasive as those in the control group.

DISCUSSION

The results of this study provide evidence that in mice with orthotopic human pancreatic adenocarcinoma, rofecoxib significantly slows that rate of tumor growth without evidence of host systemic toxicity. To date, the majority of animal studies in which
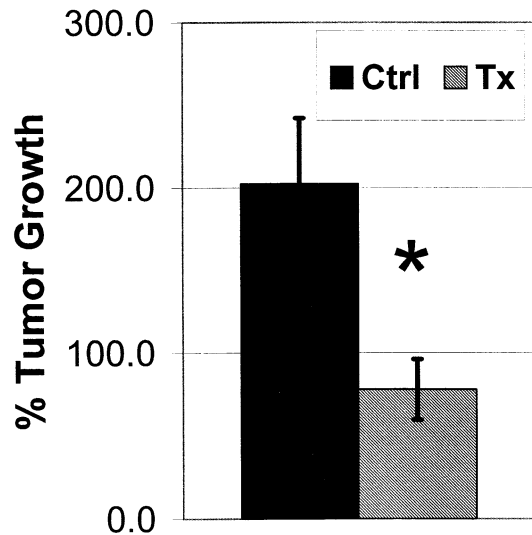
Fig. 3. In vivo tumor growth with rofecoxib treatment. Rate of tumor growth was significantly reduced in treated (n = 7) vs. control (n = 6) mice (*$P < 0.05$, Student's *t* test). Mice receiving 12.5 mg/kg/day of rofecoxib had an average 77.8% ± 20.8% increase in tumor size vs. 202.6% ± 39.6% increase in mice receiving no drug.

COX-2 inhibitors are used have been designed to evaluate prevention of carcinogenesis or malignant progression from precursor lesions, particularly in colorectal cancer.[4] One report also shows that the use of the nonselective COX inhibitors, indomethacin and phenylbutazone, reduces development of pancreatic cancer in a hamster model of toxin-initiated carcinogenesis.[17] A review of the literature suggests that the current study is the first to demonstrate the in vivo efficacy of rofecoxib, a selective COX-2 inhibitor, against established human pancreatic cancer.

We observed that the treatment efficacy of rofecoxib in pancreatic cancer occurred without obvious signs of systemic toxicity in mice at clinically useful drug dosages. The dosage regimen used in the treatment group (12.5 mg/kg/day) was chosen based on results of a previous study that demonstrated comparable plasma concentrations in mice receiving 14.7 mg/kg/day rofecoxib and in patients taking 25 mg/day for osteoarthritis.[16] Although generally safe, optimal dosages for rofecoxib are critical because supratherapeutic levels have been shown to cause both COX-2 and COX-1 inhibition.[3]

DNA microarray results of the current study suggest that rofecoxib may exert its primary antitumor effects through induction of a specific pattern of gene expression favoring cell cycle arrest. These results are in agreement with data reported earlier that in vitro treatment with an experimental COX-2 inhibitor, NS-398, leads to a significantly higher distribution of human pancreatic cancer cells in the G0/G1 phase of the cell cycle by flow cytometry.[14] This effect was found to be independent of COX-2 expression. Western blot analysis demonstrated that at the protein level, treated cells showed quantitatively lower amounts of several cell cyclins including cyclin D1/PRAD1. In addition, NS-398 increased the protein levels of p27, a member of the same tumor suppressor family as p33/ING1.[14] In our study, cyclin D1/PRAD1 expression was decreased 2.2-fold and p33/ING1 expression was increased 3.3-fold in rofecoxib-treated vs. control pancreatic cancer cells.

Two of the cell cycle genes affected by rofecoxib, cyclin D1/PRAD1 and p21/WAF1, are known to play a critical role in human pancreatic cancer.[18] Cyclin D1 is responsible for transition to the S phase (DNA synthesis) of the cell cycle, and overexpression is associated with malignant transformation. Overexpression of cyclin D1 has been shown to correlate with decreased median survival in patients with pancreatic cancer.[19] p21/WAF1 is directly activated by p53 and functions in arresting the cell cycle at G1. p21/WAF1 may also be responsible for the tumor suppressor effect of DPC4, a gene deleted or mutated in up to 50% of pancreatic cancers.[18]

Gene expressions of p33/ING1, GADD34, and GADD45 were also significantly altered with rofecoxib. Although these cell cycle genes have been extensively studied in other human malignancies, little is known about their direct role in pancreatic cancer. p33/ING1 is one of four tumor suppressor genes that bind to and activate p53. In addition to growth control, p33 has also been shown to play a role in promoting senescence and apoptosis.[20] Downregulation of GADD34 and GADD45 is postulated to be one common mechanism by which many types of cancer cells can escape growth arrest.[21] GADD45 may also act in concert with p33/ING1.[20] Together the downregulation of cyclin D1 and upregulation of p21/WAF1, p33/ING1, GADD34, and GADD45 may represent a novel pattern of cell cycle gene expression in pancreatic cancer induced by rofecoxib. Whether or not this pattern is specific to COX-2 inhibition or represents an alternative COX-2 independent pathway is unknown.

It is important to note that in this study, although rofecoxib significantly slowed tumor growth in drug-treated mice, it did not reduce tumor volume. The DNA microarray results also indicate that selective COX-2 inhibitor treatment induced possible compensatory, tumor growth–promoting responses in pancreatic cancer cells. In addition to cell cycle genes, statistically significant changes in gene expression were observed in proliferation responses (increased c-myc), antiapoptosis (increased bcl-2, decreased TNF-R), and angiogenesis (increased VEGF) (see Fig. 2). Reg-

ulatory cascades induced downstream of these individual gene responses remain to be determined. Our earlier results suggested that rofecoxib increases VEGF gene expression, but secreted VEGF protein is actually decreased.[13] In the current study, however, the in vivo results indicate that the overall net effect of the gene expression changes induced by rofecoxib is a reduction in tumor growth in rofecoxib-treated vs. control mice.

CONCLUSION

Rofecoxib, a selective COX-2 inhibitor, effectively decreases progression of human pancreatic tumors in vivo and may accomplish this primarily by inducing changes in gene expression that favor cell cycle arrest. Whether or not these effects are dependent on COX-2 expression is unknown. Recent reports have suggested synergistic effects of COX-2 inhibitors with gemcitabine[14] and ionizing radiation.[22] Whether alone or in combination therapy, rofecoxib may have clinical utility in the management of patients with unresectable or metastatic pancreatic cancer.

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Discussion

Dr. G Chandler (Boulder, CO): I would like to add some perspective to Dr. Tseng's nice presentation. Clinical interest in nonsteroidal anti-inflammatory agents as modulators of neoplastic growth was initiated 25 years ago by Dr. William R. Waddell, the second full-time surgical chairman at Colorado University. Dr. Waddell was using sulindac, a long-acting analogue of indomethacin, to treat a desmoid that had developed in the abdominal wall incision following a total colectomy and ileoproctostomy for Gardner's syndrome. The desmoid shrunk and, unexpectedly, all polyps in the residual rectum completely disappeared. Dr. Waddell then used sulindac in several additional patients with Gardner's syndrome, or familial polyposis alone, including some patients who had not had a colon resection. Sulindac caused remarkable polyp regression, most strikingly in the patients with an intact colon. When the drug was discontinued, the polyps tended to regrow, but regressed again if the sulindac was restarted. Dr. Waddell postulated that prostaglandins created a permissive environment for adenomatous cell division that was abated by cyclooxygenase blockade without directly affecting the underlying neoplastic process, hence the reappearance of growth when prostaglandin synthesis was allowed to resume.

Dr. M. Jaffe (New Orleans, LA): I was curious to know if you had carried out any studies in the in vivo model to assess whether or not there had been a change in the amount of inflammation as a result of the Vioxx. Were these purely changes in tumor cell volume or were they inflammatory changes that were decreased and thus accounted for a substantial decrease in the size of the tumors?

Dr. W.W. Tseng: No, we did not look at that. This is a very interesting point that we can look at in future studies.

Dr. T. Brentnall (Seattle, WA): You presented some really interesting data; there are some other poster presentations at this meeting that combine what you discussed here and the role of COX-2. Some of the posters suggest that as many as one third of pancreatic cancers are devoid of COX-2 overexpression and that perhaps these cancers use a different pathway. I was wondering if you had contemplated that and whether you might do a chip that might look at this alternative pathway in COX-2–negative pancreatic cancers?

Dr. Tseng: That is a very interesting point. There have been numerous studies, some of which have already been published, showing that growth inhibition can be achieved in different kinds of cancer cell lines that are also COX negative, and this is something that we definitely intend to look into in our laboratory. I think what was interesting, though, was that the two prevailing hypotheses are that COX-2 inhibition occurs through apoptosis, as well as angiogenesis, and if you study the findings that we obtained on the microarray, the individual gene changes, they would not support that theory, and we actually observed a predominant change in the cell cycling gene. So I think we could use a microarray as a way to kind of guide us into future experiments.

Dr. K.G. Billingsley (Seattle, WA): I have two questions. One, did you look at the possibility of using COX-2 to mediate the regression of established tumor? The other question, I am struck by the fact that this may in real-world clinical terms serve as a low-toxicity and potent radiosensitizing mechanism. Did you look at all at the possibility of using COX-2 inhibition as a radio sensitizer in mice with established tumors, or is that something you plan to do in the future?

Dr. Tseng: In answer to your second question, no, we have not looked at that. I know there have been articles that have demonstrated synergistic inhibition with the use of a COX-2 inhibitor, as well as radiation therapy, and they have shown an inhibition in angiogenesis. So that is definitely an interesting point to study. In answer to your first question, this model was set up so that we could look at growth inhibition in established pancreatic cancer. I am not sure where your question was leading exactly. Our model was set up so that we began treatment after the mice had already developed tumors at approximately 400 mm^3 in size, and the rationale for that was just because it would be more relevant to what occurs in clinical situations. In some studies that I have seen, treatment was actually begun immediately after the establishment of pancreatic cancer, which in my mind would be more a study of the development of pancreatic cancer, more like carcinogenesis, rather than treating an established tumor. So I am not sure if that answers your question.

Quality of Life After Bilateral Thoracoscopic Splanchnicectomy: Long-Term Evaluation in Patients With Chronic Pancreatitis

Thomas J. Howard, M.D., John B. Swofford, D.O., Dennis L. Wagner, M.D., Stuart Sherman, M.D., Glen A. Lehman, M.D.

We prospectively evaluated quality of life and visual analogue scale pain scores after bilateral thoracoscopic splanchnicectomy in 55 patients with small-duct chronic pancreatitis and abdominal pain. The perioperative morbidity rate was 11% and there were no perioperative deaths. Four late deaths occurred (7%), and three patients were lost to follow-up. Patients were divided into those who had prior operative or endoscopic interventions (N = 38) and those who did not (N = 17). Preoperatively there were no significant differences between the two groups with regard to age, sex, etiology, pain score, or narcotic use. Pain score, narcotic use, and symptoms scales improved significantly in both groups at 3 and 6 months postoperatively ($P < 0.0001$). The group with no prior surgical or endoscopic intervention did significantly better initially ($P < 0.007$), and the improvements in their quality-of-life and pain scores continued for the remainder of the study. In contrast, quality-of-life and pain scores in patients who had undergone prior surgical or endoscopic intervention returned to baseline by 12 months postoperatively and remained poor throughout the remainder of the study. Bilateral thoracoscopic splanchnicectomy appears to work best in patients who have had no prior operative or endoscopic interventions. (J GASTROINTEST SURG 2002;6:845–854.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Chronic abdominal pain, chronic pancreatitis, thoracoscopic splanchnicectomy

Relief of abdominal pain in patients with chronic pancreatitis is a complex clinical problem that remains a major focus for disease management decisions.[1] Pancreatic denervation to interrupt the flow of painful stimuli from the pancreas through the central nervous system has conceptually intrigued surgeons since it was first reported by Mallet-Guy[2] in 1943. The ability to relieve pain from chronic pancreatitis with a single therapeutic intervention without disrupting the pancreas or digestive tract remains an extremely attractive option for a large segment of patients with chronic pancreatitis. Despite the "Stradivarius"-like quality of never being adequately reproduced, Mallet-Guy's work remains the "gold standard" for pain relief after splanchnicectomy in patients with chronic pancreatitis.[3]

In 1993 two groups combined the theories of Mallet-Guy with the burgeoning technology of video thoracoscopy to report on the use of thoraco-scopic splanchnicectomy to treat the pain from pancreatic cancer.[4,5] This marriage, combining the mystique of neuronal ablation with minimally invasive surgery, re-energized the surgical community who was in search of a simple, straightforward procedure to address the vexing problem of pain from chronic pancreatitis. The operation was extended to patients with chronic pancreatitis,[6] modified to include bilateral nerve ablation through a posterior approach,[7] and patients were screened by a differential epidural anesthetic (DEA) to identify those with a high likelihood of success with the procedure.[8] Early reports confirmed the feasibility of the operation. However, despite its initial success in alleviating pain, ultimate therapeutic efficacy required larger studies with long-term clinical follow-up.[6–9] The aim of this paper is to report on health-related quality of life in 55 patients with small-duct chronic pancreatitis who have been prospectively followed for a median of 32

months after bilateral thoracoscopic splanchnicectomy to determine its long-term efficacy.

MATERIAL AND METHODS

From May 1997 to July 2001, a total of 64 patients with small-duct chronic pancreatitis and narcotic-dependent abdominal pain were evaluated. Patients were diagnosed with chronic pancreatitis by endoscopic retrograde cholangiopancreatography (ERCP) and/or computed tomography (CT) according to standard published criteria.[10] No patient had a structural lesion (i.e., pancreatic duct stricture, pseudocyst, or distal common bile duct stricture) that was amenable to either endoscopic or surgical treatment. A small pancreatic duct for this study is defined as a main pancreatic duct on ERCP or CT imaging measuring 5 mm or less in diameter in the head and 4 mm or less in the body. All patients were treated initially by medical therapy that included the following: low-fat diet, pancreatic enzyme supplementation, oral analgesics and antiemetics, and endoscopic ultrasound or CT-directed celiac plexus block. A small portion of patients (N = 14) also failed a trial of therapy with octreotide in escalating doses.

All patients who failed initial medical treatment were given a DEA as previously described.[8] By recording the subjective sensation of pain after injections of either normal saline solution (placebo control) or local anesthetic, pain perceived by the patient can be characterized as visceral, nonvisceral, or central in origin. When significant improvement in pain occurred after placebo injection alone, patients were considered "placebo responders." Patients experiencing a marked reduction in pain after documented sympathetic blockade were considered examples of visceral pancreatic pain. Patients who obtained significant pain relief only after complete epidural blockade were felt to have somatic nonpancreatic pain. Patients without pain relief after complete epidural blockade were considered to have centralization of pain (including psychogenic pain). Only patients with documented visceral sympathetic-mediated pain pathways were considered candidates for thoracoscopic splanchnicectomy in this study. Four patients were found to be placebo responders, and five were diagnosed as having nonvisceral or centralization of their pain, making them unlikely to benefit from thoracoscopic splanchnicectomy; these patients were not offered an operation and were excluded from study. This left 55 patients (19 men and 36 women) whose mean age was 38 years (range 19 to 59 years) who qualified for study and agreed to the operative procedure.

The operative technique used was similar to that originally described by Cuschieri et al.[7] with minor modifications. Briefly, the procedure is carried out under general endotracheal anesthesia with double-lumen intubation for single-lung ventilation. The patient is placed in the full prone jack-knife position. Operative access is established by means of a three-port technique: a 10 mm port (camera port) in the sixth intercostal space at the posterior axillary line, and two 5 mm ports (working ports) in the fourth and eighth intercostal spaces, approximately half the distance between the posterior axillary line and the spine. The sympathetic chain was identified running along the posterior chest wall, the mediastinal pleura was opened, and the greater splanchnic nerve emanating from the fifth and eighth vertebrae was identified. The greater splanchnic nerve was then dissected along its entire course down to the insertion of the diaphragm, clipping the proximal and distal segments. All rami communicans from the sympathetic ganglia to this nerve were interrupted using the hook electrocautery. Rami communicans from the eighth to eleventh intercostal space (lesser and least splanchnic nerves) were occasionally disrupted when identified easily. Hemostasis was obtained, and the lungs were reinflated under direct vision. The chest was closed in layers, and an upright chest x-ray film was obtained in the operating room while the patient remained anesthetized and intubated. A small-bore chest tube was inserted if a significant pneumothorax was identified on this film.

A European Organization for Research and Treatment of Cancer (EORTC) quality-of-life questionnaire (QLQ) that has been validated for chronic pancreatitis and a visual analogue pain scale were administered to the patients on their first preoperative clinic visit and at 3, 6, 12, 24, and 36 months postoperatively.[11,12] The EORTC questionnaire incorporates nine multi-item scales including the following five functional scales: physical (items 1 to 5), role (items 6 and 7), cognitive (items 20 and 25), emotional (items 21 to 24), and social (items 26 and 27). There are also three symptoms scales including fatigue (items 10, 12, and 18), pain (items 9 and 19), and nausea and vomiting (items 14 and 15), and a global quality-of-life scale (items 29 and 30). We included a visual analogue pain scale (VAS), which ranged from 0 = no pain to 10 = the worst pain imaginable. Narcotic analgesics were recorded and converted into an equianalgesic dose based on multiplying the number of tablets a patient takes per day by the assigned factor: propoxyphene = 0.75; codeine = 1.0; hydrocodone = 2; oxycodone = 4; hydromorphone = 6; and fentanyl patch = 8. A secretary administered the initial QLQ preoperatively in

the surgical outpatient clinic area. Subsequent post-operative follow-up questionnaires were mailed to patients at the appropriate times with a self-addressed stamped envelope for easy return. If a questionnaire was not returned within 2 weeks, a second letter was sent. If there was still no response, a call was made to the patient inquiring about his or her ability to fill out the questionnaire, and oftentimes a third questionnaire was sent. Administering the QLQ to eight patients twice preoperatively within a 7-day period assessed test-retest stability. This showed an 89% correlation between answers. We found no significant differences between the preoperative QLQ and the VAS pain score in those patients who did not qualify for study (N = 9) vs. those who did (N = 55). Answers were coded and kept on an Excel spreadsheet until final analysis.

For ease of interpretation and by prior convention,[11,13] all scales were linearly transformed to a 0 to 100 scale. A two-tailed t test was used to analyze the significance of the difference between the mean of two samples. Fisher's exact test or chi-square test was used to evaluate nominal data. The Wilcoxon rank test was used for ordinal data to estimate the significance of differences between QLQ scores. $P < 0.05$ was taken as significant for all data.

RESULTS

Patients were divided into those with prior operative or endoscopic interventions (N = 38) and those without (N = 17). The group who had prior surgical intervention included two patients who had undergone distal pancreatectomy, two patients who had a Whipple-type pancreaticoduodenectomy, and two patients who had a Frey-type duodenal-sparing pancreatectomy. Thirty-two patients (58%) had prior endoscopic interventions including endoscopic sphincterotomy ± pancreatic duct stent placements. Preoperatively there were no differences between the two groups with regard to age, sex, etiology, pain scores, or preoperative narcotic use (Table 1). Of the 32 patients who had prior endoscopic intervention, 28 (88%) had sphincterotomy and pancreatic duct stent placement. Of these 28 patients who received one or more pancreatic duct stents, the reasons for stent placement were as follows: ethanol abuse in eight (29%); pancreas divisum in 11 (39%); idiopathic pancreatitis in four (14%); and sphincter of Oddi dysfunction in five (18%). All eight patients with ethanol abuse, two patients with pancreas divisum, and four patients with idiopathic pancreatitis had radiographic evidence of chronic pancreatitis, as noted by main duct strictures with or without pancreaticolithiasis on ini-

tial ERCP investigation. Nine patients with pancreas divisum and all five patients with sphincter of Oddi dysfunction developed ERCP changes consistent with a diagnosis of chronic pancreatitis only after sphincterotomy and therapeutic stenting had been initiated (14/32 = 44%). The median time interval between the onset of pancreatitis and thoracoscopic splanchnicectomy was longer in the prior surgical intervention group (mean = 5.4 years [range 3 to 17 years]) compared to the no-intervention group (mean = 2.8 years [range 1.5 to 9 years]). In 18 patients pancreas divisum was considered to be the cause of chronic pancreatitis, 17 patients used ethanol excessively, 11 patients had idiopathic pancreatitis, seven had sphincter of Oddi dysfunction, and two patients had hyperlipidemia.

The median follow-up was 32 months (range 6 to 48 months). Perioperative morbidity rate was 11% (chylothorax in 3, wound infections in 2, and pneumonia in 1), and the average postoperative hospital stay was 3.6 days (range 2 to 27 days). Two patients required reoperation, one who had a thoracotomy and one who underwent repeat thoracoscopy on postoperative days 23 and 26, respectively, for persistent chylothorax recalcitrant to closure by means of standard medical treatment.[17] Six patients (11%) had postoperative intercostal neuralgia persisting for more than 30 days postoperatively. Five of the six eventually had pain relief, 3 months after the operation on average. One patient had continued neuralgia requiring re-referral to our pain clinic for evaluation and intercostal nerve blocks. There were no deaths related to the operative procedure. There were four late deaths and three patients lost to follow-up. Overall compliance of study patients with the information requested on the QLQ within 8 weeks of the scheduled follow-up evaluation time was 83%.

During follow-up we identified significant differences between the two groups of patients both in the magnitude of the initial pain relief and the duration of this effect. When stratified in terms of the magnitude of the initial pain relief, seven patients (18%) with prior intervention had no benefit (defined as a change in the VAS < 2 units from baseline), 26 patients (68%) had a moderate benefit (improved VAS 2 to 4 units), and only five patients (13%) had a marked benefit (VAS > 4 units) from splanchnicectomy (Fig. 1). In contrast, in patients who had no intervention, only one patient (6%) had no immediate benefit, seven patients (41%) had a moderate benefit, and nine patients (53%) had a marked benefit from splanchnicectomy ($P = 0.007$). Initial pain scores (3 months postoperatively) were significantly decreased from preoperative levels in both groups of patients

Table 1. Patient demographics, etiology of pancreatitis, preoperative pain score, and preoperative narcotic use in 55 patients with chronic small-duct pancreatitis

	Prior intervention (n = 38)	No intervention (n = 17)	*P* value*
Age (yr)	40 ± 9	37 ± 6	0.83
Females	25 (66%)	11 (65%)	0.82
Etiology			
Ethanol abuse	11 (29%)	6 (35%)	0.89
Pancreas divisum	13 (34%)	5 (29%)	1.00
Idiopathic	7 (18%)	4 (24%)	0.92
SOD	5 (13%)	2 (12%)	0.76
Hyperlipidemia	2 (5%)	0	0.86
Preoperative pain score	7.6 ± 1.2	6.9 ± 1.8	0.29
Preoperative narcotic use[†]	9.1 ± 2.5	8.4 ± 2.7	0.48

SOD = sphincter of Oddi dysfunction.
*Fisher's exact test for nominal data; Student's *t* test for continuous data.
[†]Equianalgesic narcotic dose based on multiplying the number of tablets taken per day by the assigned factor: propoxyphene = 0.75; codeine = 1; hydrocodone = 2; oxycodone = 4; hydromorphone = 6; fentanyl patch = 8.

(Fig. 2). Patients with no intervention, however, had significantly lower pain scores than patients with prior intervention (4.5 ± 1.2 vs. 2.6 ± 0.8; *P* = 0.002). Over the entire time course of the observation, the initial benefits of splanchnicectomy were quickly extinguished by 12 months in 31 (82%) of 38 patients who had prior intervention. The average VAS pain score at 12 months in this group was not significantly different from preoperative values, and these values remained not significantly different from preoperative values for the remainder of the study period. Seven patients (41%) who had no intervention reported continued pain relief at 36 months' follow-up, and although there was a slow erosion of the initial excellent VAS pain scores in this group, the mean VAS pain score of 3.5 at 36 months remained significantly different from preoperative values (*P* < 0.0001).

This long-term observation on pain control is supported when patients reported their analgesic use on the QLQ. Of patients who were observed for more than 24 months, only two (22%) of nine patients with no intervention required daily narcotic analgesics, whereas 13 (72%) of 18 patients with prior intervention required these medications, albeit at a slightly lower equianalgesic dose (6.3 ± 3.2 vs. 9.1 ± 2.5; *P* < 0.01) than was taken preoperatively. During follow-up, two patients (12%) with no prior intervention went on to have additional procedures directed at pain control. One patient had a Wallstent pancreatic duct dilatation followed by a Peustow drainage procedure, and in the second patient an intrathecal morphine pain pump was placed. In contrast, 14 patients (37%) with prior interventions went on to have further procedures directed at pain

control: six had intrathecal morphine pumps placed, four had total pancreatectomy and islet cell transplantation, two had enteral feeding tubes, and two had pancreatic resections.

Table 2 summarizes the results of the EORTC QLQ functioning scale scores and overall quality-of-life score preoperatively and at 12 months postoperatively. For ease of interpretation, all scale and item scores have been linearly transformed into a scale ranging from 0 to 100 points with a higher score in-
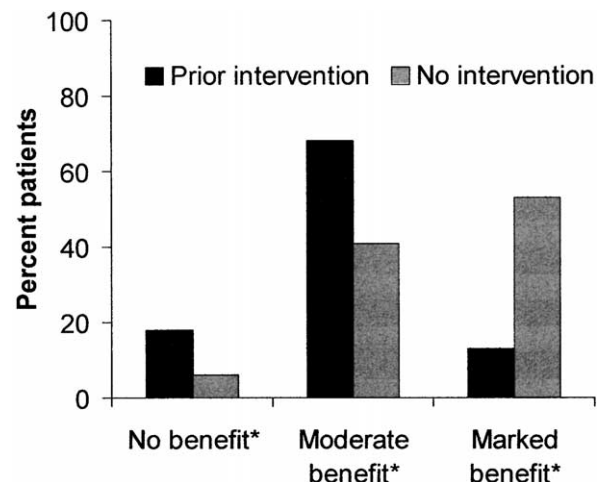
Fig. 1. Quantitation of initial pain relief (3 month) in patients after thoracoscopic splanchnicectomy. No benefit = improvement <2 units in VAS over preoperative score; moderate benefit = improvement 2 to 4 units in VAS over preoperative score; marked benefit = >4 units improvement in VAS over preoperative score. Chi-square test, *P* = 0.007, prior intervention vs. no intervention.
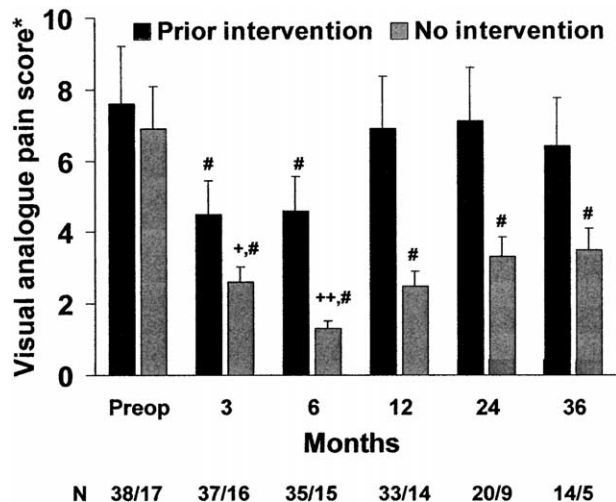
Fig. 2. Average VAS pain score for both groups of patients during 36-month follow-up. VAS was assessed at appropriate time period whether or not the patients were taking narcotic analgesics. The pain score was not assessed after the patient had a further therapeutic intervention (i.e., pancreatic surgery, intrathecal pain pump). #$P < 0.0001$ vs. preoperative score; +$P = 0.002$ vs. prior intervention; ++$P < 0.0001$ vs. prior intervention.

dicating a better level of functioning. At 12 months we found no significant difference between preoperative and postoperative values for all functioning scale scores and global quality of life in patients with prior interventions, reinforcing the findings on the VAS pain score. In contrast, patients with no prior intervention reported significantly higher levels of physical, role, cognitive, and social functioning ($P < 0.01$). Their estimation of emotional functioning was not significantly increased; however, they reported a

significantly higher global quality of life compared to preoperative values ($P < 0.01$).

DISCUSSION

Treatment of pain in patients with chronic pancreatitis remains poorly understood. There are numerous reasons for our lack of fundamental knowledge on this important topic including differing morphologic subtypes of chronic pancreatitis, multiple etiologic factors, a wide spectrum of anatomic abnormalities, particularly duct size, head size, and strictures, and the confounding problems of a variable disease progression and clinical course.[14–16] Mirroring this wide breadth of disease states, treatment options range from behavior modification techniques, prescription medications, endoscopic therapy, and minimally invasive nerve ablation to extensive pancreatic resections. The choice of which treatment option to use in which particular patient is often empiric, as there is currently no systematic evidence-based approach to choosing a particular course of therapy in these patients.[1,17,18]

This study was undertaken to assess the long-term efficacy of bilateral thoracoscopic splanchnicectomy in a limited cohort of patients, namely, those with small-duct chronic pancreatitis and severe pain requiring narcotic analgesics, all of whom had been judged failures on medical therapy. We chose to analyze our data by separating patients into those with prior intervention (endoscopic or surgical) and those without. This subgrouping of patients was based on several factors; the first of which were observations made by Dumonceau et al.[19] that endoscopic therapy for chronic pancreatitis has the best results when performed early in a patient's clinical course. In their experience, subsequent therapeutic interventions af-

Table 2. Preoperative and postoperative (12-month) functioning scale scores and global quality-of-life scores for 55 patients with chronic small-duct pancreatitis treated by bilateral thoracoscopic splanchnicectomy

	Prior intervention (n = 38)		No intervention (n = 17)	
Functioning scale*	Preoperative score	Postoperative score	Preoperative score	Postoperative score
Physical status (items 1–5)	60	70	60	90‡
Role (items 6,7)	52	60	50	85‡
Cognitive (items 20, 25)	60	67.5	62.5	82‡
Emotional (items 21–24)	55	60	60	70
Social (items 26, 27)	60	65	70	92‡
Global QOL (items 29, 30)	50	62.5	55	85‡

*Scores range from 0 to 100, with a higher score representing a higher level of functioning.
†Cronbachs coefficient α for scale reliability was >70 for all scales.
‡$P < 0.01$ vs. preoperative score; Wilcoxon rank test.

ter the initial therapeutic benefit are frequently less rewarding. This endoscopic clinical experience mimics our own. Second, Maher et al.[6] made the observation, in their series of patients with chronic pancreatitis treated by splanchnicectomy, that all three of their treatment failures occurred in patients who had undergone prior pancreatic surgery. This experience echoed the findings of Cruschieri et al.[7] who reported that the benefit of splanchnicectomy seemed to vary with the severity of disease, with the best results being observed in patients with minimal-change pancreatitis. These data, taken collectively, suggested a difference in outcome between patients with a long clinical course and prior therapeutic intervention and those without. To further distill our study population, we operated only on patients with evidence of an active visceral afferent pain pathway based on their response to a DEA.[8]

We found that bilateral thoracoscopic splanchnicectomy, as described, is a minimally invasive technique with quite acceptable morbidity and mortality in our hands, and a reasonably rapid learning curve of approximately 15 procedures. These results confirm the reported experiences of others.[6–9,20] Both instances of chylothorax and many of our minor postoperative complications occurred within the first 20 patients in our series.[21] Of these initial patients, 15 (75%) had prior intervention and five (25%) had no intervention, a percentage similar to the overall group distribution of 69% with prior intervention and 31% with no intervention. There were no significant differences between the 3-month VAS pain score and overall quality-of-life score between the first 20 patients and the subsequent 35 patients (when analyzed by group), implying that the small technical improvements in the operation that occurred over time failed to significantly alter our long-term results. We applied a validated EORTC QLQ in addition to a VAS pain score to patients prior to intervention and at 3, 6, 12, 24, and 36 months postoperatively. The intimacy of the study (single institution, small patient numbers) and the fact that many of these patients are closely followed by one or more of the coauthors resulted in an 83% success rate in obtaining QLQ responses during follow-up and maintaining a tightly controlled clinical entry criteria. These same factors, single-institution accrual and small numbers of patients, are also the principal limitations for this study. Inherent biases generated by our patient referral pattern limit the generalizability of our findings, and the small sample size increases the possibility of a type II statistical error in the analysis of our data.

Our main finding is that the overall efficacy of this operation depends in large part on the type of pa-

tient to which it is applied. This result seems intuitive to those physicians and surgeons who treat large numbers of patients with chronic pancreatitis and recognize large duct vs. small duct variants, alcoholic vs. idiopathic types, and constant vs. intermittent pain syndromes.[18] Less apparent is why patients in our series who have had prior intervention, whether it be endoscopic or surgical, have a significantly blunted response in both magnitude and duration of pain relief after bilateral thoracoscopic splanchnicectomy compared to patients without prior intervention. Although these findings were alluded to earlier in the reported series of Maher et al.[6] and Cuschieri et al.,[7] recent large clinical series have not broken down and analyzed their data in this way.[20,22] Ihse et al.[22] reporting on 21 patients with chronic pancreatitis in whom an operative technique quite similar to ours was used, found clear evidence that the operation has no significant effect on measured parameters of pancreatic endocrine or exocrine function. In their patient population, after a mean follow-up of 43 months, the VAS pain score remained decreased by 50% from baseline, and they found a concomitant decrease in patients' narcotic analgesic use. The difference in outcome between their study and ours is most likely related to differences in the two study populations. In contrast to our own study group, which was heavily weighted toward patients with pancreas divisum, idiopathic pancreatitis, and sphincter of Oddi dysfunction (84% of all patients studied), the majority of patients in their group had alcohol-induced chronic pancreatitis (57% of all patients studied). Although many of their patients had prior interventions (N = 20), the exact number of these prior interventions (i.e., cholecystectomy, gastric resection, exploratory laparotomy) that were primarily directed at pain relief is unknown. Furthermore, although 54 (98%) of 55 of our patients were taking narcotic analgesics preoperatively at a rather generous equianalgesic narcotic dose (8.7 ± 2.5), only 14 patients (67%) in their series were taking narcotic analgesics regularly prior to operation (Table 3).

Despite the use of a slightly different surgical technique to ablate the splanchnic nerves (harmonic scalpel), the recently published series by Buscher et al.[20] seems to more closely resemble our own. Of the 44 patients with chronic pancreatitis whom they studied, the majority had small-duct disease and 28 (64%) had undergone one or more prior surgical or endoscopic interventions directed at their pain. This patient population was composed of a majority of patients with alcohol-induced chronic pancreatitis (55%); however, they also had a significant proportion of patients (32%) with either pancreas divisum or idiopathic pancreatitis. In their experience, de-

Table 3. Selected review of recent reports of bilateral thoracoscopic splanchnicectomy in patients with chronic pancreatitis

Study	No.	Main etiology of pancreatitis	Preoperative opiod use	Success rate (%)	Median follow-up (mo/y)
Moodley et al.[9]	17	ETOH (100%)	8/17	94%	12
Ihse et al.[22]	21	ETOH (57%)	14/21	75%	42
Buscher et al.[20]	44	ETOH (55%)	36/44	46%	36
Present study	55	PD (33%)	55/55	31%	32

ETOH = ethyl alcohol; PD = pancreas divisum.

spite an early (6 months postoperatively) significant improvement in pain scores, after a median follow-up of 36 months, 2 (50%) of 44 patients had a return of their abdominal pain, which in 19 patients (86%) required narcotic analgesics. In our series we also saw excellent clinical results early (3 months postoperatively), which were characterized by a significant lowering of the VAS pain score, less narcotic use, and improvement in overall quality of life. After a median follow-up of 32 months, however, 38 (69%) of 55 patients reported a return of abdominal pain severe enough to require narcotic analgesics or to undergo another therapeutic intervention (intrathecal pain pump, pancreatic drainage, or resection) aimed at controlling the abdominal pain.

Based on our data, bilateral thoracoscopic splanchnicectomy has long-term efficacy in patients with chronic small-duct pancreatitis and abdominal pain who have failed medical treatment, have not undergone prior interventions, and respond appropriately to preoperative DEA. Obviously this is a fairly limited subset of all patients with chronic pancreatitis and abdominal pain. The use of splanchnicectomy in patients who have had prior operative or endoscopic interventions may provide short-term symptomatic relief (<12 months); however, in our experience, a long-term therapeutic benefit is unlikely. The reasons for recurrence of abdominal pain after bilateral thoracoscopic splanchnicectomy remain elusive. All patients in this series were operated on using a standard technique with a single surgeon, and all had both visual and histopathologic confirmation of greater splanchnic nerve removal. Nerve regeneration in the setting of complete nerve removal would seem unlikely. A more plausible explanation is the anatomic variability found in the splanchnic nerves, both in terms of the number of ganglion roots involved and their level of takeoff from the thoracic sympathetic nerve chain ganglia.[23] Incomplete nerve sectioning would explain the early failures of splanchnicectomy, but fails to adequately account for the much larger percentage of patients we identified as late (6 to 12 months) failures. Concomitant vagot-

omy with splanchnicectomy has been advocated by some investigators[24] because of both the possibility of visceral sensory afferent nerve transmission through this nerve and its possible role in decreasing the incidence of recurrent attacks in patients with alcoholic pancreatitis. Despite these perceived benefits, given the untoward side effects that vagotomy has on gastrointestinal motility and secretion, enthusiasm for this approach has been lacking without clear-cut evidence of its benefits.

In a more global sense, the inconsistency of published results by multiple investigators over six decades, using various techniques of nerve transection, implies that our classic neuroanatomic paradigm of pain impulse transmission from the pancreas through visceral afferent fibers to the spinal cord and on to the brain may not be an adequate model to explain abdominal pain in the setting of chronic pancreatitis. Recent experimental findings suggest that in setting of local inflammation, classic nerve transmission through the spinal cord to the brain may not be the primary means of altered sensation.[25] Chemically induced inflammation in the hind paws of rats cause a rapid, large, and long-lasting increase in the concentration of interleukin-1β in the central nervous system that appears to modulate the sensation of pain. This effect can only be blocked by injecting drugs directly into the central nervous system, not peripherally, implying that classic neural pathways may not be involved. Additional investigation has shown that pain is a complex experience encompassing sensory, affective, and cognitive elements within the central nervous system. The activation of μ-opiod receptor in specific brain regions appears to be involved in the attenuation of sensory and pain-specific affective responses to a sustained painful stimulus.[26] Disruption of this central mechanism of pain attenuation may play a role in chronic pain syndromes.

Clearly, more work on the treatment of pain in chronic pancreatitis needs to be done. Future studies should focus on careful patient classification, with randomization to different medical and surgical interventions and appropriate control groups, and attention

should be paid to the role of the central nervous system in these chronic pain syndromes. Well-designed, multi-institutional studies with validated disease-targeted health-related quality-of-life instruments and sufficient long-term follow-up would go a long way toward answering the basic questions still unresolved in our understanding and treatment of patients with abdominal pain from chronic pancreatitis.

CONCLUSION

Bilateral thoracoscopic splanchnicectomy is a safe, minimally invasive technique that provides long-term pain relief and improved quality of life in patients with chronic small-duct pancreatitis who respond appropriately to differential epidural anesthesia and have not undergone prior surgical or endoscopic interventions directed at pain control. Patients with chronic small-duct pancreatitis who have had multiple prior interventions realize only short-term benefits from this operation that are quickly extinguished by 12 months' follow-up.

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Discussion

Dr. C.F. Frey (Sacramento, CA): This was a well-designed, well-executed study. Could you explain what you meant by small ducts? Could you tell us if there was evidence of disease of the parenchyma, or ducts in your patients?

Dr. T.J. Howard: Your work in this field is well recognized and has inspired us to provide careful, long-term follow-up of these patients. By definition, our small-duct disease is ducts less than 4 mm in the head and less than 5 mm in the body of the pancreas. Almost all patients in this series had ERCPs and all had Marseille criteria to classify them as having chronic pancreatitis.

Dr. J. DiSario (Salt Lake City, UT): Why do you suppose that the patients who had prior interventions did not do well?

Dr. Howard: I have thought about this question quite a bit and, if I may rephrase your question, the central point of this paper is why does their pain recur? Almost all physicians who work with these patients recognize there is pain relief initially, but it is the recurrence of the pain over time that is the problem. My feeling is that the sensation of pain is much more complex neuroanatomically than what appears in the simple autonomic nervous system innervation diagram that I presented. What we have not addressed adequately is the central nervous system in these patients, and I think the real gains long-term will be in understanding this interaction more completely.

Dr. DiSario: Do you think psychological factors are involved?

Dr. Howard: Yes and no. I wouldn't say it is only psychological factors because that implies a specific psychiatric diagnosis. I do believe there are neuroanatomic connections in these patients that we do not completely understand. There are some recent data that suggest pain transmission in inflammatory diseases may not be transmitted through the nerves but is instead modulated by chemical mediators, produced at the site of inflammation, that affect interleukin production in the brain. It may have little to do with the splanchnic nerves or any similar neural connection.

Dr. N.J. Soper (St. Louis, MO): That was an excellent presentation. You do have an unusual group of patients that was considered for this type of treatment. If the groups were divided in some other way, for example, by diagnostic group rather than the presence or absence of prior intervention, would there be differences in outcomes after this treatment?

Dr. Howard: What do you mean by diagnostic group?

Dr. Soper: Sphincter of Oddi dysfunction, pancreas divisum, or pancreatitis?

Dr. Howard: We have not done that. These are the types of patients that we see in our practice who have small-duct pancreatitis and very few other therapeutic options for pain control. We chose to focus on patients who had a diagnosis of chronic small-duct pancreatitis, regardless of etiology, to see if we could help them achieve long-term pain relief with this procedure.

Dr. L.W. Traverso (Seattle, WA): I suggest that you and Dr. Frey get together to work out some credible entrance criteria for this study because, as is the case with any operation for chronic pancreatitis, the anatomy is almost everything, and a determination of the exact criteria for entrance into this study is what is needed for a successful formula. Many of these patients who have normal ducts come to us seeking pain relief, but they do not have chronic pancreatitis. For those of us who work in this area, this is a frequent first hurdle to overcome. I noticed you had a large number of patients with pancreas divisum in your group, and these are the kind of patients who are entered into a study who really have sphincter dysfunction and not chronic pancreatitis. Perhaps you and Dr. Frey could collaborate on some reliable entrance criteria for this study starting with an anatomic classification such as the Cambridge classification of 1984.

Dr. L.W. Way (San Francisco, CA): Without morphologic criteria on which to base a diagnosis of pancreatitis, I think that patients who are seen in gastrointestinal practices for abdominal pain attributed to sphincter of Oddi dysfunction and pancreas divisum are very questionable without anatomic evidence. So it is a difficult field.

Dr. Howard: All of these patients had ERCP and CT criteria for chronic pancreatitis to gain admission to the study. We did have a large number of patients who were women with either sphincter of Oddi dysfunction or pancreas divisum, but that is the nature of our practice, and these are precisely the types of patients with chronic small-duct pancreatitis who have no other viable therapeutic options for pain control. I would echo Dr. Way's comments that this is a difficult field but one that we must continue to study carefully.

Invited Discussion—Expert Commentator

Dr. David W. Rattner: Dr. Howard and his colleagues have made a valuable contribution to the management of a very difficult group of patients—those with intractable pain from chronic pancreatitis. Their study is notable for its size (55 patients), clearly defined selection criteria, duration of follow-up (median 32 months), and completeness of follow-up with 83% of the patients completing the quality-of-life instrument—truly a remarkable achievement in this patient population. Although bilateral thoracoscopic splanchnicectomy is billed as a minimally invasive procedure, clearly it is not without hazard as two patients required reoperation and six developed intercostal neuralgias. Nonetheless, the intervention was successful in relieving pain in most patients. Unfortunately, the pain re-

lief was not durable, as more than two thirds of the patients were back on narcotics at the median follow-up time of 32 months. Although this is not the first study to demonstrate efficacy of splanchnicectomy for relief of pancreatic pain, it provides several key insights that should be used as the roadmap for further work in this area. The first insight is that splanchnicectomy is most effective if the antecedent duration of disease is short. One might conclude, on the basis of Dr. Howard's work, that for patients with small-duct disease, both resection and endoscopic therapy are meddlesome. Perhaps spanchnicectomy ought to be offered as a first-line therapy rather than reserved to salvage patients who have failed years of medical therapy. The second insight, a late failure rate of 69%, however, forces one to temper the enthusiasm for splanchnicectomy as first-line therapy. As the authors point out, the reasons for the return of pain in these patients are mysterious and pose challenging questions that must be answered in the ongoing quest to provide effective therapy for the number one complaint among patients with chronic pancreatitis.

National Trends in Utilization and In-Hospital Outcomes of Bariatric Surgery

George Darby Pope, M.D., John D. Birkmeyer, M.D., Samuel R.G. Finlayson, M.D., M.P.H.

In view of recent enthusiasm for surgery to treat morbid obesity, we examined national changes in utilization and in-hospital outcomes of bariatric surgery over time. With the use of International Classification of Diseases (ICD-9) codes, we identified all bariatric procedures (n = 12,203) performed on adults from 1990 to 1997 in hospitals participating in the Nationwide Inpatient Sample. We then applied sampling weights and United States Census data to calculate the national population-based rates of bariatric surgery procedures for each year and examined secular trends in utilization. We further evaluated changes in patient characteristics and in-hospital mortality and complications. From 1990 to 1997, the national annual rate of bariatric surgery increased from 2.7 to 6.3 per 100,000 adults ($P < 0.001$). The percentage of bariatric procedures performed by gastric bypass increased from 52% to 84% ($P < 0.001$). Patients were slightly older (38.1 years vs. 40.3 years; $P < 0.001$) with more comorbid conditions (20.9% vs. 31.6%; $P < 0.001$) in 1997 vs. 1990. In-hospital mortality was 0.37% overall and remained stable. Rates of pulmonary emboli, early reoperation, and pulmonary complications declined significantly over time. Between 1990 and 1997, the annual rate of bariatric surgery in the United States more than doubled, without substantial changes in perioperative morbidity or mortality. This trend was largely associated with an increase in the use of gastric bypass procedures. (J GASTROINTEST SURG 2002;6:855–861.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Bariatric surgery, gastric bypass, morbid obesity, outcomes, utilization

The prevalence of obesity in the United States population is increasing.[1–3] More than 1 million patients in the United States are morbidly obese, as defined by the National Institutes of Health. Among health care professionals, there is growing understanding of the health risks associated with morbid obesity. In view of this, bariatric surgery has gained popularity as the only treatment to produce sustained weight loss in persons who are morbidly obese. Several studies of surgical weight loss have demonstrated reductions in obesity-related morbidity including diabetes, hypertension, obesity hypoventilation, sleep apnea, and gastroesophageal reflux.[4–21] Among morbidly obese patients, there is also increased enthusiasm for bariatric surgery, possibly related to favorable reports in the lay press, including testimonials from successfully treated high-pro-file celebrities. The availability of new, minimally invasive techniques has also added to enthusiasm for bariatric surgery.

Although bariatric surgery has received increased publicity and newfound interest, secular trends in the use of bariatric surgery in the United States have not been examined. Furthermore, although numerous surgical case series have described relatively low perioperative risks associated with bariatric surgery, whether similar results are achieved in the broader population is unknown.

In this study we used administrative data from the Nationwide Inpatient Sample (NIS), the largest source of all payer discharge information in the United States, to evaluate secular trends in the use of bariatric surgery. Specifically, we sought to determine how much population-based rates of surgery

for morbid obesity changed between 1990 and 1997. We also examined trends in patient characteristics and in-hospital outcomes over this time period.

MATERIAL AND METHODS
Database

Discharge data from the NIS for the years 1990 to 1997 were obtained from the Healthcare Cost and Utilization Project of the Agency for Healthcare Research and Quality. NIS data consist of all patient-level discharge abstracts from a 20% stratified probability sample of acute care, nonfederal hospitals in the United States (approximately 1000 hospitals in 1997).[22]

Patient Selection

We used International Classification of Diseases (ICD-9) procedure and diagnosis codes to identify all hospitalizations during which a bariatric surgical procedure was performed. Bariatric surgery discharges were identified by the presence of a code for gastric bypass (44.31, 44.39) and/or gastroplasty (44.69) and an accompanying ICD-9 diagnostic code for obesity (278.0, 278.00, 278.01, 278.1, 278.8). For discharges coded for both gastric bypass and gastroplasty (<4% average overlap), gastric bypass was assumed. To increase the homogeneity of the cohort, we excluded all patients with a diagnostic code for abdominal neoplasm (150.0 through 159.9).

Statistical Analysis

The national population-based rate of bariatric surgery was calculated for each year from 1990 to 1997. The annual number of procedures performed in the United States (numerator) was estimated using sampling weights provided with the NIS data. Adult (age >17 years) population estimates (denominator) were obtained from the United States census.

We evaluated demographic characteristics (patient age, sex, and comorbidity) and perioperative outcomes (in-hospital mortality, pulmonary embolus, reoperations, respiratory complications, and median length of stay) from the NIS cohort for each year. The Charlson comorbidity index was used to measure severity of illness.[23,24] This index is a weighted score of patient comorbidity based on ICD-9 diagnostic codes for prior myocardial infarction (412), peripheral vascular disease (440.0 to 443.9), chronic pulmonary disease (415.0, 416.8, 416.9, 491, 491 to 494, 496), dementia (290.0 to 290.9, 331.0, 331.2), diabetes mellitus (250.0 to 250.39), diabetes mellitus with complications (250.4

to 250.99), mild liver disease (571.2, 571.5, 571.6, 571.8, 571.9), severe liver disease (572.2 to 572.4, 456.0 to 456.29), chronic renal failure (585.0 to 586.9, V420, V451, V56.0 to V56.9), various cancers (140.0 to 171.9, 174.0 to 195.9, 200.0 to 208.8, 273.0 to 273.3, V104.6), and metastatic solid tumor (196.0 to 199.9). Complications were identified by the presence in the discharge abstract of specific ICD-9 codes for pulmonary embolus (415.1); reoperation for hemorrhage, anastomotic leakage, abscess, or dehiscence (54.11, 54.12, 54.19, 54.61); and respiratory complications including prolonged mechanical ventilation for more than 96 hours (96.72), tracheostomy (519.0, 519.00, 519.01, 519.02, 519.09, 31.1, 31.2, 31.21, 31.29, 96.55, 97.23), pneumonia (519.8, 997.3), respiratory failure (518.5, 518.81, 518.82, 518.84), and respiratory arrest (799.1).

Secular trends were tested for statistical significance using logistic regression for dichotomous variables (sex, type of procedure, in-hospital mortality, pulmonary embolus, reoperation, and respiratory complications) with year as a continuous independent variable. Nonparametric rank-sum tests were used to test secular trends for continuous dependent variables (patient age, comorbidity, and median length of hospital stay).

Multiple logistic regression was used to calculate adjusted mortality rates for each year.[25] Age and sex were selected as covariates for the final model following stepwise comparison of nested models. Although race showed significant changes over time (5.0% black in 1990 vs. 11.5% in 1997; $P < 0.001$), this variable was excluded from the regression analysis because of systematically missing data from certain states.

Population sampling weights from the NIS were applied to all statistical computations to account for the stratified, clustered survey design. Significance for all tests was set at a P value of less than 0.05. All P values are two tailed. The analyses were all performed using statistical computer software (STATA 7.0, STATA Corp., College Station, TX).

RESULTS
Rates of Surgery

From 1990 to 1997, the national annual rate of bariatric surgery more than doubled from 2.7 to 6.3 per 100,000 adults ($P < 0.001$) (Fig. 1). We observed a shift toward the use of gastric bypass during this time period. National gastric bypass utilization rates increased nearly fourfold from 1.4 to 5.4 per 100,000 adults, whereas gastroplasty utilization declined significantly, as seen in (Fig. 2). As a proportion of all
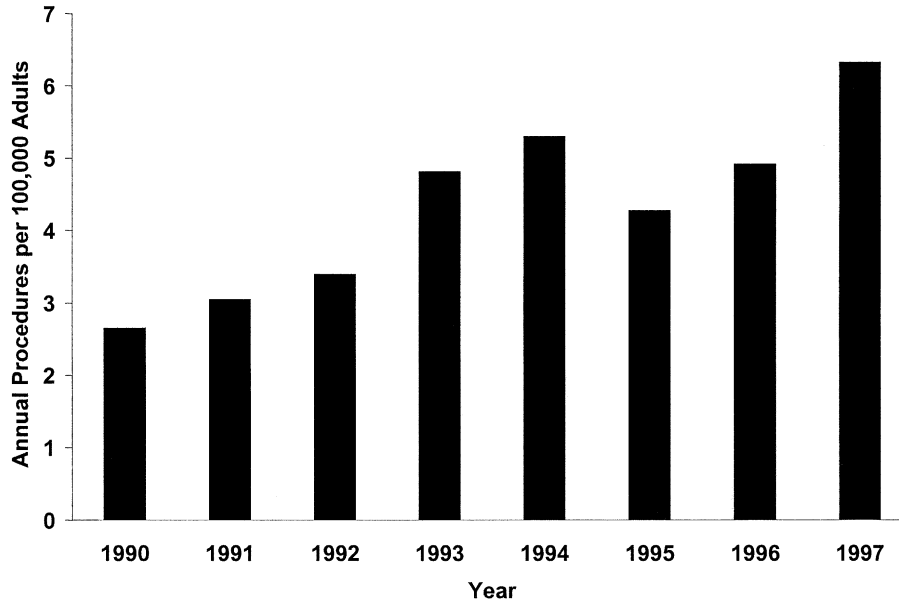
Fig. 1. Annual population-based rates of bariatric surgery in the United States, 1990 to 1997.

bariatric procedures, gastric bypass thus increased from 52% in 1990 to 84% in 1997 (Table 1).

Patient Characteristics

We observed slight increases in mean patient age (38.0 years in 1990 to 40.2 years in 1997; $P < 0.001$) and the proportion of patients with one or more major comorbid conditions (20.8% to 31.4%; $P < 0.001$). The proportion of procedures performed in

female vs. male patients did not change significantly (see Table 1).

In-Hospital Outcomes

The unadjusted in-hospital mortality rate showed a slight upward trend from 0.3% in 1990 to 0.5% in 1997 ($P = 0.08$). After adjustment for age and sex, mortality remained the same without an observed trend ($P = 0.3$). From 1990 to 1997, there were significant decreases in overall rates of reoperations for bleeding, abscess, and
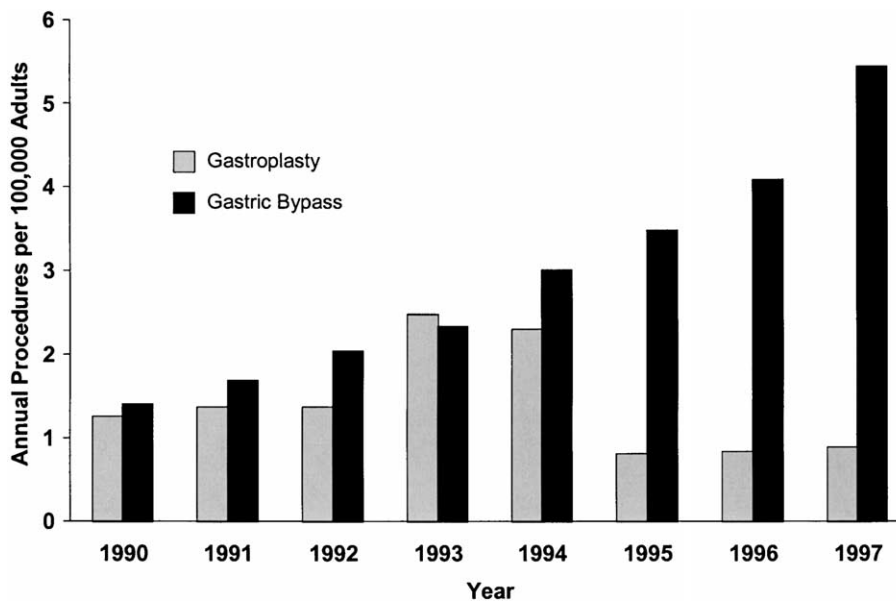

Fig. 2. Annual population-based rates of gastroplasty and gastric bypass procedures, 1990 to 1997.

Table 1. Summary of procedures, patient characteristics, and perioperative outcomes after bariatric surgery in the United States, 1990 to 1997

	1990	1991	1992	1993	1994	1995	1996	1997	Trend (P value)
Procedures									
Total	4,925	5,710	6,425	9,189	10,204	8,314	9,651	12,541	
Rate/100,000 adults	2.7	3.1	3.4	4.8	5.3	4.3	4.9	6.3	
Gastric bypass (%)	52.9	55.3	59.9	48.6	56.6	81.3	83.1	86.1	<0.001
Gastroplasty (%)	47.1	44.7	40.1	51.4	43.4	18.7	16.9	13.9	<0.001
Patient characteristics									
Median age (yr)	37	39	38	39	38	39	39	40	<0.001
Sex (% female)	85.5	83.2	86.2	85.4	82.9	83.2	84.5	83.6	0.2
One or more comorbid conditions (%)*	20.8	19.9	27.1	28.1	26.7	30.9	30.8	31.4	<0.001
Perioperative outcomes									
Crude mortality (%)	0.31	0.09	0.16	0.60	0.36	0.26	0.61	0.51	0.08
Adjusted mortality (%)	0.29	0.08	0.20	0.57	0.31	0.24	0.56	0.50	0.3
Pulmonary embolus (%)	0.24	1.2	0.34	0.29	0.35	0.13	0.0	0.07	<0.01
Reoperations (%)	2.2	3.1	2.6	2.0	1.5	2.0	1.8	1.4	<0.01
Respiratory complications (%)	7.4	6.7	10.4	10.5	7.2	5.7	6.5	5.9	<0.001
Median length of stay (days)	5	5	5	5	5	5	4	4	<0.01

All data except median length of stay are national estimates based on sampling weights from the NIS.
*Percentage of patients with a Charlson comorbidity index ≥ 1.

dehiscence (2.2% to 1.4%; $P < 0.01$). Similarly, declines were observed in rates of respiratory complications (7.4% to 5.9%; $P < 0.001$), which include prolonged ventilation, tracheostomy, pneumonia, respiratory failure, or respiratory arrest. Pulmonary emboli followed a declining trend but occurred infrequently (0.24% in 1990 to 0.07% in 1997; $P < 0.01$). Median length of stay declined (5 to 4 days; $P < 0.01$), whereas the proportion of patients with hospital stays longer than 14 days (2.9% overall) did not change significantly.

DISCUSSION

Bariatric surgery utilization rates in the United States have increased more than twofold between 1990 and 1997, from 2.7 to 6.3 per 100,000 adults. This trend was associated with a shift toward the use of gastric bypass. On average, patients undergoing bariatric surgery tended to be slightly older, with more comorbid conditions toward the end of the study period. Perioperative outcomes such as pulmonary emboli, reoperations, and respiratory complications improved over this time, whereas in-hospital mortality remained stable.

Comparison to a Population-Based Study

We are aware of only one other population-based study of bariatric surgery. This national study from Sweden showed increasing surgery rates from 7.7 to 13.6 per 100,000 adults (age >17 years) between 1990 and 1996.[26,27] Swedish bariatric surgery rates are more than twice as high as those seen in our study but increase at a similar rate over a corresponding period of time.

The Swedish study also demonstrated a decline in the use of bariatric surgery from 1994 to 1995.[26] We suspect that the decreases in utilization between 1994 and 1995, observed both in our study and the Swedish study, can be explained by the popularization of the "magic bullet" anorectic combination fenfluramine-phentermine (fen-phen). The number of fenfluramine prescriptions grew from 50,000 per year in 1994 to more than 1 million in 1995.[28] Bariatric surgery utilization in the United States began to rebound in 1996, even though more than 7 million prescriptions for fenfluramine were written that year. Utilization then rose sharply the next year during which fenfluramine was taken off of the market because of the potential for severe side effects.

The report from Sweden is also the only previously published population-based study of in-hospital mortality after bariatric surgery.[26] The overall in-hospital mortality rates reported in the Swedish study were identical to our findings of 0.37% (95% confidence interval 0.26% to 0.48%).

Comparison to Clinical Series

In-hospital outcomes of bariatric surgery were found to be similar to those reported in published clinical series. Operative mortality in large clinical series averaged 0.24% after vertical banded gastroplasty (VBG)[29] and 0.4% after both gastric bypass

and biliopancreatic diversion,[30,31] with a range of 0% to 2% across a large volume of clinical literature.* The surgical literature describes early perioperative complications of pulmonary embolus, gastric leakage, gastrointestinal hemorrhage, major and minor wound problems including infection and dehiscence, pulmonary embolus, atelectasis, and reoperation after bariatric procedures. The in-hospital complication rates that we were able to identify using administrative data were consistent with the published complications rates of other studies.† Pulmonary emboli occurred in up to 0.5% of patients approximately in the literature as compared to yearly incidence rates ranging from 0 to 1.2% in our data.[14,17,19,33,35] Published reoperation rates ranged from 1% to 2.8%, whereas we observed rates ranging from 1.4% to 3.1%.[14,17,19,44] Finally, respiratory complications are rarely reported in the literature on bariatric surgery. Atelectasis was noted as an early complication in 5.8% of patients in a series by Yale.[33] Our broader definition of respiratory complications included prolonged ventilation, pneumonia, tracheostomy, and respiratory failure and arrest. We found higher rates of these respiratory complications (5.9% to 10.5%), but we did not find any studies reporting these outcomes.

Limitations

The limitations of this study are related to the use of administrative data. First of all, our ability to identify bariatric surgical procedures was limited by the absence of specific ICD-9 procedure codes for simple gastric restrictive procedures such as VBG or gastric banding, malabsorptive procedures such as biliopancreatic diversion, or laparoscopic bariatric procedures. We could easily identify gastric bypass procedures (44.31), but identification of other bariatric surgical techniques required including less specific codes, such as "Gastroplasty not elsewhere classifiable (NEC)" (44.69) and "Other gastroenterostomy bypass" (44.39), plus the presence of a diagnostic code for obesity. We believe this may have led to an underestimate of the number of bariatric procedures if diagnosis codes for obesity were not consistently used by hospitals.

Second, our conclusions regarding secular changes in mortality and other outcomes are limited by our ability to adjust for case-mix differences other than patient age, sex, and coded comorbid conditions. Information regarding patients' baseline operative risk is difficult to ascertain from administrative data.

Finally, the NIS data is limited to in-hospital outcomes. Most surgical literature considers early outcomes as in-hospital and less than 30 days postoperatively. However, we were unable to capture postdischarge adverse events.

Implications

Most of the increased use of bariatric surgery appears to be associated with increasing preference for gastric bypass procedures vs. gastroplasty procedures. Jones[21] reports similar shifts in choices of primary bariatric operations among members of the American Society of Bariatric Surgery between 1989 and 1998, with increases from 42% to 82% in malabsorptive operations (e.g., gastric bypass or biliopancreatic diversion) and decreases from 54% to 15% in gastric restrictive procedures (e.g., VBG).

Minimally invasive surgery had not become a significant factor in the increased utilization of bariatric surgery over the period of our study because advanced laparoscopy was still in the experimental stages during most of the 1990s. However, laparoscopy may play a role in even greater post-1997 increases in bariatric surgery utilization as patients are attracted by the promises of lower morbidity and quicker recovery.

Even greater increases in utilization of bariatric surgery are likely due to an increasing number of providers offering bariatric surgery, a continued rise in the prevalence of obesity in the United States population,[1–3] and greater patient acceptance of bariatric surgery. However, bariatric surgery utilization appears to be greatly influenced by patient preference and competing options. As our data show, rates of bariatric surgery in the future could be greatly affected by the emergence of a new weight loss "miracle drug."

CONCLUSION

Annual rates for bariatric surgery in the United States between 1990 and 1997 more than doubled, with no substantial changes in perioperative morbidity or mortality. This trend was largely associated with an increase in the use of gastric bypass procedures. Rates of bariatric surgical procedures may continue to increase with the recent application of minimally invasive techniques, widespread publicity, and a large reservoir of potential patients.

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†References 14, 17, 19, 31, 33–35, 42–44.

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Discussion

Dr. L.W. Traverso (Seattle, WA): I enjoyed this paper and would like to ask a very specific question. The Institute of Medicine in its report to Congress called on all surgical organizations to begin compiling their outcomes. This is not being done currently for many reasons. Do you know of any outcomes recording in the bariatric surgery community that are being compiled nationally? You might know that the SAGES Outcomes Morbid Obesity tool has been available since January 1 of this year and is compiling these items on a national basis, but do you know of any other?

Dr. G. Pope: Yes. The BAROS bariatric surgery registry through the American Society of Bariatric Surgeons is collecting outcomes, which also include patient satisfaction before and after their surgery, and actually much of our data corresponds well with what they have found among their members.

Dr. L.F. Rikkers (Madison, WI): I would just comment that so often as we see technology rapidly proliferate, as we have seen in the past with other minimally invasive operations, it is encouraging that as this type of surgery is extended to many medical centers complica-

tions and mortality have actually gone down rather than a bit up.

Dr. H.J. Sugerman (Richmond, VA): I would just like to comment, as I heard this presented yesterday to the news media. It is an estimate that in 1999 there were about 20,000 operations performed. According to the Bariatric Surgical Society it was estimated there were 40,000 performed in 2000 and that number approached 75,000 in 2001. Clearly, the popularity of this procedure is exploding. And I hope we are not jumping onto a "bandwagon" that is going to end up being negative. I hope it is a positive bandwagon, and that it is going to be wonderful for patients who are desperately in need of these procedures. I am also hoping that we will not have a problem in the future with the explosion of laparoscopic procedures for common duct-type injuries with the initial explosion of laparoscopic cholecystectomy. I am hoping that all of us who get involved with the laparoscopic approach of this procedure will be doing it appropriately and safely with adequate guidance and training, not only in terms of the technique of the procedure but in terms of the postoperative management of these patients, which requires a lot of work.

Surgical Management of Abdominal Tuberculosis

Imran Hassan, Emmanouil S. Brilakis, Rodney L. Thompson, Florencia G. Que, M.D.

Recent reports suggest an increased incidence of abdominal tuberculosis in the United States, particularly in high-risk groups. The aim of this study was to review the spectrum of abdominal tuberculosis and its surgical management at a tertiary referral center in the United States. The medical records of patients treated for abdominal tuberculosis at our institution between January 1992 and June 2001 were retrospectively reviewed. Eighteen patients were diagnosed with abdominal tuberculosis by microbiologic and/or histologic examination. The 10 men and eight women had a mean duration of symptoms of 4 months (range 1 to 24 months). Five were born in the United States, and 13 were foreign born (7 Asians and 6 Africans). The United States–born patients with abdominal tuberculosis, as compared to the foreign-born patients, were older (mean age 74 years *vs.* 35 years), more likely to have chronic medical illnesses (80% *vs.* 7%), and had concomitant pulmonary tuberculosis (60% vs. 0%). Computed tomography was the most frequent imaging modality (88%); findings suggestive of abdominal tuberculosis were mesenteric/omental stranding (50%), ascites (37%), and retroperitoneal lymphadenopathy (31%). Seventeen of the 18 patients required operative intervention, and one patient underwent CT-guided drainage of a psoas abscess. Laparoscopy was useful for diagnosis in eight patients; laparotomy was performed for complications of abdominal tuberculosis in six patients and to obtain a tissue diagnosis in three patients. Abdominal tuberculosis continues to represent a diagnostic challenge to clinicians. Among native-born white Americans, abdominal tuberculosis is primarily a disseminated disease of elderly, debilitated patients with chronic illnesses. Among foreign-born individuals, abdominal tuberculosis occurs in young, immunocompetent patients from endemic areas. Characteristic CT findings should be evaluated for abdominal tuberculosis in the appropriate clinical setting. Laparoscopy is an effective modality for diagnosis of peritoneal tuberculosis. (J GASTROINTEST SURG 2002;6:862–867.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Abdominal tuberculosis, United States, management, laparoscopy, CT scans

During the second half of the twentieth century, as a result of improved nutrition, reduced crowding, public health measures, and effective chemotherapy, a dramatic decrease in the incidence of tuberculosis was seen in the United States. However, with the advent of the human immunodeficiency virus (HIV) in the 1980s and an increase in the immigrant population in the 1990s, there has been a resurgence of tuberculosis in this country.[1] HIV is a major risk factor for the development of clinical tuberculosis. It has been estimated that a person who is infected with both HIV and tuberculosis has a 7% to 10% chance per year of developing active tuberculosis, as opposed to the 10% lifetime chance of someone who is infected with tuberculosis alone.[2] At the same time, epidemiologic figures have shown an increase in the number of foreign-born persons with tuberculosis. In 1997, 39% of the cases of tuberculosis in the United States were in foreign-born individuals as compared to 27%, 5 years earlier.[3] Several reports have also shown a relatively high incidence of extrapulmonary disease among HIV-infected individuals and the immigrant population with tuberculosis.[4] As a result, the proportion of cases of tuberculosis involving extrapulmonary sites including the abdomen has steadily increased in the United States from 8% in 1964 to 26.6% in 1998.[3,5]

Abdominal tuberculosis has protean manifestations and is known to imitate a variety of intra-ab-

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dominal disorders.[6] Unless there is a high index of suspicion, this diagnosis is often overlooked, resulting in significant morbidity and mortality. Recent studies on the surgical management of abdominal tuberculosis in the United States have come from regions where the incidence of HIV is high and where there are large immigrant populations.[5,7] The purpose of our review was to determine the spectrum of abdominal tuberculosis and its surgical management in an area where these groups are not as prevalent.

PATIENTS AND METHODS

From January 1992 to June 2001, 18 patients were diagnosed with abdominal or pelvic tuberculosis at our institution. Traditionally, reports of abdominal tuberculosis included infection of the gastrointestinal tract, peritoneum, mesentery, abdominal lymph nodes, liver, spleen, and pancreas. In addition to these sites, we also included patients with pelvic disease in this series. The diagnosis of abdominal tuberculosis was made if tissue obtained during laparotomy or laparoscopy yielded positive cultures for *Mycobacterium tuberculosis* complex and/or acid-fast bacilli were seen on histologic examination of the specimen along with strong clinical evidence. Patients with *Mycobacterium avium* intracellulare (MAC) infections were excluded. Details of culture techniques have been described in detail previously[8] and will be summarized as follows. Specimens were treated with equal volume of BBL MycoPrep Reagent (BD Diagnostic Systems, Sparks, MD) containing 2% NaOH and *N*-acetyl-L-cysteine mixed and incubated at 25° C for 15 minutes. After the addition of MycoPrep buffer and centrifugation, the supernate was removed and the sediment was suspended in 3 ml of MycoPrep phosphate buffer. Cultures were performed by inoculating 0.5 ml of sediment into a MGIT tube and onto each side of a biplate that contained Middlebrook 7H10 agar and Middlebrook 7H11 selective agar with antimicrobials (Remel, Lenexa, KS). After inoculation, tubes were placed into the BACTEC MGIT 960 System and incubated at 35 to 37° C for 6 weeks. Biplates were sealed in polyethylene bags and incubated at 37° C in the presence of 5% to 7% CO_2, and observed weekly for the presence of growth over an 8-week incubation period.

Patient histories including the surgeon's operative notes, hospital records, and follow-up data were reviewed for variables including patient age, sex, nationality, presenting symptoms and duration, history of tuberculosis exposure, preoperative laboratory values, radiographic investigations, type of operation, micro-

biological stains, cultures and susceptibilities, postoperative course, and follow-up.

RESULTS
Patient Demographics

There were 10 men and eight women, whose average age was 44 years (range 16 to 88 years); the mean duration of symptoms was 4 months (range 1 to 24 months) in the symptomatic patients. Five patients were born in the United States and 13 were foreign born. Among the foreign-born patients, there were seven immigrants: six from Africa (4 from Somalia, 1 each from Ethiopia and Kenya) and one from India. The average duration of United States residence of the immigrants prior to presentation was 60 months (range 14 to 134 months). Six patients had come from abroad for medical treatment, including four patients from the United Arab Emirates and one patient each from Saudi Arabia and Kuwait. Therefore there were three main groups of patients identified in our cohort. The first group (n = 5) was comprised of patients who were native-born white Americans and were, on average, older than the rest of the patients (mean age 74 years). The second group (n = 7) included patients who had emigrated to the United States. These individuals were younger (mean age 26 years) than the others and were otherwise healthy. The third group (n = 6) of patients were those who had come to our institution for treatment from abroad and were of an intermediate age group (mean age 41 years). There was no difference in the duration of symptoms prior to diagnosis among the three groups.

Clinical Presentation

Abdominal pain (76%), weight loss (64%), fever (35%), and abdominal distention (24%) were the most common presenting signs and symptoms. One patient was asymptomatic at presentation (Table 1). Five patients had concomitant extra-abdominal sites of tuberculosis: three patients with pulmonary tuberculosis and one patient each with cervical lymph nodes and central nervous system involvement. Tuberculosis was suspected preoperatively in 12 (66%) of 18 patients. The preoperative diagnoses in the remaining patients included lymphoma in three, ovarian cystic mass in two, and disseminated intra-abdominal malignancy in one. Five patients (27%) had a history of prior tuberculosis exposure (two patients each from groups 1 and 3 and one patient from group 2).

Associated comorbid conditions included hematologic malignancies in two patients (chronic lymphocytic leukemia and myelodysplastic syndrome in one

Table 1. Presenting signs and symptoms in patients
with abdominal tuberculosis

Signs and symptoms	No. of patients (%) n = 17*
Pain	13 (76)
Weight loss	11 (64)
Fever	6 (35)
Nausea/emesis	5 (29)
Fatigue	4 (24)
Abdominal distention	4 (24)
Anorexia	3 (18)
Abdominal mass	3 (18)
Night sweats	3 (18)
Abdominal tenderness	3 (18)

*One patient was asymptomatic.

patient each), diabetes mellitus, and long-term steroid use for polymyalgia rheumatica and alcoholism with cirrhosis in one patient each.

Laboratory Investigations

Seventeen patients (94%) were anemic preoperatively. The average hemoglobin concentrations for men and women were 12 g/dl and 10.6 g/dL, respectively (normal value for men, 13.5 mg/dl; normal for women, 11.5 mg/dl). Three patients had leukocytosis ($>10 \times 10^9$/L), and none had significant monocytosis or lymphocytosis. Ten patients (55%) had hypoalbuminemia (serum albumin <3.5 g/dl). More than twice the normal elevation in transaminases and alkaline phosphatase levels was seen in six patients (33%). Elevation (>30 mm/hr) in the erythrocyte sedimentation rate was seen in nine patients (50%). Results of tuberculin skin testing were available for 14 patients; intermediate purified protein derivative (PPD) was positive in six patients and negative in eight. HIV serology in all 10 patients who were tested was negative.

Radiologic Findings

Six (33%) of the 18 patients had an abnormal chest radiograph. Abnormalities noted included pleural effusion (2 patients), pulmonary infiltrate (2 patients), apical opacity (1 patient), and soft tissue prominence around the gastroesophageal junction (1 patient). The latter was subsequently found to represent asymptomatic retroperitoneal lymphadenopathy around the celiac artery and the aorta. All three patients with active pulmonary tuberculosis had an abnormal chest radiograph.

CT scanning was the most frequently used abdominal imaging modality (16 patients) followed by gastrointestinal contrast studies (4 patients), colon-

oscopy (3 patients), and abdominal ultrasonography (3 patients). The most common CT findings in abdominal tuberculosis were ascites, lymphadenopathy, and omental/mesenteric stranding (Table 2).

Operative Findings and Management

Seventeen patients underwent surgical procedures, whereas one patient had CT-guided drainage of a psoas abscess. All operations were performed electively, with the exception of one emergent surgery for a tuboovarian abscess. Of the patients requiring surgery, eight underwent a laparoscopic exploration, whereas nine underwent a laparotomy. Seven (out of 8) patients undergoing laparoscopy had peritoneal tuberculosis, with diffuse involvement of the visceral and parietal peritoneum and innumerable peritoneal nodules or plaques, usually with ascites. In these patients, laparoscopic peritoneal biopsies were obtained to confirm the diagnosis.

Among the patients who underwent open exploratory operations, two had pelvic tuberculosis with tuboovarian abscesses and required salpingo-oophorectomy. Two other patients had gastric outlet obstruction from retroperitoneal and gastroduodenal lymphadenopathy causing compression of the pylorus. One patient required a gastrojejunostomy, whereas the other had a venting gastrostomy and a feeding jejunostomy. Two other patients were found to have an ileocecal mass from lymphadenopathy in the intestinal mesentery, resulting in narrowing of the bowel lumen and abdominal pain. Both of these patients underwent a right hemicolectomy with primary anastomosis. One patient each had lymphadenopathy in the small bowel mesentery forming a mass, retroperitoneal lymphadenopathy along the celiac artery, hepatic artery, and aorta, and diffuse hepatic disease, respectively (Table 3). These three patients underwent excisional biopsies to establish a tissue diagnosis.

There was no significant operative morbidity or mortality among the patients who underwent surgery.

Table 2. CT scan characteristics of patients with abdominal tuberculosis

CT findings	No. of patients (%) n = 16
Ascites	6 (37)
Retroperitoneal lymphadenopathy	5 (31)
Mesenteric stranding	4 (25)
Omental stranding	4 (25)
Mesenteric mass/lymphadenopathy	3 (19)
Bowel wall thickening	2 (13)

Table 3. Intra-abdominal sites of tuberculosis involvement

Site of involvement	No. of patients (%) n = 18
Peritoneal	8 (44)
Gastroduodenal	2 (11)
Ileocecal	2 (11)
Tuboovarian	2 (11)
Retroperitoneum	2 (11)
Liver	1 (6)
Mesenteric	1 (6)

Pathology, Microbiology, and Chemotherapy

Pathologic examination of operative specimens showed necrotizing and non-necrotizing granulomas consistent with granulomatous disease in all patients. Patients were started on the standard four-drug antituberculous regimen after a clinical diagnosis of abdominal tuberculosis, based on operative and pathologic findings. *Mycobacterium tuberculosis* organisms were subsequently cultured in 17 patients and were fully sensitive to isoniazid, rifampin, ethambutol, pyrazinamide, and streptomycin in all cases. Drug regimens and duration of treatment were then adjusted according to the microbiological sensitivities, extent of extra-abdominal involvement, and resolution of symptoms. The mean length of proposed treatment was 6 months (range 6 to 18 months). Routine follow-up abdominal studies were not performed in patients who responded appropriately to chemotherapy and were doing well clinically once they completed their treatment.

Two patients developed significant liver function abnormalities after chemotherapy was initiated, necessitating temporary discontinuation of therapy. Treatment was reinstituted at a lower dosage once hepatic enzyme levels normalized.

Follow-Up

Mean follow-up was 18 months (range 1 to 60 months). One patient died 4 years after diagnosis of abdominal tuberculosis from his underlying hematologic malignancy. One female patient who had pelvic tuberculosis developed infertility and is currently undergoing evaluation for in vitro fertilization.

DISCUSSION

Our review highlights several of the tribulations that the diagnosis and treatment of abdominal tuber-culosis pose for physicians in the United States. In the first group, which consisted of native-born patients, abdominal tuberculosis was suspected preoperatively in only one of them. The majority had concomitant active pulmonary disease and associated chronic medical illnesses. These findings are similar to those of previous studies on tuberculosis among the native-born white American population. Reactivation disease is mainly seen in elderly debilitated patients with chronic illnesses, and these patients are more likely to have disseminated multiorgan disease.[9]

The presenting signs and symptoms, as well as the physical findings in our series, were nonspecific and nondiagnostic, and this resulted in a significant delay in diagnosis. This observation is similar to those in previously published reports on this condition.[9–11] Patients had anemia and hypoalbuminemia, reflecting a chronic malnourishing process, although laboratory investigations could not reliably distinguish abdominal tuberculosis from other chronic disease processes. HIV infection was not identified in our cohort and has a low prevalence in our patient population. All patients in this country who present with tuberculosis, however, must be screened for HIV. The incidence of liver enzyme abnormalities was not as high, as was reported in a recent series.[7] This most likely reflects differences in study populations, since their cohort had several patients with HIV infection in whom liver enzyme abnormalities may have been the result of the underlying disease, its complications, or its treatment. It is important to monitor hepatic function in these patients during treatment for tuberculosis, because several first-line antitubercular drugs are hepatotoxic.

Tuberculin skin tests were positive in only 42% of the patients and could not be used as a dependable predictor of disease. This test has been shown to have a lower specificity for abdominal disease as compared to pulmonary tuberculosis.[11] Particularly in areas where tuberculosis is endemic, it has been found to have a high false positive rate.[11] Furthermore, the tuberculin skin test cannot accurately differentiate between active disease and previous sensitization by contact or vaccination.[10] In general, experience suggests that a positive PPD in a patient from an area where tuberculosis is endemic should be attributed to exposure and infection, not vaccination, whereas a negative PPD does not rule out disease in any case. The incidence of concurrent active pulmonary disease in patients with abdominal tuberculosis was low (16%) in our series. Although some investigaors have noted a higher incidence of active pulmonary involvement, there have been several recent reports showing a similar low incidence of active pulmonary tuberculosis in patients with abdominal tuberculo-

sis.[6] The pathogenesis of abdominal tuberculosis is presumed to involve hematogenous spread from a primary focus in the lung, ingestion of infected sputum, or local reactivation of disseminated infection at a much later date. It has therefore been suggested that these patients may have had previous undiagnosed pulmonary tuberculosis, which has either resolved or is just not radiologically apparent.[1] It is necessary to consider the diagnosis of abdominal tuberculosis, despite the absence of clinical or radiologic evidence of active pulmonary tuberculosis.

CT scanning was the most common imaging study used in our series. Although CT was unable to specifically differentiate this condition from other intra-abdominal disorders, it was sensitive in detecting various intra-abdominal abnormalities that are typical of abdominal tuberculosis. In the appropriate clinical settings, patients with a constellation of characteristic CT findings such as retroperitoneal lymphadenopathy, bowel wall thickening, ascites, or omental and mesenteric stranding should be suspected of having abdominal tuberculosis and should be evaluated accordingly. Colonoscopy and gastrointestinal contrast studies were useful adjuncts in diagnosing patients with abdominal tuberculosis involving the gastrointestinal tract. Ultrasound was able to detect abnormalities in the pelvis in both patients with pelvic tuberculosis that presented as tuboovarian abscesses. It is recommended that these diagnostic modalities be considered in conjunction with clinical findings, laboratory investigations, and CT scans to establish a definitive diagnosis.

The role of surgery has traditionally been described as being limited to managing complications from abdominal tuberculosis.[9,12] In our series, complications resulting from abdominal tuberculosis were seen in only six patients (33%). These included gastric outlet obstruction from gastroduodenal retroperitoneal lymphadenopathy, intestinal obstruction and pain from an ileocecal mass, and tuboovarian abscesses. Surgery, and laparoscopy in particular, however, was more commonly used as a diagnostic modality especially in patients with peritoneal tuberculosis. The preoperative investigations and radiographic findings in these patients are nonspecific, and it is often not possible to differentiate peritoneal tuberculosis from a disseminated malignancy or primary liver disease. Ascitic fluid, which is usually present in these patients, can be obtained via paracentesis, but the isolation rate for *Mycobacterium tuberculosis* is only 20%,[13] whereas the sensitivity and specificity of various chemical tests on the ascitic fluid, such as the adenosine deaminase assay, are still approximately 80%.[13] In these patients, laparoscopic exploration provides an ideal opportunity to examine the intra-abdominal contents and establish a clinical diagnosis while obtaining sufficient tissue for histologic and microbiologic examination.

Pelvic tuberculosis is not conventionally included in discussions of abdominal tuberculosis. We believe that involvement of the pelvic organs is a continuum of the abdominal process demonstrating the potential for this disease to affect any organ in the body. The significance of pelvic tuberculosis lies in its long-term consequences with regard to the fertility of female patients. A high incidence of infertility has been reported in patients with pelvic tuberculosis,[14] and hence this should be kept in mind when treating and counseling these patients.

With the increased incidence of tuberculosis in certain high-risk groups such as immigrants and HIV-infected individuals, the number of patients with abdominal tuberculosis will increase in this country. However, because of its nonspecific presentation and the lack of reliable diagnostic tests, abdominal tuberculosis will continue to present a challenge to the clinicians evaluating these patients. Immigrants or visitors from areas where tuberculosis is endemic who have characteristic CT findings and symptoms should always be evaluated for the possibility of abdominal tuberculosis. Laparoscopy is an effective modality for diagnosis of patients with peritoneal tuberculosis and should be considered in cases where there is a clinical suspicion of the disease. Mortality in these patients is related to the underlying disease process and not to the abdominal tuberculosis itself. The outcome of immunocompetent patients with abdominal tuberculosis is favorable, but long-term follow-up is important.

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Adenomatous Polyposis Coli Truncation Alters Cytoskeletal Structure and Microtubule Stability in Early Intestinal Tumorigenesis

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Partial loss of function of adenomatous polyposis coli (APC) protein by truncation of its carboxy (C)-terminus is an early factor in the development of many sporadic colorectal cancers. In the C57BL/6J Min/+ (Min/+) mouse, an animal with a germline mutation of *Apc*, we found that APC truncation was associated with reduced enterocyte migration and loss of association and membrane expression of adherens junction proteins. We hypothesized that these defects were related to changes in cytoskeletal function resulting from truncation of the APC C-terminus, which contains microtubule binding regions, as well as putative sites for indirect actin binding. We investigated this further by determining whether APC truncation produced in vivo changes in actin cytoskeletal structure and microtubule stability. The actin cytoskeleton of histologically normal enterocytes from Min/+ mice was compared to that of *Apc+/+* (wild-type) mice by confocal indirect immunofluorescence microscopy. We found a significant loss of actin localization at the apical plasma membrane in Min/+ enterocytes. In addition, immunoblotting revealed increased levels of both unstable Tyr-tubulin and α-tubulin turnover in Min/+ enterocytes, indicating an alteration in microtubule dynamics. These studies suggest that loss of actin localization and changes in microtubule dynamics may be dominant negative effects of truncated APC. These changes are consistent with the defects in enterocyte migration and junctional complex formation observed in the Min/+ model of early APC-associated colorectal tumorigenesis. (J GASTROINTEST SURG 2002;6:868–875.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: APC, cytoskeleton, colorectal cancer

The adenomatous polyposis coli (*APC*) gene encodes a 310 kD cytoplasmic protein with numerous functional domains[1] (Fig. 1). The major influence of APC on intestinal cell growth is best illustrated by the phenotype of patients with germline mutations of *APC*, a familial tumor syndrome known as familial adenomatous polyposis. These individuals develop hundreds of lower intestinal adenomas and also exhibit neoplastic transformation of the duodenal mucosa. The great majority of germline *APC* mutations found in familial adenomatous polyposis produce truncations of the APC protein. Similar mutations are also frequently observed as an early event in sporadic colon tumors.[2,3] The best known function of APC is its role as one member of a multi-protein degradation complex that regulates the intracellular concentration of the oncoprotein, β-catenin. Loss of APC protein increases free β-catenin, which in turn promotes its nuclear association with Tcf-4, thus creating a transcriptional activator of growth-related genes.[4] More recently, APC has been recognized for its multifunctional role in diverse cellular processes including cytoskeletal regulation.
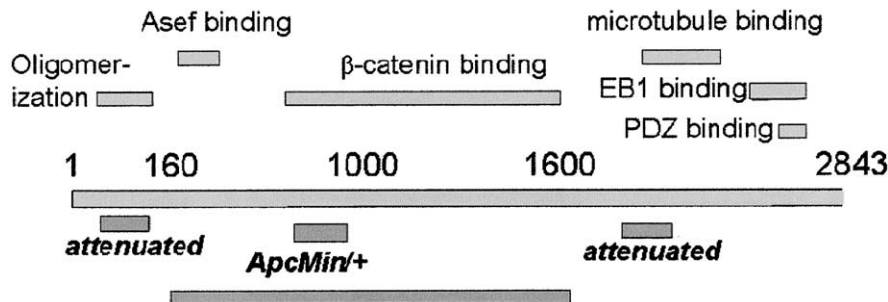
One of the earliest discernable consequences of APC loss is an outpocketing and accumulation of intestinal epithelial cells above the crypt, despite unchanged levels of proliferation.[5] This abnormal architecture could represent the effect of inappropriate activation of β-catenin–Tcf transcriptional activation resulting

Fig. 1. Domain structure of the APC protein. The *Apc* gene encodes a 310 kD protein product with multiple binding domains. The amino (N)-terminus contains heptad repeats that form coil-coil domains involved in oligomerization (Joslyn G, et al. Proc Natl Acad Sci 90:11109–11113, 1993). ASEF binds to the APC N-terminus. The central portion of the APC protein interacts with members of the Wnt signaling pathway, including β-catenin, GSK3β, and axin. The C-terminus mediates interaction with scaffolding proteins through its PDZ binding domain located in the last 15 aa's. Binding sites for EB-1 and microtubules are also shown. Mutation sites common in both "classical" and "attenuated" familial adenomatous polyposis, as well as the site of the $Apc^{Min/+}$ mutation at codon 850, are shown.

from "second-hit" loss of wild-type APC expression. However, previous work in our laboratory suggests that these early changes may reflect a failure of intestinal cell adhesion or migration.[6–8] We examined the intestinal mucosa of the C57BL/6J-Min/+ (Min/+) mouse, an animal model of familial adenomatous polyposis. These animals carry a germline mutation at codon 850 of murine *Apc* that yields an APC protein truncated at its carboxy (C)-terminus. Min/+ mice develop approximately 20 to 30 intestinal tumors by 4 months of age. We found that the preneoplastic, histologically normal–appearing enterocytes in the small intestine of Min/+ mice are also abnormal, as they show a 25% reduction in migration rate compared to their wild-type (*Apc*+/+) littermates.[9] Enterocyte cell-cell adhesion is also markedly altered in these animals, as they exhibit reduced association between the adherens junction proteins, E-cadherin and β-catenin. In addition, electron micrographs demonstrate a visible separation of the plasma membranes at sites of cell-cell contact[10] (unpublished data). Together these observations suggest that *Apc* mutation alters the architecture of the polarized intestinal epithelium. Directed cell migration, adhesion, and the establishment of cell polarity are processes that define cellular architecture. All require spatial and temporal coordination of intact actin and microtubule networks. Based on the defects observed in the Min/+ intestine, we predicted that enterocytes from these animals would exhibit abnormalities in cytoskeletal function.

MATERIAL AND METHODS

Approximately 3-month-old C57BL/6J $Apc^{Min/+}$ and wild-type littermate (C57BL/6J) mice were ob-

tained from Jackson Laboratories (Bar Harbor, ME). AIN-76A chow was prepared by Research Diets (New Brunswick, NJ). Antibodies directed against tyrosinated tubulin (clone TUB-1A2), α-tubulin (clone B-5-1-2), acetylated tubulin (clone 6-11-B-1), and β-actin (clone AC-40), the proteasome inhibitor N-acetyl-Leu-Leu-norleucinal (ALLN), and nocodazole were purchased from Sigma (St. Louis, MO). Antibody directed against the C–terminus of APC (C-20) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescent-labeled (Alexa-488) phalloidin and (Texas Red) goat antirabbit immunoglobulin G were purchased from Molecular Probes (Eugene, OR). Vectashield 4′6-diamidino-2-phenylindole-2HCl (DAPI) was obtained from Vector Laboratories (Burlingame, CA). Reagents and materials for immunoblot analyses were as described previously.[10]

Enterocyte Isolation

Adult Min/+ and wild-type mice were fed AIN-76A diet and tap water ad libidum. All mice were killed and their intestinal tracts were removed and washed with cold Dulbecco's phosphate-buffered saline (PBS) solution containing physiologic concentrations of $MgCl_2$ and $CaCl_2$. For assays that used drug-treated tissue, segments of small intestine were infused with warm Dulbecco's modified Eagle medium (DMEM) or DMEM containing nocodazole (1 μmol/L) and incubated in a 5% CO_2 incubator at 37° C for 60 minutes. Tumors were separately excised and excluded from analyses of Min/+ intestinal tissue. Enterocytes from macroscopically normal tissue were then removed by scraping the lumen surface using a glass microscope slide and washed twice with PBS before further processing.

Lysate Preparation and Immunoblot Analyses

Cold lysis buffer containing ALLN was added to enterocytes or adenomas from the mouse intestine and homogenized by 10 strokes in chilled dounces.[10] All subsequent steps were performed at 4° C. Samples were centrifuged at 12,000 rpm for 10 minutes, and protein concentrations of the total cell lysates were measured by Bradford assay. Normalized aliquots of each lysate were stored in Laemmli buffer at −70° C. Samples were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), electrotransferred to nitrocellulose membrane, and processed for immunoblot analyses as detailed in prior studies.[11]

## Confocal Immunofluorescence Microscopy

Sections 4 μm thick of wild-type ($Apc^{+/+}$), Min/+ ($Apc^{Min/+}$), and adenomas ($Apc^{Min/−}$) formalin-fixed, paraffin-embedded small intestine were deparaffinized and rehydrated. Antigen retrieval was performed by boiling in 10 mmol/L citrate buffer (pH 6.4). Slides were then washed in PBS with 0.1% Triton-X. Prepared sections were incubated in primary rabbit anti-APC antibody (diluted in PBS with 1% bovine serum albumin) and/or Alexa-488 phalloidin for approximately 2 hours at room temperature, protected from light. Slides were then washed again in PBS/0.1% Triton-X. If primary anti-APC had been applied, slides were incubated in goat antirabbit Texas Red–labeled antibody for 30 minutes to 1 hour at room temperature. Slides were again washed in PBS and mounted with Vectashield DAPI. Images were obtained using a Zeiss LSM410 confocal microscope at ×63 magnification.

RESULTS
Tubulin Dynamics Are Altered in $Apc^{Min/+}$ Enterocytes

The truncation produced by the *ApcMin* allele causes loss of the microtubule-binding portion of the protein. We therefore determined the effect of APC truncation on microtubule characteristics in vivo. At least two populations of microtubules have been distinguished in interphase cells: short-lived, or dynamic, microtubules ($t_{1/2}$ = 5 to 10 minutes) and long-lived, or stable, microtubules ($t_{1/2}$>1 hour).[12] Dynamic mi-
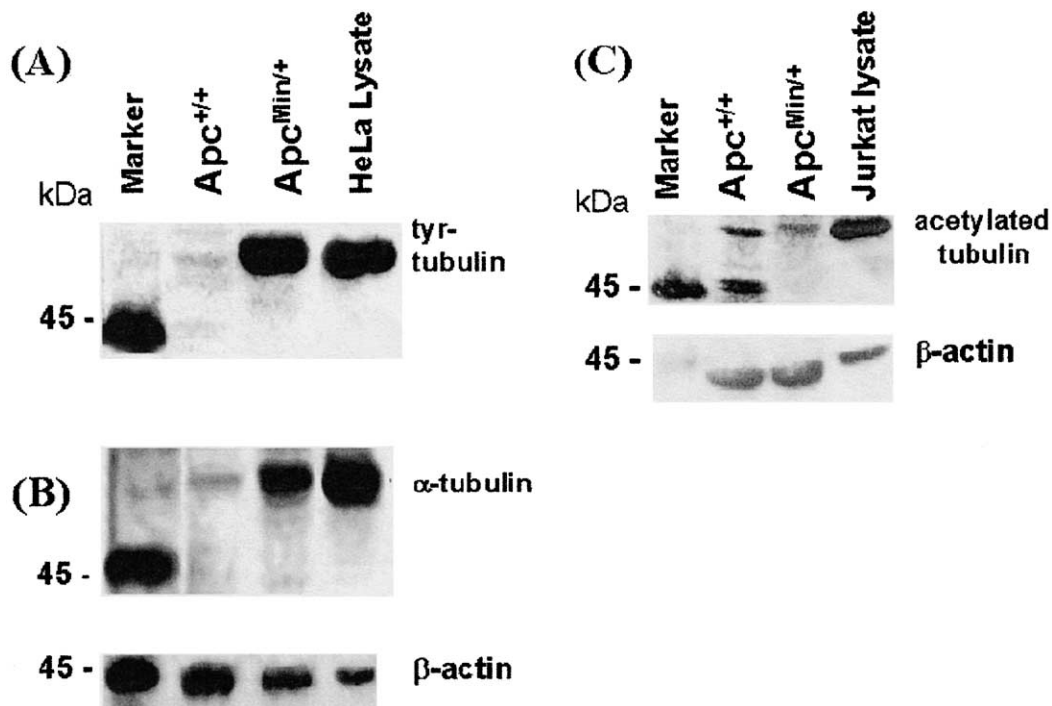
Fig. 2. Tubulin isoform expression is altered in $Apc^{Min/+}$. In vivo expression levels of α-tubulin isoforms in $Apc^{+/+}$ and $Apc^{Min/+}$ enterocytes were compared by immunoblot analyses using total cell lysates. Proteins were resolved by 12% SDS-PAGE and electrotransferred to a membrane. Each membrane was stripped and reprobed with antiactin antibody as a control. **A,** Immunoblot using monoclonal anti-Tyr tubulin antibody. **B,** Immunoblot using monoclonal anti-α–tubulin. **C,** Immunoblot using monoclonal antiacetylated tubulin. The band corresponding to 50 Dka tubulin in a HeLa or Jurkat cell lysate serves as a standard.

crotubules predominate in undifferentiated cells, whereas stabilized microtubules are found at an elevated level in polarized and differentiated cells.[13–15] Post-translational modifications of tubulin do not alter the stability of microtubules but serve as markers of the current state of microtubules within the cell. Dynamic, unstable tubulin is comprised predominantly of tyrosinated tubulin (Tyr-tubulin),[14] and acetylation is a post-translational modification that is a marker for stable tubulin available to serve as a substrate for microtubule assembly. Alpha (α)-tubulin is reflective of turnover of the entire tubulin population.[15,16]

We examined the relative expression of tubulin forms by immunoblot analyses of $Apc^{+/+}$ (wild-type) and $Apc^{Min/+}$ enterocyte lysates. We found that the level of unstable Tyr-tubulin was markedly elevated in $Apc^{Min/+}$ relative to $Apc^{+/+}$ enterocytes, suggesting that an increase in dynamic instability is associated with the presence of truncated APC protein (Fig. 2). The level of stable, acetylated tubulin was also reduced in $Apc^{Min/+}$ relative to $Apc^{+/+}$ enterocytes. Together these results demonstrate a notable alteration in microtubule dynamics associated with APC truncation.

Nocodazole Treatment of Wild-Type Intestine Mimics the $Apc^{Min/+}$ Phenotype

To determine whether changes in microtubule dynamics in Min/+ enterocytes were associated with disruption of normal microtubule assembly, we treated whole mucosa enterocyte preparations of wild-type mice with 1 μmol/L nocodazole, an antimitotic agent that depolymerizes microtubules. After a 1-hour incubation in the presence of nocodazole, marked similarities were noted between the tubulin expression profiles of Min/+ mice and wild-type mice given nocodazole (Fig. 3). Both expressed high levels of Tyr-tubulin that exceeded the level found in $Apc^{+/+}$ (wild-type) enterocytes, providing further evidence that Apc mutation in Min/+ is associated with fundamental defects in microtubule structure and/or function.

Apc Truncation Is Associated With Disruption of Actin Cytoskeletal Structure In Vivo

In a previous study we found that the normal adherins junction structure is disrupted as an effect of the Min mutation.[1] In $Apc^{Min/+}$ enterocytes, we observed reduced association between E-cadherin and β-catenin at the plasma membrane along with increased cytosolic E-cadherin. The targeted delivery of such basolateral membrane proteins requires an intact actin cytoskeleton.[17–19] We therefore investigated the effects of the Min mutation on the actin cytoskeletal structure of enterocytes, using the technique of indirect immunofluorescence confocal microscopy. In wild-type enterocytes, a prominent band of actin fibers was visualized beneath the apical brush border (Fig. 4, *A*). In Min/+ cells the specific localization of actin at the apical region of the cell was lost, and actin staining appeared more diffuse overall. We next examined the localization of APC in wild-type and
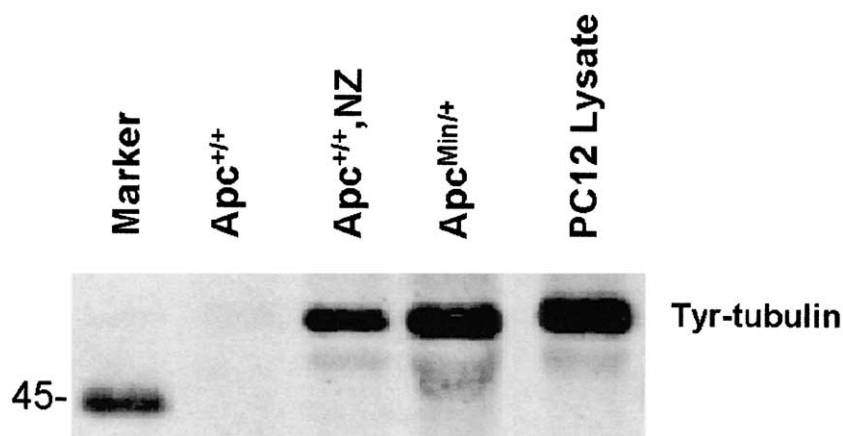
Fig. 3. Nocodazole (*NZ*) treatment of $Apc^{+/+}$enterocytes mimics the $Apc^{Min/+}$phenotype. $Apc^{+/+}$ intestine was treated ex vivo with 1 μmol/L NZ in Dulbecco's modified Eagle medium for 1 hour at 37° C in a 5% CO_2 incubator. NZ at micromolar concentrations depolymerizes microtubules. In parallel, untreated $Apc^{+/+}$ and $Apc^{Min/+}$ intestine were incubated in DMEM. Enterocyte lysates prepared from these samples were then subjected to immunoblot analyses for Tyr-tubulin using a monoclonal anti-Tyr tubulin antibody. Proteins were resolved by 12% SDS-PAGE. Immunoblotting for actin was used as a loading control. The 50 kDa band in the HeLa cell lysate serves as a standard.
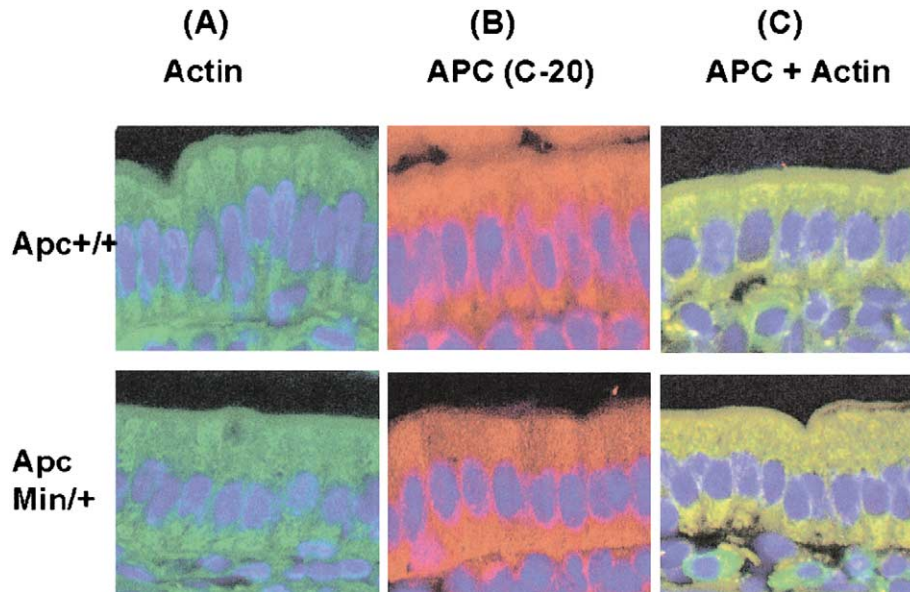
Fig. 4. APC and actin staining in enterocytes isolated from mouse small intestine. Confocal immunofluorescence microscopy at ×63 magnification was used to examine APC and actin localization in both $Apc^{+/+}$ and $Apc^{Min/+}$ enterocytes. **A,** Alexa-488 phalloidin (Molecular Probes) was used to stain for actin. The image demonstrates that in $Apc^{+/+}$ enterocytes, actin exhibits prominent apical membrane localization, a feature that is not observed in $Apc^{Min/+}$ enterocytes. **B,** Texas Red–labeled goat antirabbit antibody was used to bind primary antibody directed against the APC C-terminus. The image demonstrates that APC protein in $Apc^{+/+}$ tissue exhibits apical plasma membrane staining as well as cytoplasmic staining. In $Apc^{Min/+}$, APC staining is limited to the cytoplasm. **C,** Double staining revealed colocalization of APC and actin to the apical plasma membrane in $Apc^{+/+}$, which is lost in $Apc^{Min/+}$.

Min/+ enterocytes. Although the majority of APC staining in wild-type cells was cytoplasmic, APC also localized to the apical plasma membrane. This apical membrane distribution was not observed in Min/+ enterocytes (Fig. 4, *B*). In both actin and APC immunofluorescence studies, negative control experiments were performed in the absence of phalloidin and anti-APC antibodies, respectively, and only faint, nonspecific autofluorescence was observed. Finally, we observed co-localization of actin and APC in wild-type enterocytes that was lost in Min/+ (Fig. 4, *C*). Together our data suggest that APC protein imparts some regulatory control over the actin network.

DISCUSSION

The cytoskeleton is an essential mediator of cell function, governing processes such as migration, cell adhesion, membrane polarity, and differentiation. These activities are dynamic and require coordinated cross-talk between different cytoskeletal elements, including actin, microtubules, and their associated binding partners.[19,20] APC is physically associated with the cytoskeleton, and may regulate patterns of normal cell migration and differentiation by establishing links between cytoskeletal proteins and plasma membrane–associated proteins. The C-terminus of APC contains regions that interact with several structural proteins, including a basic 200-aa region thought to directly bind microtubules, and a 170-aa sequence further downstream that binds to EB-1, a microtubule-binding protein.[21] A PDZ domain, forming a binding region for scaffolding proteins, resides in the last 15-aa residues and may directly connect APC to actin.[22] The central region of APC also interacts indirectly with the cortical actin cytoskeleton via its link to β-catenin and α-catenin.[23] Finally, the N-terminus of APC contains a site of association with APC-stimulated exchange factor (ASEF), which is a guanine nucleotide exchange factor involved in regulating actin dynamics.[24]

In cultured MDCK cells, subcellular localization of APC protein demonstrates clusters of puncta near the (+) ends of microtubules at peripheral membrane sites of migrating edges, suggesting that APC plays a role in directed growth through stabilization of microtubules.[7–9] APC has been shown in vitro to track along microtubules and to accumulate at their distal (+) ends, where it facilitates tubulin assembly.[25–27] In addition, biochemical analyses have indicated that when

APC protein directly binds microtubules via its C-terminus, it promotes microtubule polymerization in vitro.[22,25,28] Various microtubule-associated proteins have been characterized, but APC appears to be unique in that its microtubule association is restricted to specialized areas of cells, a factor that may be important for cellular morphogenesis.

Our data suggest that the cytoskeleton-modulating activities of APC may play a role in early tumorigenesis. It is fairly clear that loss of function of both APC alleles is required for unregulated Wnt signaling via β-catenin-Tcf transactivation, and that this condition is associated with adenoma formation. The more subtle phenotype observed in $Apc^{Min/+}$ cells, however, is one of altered cell migration, cell-cell adhesion, and membrane polarity, suggesting that the presence of a truncated APC protein alters cytoskeletal function in the preadenoma stage. Our results show that there are increased levels of unstable tubulin isoforms and slightly decreased levels of stable tubulin in Min/+ relative to wild-type enterocytes. In $Apc^{Min/+}$ enterocytes, levels of Tyr-tubulin are markedly elevated compared to wild-type cells. Although tyrosination in and of itself does not cause microtubule instability, it is a marker for microtubules that are susceptible to depolymerization.[29] Therefore this result indicates that a greater proportion of tubulin in $Apc^{Min/+}$ enterocytes is dynamic, or unstable. Levels of total α-tubulin are also increased in $Apc^{Min/+}$ relative to wild type cells, reflecting an overall increase in tubulin turnover in the enterocytes containing truncated APC. This tubulin profile is analogous to that seen in nocodazole-treated wild-type intestine, suggesting that increased depolymerization of microtubules occurs in Min/+, potentially as a result of APC dissociation from MT (+) ends. Because dynamic microtubules predominate in undifferentiated cells,[15] these findings suggest that, by altering microtubule dynamics, truncation of APC produces a phenotype more characteristic of an undifferentiated cell.

One mechanism by which full-length APC may stabilize microtubules is by "capping" of microtubule ends. APC is targeted specifically to the distal tips of microtubule (+) ends, possibly via its association with kinesin, an (+)-end directed microtubule motor protein, and KAP3A, a kinesin-associated protein.[30] Once at the distal tips, APC may promote tubulin assembly and stabilization through its association with EB-1 or by linking microtubules to actin and/or the plasma membrane. Alternatively, APC may secondarily affect microtubule stability by promoting adherens junction structural integrity. In polarized epithelial cells, microtubules run along an apicobasal axis, with (−) ends at the apical and (+) ends at the basal membrane. In epithelial cells, the (−) ends undergo short-ening, or depolymerization, unless stabilized. Unlike fibroblasts, the end of which (−) are capped by the centrosome, the (−) ends of microtubules in epithelial cells are capped by an unknown mechanism independent of the centrosome.[31] E-cadherin signaling has been shown to stabilize microtubule (−) ends, an effect that is reversed on disruption of cell-cell contacts.[31] Because APC truncation is known to disrupt the formation of adherens junction, this may have a secondary negative effect on microtubule stabilization.

Coordination between microtubules and the actin network is essential for epithelial cell function, but the processes governing the interactions of these cytoskeletal components in a migratory epithelium in vivo are not well understood. APC co-localizes with actin in vitro to the apicolateral plasma membrane.[27,32] We also found that in vivo, APC and actin co-localize to the apical plasma membrane, an association that is lost in Min/+ enterocytes. Although the majority of APC appears to be cytoplasmic, a membrane-localized fraction of APC may serve as a functional link between cytoskeletal scaffolding proteins and adhesion complexes. Adherens junction formation and cell-cell contact are important prerequisites for cortical actin localization.[33] The lack of actin structure observed in Min/+ enterocytes in this study is therefore not surprising, given our previous work showing that these enterocytes exhibit deficient adherens junction formation and a physical separation of the lateral membranes. Future studies in our laboratory aim to better characterize actin localization at the apical plasma membrane and at tight junctions through the use of electron microscopy and indirect immunofluorescence microscopy utilizing antibodies directed against actin-binding proteins. Alterations in actin structure at both the apical membrane and at tight junctions resulting from APC truncation may lead to the changes in migration and adherens junction complex formation that we have previously described in Min/+ enterocytes. In addition, full-length APC protein may modulate actin dynamics by stimulating Rho family GTPases, which play an integral role in the reorganization of actin during cell migration and the formation of cell contacts.

CONCLUSION

Our results provide further evidence that both a structural and a functional link exist between APC and the cytoskeleton. Epithelial cell migration and adhesion depend on the spatial and temporal regulation of the actin and microtubule cytoskeletal networks. We suggest that the tumor-promoting effect

of the *Apc Min* mutation involves dysregulation of the cytoskeleton, and that this effect comprises one of the earliest changes observed in intestinal tumorigenesis. Further studies to characterize the tumor suppressor role of APC will be important for understanding normal enterocyte growth and differentiation, as well as the cellular biology of early intestinal tumorigenesis.

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Discussion

Dr. M.D. Duncan (Baltimore, MD): I am more familiar with actin cytoskeleton than tubulin, and I was wondering if you had differentiated the polymerization state of actin, looking at F and G actin, because by disrupting and overpolymerizing actin you can achieve a state where karyokinesis is permitted but cytokinesis is inhibited, thus leading to antiploid-polyploid tumors. Have you looked at the subclassification of the actin cytoskeleton?

Dr. S.A. Hughes: We have not yet done that, but we would be interested in doing so. We are going to have to come up with better fixation methods, as well, to characterize the differences between those two forms of actin.

Dr. M. Sarr (Rochester, MN): Give us your thoughts on whether this is an epiphenomenon or a phenomenon that is directly related to adenoma formation.

Dr. Hughes: We think it is actually a phenomenon that results or helps lead to adenoma formation. We do not know whether the effects on cell adhesion by APC come first and lead to changes in the cytoskeleton or cytoskeletal changes, in effect, turn to defects in cell adhesion, but we do not believe that it is one of the effects that leads to adenoma formation, not a consequence of it.

Dr. Sarr: Do you think this change in microtubular instability and interaction between APC and actin is important in the migration of the enterocyte? You presented some data that suggests the Min/+ mouse has a decrease or an increase in migration. Can you explain this?

Dr. Hughes: The decrease is actually in the preneoplastic enterocytes. These Min/+ intestinal enterocytes show a profile that is closer to that of a de-differentiated cell or a nonmigrating cell. You will notice that the expression in the tumor actually is more similar to that of the wild type, and it is possible that in order for the tumor cells to regain function of the wild type, their tubulin has to be able to function as a wild-type cell to migrate and invade and divide.

Dr. J.B. Matthews, (Cincinnati, OH): You showed us evidence of profound perturbation of tubulin biochemistry. Do you see marked disruption of microtubular networks by microscopy to correlate with these changes? Or is what you are seeing a perturbation in a limited pool of labile tubulin? One would expect such a global disruption in the microtubular cytoskeleton to perturb a variety of cellular functions, such as membrane trafficking, cell polarity, delivery of proteins to the apical or basolateral surfaces, and so forth. Have you looked at that?

Dr. Hughes: We did indeed look at that. It has been shown by others, including Waterman-Storer and Salmon, that the tubulin population is different depending on the site within each cell, and whether it is a migratory edge of the cell, a leading edge, or at the basal membrane. So we did look at immunohistochemical staining, mainly through confocal immunofluorescence microscopy, a tubulin within the cell, staining for either tyrosine or α-tubulin. We did not really show a difference in the appearance of the tubulin within the cells. What was different was mainly the level of intensity of the staining. These findings were not sufficiently conclusive to present today.

Invited Discussion—Expert Commentator

Dr. Richard A. Hodin: The paper on APC truncation represents what I believe is an excellent example of where things need to go when it comes to understanding the neoplastic process. We have seen some recent examples of new therapies against cancer that were developed only after the biology of a specific gene mutation became understood (e.g., Gleevac). In other words, it is one thing to identify an important cancer gene, but it is entirely different to figure out how that specific mutation in that gene leads to cancer. This second step, which is the mechanism part of the puzzle, is critical, because it allows one to then design a drug that targets a specific pathway.

The APC gene has clearly been shown to play an important role in the development of many human colon cancers, but the precise mechanism by which mutant APC proteins lead to cancer has not yet been determined. The present work took advantage of the powerful mouse model (Min) in which there is a germline APC mutation. Compared to wild-type mice, the heterozygous Min mice were shown to exhibit significant alterations in both the actin cytoskeleton and microtubule stability.

It is worth noting that the cytoskeleton was once thought to provide a mere structural framework for the important inner workings of the cell. But we now know that this framework actually provides a vital functional and regulatory role in diverse cellular processes. The cytoskeleton is a key link in the cell's interaction with its external environment. The present studies raise the provocative notion that the APC mutant phenotype leads to cancer by virtue of the cell having an abnormal interaction with its surrounding, extracellular environment. This work provides intriguing clues as to how colon cancer might develop. Such studies will be absolutely critical as we attempt to design cancer therapies in an intelligent and targeted manner.

Activation of Intestinal Arginine Transport by Protein Kinase C Is Mediated by Mitogen-Activated Protein Kinases

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L-Arginine uptake by the small intestine can play a pivotal role in regulating nitric oxide synthesis and immune functions in catabolic states. We previously showed that protein kinase C (PKC) activation stimulates intestinal brush-border membrane arginine transport. However, the signaling pathways implicated in this activation have not been studied. The purpose of this study was to investigate the intracellular signal transduction pathways involved in the protein kinase C stimulation of arginine transport across the apical membrane of intestinal epithelial Caco-2 cells. [^{3}H]-L-arginine transport activity, Northern blot analysis of mRNA levels of the intestinal arginine transporter CAT1, and Western blot analysis of the mitogen-activated protein (MAP) kinases phospho-p44/42 activity and phospho-MEK1/2 were measured in cultured Caco-2 cells treated with phorbol ester (phorbol 12-myristate 13-acetate, TPA; 0 to 0.5 μmol/L), and the MEK1 inhibitor PD 98059 (0 to 50 μmol/L). Phorbol ester stimulated intestinal arginine transport activity. Arginine transporter gene CAT1 mRNA, phospho-p44/42, and phospho-MEK1/2 levels were stimulated in phorbol ester–treated cells, compared with the control group. Phorbol ester stimulation of arginine transport activity and transporter CAT1 mRNA levels was blocked by PD 98059. These data suggest that phorbol ester stimulates arginine transport in Caco-2 cells via signaling pathways that lead to increased transcription and/or stabilization of CAT1 mRNA. Protein kinase C and MAP kinases MEK1/2 and p44/42 are key intracellular regulators involved in this signal transduction cascade. (J Gastrointest Surg 2002;6:876–882.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Arginine transport, intestine, protein kinase C, mitogen-activated protein kinase

L-Arginine is a conditionally essential amino acid in humans during periods of rapid growth and development, and an essential amino acid in weaning cats and dogs.[1–3] As a precursor for nitric oxide (NO) synthesis, arginine has profound effects on host immune function and host growth.[4,5] Recent clinical trials have demonstrated that enteral arginine-supplemented nutrition improves immune functions and reduces septic complications and hospital costs.[6–9] Arginine absorbed from the intestinal lumen is the starting point for a variety of nitrogen intermediary metabolism reactions within enterocytes[10]; the intestinal mucosa supplies the systemic circulation with arginine metabolites or free arginine. Transport of luminal arginine across the intestinal epithelial brush/border membrane is accomplished by discrete membrane transport systems such as system y$^+$, system L, or system b$^{0,+}$.[11,12] Intestinal arginine transport is regulated by local as well as systemic factors such as growth factors, differentiation states, and luminal substrates.[12–16] Protein kinase C (PKC) mediates many biological responses in the intestine including epidermal growth factor stimulation of intestinal amino acid transport.[13,17–19] The human intestinal epithelial cell line used in this study, Caco-2, is widely accepted as an in vitro model for transport studies of small intestinal epithelium.[20,21] The uptake of glucose, cationic and neutral amino acids, and other solutes in this cell line is comparable to uptake phenomena occurring in the intact small intestine.[13,16,19,22,23] In earlier studies, we char-

acterized the apical membrane arginine transport system y^+ (70%) and system^{y+L} or system $b^{0,+}$ (30%) in Caco-2 cells.[13] These studies identified PKC as an important mediator of growth factor stimulation of system y^+ activity.[15] However, the intracellular signaling pathways that mediate PKC stimulation of intestinal arginine transport remain unclear. In the present study, we explored the intracellular signaling pathways involved in regulation of intestinal arginine transport by PKC activation in Caco-2 cells.

MATERIAL AND METHODS
Caco-2 Cell Cultures

The human intestinal epithelial Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD) at passage 16. Cells were grown in a humidified incubator at 37° C in 10% CO_2/90% O_2. Cells were routinely grown in Dulbecco's modified Eagle medium (DMEM) containing 25 mmol/L glucose, 4 mmol/L glutamine, and 0.4 mol/L sodium bicarbonate, supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 1% nonessential amino acids. Caco-2 cells were passaged weekly after treatment with 0.05% trypsin and 0.02% EDTA. Cells were reseeded at a density of 4.5×10^6 cells per 100 mm dish for future subculturing, seeded in six-well cluster Costar tissue culture plates at a density of 10^5 cells per well for Northern blot or Western blot analysis, or seeded in 24-well cluster Costar tissue culture plates at a density of 10^4 cells per well for transport experiments.[14] Confluent cells (day 7, passages 20 to 40) were used for experiments. The day of seeding was designated as day 0. The growth medium was changed daily, and cultures were inspected daily using a phase-contrast microscopy.

Cell Treatments

To treat cells, growth medium was first replaced with serum-free media (i.e., DMEM containing amino acids, penicillin, and streptomycin, but lacking fetal bovine serum) for 2 hours at 37° C. The cell monolayer was washed three times in serum-free media. The cells were then exposed to each agent for various times and concentrations as described below. Treatment media were replenished every 6 hours to ensure a consistent concentration and to minimize possible paracrine effects. Cells were treated individually with phorbol 12-myristate 13-acetate (PMA; 0 to 10 μmol/L) for various times (minutes to 48 hours) in a 37° C 10% CO_2/90% air humidified incubator. Dimethyl sulfoxide (DMSO) served as control medium. Cells were also treated with individual inhibi-

tors: PD 98059 (0 to 100 μmol/L; DMSO as control medium) for mitogen-activated protein (MAP) kinase MEK 1, chelerythrine chloride (CHEL; 0 to 6.6 μmol/L, DMSO as control medium) for PKC for various periods of time (30 seconds up to 24 hours). Caco-2 cells remained healthy (viability >99% by dye exclusion) during at least 48 hours to exposure to serum-free media.

L-Arginine Uptake Measurements

L-Arginine transport activity was measured at 37° ± 1.0° C. After pretreatment of cells with various agents (described above), cells were rinsed with "uptake buffer" (37° C) comprised of 137 mmol/L choline chloride, 10 mmol/L HEPES/Tris buffer (pH 7.4), 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, and 2.5 mmol/L $CaCl_2$. Transport was initiated by adding 1 ml of this buffer, which also contained L-[^{3}H]arginine (2 μCi/ml, 1 μmol to 10 mmol/L). Cell culture plates were continuously shaken using an orbital shaker (1 Hz) during the uptake period. Uptake was arrested by discarding the uptake buffer and washing the cells three times with ice-cold uptake buffer lacking ^{3}H-labeled substrate. At the end of each uptake period, the radioactivity of the isotope trapped in the cells was extracted by lysing cells with 1 ml 1N NaOH that was neutralized with acetic acid. The radioactivity of the isotope was then assayed by liquid scintillation spectrometry. Protein in the NaOH extract was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).[11] Arginine uptake was linear up to 10 minutes, and a 2-minute uptake period was selected for all of the experiments in this study with zero time points serving as blanks.[15] Initial rates of transport activity were determined during the linear uptake period (2 minutes).[15,16] Uptake rates are expressed as picomoles glutamine per minute per milligram cell protein. System y^+ is defined as sodium-independent, 10 mmol/L leucine-insensitive uptake of L-[^{3}H]arginine.[15]

Northern Blot Analysis of System y^+ CAT1 mRNA

After pretreatment of cells with various agents (described above), cells were rinsed three times with phosphate-buffered saline. All procedures were performed under RNase-free conditions. Total RNA was isolated from control and treated Caco-2 cells using the "Totally RNA" isolation kit (Ambion, Austin, TX). Total RNa (10 μg) was separated on a 1% formaldehyde gel and transferred to GeneScreen membrane (PerkinElmer Life Sciences, Inc., Boston, MA) in 20× standard sodium citrate. The membrane was

hybridized with an antisense oligonucleotide probe specific to human CAT1 (5′-AGTGCCAATGGA-CATGAGGTCCACCA-3′), and then stripped and rehybridized with an oligonucleotide probe specific for 18S ribosomal RNA (5′-GTTATTG-CTCAATCTCGGGTG-3′). Autoradiographs were scanned with a laser densitometer, and the MCAT1 signal was normalized to 18S RNA. The CAT1 probe was 3′ end-labeled using terminal transferase and ^{32}P-dATP, and the 18S probe was 5′ end-labeled using T$_4$ polynucleotide kinase and ^{32}P-ATP.

Western Blot Analysis of Phospho–Protein Kinase C and Mitogen-Activated Protein Kinases

After cells were pretreated with various agents (described above), these cells were rinsed three times with phosphate-buffered saline. Total Caco-2 cell lysate was obtained by incubating cells in lysis solution (50 mmol/L HEPES, 150 mmol/L NaCl, 1.5 mmol/L MgCl$_2$ 1.0 mmol/L EGTA, 100 mmol/L NaF, 0.2 mmol/L Na$_3$VO$_4$, 1 mM phenylmethane sulfonyl fluoride, and 10 μg/ml aprotinin) for 30 minutes on ice and collecting supernate.[15] Equal amounts of protein from control and treated cells were separated on an SDS-PAGE gel and transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA). The membrane was then incubated with phospho-PKC (pan) antibody, phospho-MEK1/2, or p44/42 antibodies (1:1000; Cell Signaling Technology, Beverly, MA) overnight at 4° C and then incubated with horseradish-peroxidase–conjugated secondary antibody (1:50,000). Phospho-PKC (pan), MEK1/2, and p44/42 proteins were detected using the enhanced chemiluminescence system (Amersham Biosciences Corp., Piscataway, NJ). Autoradiographs were scanned with a laser densitometer.

Statistical Analysis

All experiments were conducted in triplicate (including the zero-time blanks), and all experiments were confirmed using at least two independent generations of cells. Experimental means are reported ± SEM. Comparison of means was made by analysis of variance with pairwise multiple comparisons by the Newman-Keuls method at $P < 0.05$.

RESULTS

In an earlier study,[13] we showed that phorbol ester TPA stimulated Caco-2 cell apical membrane arginine transport activity in a dose- and time-dependent

fashion. Arginine transport activity was stimulated by incubation with TPA for 6 hours or longer. Based on that study, the 24-hour incubation time point of TPA (0 to 0.5 μmol/L) was selected for the present study. Under this condition, TPA stimulated arginine transport activity and PKC activity, as indicated by Western blot analysis.[13] To assess the effect of TPA on expression of the predominant arginine transporter system y$^+$ (CAT1) gene, we performed Northern blot analysis of CAT1 mRNA in control cells (treated with DMSO) and cells incubated with TPA (0.5 μmol/L) for 24 hours. As shown in Fig. 1, TPA stimulated the CAT1 mRNA levels ninefold, suggesting that TPA stimulates arginine transport by augmenting system y$^+$ mRNA CAT1 expression. The elevation of transporter system y$^+$ CAT1 mRNA after TPA treatment indicates that TPA stimulates system y$^+$ arginine transport activity by either specifically enhancing the transcription of the system y$^+$ CAT1 gene or stabilizing the transcribed mRNA.

To assess the effect of TPA on intracellular MAP kinases, phospho-MEK1/2 activity was measured by Western blot analysis in both control and TPA-treated Caco-2 cells using a commercially available monoclonal phospho-MEK1/2 antibody. As shown in Fig. 2, phospho-MEK1/2 levels were elevated by TPA, compared to the control group, suggesting that TPA stimulated phospho-MEK1/2 activity. The PMA-induced phospho-MEK1/2 increase was completely blocked by the specific PKC inhibitor CHEL, suggesting that PKC activation mediated the TPA-induced phospho-MEK1/2 activation.

To further define the effects of TPA on the MAP kinase MEK1/2 cascade, we measured the p42/p44 and phospho-p44/42 (the active form of MAP kinase p44/42) by Western blot analysis in control and TPA-treated cells using commercially available monoclonal p42/p44 and MAP kinase phospho-p44/42 antibodies. TPA increased the phospho-p44/42 levels without increasing the p44/42 levels (Fig. 3), suggesting that TPA specifically increased the phospho-p44/42 activity.
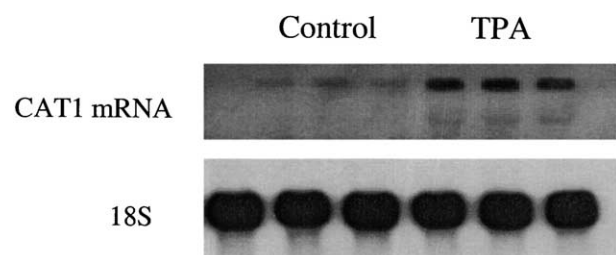
Fig. 1. Northern blot of system y$^+$ CAT1 mRNA. Arginine transporter system y$^+$ CAT1 mRNA levels were measured in cells incubated with phorbol ester (TPA, 0 to 0.5 μmol/L) for 24 hours.
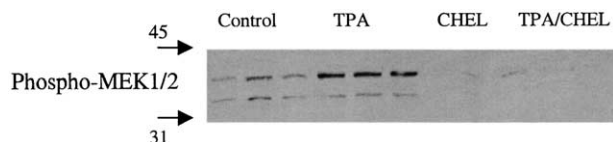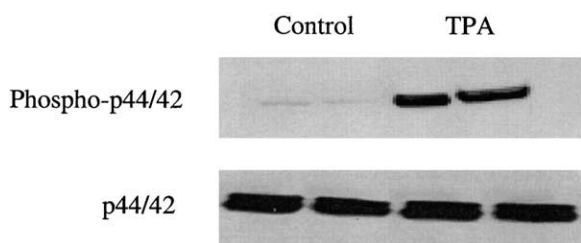
Fig. 2. Western blot of MAP kinase phospho-MEK1/2. Whole-cell phospho-MEK1/2 levels were measured using monoclonal phospho-MEK1/2 antibody in cells incubated with phorbol ester (TPA, 0 to 0.5 μmol/L) for 24 hours.

To further define the role of MAP kinase in the TPA activation of system y^+ arginine transport activity, Caco-2 cells were incubated in TPA (0 to 0.5 DMSO as control medium) with or without coincubation of the MEK1 inhibitor PD 98059 (0 to 50 μmol/L, DMSO as control medium). As shown in Fig. 4, PD 98059 blocked the TPA-induced activation of arginine transport without affecting the control cells (Fig. 4). Similarly, Northern blot analysis of system y^+ mRNA (CAT1) showed that PD 98059 attenuated the TPA-induced CAT1 mRNA levels (Fig. 5).
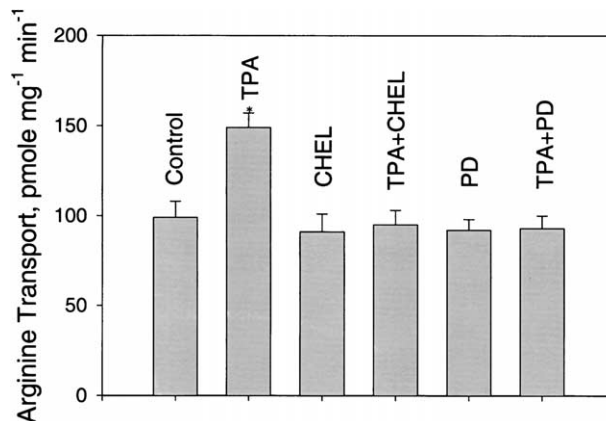
These data demonstrate that TPA stimulates the MAP kinases MEK1/2 cascade that mediates the TPA stimulation of system y^+ arginine transport activity and the transporter CAT1 mRNA in Caco-2 cells.

DISCUSSION

In the present study, we explored the intracellular signaling pathway of PKC stimulation of amino acid arginine transport in cultured intestinal epithelium. L-Arginine is a semiessential amino acid in humans under normal conditions. It is an essential amino acid in weaning cats and dogs,[1–3] and becomes essential in humans during trauma where demand is high.[4,5] Arginine has a profound effect on host immune function and host growth. It exerts its biological activities via two metabolic pathways. It is metabolized by arginase to urea and ornithine, which in turn stimulates

Fig. 4. Effect of inhibitors of PKC and MAP kinase MEK1 on TPA stimulation of system y^+ arginine transport activity. Uptake of arginine (50 μmol/L) was measured in cells incubated with phorbol ester (TPA, 0 top 0.5 μmol/L) $\pm$ PKC inhibitor chelerythrine chloride (CHEL, 6.6 μmol/L) and MAP kinase MEK 1 inhibitor PD 98059 (PD, 50 μmol/L). Transport values are means $\pm$ SEM (n = 9; $P < 0.01$).

polyamine synthesis, in addition to being a preferred fuel for lymphocytes, macrophages, and fibroblasts. It is metabolized to NO via NO synthetase. NO exerts numerous effects on inflammatory and immunologic responses. As the sole precursor for NO synthesis, membrane arginine transport has been demonstrated to be the rate-limiting step in NO synthesis in many tissues. Furthermore, arginine has multiple secretagogue activities on endocrine systems including the pituitary gland (growth hormone and prolactin), the pancreas (insulin, glucagon, and somatostatin), and the adrenal (catecholamine) glands.[1–5] Dietary arginine supplementation enhances natural killer cells and lymphokine-activated killer cell cytotoxicity.[6] Interestingly, the gene encoding system y^+, CAT-1, has been determined to be the same gene that encodes for a receptor of murine leukemia virus in mouse cells,[24,25] and system y^+ substrates such as arginine or lysine may participate in blocking entry of
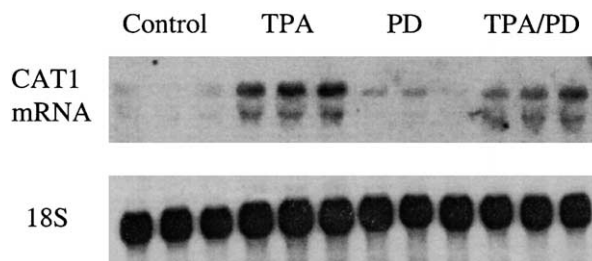

Fig. 3. Western blot MAP kinases p44/42 and phospho-p44/42. Whole-cell p44/42 and phospho-p44/42 levels were measured using monoclonal MAP kinase p44/42 and MAP kinase p44/42 antibodies in cells with phorbol ester (TPA, 0 to 0.5 μmol/L) for 24 hours.

Fig. 5. Northern blot of system y^+ CAT1 mRNA. Arginine transporter system y^+ CAT1 mRNA levels were measured in cells incubated with phorbol ester (TPA, 0 to 0.5 μmol/L) $\pm$ MAP kinase MEK1 inhibitor PD 98059 (PD, 50 μmol/L) for 24 hours.

murine retroviruses. In murine blastoma–bearing mice, arginine supplementation retards sarcoma growth and improves survival. Dietary arginine improves the body's immune function and decreases bacterial translocation and mortality under different insults, such as radiation injury, sepsis, or burns, as shown in numerous animal studies. Recent clinical trials in trauma patients, those in the ICU, and cancer patients have demonstrated that enteral feeding supplemented with arginine improves gut function, and reduces septic complications and hospital costs.[7–9]

Arginine absorbed from the intestinal lumen is the starting point for a variety of nitrogen intermediary metabolism reactions within the enterocyte and for the entry of dietary arginine into the systemic circulation.[10] Transport of luminal glutamine across the intestinal epithelial brush-border membrane is accomplished by discrete membrane transport systems such as system y^+, system L, or system $b^{0,+}$. In previous studies with Caco-2 cells, we characterized system y^+ arginine transport and investigated the regulation of the system y^+ arginine transport activity by cell differentiation state and epidermal growth factors.[15] In the present study we explored the intracellular signaling pathways involved in the regulation of intestinal arginine transport initiated by PKC activation in Caco-2 cells. Cultured Caco-2 cells undergo spontaneous differentiation in cell culture, and the differentiated cells display small intestinal epithelial characteristics such as polarized cell membrane with specific membrane marker enzymes such as alkaline phosphatase, sucrase, and sodium-potassium ATPase.[26,27] Caco-2 cells have been widely used as the in vitro small intestinal epithelia model for nutrient transport and drug transport studies.[26,27] The uptake of glucose, cationic and neutral amino acids, and other solutes in this cell line is comparable to uptake phenomena occurring in the intact small intestine.[13,16,22,23]

The intestinal epithelium is continuously exposed to various stimuli such as bacteria and associated products (endotoxin), dietary components, luminal growth factors, and inflammatory mediators (cytokines). The gut reacts to these stimuli by increasing expression of a variety of genes via activation of intracellular kinases.[28,29] The response of intracellular signaling pathways within the intestine to septic insult has been studied extensively.[30–32] Binding of endotoxin, growth factors, or cytokines to membrane receptors activates a series of intracellular signaling cascades, including families of different PKC isozymes and MAP kinases such as extracellular related kinase (ERK), MEK, Jun amino–terminal kinase (JNK), or p38, ultimately resulting in various gene expression and biological responses.[33–38] These mechanisms are designed to allow the enterocyte to maintain intestinal homeostasis. One

characteristic response is upregulation of arginine uptake. Information on cellular regulation of intestinal arginine transport remains limited.

PKC is a family of intracellular enzymes that controls diverse biological functions including intestinal mucosal repair induced by epidermal growth factor and intestinal dysfunction caused by endotoxin/sepsis.[17,18,28,29] Tumor promoter phorbol esters such as TPA can substitute for 1,2-diacylglycerol (DAG) and directly activate intracellular PKC. Phorbol ester is a useful tool to study intracellular PKC activation, a common signaling pathway for regulating intestinal mucosal functions including amino acid transport.[13,19]

Previously we showed that prolonged incubation (>6 hours) with TPA was required to stimulate the arginine transport activity in Caco-2 cells. Prolonged continuous TPA incubation (24 hours, 0.5 μmol/L) stimulates both arginine transport activity and PKC activity as indicated by Western blot analysis in Caco-2 cells.[39] As shown in Fig. 1, prolonged (24 hours) phorbol ester TPA incubation increases system y^+ transporter CAT1 mRNA levels. The elevation of transporter system y^+ mRNA CAT1 after TPA treatment indicates that TPA stimulates system y^+ arginine transport activity by either specifically enhancing the transcription of the system y^+ CAT1 transcription or stabilizing the transcribed mRNA. Two activation mechanisms could account for the TPA-induced system y^+ arginine transport activity: an increase in transporter units or modulation of existing transporter configuration. TPA stimulated system y^+ arginine transport activity by increasing the system y^+ transport capacity (V_{max}) without affecting transport affinity (K_m).[13] An increase in transport maximal capacity in the absence of a change in transport affinity indicates an increase of functional transporter units. The prolonged TPA exposure (>6 hours) required for this upregulation and the involvement of mRNA synthesis preclude the rapid (minutes) post-translational modifications such as phosphorylation of existing transporter polypeptides. The phorbol ester upregulation of system y^+ arginine transport activity also involved de novo protein synthesis, as indicated by the blockage by cycloheximide.[13] These data suggest that this TPA-induced system y^+ V_{max} increase was most likely due to de novo new transporter protein synthesis. A specific antibody to the system y^+ transporter would allow investigation of the synthesis and trafficking of transporter protein and determination of whether the de novo synthesized protein is a system y^+ transporter regulatory protein or the transporter protein itself. Because of the time frame, the translocation of preexisting system y^+ transporters or modulation of an existing

transporter configuration is an unlikely mechanism in this TPA stimulation of arginine transport.

MAP kinases are a family of kinases that mediate various biological activities and regulate gene expression in response to various stimuli.[40–42] There are at least four distinctly regulated groups of MAP kinases: ERK1/2, JNK1/2/3, p38 protein (p38α/β/γ/δ), and ERK5. Each group is activated by specific MAP kinases such as MEK1/2 for ERK1/2, MKK3/6 for p38, and so forth. PKC is a well-known upstream activator of MAP kinases. Phorbol ester has been shown to activate MAP kinase KK Raf, which in turn activates a second protein kinase, MEK, by phosphorylating it at two serine residues. This modification activates MEK to phosphorylate and activate ERK1/2. The intracellular signaling cascade for PKC-induced system y^+ arginine transport is unknown. Western blot analysis of phospho-MEK1/2 showed that phospho-MEK1/2 levels were elevated by TPA, compared with a control group, suggesting that TPA stimulates phospho-MEK1/2 activity (see Fig. 2). The TPA-induced phospho-MEK1/2 increase was completely blocked by the specific PKC inhibitor CHE,[43] indicating that the TPA-induced phospho-MEK1/2 activation is mediated by PKC activation.

To further define the effect of TPA on MEK1/2 cascade, we measured the MAP kinases p42/44 and phospho-p44/42 (the active form of p44/42) by Western blot analysis in control and TPA-treated cells using commercially available monoclonal p42/p44 and phospho-p44/42 antibodies. TPA increased the phospho-p44/42 levels without increasing the p44/42 levels (see Fig. 3), suggesting that TPA specifically activated the phospho-p44/42 activity. These data demonstrate that TPA is an activator of the MEK cascade but do not establish a linkage between the MAP kinase and arginine transport. To define the relationship among TPA, MAP kinase, and system y^+ arginine transport, we measured Caco-2 system y^+ arginine transport activity and system y^+ CAT1 mRNA in the presence and absence of the MAP kinase MEK1 inhibitor 2'-amino-3'-methoxyflavone (PD 98059). PD 98059 is a potent, cell-permeable and selective inhibitor of the MAP kinase ERK kinase 1 (MEK1).[44] This inhibitor blocks the activation of MEK1, therefore inhibiting the subsequent phosphorylation and activation of MAP kinases such as ERK and biological responses. As shown in Fig. 4, PD 98059 blocked the TPA-induced activation of arginine transport without affecting the control cells (see Fig. 4). Similarly, Northern blot analysis of system y^+ mRNA (CAT1) showed that PD 98059 attenuated the TPA-induced CAT1 mRNA levels (see Fig. 5). These data suggest that the TPA stimulation of system y^+ arginine transport activity and transporter CAT1 mRNA is mediated by MAP kinase ERK cascade.

In summary, phorbol ester stimulates Caco-2 intestinal system y^+ arginine transport activity and transporter CAT1 mRNA expression. This stimulation is the result of an increase in transporter units rather than a modification of transporter affinity, most likely because of de novo synthesis of new transporters. Furthermore, this TPA/PKC-activated system y^+ arginine transport is mediated by an intracellular MAP kinase MEK1/2 pathway.

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Adequacy of Nodal Harvest in Colorectal Cancer: A Consecutive Cohort Study

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The presence of nodal metastasis is a critical component of staging in colorectal cancer. Accurate assessment of nodal status requires sufficient node sampling, although the number of such nodes is controversial, with recommendations ranging from 6 to 17 nodes. The purpose of this study was to describe the nodal harvest in colorectal cancer and to identify factors associated with adequate lymph node harvest. Pathology reports from consecutive patients with newly diagnosed colorectal cancer undergoing resection between January 1997 and December 2000 at a tertiary care academic institution were reviewed. Identification of 12 or more lymph nodes was considered to be an adequate nodal harvest based on the current American Joint Committee on Cancer recommendations. Among the 579 consecutive specimens, the number of nodes identified was not stated for 10 (1.7%). Of the remaining 569 specimens, 4700 nodes were identified with a mean of 8.3 nodes per patient (median 7, range 0 to 60). Nodal metastases were identified in 219 patients (38.5%). Patients with one or more positive nodes had greater nodal harvest than those with negative nodes (9.5 vs. 8.2, respectively; $P = 0.03$). Only 22.4% of patients were found to have an adequate nodal harvest ($\geq$12 nodes). Right-sided resections, high surgeon volume, and gross examination of specimens by a staff pathologist were associated with higher nodal harvests, compared to left-sided resections, low surgeon volume, and gross examination of specimens by a pathology resident/technologist, respectively. There was no association with pathologist volume. In this study, nodal harvest in patients undergoing resection for colorectal cancer was highly variable. This problem appears to be multifactorial, and is related to patient, pathologic, and surgical factors. (J GASTROINTEST SURG 2002;6:883–890.)   © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Colon cancer, rectal cancer, lymph nodes, staging

Accurate assessment of lymph node status is a critical component in the staging of colorectal cancer. The presence of lymph node metastases often determines the use of adjuvant therapy; such adjuvant therapies have been shown unequivocally to provide a disease-free and overall survival benefit in patients with node-positive disease.[1,2] Furthermore, the presence of nodal metastases provides important prognostic information.[3] The number of lymph nodes required for accurate staging of patients is controversial with recommendations in the literature ranging from 6 to 17 nodes.[4–11] Current guidelines from the American Joint Committee on Cancer (AJCC) recommend assessment of 12 nodes or more for accurate staging.[7] Many of the previous recommendations were based on studies of node-positive rates in relation to the number of nodes examined rather than survival data. Only recently have data from large clinical trials demonstrated a correlation between nodal harvest and long-term survival in patients with node-negative disease.

Studies have consistently demonstrated that nodal harvest from colorectal cancer specimens is highly variable, although this variability is poorly understood. Although anatomic differences in the amount of nodal tissue, type of surgical resection, use of neoadjuvant therapy, and methods of nodal assessment have all been implicated as determinants of nodal harvest,[4,5,11–13] surgeon and pathologist factors have not been well studied. Furthermore, recent studies examining nodal harvest in colorectal cancer have been based on the analysis of clinical trial data, and

thus may not be generalizable for standard clinical care. The purpose of this study was to examine the adequacy of nodal harvest in a "standard practice" setting. Specifically, we sought to identify factors that may be associated with an inadequate harvest.

METHODS

The pathology reports from all primary colorectal cancer resection specimens between January 1, 1997 and December 31, 2000 at a tertiary care teaching institution were reviewed. Transanal resections and recurrent cancers were excluded. Surgical factors including surgeon, procedure, and length of bowel were recorded. Resections were grouped as right sided (right hemicolectomy, extended right hemicolectomy, and transverse colon resection), left sided (left hemicolectomy, anterior resection, low anterior resection, abdominoperineal resection, and pelvic exenteration), total colectomy (abdominal colectomy and proctocolectomy), and unknown. Pathologic factors included tumor size, gross resection margins, tumor (T) stage, differentiation, lymphatic/vascular invasion, number of nodes identified, and number of positive nodes. In addition, the individual pathologist reporting on a particular case, as well as the person (staff pathologist, resident, or technologist) examining the gross specimen and thus identifying potential lymph nodes, was recorded. If any uncertainty existed, the reporting pathologist was assumed to have performed the gross examination. No standardized harvesting technique for pathologic examination of lymph nodes was used, and no strategy of standardized pathology reporting was used at any time during the study period.

Synchronous lesions were treated as individual specimens if two separate segments of colon were resected. If more than one tumor was present in a single specimen, tumor data were collected from the most pathologically advanced lesion. Identification of 12 or more nodes was considered an adequate nodal harvest in keeping with current recommendations from the American Joint Committee on Cancer (AJCC) for the management of colorectal cancer.[7] Subsequent analysis altering the threshold of an adequate nodal harvest from six or more nodes to 17 or more was also performed, based on extremes in the literature.[4,10]

All data were collected and entered into a computer database. Categorical variables were compared by means of chi-square analysis, with the Wilcoxon rank-sum test used to examine differences in continuous variables.[14] To control for potential confounding, multivariate analysis by logistic regression was performed to identify factors independently associated with an adequate nodal harvest. All factors examined on univariate analysis were initially included in the model, and a forward stepwise technique was used; a value of $P < 0.10$ was established as the criteria for entry into the model.[15] All analyses were performed using SPSS for Windows 9.0 (Chicago, IL), and statistical significance was set at $P \leq 0.05$.

RESULTS

A total of 579 specimens were eligible for review. Ten pathology reports (1.7%) did not state the number of lymph nodes identified and were excluded from the study. Among the remaining 569 specimens comprising the study cohort, the mean patient age was 69 years (median 70 years, range 30 to 99 years), with 308 men (54%) and 261 women (46%). Over the 4-year study period, 184 right-sided resections (32%), 346 left-sided resections (61%), and 24 colectomies (4%) were performed. The type of resection performed was not stated in 15 reports (3%). Surgical procedures were performed by 28 surgeons, with two surgeons performing more than half of the operations. Pathologic assessments were performed by 18 pathologists, with five pathologists performing half of the procedures. Gross examination of the specimen was performed by a resident or technologist in 191 cases (34%), with the staff pathologist responsible for the gross examination in the remaining 378 specimens (66%).

Adequate Harvest

Among the 569 specimens, 4700 lymph nodes were identified (mean 8.26 nodes/patient, median 7 nodes/patient, range 0 to 60 nodes). No lymph nodes were identified in 28 cases (4.9%). Based on the current AJCC recommendation of 12 or more nodes, only 128 patients (22%) underwent an adequate harvest. Using the most conservative recommendation in the literature of six or more nodes, 342 patients (60%) had an adequate harvest, whereas only 46 (8%) had an adequate harvest if the most aggressive recommendation of 17 or more nodes was followed.

Lymph Nodes Metastases

A total of 219 patients (38%) had one or more positive lymph nodes identified. On average, patients with nodal metastases had greater nodal harvests compared to those patients with negative nodes (9.5 nodes/patient vs. 8.2 nodes/patient, respectively; $P =$

0.03). There was a strong association between the number of nodes, categorized into quartiles, and the presence of nodal metastases ($P = 0.002$; Table 1). The incidence of identifying nodal metastases according to harvest thresholds of six or more, 12 or more, and 17 or more nodes was also examined (Table 2). Patients with nodal harvests of six or more and 12 or more nodes were more likely to have nodal metastases compared to patients with lesser harvests ($P = 0.003$ and $P = 0.04$, respectively). A nodal harvest of 17 or more nodes was not associated with a significantly higher rate of node positivity.

Factors Associated With Lymph Node Harvest

For harvest thresholds of six or more, 12 or more, and 17 or more nodes, right-sided resections were associated with a higher nodal harvest compared to left-sided specimens ($P < 0.0001$, $P = 0.008$, and $P = 0.003$, respectively). On average, right-sided resection specimens were longer than left-sided specimens (mean 27.2 cm vs. 19.3 cm, respectively; $P < 0.000$). There was a strong association between the length of bowel and the number of lymph nodes identified ($P = 0.005$; Table 3). There was no association between tumor size or grade and either the adequacy of nodal harvest or the number of lymph nodes identified. Similarly, the small number of patients who received neoadjuvant chemoradiation for rectal cancer (n = 19) had similar nodal harvests compared to other patients who underwent left-sided resection without preoperative therapy (mean 7.0 vs. 7.2 nodes, respectively; $P = $ NS).

The relationship between nodal harvest and surgeon volume was examined by comparing the two surgeons who performed the upper fiftieth percentile of cases to the remaining 26 surgeons. When nodal harvest was examined by quartiles, there was a significant relationship between surgeon volume and nodal harvest ($P = 0.03$; Table 4). We then examined the association between nodal harvest, at various literature-based thresholds, and surgeon volume.

Compared to surgeons in the lower fiftieth percentile, surgeons in the upper fiftieth percentile were more likely to obtain a nodal harvest of six or more nodes ($P = 0.03$), but not 12 or more ($P = 0.08$) or 17 more ($P = 0.55$) nodes.

Similarly, pathologist volume was studied by comparing the five pathologists who performed the upper fiftieth percentile of cases to the 13 who performed the remainder of the procedures. There was no significant association between nodal harvest and pathologist volume when nodal harvest was examined by quartile ($P = 0.9$) or according to harvests of six or more, 12 or more, or 17 or more nodes ($P = 0.51$, $P = 0.22$, and $P = 0.17$, respectively). When nodal harvest was examined according to the person who performed the gross examination of the specimen, there was a significant correlation between gross examination performed by a staff pathologist and a nodal harvest of six or more ($P = 0.005$) and 12 or more nodes ($P = 0.03$) but not for 17 or more nodes ($P = 0.19$; Table 5).

On multivariate analysis, factors independently associated with an adequate nodal harvest, at various nodal harvest thresholds, are presented in Table 6. Findings on univariate and multivariate analyses were similar. Right-sided resections, higher surgeon volume, and gross examination by the staff pathologist were independently associated with a higher likelihood of achieving a nodal harvest of six or more nodes.

DISCUSSION

The prognostic and adjuvant therapy-related consequences of nodal metastases make the accurate assessment of lymph node status an essential component of colorectal cancer staging. Despite recent interest in this subject, the number of nodes required to accurately stage patients is controversial, and considerable variation exists among studies. Previous recommendations of nodal harvest have been based on retrospective studies of node positivity rates according to the number of harvested lymph nodes and not on survival data. These studies, most of which had a sample size of fewer than 200 patients, recommended harvests of 6, 12, and 14 nodes.[4,9,11] The only large study of this type examined 750 patients with T3 colorectal cancer and recommended a harvest of 17 nodes.[10]

Recently data from large clinical trials have been used to examine the impact of nodal harvest. A significant improvement in survival at a mean follow-up of 3.6 years was observed for patients with node-negative colon cancer who had seven or more nodes

Table 1. Association between incidence of nodal metastases and lymph node harvest by quartiles

Lymph node harvest	No. of patients	% With ≥1 positive node(s)
0–3	130	26.2
4–6	138	39.9
7–10	149	41.6
>10	152	44.7

$P = 0.003$.

Table 2. Node positivity rates for colorectal cancer specimens for varying thresholds of "adequate" nodal harvest

Recommended lymph node harvest	"Inadequate" harvest (% with ≥1 positive nodes)	"Adequate" harvest (% with ≥1 positive nodes)	P value
≥6 nodes	31.3	43.3	0.003
≥12 nodes	36.5	45.3	0.045
≥17 nodes	37.9	45.7	0.19

compared to those who had less than seven nodes identified.[5] Two studies of node-negative rectal cancer have demonstrated similar findings. A nodal harvest of 14 or more nodes was associated with a significant improvement in 7.5-year survival in patients with T3/T4 node-negative rectal cancer compared to patients with fewer nodes identified (80% vs. 60%).[8] A second study of patients with T3N0 rectal cancer also showed an improved overall and disease-free survival at a mean follow-up of 5 years for patients with more than 10 nodes identified.[6]

Regardless of the actual literature-based minimum nodal harvest, an implication from these studies was that patients with colon cancer were being staged inaccurately as node negative and therefore were not offered appropriate chemotherapy. For patients with T3 and T4 rectal cancers, nodal status will not typically influence the use of adjuvant chemoradiation therapy but will directly affect the prognostic information given to patients and their families. To improve staging and minimize the consequences of patient understaging, a better understanding of the factors that influence nodal harvest is essential.

Despite the fact that most of the patients in this study did not undergo an adequate harvest, according to current clinical guidelines, the overall rate of nodal metastases (38%) was comparable to that reported in the literature using standard manual techniques for gross examination of the specimens.[8,10] Contrary to other reports, this study found only

small incremental increases in node positivity rates among harvest thresholds of 6 or more, 12 or more, and 17 or more nodes. At first glance this would seem to suggest that a nodal harvest of six is perhaps adequate. However, because this study was not designed to specifically address this issue and was not based on survival data, we believe that this threshold of six or more nodes is likely inappropriate. Specifically, the small number of patients in this study with harvests of 12 or more and 17 or more nodes limits the analysis and interpretation of these subsets. An explanation of the relative high rate of positive nodes with a low harvest threshold (6 to 12) is unclear. It may reflect the fact that, in some patients, once the pathologist identified nodes that were obviously involved at the time of gross examination, more nodes were not sought. The ability to identify positive nodes on gross examination of the surgical specimen, however, would require frank malignant features because size is not a reliable indicator of nodal metastases.[16]

Although this study and most others have demonstrated that increasing nodal harvest is associated with higher node-positive rates,[4,9–11] the factors contributing to nodal harvest are not well studied. Three variables must be considered when examining the issue of nodal harvest: patient factors, surgical factors, and pathologic factors. Patient factors may reflect anatomic or individual variability in nodal harvest.[12] This study and others demonstrated that right-sided resections are associated with a greater nodal harvest than left-sided resections.[4,5] This likely reflects the

Table 3. Association between nodal harvest and length of bowel

	0–3 nodes	4–6 nodes	7–10 nodes	>10 nodes
Mean length of bowel (cm)	20.9	23.2	25.1	27.8

P = 0.005.

Table 4. Nodal harvest according to surgeon volume

	0–3 nodes	4–6 nodes	7–10 nodes	>10 nodes
Upper 50th percentile	22.7%	20.5%	28.3%	29.5%
Lower 50th percentile	24.3%	29.1%	23.5%	2.1%

P = 0.03.

Table 5. Adequacy of nodal harvest, for varying thresholds, according to person who performed gross examination of the specimen

Recommended lymph node harvest	Staff pathologist (% adequate harvest)	Resident/technologist (% adequate harvest)	P value
≥6 nodes	64.0	52.4	0.005
≥12 nodes	24.9	17.8	0.03
≥17 nodes	9.0	6.3	0.19

fact that right-sided specimens were longer and had more mesenteric tissue. It is possible that other patient-related factors such as body mass index are also important, although these data were not available in this study.

The impact of the pathologist and surgeon as it pertains to colorectal lymph node harvest is largely unreported. This study found no correlation between pathologist volume and nodal harvest. However, when lymph node harvest was examined in relation to the person who performed the gross examination (staff pathologist vs. resident/technologist), an association between improved nodal harvest and staff pathologist emerged. The fact that a wide variety of persons are often involved in the gross examination of specimens not only compounds the difficulty involved with examining variability in nodal harvest, it may contribute to it.

Several techniques of pathologic nodal assessment have been described, most of which involve some type of fat clearing, which has been shown to increase nodal harvest.[17–21] The nodal harvests described in these studies range from 20 to 58.2 nodes per patient, which is markedly superior to nodal harvests described in this study. However, these techniques have not been widely applied as standard practice, largely because they are typically more labor intensive. The absence of the use of these techniques in our study cohort, although likely detrimen-

tal in terms of nodal harvest, is thought to be representative of most North American centers.

In contrast to pathologist volume, surgeon volume appeared to be associated with nodal harvest. However, this association was modest and was not present throughout all thresholds of nodal harvests examined. As opposed to pathologic gross examination, the impact of surgical resident involvement was not analyzed in this study because it could not be reliably determined retrospectively.

Based on current recommendations, the majority of our patients in a "standard care" setting did not have an adequate nodal harvest. Consequently we have asked the following questions: (1) How many of the patients in this study were understaged; (2) what are the implications of understaging patients; and (3) what can be done to improve nodal harvest? Based on a nodal harvest of 12 or more nodes, with an observed node-positive rate of 45% in those with an adequate harvest and a node-positive rate of 37% in those with an inadequate harvest, we estimate that 39 node-negative patients (14%) were understaged. Although a small proportion of these patients would have T3/T4 rectal cancers and thus still receive adjuvant therapy, in most of them chemotherapy would not have been offered because of the inaccurate diagnosis of node-negative colon cancer. The major implication is the loss of potential survival benefit from such chemotherapy. There is clearly room for im-

Table 6. Multivariate analysis of factors associated with an adequate nodal harvest, at various thresholds*

Threshold	≥6 nodes		≥12 nodes		≥17 nodes	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Right-sided resection	2.6 (1.8–3.9)	<0.0001	1.8 (1.2–2.8)	0.008	2.7 (1.4–5.2)	0.004
Upper 50th percentile surgeon	1.6 (1.1–2.3)	0.01	1.5 (0.9–2.3)	0.09	NIM	—
Staff pathologist performing gross examination	1.5 (1.0–2.2)	0.05	NIM	—	NIM	—

NIM = not in model; OR = odds ratio; CI = confidence interval.
*Excludes patients who underwent total colectomy.

provement and, to this end, recognition of this problem by the surgeon and the pathologist is essential. Standardization of gross pathologic examination and reporting may also improve outcomes, although further investigation of this is required.[22] Standardization of surgical technique, perhaps by routine marking of vascular pedicles for subsequent lymph node retrieval, may also improve outcomes.

CONCLUSION

In this study of a consecutive cohort, we described nodal harvest in colorectal cancer and examined the factors that may lead to an inadequate nodal harvest. Most of the patients in this study did not have an adequate nodal harvest based on current guidelines. Right-sided resections, high surgeon volume, and examination of gross specimens by a staff pathologist were associated with improved nodal harvest. Nodal harvest was highly variable; this problem appeared to be multifactorial and was related to patient, pathologic, and surgical variables. Further investigation regarding surgical and pathologic standardization is needed with the goal of reducing variability and thus permitting more consistent staging of patients with colorectal cancer.

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Discussion

Dr. K. Mayer (Sacramento, CA): I am wondering whether your pathologists have any guidelines as to how they handle the mesentery? Do they "de-fat" the mesentery? At least at our institution it seems to be somewhat pathologist related in that respect.

Dr. G. Porter: There was no standardization in either the performance of the gross examination or the reporting of the pathologic findings within this cohort. Specifically, there was no routine de-fatting. However, we believe this represents what occurs at many centers, making the results of this study important. Clearly, routine de-fatting would likely increase the nodal harvest, although a consistent, standardized approach is critically important.

Dr. D. Nora (Santa Monica, CA): You mentioned that in 5% of your patients no lymph nodes were found. Did you look at those patients and see what factors were involved in terms of not finding lymph nodes at all?

Dr. Porter: That is a good question. Unfortunately, this was a small subgroup of patients, and, thus, we were unable to identify any factors associated with not identifying any lymph nodes.

Dr. J.P. Hoffman (Philadelphia, PA): I am sure you must have looked at the outcome of recurrence and nodes harvested. Would you tell us what you saw in terms of local recurrence or systemic recurrence?

Dr. Porter: We have not looked at recurrence and survival. Five-year follow-up is not available for many of these patients who underwent resection between 1997 and 2000. However, secondary analysis of clinical trial data and registry data has demonstrated a linear relationship between survival and the number of nodes harvested in "node-negative" patients. This study likely may lack sufficient power to do this well, but obviously over time we probably will examine this.

Dr. M. Dayton (Salt Lake City, UT): How much of the variability that you observed was technique dependent; in other words, how often was an operation done in which an adequate lymphadenectomy was not performed, and how much of this might have been related to the variability in the number of lymph nodes that an individual may have? Are there any studies that have looked at this?

Dr. Porter: This is a complex issue, and you bring up a good point. We can categorize factors as patient related, such as anatomic issues, pathologic factors, and surgical factors. It is very difficult retrospectively to assess the quality of "amount" of surgery. The only way we thought we could do this was to look at a very crude measure—the length of bowel resected. In this study, right-sided resections were associated with longer lengths of bowel, as has been shown in other studies, as well as greater nodal harvest. Interestingly, however, within each type of resection, the length of bowel was not associated with nodal harvest.

Dr. R. Hodin (Boston, MA): It is well recognized that the harder a pathologist looks for nodes, the more nodes they are going to find. Would you agree with that?

Dr. Porter: I think this is likely very important, although it is difficult to document, even prospectively, how "hard" a pathologist looks for lymph nodes. However, this study suggested that surgical and patient factors also play a role in nodal harvest.

Dr. Hodin: I am wondering whether it is fair to say that an inadequate nodal harvest was performed in those cases where one or more positive nodes were found. I say this because, once a pathologist sees a positive node, he or she may be less likely to look for a large number of nodes. Do you see what I am getting at?

Dr. Porter: I think I understand your point. First of all, the question is, are some of these patients having an inadequate harvest because they have a node that appears to be positive on gross examination, and thus the pathologist is not searching any further? That is certainly a possibility. I think that represents the minority of patients. As we know, in most patients with nodal metastases, those nodes are in fact less than 5 mm in size. The issue of whether it matters once a patient is found to be node positive or not is a good point. Certainly there is prognostic information to be gained from patients who have four or more nodes, as opposed to those patients who have one to three nodes. But you are correct. Certainly, if the patient is deemed simply node positive, is a large nodal harvest really necessary? I think that question is a little bit up in the air.

Invited Discussion—Expert Commentator

Dr. Robin S. McLeod (Toronto, Ontario): It is recognized that surgical technique has a great effect on the rate of local recurrence following surgery for rectal cancer. After acceptance of total mesorectal excision, the reported local recurrence rate dropped to 5% to 10% instead of 30% to 50%. Recent reports have suggested that outcome in patients undergoing a resection for colon cancer may vary depending on the adequacy of the surgical resection. Spe-

cifically, outcome may vary depending on the number of nodes that are retrieved in the specimen.

Dr. Johnson and his colleagues examined the adequacy of nodal retrieval in colon cancer specimens. Their hypothesis was that perhaps if surgeons do a better resection and pathologists examine an adequate number of nodes, patients would be more likely to be correctly staged. The authors state that a minimum of eight nodes should be ex-

amined, although recently the National Cancer Institute has published guidelines indicating that 12 should be the minimum. In this study 409 patients had surgery at a tertiary care institution (not population based as stated). The pathology reports of these patients were reviewed retrospectively. The mean number of nodes examined was 7.8 per specimen, but interestingly 19% had less than three nodes examined and only 43% had more than eight nodes per specimen examined. Patients with positive lymph nodes had a greater lymph node harvest (8.5 vs. 7.4). Interestingly, surgeon volume and pathologist volume were predictors of adequate nodal harvest, as was the type of resection. Other studies have replicated these results. At the University of Toronto, a similar study based on population data from the province of Ontario was conducted by Wright et al.[1] and showed that nodal harvest was greater at academic centers, but there was no volume effect.

What may be most important about the attention to adequate nodal identification is not the identification of nodes, per se, but improved institutional management of patients with colon cancer. Given the following: that approximately 50% of patients will be staged as having stage I or II cancer; that only a fraction of them may be staged incorrectly; and that adjuvant chemotherapy results in an absolute improvement in survival of perhaps 5%, it is unlikely that more accurate staging will improve overall survival by more than a few percentage points. Increased node retrieval may simply be a surrogate marker for improved care for these patients by the multidisciplinary team. This would be supported by the finding of a volume effect in this study by Dr. Johnson and his colleagues.

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Pathogenesis of Pigment Gallstones in Western Societies: The Central Role of Bacteria

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Bacteria are traditionally accorded a greater role in pigment gallstone formation in Eastern populations. Stone color is thought to predict the presence of bacteria; that is, black stones (Western predominant) are supposedly sterile and brown stones (Eastern predominant) contain bacteria. We previously reported that, regardless of appearance, most pigment gallstones contain bacteria. This study examined, in a large Western population (370 patients), the incidence, appearance, and chemical composition of pigment stones, and the characteristics of gallstone bacteria. One hundred eighty-six pigment stones were obtained aseptically. Bacteria were detected by means of scanning electron microscopy and gallstone culture. Chemical composition was determined by infrared spectroscopy. Bacteria were tested for slime and β-glucuronidase production. Seventy-three percent of pigment stones contained bacteria. Choledocholithiasis was associated with gallstone bacteria. Ca-bilirubinate was present in all pigment stones. Ca-palmitate was characteristic of infected stones, and more than 75% Ca-carbonate was characteristic of sterile stones. Neither chemical composition nor stone appearance predicted the presence of bacteria. Ninety-five percent and 67% of infected pigment stones contained bacteria that produced slime and β-glucuronidase, respectively. Most pigment stones contained bacteria that produced β-glucuronidase, slime, and phospholipase, factors that facilitate stone formation. Thus bacteria have a major role in Western pigment gallstone formation. Furthermore, gallstone color did not predict composition or bacterial presence. (J GASTROINTEST SURG 2002;6:891–904.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Pigment gallstones, slime, biofilm, gallstone pathogenesis, gallstone composition, infrared spectroscopy

There is no consensus with regard to many of the basic aspects of pigment gallstone pathogenesis. Most experts believe that bacteria have a role in the formation of pigment stones in Eastern populations, but there is less agreement with regard to Western populations. It is currently accepted by many that the presence of bacteria in pigment gallstones can be predicted on the basis of stone color; that is, bacteria are thought to be a component of brown but not black stones. There are no data to support this contention, however, and standard criteria for reproducibly distinguishing black from brown stones have not been reported. Some investigators have included other stone characteristics, such as hardness or size, in helping to make the distinction. Stones that contain both black and brown pigments are variably called black or brown; many brown stones appear black when wet, and some investigators have actually classified dark-brown stones as black.[1–18] Regardless of these inconsistencies, it is still generally believed that black pigment stones are sterile and that they are the predominant type of pigment stone in Western populations.[1–18] Thus bacteria are considered to play a minor part in the formation of pigment stones in these societies. The data in this study contradict these conventional ideas.

We previously reported that most pigment gallstones in typical Western societies contain bacteria

that can be detected by scanning electron microscopy, which suggested a significant causal role for bacteria in the formation of pigment gallstones.[19] The presence of bacteria in the stones also correlated with clinical infection.[20] The most commonly accepted relationship between bacteria and pigment stone formation involves deconjugation of bilirubin by bacterial β-glucuronidase with the precipitation of Ca-bilirubinate.[7] For the precipitate to evolve into a macroscopic stone, an anionic glycoprotein is needed.[7] We reported that most bacteria recovered from gallstones produced slime (i.e., glycocalyx), an anionic gyocoprotein that can aggluntinate the Ca-bilirubinate into stones.[21]

In the current study of a large unselected Western population, we examined pigment gallstones for visual appearance, chemical composition, and bacterial possession. The pathogenesis of pigment gallstone formation was studied by correlating the presence of bacteria in the stone, the ability of the bacteria to make β-glucuronidase and slime, the chemical composition of the stone (defined by Fourier-transformed infrared [FTIR] spectroscopy), and the visual appearance of the stone.

METHODS

A total of 397 unique stones from 370 patients were studied. When available, stones from both the gallbladder (GB) and common bile duct (CBD) in a single patient were examined separately. Stones were obtained from the following three sources: a university hospital—UCSF Moffitt-Long Hospital (98 patients, 27%); the San Francisco Veterans Medical Center (248 patients, 67%); and Norwich Hospital, Norwich, England (24 patients, 6%). Twenty-three percent of the patients were women and 77% were men. The average age was 61 years (range 17 to 104 years). Seventy-four percent of the patients were white, 10% were Hispanic, 9% were black, 6% were Asian, and 1% were of Middle-Eastern descent. The 186 pigment stones (from 169 patients) constituted the study group. Henceforth, whenever the term gallstones or stones is used in this report, it refers to pigment gallstones unless otherwise specified.

The stones were photographed and classified according to their visual appearance (i.e., stone color). Only the color was considered, not any other gross characteristics, stone locations, or associated clinical findings. The stones were separated into the following three groups: (1) black stones, (2) stones with both black and brown pigments, and (3) brown stones. For stones with both black and brown pigments, the exterior color and the predominant color were also recorded.

The stones were obtained under sterile conditions at the time of surgery. The presence of bacteria in the stones was determined by stone culture and by examination under a scanning electron microscope. The latter can reliably demonstrate bacterial microcolonies or bacterial casts.[19] The scanning electron microscopic studies were performed using previously described techniques.[19] For culture, the stones were rinsed with normal saline solution, crushed, and cultured in Tryptic-Soy broth for 24 for 48 hours. One hundred eighteen stones were cultured, 125 stones were examined by scanning electron microscopy, and 59 stones were both cultured and examined by scanning electron microscopy. Stones were judged to contain bacteria if the stone culture was positive or bacterial microcolonies were seen on scanning electron microscopic examination. Bile cultures, available in 67% of patients with pigment stones, were correlated with the results of scanning electron microscopy and gallstone cultures.

The chemical composition of the stones was determined using FTIR in 84 representative stones. For this test, the stones were air-dried, ground in an agate mortar and pestle, mixed with potassium bromide in a shaking device, pressed into pellets at 3000 psi, and then examined under FTIR. Stone composition was determined by comparing their infrared spectra with those of standards using techniques previously described.[3,11] Standards of varying composition were made using commercially available cholesterol, conjugated bilirubin (bilirubin isomers; Sigma, St. Louis, Missouri), Ca-palmitate, Ca-carbonate and Ca-phosphate, and synthesized Ca-bilirubinate. Ca-bilirubinate was synthesized using the method of Edwards et al.[9] Multiple standards were made to fully bracket the range of compositions of the gallstones. The bacteria obtained from the stones were also tested for slime and β-glucuronidase production using previously described methods.[21]

RESULTS

There were a total of 169 patients (4%) from whom we obtained 186 pigment stones from different locations (i.e., GB, CBD). The average age of the patients with pigment stones was greater than that of our patients with cholesterol or mixed stones (63 years vs. 56 years, respectively, $P < 0.0001$, t-test), although the age ranges overlapped. The age distribution of patients with infected and sterile pigment stones is shown in Fig. 1. Pigment stones were more prevalent in the elderly, but were present throughout all age groups, and infected pigment stones predominated over sterile pigment stones in all age groups.
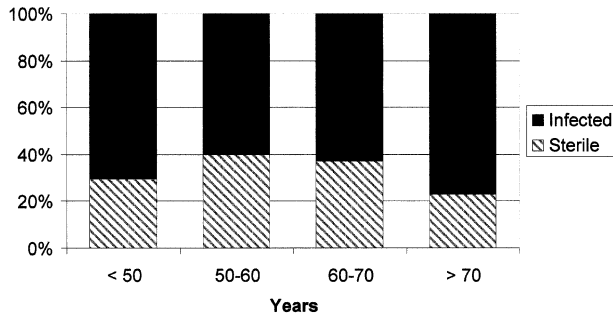
Fig. 1. Age distribution of patients with pigment stones and the proportion of infected (*solid bars*) and sterile (*cross-hatched bars*) stones in each age group.

The proportion of men was higher among patients with pigment stones than among those with cholesterol or mixed stones (86% vs. 75%, respectively; $P = 0.029$, chi-square test).

Gallstone Culture and Scanning Electron Microscopy Findings

A total of 136 (73%) of the 186 pigment stones contained bacteria, and bacteria-containing stones were found in 71% of these patients. One hundred thirty-five bacterial isolates were recovered from the 118 stones cultured from 109 patients. The stones from 39% of these patients contained one bacterial species, 31% contained two bacterial species, and 30% contained three to five bacterial species. Table 1 lists the bacterial species cultured and the incidence of slime and β-glucuronidase production for each. Most bacteria (83%) recovered from these stones produced slime, but only 44% produced β-glucuronidase. Because more than one organism was recovered from 57% of stones, 67% of patients had gallstone bacteria that produced β-glucuronidase and 95% had gallstone bacteria that produced slime. Infected pigment stones more commonly contained bacteria that produced β-glucuronidase than did infected stones that were predominantly cholesterol (67% vs. 42% for pigment- and cholesterol-containing stones, respectively; $P = 0.033$, chi-square test).

Bile cultures were obtained in 67% of patients. Stone culture agreed with bile culture in 87% of instances. In 9%, bacteria were detected in stones but not in bile, and in 4% bacteria were detected in bile but not in stones. Scanning electron microscopy was more sensitive than bile culture in that it detected bacteria not recovered from bile in 23% of cases and agreed with bile culture in 74% of cases. In 3% of cases, bacteria were recovered from bile but were not detected on scanning electron microscopic examination. Bacteria were present in the stones of 88% of patients in whom bile cultures had not been performed (43% of whom had clinical evidence of biliary infection).

Seventy-five percent of pigment stones examined by scanning electron microscopy demonstrated bacteria. Representative scanning electron micrographs and photographs of the stones are shown in Figs. 2 to 7. Scanning electron microscopy detected bacterial microcolonies in all stones with positive cultures. Of stones both cultured and examined under a scanning electron microscope, 35% had negative cultures and no bacteria on scanning electron microscopic examination, 53% had positive cultures and bacterial microcolonies on scanning electron microscopy examination, and 12% had negative gallstone cultures

Table 1. Bacterial species cultured from pigment gallstones: Distribution, slime production, and β-glucuronidase production

Bacterial species	No. of isolates	Slime production (%)	b-Glucuronidase production (%)
E. coli	30	100	93
Enterococcus	27	85	0
Klebsiella	21	70	50
Enterobacter	10	63	38
Pseudomonas	8	100	60
Citrobacter	7	75	33
Xanthomonas maltophilia	5	100	0
Non–Enterococcus streptococcus	7	100	0
Staphylococcus species	4	100	0
Other (*Aeromonas hydrophila, Bacteroides, Clostridium, Serratia, Proteus*, nonspeciated gram-negative rods, yeast)	16	—	—
TOTAL	135	83	44
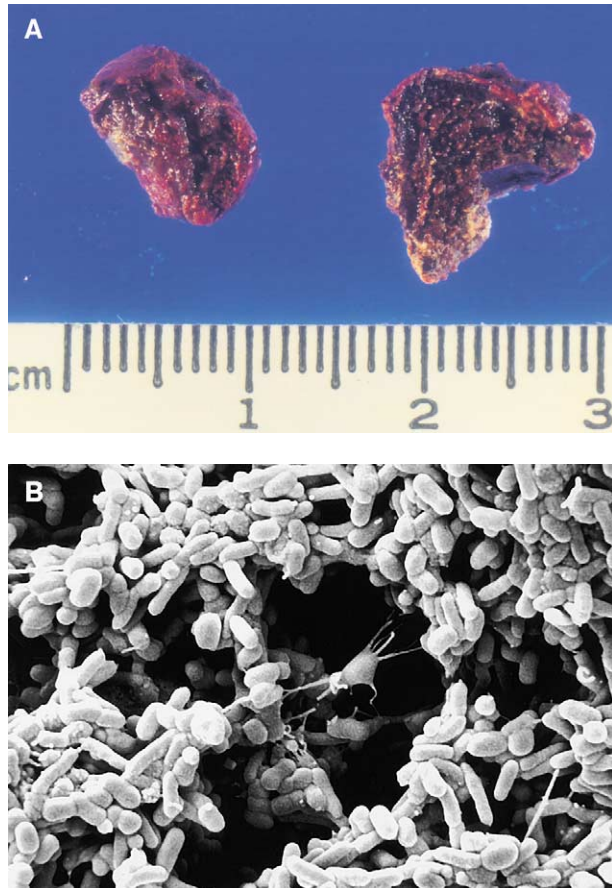
Fig. 2. A, Pigment gallstones. These stones are predominantly brown, with some black pigment as well. They contained both Ca-bilirubinate and Ca-palmitate. **B,** Scanning electron micrograph of a stone demonstrating bacterial microcolonies. Note bacterial bridges and three-dimensional nature of the biofilm.

but demonstrated bacterial microcolonies on scanning electron microscopy. Thus the two tests agreed in 88% of cases. In the remaining 12%, scanning electron microscopy was more sensitive than stone culture in detecting gallstone bacteria.

Bacterial morphology (rods or cocci) seen on scanning electron microscopy correlated well with the morphology of the bacterial species recovered from stone culture. In 75%, scanning electron microscopy and culture demonstrated the same findings (39% had the same morphology on scanning electron microscopy as the cultured pathogens, 36% were sterile on both tests); 10% showed the same morphology on scanning electron microscopic examination as the cultured pathogens and demonstrated additional bacterial morphologic types on scanning electron microscopy that were not recovered from stone culture; 3% showed some, but not

all, of the cultured bacterial morphologic types on scanning electron microscopy; and 12% had negative gallstone cultures but demonstrated bacterial microcolonies on scanning electron microscopy. Thus, morphologically, scanning electron microscopy demonstrated the same pathogens that were recovered from culture in 88% of cases, it showed additional pathogens not recovered from culture in 21% of cases, and failed to image some bacterial species recovered from culture in 3% of cases.

Gallstone Location and Presence of Bacteria

The location of the stones correlated with the presence of bacteria (Fig. 8). Stones from the CBD (secondary or primary) or intrahepatic ducts more commonly contained bacteria than did stones located exclusively in the GB ($P < 0.0001$, chi-square test). As shown in Fig. 8, 52% of the stones confined to the GB contained bacteria. When stones were present in both the GB and CBD, 82% contained bacteria, and all primary CBD stones and intraheptic duct stones (i.e., patients without GB stones) contained bacteria. Among patients with gallstones in both the GB and CBD, there was no difference in the presence of bacteria in stones located in the GB or CBD.

Gallstone Composition and Presence of Bacteria

The average composition of pigment stones that contained bacteria (i.e., infected stones) and sterile stones is shown in Table 2. The stones were composed of varying amounts of Ca-bilirubinate, conjugated bilirubin, Ca-carbonate, Ca-palmitate, Ca-phosphate, and sometimes cholesterol (18% of pigment stones). Not all components were present in every stone. The prevalence of the different chemical constituents in sterile and infected stones is also shown in Table 2. Ca-bilirubinate was present in all but one pigment stone (which was 100% Ca-carbonate). Conjugated bilirubin, when present, was always found in combination with Ca-bilirubinate. There were no differences between the amount of Ca-bilirubinate or conjugated bilirubin in sterile compared with infected stones. Among stones composed exclusively of Ca-bilirubinate and conjugated bilirubin, 68% were infected and 32% were sterile, but there were no differences in the composition of sterile and infected stones ($P = 0.445$, analysis of variance [ANOVA]). In addition, there was no difference in the amount of Ca-bilirubinate in infected stones containing bacteria that did or did not produce β-glucuronidase ($P = 0.504$, ANOVA).

The only significant compositional difference between sterile and infected stones was the amount of
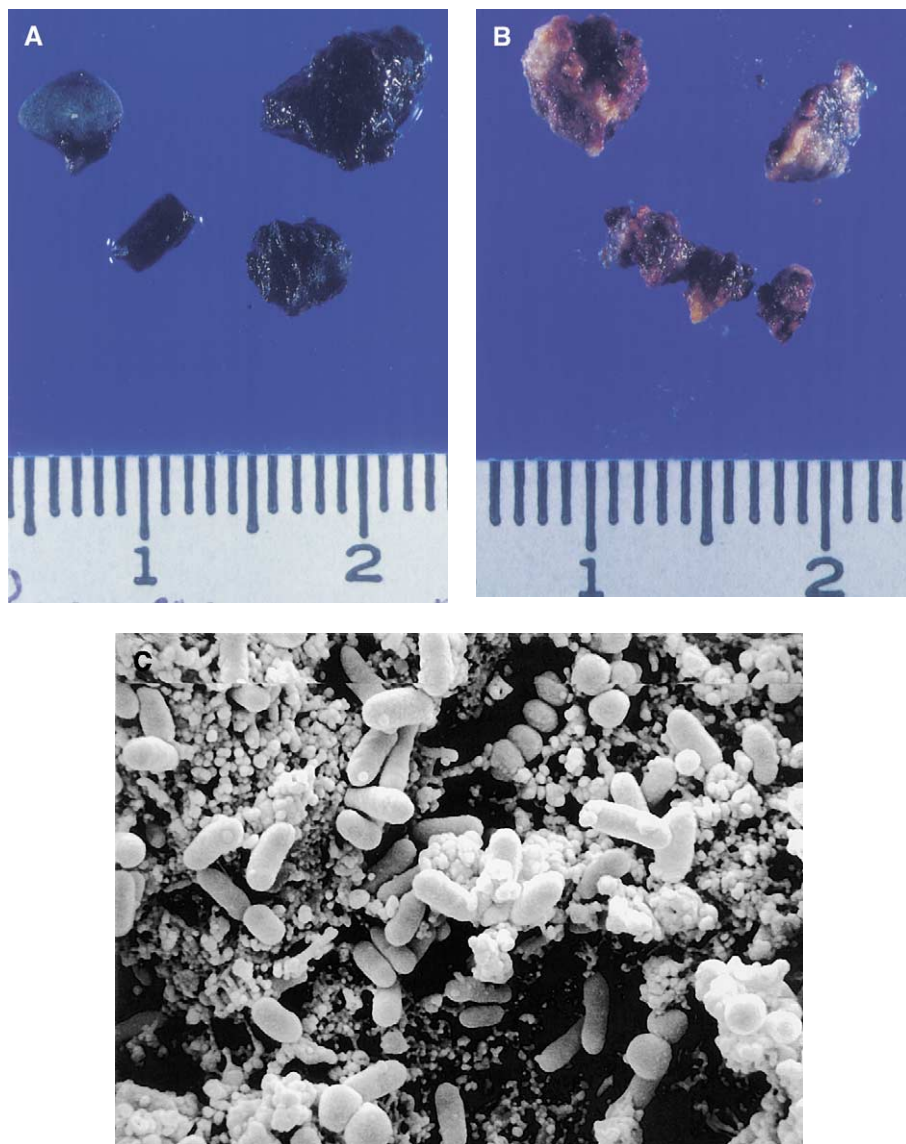
Fig. 3. A, Black pigment gallstones. These stones contained Ca-bilirubinate, Ca-palmitate, and cholesterol. **B,** Black and brown common duct stones from the same patient. These stones contained Ca-bilirubinate, Ca-palmitate, and cholesterol. **C,** Scanning electron micrograph of a stone demonstrating bacterial microcolonies with pigment solids attached to the bacteria.

Ca-palmitate and Ca-carbonate. On average, infected stones had a higher proportion of Ca-palmitate (15% vs. 0%, infected and sterile stones, respectively; $P < 0.0001$, ANOVA), whereas sterile stones had a higher proportion of Ca-carbonate (40% vs. 5%, sterile and infected stones, respectively; $P < 0.0001$, ANOVA). The presence of Ca-palmitate correlated strongly with the presence of bacteria: 98% of stones containing any amount of Ca-palmitate contained bacteria and 65% of infected pigment stones contained Ca-palmitate (Table 2). Ca-carbonate was more commonly present in sterile stones.

Fifty percent of sterile stones contained Ca-carbonate, and 57% of pigment stones containing any amount of Ca-carbonate were sterile. The correlation was best among stones with high concentrations of Ca-carbonate; 88% of pigment stones that contained more than 75% Ca-carbonate were sterile.
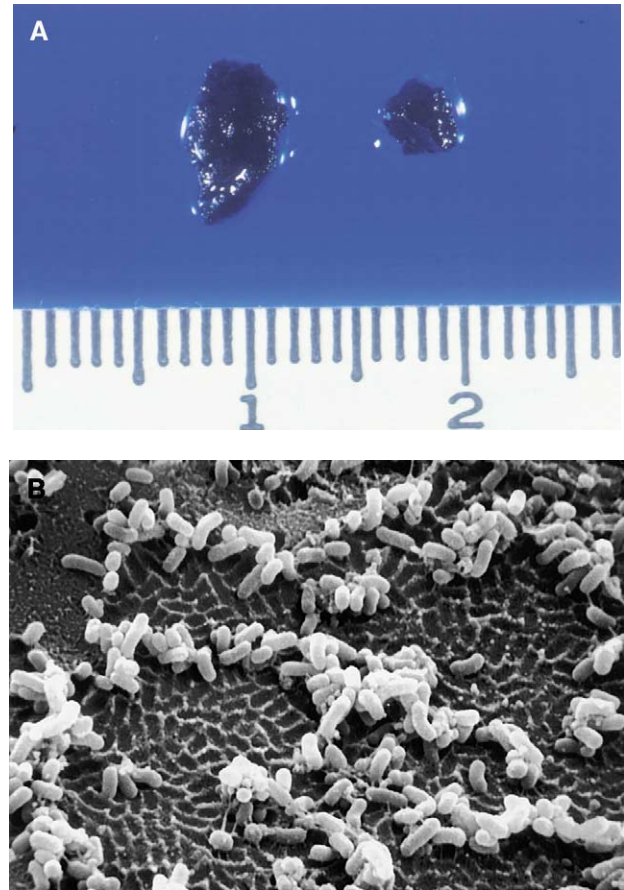
Interaction of Bacterial Pigment Stone–Forming Factors

The relationship between the various bacterial factors that promote pigment stone formation is
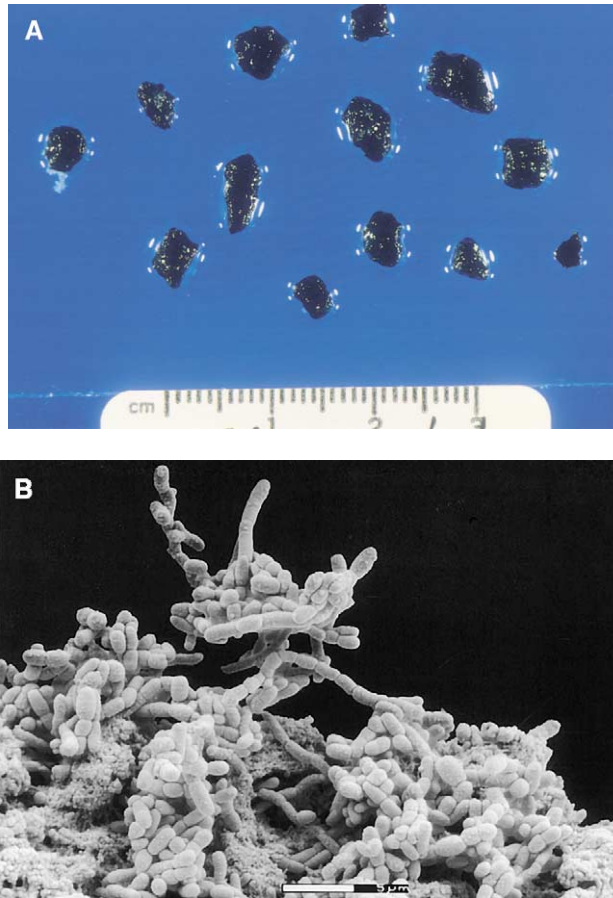
Fig. 4. A, Black pigment gallstones. **B,** Scanning electron micrograph of a stone demonstrating bacterial microcolonies. Note the bacterial bridges and three-dimensional nature of the biofilm.

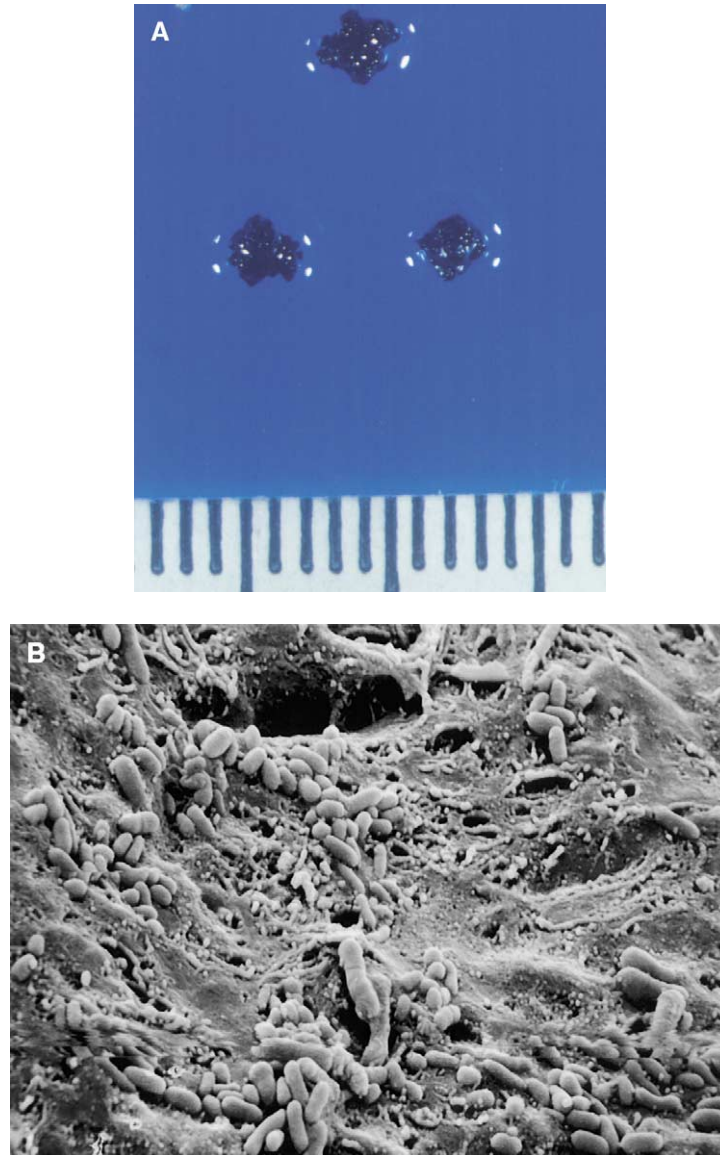
Fig. 5. A, Black pigment gallstones. These stones contained Ca-bilirubinate, conjugated bilirubin, and Ca-phosphate. **B,** Scanning electron micrograph of a stone demonstrating bacterial microcolonies. Note the honeycomb imprint next to the bacteria, which represents the presence of residual slime deposit where the biofilm was pulled apart as the stone was cracked for examination.

shown in Fig. 9. The bacterial factors slime, β-glucuronidase, and phospholipase (inferred from the presence of Ca-palmitate in the stone) are graphed on the three axes, with the percentage of stones containing bacteria that produced each factor shown. The incidence of sterile stones, in that they contained no bacteria, are at the zero point for the bacterial factors. The figure shows that there were no infected stones whose bacteria did not produce one of the factors. Twenty-eight percent of all stones (38% of infected stones) possessed bacteria that produced all three factors (slime, β-glucuronidase, and phospholipase). In the remainder, the bacteria produced β-glucuronidase or the stones contained Ca-palmitate, but always in combination with slime-producing bacteria; or the bacteria only produced slime. Slime was the common denominator present in nearly all infected stones (shown as the shaded plane). Few stones (3%) contained Ca-palmitate without either slime or β-glucuronidase–producing

bacteria. Furthermore, the prevalence of sterile stones was low (27%). A proposed mechanism for the formation of infected pigment stones that incorporates these observations is presented in Fig. 10.

Stone Appearance and Presence of Bacteria

Visually classifying pigment stones is a subjective process, which in our experience exhibits substantial interobserver and intraobserver variability. The variation in appearance spans a spectrum; there are not just two distinct groups. Most stones contained both black and brown pigments and could be classified differently depending on how, when, and by whom they were viewed. Many stones with both black and brown pigments were black on the outside; many had a very small amount of brown pigment; and dark

Fig. 6. A, Black pigment gallstones. These stones were pure Ca-bilirubinate. **B,** Scanning electron micrograph of a stone. Note how the bacteria are incorporated into the matrix of the stone, encased by the bacterial slime. *Pseudomonas aerginosa* was cultured from this stone, which is a lush slime-forming species.

brown stones often appeared black when wet (as viewed when freshly removed from the patient). If the stone was cut to expose the center, and then dried, the presence of brown pigment became more obvious. After viewing and classifying nearly 400 gallstones, one member of our group could fairly reliably predict which pigment stones would be sterile. Approximately 80% of stones with the following features were sterile: jet black (even when dry), no brown pigment whatsoever, and brittle (glass-like) when cut. These obsidian-like stones constituted 32% of the pigment stones (79% were located in the GB, 21% were in the GB and CBD, 22% had associated positive bile cultures, and 20% contained bacteria). Nevertheless, many totally black stones contained bacteria. The distribution of stones of different colors is shown in Fig. 11 for sterile and infected stones. Although there was considerable variation, all stones that were brown and 96% of stones that were black and brown contained bacteria, whereas 45% of black stones contained bacteria. Only 8% of patients with pigment stones had underlying hemolysis or cirrhosis. Among this small group, 77% of the stones were black and 54% were sterile.
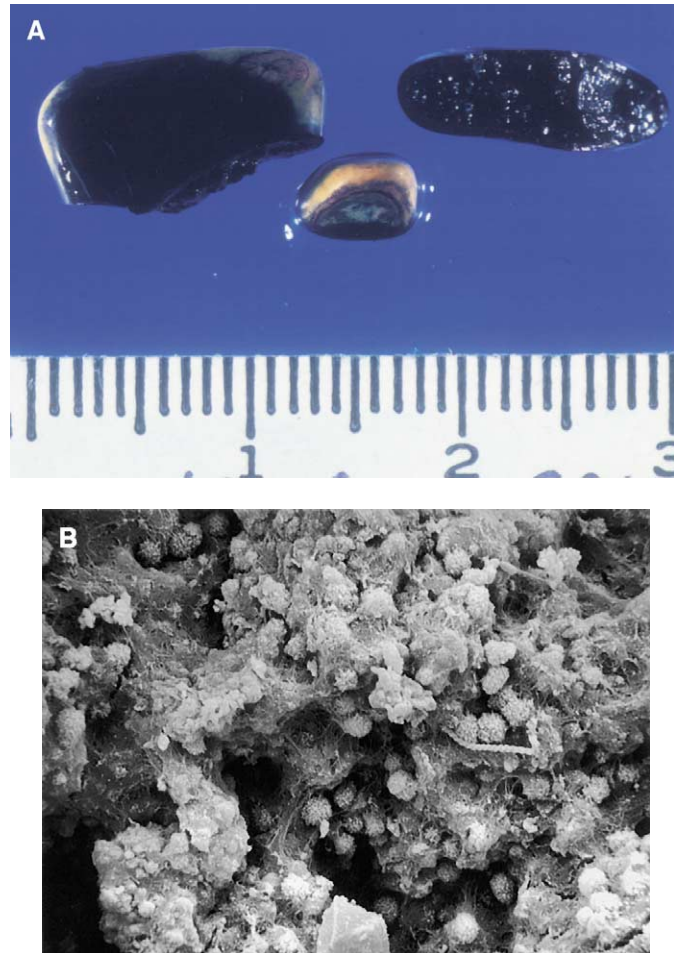
Fig. 7. A, Black pigment gallstones that were sterile. These stones contained Ca-bilirubinate, Ca-carbonate, and Ca-phosphate. **B,** Scanning electron micrograph of a stone. Note the absence of a bacterial biofilm.

Stone Appearance and Chemical Composition

Most stones (70% to 80%) containing Ca-carbonate or Ca-phosphate were black. Approximately 50% of stones containing conjugated bilirubin or Ca-bilirubinate were black, whereas the rest were either brown or black and brown. Only 25% of stones containing Ca-palmitate or cholesterol were black, whereas the rest were either brown or black and brown. All pure Ca-bilirubinate stones were black.

DISCUSSION

Previous reports have characterized pigment gallstones as either black or brown and inferred a host of generalizations based on this simple classification. Black stones have been variably described as black amorphous stones, stones that are both brown and black, or brown to black.[1–18] They are said to occur in the gallbladder only, are small (approximately 3 mm), and are the kind of stones found in patients with hemolytic disease, cirrhosis, or alcoholism. Black stones are believed to contain Ca-bilirubinate (reportedly polymerized to a greater extent than in brown stones), and sometimes Ca-carbonate or Ca-phosphate. These black stones, which are thought to occur in patients without biliary infection, are believed to be the predominant kind of pigment stone in Western populations.[1–18]

Conversely, brown stones are conventionally associated with biliary infection and are more common in the elderly. They are described as soft and lusterless, often containing alternating layers of different shades of brown. Some investigators have said that stones containing both black and brown pigments are associated with biliary infections,[13] which would put them into the brown stone category, but more
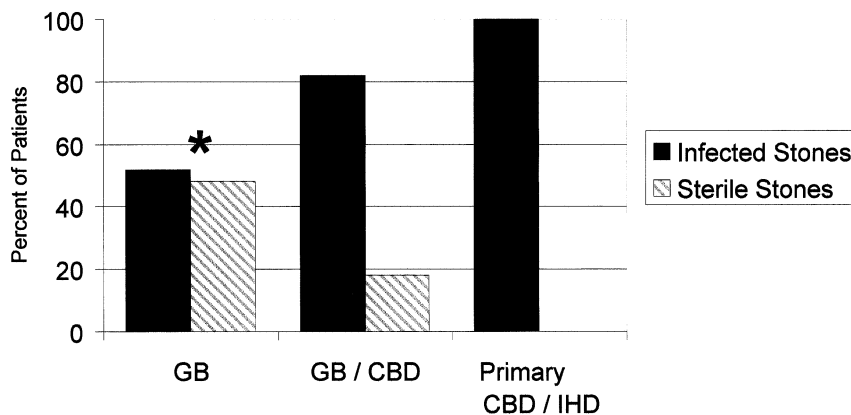
Fig. 8. Distribution of sterile (*cross-hatched*) and infected (*solid bar*) stones by stone location and mode of formation (GB = gallbladder; GB/CBD = stones in both the gallbladder and common bile duct; primary CBD/IHD = stones formed primarily in the common bile duct or intrahepatic ducts). Differences in the proportion of infected and sterile stones between GB and GB/CBD or primary CBD/IHD were significant (*$P < 0.0001$, chi-square test).

often such stones are classified as black.[1] Brown stones are reported to usually come from the CBD, often in association with juxtapapillary duodenal diverticula, and often as primary CBD stones. Brown stones have been reported to contain Ca-bilirubinate, often (or even always) in combination with Ca-palmitate, and usually with small amounts of cholesterol. Finally, brown stones are thought to be the predominant stone in Eastern (i.e., Asian) populations, but are presumably uncommon in Western populations.[1–18]

These color-based stereotypes were codified at a consensus conference held in 1982,[2] and the terminology and its outdated assumptions are still being used.[1,3–18] Several experiments have investigated the chemical composition, morphologic characteristics, and bile culture results of gallstones classified in this way.[2–18] Fourteen years ago, we reported finding bacterial microcolonies in most pigment gallstones obtained from patients in the United States and En-

gland.[19] Other workers, using similar scanning electron microscopy techniques, also found bacterial microcolonies in what they referred to as brown stones (or those with both brown and black pigments).[13–15] Infrared spectroscopy of selected stones was reported to have detected compositional differences between brown and black stones.[13] One group found bacteria in most pigment stones,[13] but because so few stones were examined,[14] and they were highly selected,[13] whether these findings can be considered representative is questionable. Furthermore, bile cultures, not stone cultures, were used to confirm the presence of gallstone bacteria,[12–14] which is an insensitive technique. Another study[15] that used scanning electron microscopy/electrodiagnosis to determine gallstone composition found Ca-palmitate in 31% of black stones in agreement with our data.

The current study examined, in a large unselected Western population, the prevalence of bacteria within pigment stones. This study differed from oth-

Table 2. Average gallstone chemical composition and prevalence of chemical components in infected and sterile pigment stones

	Average amount of chemical component in stone (%)		Prevalence of chemical component (%)	
	Infected stones	Sterile stones	Infected stones	Sterile stones
Cholesterol	7	6	16	6
Ca-bilirubinate	56	38	100	93
Conjugated bilirubin	14	11	38	31
Ca-palmitate	15*	0	65†	6
Ca-carbonate	5	40*	9	50†
Ca-phosphate	3	5	6	19

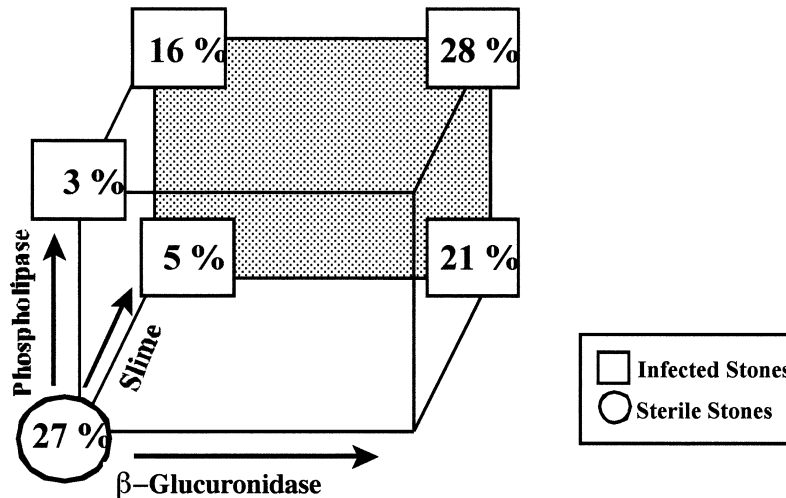*$P < 0.0001$, one-way ANOVA.
†$P < 0.0001$, chi-square test.

Fig. 9. Interaction and prevalence of various factors that lead to pigment stone formation. The bacterial factors, slime, β-glucuronidase, and phospholipase, are shown on the three axes, with the percentage of stones at each formulation shown in the boxes. The percentage of sterile stones, shown in the circle, is plotted at the zero point for bacterial factors. Notice that nearly all infected stones have a formulation that includes slime (*shaded plane*).

ers in that the presence of bacteria in the stones (not bile) was ascertained by two methods (gallstone culture and scanning electron microscopy), gallstone bacteria were tested for β-glucuronidase and slime production, the chemical composition of the stone was determined, and the visual appearance was recorded. There are several important findings. Foremost is that most (73%) pigment stones contained bacteria and that neither stone appearance nor stone composition alone could exclude the presence of bacteria. Several factors, such as brown or brown-and-black color and the presence of Ca-palmitate, were common in stones containing bacteria. Nevertheless, 26% of pigment stones containing Ca-palmitate were black, only 65% of infected stones contained Ca-palmitate, and nearly half the pigment stones were black, not brown (as would be expected by the high number that contained bacteria). Further, many stones containing bacteria either did not contain Ca-palmitate (62%) or were black (45%). Even black stones that did not contain Ca-palmitate were sterile in only 52% of cases. Thus, without stone culture or scanning electron microscopic examination, the presence of gallstone bacteria could not be predicted from other findings, most important, the gross features of the stone (e.g., color).

Stone culture and scanning electron microscopy were essential to detect bacteria in gallstones. These tests were more sensitive than bile cultures, and the results of bile cultures differed from the results of stone cultures or scanning electron microscopy in 26% of cases. The results of scanning electron microscopy and stone culture agreed 88% of the time,

even to the extent that they demonstrated the same bacterial morphologic types (rods or cocci) on both tests. Furthermore, 88% of patients in whom bile cultures were not obtained had bacteria-laden gallstones, but only 43% of them had clinical manifestations of biliary infection. Thus relying purely on clinical findings, with or without bile cultures (i.e., no stone cultures or scanning electron microscopy), would substantially underestimate the prevalence of bacteria in gallstones.

Stone color did not reflect stone composition in any predictable way. Otherwise similar-appearing black stones might contain different amounts of the various constituents. The proportion of conjugated bilirubin did not differ between black stones, black and brown stones, or brown stones. Even jet-black obsidian-like stones were not compositionally uniform. They contained Ca-carbonate in approximately half the cases, or were composed primarily of Ca-bilirubinate alone or in combination with conjugated bilirubin, and sometimes Ca-phosphate. Stones of similar composition might look different, and infected, and sterile stones (from different patients) might have similar compositions. Furthermore, stone appearance varied widely from black, to mostly black, to black on the outside, to evenly brown and black, to mostly brown, and finally to completely brown. We only classified stones that appeared to be totally black throughout as black stones. But many stones that were a heterogeneous mixture of black and brown (but predominantly black) would probably have been called black by most observers. Even so, there was not a close correlation between sterile
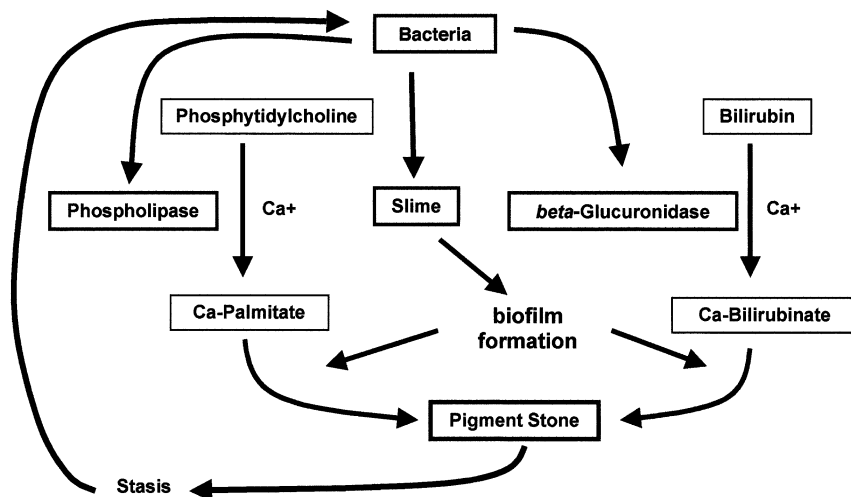
Fig. 10. Proposed mechanism of pigment gallstone formation by bacteria. This figure incorporates the contributions of slime, β-glucuronidase, and phospholipase in the process of stone formation.

stones and black color. Brown stones or brown and black stones also exhibited various compositions. Stones containing Ca-palmitate or cholesterol were more commonly brown or brown and black, but cholesterol, which is said to be present in brown stones, was infrequently found in our pigment stones. Moreover, Ca-palmitate and bacteria were present in stones of all colors. Finally, although there were differences between infected and sterile pigment stones, as noted previously, composition did not strongly correlate with the visual appearance of the stone.

Patients with hemolysis or cirrhosis represented a small fraction of the entire group with pigment stones. Patients with infected stones were older and more commonly men than were those with sterile stones. Bacteria in the stone correlated with gallstone location and choledocholithiasis.

These data strongly support the theory that bacteria have a role in the pathogenesis of most cases of pigment gallstone disease in the West: 73% of pigment stones contained bacteria, 95% of infected stones contained bacteria that produced slime, 67% contained bacteria that produced β-glucuronidase, and 71% of pigment stones contained Ca-palmitate. Thus the predominant pigment stone in the West, irrespective of its color, contained bacteria along with factors known to participate in pigment stone formation.

The importance of β-glucuronidase in pigment stone formation has been appreciated ever since Maki's description[7] of the deconjugation of bilirubin by bacterial β-glucuronidase leading to the precipitation of Ca-bilirubinate. Because nearly all pigment stones contain Ca-bilirubinate, β-glucuronidase seems

to be important in pigment stone formation. β-Glucuronidase is also present in bile, although the pH optimum for the bile form of the enzyme is lower than that of the bacterial enzyme.[21] We previously reported that only 38% of bacteria recovered from gallstones produced β-glucuronidase, whereas 47% of patients with infected stones had one or more bacterial species that produced β-glucuronidase.[21] The data included bacteria from pigment and predominantly cholesterol stones. Interestingly, a higher proportion (67%) of infected pigment stones contained bacteria that produced β-glucuronidase compared with predominantly cholesterol stones ($P = 0.033$, chi-square test). These findings support the bacterial β-glucuronidase theory.

Ca-palmitate is formed by the action of bacterial phospholipase on biliary phosphytidylcholine, resulting in the release of palmitic acid, which then combines with ionized calcium to form Ca-palmitate. Concentrations of free fatty acids in bile are higher in the presence of bacterial infection, and most bacterial strains isolated from bile have phospholipase activity.[22,23] Others have said that all brown pigment stones contain Ca-palmitate, which would imply that all infected pigment stones contain Ca-palmitate.[1,4–6,10–15] Although we found a strong correlation between the presence of Ca-palmitate and bacteria in the stones, the two were not universally associated. These differences are probably due to our use of stone culture and scanning electron microscopic examination to find bacteria, not just the appearance of the stones. In any case, the precipitation of Ca-palmitate by the action of bacteria produces additional solids that can be incorporated into the stone.
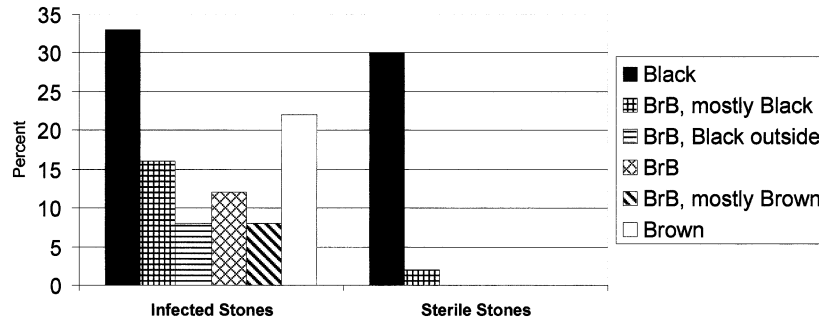
Fig. 11. Variation in pigment stone appearance of infected and sterile stones. The variation of black and brown stones is shown. BrB = brown and black pigments.

Although Maki[7] found that Ca-bilirubinate crystals were readily formed by the action of β-glucuronidase on bile, an anionic glycoprotein, sodium alginate, was also needed before macroscopic stones would form. Certain bacteria produce an anionic glycoprotein (slime, or glycocalyx), which allows them to form bacterial microcolonies or biofilms. This creates a protected environment that allows the bacteria to resist phagocytosis, antibodies, surfactants, and antibiotics while acting as an ion exchange resin for nutrients. Bacterial biofilms are involved in many chronic infections, foreign body infections, and the blockage of biliary stents.[24–27] We previously posited a key role for bacterial slime production in the pathogenesis of gallstones, and noted that slime-producing bacteria could be recovered from 82% of infected stones, 90% of common duct stones, and 100% of primary CBD (or intrahepatic) stones.[21] In the current data, 95% of infected pigment stones possessed bacteria that produced slime, further supporting this theory. The slime acts as an agglomerating substance (much the same as the sodium alginate used by Maki[7]) that cements together the precipitated Ca-bilirubinate and Ca-palmitate to form a pigment stone. Slime was the common denominator of nearly all pigment stones. Although approximately one third of the stones lacked bacteria that produced β-glucuronidase or phospholipase (as evidenced by the lack of Ca-palmitate), nearly all possessed bacteria that produced slime.

Linking the presence of gallstone bacteria and factors made by these bacteria with stone composition allowed an algorithm (Fig. 10) to be developed for pigment stone formation. The algorithm highlights the importance of slime-to-pigment stone pathogenesis and bacteria that produce β-glucuronidase or phospholipase. Infected stones without Ca-palmitate or with bacteria that did not produce β-glucuronidase all contained slime-producing bacteria. Nevertheless, infected stones containing bacteria that only produced slime (without β-glucuronidase or phospholipase production) constituted a small proportion of the entire group (7%). Thus not only was slime (the glue) important, but a bacterial mechanism was essential to the formation of pigment solids (Ca-bilirubinate or Ca-palmitate). Because all infected pigment stones contained Ca-bilirubinate, and only 67% contained bacteria that produced Ca-bilirubinate, bile β-glucuronidase may participate as well. In summary, bacteria facilitate pigment stone formation by bringing about the precipitation of Ca-palmitate (via the action of phospholipase) and the precipitation of Ca-bilirubinate (via the action of β-glucuronidase), and the precipitates are agglomerated into macroscopic stones by slime (see Fig. 10). In the process, the bacteria become incorporated into the stone matrix from which they can be cultured, indicating that they are alive. They are also easily detected on scanning electron micropic examination.

CONCLUSION

The majority of pigment stones contained bacteria that are easily detected by scanning electron microscopic examination and gallstone culture. These bacteria were able to produce β-glucuronidase, slime, and phospholipase, which contribute to the formation of pigment stones. Neither the color of the stone nor its chemical composition accurately predicted the presence or absence of gallstone bacteria, although certain components were more common in infected stones (Ca-palmitate) and sterile stones (>75% Ca-carbonate). Stone location was a predictor of gallstone bacteria (usually associated with choledocholithiasis), as were age and sex. Hemolysis or cirrhosis was infrequent. The previously accepted mechanism of Western pigment gallstone pathogenesis should be modified in accordance with these data. Bacteria have a major role in Western populations, as has been accepted for Eastern populations.

Finally, classifying pigment stones by color is an invalid avenue to understanding their composition, etiology, or clinical significance.

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Discussion

Dr. M. E. Zenilman (Brooklyn, NY): I think that a much more sensitive way of identifying and localizing bacteria within stones would be molecular, either with a marker or by polymerase chain reaction (PCR). You could also easily construct a karyotype of the type of bacteria present.

Dr. L. Stewart: The PCR has been used mostly in cholesterol-containing stones, and that is not relevant to this paper. Bacteria are abundant in pigment stones, using PCR here would be similar to using it to detect bacteria in stool. However, if you look at the papers that have used PCR to study cholesterol-containing stones, they start with the supposition that the presence of bacteria in the stone can be defined by the stone's composition, and these papers state that stones that are more than 90% cholesterol do not contain bacteria. In fact, we have found in our studies that this is not the case. I am not talking about cholesterol stones today, but I can tell you that you can easily culture bacteria from stones that are more than 90% cholesterol. We have done it; I have the data. So PCR is not

needed to find bacteria. They can be cultured, and they can be seen very easily on scanning electron microscopy. We have the bacteria, and we have done more than construct a karyotype of them. We have them in our freezer, we have studied them, and we will continue to study them.

Dr. C. W. Deveney (Portland, OR): You have talked about pigment stones today. What percentage of patients have predominantly pigment stones and what percentage have predominantly cholesterol stones?

Dr. Stewart: Forty-six percent of our patients had pigment stones.

Dr. M. G. Sarr (Rochester, MN): I think what Dr. Zenilman was asking was, did you identify all the bacteria? You have shown us data for one organism and for two organisms. We understand you can see the bacteria with a scanning electron microscope, but did you identify all of them?

Dr. Stewart: This question is actually answered in our presentation. A number of bacteria were cultured; there were 135, they are all listed in the manuscript, and their slime and β-glucuronidase production are also listed, and yes we did identify them.

Dr. Sarr: I understand that you were able to culture them; we are not arguing with you. But were you unable to culture some bacteria that were present that could only be detected by a PCR? For instance, you told us there was *Pseudomonas*. Were there some *E. coli* that you did not identify simply because they did not grow?

Dr. Stewart: I do not know that I can say for sure that there are no bacteria there. I will tell you that on scanning electron microscopy, we looked at the bacterial morphology that we saw on SEM, and we compared it to what we cultured, and those agreed very well.

In terms of PCR, my point is I do not think PCR is needed to detect these bacteria. They are readily detectable with scanning electron microscopy and/or SEM plus culture.

In answer to your question of whether we would find other bacterial species if we performed a PCR, I cannot say. With regard to these stones, 60% had two or more bacteria, and 30% had three to five bacteria. I think our collection of bacteria was more representative than potential PCR data.

Portal Vein Embolization vs. Portal Vein Ligation for Induction of Hypertrophy of the Future Liver Remnant

Dieter C. Broering, M.D., Christian Hillert, M.D., Gerrit Krupski, M.D., Lutz Fischer, M.D., Lars Mueller, M.D., Eike G. Achilles, M.D., Jan Schulte am Esch, M.D., Xavier Rogiers, M.D.

The objective of this study was to assess the efficacy of right portal vein embolization (PVE) vs. right portal vein ligation (PVL) for induction of hypertrophy of the left lateral liver lobe before extended right hepatectomy. Thirty-four patients with primary or secondary liver tumors and estimated remnant functional liver parenchyma of less than 0.5% of body weight underwent either right PVE (transcutaneous, n = 10; transileocolic, n = 7) or right PVL (n = 17). Liver volume was assessed by CT scan before occlusion of the right portal vein and prior to resection. There were no deaths. The morbidity rate in each group was 5.8% (PVE, 1 abscess; PVL, 1 bile leak). The increase in liver volume was significantly higher after PVE compared with PVL (188 ± 81 ml vs. 123 ± 58 ml) ($P = 0.012$). Postoperative hospital stay was significantly shorter after PVE in comparison to PVL (4 ± 2.9 days vs. 8.1 ± 5.1 days; $P < 0.01$). Curative liver resection was performed in 10 of 17 patients after PVE and 11 of 17 patients after PVL. PVE and PVL were found to be feasible and safe methods of increasing the remnant functional liver volume and achieving resectability for extended liver tumors. PVE results in a significantly more efficient increase in liver volume and a shorter hospital stay. (J GASTROINTEST SURG 2002;6:905–913) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Portal vein embolization, portal vein ligation, extended liver resection, remnant liver volume

Complete resection of hepatic tumors remains the first choice for curative treatment of primary and secondary liver malignancies, giving the patient the only chance of long-term survival. In up to 45% of primary and secondary liver tumors, extended liver resection is necessary to achieve clear resection margins.[1–6]

The reason for unresectability is that often the remnant liver is of insufficient volume to support postoperative liver function, which itself is still the principal cause of postoperative death after major hepatectomy.[7] The mortality rate after major liver resection ranges from 3.2% to 7% in noninjured liver parenchyma and increases up to 32% in patients with cirrhosis.[4,8–12] It has been demonstrated that liver failure is directly related to the amount of remnant functional liver volume,[4] and various procedures have been developed to induce liver regeneration.[11,12] Preoperative occlusion of the portal vein branches feeding the hepatic segments to be resected reduces the risk of postoperative liver failure after major liver resection and increases the number of resectable patients.[13]

In 1920, Rous and Larimore[14] first reported occlusion of the portal branch. Ligation of the portal vein branches in rabbits led to hypertrophy of the contralateral hepatic lobe and atrophy of the ipsilateral lobe. Takayasu et al.[15] observed hypertrophy after portal occlusion resulting from tumor invasion, and Honjo et al.,[16] in 1975, first reported portal ligation in patients with hepatocellular carcinoma. It was Makuuchi et al.[17,18] who first reported a novel approach for routinely inducing atrophy and contralateral hypertrophy in patients with cholestatic liver disease, chronic hepatitis, or cirrhosis. To increase the

number of patients amenable to curative surgery, they performed a preoperative portal vein embolization in patients with hilar bile duct carcinomas before major hepatic resection. Since then, several investigators have extended the indications for portal occlusion to secondary hepatic tumors[19] and to patients with hepatocellular carcinoma in injured livers.[20]

In the literature, no data are available regarding the type of portal vein occlusion leading to the greatest increase in size in the contralateral liver lobe. Therefore we performed a prospective study to assess the feasibility, safety, and efficacy of preoperative portal vein embolization (PVE) vs. portal vein ligation (PVL) in the induction of hypertrophy of the future remnant liver before major hepatectomy.

MATERIAL AND METHODS

Prospective data were collected between August 1995 and November 2000 from 34 patients with primary or secondary liver tumors who underwent occlusion of the right portal branch before major liver resection. The median age of the patients was 64 years (range 46 to 78 years). There were 21 men and 13 women (Table 1).

Curative liver resection was technically feasible in all 34 patients. In 31 patients (88%), portal vein occlusion was performed because the amount of remnant functional liver parenchyma was estimated to be less than 0.5% of body weight. In three patients (12%), potential impairment of the remnant liver function due to the abnormal quality of the parenchyma (e.g., liver fibrosis) was the indication for the procedure. In all patients, a preoperative workup had excluded any evidence of extrahepatic tumor spread.

The volume of the left lateral lobe (remaining liver) was calculated from serial transverse CT scans from each patient before segmental occlusion of the portal vein and before extended hepatectomy. In patients with obstructive jaundice, biliary endoscopic stenting was conducted until the total bilirubin concentration declined to at least 5 mg/dl.

Portal Vein Embolization

Seventeen patients underwent embolization of the right portal vein system, prior to surgery, by transcutaneous puncture of the contralateral portal vein under ultrasound guidance and local anesthesia (n = 10) or via a transileocolic vein after minilaparotomy under general anesthesia (n = 7). A 3F/5F polyethylene catheter was placed in the main right portal branch. After portography had defined the intrahepatic portal anatomy and correct placement of the catheter, the portal venous branch of the right lobe was embolized under fluoroscopic control. Embolization material consisted of a mixture (1:1-2) of 4 to 8 ml cyanoacrylate (Histoacryl; Braun, Melsungen Laboratories, Melsungen, Germany) and iodized oil (Lipiodol Ultrafluid; Andre Guerbet, Aulnay Soubois, France). Embolization was performed for each portal branch under constant fluoroscopic surveillance. Success of the portal branch embolization was confirmed by final portography. In 10 patients (59%), partial or complete occlusion of the segment IV branches was also performed.

Portal Vein Ligation

Ligation of the portal vein was performed in 17 patients. In nine of them, preoperative staging revealed no contraindications for primary liver resec-

Table 1. Patient characteristics

Category	PVL (n = 17)	PVE (n = 17)	P values*
Males/females (n)	8/9	13/4	NS
Age (yr)	63.8 ± 9.2	64.4 ± 6.3	NS
Body size (cm)	171 ± 9	172 ± 7	NS
Body weight (kg)	73.8 ± 16	78.9 ± 9.5	NS
Body mass index (kg/m²)	24.9 ± 3.9	26.5 ± 3.5	NS
Diagnosis			
Colorectal metastasis	9	8	
Hepatocellular carcinoma	1	1	
Cholangiocarcinoma	6	7	
Others	1	1	
Previous chemotherapy (n)	2	4	NS
Total bilirubin (mg/dl)	1.25 ± 1.34	0.97 ± 0.77	NS
Prothrombin time (%)	106 ± 7	107 ± 17	NS

*Mean ± standard deviation; NS = not significant.

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tion, but intraoperative findings indicated the need for portal occlusion because the liver remnant would have been too small after resection. In seven patients, abdominal staging laparotomy was necessary to (1) exclude extrahepatic tumor spread or (2) in situations of questionable technical operability. In one patient, PVE failed both percutaneously and via minilaparotomy, requiring an exploratory operation and ligation.

In 16 cases the indication for induction for contralateral hypertrophy was a large right-sided tumor with involvement of segment IV or the hilum, which would have required extended right liver resection with less than 0.5% of body weight for the remnant liver parenchyma. In one patient the beginning of cirrhosis was detected, which prohibited immediate curative resection of the tumor because of possible impaired liver function, even though more than 0.5% of body weight was calculated for the remnant liver volume.

All 17 patients underwent dissection of the portal bifurcation and ligation of the right main portal vein during intraoperative exploration. In five patients (29%), partial or complete occlusion of the segment IV branches was performed additionally.

Follow-Up After the Procedure

Liver volume was assessed by CT scan volumetry before and at 4-week intervals after portal branch occlusion. Serial transverse scans at 1 cm intervals were obtained, with enhancement by intravenous bolus injection of contrast medium. The volume of the left lateral segments was obtained by adding the volumes of individual slices. Volumetry was performed by the same radiologist (G.K.). For practical reasons we assumed equivalency between liver volume and liver weight. Volume was measured in milliliters and ratio in relation to body weight.

Statistics

Data are expressed as mean ± standard deviation unless stated otherwise. Survival analysis was estimated according to Kaplan-Meier analysis and included in-hospital deaths. Comparison of patient survival in different groups was performed with the use of the log-rank test. Categorical and continuous variables were compared by using Fisher's exact test and the Mann-Whitney U test, respectively. $P < 0.05$ was considered statistically significant.

RESULTS

Mean follow-up was 20 ± 17 months. With the exception of the sex ratio, none of the patient characteristics were significantly different in either group. Preoperative bilirubin levels and prothrombin time were comparable in both groups (Table 1). There were no deaths related to the procedure. The overall morbidity rate was 5.8% in each group (one subphrenic abscess after transileocolic puncture and one bile leak after PVL from a segment IV branch after dissection of the portal vein branch of segment IV). In two patients, the percutaneous approach was unsuccessful because of technical reasons. The patients were embolized via minilaparotomy and a transmesenteric vein 2 days later. In one patient, PVE failed both percutaneously and via minilaparotomy, requiring operative exploration and ligation. Occlusion of additional segment IV branches was performed in 10 (59%) of 17 patients in the PVE group and 5 (29%) of 17 patients in the PVL group. The mean postoperative hospital stay was significantly shorter in the PVE group (4 ± 2.9 days) compared to the PVL group (8.1 ± 5.1 days; $P<0.01$) (Table 2). Most of the patients experienced low-grade fever following portal vein occlusion. The mean peak of aspartate aminotransferase (AST) after portal vein ligation was 45.2 (±32.1) U/L compared to 50.3 (±31.3) U/L after portal vein embolization (NS) and returned to normal within 2 days after the procedure. Similar results were found for alanine aminotransferase (ALT) in both groups.

After portal vein occlusion, the left lateral liver lobe showed a substantial increase in volume in all but four patients. Three patients were not eligible for extended hepatic resection after PVL because of insufficient growth of the left lateral lobe. After PVE, one patient with insufficient growth of the contralateral liver lobe 4 weeks after right portal vein embolization underwent additional embolization of segment IV portal vein branches resulting in adequate regeneration of the left lateral lobe.

The estimated volume of remnant functional liver parenchyma increased from 287 (± 60) ml to 411 (± 80) ml in 83 (± 44) days after PVL. After PVE, the contralateral liver parenchyma increased from 271 (± 95) ml to 459 (±113) ml in 72 (±49) days (Fig. 1). There was a significant difference between the two groups with regard to the gain of the left lateral liver volume with 188 (±81) ml after PVE and 123 (±58) ml after PVL ($P = 0.012$). The daily increase in liver volume after portal branch occlusion showed significantly better results for PVE (3.89 ± 2.69 ml/day) than for PVL (1.72 ± 0.85 ml/day) ($P = 0.012$) (Fig. 2). Maximum tumor size increased from 4.66 ± 3.18 cm to 5.27 ± 3.47 cm after PVL vs. 3.53 ± 2.46 cm to 5.14 ± 2.48 cm after PVE (NS) (Table 3).

Before surgery, all liver function tests returned to baseline. PVE and PVL allowed curative resection in 21 (62%) of 34 patients. Curative extended liver re-

Table 2. Portal vein occlusion

| Category	PVL (n = 17)	PVE (n = 17)	P values*
Before occlusion			
Volume segments II and III (ml)	287.8 ± 60.1	271.8 ± 95.8	NS
Segments II and III/body weight ratio (%)	0.39 ± 0.06	0.38 ± 0.10	NS
Maximum tumor size (cm)	4.66 ± 3.18	3.53 ± 2.46	NS
Occlusion			
Technique			
Ligation	17		
Percutaneous embolization		10	
Transileocolic embolization		7	
Occlusion segment IV (n)	5	10	0.02
Major complications (n)	1 bile beak	1 abscess	NS
Length of hospital stay (days)	8.14 ± 5.15	4 ± 2.9	0.003

*Mean ± standard deviation; NS = not significant.

section was performed in 10 (59%) of 17 patients with PVE after 64 ± 29 days and 11 (65%) of 17 patients with PVL after 84 ± 30 days. In 19 patients, extended right hemihepatectomy (segments I and IV to VIII) was performed. Two patients underwent an anatomic segmental resection within the right lobe. After PVL, the mean specimen weight was 925.4 ± 357.9 g vs. 859.7 ± 253.9 g for PVE. The maximum tumor diameter in the final histology report was measured as 5.3 ± 3.9 cm for PVL and 4.85 ± 2.78 cm after PVE (NS). All but one of the PVE patients (n = 9 of 10; 90%) and all of the patients resected after PVL had negative resection margins (NS).

Thirteen (38%) of 34 patients were not resectable. One patient after PVE and two patients after PVL developed extrahepatic tumor spread detected in preoperative CT scans and were considered for palliative chemotherapy. In 10 of 31 scheduled operations, the decision not to resect was made during operative exploration. In four cases after PVE, progression of malignancy was observed with infiltration of the malignant process into the left portal and arterial branch in one case, segment 3 in two cases, and peripancreatic lymph nodes in one case. In two patients after PVE, liver fibrosis combined with insufficient hypertrophy was detected, prohibiting a curative extended resection. After PVL, two patients showed progression of their malignancies with infiltration of segment 3 and peritoneal carcinosis. In two patients after PVL, portal liver fibrosis causing insufficient hypertrophy was detected making resection impossible.

Postoperative intensive care and hospital stay were not significantly different in either group (Table 4). One of the patients in the PVE group died after extended right resection due to liver failure. The mortality rate in the PVL group was 0% (Table 4). The morbidity rate for major complications was 27% (n = 3) in the PVL group and 40% (n = 4) in the PVE group. The overall morbidity rate for resected patients was 33%. We noticed two bile leaks and one subphrenic hematoma in the PVL group and one deep wound infection, one subphrenic abscess, one necrosis at the resection site, and one subphrenic hematoma in the PVE-group. Postoperative survival was significantly longer in patients undergoing curative resection compared with nonresectable patients. The 1-, 2-, and 5-year survival rates were 86%, 63%, and 63%, respectively, for curative resections and 64%, 49%, and 0% respectively for the noncurative ones (Fig. 3) (P < 0.05).
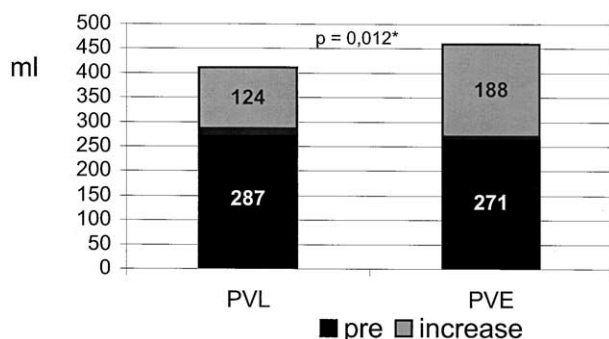
Fig. 1. Mean volume of the remnant liver (segments II and III) before and after different techniques of portal vein occlusion. PVL = portal vein ligation (n = 17); PVE = portal vein embolization (n = 17); * Mann-Whitney U test.

DISCUSSION

In the present study we compare different approaches for right portal vein occlusion for the in-

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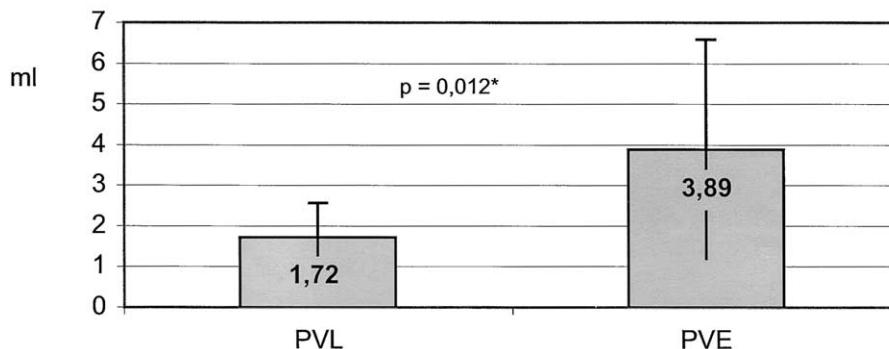
Fig. 2. Mean (±SD) daily increase in remnant liver volume (segments II and III) after different techniques of portal vein occlusion. PVL = portal vein ligation (n = 17); PVE = portal vein embolization (n = 17); * Mann-Whitney U test.

duction of contralateral hypertrophy before major liver resection. In the past, the techniques of portal vein occlusion have been mostly applied in patients with liver metastases and hilar cholangiocarcinoma because a large amount of hepatic parenchyma must be removed once segment IV, the middle hepatic vein, or the hilum is infiltrated.[17,19,20] Faced with the preoperative finding that the remnant liver would been too small, our main concern was whether ligation or embolization of the right portal branch was the preferred approach.

Preoperative CT volumetry provides the primary method for determining which patients are able to undergo extended liver resection and which patients may require portal vein occlusion.[21] It permits detailed cross-sectional radiographic visualization of the liver and estimation of the future liver remnant ±3% to 5%.[22]

The liver to body weight ratio gives an accurate idea of the liver volume required. For practical reasons we assumed equivalency between liver volume and liver weight. The difference between volume and weight of the liver tissue is, according to Heinemann et al.,[23] minimal and the formula that has been published for standard liver volume in Caucasians (liver volume [ml] = 1072.8 * body surface area $[m^2] - 345.7$) as the most accurate method to calculate the liver volume seems not to be practicable for clinical routine.

There is still no consensus as to how much of the liver can be safely resected with resections ranging from 60%[24] to 80%.[25] This disagreement results from a lack of standardization in the volumetry and liver function assessment of the liver before major liver resection. We defined the minimal acceptable remnant liver volume as 0.5% of the liver to body weight ratio, taking into consideration that the normal standard liver volume is approximately 2% to 2.5% of body weight. Therefore 0.5% of body weight is equivalent to approximately 25% to 20% of total liver volume, which is in agreement with the lower end in the literature (range 40%[24] to 20%[25]). Approaching this critical margin or decreasing it is

Table 3. Follow-up after portal vein occlusion

Category	PVL (n = 17)	PVE (n = 17)	P values*
After occlusion			
Time interval to final CT (days)	83.9 ± 44.19	72.7 ± 49.9	NS
Volume of segment II and III (ml)	411 ± 80.5	446.1 ± 134.32	NS
Segments II and III—% of body weight	0.57 ± 0.12	0.59 ± 0.16	NS
Maximum tumor size (cm)	5.27 ± 3.47	5.14 ± 2.48	NS
Gained volume			
Segments II and III—total (ml)	123.9 ± 94	188.8 ± 81	0.012
Segments II and III—per day (ml)	1.72 ± 0.85	3.89 ± 2.69	0.012
Segments II and III—% of body weight	0.18 ± 0.09	0.25 ± 0.13	NS
Nonresectability			
Nonresectable due to progression of malignancy (n)	6	7	NS

*Mean ± standard deviation; NS = not significant.

Table 4. Operation/follow-up

| Category	PVL (n = 11)	PVE (n = 10)	*P* values*
Operation			
Time from occlusion to operation (days)	83 ± 30	64.1 ± 29	0.024
Type of resection			
Extended right hepatectomy	10	9	
Segmentectomy	1	1	
Maximum tumor size in final histology report (cm)	5.3 ± 3.9	4.85 ± 2.78	NS
Specimen weight (g)	925.4 ± 357.9	859.7 ± 253.9	NS
Negative margins (n)	11/11 (100%)	9/10 (90%)	NS
Intensive care postoperative (days)	2.7 ± 2.2	4.6 ± 4.8	NS
Postoperative hospital stay (days)	12.2 ± 6.72	19.8 ± 19	NS
Mortality (n)	0	1 (10%)	NS
Morbidity (n)	3 (27%)	4 (40%)	NS

*Mean ± standard deviation; NS = not significant.

combined with increasing morbidity and mortality, especially in association with postoperative liver insufficiency, bile leakage, and liver failure.[7,26,27] The formula should not be used in patients with impaired liver due to fibrosis or cirrhosis. In those instances the experience of the surgeon, with regard to the histologic findings, liver function, general condition of the patient, tumor stage, and prospective liver remnant, is decisive. Patients with cholestatic liver disease should undergo preoperative drainage because liver resection in patients with cholestasis is followed by increased morbidity and mortality.[28]

Many surgeons prefer the percutaneous transhepatic approach over the transileocolic approach when performing PVE because it does not require general anesthesia. The procedure is fast and safe to perform

and avoids the risk of postoperative adhesions and complications related to general anesthesia.[29,30] Imamura et al.[31] reported severe complications with the use of the transileocolic approach. In a series of 78 patients undergoing transileocolic PVE, two patients developed bowel obstruction after the procedure, one of whom required surgery to relieve the obstruction.

At present, there is still no agreement on the ideal approach for percutaneous PVE. Abdalla et al.[13] and Nagino et al.[30] recommend an ipsilateral approach to avoid injury and thrombosis of the contralateral portal vein. They state that occlusion of the segment IV branches is easier than with the contralateral approach. We prefer the contralateral approach to occlude the right portal vein system completely. The left side is easier to access and the occlusion of the
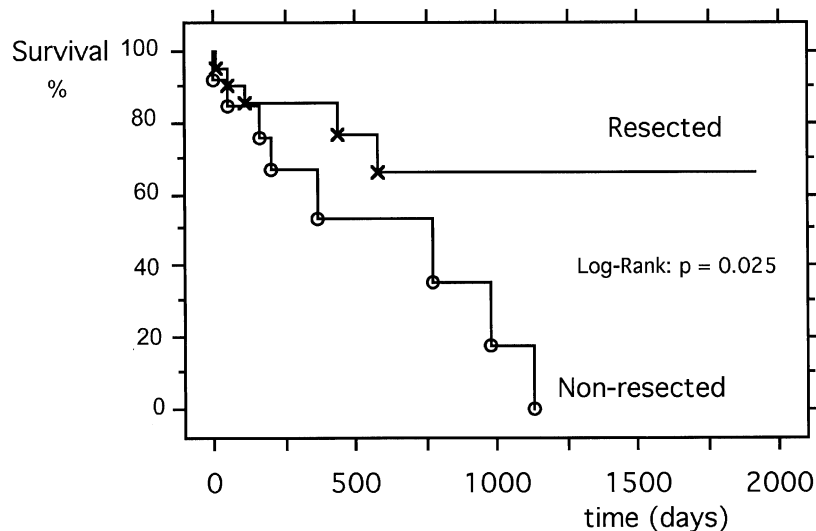
Fig. 3. Survival after curative resected (n = 20) and nonresected patients (n = 13) after preoperative portal vein occlusion either by PVL (n = 17) or PVE (n = 17).

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peripheral right portal vein system with cyanoacrylate (Histoacryl), using the physiologic portal flow, is complete, safe, and prevents incidental occlusion of the left or main portal vein branch. We have not observed portal vein thrombosis or vascular injury, and with further experience we were able to occlude the segment IV branches from the left-sided approach in more than 90% of the cases.

In our study we found PVE to be superior to PVL in terms of volume gain in a shorter time, shorter hospital stay, and fewer adhesions during major hepatectomy. The percutaneous approach does not induce adhesions, and the hilum will be preserved "untouched" for the intended resection, preventing tumor spread. On the other hand, laparotomy allows immediate resection and gives the surgeon the opportunity to assess hepatic or extrahepatic tumor extension and liver tissue quality that would exclude the patient from curative resection.[31] Especially in patients with hilar cholangiocarcinoma, operative exploration often is the only means of exact staging and documentation of resectability. Tsuge et al.[32] described the transileocolic approach via laparoscopy to assess exact preresection staging and to select the ideal branch for embolization.

In the beginning of our series, only segment IV branches from the right portal vein were occluded routinely either with ligation or embolization. Branches to segment IV from the left portal vein were only considered for occlusion if anatomically feasible, to minimize the risk of incidental occlusion of portal veins supplying the left lateral lobe. Intraoperative ligation of segment IV branches required dissection of the left portal vein, causing severe postoperative adhesions for the second operation, and dissection of the hepatoduodenal ligament in patients with central cholangiocarcinoma can be dangerous in terms of touching the tumor. Nagino et al.[33] demonstrated the importance of occlusion of segment IV branches before resection. The volume gain of patients with segment IV embolization was significantly larger than that in patients with isolated embolization of the main right portal branch after a time interval of only 2 to 3 weeks before the planned resection. The volume gain of the left lateral segments increased very significantly from 66 ± 35 cm^3 without occlusion of segment IV veins to 122 ± 39 cm^3 with occlusion of segment IV veins ($P < 0,0001$). These impressive results and our own experiences led us to initiate complete embolization of segment IV branches routinely if it seemed anatomically feasible.

In the present study we were able to perform a complete resection in 20 (58%) of 34 patients with an overall mortality of less than 5% and morbidity of 33%, which is comparable to rates in other series af-ter major liver resection with[17,20,33] and without[26,34] previous segmental portal occlusion. One patient with gallbladder carcinoma and hypertrophy of the left lateral segments from 204 to 452 ml in 66 days after transileocolic embolization died of liver failure 11 days after major liver resection.

One of the reasons for larger increase in liver volume are porto-portal collateral vessels, which have been described by Denys et al.[35] and which can be occluded by embolization more sufficiently compared with a central ligation. We used iodized oil (Lipiodol) and cyanoacrylate (Histoacryl) routinely with a highly dilute mixture (1:1-2) of 4 to 8 ml cyanoacrylate and iodized oil as the embolizing material for PVE throughout the study period to achieve irreversible obstruction, even in the peripheral branches, to minimize the development of porto-portal collateral vessels. The additional use of Lipiodol allows visualization of the embolized portal cast on plain radiographs. The functional burden with the permanent obstructing effect placed on the liver by PVE using this material is thought to be minimal and transient, as reflected in the only slight elevation in the transaminase levels. Peribiliary fibrosis has been reported when cyanoacrylate is used.[36] This might be of concern in those patients who cannot undergo major liver resection after portal vein occlusion, resulting in a possible loss of functioning liver parenchyma. On the other hand, in hepatocellular carcinoma, PVE helps to prevent metastatic spread by the portal vein.[37] Furthermore, the present results are in line with observations in animal models of portal vein ligation with a mild and transient necrotic response that is promptly masked by the emergence of apoptosis.[38]

Elias et al.[39] reported on a potential risk after PVE or PVL for the induction of rapid growth of potential tumors in the hypertrophic left lateral segments and of the tumor to be resected.[39] Four patients with liver metastasis showed an increase in normal liver parenchyma from 59% to 127%, compared with 60% to 970% for the liver metastasis within a median of 34 days (range 28 to 40 days) after PVE. The ratio between the grow rate of the left lobe and the liver metastasis varied from 1.0 to 15.6. In contrast Azoulay et al.[19] reported the first long-term results in patients after major hepatic resection of colorectal metastases with and without portal vein embolization. The survival rates of both groups were not statistically different.

CONCLUSION

PVE and PVL are feasible and safe methods of increasing the remnant functional liver volume and

achieving resectability of extended liver tumors without increasing mortality and morbidity. PVE results in a significantly greater increase in remnant liver volume and a shorter hospital stay than PVL. Because of high interindividual variability in the left-to-right lobar volume ratio and in the degree of hypertrophy induced in the nonembolized liver segments, we changed our policy based on the results of this study. We now perform preoperative volumetry routinely in all patients for whom major liver surgery greater than or equal to an anatomic right or left hepatectomy is planned. In case of less than 0.5% of body weight for the estimated uninjured liver remnant, our current strategy is to perform transcutaneous portal vein embolization as the first approach or operative exploration and transmesenteric embolization for patients with questionable staging. The ligation of the portal branch should be reserved for those patients in whom embolization is not possible because of anatomic factors. In any case, occlusion of the segment IV branches should be performed.

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Discussion

Dr. J.N. Vauthey (Houston, TX): I congratulate you on studying a new preoperative technique that may improve the resectability rate and outcome of a group of patients who are borderline candidates for extended liver resections. We too have used this technique, and I think it is very important that before we proceed with any new technique, we consider it very carefully and only in select patients. My first question relates to the 0.5% body weight you used as future liver remnant volume. Did you study that in a prospective manner before deciding on a 0.5% cutoff?

Dr. C. Hillert: We did not conduct a prospective study on this, but we approached it based on experiences with split-level and reduced-size transplantations, and it is comparable to around 25% of the standard liver volume, which is actually known to be the critical margin of liver volume needed to be left in the patient.

Dr. Vauthey: My second question relates to your planning before your procedures. You mentioned that you embolized or ligated the branches to segment IV. There are at least two theoretical issues here: (1) you want to maximize regeneration and (2) you do not want accelerated tumor growth because you do an extended right hepatectomy for patients who presumably have tumor in segment IV. Have you embolized or ligated these branches routinely?

Dr. Hillert: We have not. It was kind of a learning curve, so we now do it more often than we did in the beginning of our study. Embolization of segment IV is always associated with a slight risk of embolization of the left main branch, so it is critical that the radiologist be really experienced. Now we use it almost routinely to achieve the maximal increase in volume, at the beginning of the study we did not.

Dr. Vauthey: I have a third question. I am not surprised that ligation did not work well because there are anatomic variants of the right portal vein in 17% and you may have

missed them, and porto-portal collateral vessels may also form as a result of recanalization of the portal vein. Did you study the patients who did not show good regenerization, particularly within the group of patients who underwent ligation?

Dr. Hillert: We did not check on this, but we performed experimental studies concerning this topic, and we also found embolization to be superior to ligation, especially because of shunts between segment IV and the right segments causing an inadequate hypertrophy.

Dr. E. Klar. (Heidelberg, Germany): Most probably you induce shedding of growth factors by this technique, so the tumor might be affected, as well, with regard to an increase in tumor growth. So what is your protocol to bridge the time you wait for regeneration of the liver to keep tumor growth low?

Dr. Hillert: Until now there has actually not been any policy or special treatment to bridge this time. Special chemotherapy is known to block the increase in parenchyma, but we are introducing a study to check on this. On the other hand, a recent study comparing outcomes in patients who were not resected and after embolization and patients who were resected and had no embolization showed no difference. So for colorectal metastasis probably there is no clinical impact.

Dr. H. Orozco (Mexico City, Mexico): How long do you wait after the ligation or embolization before you resect the tumor?

Dr. Hillert: It varies. The mean waiting time ranged from 72 to 83 days. So we checked a time interval of 3 to 4 weeks routinely with CT scan volumetry and, depending on the increase in volume, we decide when the best point of resection will be.

Announcements

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Bariatric Surgery, January 24–25, 2003; May 30–31, 2003; September 26–27, 2003; The University of Texas Southwestern Medical Center at Dallas. Cost: physicians ($300, lecture only; $1050, lecture and lab); UTSW and SCMIS Alumni ($250, lecture only; $950, lecture and lab); nurse ($175, lecture only; $375, lecture and lab). For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

14th Annual Colorectal Disease Symposium, An International Exchange of Medicinal and Surgical Concepts, February 13–15, 2003, Marriott's Harbor Beach Resort, Fort Lauderdale, Florida. Sponsor: Cleveland Clinic Florida. Symposium director: Steven D. Wexner, M.D. CME credit: 26 Category 1. Contact information: Cleveland Clinic Florida, Department of Continuing Education, 2950 Cleveland Clinic Boulevard, Weston, FL 33331. Phone: 954-659-5490; toll free: 866-293-7866, ext. 55490; fax: 954-659-5491; e-mail: cme@ccf.org

Surgery of the Foregut, February 17–18 2003, Biltmore Hotel, Coral Gables, Florida. Meeting sponsor: Cleveland Clinic Florida. For further information contact: Cleveland Clinic Florida, Office of CME, 2950 Cleveland Clinic Boulevard, Weston, FL 33331. Phone: 954-659-5490; toll free: 866-293-7866 ext. 55490; fax: 954-659-5491; e-mail: cme@ccf.org

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Bariatric Surgery Mini-Fellowship Program, February 23–28, 2003; April 20–25, 2003; June 22–27, 2003; August 24–29, 2003; October 26–31, 2003; The University of Texas Southwestern Medical Center at Dallas. Cost: $12,500 (team of 2 physicians and 1 nurse); $6,250 (physician); $1,000 (nurse). For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Ventral Hernia, March 7–8, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Female Pelvic Floor Disorders, March 14–16, 2003, Sheraton Yankee Trader Beach Hotel, Fort Lauderdale, Florida. Meeting sponsor: Cleveland Clinic Florida. For further information contact: Cleveland Clinic Florida, Office of CME, 2950 Cleveland Clinic Boulevard, Weston, FL 33331. Phone: 954-659-5490; toll free: 866-293-7866 ext. 55490; fax: 954-659-5491; e-mail: cme@ccf.org

Southwestern Center for Minimally Invasive Surgery (SCMIS): Diagnostic Laparoscopy & Ultrasonography, April 11–12, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Management & Percutaneous Ablation of Small Retinal Tumors, July 25–26, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Management of CBD Stones, August 15–16, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu

Southwestern Center for Minimally Invasive Surgery (SCMIS): Laparoscopic Splenectomy, November 14–15, 2003; The University of Texas Southwestern Medical Center at Dallas. For further information contact: Jennifer Leedy, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9059. Phone: 214-648-3792; fax: 214-648-2317; e-mail: jennifer.leedy@utsouthwestern.edu