J Gastrointest Surg (2007) 11:1233–1241
DOI 10.1007/s11605-007-0229-5

Presidential Address: Adjusting the Art and the Science of Surgery

L. William Traverso

Received: 5 June 2007 / Accepted: 15 June 2007 / Published online: 25 July 2007

Abstract Why are there so many opinions for surgical treatments? Why do surgeons not agree on the same definitions? To adjust the art and science of surgery, we should understand the reason behind this Tower of Babel and ourselves by grasping the three biological lessons of history. These lessons are instincts of man – our instincts have not changed for as long as there has been recorded history. The lessons were elucidated by Will and Ariel Durant and these are competition, selection, and reproduction. How might they be applied to improving our surgical science?

First, *competition* has always forced individuals or small groups to strengthen themselves with cooperation. Cooperate or not survive. Cooperation increases with social development and technology. Next, we must realize that nature relishes diversity. We are all born unequal and diverse. The second biological lesson is *selection*; which individual among a diverse group of individuals will succeed (by improving)? Therefore, by nature, man's instincts provide diverse opinions and bias. This creates a myopic view when surgeons try to discern the truth. The results are the trendy bandwagons that divert us, like tonsillectomy. Too much diversity is bad, and a balance is required. Man's third lesson of history is *reproduction*. Better stated is that nature loves quantity. We naturally give priority to quantity over quality. To obtain quality rather than just quantity, we need the antidotes for competition and diversity – that would be cooperation using the Deming guidelines of leadership, profound knowledge, and technology. One example of this urge for quantity and diversity is our lack of standardized definitions. These three biological lessons can be summarized by viewing competition as an impediment for quality improvement in the complex challenges of modern healthcare. Cooperation (trust) is the antidote to the bandwagon effect of unproven treatments. Cooperation and technology can be joined to establish a successful team using the global technology of the internet ("Club Web"). To improve, we must measure real cases in a registry and generate a standard set of definitions and benchmarks. A focus group that trusts each other through the common interest of a disease or organ could succeed. Only then does comparison (and improvement) become possible.

Keywords Quality · Outcomes · Cost · History · Deming · Surgical procedures · Operative · Surgical · Pancreaticoduodenectomy · Economics · Costs and cost analysis · Cost

Presented at the 48th Annual Meeting of the Society for Surgery of the Alimentary Tract, Washington, D.C., May 19–24, 2007

L. W. Traverso (✉)
Department of General, Thoracic, and Vascular Surgery,
Virginia Mason Clinic,
1100 Ninth Avenue (C6-GSURG), P.O. Box 900, Seattle,
WA 98101-2799, USA
e-mail: gtslwt@vmmc.org

Accolades

This morning is the culmination of a great year, which has permitted me the utmost honor of presenting this presidential address to you, the members and guests of this society, as we start the 48th Annual SSAT Program. To begin, I must establish priorities of gratitude. I learned much from Morgan Wootten, the SSAT guest orator at the 46th Annual SSAT meeting. He is one of our nation's best-ever high school basketball coaches, and his lecture "Touching People's Lives," was inspiring. He directed his players to establish self-priorities in the following manner—God, family, education, and then basketball. In this surgeon's case, I substituted surgery for basketball. My personal

spiritual tribute has already been given, and I will begin this lecture with a tribute to my family.

Consider this slide, which has been constructed from six pictures of my wife, Adele. These action pictures are a tribute to a woman who is smart, independent, and yes, supportive. My wife is the most important factor in the success I have enjoyed. How did this happen? First, she allows me to take risks. I grew up in Reno, NV, where I learned to take risks but not gamble. However, those who take no risks will rarely succeed. By providing a stable family life for me and our children, Adele gives me the confidence to take the risks necessary to achieve unreachable goals. Most of us need some sort of stability. If our personal lives are disheveled, it is hard to make our professional lives work. Remember the Robert F. Kennedy quote, "Only those who dare to fail greatly can ever achieve greatly." I have a wonderful partner who is there to catch me when I fail. Second, she provides self-confidence. By praising what I do and not demeaning my ideas, Adele has given me the confidence to succeed. Third, she offers solid support. Thanks to Adele's emotional and spiritual strength, I can fulfill professional goals and refuel my emotional tank. She has done this while placing her own career as a surgeon on the back burner. Fourth, she publicly provides support. Adele champions my ideas. She does this, although she may not believe in all of them. Therefore, ladies and gentlemen, my wife gives me the perspective that the world is a better place because of faith, family, friends, and you, my colleagues.

Finally, this year would not have been possible without many great members of our SSAT team—Barbara Bass and the Board of Trustees, the Committees, and at the SSAT National Office with Bob Jones, Jon Blackstone, and Heather Wood. I really appreciate working with each of you.

Guidance: Bandwagons to Club Web

For this lecture, I sought much guidance from mentors, past SSAT presidents (many of whom were also my mentors and role models), and several diverse, yet august, intellects that have provided me a framework to understand the instincts of man himself. I have made some giant assumptions that only a fool would attempt—but I shall proceed.

Table 1 Past Addresses By SSAT Presidents

Fromm categories[1]	President addresses	Years
Clinical-historical	13	1968–1985
Political-philosophical	6	1974–1998
Research education	7	1966–1995
SSAT topics	8	1963–1997

Initially, this lecture must rely heavily on guidance from three past SSAT presidents—David Fromm, Robert E. Herman, and Layton "Bing" Rikkers. In 1999, to place some order into past addresses by SSAT presidents, Fromm categorized them as listed in Table 1.[1] He noted that the most popular topic with earlier presidents before 1985 was the "historical-clinical" category where these surgeons reviewed a surgical topic and its history. Because this has recently become less popular, I decided to obtain guidelines from history—not just about a surgical disease, but of man himself.

Ten years earlier, in 1989, Bob Hermann had categorized these lectures into three types: the most popular was a review of a surgical topic, then the historical review (SSAT topic or surgical disease), and finally, a major issue of the day and its solution.[2] For me, all of the Hermann lecture-types sounded appealing to weave into a lecture, especially "the solution" to a major issue of the day.

Do you remember the developing trend in the late 1990s of evidence-based medicine (EBM)? During his presidential address in 2002, "Bing" Rikkers suggested EBM as an antidote to the "Bandwagon Effect."[3] Because today's meeting is held in Washington, D.C., it seems only fitting to define a medical bandwagon using the death of our first president, George Washington, on December 14, 1799. Using accepted medical treatments of the day, his physicians, staff, and Washington himself, directed four phlebotomies. In 12 h, the General was drained of 2,365 ml of blood.[4] He was most likely suffering from acute epiglottis. Did he die from the disease or the treatment? Besides phlebotomy, other medical bandwagons have been tonsillectomy and for breast cancer, radical mastectomy. The nature of the bandwagon is an enthusiastic trend without proof. Many people jump on the bandwagon for economic or academic advancement. To avoid unproven enthusiasm for a new treatment, we need to listen to Dr. Rikkers' suggestion to use EBM as an antidote. More recently, aided by level one evidence, extended lymphadenectomy for pancreatic cancer has been added to the list of bandwagons—enthusiasm for radical surgery but without benefit. The main hurdle to avoid a medical bandwagon is most adequately summarized by Walt Kelly's Pogo, "We have met the enemy, and he is us." With this hurdle being man's nature of enthusiasm without proof, how do we adjust the art and the science of surgery? My suggestion for a "solution to a modern-day problem" is based on a review of man's history and what I will later term the "Club Web."

Origin: Adjust the Art and Science of Surgery

The title of today's lecture was conceived through a speech given almost 40 years ago by one of my mentors and role models in American surgery. The idea came to me at the

end of William P. Longmire Jr.'s presidential address to the American Surgical Association in 1968 titled "Some Wise Men In American Surgery."[5] The title may be misleading as the lecture dealt with how four wise men and two brothers conceived methods to improve surgical treatment, a goal I have pursued for the last two decades along with others in measuring surgical outcomes. Dr. Longmire traced the Halstedian surgical residency in the early part of the twentieth century and then the conception of the Council on Medical Education of the American Medical Association in 1908 by Arthur Dean Bevan, a surgeon and the 51st President of the American Surgical Association. That Council began by implementing the Flexnor Report through the guidance of Dr. Bevan, its first chairman from 1908 to 1928.

In addition to improving surgical training (Halsted) and medical schools (Bevan), Dr. Longmire proceeded to discuss two other ingredients in American surgery that led to improvement in surgical quality performance. These were the inception of the American Board of Surgery in 1935 by William Archibald (and others before him) and the amazing story of the genesis of the Veteran's Administration Hospital system by Paul B. Magnuson with the help of Generals Omar Bradley and Paul Hawley in 1945. In addition to the contributions of these four men that were designed to improve surgical care, Dr. Longmire paused to recognize another event in 1887: the founding of the "clinic in the cornfield" by the Mayo brothers. The purpose of this "group practice" was to improve the surgical care to the patient.

In the last paragraph of Dr. Longmire's presidential lecture, he proclaimed, "Let us therefore strengthen our efforts to insure that *the science and the art of surgery adjust* to the changes in social relationships. Let us strive to blend our science into the pattern of life of the people of this country so that its support does not rest primarily on fear but on understanding." The goal of today's SSAT lecture in 2007 is to give a fresh perspective on how well we can use new technology to further adjust our science and art of surgery.

Surgeons all over the world are focused on the continuing evolution of two aspects of surgery that lead to delivery of high quality care—the training of surgeons and the daily care delivered by surgeons. We have moved beyond core curriculums and CME as illustrated by the new concept that embodies both: maintenance of certification (MOC). Frank Lewis outlined MOC in a table reproduced for simplification (Table 2), where I direct your attention to part 4.[6] The evaluation of performance is where the "rubber meets the road." To adjust any aspect of surgery, we must have one ingredient and that is the outcome. If there are no outcomes, then there is nothing to adjust. In general, there are three items that measure quality: structure, process, and outcome. The hardest to measure and the most important to assess is the outcome.

Table 2 MOC Outline

Four components of MOC[a16]
1. Professional standing
2. Lifelong learning and self-assessment
3. Cognitive expertise
4. Evaluation of performance in practice

[a] To assess physician competencies on a continual basis

The Automatic Pilot of Surgical Care

I next sought guidance from Henry Hebeler, author of a current best-selling self-help book on retirement plans. In this book, "Your Winning Retirement Plan," the author outlined his unique method of success by continually adjusting your assets.[7] Mr. Hebeler has been president of Boeing engineering, Boeing Electronics, Boeing Aerospace Company, and the Corporate Vice President of Planning at Boeing. With this background, it is not surprising that Mr. Hebeler conceived a retirement program based on the automatic pilot of an airplane. The airplane must measure six outcomes and continually adjust its course to reach the desired destination. Modern technology allows the airplane to adjust its course every second, utilizing global positioning satellites (GPS) and a map that considers every square inch of the earth's surface. The six dimensions (outcomes) measured, and then used, by the automatic pilot are roll, pitch, altitude, latitude, and longitude. In an anthropomorphic sense, the airplane knows where it is, and where it is going every second. It does this by constantly adjusting, based on the measurement of outcomes. With new technology, this is the solution to improving surgical care. Let me explain further, but first, we have to examine the history of man's instincts.

Biological Lessons of History

Let us begin with the most often quoted directive by George Santayana, the Spaniard from Harvard, at the beginning of the twentieth century. Professor Santayana said "those who cannot remember the past are condemned to repeat it."[8] With that quote in mind, I sought guidance from the incredible intellects of Will and Ariel Durant, the coauthors of the 11-volume text titled "The Story of Civilization."[9] This work contains 2 million words. The authors received the Pulitzer Prize in 1967 for Volume X, "Rousseau and Revolution." Both authors received a Presidential Medal of Freedom in 1977. Will Durant avoided using the word "history" in the title of this 11-volume set because he wanted the reader to be attracted to the "stories" he had read since the beginning

of recorded time. After 75 years of reading the stories of man and the repetitive patterns followed by these civilizations, the Durants concluded that man has not changed in 20,000 years. His habits may have changed, but his instincts have not changed one iota. Further, they suggested by their observations that man's instincts were ruled by three biological principles. Therefore, these biological laws are the fundamental lessons of history. Will Durant liked to say that "the past is the present unrolled for understanding" and that the present is so brief that one must explain this moment in the light of the past. He also liked to use a similar statement that "the present is the past rolled up for action."[10]

According to the Durants, the three biological principles of history are competition, selection, and reproduction. When delving into the definitions of these principles, a surgeon can begin to glimpse how man's instincts can be addressed to adjust and improve our surgical outcomes. *Competition* between individuals, tribes, or nations is required for each group to strengthen itself. This requires intragroup cooperation, and the Durants noted that cooperation increased with social development. War was the ultimate competition because it promoted cooperation. The next lesson of history is *selection*. Man is born free and unequal, and the Durants observed the fact that nature relishes diversity. Through competition, man promoted this inequality. Free men will multiply their inequality until liberty is sacrificed, e.g., the Bolshevik Revolution. The third biological lesson of history is *reproduction*. Nature also relishes quantity as a prerequisite to quality, e.g., to ensure quantity, nature provides millions of spermatozoa for one ovum. Those groups that can produce more individuals or quantity of themselves ensure survival. Nature controls quantity using pestilence, famine, and war. My own perspective of these biological lessons of history is that, to achieve quality, man's instincts must be balanced by controlling diversity (every randomized controlled trial has a different set of definitions) and combating competition with cooperation (surgeons working together to achieve a standard set of definitions).

Further Guidance

To support my perspective, I next sought further guidance from other SSAT presidents. In his presidential address titled "Gut Reactions," given at the 28th Annual SSAT meeting in Chicago, Ronald K. Tompkins provided an example of competition in medicine.[11] He listed the three Ts as threats to surgical training programs. These were turf, technology, and training. As new technology allowed other specialties to treat traditional surgical diseases, a threat was provided to training programs of gastrointestinal surgeons. For example, common bile duct stones could be treated with endoscopic methods, thereby a "turf war" began. An interventional radiologist could drain an abdominal abscess. The result was gerrymandering, and the turf wars provided a threat to surgical residencies. To combat the competition, President Tompkins turned to intragroup cooperation and established a permanent SSAT Committee on Research and Education. This antidote helped training programs cope with the diversity of new technologies. From our perspective almost 20 years later, we can see that cooperation between specialties has allowed us to improve quality in the delivery of treatments for common bile duct stones and intraabdominal abscesses. A balance occurred.

However, let us not proceed too quickly. Isidore Cohn, SSAT President at the 18th Annual meeting in Toronto, provided the balance to our instinct of competition. In his presidential lecture titled "Gastrointestinal Cancer, Surgical Survey of Abdominal Tragedy," he provided the following warning to our natural overzealousness by defining what he meant by "tragedy."

> There is so much room for improvement in our results, and there is such an ever-present danger that we will delude ourselves, and our patients and our public, by quoting only the results of very large and very selected series, rather than observing the whole picture of that particular disease.... in our zeal to impress people with how well we are doing, we run the very real risk of emphasizing the optimum results obtained under the best conditions.[12]

Long before the emphasis of measuring surgical quality, Professor Cohn warned us to measure the average of the average as well as the best of the best. For instance, the single-surgeon, high-volume database can provide us with the best of what we can do with current technology. However, the reality is where the "rubber meets the road." We must measure what is actually happening, i.e., the average and the range around that average. Isidore Cohn was ahead of his time. He was actually visualizing the current NSQIP (National Surgical Quality Improvement Program) approach to identifying the best and the worst outliers and the concept of the benchmark. Here is where I really needed further guidance, and I found it in the writings of W. Edwards Deming.

Dr. Deming and Continuous Quality Improvement—The Autopilot for Healthcare

Dr. Deming (1900–1993) grew up in small town Powell, Wyoming, and trained to be an electrical engineer, mathematician, and a physicist. His mathematical logic is very attractive to my obsessive-compulsive surgical nature. Deming made a significant contribution to Japan, becoming renowned for producing innovative high-quality products.

Table 3 Dr. Deming's View on Profound Knowledge, Process, and Outcomes

Deming quotes[14]
Knowledge
Information is not knowledge. Let us not confuse the two
You cannot hear what you do not understand
I will continue to work with you if you are willing to share what you have learned
Process (System)
We should work on processes not the outcomes of processes
Management does not know what a system is
A system must be managed, it will not manage itself
When people try to do what they cannot do, they wish to give up
If you destroy the people of a company, you do not have much left
Outcomes
They are just doing their best, but how do they know?
Nobody should try to use data unless he has collected data
A goal without a method is nonsense
Have you ever known a golfer who is happy?

He is regarded as having had more impact upon Japanese business and manufacturing than any other non-Japanese person. One of my favorite Deming quotes is "You can't improve something you can't measure." I tried to find this quote in his writings but could not. The Deming Institute (http://www.deming.org) indicated to me that Dr. Deming probably never said this statement; more likely, his students summarized his teaching with this informative statement. If you agree with this statement, then you are beginning to understand the importance of having a benchmark, a standard for comparison. In his book the "New Economics," Deming outlined for us that only two ingredients are required for progress to achieve quality: leadership and profound knowledge of the subject.[13] The combination of these two ingredients can yield the best benchmarks for an individual operation that provides a mechanism for comparison. If only the authors of randomized controlled trials would avoid diversity by listening to Deming! To improve the quality of evidence-based medicine, we first need a standard set of definitions for these unique benchmarks. I have selected a number of actual quotes from Dr. Deming to illustrate his view on profound knowledge, process, and outcomes (Table 3).[14]

In regards to knowledge, note that Deming is really fostering cooperation between groups that are competing. In regards to process, Deming warns us to avoid regulating diversity to the point where nothing remains. The Durants warned us that balance is required between diversity and its regulation. The proper balance will allow free men to develop their potential abilities, and this will have a survival advantage in the competition of groups. Following their lead, the survival of our healthcare system depends on this balance. In summary of the Deming writings, I especially like the following—"they are just doing their best, but how do they know?"

Pancreatic Cancer—Stop the Bandwagon with Club Web

In the past year, I have been asked to give two named lectures on pancreatic cancer: the Bevan Lecture for the Chicago Surgical Society and the Ben Park lecture at the Weill Medical College of Cornell University. The titles I chose were, respectively, "Continuous Quality Improvement of the Whipple Operation" and "Pancreatic Cancer: A Pathway That Leads to Improved Outcomes." These titles are loaded with quality terms such as "pathway," which is really another name for process, and "improved," which can be determined only after measurement of outcomes. I will use some of the contents of these lectures to illustrate the Deming principles of profound knowledge and leadership.

With a knowledge of pancreatic cancer gleaned from caring for these patients, I would like to take that knowledge and exhibit some leadership by showing you a potential solution within this current medical problem. I will use this knowledge to develop a hypothesis that the concept of "Club Web," a form of new technology promoting cooperation, will allow us to conform to the three biological principles of history yielding a database methodology that will facilitate, simplify, and supplement randomized controlled trials.

What do the terms "continuous quality improvement" or "improved outcomes" really mean when discussing pancreatic cancer treatments? Does that mean safer treatments, improved quality of life, improved survival, or all of the above? I believe most of you would answer "all of the

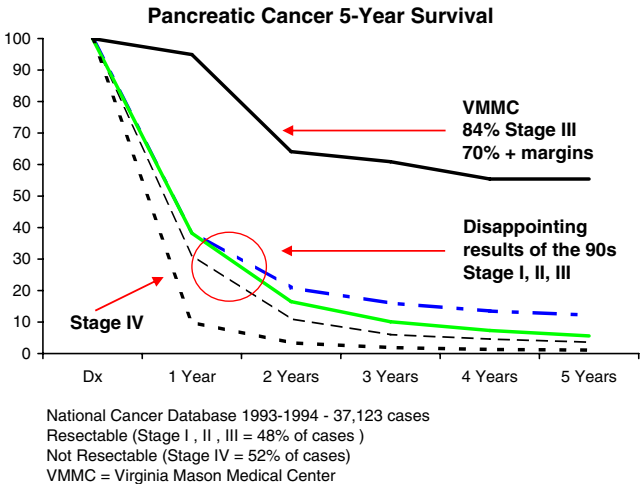

Figure 1 Survival curves supplied by the National Cancer Database of the American College of Surgeons for patients enrolled between 1993 and 1994 are depicted for *Stage I* to *IV*, using the fifth edition of the AJCC Staging Manual. Superimposed on these survival curves is the survival curve of the Virginia Mason Medical Center (*VMMC*) adjuvant study by Picozzi et al. of resected head pancreatic adenocarcinoma between 1995 and 2002. Most of these VMMC patients were Stage III with positive surface margins (not necessarily cut surgical margins). Are the better results a product of selection or the pathway?

above," but the literature would not agree, as it is almost wholly concerned with survival. In Seattle, our survival outcome after resection for Stage III pancreatic cancer of the head of the pancreas was surprisingly better than the survival curves of the National Cancer Database (NCDB, Fig. 1).[15] Was this another medical bandwagon to avoid? Was there something in the pathway (process) that allowed these patients to survive longer? I believe that surgeons have been part of the problem because of the often-mentioned slogan "the only chance for cure is with surgical resection." This statement is misleading because it focuses on surgery alone. This statement should be changed to "the best chance for care *begins* with surgical resection." I firmly believe that surgery alone is not enough to provide the best chance for survival.[16] In all countries, the medical bandwagon for extended lymphadenectomy has proceeded with our ears being deaf to Pogo's statement of the enemy within us. I began to see the enemy within me after three decades of assessing my own results and the medical literature's perspective on pancreatic cancer. Within this information, I had been focused in the wrong place. Being focused on survival results, I totally ignored the enormous diversity between studies for the same outcomes within this literature, like estimated blood loss or use of blood transfusions. What follows is my own reinterpretation of the following four broad categories that led to the belief that "surgery alone is not enough." These categories are:

- A comparison of controlled trials of the classic Whipple versus the pylorus preserving Whipple (PPW)
- The message brought to us by laparoscopic staging of unresectable patients with pancreatic cancer
- The negative influence of blood loss and blood transfusion associated with surgical resection
- The positive influence of adjuvant chemoradiotherapy given in a high-volume center.

The details of each category has been reviewed in detail elsewhere,[16] but the following brief summary might help to illustrate my proposed solution for improving the surgical care of pancreatic cancer patients using the Club Web

After review of the randomized controlled trials comparing the classic Whipple to the PPW, it became apparent that the survival between the two operations was not different. To me, this meant that the focus should not be on the extent of the resection but on other factors that might positively or negatively influence survival. This concept was additionally supported by the randomized controlled trials that found no difference between extended lymphadenectomy versus standard resection.

Next, consider the common group of patients that have locally extending tumors by computed tomography (CT) scan. The tumor is involving the major vessels, and the surgeon deems the patient unresectable by CT. Our results in patients with locally extending pancreatic cancers that were not resectable by CT showed that staging laparoscopy and peritoneal lavage for cytology upstaged the CT staging from Stage III to IV in one quarter of the patients with head cancers and one half of the patients with body/tail tumors.[17] Pancreatic cancer was clearly a systemic disease that was only partially treated by the local approach of surgical resection. As I tell my patients, the best results will be obtained when all of the tumor is treated at the same time. Surgery alone is not enough. Something else is needed.

The negative effect on survival by blood transfusion has been well documented in the following cancers—colorectal cancer, breast, prostate, cervix, lung, pancreas, and kidney cancer. The best illustration, based on the largest number of cases reported for pancreatic cancer, comes from the Johns Hopkins group.[18] Here, a high-volume, multisurgeon single-center report of 616 cases of pancreatic cancer resection showed a negative influence on survival with the use of blood transfusion or an estimated blood loss of greater than 750 ml. This effect is even more striking when observing the survival after pancreaticoduodenectomy for the less-aggressive ampullary cancer.[19] This report from Korea showed that blood transfusion in the preoperative or intraoperative period had its negative effect more than in the postoperative period. Because blood loss is a function of experience, this appears to be an opportunity and a technical challenge where surgeons could improve through cooperative effort and knowledge of why it is important.

There are other items within the pathway of treatment that can allow us to improve. High-volume centers provide surgical experience and proven operative technique. Yes, the hospital-based experience also contributes. However, let us not focus just on increased surgical experience. How about high volume chemotherapy and radiotherapy? Is this why centers in Europe do not see improved results with the

Table 4 Factors Influencing Survivorship For Pancreatic Cancer

Survival after pancreaticoduodenectomy	
Negative prognostic:	Positive prognostic factors:
Large tumor size	Balanced resection
Positive lymph nodes	Exocrine/endocrine/GI
Margin status—R1, R2	Chemo±radiation
Postop complications	High volume center
Blood transfusion	↑ Surgeon experience
Pre/postop nutrition	↓ Complications
	↓ Transfusion
	Critical pathway
	✓Ticket home
	Chemo infusion units
	✓Supportive pathway
	Radiotherapy expertise

Table 5 Comparison Of Estimated Blood Loss (EBL) Between The Classic Whipple Versus The PPW

EBL median				
	Std W (ml)	Number	PPW (ml)	Number
DiCarlo et al.[20] case series	X	–	X	–
Lin and Lin[21] RCT	687	15	451	16
Seiler et al.[22] RCT	2,096	40	1,453[a]	37
Yeo et al.[24] RCT (86% PPW)	700	148	600	146
Tran et al.[23] RCT	2,000	83	2,000	87

X not reported
EBL estimated blood loss, *Std W* standard Whipple, *PPW* pylorus preserving Whipple, *RCT* randomized controlled trial
[a] Significantly different Std W versus PPW

addition of adjuvant radiotherapy? In addition to experience, the high-volume center is more likely to use an efficacious treatment protocol. In regards to chemoradiotherapy, treating to toxicity will not be associated with increased survival, unless the chemotherapy and/or radiotherapy has increased efficacy. It is possible that the results shown in Fig. 1 could be exhibited only in a high-volume center that has supportive pathways for surgery, chemotherapy, and radiotherapy. Yes, the surgeon's responsibility is to provide a healthy patient in a timely fashion for the adjuvant therapy 6 weeks after resection. However, why not also include under this scrutiny the other modalities of treatment? Because of this reasoning, we believe adjuvant therapy should be the focus of upcoming controlled trials but only in centers experienced in surgery and adjuvant treatments. Ideally, the participating centers will have pathways proven to minimize the negative factors and maximize the positive influences on survivorship for pancreatic cancer as summarized for you in Table 4.

Evidence-Based Medicine, the Whipple, and the Antidote to Diversity

For improvement, the logic and the outcomes favor an experienced group that uses a maximized pathway. Next, we must also have a set of standardized definitions for future trials so that RCTs can be compared to each other. In this year's SSAT postgraduate course, we examined the available evidence-based literature, and how it has assisted the decision-making for the treatment of pancreatic cancer. This review observed four major problems with controlled trials. First and second, we observed items of quantity. There are not many RCTs, and second, they are underpowered. Additionally, we observed issues of low quality. Like all of the literature, there are no standardized definitions. Considering the first three items, it is not surprising that the fourth major problem was that comparison of trials showed diverse results and conclusions. Nature loves diversity, and this is certainly supported in our evidence-based medicine literature. Let us examine the diverse outcomes of five major reports that compare estimated blood loss (EBL) between the classic Whipple versus the PPW (Table 5).[20–24] First, one group did not measure blood loss. Second, when EBL was measured, we see a large diversity between reports but no difference between operations. Could this be due to a variety of definitions for estimated blood loss? Could blood loss of two liters be accurate? If accurate, is this not more than the 750 ml that the Hopkins group observed to be associated with decreased survival? Is this EBL not excessive? How could we use technology to standardize definitions, allowing comparison using the same definition, and therefore imparting an accurate assessment or knowledge that we are the best or the worst?

The answer might be Club Web. A Club Web-based registry is comprised by a group of investigators who belong to a "Club" as a result of an intense interest in a disease, operation, or organ. The club members already know each other on a global basis. The love of a similar area of medicine

Table 6 Ranking Of Databases By Study Reliability

Potential sources by reliability				
Databases	Surgeon variable	Number of Pts	Data points	Std data structure
Single surgeon, high volume	No	↔	↑	No
Single center, high volume	Yes	↑	↑	No
Multicenter RCT	Yes+	↑↑	↑	Yes[a]
Large administrative DB	Yes++	↑↑↑	↓	NA
Club web-based registries	Yes	↑↑	↑	Yes
Individual case log	No	↔	↑	Yes

RCT Randomized controlled trial
[a] Not between RCTs

is a bond that generates trust and cooperation within the group. The opportunity to work together becomes possible. The Web allows the cooperation. The cooperation generates an experimental design based on the same definitions. What occurs is the potential to collect a large number of cases, more than could ever be generated by a high-volume single center study. Club Web means that the data is gathered through a password-protected web interface and stored in a secure server. Anonymity for both patient and surgeon is assured by the third party data manager. A side benefit is the opportunity to compare one's own results to the rest of the database, anonymously, and at any time, with the calculations of the "24/7" internet.

Taking the lead from the previous table of RCTs, other types of databases are ranked in Table 6 by study reliability, i.e., how meaningful are the conclusions of studies from each of these databases? In this table, the influence on reliability is compared in regards to surgeon variable, the number of patients that can power the database, the number of data points that can be reliably measured, and whether a standard database structure can be used by all investigators. The Club Web-based registry has similar advantages to a multicenter RCT because of the large number of patients that can be gathered, the large number of data points that can be measured, and a standard database structure that can be implemented. Yes, surgeon variables are present in both RCTs and the Club Web-based registry, but the RCT has two disadvantages, besides being difficult and expensive to accomplish. RCTs are not comparable to each other because each has a different set of definitions for their database structure. In contrast, the Club Web-based registry will have the same set of definitions for all participants. The RCT does not allow for anonymous self-comparison between surgeons and/or institutions.

As alluded to earlier in this lecture, the single-surgeon, high-volume database is a jewel that shows the best of what we can be, and it delivers a large number of data points without the surgeon variable. However, the literature (as well as program committees) does not value these single-surgeon databases very highly. Of the 40 oral presentations given during this current SSAT annual meeting, only two are in this category, and only one is the Club Web variety of study. All three of these presentations follow this lecture, and I hope you will observe them to see how the literature can be improved from the information in these single-surgeon "best of the best" and Club Web high volume "average of the average" databases.

The single-center, high-volume database is more popular and is a tremendous reservoir of information. In the SSAT scientific sessions, 23 of the 40 oral presentations will be from this type of database. It may not have a standard database structure, and it does have surgeon variables, but these variables are minimized because these surgeons operate within the same institution utilizing the same critical pathway. To a program committee, these studies are very popular, second only to controlled trials. However, once again, single-center, high-volume databases cannot be compared well to other single-center studies because of a lack of standardized definitions and database structures. They have the same weakness that controlled trials have—not comparable because of different definitions. I would propose that even the sophisticated statistical science of metaanalysis cannot overcome the diversity of definitions between multicenter randomized controlled trials.

If the Club Web-based registry has several advantages over our other commonly used databases, why is it not more popular? First, the technology has only recently been available. Second, we are just waiting for the leadership, influence, and profound knowledge to utilize the technology. The third reason is the dependence on cooperation (the antidote is leadership). The web-based registry depends on cooperation to decide on the definitions and data structure beforehand. Cooperation is an antidote to diversity and represents the pathway to standardize definitions and database structures. The Durants have observed a lack of cooperation for 20,000 years in the story of civilization; it is no wonder that we see this today among surgeons. A caveat here—Will and Ariel Durant cautioned against too much regulation that might stifle innovation, such as the Health Insurance Portability and Accountability Act, HIPAA. Therefore, the creative potential of our surgical science might work best among people who trust each other. That is why I choose to call this type of database "Club Web." The friendship within the Club extends around the world, and with the new technology of the "Web," some of these surgeons can become united to control diversity and optimize creativity. In addition, only the web-based registry and the individual case log allow comparison in an anonymous, self-assessment fashion. There are two of these individual databases that are free to surgeons, if you are a member of the American College of Surgeons or the Society of Gastrointestinal Endoscopic Surgeons. The attraction for voluntary participation in these programs is that they will soon be linked to practice based-learning and improvement, as will be illustrated later this morning by our Guest Orator, Ajit Sachdeva, Director of the Division of Education, American College of Surgeons. An example of Club Web is the international registry of outcomes after the Whipple operation (http://www.pancreaticdata.org).

The web-based registry and the individual case log, I believe, will be an avenue that will help adjust the art and the science of surgery as envisioned by Dr. Longmire almost 40 years ago. In April of 2007, anticipating the future demand for cooperative registries, the Agency for Healthcare Quality and Research (AHRQ) published on their website "Registries for Evaluating Patient Outcomes: A User's Guide" (http://effectivehealthcare.ahrq.gov/repFiles/PatOutExecSumm.pdf).

Summary

There are three biological lessons of history. First, *competition* allows groups to strengthen themselves with cooperation. This increases with social development, such as technology and the internet. Second is *selection* among a vast array of different individuals or groups. Nature relishes diversity. The antidote for diversity is to standardize the definitions within our experimental designs. A group that allows potential abilities to develop and function will have a survival advantage—such as those that exhibit profound knowledge of the subject and leadership. Third is *reproduction*. Nature loves quantity and gives it a priority over quality. To obtain quality rather than just quantity, we need technology, leadership, profound knowledge, and standardized definitions. The Durant concept of competition impedes our efforts for quality improvement in the complex challenges of modern healthcare. Cooperation is the antidote to the bandwagon effect. Trust is required. The global technology of the Web may allow a disease or an operation to be benchmarked in a more streamlined fashion by using a large number of cases and standardized definitions, i.e., the Club Web. A focus group that trusts each other could succeed but must have the Deming qualities of profound knowledge and leadership. Only then, does comparison become possible. Only then, can the automatic pilot of surgical healthcare begin to function.

Measure to compare. The rest is history.

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J Gastrointest Surg (2007) 11:1242–1252
DOI 10.1007/s11605-007-0245-5

Trends and Disparities in Regionalization of Pancreatic Resection

Taylor S. Riall · Karl A. Eschbach · Courtney M. Townsend Jr. · William H. Nealon · Jean L. Freeman · James S. Goodwin

Received: 10 May 2007 / Accepted: 11 July 2007 / Published online: 13 August 2007

Abstract
Background The current recommendation is that pancreatic resections be performed at hospitals doing >10 pancreatic resections annually.
Objective To evaluate the extent of regionalization of pancreatic resection and the factors predicting resection at high-volume centers (>10 cases/year) in Texas.
Methods Using the Texas Hospital Inpatient Discharge Public Use Data File, we evaluated trends in the percentage of patients undergoing pancreatic resection at high-volume centers (>10 cases/year) from 1999 to 2004 and determined the factors that independently predicted resection at high-volume centers.
Results A total of 3,189 pancreatic resections were performed in the state of Texas. The unadjusted in-hospital mortality was higher at low-volume centers (7.4%) compared to high-volume centers (3.0%). Patients resected at high-volume centers increased from 54.5% in 1999 to 63.3% in 2004 (P=0.0004). This was the result of a decrease in resections performed at centers doing less than five resections/year (35.5% to 26.0%). In a multivariate analysis, patients who were >75 (OR=0.51), female (OR=0.86), Hispanic (OR=0.58), having emergent surgery (OR=0.39), diagnosed with periampullary cancer (OR=0.68), and living >75 mi from a high-volume center (OR=0.93 per 10-mi increase in distance, P<0.05 for all OR) were less likely to be resected at high-volume centers. The odds of being resected at a high-volume center increased 6% per year.
Conclusions Whereas regionalization of pancreatic resection at high-volume centers in the state of Texas has improved slightly over time, 37% of patients continue to undergo pancreatic resection at low-volume centers, with more than 25% occurring at centers doing less than five per year. There are obvious demographic disparities in the regionalization of care, but additional unmeasured barriers need to be identified.

Keywords Pancreatic resection · Volume–outcome relationship · Regionalization of care

Introduction

As for many complex surgical procedures, a strong volume–outcome relationship has been demonstrated in patients undergoing pancreatic resection. Whereas the definition of "high-volume" has varied, multiple studies have shown that surgical mortality, length of stay, hospital charges/costs, and long-term mortality are all decreased when such procedures are performed at high-volume centers[1–11] or by high-volume surgeons.[2,12,13]

Because of the strongly observed volume–outcome relationship, pancreatic resection is often evaluated despite it being a relatively uncommon surgical procedure. As pointed out by Birkmeyer,[14] the heavy scrutinization of pancreatic resection is also partly attributable to the high baseline risks associated with the procedure and its usefulness as a prototype for other complex surgical procedures.

Work supported by the Society of University Surgeons_Wyeth Clinical Scholars Award and the Dennis W. Jahnigen Career Development Scholars Award.

T. S. Riall (✉) · C. M. Townsend Jr. · W. H. Nealon
Department of Surgery, University of Texas Medical Branch,
301 University Boulevard,
Galveston, TX 77555-0542, USA
e-mail: tsriall@utmb.edu

K. A. Eschbach · J. L. Freeman · J. S. Goodwin
Department of Internal Medicine,
The University of Texas Medical Branch,
Galveston, TX, USA

As health care reform becomes an increasingly important issue, regionalization of care to high-volume centers specializing in specific complex procedures will be a topic of debate. Regionalization is defined as the delivery of care at a limited number of selected provider sites. Based on the volume-outcomes data for pancreatic resection,[1–10] regionalization has been recommended for this procedure. The Leapfrog group, which is a coalition of greater than 150 large public and private health care purchasers, is making efforts to concentrate selected surgical procedures in centers that have the best results.[15] In January 2004, pancreatic resection was added to Leapfrog group's list of procedures targeted for evidence-based referral. For pancreatic resection, the Leapfrog group's standard for evidence-based referral is strictly based on the process measure of annual volume of procedures performed. They recommend a minimum volume of greater than 10 cases per year.

In the studies evaluating the volume–outcome relationship for pancreatic resection, the percentage of patients resected at low-volume centers ranges from 24% to 77%.[1–4,6] As the data supports regionalization and a large percentage of patients are still being resected at low-volume centers, we sought to evaluate trends and disparities in regionalization of pancreatic resection subsequent to the introduction of the concept in the mid-1990s.

This study uses the Texas Hospital Inpatient Discharge database to evaluate temporal trends in the percentage of patients undergoing pancreatic resection at high-volume hospitals throughout the state over the time period 1999 through 2004. Texas was chosen as it serves as a good model for regionalization throughout the United States. Texas has the largest rural population in the United States,[16] the highest percentage of people without health insurance,[17] and no ethnic majority. One-fifth of the state's population lives in counties where the whole county has been designated by the U.S. Health Resources and Services Administration as medically underserved.[18] As a result, patients often travel large distances to medical centers. We confirmed the volume–outcome relationship for pancreatic resection in Texas by comparing the in-hospital mortality, perioperative lengths of stay, and total charges between low and high-volume hospitals. In addition, we evaluated geographic patterns of referral and regionalization to high-volume centers and performed a multivariate analysis to determine the factors that predict resection at high-volume centers.

Methods

Data Source

Data from the Texas Hospital Inpatient Discharge Public Use Data File (PUDF) for the years 1999 through 2004 are used for this study. The data are collected by the Texas Department of State Health Services, Texas State Health Care Information Center (THCIC), Center for Health Statistics to develop administrative reports on the use and quality of hospital care in Texas.[19] The database includes all discharge records for 466 participating non-federal hospitals in Texas. It has 205 data fields in a base data file and 13 data fields in a detailed charges file. The data include patient demographics, hospital information, lengths of stay, ICD-9 diagnosis codes, ICD-9 procedure codes, hospital day of procedure, hospital charges, payer information, and discharge status.

Study Population/Patient Characteristics

For the years 1999 through 2004, all discharges with a primary procedure code for pancreatic resection (ICD-9 procedure codes, 52.6, 52.7, 52.51, 52.52, 52.53, and 52.59; see Table 1) were selected. ICD-9 procedure codes 52.6, 52.7, 52.51, 52.53, and 52.59 were considered pancreatic head resections. ICD-9 procedure code 52.52 was considered distal pancreatic resection and 52.59 was considered pancreatectomy, not otherwise specified. Pancreatic resection for any reason including periampullary adenocarcinoma, chronic pancreatitis, and other benign and malignant diseases of the pancreas were included. Patients were classified as having periampullary adenocarcinoma (ICD-9 diagnosis codes 152.0–157.9, see Table 1) or

Table 1 ICD-9 Procedure and Diagnosis Codes

Code	Definition
ICD-9 Procedure code	
52.6	Pancreatectomy (total) with synchronous duodenectomy
52.7	Pancreaticoduodenectomy, radical (one-stage; two-stage)
52.51	Proximal pancreatectomy (head; with part of body; with synchronous duodenectomy)
52.52	Distal pancreatectomy (tail; with part of body)
52.53	Radical / subtotal pancreatectomy
52.59	Pancreatectomy / Pancreaticoduodenectomy partial NEC
ICD-9 Diagnosis code	
152.0	Malignant neoplasm of the duodenum
156.0	Malignant neoplasm of gallbladder
156.1	Malignant neoplasm of extrahepatic bile ducts
156.2	Malignant neoplasm of ampulla of Vater
156.8	Malignant neoplasm other specified sites of gallbladder and extrahepatic bile ducts
157.0	Malignant neoplasm of head of pancreas
157.1	Malignant neoplasm of body of pancreas
157.2	Malignant neoplasm of tail of pancreas
157.3	Malignant neoplasm of pancreatic duct
157.8	Malignant neoplasm of other specified sites of pancreas
157.9	Malignant neoplasm of pancreas, part unspecified

having other pancreatic diseases (all other ICD-9 diagnosis codes).

To evaluate trends in regionalization in Texas, patients living out of state (or country) were excluded from the analysis. In addition, patients less than 18 years of age were excluded from the analysis. Age was defined as age groups based on the available data: 18–44 years, 45–54 years, 55–64 years, 65–74 years, and >75 years. These inclusion and exclusion criteria provided a cohort of 3,189 patients who underwent pancreatic resection in Texas between 1999 and 2004, inclusive.

For all patients with zip code data available (n=3,161), we calculated the following distances: 1) the distance to the hospital at which the surgery was performed, 2) the distance to the nearest high-volume hospital, and 3) the distance to the nearest low-volume hospital.

Independent variables examined in the analysis included patient age group, gender, race/ethnicity (Hispanic, non-Hispanic white, non-Hispanic black, and other), diagnosis (periampullary cancer or other), procedure (distal pancreatectomy, pancreatic head resection, vs. other), year of diagnosis, admission type (emergent or elective), insurance status (uninsured, Medicare/Medicaid, other insurance), and distance to nearest high-volume facility. To control for patients' comorbidities we used a variable included in the discharge data public use file called "Severity of Illness." This variable is based on the all-patient refined diagnosis-related grouper (DRG) and considers comorbidity, age, and certain procedures to calculate the "severity of illness" (on a 0–4 scale), with 4 being the most severe. As only two patients had illness severity scores of 0, these were combined with the scores of 1 for the purpose of the analysis.

Hospital Volume/Hospital Characteristics

A Texas hospital was included in the analysis if at least one pancreatic resection was performed there in the 6-year time period. Pancreatic resections performed on patients from out of the state or country were included when determining a hospital's volume status. Hospitals were then classified into high-volume and low-volume providers based on the 2004 Leapfrog criteria,[14] greater than 10 cases per year.

The number of pancreatic resections performed by each hospital each year was examined. The criteria to qualify as a high-volume provider were a minimum volume of more than 10 pancreatic resections per year for 3 of the 6 years of the study and an average volume during the 6-year period of >10 pancreatic resections. Only two hospitals did greater than 10 cases per year for 3 years, but did not meet the average volume requirements to be considered high-volume hospitals. Hospital volume was determined before removing non-Texas residents.

To provide more detail for some analyses on the distribution of pancreatic resections throughout the state, the volume criteria were further subdivided into hospitals that performed less than five resections per year, 5–10 resections per year, 11–19 resections per year, and >20 resections per year. Besides resection at a high-volume center, other outcome variables of interest included in-hospital mortality, the lengths of hospital stay (total and postoperative), and the total hospital charges. Given the nature of the dataset, 30-day mortality could not be determined.

Statistical Analysis

SAS Statistical Software, version 9.1.3 (Cary, NC) was used for all statistical analyses. The percentage of patients undergoing surgical resection at high-volume hospitals each year was calculated. Trends were evaluated for statistical significance using the Cochran-Armitage test for trend.

The patient characteristics, hospital characteristics, and outcome variables were compared between high- and low-volume providers. The primary outcome variable of interest was resection at a high-volume center. Bivariate analyses were used to determine which independent variables were associated with resection at a high-volume center. Significance was accepted at the $p<0.05$ level. All means are expressed as mean+standard deviation and all proportions are expressed as percentages. Chi-square analysis was used to compare proportions for all categorical data and t tests were used to compare all continuous variables between the high- and low-volume providers.

A logistic regression model was used to estimate the odds ratio for receipt of surgical resection at a high-volume center. Year and distance to the nearest high-volume center were modeled as a continuous variable. Patient age group, gender, race, diagnosis, illness severity, admission status, insurance status, type of resection, and distance to a high-volume center were used as covariates to determine the independent predictors of surgical resection at a high-volume center. Categorical variables were modeled as a series of binary variables referenced to a single group specified for each variable.

Results

From January 1999 through December 2004, 3,189 pancreatic resections were performed on Texas residents at 157 hospitals throughout Texas. The number of resections per year increased from 409 resections in 1999 to 624 resections in 2004 (Fig. 1). A total of 1,254 (87.8%) resections were performed at hospitals that were members of the Council of Teaching Hospitals.

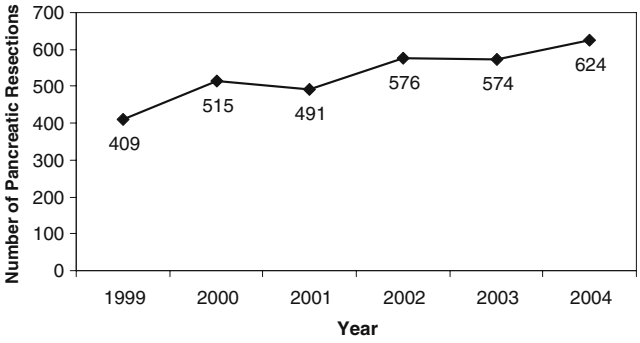

Figure 1 The number of pancreatic resections per year of the study in Texas, 1999–2004.

Overall Cohort

The patient demographic factors, procedure type, diagnosis, illness severity, and mortality risk are summarized in Table 2. Patients aged 55–64 accounted for 24.9% of patients undergoing pancreatic resection and patients aged 65–74 accounted for 24.4%. The gender distribution was nearly equal, with 1,476 male patients (48.4%). The majority of patients (62.9%) were non-Hispanic White. Hispanic patients comprised 18.9% of the cohort and non-Hispanic Black patients accounted for 11.2% of the cohort. Some 228 (7.2%) patients undergoing pancreatic resection were uninsured. Of the insured patients, 1,366 (42.9%) were insured by Medicare/Medicaid and 1,589 (49.9%) had other types of insurance including private insurance and health maintenance organization (HMO) coverage. Based on the APR-DRG Grouper, version 20, patients were assigned "severity of illness" scores on a 1–4 scale. The distribution of "severity of illness" scores is detailed in Table 2.

The most common reason for pancreatic resection was periampullary adenocarcinoma, in 57.8% of patients, followed by chronic pancreatitis in 13.6%, other benign disease processes in 14.5%, and other malignant disease processes in 14.1%. 71.6% of resections were performed electively. Distal pancreatectomy was performed in 24.5% of patients (ICD-9 Procedure Code 52.52), whereas pancreaticoduodenectomy was performed in the remaining 75.5% (ICD-9 Procedure Codes 52.51, 52.53, 52.59, 52.6, 52.7; see Tables 1 and 2).

Trends in Resection at High-Volume Centers

Of the 3,189 pancreatic resections, 1,849 (58.0%) were performed at the 14 high-volume centers in Texas, as defined by the leapfrog criteria of greater than 10 resections per year, and 1,340 (42.0%) were performed at 143 hospitals performing <10 pancreatic resections per year. As shown in Fig. 2, 994 cases were (31.2%) performed at centers doing fewer than five resections per year, 346 (10.9%) were performed at centers doing 5–10 resections per year, 818 (25.6%) were performed at centers doing 11–19 resections per year, and 1,031 (32.3%) were performed at centers doing 20 or more resections per year.

Table 2 Overall Cohort (n=3,189)

	N	Percent (%)
Age group		
18–44 years	526	17.6
45–54 years	570	17.9
55–64 years	793	24.9
65–74 years	780	24.4
>74 years	484	15.2
Gender		
Male	1,476	48.4
Female	1,572	51.6
Race/Ethnicity		
Non-Hispanic White	2,006	62.9
Non-Hispanic Black	356	11.2
Hispanic	602	18.9
Other	225	7.0
Insurance type		
Medicare/Medicaid	1,366	42.9
Other insurance	1,589	49.9
Uninsured	228	7.2
Severity of illness		
Score=1	159	5.0
Score=2	553	17.3
Score=3	1,367	42.9
Score=4	1,110	34.8
Diagnosis		
Periampullary adenocarcinoma	1,841	57.7
Chronic pancreatitis	435	13.6
Other malignant disease	450	14.1
Other benign disease	463	14.5
Admission type		
Elective	1,911	71.6
Emergent	757	28.4
Type of operation		
Pancreaticoduodenectomy	2,189	68.6
Distal pancreatectomy	780	24.5
Pancreatectomy not otherwise specified	220	6.9

From 1999 to 2004, the percentage of patients resected at high-volume centers increased from 54.5% to 63.3%

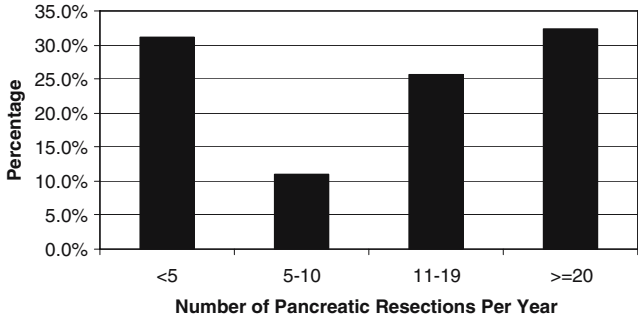

Figure 2 The percentage of pancreatic resections performed at centers in Texas performing <5, 5–10, 11–19, and >20 pancreatic resections per year.

(Fig. 3, P=0.0004 for trend). Much of this increase was accounted for by decreased volume at the centers performing fewer than five pancreatic resections per year (very-low-volume centers). In 1999, 35.5% of resections in Texas were performed at centers doing fewer than five resections per year, whereas in 2004 only 26.0% were done in very-low-volume centers.

Comparison of High- and Low-Volume Centers

As shown in previous studies evaluating volume–outcome relationships after pancreaticoduodenectomy,[1–10] high-volume centers had lower unadjusted mortality rates (3.3% vs. 7.4%, P<0.0001), shorter lengths of hospital stay (median 12 vs. 14 days, P=0.0004), and lower total hospital charges (median $55,000 vs. $67,000, P=0.008, Table 3). Over the study period, the overall crude mortality rates after pancreatic resection decreased from 6.6% in 1999 to 3.9% in 2004 (P=0.01). The mortality at low-volume hospitals did not change, whereas the mortality at high-volume hospitals decreased over the same time period from 6.7% to 2.3% (P=0.003).

Patients undergoing resection at high-volume centers were more likely to be male (50.3 vs. 45.9%, P=0.02), non-Hispanic white (66.7% vs. 57.6%, P<0.0001), have non-federal insurance (52.4% vs. 46.5%, P<0.0001), undergo pancreatic head resection (71.2% vs. 64.1%, P<0.0001), and to be undergoing elective procedures (79.5% vs. 60.6%, P<0.0001, Table 3). They were less likely to have periampullary cancer (56.2% vs. 59.9%, P=0.039). Patients resected at high-volume hospitals had higher "severity of illness" scores (P=0.0012).

Distance to High-Volume Hospital and Hospital of Surgical Procedure

Data on the distance from a patient's home zip code to: 1) the hospital performing their surgery and 2) the nearest high-volume hospital were available on 3,161 of the 3,189 patients. Of the patients, 24.9% lived within 10 mi from a high-volume provider; 50.1% lived within 25 mi of a high-volume provider; 61.5% lived within 50 mi of a high-volume provider; and 73.7% lived within 75 mi of a high-volume provider. For the overall cohort, patients traveled a mean distance of 41.9+70.7 mi (median=14.1 mi, range 0.3–678 mi) to the hospital where their surgery was performed. The mean distance to the nearest high-volume provider was 67.3+94.9 mi (median=26.0 mi) and to the nearest low-volume provider was 9.9+13.1 mi (median=4.5 mi). Of the patients resected at low-volume centers, 19% traveled a distance further than the distance to the nearest high-volume center to have their surgery performed.

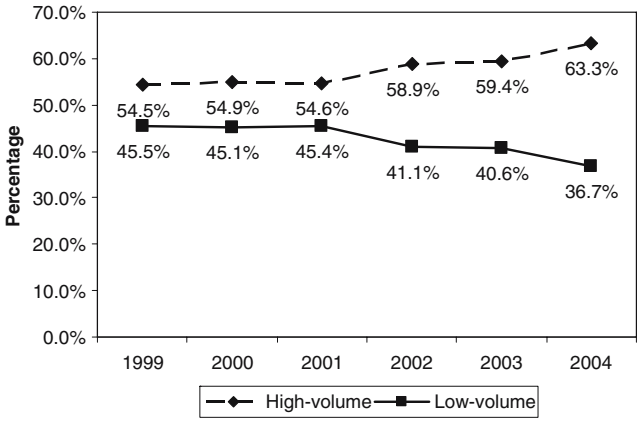

Figure 3 Trends in the percentage of patients undergoing resection at high-volume centers, shown by the *dotted line with diamonds*, and low-volume centers, shown by the *solid line with squares*.

Table 3 Bivariate Comparison of Low- and High-Volume Centers[a]

	Low Volume %	High Volume %	P value
Unadjusted in-hospital mortality	7.4%	3.3%	<0.0001
Total length of stay (median)	14 days	12 days	0.0004
Total hospital charges	$67,000	$55,000	0.008
Age group			
18–44 years	16.9%	18.2%	0.006
45–54 years	16.4%	18.9%	
55–64 years	24.4%	25.2%	
65–74 years	24.5%	24.5%	
>74 years	17.8%	13.2%	
Percent male	45.8%	50.3%	0.02
Race/Ethnicity			
Non-Hispanic white	57.6%	66.8%	<0.0001
Non-Hispanic black	10.4%	11.7%	
Hispanic	26.6%	13.3%	
Other	5.4%	8.2%	
Insurance type			
Medicare/Medicaid	45.4%	41.1%	0.0024
Other insurance	46.5%	52.4%	
Uninsured	8.1%	6.5%	
Severity of illness			
Score=1	6.3%	4.1%	0.0012
Score=2	17.5%	17.2%	
Score=3	39.6%	45.3%	
Score=4	36.6%	34.4%	
Diagnosis			
Periampullary adenocarcinoma	59.8%	56.2%	0.039
Other	40.2%	43.8%	
Elective admission	60.6%	79.5%	<0.0001
Type of operation			
Pancreaticoduodenectomy	64.1%	71.9%	<0.0001
Distal pancreatectomy	28.8%	21.3%	
Pancreatectomy not otherwise specified	7.1%	6.8%	

[a] High-volume defined as >10 cases/year

Figure 4 divides the patient population into 20 equal-size groups based on progressive distance from the nearest high-volume hospital, then graphs the percentage of patients undergoing resection at a high-volume center for each group.

To further explore factors affecting utilization of high-volume hospitals, we stratified patients by whether they lived within 75 mi of a high-volume hospital. When evaluating the 73.7% of patients who lived within 75 mi (n=2,329) of a high-volume hospital, 34% (n=792) were resected by at a low-volume hospital and 66% (n=1,537) were resected at a high-volume center. Patients resected at a high-volume center traveled further than patients resected at low-volume centers (mean 32.9 vs. 13.1 mi, median 17.8 vs. 7.4 mi, P<0.0001). In addition, patients resected at high-volume centers often traveled to a high-volume center that was not the closest to their home, with a mean distance of 32.9+42.6 mi to the hospital performing the surgery and mean distance of 22.7+20.3 mi to the nearest high-volume hospital.

Of the 832 patients who lived more than 75 mi from a high-volume center, they traveled a mean distance of 86.1+111.2 mi (median=37.3 mi) to get to the hospital performing their surgery. The mean distance to a high-volume hospital was 191.7+109.8 mi (median=145.6 mi). Only 36% were resected at high-volume hospitals. Those resected at low-volume hospitals traveled 27.8+54.8 mi (median=8.6 mi) to have their surgery, whereas those resected at high-volume hospitals traveled 188.4+111.2 mi (median=150.6). On average, patients resected at high-volume centers lived closer to the nearest high-volume center that those resected at low-volume centers (149.2 mi vs. 215.9 mi, P<0.0001).

Figure 5 is a thematic map of Texas showing the location of the 14 high-volume hospitals and the percentage of resections that are at high-volume hospitals by hospital service area. We noticed several patterns in the distance data (refer to map in Fig. 5). The 14 high-volume providers are located in six of Texas's 254 counties: Dallas, Tarrant, Bell, Harris, Bexar, and Galveston. These counties encompass the major cities of Dallas (Dallas County), Fort-Worth (Tarrant County), Temple (Bell County), Houston (Harris County), San Antonio (Bexar County), and Galveston (Galveston County). Patients in these counties are more likely to go to a high-volume provider (66.2%) than those in other counties (52.1%, P<0.0001). However, within these counties, high-volume centers have varying levels of monopoly. Of patients living in Galveston county, 96.7% were resected at high-volume centers, followed by 77.4% in Bell county, 71.0% in Dallas county, 66.3% in Harris County, 57.4% in Bexar County, and 13.6% in Tarrant county (P<0.0001).

Multivariate Logistic Regression Analysis

We fit a logistic regression model to predict resection at a high-volume center. An odds ratio (OR) of greater than 1 implies increased likelihood of being resected at a high-volume center. The final model is shown in Table 4. As a patient's age group increased, the likelihood of being resected at a high-volume center decreased. Likewise, Hispanic patients, patients with periampullary cancer, patients undergoing emergent procedures, and patient's undergoing distal pancreatic resections (compared to head of pancreas resections) were less likely to be resected at high-volume centers. The distance to the nearest high-

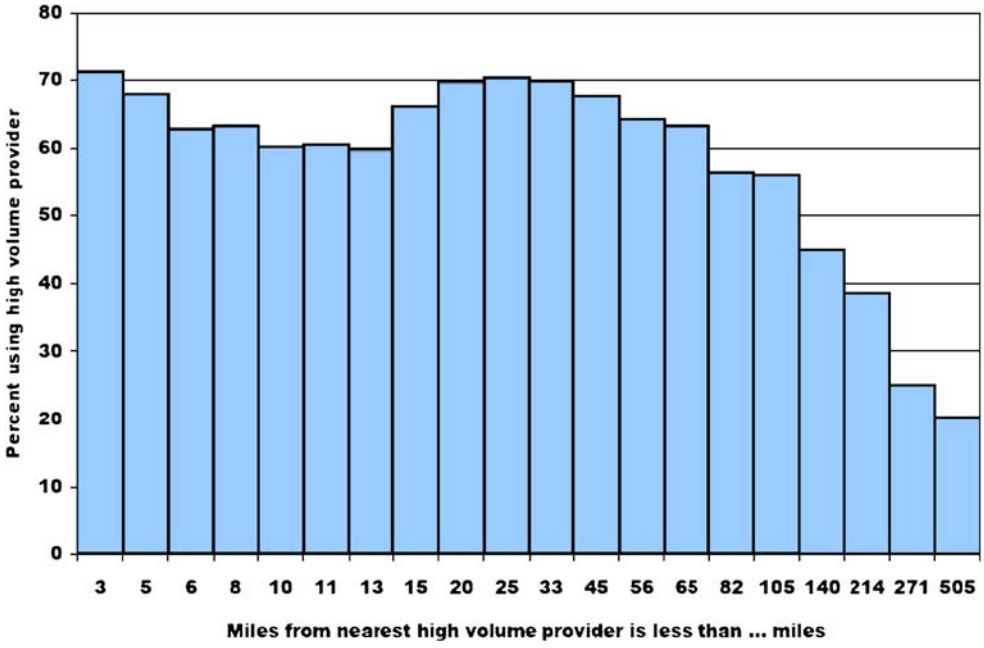

Figure 4 Patients were divided into 20 equal-sized groups based on distance to the nearest high-volume provider. This graph shows the percentage of patients using a high-volume provider (x-axis) based on their distance in miles from the nearest high-volume provider (y-axis). There is a dip and then rise in the use of high-volume providers based on distance.

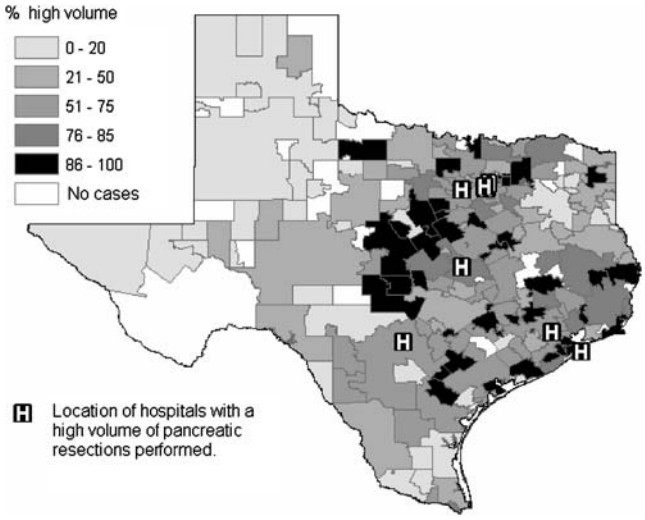

Figure 5 A thematic map of Texas showing the location of the 14 high-volume hospitals and the percentage of resections that are at high-volume hospitals by hospital service area. An *H* on the map denotes each high-volume provider. Dallas, Tarrant, Bell, Harris, Bexar, and Galveston counties, the six counties containing the 14 high-volume centers are labeled.

volume center was a significant predictor of resection at a high-volume center. Compared to patients living within 10 mi of a high-volume center, the odds of resection at a high-volume center decreased by 7% with each 10-mi increase in distance. In the final model, the year of surgery was an independent predictor of resection at a high-volume center, with a 6% increase in likelihood per advancing year. Patients with increased illness severity were more likely to be resected at high-volume centers, although the ORs are not significant for each illness severity category. Insurance status was not a significant predictor of resection at a high-volume center.

We tested for interactions between "distance to a high-volume hospital" and other covariates, and none were significant. As no significant interactions were identified, we did not stratify patients by distance in the multivariate models.

Table 4 Logistic Regression Analysis[a]

Factor	Odds Ratio	95% Confidence Interval
Age group		
18–44 years	1.00	–
45–54 years	0.88	0.65–1.81
55–64 years	0.75	0.56–1.00
65–74 years	0.75	0.53–1.06
>74 years	0.51	1.00–1.12
Year of diagnosis	1.07	1.02–1.13
Distance to nearest high-volume hospital (by 10 mile increment increases)	0.93	0.92–0.94
Gender		
Male	1.00	–
Female	0.86	0.72–1.03
Race/Ethnicity		
Non-Hispanic White	1.00	–
Non-Hispanic Black	0.79	0.59–1.06
Hispanic	0.58	0.46–0.74
Other	1.14	0.80–1.60
Insurance type		
Uninsured	1.00	–
Medicare/Medicaid	1.43	0.96–2.14
Other insurance	1.18	0.82–1.70
Severity of illness		
Score=1	1.00	–
Score=2	1.20	0.78–1.85
Score=3	1.56	1.01–2.41
Score=4	1.20	0.77–1.81
Diagnosis		
All other diagnoses	1.00	–
Periampullary adenocarcinoma	0.68	0.56–0.83
Admission type		
Elective	1.00	–
Emergent	0.39	0.32–0.48
Type of operation		
Pancreaticoduodenectomy	1.00	–
Distal pancreatectomy	0.53	0.41–0.69
Pancreatectomy not otherwise specified	0.66	0.45–0.95

[a] Models the probability of undergoing resection at a high-volume center. OR>1, increased likelihood; OR<1, decreased likelihood

Discussion

Regionalization of medical and surgical procedures, especially those procedures that involve large costs and require considerable technical and professional skills, can be expected to improve the quality of medical care and save money. However, the benefits of regionalization must be weighed against the potential detriments including inconvenience to patients (increased travel costs, loss of time from work, constraints on the places where one can receive care),[20] the potential for overwhelming of high-volume centers, increased mortality at low-volume hospitals as a result of regionalization, the decreasing quality of urgent related procedures at low-volume hospitals, and reduced access to surgical care if low-volume hospitals cannot recruit qualified surgeons.[14]

As discussed in his editorial, Birkmeyer[14] points out that these concerns are "not very persuasive in the case of pancreaticoduodenectomy" or pancreatic resection in general. Pancreatic resection is an ideal model for regionalization of care for several reasons. First, there is a well-demonstrated, strong volume–outcome relationship. Second, the volume of pancreatic resections performed in this country in a

given year are low enough such that high-volume centers would not be overwhelmed. Similarly, the volume lost from shifting these procedures away from low-volume centers would not be detrimental to the low-volume centers, as they occur so infrequently and often cost the hospitals money.

In Texas, the regionalization of pancreatic resection has improved between 1999 and 2004. Of the patients, 54.5% had their pancreatic resection performed at a high-volume center (>11 cases/year) in 1999, and this percentage increased to 63.3% by 2004. This extent of regionalization of pancreatic resection to high-volume centers is similar to the rates seen elsewhere.[1,3,6,7,21] The studies are difficult to compare as the volume cutoffs vary. In a 2000 paper by Gouma and colleagues,[7] 40% of patients were resected at a hospital performing fewer than five pancreatic resections per year. Likewise, in a Maryland study by Gordon et al,[1] 45.9% of patients were resected at hospitals performing fewer than 20 resections per year. Worse, in a 2003 study of the California and Florida data by Ho and colleagues,[3] 77% of resections were performed in hospitals doing fewer than 10 resections per year. The Netherlands experience has been similar, with 65% of patients in 1994–1995 undergoing surgery at centers performing 10 or fewer resections per year. In the Netherlands, a plea for regionalization was made, but they were only able to decrease the percentage of patients resected at low-volume centers (<10 cases/year) to 57% in the time period 2000–2003. In a 2002 analysis of the Nationwide Inpatient Sample (NIS),[6] the mean number of resections performed at any given hospital in the sample was only 1.5 cases per year. In a more recent analysis of the NIS,[21] 34.4% of patients were resected at a hospital performing fewer than five resections per year.

Whereas regionalization has increased significantly over the time period of the study, it is still a matter of concern that 26.6% of pancreatic resections in Texas in 2004 were performed at centers doing fewer than five cases per year, and 36.7% were performed at hospitals doing 10 or fewer resections per year. In addition, 19% of patients who were operated on at a low-volume center traveled farther than the distance to the nearest high-volume center.

We also observed interesting geographic patterns in the likelihood of traveling to high-volume centers (see Fig. 5), which are likely applicable to the United States as a whole. The 14 high-volume providers are located in six of Texas's 254 counties: Dallas, Tarrant, Bell, Harris, Bexar, and Galveston. However, within these counties, high-volume centers have varying levels of monopoly, with people living in Galveston, Bell, and Dallas counties being the most likely to get resected at high-volume centers. The dip and then rise in percentage of patients undergoing resection at a high-volume center based on distance seen in Fig. 4 is likely real. We theorize that big counties with high-volume providers also generate more low-volume providers. As a result, they may be more likely to go to or be referred to one of these providers. However, in the mid-distance suburbs, where fewer low-volume providers exist, referring physicians may be more likely to tell patients that they don't do such complex procedures and refer them to the high-volume centers in surrounding counties. Therefore, both distance, and referral patterns affect the extent of regionalization.

Outside the principal counties, there are four different situations: (1) suburban rings around the principal counties, (2) middle distance places (such as Texarkana, east Texas, Austin Hill County), (3) remote places (such as south Texas and San Angelo), and (4) very remote west Texas. For the suburbs and middle-distance places, the existence of a local middle-volume provider is key (5–10 cases/year). For example, in Brownsville (South Texas) there is no middle-volume provider. As a result, Brownsville patients are more likely to travel to high-volume providers. McAllen, close to Brownsville, has a middle-volume provider, and few of their people travel to high-volume hospitals. In addition, Brownsville does not refer to McAllen despite its proximity. Beaumont, in East Texas, has a similar situation to Brownsville, with no middle- or high-volume provider, and these patients tend to travel. From San Angelo west, low-volume providers in El Paso, San Angelo, and Lubbock monopolize the market. Here, the very long distance to high-volume providers seems to be a key factor.

Many studies use quartiles or quintiles to establish the volume cutoffs so as to have equal group sizes for statistical analysis when comparing outcomes such as in-hospital mortality, charges, and lengths of stay. For our analysis, we chose to use the Leapfrog group's minimal volume cutoff for pancreatic surgery to evaluate the extent of regionalization, as this is the current recommendation by a large coalition of payers. Based on the definition we used for "high-volume", only two hospitals shared the time period with fewer than 11 cases per year, but met the high-volume criteria. Several cities within Texas have middle-volume providers that do not meet the minimum volume requirements, but are clearly referral centers for the geographic area (e.g., Tyler, Lubbock). In these areas, concentration of patients from surrounding very low volume centers would likely bring these centers up to minimum volume standards.

Although we based our volume standards on all resections performed in the state, we eliminated patients who were not from Texas in analyzing the trends in regionalization (although these resections were included in determining a hospital's volume status). By virtue of the fact that these patients traveled out of state (or country) to have the procedure performed, it is implied that they were an inherently different group of patients. There is also a potential for bias if patients in Texas traveled to nearby

cities outside of the state to have their pancreatectomy performed, which we cannot identify. For example, it may be closer for patients in west Texas to travel to Denver, Albuquerque, or Oklahoma City instead of an in-state high-volume provider.

Despite the evidence that regionalization of pancreatic resection is warranted, Texas (and likely other states) are only achieving partial regionalization of care, with greater then 25% of patients being operated on at low-volume centers. In the multivariate analysis of the Texas Data, Hispanic patients were less likely to be resected at high-volume centers. This may be a result of a lack of education regarding the importance of volume for this procedure to this largely Spanish-speaking or bilingual population. The same was not true for Blacks. Older age also seemed to be a barrier to regionalization. Older people may be more reluctant to travel even a minimally further distance to get the best care. However, in the elderly population it is even more critical for the procedure to be performed at high-volume centers so as to minimize complications.

Patients with a diagnosis of periampullary cancer were less likely to be resected at high-volume centers, despite the fact that the highest volume center in Texas is a designated cancer center. This may be the result of hurried decision making and concern of delay when this uncommon diagnosis is made at low-volume centers. The same holds true for emergent procedures, which are far more likely to occur at low-volume centers. Especially in patients with cancer, regionalization to specialized centers will improve both their short- and long-term outcomes. Moreover, there are very few urgent or emergent indications for pancreatic resection and such resections should be minimized.

One of the biggest barriers to regionalization seems to be the distance to a high-volume center. Interestingly, this distance need not be great to influence the choice of hospital. Using the Medicare claims data, Birkmeyer et al.[22] have demonstrated that, if not set too high, hospital volume standards could be implemented without imposing unreasonable travel burdens on individuals. This is true for the Texas Discharge Data with a cutoff of 11 or more procedures per year, as 75% of patients live within 75 mi of a high-volume center. However, our study demonstrates that even when the excess travel distance required for surgery at a high-volume center is short, many patients do not go to the high-volume centers. The etiology, however, is unclear, and both patient preference and referral patterns (such as those observed in Brownsville and McAllen) likely influence whether patients go to high-volume centers.

Texas serves as a good model for the regionalization of pancreatic resection. Unlike smaller states in which all patients can easily travel to a high-volume center, Texas is large with many rural areas distant from high-volume centers and would serve as a good model for regionalization to the high-volume centers throughout the U. S. Our data demonstrate that regionalization is feasible and the detailed analysis of the barriers to successful regionalization will aid in achieving this goal. To succeed in regionalizing care for pancreatic resection, we need to change referral patterns such that the 34% of patients living within 75 mi go to high-volume versus low-volume centers. In cities throughout the Texas or the U.S. with medium volume referral centers, cases done by very low-volume providers need to be concentrated at these medium-volume providers. In addition, we can help implement process measures at middle-volume centers that improve outcomes to the level of the high-volume centers (if needed), thereby removing the travel burden for patients.

Most of the volume–outcomes literature focuses on mortality as the endpoint. Although volume is clearly important for good outcomes after pancreatic resection, it is not the whole picture. A recent study by Meguid and colleagues[23] demonstrated that volume explained less than 2% of the variance in the data on perioperative death after pancreatic resection. Other endpoints such as length of stay and total charges are also available in some of the published studies, but data on complication rates, morbidity rates, and readmission rates are not readily available. The next step is to further examine "high-volume" providers and measure outcomes more specifically, including the evaluation of common complications after pancreatic surgery including pancreatic fistula,[24–33] delayed gastric emptying,[24–27,34] intraabdominal abscess formation,[24–27] wound infections, bleeding, and others. The goal would then be to standardize care at high-volume institutions through the implementation of critical pathways (which focus on the process measures in the care of patients) designed based on the practices at the institutions with the best outcomes. These data need to be made widely available such that referring physicians, payers, and patients can make informed decisions regarding where to have their pancreatic surgery performed.

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DISCUSSION

Russell G. Postier, M.D. (Oklahoma City, OK): I want to congratulate Dr. Riall and her colleagues on an informative paper that highlights the continued difficulty of getting the right patient to the right surgeon and the right center at the right time. Your manuscript data suggest that there are a number of factors in addition to medical politics that influence where a patient requiring pancreatic resection is referred. You postulate that the patients with less knowledge about volume outcome data might be more likely to be operated on by their local surgeon in a low-volume center. I would suggest that a lack of understanding regarding the appropriate treatment of patients requiring pancreatic surgery, especially in the case of pancreatic cancer, also represents a global lack of understanding of the uniqueness of approach required by these patients in the general physician population. At this meeting a year ago you presented disturbing data as to the surprisingly high number of patients with localized and presumably resectable and potentially curable pancreatic cancer who were not offered pancreatic resection. We all see patients whose referral to pancreatic surgical specialists has been delayed by weeks or months by unsuccessful attempts at obtaining a diagnosis percutaneously and by a lack of understanding of the low surgical mortality achieved in high-volume referral centers. It appears that we have more work to do in

educating our referring physician colleagues than we may in educating our patients. I have four questions.

First of all, how do you explain the relatively good results in mortality achieved in the low-volume centers in Texas? Your operative mortality in the low-volume centers was 7.4% as compared to around 3% in your high-volume centers. This 7.4% is substantially lower than that seen in other studies, specifically the nearly 19% mortality seen in low-volume centers in Maryland.

Secondly, what is the volume necessary to achieve optimal results in pancreatic surgery? I am not sure that 10 cases, which is the Leapfrog criteria, are adequate, and I think the much higher volume than 10 per year done by the surgeons in your high-volume hospitals represents a substantially higher level of care.

Thirdly, the distal pancreatectomies, which were lumped with pancreaticoduodenectomies in your study, require the same level of expertise as do pancreaticoduodenectomies. In my view, the distal resection is a significantly less complex procedure, and a well-trained general surgeon may well be able to do that with good outcomes although they are not a high-volume provider. Do you have any data on this question or do you know of data?

And finally, you looked at hospital volume, not surgical volume. Do you think that low-volume surgeons operating in medium- or high- volume centers independently, without being mentored by high-volume surgeons, can achieve results equivalent to those of high-volume surgeons?

I think this is an important paper and I congratulate you and your colleagues for excellent work and you on a fine presentation. Thank you.

Taylor S. Riall, M.D. (Galveston, TX): Thank you, Dr. Postier. Your first question was regarding the good results at low-volume centers in Texas with a mortality rate of 7.4%. Our volume cutoff, as you mentioned, is arbitrary. If you made the volume cutoff at different points, you can see that mortality goes from as high as 12 or 13% to as low as 5%. It depends on where you make the cutoff, which brings me to your next question: what is the volume of pancreatic resections necessary and why did we choose a criteria of greater than 10?

We chose the Leapfrog criteria of greater than 10 resections per year primarily because organizations like the Leapfrog group and other health care purchasers are going to be the ones who are deciding where patients go. As of 2004 they are choosing greater than 10 cases as their cutoff. Also, with some of the concerns with regionalization, referring to Birkmeyer's data, you need to pick a reasonable volume cutoff so that you don't impose unreasonable travel burdens on patients. You don't want patients having to travel several hundred miles to the nearest high-volume center. For these reasons, I think greater than 10 pancreatic resections per year is a reasonable cutoff.

Also, there was a paper presented at the SUS by Dr. Meguid and colleagues at Hopkins who looked at volume cutoff for pancreatic resection. They tried to pick the best volume cutoff. No matter where they chose the volume cutoff, they observed a difference between mortality between the high- and low-volume providers. So volume alone is not the only issue.

With regard to distal pancreatectomy, there are no data looking specifically at distal pancreatectomy comparing outcomes to low- and high-volume centers, although that would be simple to do.

With regard to multidisciplinary care, these patients are better served at centers that deal with pancreatic diseases and not done by the general surgeon. While distal pancreatectomy is technically easier, they have higher rates of fistulas than proximal pancreatectomies. I certainly think they should be performed at high-volume centers as well.

The question with regard to hospital and surgeon volume comes up all the time. Our data doesn’t have the ability to look at individual surgeons, but I am certain that individual surgeon experience plays a role. In order to achieve my suggestion of making some medium-volume centers high-volume centers, such centers will need mentoring from the high-volume centers to achieve the same results. Another interesting point is that if you look at the outcomes among high-volume centers only, there is incredible variability of care among the high-volume providers. We need to standardize the care among all the providers who are considered referral centers to optimize outcomes for patients undergoing pancreatic resection for any reason.

J Gastrointest Surg (2007) 11:1253–1261
DOI 10.1007/s11605-007-0193-0

Protein Kinase C-ζ is Critical in Pancreatitis-Induced Apoptosis of Kupffer Cells

Yanhua Peng · Celia A. Sigua · Scott F. Gallagher · Michel M. Murr

Received: 8 March 2007 / Accepted: 11 May 2007 / Published online: 26 July 2007

Abstract

Protein kinase C-zeta (PKC-ζ) regulates cell death via NF-κB; therefore, we tested the hypothesis that PKC-ζ plays a critical role in pancreatitis-induced Kupffer cell apoptosis. Acute pancreatitis was induced in rats by cerulein injection 24 h later, livers were assayed for PKC-ζ, IKKα, IKKβ, IKKγ, NF-κB, Fas/FasL, and apoptosis was assessed with Caspase-3 and DNA fragmentation. Kupffer cells from unoperated rats were infected with a PKC-ζ domain-negative adenovirus (AdPKCζ-DN) to inhibit PKC-ζ, or transfected with pCMVPKC-ζ to overexpress PKC-ζ, and then stimulated with pancreatic elastase; cellular extracts were assayed for PKC-ζ, IKKα, IKKβ, IKKγ, NF-κB, Fas/FasL, Caspase-3, and DNA fragmentation. Cerulein-induced pancreatitis upregulated PKC-ζ protein and activity, IKKβ, IKKγ, NF-κB, Fas/FasL, Caspase-3 and increased DNA fragmentation in rat livers (all $p<0.001$ vs control). AdPKCζ-DN abolished elastase-induced upregulation of PKC-ζ activity, IKKβ, IKKγ, NF-κB, Fas/FasL, Caspase-3 and DNA fragmentation (all $p<0.001$ vs infection control), whereas over-expression of PKC-ζ augmented elastase-induced upregulation of IKKβ, IKKγ, Fas/FasL, Caspase-3 and DNA fragmentation ($p<0.001$ vs control). PKC-ζ plays a critical role in pancreatitis-induced Kupffer cell apoptosis via NF-κB and Fas/FasL. The ability of Kupffer cells to autoregulate their stress response by upregulating their death receptor/ligand and key proapoptotic cell signaling systems warrants further investigation.

Keywords Kupffer cells · PKC-ζ · NF-κB · Fas/FasL · Acute pancreatitis

Abbreviations

ABTS	2, 2′azino-di [3-ethylbenzthiazoline sulfonate (6)] and H_2O_2 in glycine/citric acid buffer
AdLacZ	adenovirus which expresses LacZ gene
AdPKCζ-DN	adenovirus which expresses domain negative protein kinase C zeta
DMEM	Dulbecco Modified Eagle's Medium
ELISA	enzyme-linked immunosorbent assay
FBS	fetal bovine serum
IKKα	IκB kinase α
IKKβ	IκB kinase β
IKKγ	IκB kinase γ
LPS	lipopolysaccharide
MOI	muliplicity of infection
NF-κB	nuclear factor kappa-B
NP-40	non-ionic surfactant
PCMVcDNA3.1	empty expression vector driven by cytomegalovirus (CMV) promoter
PCMVPKC-ζ	expression plasmid of protein kinase C zeta driven by CMV promoter
PKC-ζ	protein kinase C zeta
PMSF	phenylmethylsulfonyl fluoride
PRKC	primary rat Kupffer cells
RIPA buffer	PIPA buffer (50 mM Tris–HCl (pH 7.5), containing 1% Nonidet P-40, 0.05% SDS, 0.5% sodium deoxycholate, 1 mM EDTA, 150 mM NaCl, and protease inhibitors)

Presented as a poster during the Annual Meeting of the SSAT, Los Angeles, May 2006.

Y. Peng · C. A. Sigua · S. F. Gallagher · M. M. Murr
Department of Surgery, James A. Haley Veterans Affairs Medical Center, University of South Florida Health Sciences Center, Tampa, FL, USA

M. M. Murr (✉)
Tampa General Hospital,
PO Box 1289, Tampa, FL 33601, USA
e-mail: mmurr@health.usf.edu

SDS	sodium dodecyl sulfate
TNF-α	tumor necrosis factor-α

Introduction

Kupffer cells play a central role in sepsis,[1] acute pancreatitis,[2] ischemia–reperfusion, and hepatitis.[3] We have demonstrated that Kupffer cell-derived cytokines such as TNF-α and Fas/FasL mediate parenchymal liver injury and hepatocyte death during acute pancreatitis.[4,5] On the other hand, Fas/FasL induces Kupffer cell apoptosis through NF-κB transcriptional regulation,[6] thereby suggesting that stress-induced Kupffer cell apoptosis may have a protective effect on the liver during acute pancreatitis. Yet, inhibition of NF-κB within Kupffer cells did not abolish stress-induced Kupffer cell apoptosis.[7]

NF-κB activation interacts with different kinases, such as PI3 kinase and protein kinase C (PKC). Atypical PKC isoforms, such as PKC-ζ and PKC- λ, are important components of the TNF-α/IL 1-β signaling pathway that control NF-κB activation.[8,9] Moreover, PKC-ζ regulates FasL-induced apoptosis.[10] Therefore, the direct and indirect interactions of PKC-ζ and NF-κB may be critical for programmed cell death and apoptosis in immuno-competent cells. This study was undertaken to investigate the role of PKC-ζ in apoptosis of Kupffer cells during acute pancreatitis.

Materials and Methods

All experiments were conducted with the prior approval of the Institutional Animal Care and Use Committee (IACUC) at the University of South Florida.

Acute Pancreatitis Acute pancreatitis was induced in adult male Sprague-Dawley rats (240–260 g) by intraperitoneal injection of cerulein (50 mg/kg/h, ×4); saline was used as a vehicle control; (n=5 each). The animals were sacrificed 24 h after induction of pancreatitis and the livers were collected and frozen at −80°C until use. The liver homogenates were processed for PKC-ζ, IKKα, β and γ, p65/NF-κB, Fas/FasL, Caspase-3, and DNA fragmentation.

Isolation of Fresh Primary Rat Kupffer Cells (PRKC) PRKC were isolated from unoperated adult male Sprague-Dawley rats and provided by the Research Center for Alcoholic Liver and Pancreatic Diseases, University of Southern California.[11] Cells were grown in RPMI 1640 with 10% fetal bovine serum (FBS), 1% nonessential-amino acids, and 0.03% glutamine.

Over-expression of PKC-ζ PRKC were transfected with a cytomegalovirus (CMV)-promoter-driven PKC-ζ gene expression plasmid (pCMVPKC-ζ, Invitrogen, Carlsbad, CA). Briefly, 15-μg pCMVPKC-ζ or control expression vector pCMVcDNA 3.1 were separately transfected into 1×10^7 PRKC; 24–48 h later, the cells were treated with pancreatic elastase (1 U/ml, Sigma, St Louis, MO) for 4 h to simulate conditions of acute pancreatitis. The dose of elastase has been validated in our laboratory in human mononuclear cells, lung macrophages and Kupffer cells.[2,12] The experimental groups were: (1) PRKC+no treatment, (2) PRKC+elastase, (3) PRKC+pCMVcDNA3.1, (4) PRKC+pCMVcDNA3.1+elastase, (5) PRKC+pCMVPKC-ζ, and (6) PRKC+pCMVPKC-ζ+elastase. Cellular extracts were processed for PKC-ζ, IKKα, β and γ, p65/NF-κB, Fas/FasL, Caspase-3, and DNA fragmentation.

Inhibition of PKC-ζ Activity PRKC were infected with an adenovirus that expresses a domain negative PKC-ζ driven by a CMV promoter (AdPKC-ζDN). AdPKC-ζDN and control AdLacZ were kindly provided by Dr. Robert Farese, James A. Haley Veterans Affairs Medical Center, University of South Florida, Tampa, FL.

Amplification and Purification of Adenovirus Human Embryonic kidney cell line 293 (HEK-293) is a gift from William Gower, Jr., PhD, James Haley Veterans Affairs Medical Center, University of South Florida, Tampa, FL. HEK-293 cells were grown in 15-cm tissue plate at 90% confluence and infected at a muliplicity of infection (MOI) of 8–10 per cell. The infected cells were grown in Dulbecco Modified Eagle's Medium (DMEM) media with 10% FBS for 72 h until a very strong cenotaphic effect could be observed and about 75% cells were detached, and then centrifuged at 3,000 rpm for 5 min. Viral particles were released by five cycles of rapid freezing and thawing at 40–50°C, and purified using Virakit TM Adenomini-24 kit (Virapur, LLC, San Diego, CA). Determination of virus infectivity was by viral plaguing assay; concentration of viruses was measured by colorimetry, and X-gal staining was used to determine the efficiency of viral infection.

The 1×10^7 PRKC were plated overnight before infection with AdPKC-ζ DN or AdLacZ at 100 plaque-forming units (PFU)/cell or at 50–100 MOI. To enhance viral infection efficiency, FBS concentration was kept at 3% during the first 2 h and increased, to 10% afterwards. Twenty-four hours later cells were treated with pancreatic elastase for 4 h. The experimental groups were: (1) PRKC+no treatment, (2) PRKC+elastase, (3) PRKC+AdPKC-ζDN, (4) PRKC+AdPKC-ζDN+elastase, (5) PRKC+AdLacZ, and (6) PRKC+AdLacZ+elastase. Cellular extracts were processed for PKC-ζ, IKKα, β and γ, p65/NF-κB, Fas/FasL, Caspase-3, and DNA fragmentation.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Fas/FasL mRNA was measured by semiquantitative differential RT-PCR. Briefly, total cells mRNA was isolated by Trizol solution (Invitrogen). One microgram of RNA was primed using oligo (dT; Gibco, Gaithersburg, MD) and subsequently reverse transcribed with reverse transcriptase (SuperscriptII, Gibco). cDNA production was amplified in the presence of rat specific Fas, FasL, and BMG primers for 30 cycles of PCR in a UNO-Thermo block (Biometra, Tampa, FL). The sequences for primers were: Fas: sense 5′GTATGCTGTGGATCATGGC 3′ and antisense 5′AAC TTTTCGTT-CACCAG3′ (Invitrogen); FasL: sense 5′ ATG GAACTGCTTTGATCTCTGG3′ and antisense 5′TTCCT CAAAATTGATCAGAG3′; βMG: sense 5′CTCCCC AAATTCAAGTGTACTCTCG3′, antisense 5′GAGTGA CGTGTTTAACTCT-GCAAGC3′. The PCR products were separated with electrophoresis in 4% low melting temperature agarose gel containing ethidium bromide and photographed digitally (UVP, GDS 8000 Upland, CA).

Immunoblotting Cells were lysed in RIPA buffer [phosphate-buffered saline (PBS) with 0.1% sodium dodecyl sulfate (SDS), 1% NP40, 0.5% sodium deoxycholate]; 50–100 μg protein was fractionated by 10% SDS-PAGE, transferred to nitrocellulose membrane (Amersham, Pharmacia Biotech), blocked for 1 h with PBS containing 5% instant nonfat dry milk, and 0.1% Tween-20, then incubated for 2 h with antibodies to either Fas, FasL (BD Biosciences, San Diego, CA), Caspase-3, PKC-ζ, phosphorylated IKKα, β, γ, or β-actin (Cell Signaling technology, Beverly, MA). Bound primary antibody was detected by incubating with horseradish peroxide goat antimouse IgG. Membranes were developed using SuperSignal (Pierce, Rockford, IL) ECL reagent and quantified by densitometry.

PKC-ζ Activity Briefly, PKC-ζ was immuno-precipitated with a rabbit polyclonal antibody[13] (Cell Signaling Technology), collected on Sepharose-A and G beads (Amersham) and incubated for 10 min at 30°C in 100 μl $Na_4P_2O_7$, 1 mM NaF, 100 μM PMSF, 4 μg phosphatidylserine (Sigma), 50 uM[$\gamma-^{32}$ P] ATP (Amersham), 5 mM $MgCl_2$, and 40 μM serine analog of the PKC pseudosubstrate (Biosource Technologies, Camarillo, TX). After incubation, ^{32}P-ATP-labeled substrate was trapped on P-81 filter papers and counted with scintillography.

DNA fragmentation Apoptosis was determined using ELISA-based DNA fragmentation assay (Roche Molecular Biochemicals, Indianapolis, IN). Briefly, 10^4 cells were lysed, transferred to a 96-well plate, and incubated with 80 μl of immunoreagent for 2–4 h at room temperature or 4°C overnight. After adding ABTS (2, 2′azino-di [3-ethylbenzthiazoline sulfonate (6)] and H_2O_2 in glycine/citric acid buffer), the plates were left on a plate shaker at 250 rpm until color development was sufficient for photometric analysis at 425 nm against ABTS solution as a blank.

ELISA for nuclear translocation of p65/NF-κB Cell pellets were lysed in 400 μl hypotonic lysis buffer with 1% NP-40 and centrifuged for 30 s at 14,000 rpm. The supernatant (cytoplasmic extracts) was removed and 220 μl of ice cold nuclear extraction buffer was added. Protein concentration was determined by ELISA of nuclear extracts (Imgenex, San Diego, CA).

Data Analysis All experiments were repeated at least in triplicates. ANOVA was used to compare means; if $p<0.05$ then a t test was used to compare means of two different arms, e.g., control vs elastase. Bonferroni's correction was used to correct for multiple comparisons. Generally, we used six different experimental groups; therefore, the corrected p value for statistical significance was set at $p=0.05/6=0.008$. Data are mean+standard deviation (SD).

Results

Upregulation of PKC-ζ Expression and Activity in Both Rat Liver During Cerulein-induced Pancreatitis and Elastase-treated PRKC Total PKC-ζ protein and activity were increased in rat livers 24 h after cerulein-induced acute pancreatitis compared to control (all $p<0.001$, Figs. 1 and 2). Similarly, PKC-ζ protein and activity were increased in elastase-treated PRKC (elastase vs control, $p<0.001$, Figs. 1 and 2).

In vitro, as expected, AdPKC-ζ DN increased elastase-induced expression of PKC-ζ protein in PRKC ($p<0.001$ vs control, Fig. 1); however, the activity of the mutant PKC-ζ was reduced [AdPKC-ζ DN+elastase vs AdLacZ+elastase (data not shown), $p<0.001$, Figs. 1 and 2]. Viral infection control experiments demonstrated the specificity of AdPKC-ζ DN (AdPKC-ζ DN+elastase vs AdPKC-ζ DN, $p<0.001$).

In contrast, transfection of PRKC with PKC-ζ expression vector pCMVPKC-ζ further augmented the elastase-induced upregulation of PKC-ζ protein and activity (pCMVPKC-ζ+elastase vs elastase, all $p<0.001$, Figs. 1 and 2). Transfection with pCMVcDNA3.1 as a control demonstrated the specificity of pCMVPKC-ζ [pCMVPKC-ζ+ elastase vs pCMVcDNA3.1+elastase (data not shown), all $p<0.001$, Figs. 1 and 2].

Upregulation of IKKβ and IKKγ But Not IKKα in Both Rat Liver During Pancreatitis and Elastase-treated PRKC Phosphorylated IKKβ and IKKγ proteins were increased in rat livers within 24 h after cerulein-induced pancreatitis compared to sham (all $p<0.001$, Fig. 3). However, IKKα

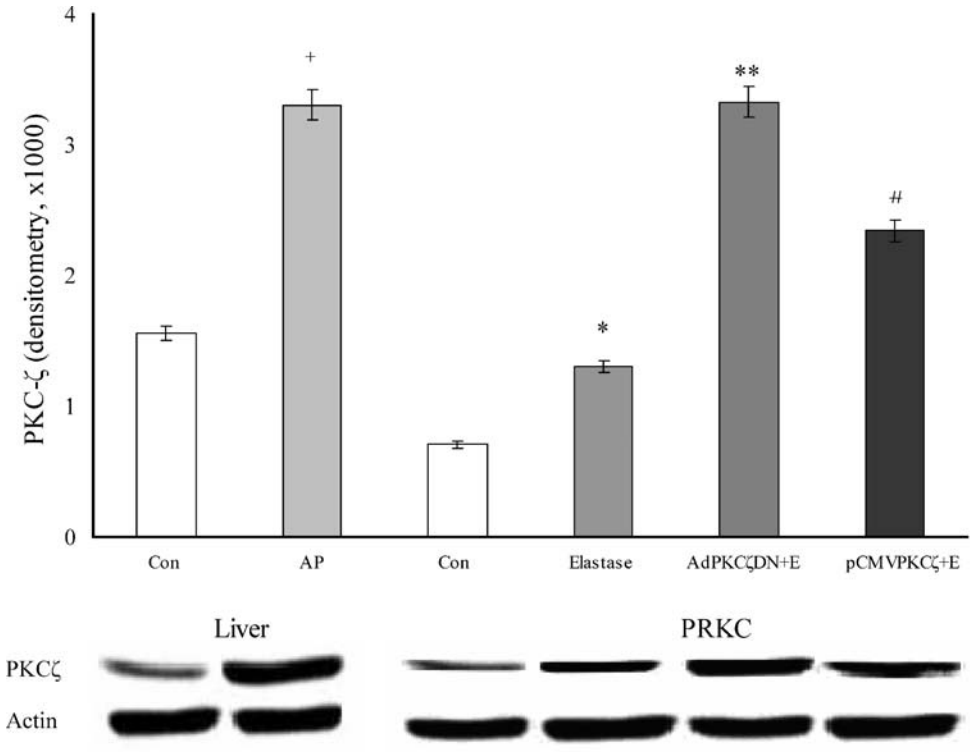

Figure 1 PKC-ζ protein increased in livers after acute pancreatitis (AP) compared to control (*Con*; *plus sign*, $p<0.001$), and in elastase-treated (*E*) PRKC (*asterisk*, $p<0.001$ vs Con). Both AdPKC-ζ DN and pCMVPKC-ζ augmented (*double asterisk*, $p<0.001$ vs AdLacZ+E; $p<0.001$, vs pCMV.cDNA3.1+E, not shown, respectively) the elastase-induced expression of PKC-ζ. Panel is a representative gel of PKC-ζ protein, the bar graph is densitometric quantification of $n=3$ immunoblots.

was not upregulated ($p>0.05$, data not shown). Similarly, phosphorylated IKKβ and IKKγ increased in elastase-treated PRKC (elastase vs control, all $p<0.001$, Fig. 3). AdPKC-ζ DN attenuated the elastase-induced upregulation of IKKβ and IKKγ [AdPKC-ζ DN+elastase vs AdLacZ+ elastase (data not shown), all $p<0.001$, Fig. 3]. Viral infection control experiments confirmed the specificity of AdPKC-ζ DN [AdPKC-ζ DN+elastase vs AdPKC-ζDN, all $p<0.001$). The pCMVPKC-ζ augmented the elastase-induced upregulation of phosphorylated IKKβ and IKKγ [pCMVPKC-ζ± elastase vs pCMVcDNA3.1+elastase (data not shown), all $p<0.001$, Fig. 3], but not IKKα.

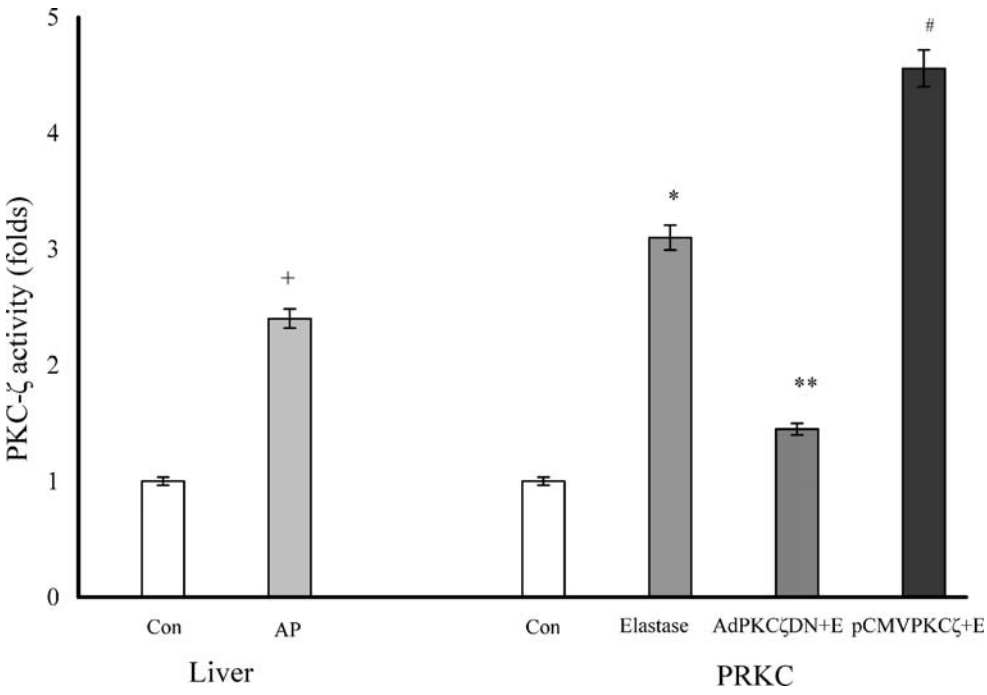

Figure 2 PKC-ζ activity increased in livers after acute pancreatitis (AP) as compared to control (*Con*); *plus sign*, $p<0.001$ and in elastase-treated (*E*) PRKC (*asterisk*, $p<0.001$ vs Con). AdPKC-ζ DN attenuated (*double asterisk*, $p<0.001$ vs AdLacZ+E, not shown), whereas pCMVPKC-ζ augmented (*number sign*, $p<0.001$ vs pCMVcDNA3.1+E, not shown) the elastase-induced upregulation of PKC-ζ activity.

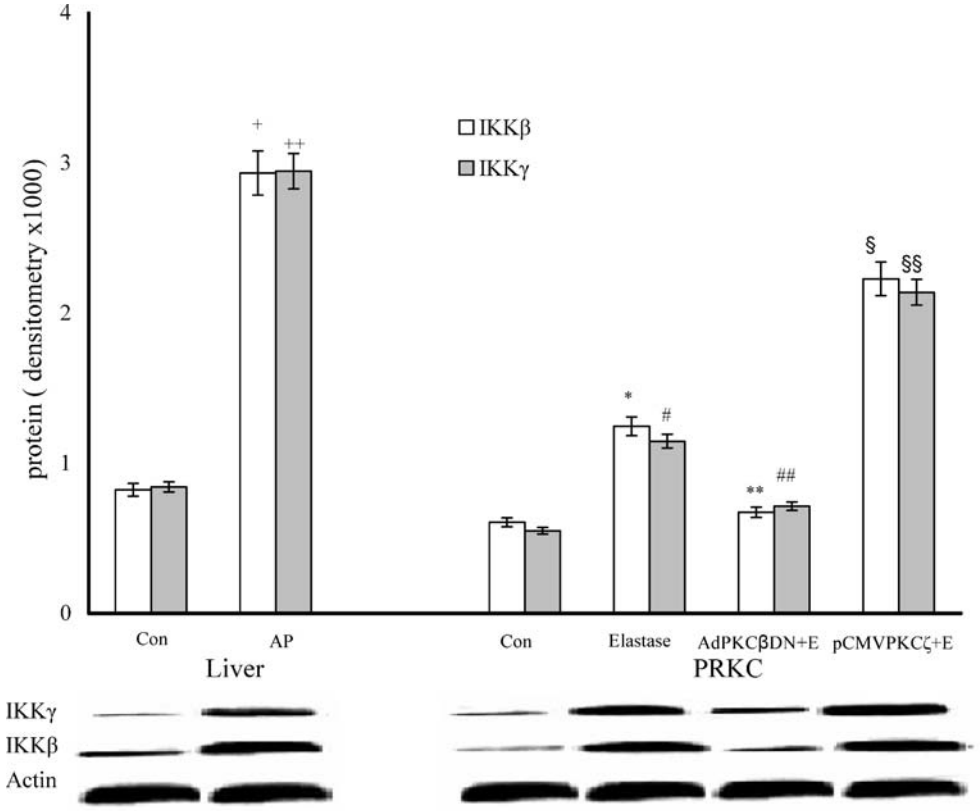

Figure 3 Phosphorylated IKKβ/IKKγ protein were increased in livers after acute pancreatitis (*AP*) as compared to control (*Con*, *plus sign*, *double plus sign* $p<0.001$) and in elastase-treated (*E*) PRKC (*asterisk*, *number sign* $p<0.001$ vs Con). AdPKC-ζ DN attenuated (*double asterisk*, *double number sign* $p<0.001$ AdLacZ+E, not shown), whereas pCMVPKC-ζ augmented (*section mark*, *double section mark* vs pCMVcDNA3.1+E, not shown) the elastase-induced upregulation of IKKβ/IKKγ. Panel is a representative gel of IKKβ/IKKγ/β-actin, the bar graph is densitometric quantification of $n=3$ immunoblots.

Nuclear Translocation of p65/NF-κ B in Both Rat Liver During Pancreatitis and Elastase-treated PRKC Nuclear translocation of p65/NF-κ B increased in rat liver during cerulein-induced pancreatitis (acute pancreatitis vs control, $p<0.001$; Fig. 4) and in elastase-treated PRKC (elastase vs control, $p<0.001$; Fig. 4).

AdPKC-ζ DN attenuated the elastase-induced nuclear translocation of p65/NF-κB [AdPKC-ζ DN+elastase vs

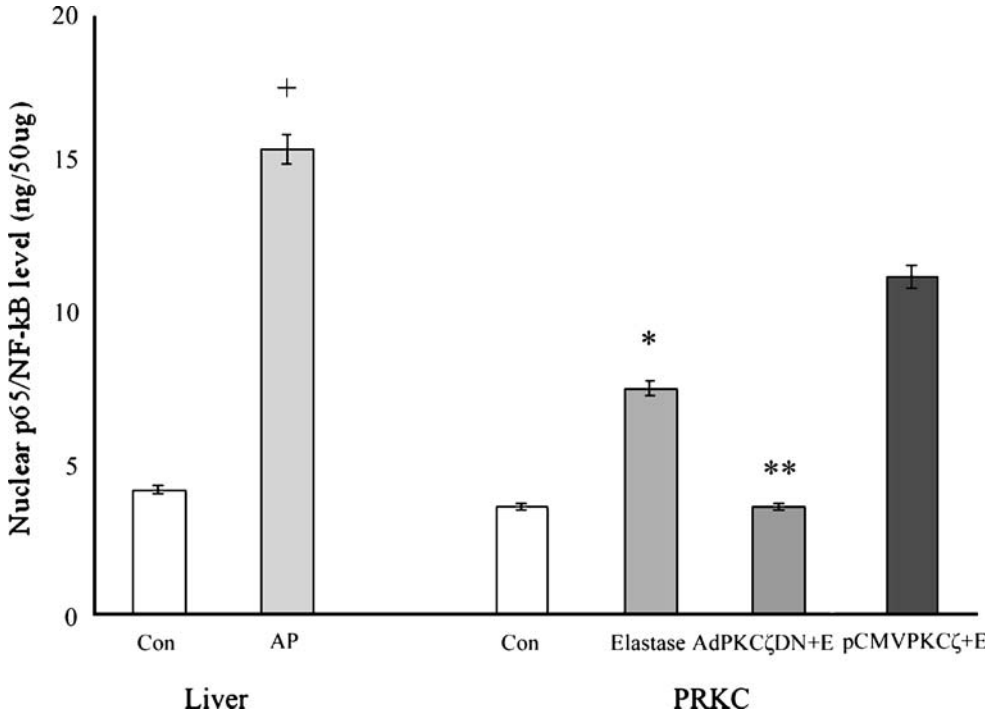

Figure 4 Nuclear translocation of p65/NF-κB increased in livers after pancreatitis (*AP*) compared to control (*Con*; *plus sign* $p<0.001$), and in elastase-treated (*E*) PRKC (*asterisk* $p<0.001$, vs Con). AdPKC-ζ DN attenuated (*double asterisk* $p<0.001$, vs AdLacZ+E, not shown), but pCMVPKC-ζ did not have any effect ($p>0.05$, vs pCMVcDNA3.1+E, data not shown) on elastase-induced activation of p65/NF-κB.

AdLacZ+elastase (data not shown), $p<0.001$; Fig. 4]. However, pCMVPKC-ζ did not augment the elastase-induced p65/NF-κB activation [pCMVPKC-ζ+elastase vs + pCMVcDNA3.1+elastase (data not shown), $p>0.05$].

Upregulation of Fas/FasL in Rat Liver During Pancreatitis and Elastase-treated PRKC Fas/FasL transcription and translation were upregulated in rat livers during cerulein-induced pancreatitis (acute pancreatitis vs control, all $p<0.001$, Fig. 5) and in elastase-treated PRKC (all $p<0.001$, elastase vs control, Fig. 5).

AdPKC-ζ DN attenuated the elastase-induced upregulation of Fas/FasL protein and mRNA in PRKC [AdPKC-ζ DN+elastase vs AdLacZ+elastase (data not shown), all $p<0.001$; Fig. 5]. In contrast, pCMVPKC-ζ did not increase elastase-induced upregulation of Fas/FasL protein ($p<0.05$) in PRKC, but increased elastase-induced Fas/FasL mRNA [pCVMPKC-ζ+elastase vs pCMVcDNA3.1+elastase (data not shown), all $p<0.001$; Fig. 5].

Upregulation of Apoptosis in Rat Liver During Pancreatitis and Elastase-treated PRKC Activated Caspase-3 was increased during cerulein-induced pancreatitis (pancreatitis vs control, $p<0.001$; Fig. 6) and in elastase-treated PRKC (elastase vs control, $p<0.001$; Fig. 6). AdPKC-ζ inhibited the elastase-induced activation of Caspase-3 [AdPKC-ζ DN+elastase vs AdLacZ+elastase (data not shown), $p<0.001$; Fig. 6]. In contrast, pCMVPKC-ζ increased the elastase-induced activation of Caspase-3 [pCMVPKC-ζ+elastase vs pCMVcDNA3.1+elastase (data not shown), $p<0.001$; Fig. 6].

DNA fragmentation was increased in rats livers during cerulein-induced pancreatitis (acute pancreatitis vs control, $p<0.001$; Fig. 7) and in elastase-treated PRKC (elastase vs control, $p<0.001$; Fig. 7). AdPKC-ζ DN attenuated the elastase-induced DNA fragmentation in PRKC [AdPKC-ζ DN+elastase vs AdLacZ+elastase (data not shown), $p<0.001$; Fig. 7). pCMVPKC-ζ increased the elastase-induced DNA fragmentation in PRKC [pCMVPKC-ζ+elastase vs pCMVcDNA3.1+elastase (data not shown); Fig. 7].

Discussion

Liver injury is an important prognostic indicator in acute pancreatitis, trauma, and sepsis. We have demonstrated that during acute pancreatitis extrapancreatic, resident macrophage-derived proinflammatory cytokines induce biochemical and histomorphologic injury in distant organs such as the lungs and liver.

Specifically, we demonstrated that Kupffer cell-derived TNF and Fas/FasL induce liver injury and hepatocyte apoptosis via NF-κB[2,4,5,14,15] in both in vivo and in vitro models of acute pancreatitis. Pancreatic enzymes that gain access to the systemic circulation through the inflamed retroperitoneum are probably the link between the localized inflammation in the pancreas and the propagation of the

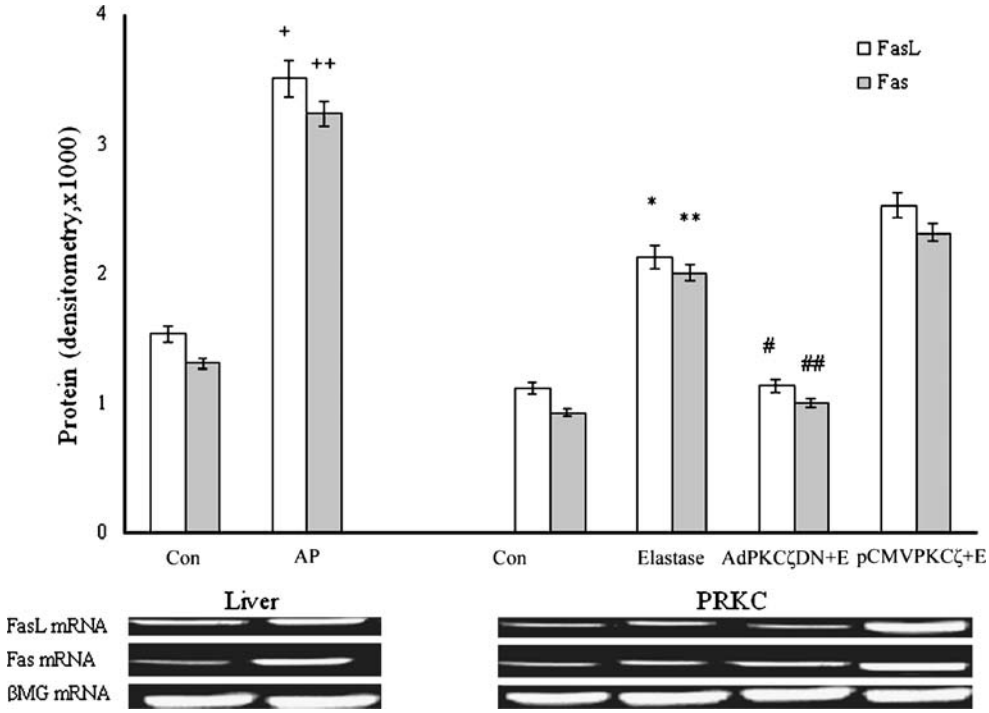

Figure 5 Fas/FasL protein and mRNA increased in livers after acute pancreatitis (*AP*) as compared to control (*Con*, *plus sign*, *double plus sign* $p<0.001$), and in elastase-treated (*E*) PRKC (*asterisk*, *double asterisk*, $p<0.001$ vs Con). AdPKC-ζ DN attenuated the elastase-induced upregulation of Fas/FasL protein and mRNA (*number sign*, *double number sign* Fas/FasL protein, $p<0.001$ vs AdLacZ+E, not shown); pCMVPKC-ζ did not augment the elastase-induced upregulation of Fas/FasL protein ($p>0.05$ vs pCMVcDNA3.1, data not shown), but augmented Fas/FasL mRNA. Panel is a representative RT-PCR, bar graph is densitometric quantification of $n=3$ immunoblots.

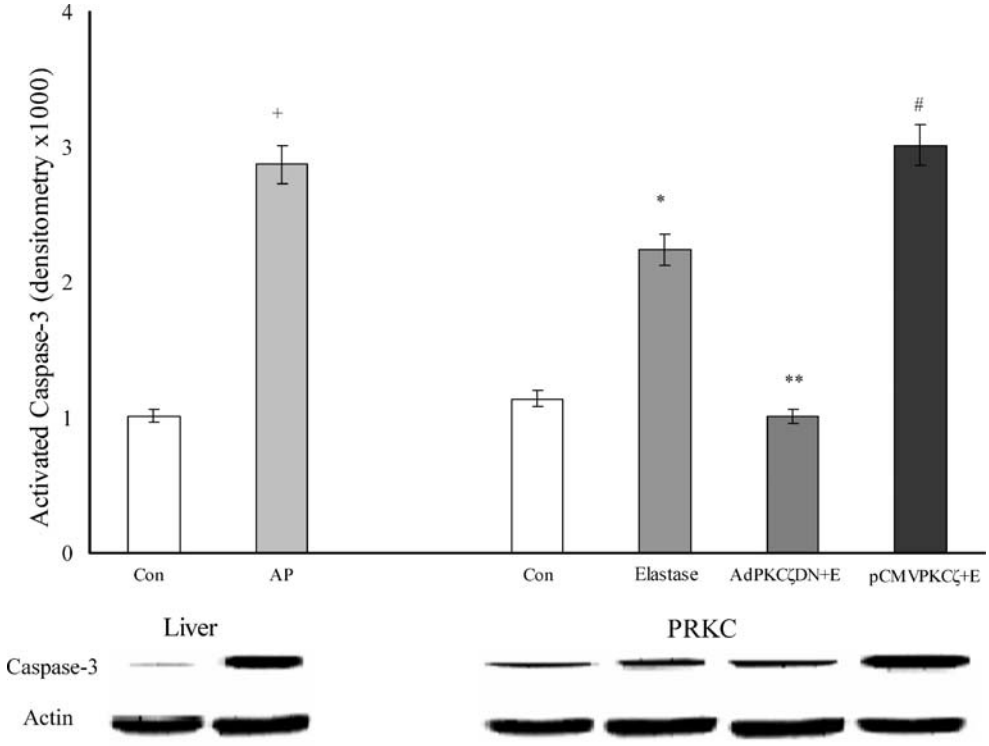

Figure 6 Activated Caspase-3 increased in livers after acute pancreatitis (*AP*) as compared to control (*Con*; *plus sign* $p<0.001$), and in elastase-treated (*E*) PRKC (*asterisk* $p<0.001$ vs Con). AdPKC-ζ DN attenuated (*double asterisk* $p<0.001$ vs AdLacZ+E, data not shown), whereas pCMVPKC-ζ augmented (*number sign* $p<0.001$ vs pCMVcDNA3.1+E, not shown) the elastase-induced Caspase-3 activation. Lower panel is a representative gel of cleaved Caspase-3; bar graph is densitometric quantification of $n=3$ immunoblots.

systemic effects of pancreatitis[16] or sepsis[17]. Pancreatic elastase induces proinflammatory cytokine production from Kupffer cells that in turn induce liver injury and hepatocyte death indistinguishable from acute pancreatitis. [2,4–7,12]

More importantly, we found that Kupffer cells undergo accelerated apoptosis and upregulate their Fas/FasL during acute pancreatitis, thereby suggesting that Kupffer cells may modulate their stress response by upregulating their own cell death receptors.[4,6,18,19] This poses a provocative hypothesis that the severity of liver injury in experimental pancreatitis is not only influenced by activation of Kupffer cells, but also by stress-induced apoptosis of Kupffer cells.

Inhibition of NF-κB transcriptional activity by over-expression of mutant IκBα or by siRNA[6,7] attenuates upregulation of Fas/FasL and reduces Kupffer cell apoptosis. Since PKC-ζ and PKC-λ are important in TNF/IL-1β signaling that regulates NF-κB, we hypothesized that upstream kinases play a central role in Kupffer cells'

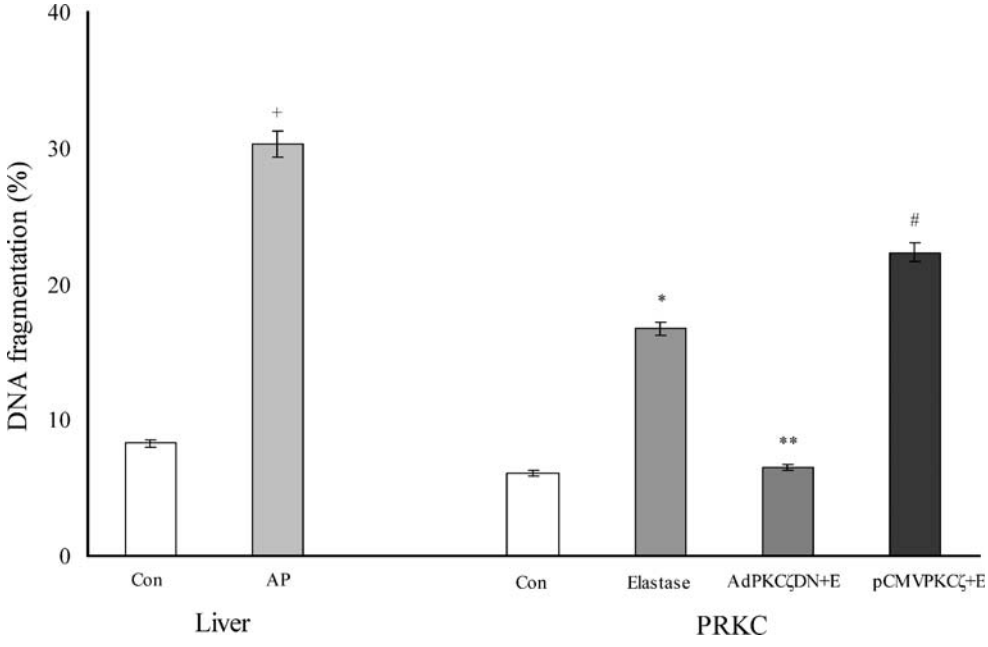

Figure 7 DNA fragmentation increased in livers after acute pancreatitis (*AP*; *plus sign* $p<0.001$ vs Con), and in elastase-treated (*E*) PRKC (*asterisk* $p<0.001$ vs control). AdPKC-ζ DN attenuated (*double asterisk* $p<0.001$ vs AdLacZ+E, data not shown), whereas pCVMPKC ζ augmented (*number sign* $p<0.001$ vs pCMVcDNA3.1+E, data not shown) the elastase-induced DNA fragmentation.

stress response and undertook this study to characterize the role of PKC-ζ in pancreatitis-induced apoptosis of Kupffer cells.

We utilized an in vivo model of cerulein-induced pancreatitis and verified our findings in vitro by using pancreatic elastase as a surrogate of pancreatitis. Pancreatic enzymes that leak into the systemic circulation induce a pattern of injury indistinguishable from that of acute pancreatitis in the lung and liver.[2,5,12,20]

Cerulein-induced acute pancreatitis upregulates PKC-ζ expression and activity, upregulation of IKKβ, IKKγ, nuclear translocation of p65/NF-κB, Fas/FasL, cleavage of Caspase-3, and DNA fragmentation in rat livers as well as Kupffer cells. To further confirm the central role of PKC-ζ in apoptosis of Kupffer cell, we used a dominant-negative adenovirus (AdPKC-ζDN) that induces a mutated and inactive PKC-ζ protein, and in other experiments, we used pCMVPKC-ζ to overexpress PKC-ζ. AdPKC-ζ DN significantly attenuated the elastase-induced upregulation of PKC-ζ activity, IKKβ, IKKγ, nuclear translocation of p65/NF-κB, Fas/FasL, Caspase-3, and DNA fragmentation. As expected, overexpression of PKC-ζ augmented the elastase-induced upregulation of PKC-ζ levels and activity, IKKβ, IKKγ, Fas/FasL mRNA, Caspase-3, and DNA fragmentation but had no effect on Fas/FasL protein or nuclear translocation of p65/NF-κB.

Our finding that PKC-ζ is upregulated in the liver and Kupffer cells is consistent with recent reports of the essential role of PKC-ζ in endotoxin-induced macrophage activation and nuclear translocation of p65/NF-κB.[21] Additionally, PKC-ζ has been implicated in the activation of NF-κB in response to several stimuli.[21–23] Others have reported that phosphorylation of RelA Ser311 by PKC-ζ is important in transcriptional activation of NF-κβ[24] and that PKC-ζ modulates IKKs activity in macrophages.[25]

Although IKKβ and IKKγ are upregulated, IKKα exhibited no change in liver or Kupffer cells. IKKα and IKKβ contain similar kinase domains with essentially identical activation loops. However, they are functionally distinct; IKKβ is essential for NF-κB activation in response to proinflammatory and innate immune stimuli, whereas IKKα is not required for such a response.[24]

Conclusion

PKC-ζ plays a central role in pancreatitis-induced Kupffer cell apoptosis via NF-κβ. The complex interplay between PKC-ζ, NF-κβ, and IKKs warrants further investigation. Autoregulation of the stress response by Kupffer cells cannot be overemphasized and may offer insight on the variability of the stress response and systemic inflammation.

Acknowledgment M.M. was supported by the Dr. Bob Haines Pancreatitis Research Fund and received a VA Merit Award.

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J Gastrointest Surg (2007) 11:1262–1267
DOI 10.1007/s11605-007-0213-0

Altered Esophageal Motility and Gastroesophageal Barrier in Patients with Jejunal Interposition After Distal Esophageal Resection for Early Stage Adenocarcinoma

Georg R. Linke · Jan Borovicka · Radu Tutuian · Rene Warschkow · Andreas Zerz · Jochen Lange · Michael Zünd

Received: 8 May 2007 / Accepted: 10 June 2007 / Published online: 12 July 2007

Abstract

Introduction Limited resection of the esophagogastric junction has been proven to be safe and oncologically radical in patients with early esophageal cancer. Reconstruction with interposition of isoperistaltic jejunal loop (Merendino procedure) is supposed to prevent gastroesophageal reflux and therefore the recurrence of intestinal metaplasia at the anastomosis. The aim of this study was to assess the frequency of acid and nonacid refluxes after Merendino procedure using multichannel intraluminal impedance-pH (MII-pH) monitoring.

Patients and Methods Between 2002 and 2005, 12 patients with esophageal adenocarcinoma underwent limited resection and jejunal interposition. Ten patients agreed to undergo a Gastrointestinal Symptom Rating Scale assessment, upper gastrointestinal (GI) endoscopy, esophageal manometry, and combined 24-h MII-pH monitoring more than 10 months postoperatively.

Results Postoperatively, 4 (40%) patients reported belching without heartburn or acid regurgitation, 3 of them having a positive symptom index during 24-h MII-pH monitoring. Upper GI endoscopy revealed no inflammation, metaplasia, or stenosis at the esophagojejunal anastomosis. Esophageal manometry showed ineffective esophageal motility in four of ten patients. Combined 24-h MII-pH monitoring revealed normal distal esophageal acid exposure (% time pH<4: 0.1% [0–1.5]), normal number of acid reflux episodes (3 [0–11]) but a high number of nonacid reflux episodes (82 [33–184]). Overall, eight patients revealed an abnormal number of nonacid reflux episodes.

Conclusion The limited resection with jejunal interposition for early esophageal cancer is efficient in controlling acid but not nonacid reflux. While the clinical relevance of nonacid reflux in the recurrence of Barrett's esophagus is currently unknown, endoscopic surveillance should be considered in these patients.

Keywords Esophagus · Early carcinoma · Merendino procedure · Gastroesophageal reflux · Multichannel intraluminal impedance and pH monitoring

Poster presented at the Digestive Disease Week, May 21, 2007, Washington DC.

G. R. Linke (✉) · R. Warschkow · A. Zerz · J. Lange · M. Zünd
Department of Surgery, Kantonsspital St. Gallen,
9007 St. Gallen, Switzerland
e-mail: gelinke@web.de

J. Borovicka
Division of Gastroenterology and Hepatology,
Department of Internal Medicine, Kantonsspital St. Gallen,
9007 St. Gallen, Switzerland

R. Tutuian
Division of Gastroenterology and Hepatology,
University Hospital of Zurich,
8091 Zurich, Switzerland

Introduction

In early stage esophageal adenocarcinoma, treatment options vary from radical esophagectomy to limited surgical resection of the esophagogastric junction and endoscopic mucosa resection. Without disregarding the principles of radical oncological resection, the limited surgical resection with regional lymphadenectomy appears to be sufficient with lower morbidity and mortality compared to radical esophagectomy.[1] After endoscopic mucosa resection, the local

recurrence rate may reach 30% as reported[2] due to remaining dysplastic or metaplastic Barrett's epithelium and persistence of gastroesophageal reflux. As well as after esophagectomy and gastric tube reconstruction, the proximal esophagus remains exposed to high amounts of gastroesophageal reflux, and new development of metaplastic esophageal columnar mucosa is described in up to 47%.[3]

Performing a reconstruction by interposition of an isoperistaltic jejunal segment after limited resection (Merendino procedure) is expected to prevent gastroesophageal reflux and is reported to achieve excellent functional results and a good quality of life.[4]

However, there are few data about the antireflux and motility properties of the jejunal interposition. Functional evaluations of the remaining esophagus after Merendino procedure were performed by fluoroscopy[5,6], alimentary scintigraphy[7], and 24-h pH metry[4,8] so far.

Recently, multichannel intraluminal impedance monitoring[9] has been validated for the detection of bolus movement in the esophagus and is able to characterize physical (liquid, gas, or mixed) or chemical (acid, nonacid) gastroesophageal refluxates.

The aim of our study was to evaluate esophageal function and antireflux characteristics of the effect of jejunal interposition after Merendino procedure by means of manometry and 24-h pH intraluminal impedance monitoring (MII-pH).

Patients and Methods

A limited resection of the distal esophagus and esophagogastric junction together with a regional lymphadenectomy and reconstruction by interposition of an isoperistaltic jejunal segment (Merendino procedure) was performed in histologically confirmed early Barrett's carcinoma (i.e., T1 carcinoma). Preoperative staging included upper gastrointestinal (GI) endoscopy with biopsy, endosonography, and computed tomography, whereas endosonography was the major criterion to determine tumor invasion into the esophageal wall.

Surgery

From October 2002 to November 2005, Merendino procedure was performed as a modification of the technique originally described by Merendino and Dillard[10] in 12 patients. After complete lymphadenectomy, the distal esophagus, esophagogastric junction, and subcardiac part of the stomach were completely mobilized and resected. The gastric resection line started 3 cm distal to the esophagogastric junction on the small curvature. The resection included the fundus and partial proximal corpus, representing about 25% of the gastric body. The vagal nerves were resected, and a pyloric dilatation was performed to optimize gastric emptying. The reconstruction was performed with an interposed retrocolic pedicled jejunal segment. Anastomoses were built end to side using circular staplers, a 25-mm stapler for the esophagojejunal anastomosis and a 28-mm stapler for the jejunogastrostomy, respectively (Autosuture™, Tyco Healthcare, Norwalk, CT, USA). The jejunogastrostomy was placed to the anterior wall of the stomach (Fig. 1).

Functional Evaluation

Ten or more months after limited resection, 10 patients [2 women, aged 60 years (43–72), BMI 24.6 kg/m^2 (19.9–31)] agreed to follow-up examination of the esophageal function and underwent stationary esophageal manometry and

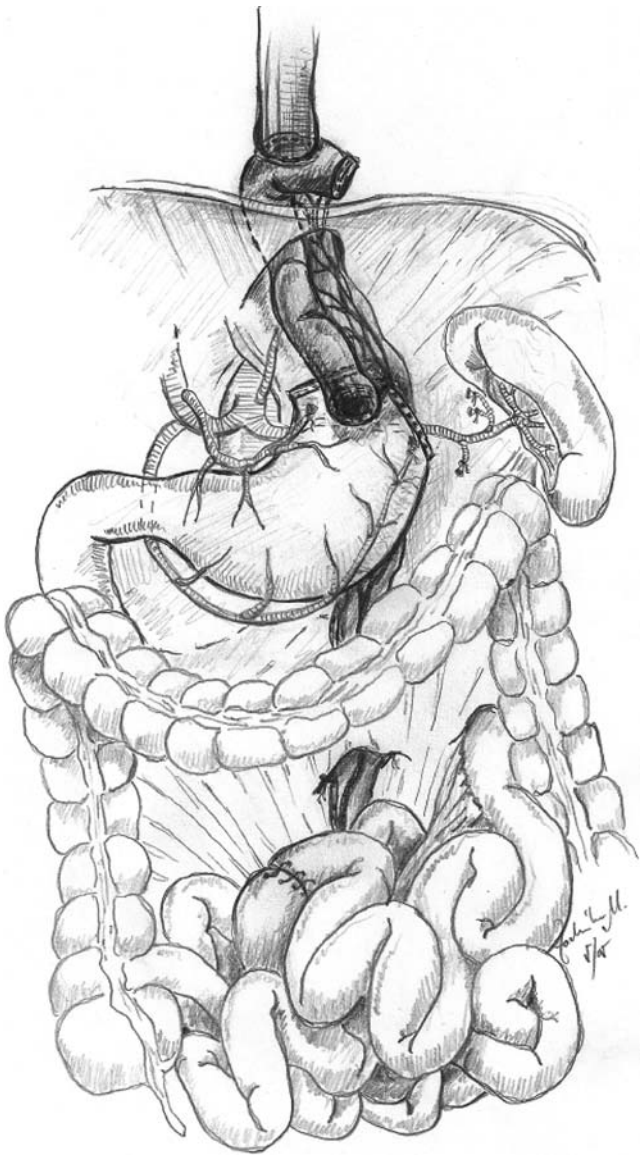

Figure 1 Representation of the limited resection of the gastroesophageal junction and reconstruction by a pedicled isoperistaltic jejunal interposition (Merendino procedure).

ambulatory 24-h MII-pH. Before these exams, an upper GI endoscopy was conducted to exclude local recurrence, to localize and measure the proximal and distal anastomosis with the length of the jejunal interposition, as well as to detect reflux lesions. Symptoms were assessed by the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire[11] containing 15 items, each rated on a 7-point Likert scale from no discomfort to very severe discomfort. The response scale was interpreted as follows: (1) no discomfort; (2) slight discomfort; (3) mild discomfort; (4) moderate discomfort; (5) moderately severe discomfort; (6) severe discomfort; (7) very severe discomfort.

Manometry

Esophageal manometry was performed with an eight-channel assembly incorporating a sleeve sensor (A-E27-LOSS-2, Dentsleeve Pty, Wayville, Australia) to record pressure at the esophagojejunal anastomosis. Intrajejunal pressure was recorded through a side hole located 1 cm beyond the distal margin of the sleeve. Side holes at the proximal margin of the sleeve and 5, 10, 15, 20, and 26 cm more proximal recorded motility in the esophageal body. The sleeve and the other esophageal side holes were perfused with degassed distilled water at a rate of 0.45 ml/min with a low-compliance pneumohydraulic capillary infusion system. Each lumen was connected to external pressure transducers (SEDIA, Freiburg, Switzerland).

The location of the anastomosis was determined by the lack of swallow-induced esophageal motility patterns during stationary pullthrough. The manometrically measured distance was then compared to the location of the anastomosis as defined by upper GI endoscopy. The difference between manometrical and endoscopical locations was estimated to be ideally 5 cm due to the different reference points (nose and teeth). Ineffective esophageal motility (IEM) was defined as the presence of more than 30% of nontransmitted or low-amplitude (i.e., distal peristaltic amplitude <30 mmHg) contractions during convential manometry as published by Richter et al.[12]

24-h MII-pH

The principle and technical issues of 24-h MII-pH were described by Tutuian and Castell[9] elsewhere. Ambulatory MII-pH monitoring was performed using a 6-impedance 1-pH catheter (Sandhill Scientific, Highlands Ranch, Colorado, USA). The design of the catheter allowed recording impedance data −2, 0, 2, 4, 10, and 12 cm above the esophagojejunal anastomosis and pH data at the esophagojejunal anastomosis. In those patients on medication, MII-pH was performed after cessation of proton pump inhibitor therapy for 10 days. The MII-pH catheter was passed transnasally and positioned with the pH probe and the second impedance rings at the esophagojejunal anastomosis as located by manometry and endoscopy. During the monitoring period, patients were asked to record the timing and consistency of ingested meals, periods of upright and recumbent position, and the type (up to three symptoms) and time of symptoms.

After a 24-h period of data acquisition, tracings were edited and initially analyzed using a software program (BioView Analysis™, Sandhill Scientific). Subsequently, MII-pH data were reviewed by an experienced investigator for artefacts and accuracy of reflux events (Fig. 2). The symptom index (SI) was calculated as the percentage of symptoms preceded by a reflux event detected by MII or drop in esophageal pH below 4 within a 5-min time frame divided by the total number of symptoms. A positive SI was defined as SI>50%. Previously published normal value data were used to assess if patients had a normal or abnormal number of reflux episodes.[13]

Statistics

Statistical analysis was performed using the SPSS 11.5.1 software (SPSS, Chicago, Illinois, USA). Data are reported as median (range). The normality of data distribution was

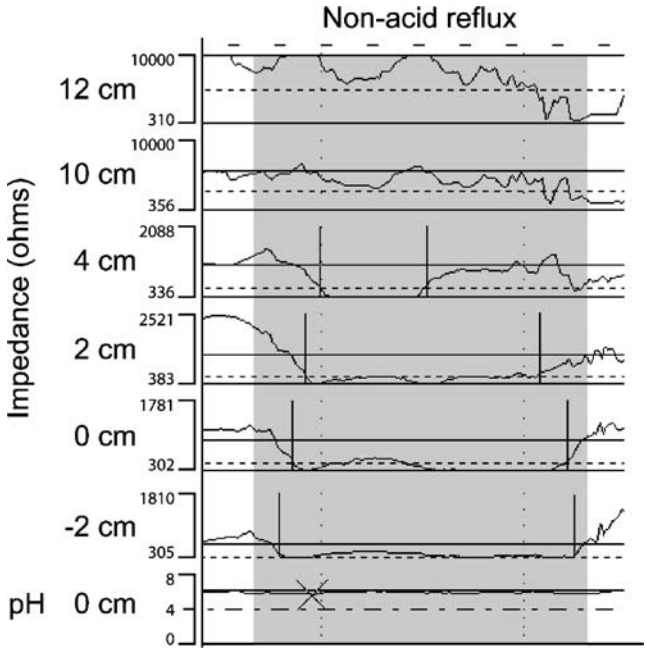

Figure 2 Example of impedance-pH recording of a nonacid reflux episode. Impedance-measuring segments are located −2, 0, 2, 4, 10, and 12 cm above the esophagojejunal anastomosis and the pH sensor at the anastomosis. Gastroesophagael reflux episodes are identified as rapid decline in impedance starting in the distal esophagus and advancing over time to the proximal esophagus. Nonacid reflux episodes are impedance-detected reflux episodes with a nadir pH above 4 (acid reflux episodes are impedance-detected reflux episodes with a nadir pH below 4).

Table 1 24-h MII-pH Monitoring

	Median (range)	*p* value
Acid exposure (%)		
Total	0.1 (0–1.5)	
Upright	0.05 (0–3.8)	
Recumbent	0 (0–0.6)	
Number of reflux events (liquid and mixed)		
Total	82 (33–184)	
Upright	53 (16–119)	
Recumbent	18 (8–78)	($p<0.05$)[a]
Number of reflux events based on pH		
Acid	3 (0–11)	
Nonacid	79 (29–175)	($p<0.005$)[b]
Number of proximal reflux events		
Total	31 (11–62)	
Upright	23 (5–41)	
Recumbent	5 (1–23)	($p<0.05$)[c]

Results of 24-h MII-pH monitoring in patients after limited resection and reconstruction by isoperistaltic jejunal interposition ($n=10$). Values are median (range).
[a] Upright vs recumbent
[b] Acid vs nonacid
[c] Upright vs recumbent

assessed by Kolmogorov–Smirnov analysis. A two-sided error probability of $p<0.05$ was considered statistically significant. Differences in mean number of reflux episodes were compared by paired *t* test.

Results

Symptoms

According to the GSRS questionnaire with the use of the 7-point Likert scale, one patient reported mild acid reflux, whereas nine out of ten patients had no reflux symptoms at all. The median reflux syndrome score was 1 (range 1–2). Three of ten patients reported moderate discomfort of diarrhea (2.3; range 1–4), and two patients suffered from moderate indigestion (2.1; range 1–4.5). One patient reported moderate abdominal pain (1.7; range 1–4), and no one suffered from clinically relevant constipation (1.7; range 1–3).

Upper GI Endoscopy

Upper GI endoscopy was performed 13 months (range 4–36) after surgery. The esophagojejunal anastomosis was located 32 cm (range 30–38) from the teeth. The length of the jejunal interposition as measured by endoscopy was 20 cm (range 10–35). In all patients, there were no endoscopic findings of erosive reflux disease. A local recurrence or stenosis was excluded in all patients.

Manometry

Distal contraction amplitudes were 41 mmHg (range 21–106). IEM was found in four out of ten patients but no case of esophageal aperistalsis.

MII-pH

MII-pH was performed 15 months (range 10–37) after surgery. The MII-pH monitoring results are shown in Table 1. Although distal esophageal acid exposure was normal, the median total number of liquid-containing (i.e., liquid and mixed) reflux events was increased. Eight out of 10 (80%) patients had an abnormal number of reflux episodes when compared to healthy volunteers (>73 reflux episodes/24 h). Reflux events occurred more often in upright position ($p<0.05$). Thirty-eight percent of reflux episodes reached the proximal esophagus 12 cm above the esophagojejunal anastomosis. These proximal reflux episodes also occurred more often in upright position ($p<0.05$).

The recorded symptoms and the calculated SI are shown in Table 2. The most frequently reported symptoms were belching (four patients) and coughing (three patients). Four patients recorded two different symptoms, four patients recorded one symptom only, and two patients recorded no symptom during the 24-h period of data acquisition.

With regards to reflux symptoms, one patient reported acid regurgitation with a negative SI of 33% and no acid reflux event. Four patients reported belching, three of them having a positive SI. All but one reflux-related symptoms were correlated with nonacid reflux.

The only patient reporting mild acid reflux within the GSRS questionnaire was that with the shortest jejunal interposition of 10-cm length and the highest number of reflux events detected by MII-pH.

Table 2 Symptoms Reported by Each Patient During the 24-h Period of 24-h Intraluminal Impedance Monitoring

Patient	Symptom 1 (RR/OC)	Symptom 2 (RR/OC)	Symptom 3 (RR/OC)
1	Epigastric pressure (0/1)	–	–
2	None	–	–
3	Belching (7/15)	Cough (11/30)	–
4	*Belching (1/1)*	–	–
5	*Belching (33/52)*	*Cough (11/18)*	–
6	*Belching (22/26)*	*Pain (1/1)*	–
7	Acid regurgitation (1/3)	–	–
8	*Epigastric pressure (1/1)*	–	–
9	None	–	–
10	Abdominal pain (0/2)	Diarrhea (0/1)	–

The symptom index (SI) is calculated as the percentage of symptoms preceded by a reflux event (RR) divided by the total number of symptoms (OC). A positive SI (RR/OC>50%) is marked by italicized letters.

Discussion

Limited resection and isoperistaltic jejunal interposition have been reported to be well tolerated and to prevent gastroesophageal reflux.[4–7] Using MII-pH, we could show for the first time that reflux (mainly nonacid reflux) is still present after the operation despite the jejunal interposition. However, as shown by others before[4–6,14], reflux symptoms in these patients after Merendino procedure are rare due to the lack of acid refluxates. During the MII-pH recording period, only one patient reported acid regurgitations, whereas the most frequently noted symptoms were belching and coughing. Wright and Cuschieri[14] assessed 19 patients after jejunal interposition for benign esophageal disease and found episodes of eructation of foul-smelling air as the only complaint by two patients. This subjective information correlates well with our MII-pH data demonstrating normal esophageal acid exposure in all patients. Similarly, Stein et al.[4] found no evidence of pathologic gastroesophageal reflux in six patients who volunteered for postoperative pH monitoring. In contrast, Gutschow et al.[8] reported increased acid reflux in two patients after a Merendino procedure with preservation of the vagus.

Previous studies have evaluated the function of jejunal interposition by fluoroscopy and scintigraphy and found no evidence of reflux into the distal esophagus. In six patients studied by barium swallow under fluoroscopy, backward movement of the bolus was at maximum 8 cm[5] within a jejunal interposition of 15-cm length. Using scintigraphy, Stier et al.[7] found no evidence for bolus reflux from the jejunal loop into the distal esophagus during the investigation of eight patients.

We used the new technique of MII-pH monitoring to detect even the smallest bolus movements and reflux events independent of pH. The conflicting results with barium swallow and scintigraphy are explained by the low sensitivity of both methods to detect reflux. Using MII-pH, we documented an abnormal number of reflux events in eight of the ten patients with an increase in reflux events compared to healthy volunteers. Correlating the reflux episodes with concomitant detected intraluminal pH identified the majority as nonacid. Two reasons may explain the lack of acidity. First, the reduction in acid secretion by vagotomy and proximal gastric resection, and second, the length of the jejunal interposition. Additionally, the alkaline secretions in the jejunal interposition may buffer acid reflux.

In our patients after Merendino procedure, reflux events occurred more often in the upright than in the recumbent position. Previous studies using MII-pH in healthy volunteers also found reflux events to be more frequent in upright position.[13] In contrast, Oberg et al.[3] evaluated 32 patients after esophagectomy and gastric tube reconstruction by means of 24-h pH monitoring and found increased cervical acid exposure mainly due to reflux in recumbent position. This indicates that the isoperistaltic jejunal interposition behaves like a physiological sphincter although by different mechanism. In healthy volunteers, upright reflux is supposed to be a result of frequent transient lower esophageal relaxations.[15] After Merendino procedure, the gradient between positive intraabdominal pressure and negative intrathoracic pressure as well as the loss of the hiatal sling and of the angle of His should be mostly responsible to promote reflux across the interposition.[16] In the recumbent position, reflux is prevented effectively by the isoperistaltic jejunal interposition.

In our patient collective, the jejunal interposition was at median 20 cm ranging from 10 to 35 cm measured by endoscopy. Skinner and Merendino originally described a jejunal segment of 15 cm as adequate.[10] In the following, others employed jejunal segments varying from 8 to 30 cm.[7,14] Although the data were not statistically significant, we observed a negative correlation between proximal reflux events and the total of reflux episodes with the length of the jejunal interposition. The only patient reporting mild acid reflux within the GSRS questionnaire was that with the shortest jejunal interposition of 10-cm length and the highest number of reflux events detected by MII-pH. These results confirm the observations by Moylan et al.[5] that the length of the jejunal interposition should be at least 15 cm.

In 32 patients who have undergone esophagectomy and gastric tube reconstruction, Oberg et al.[3] found 15 patients with recurrent Barrett's epithelium having a significantly higher prevalence of acid exposure. Another study found recurrent Barrett's epithelium in 18% after esophagectomy for treatment of high-grade dysplasia or localized adenocarcinoma.[17] After limited resection and jejunal interposition, Stein et al. found in 88% of 49 patients with a mean follow-up of 41 months no evidence of esophagitis on endoscopy.[18] In our patient collective with a short follow-up period of 13 months, endoscopic surveillance revealed no esophageal erosions or recurrent Barrett's epithelium. To which extent nonacid reflux carries bile acids was not assessed in this study. Further endoscopies at defined time intervals will be required to rule out recurrence of metaplasia and neoplastic disease at the esophagojejunal anastomosis.

Conclusion

Based on our findings, we conclude that jejunal interposition after limited resection for early stage adenocarcinoma is efficient in controlling acid but not nonacid reflux. Thus, a routine therapy with proton pump inhibitors is not necessary postoperatively. Still, while the clinical relevance of nonacid reflux in the recurrence of Barrett's esophagus is currently unknown, endoscopic surveillance should be considered in these patients.

Acknowledgment Georg Linke and Jan Borovicka contributed equally to this work. The authors thank Dr. M. Zadnikar for drafting the manuscript figure.

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J Gastrointest Surg (2007) 11:1268–1274
DOI 10.1007/s11605-007-0241-9

Pneumatosis Intestinalis in Adults: Management, Surgical Indications, and Risk Factors for Mortality

Alexander J. Greenstein · Scott Q. Nguyen · Ana Berlin · Jacqueline Corona · Jonathan Lee · Everlyn Wong · Stephanie H. Factor · Celia M. Divino

Received: 12 June 2007 / Accepted: 4 July 2007 / Published online: 9 August 2007

Abstract
Background Pneumatosis intestinalis (PI) is an unusual finding that can exist in a benign setting but can indicate ischemic bowel and the need for surgical intervention. We present a series of cases of PI in adults to illustrate factors associated with death and surgical intervention.
Methods We reviewed the radiology database of the Mount Sinai Medical Center for cases of PI between 1996–2006 in adult patients. Chi-square and multivariable logistic regression analyses were used to identify factors significant for surgery and death.
Results Forty patients developed PI over a 10-year span. The overall in-hospital mortality rate was 20%, and the surgical rate was 35%. Factors independently associated with surgical management on multivariable analysis were age≥60 years (p=0.03), the presence of emesis (p=0.01), and a WBC>12 c/mm^3 (p=0.03). Pre-existing sepsis was independently associated with mortality (p=0.03) while controlling for surgery.
Conclusion Patients with the concomitant presence of PI, a WBC>12 c/mm^3, and/or emesis in the >60-year-old age group were most likely to have surgical intervention, whereas PI patients with sepsis had the highest risk for death. A management algorithm is proposed, but further research will be needed to determine which patients with PI may benefit most from surgery.

Keywords Pneumatosis · Intestinalis · Gas · Cyst · Pneumoperitoneum · Ischemia

Introduction

Pneumatosis intestinalis (PI), characterized by subserosal or submucosal gas-filled cysts of the GI tract that are typically diagnosed on CT imaging or gross pathology, is a rare but concerning condition. In some cases, PI is an inconsequential finding, but in others, it represents a life threatening intra-abdominal condition. As many patients are asymptomatic and do not come to clinical attention, the overall incidence of PI is difficult to ascertain. In the years since its discovery by DuVernoi during a cadaveric dissection in 1730,[1] there has been an evolution in the understanding of this entity, but as it is encountered in such a diverse array of clinical settings, its implications are still often misinterpreted.

With a foundation based on Koss' review of 213 cases from 194 papers in 1952,[2] PI has been descriptively divided into primary (idiopathic) and secondary pneumatosis, with approximately 85% of cases considered to be of secondary nature. Primary PI typically consists of colonic cystic collections of air and can be identified either radiologically or pathologically. Secondary PI has been attributed to a host of clinical diseases with a wide range of severity and is identified by linear or circumferential air in any part of the gastrointestinal (GI) tract.[3]

Categories of associated conditions are mechanical, inflammatory, autoimmune, infectious, pulmonary, and

A. J. Greenstein · S. Q. Nguyen · A. Berlin · J. Corona · J. Lee · E. Wong · C. M. Divino (✉)
Department of Surgery, The Mount Sinai Medical Center, 5 E. 98th Street, 15th Floor, P.O. Box 1259, New York, NY 12029, USA
e-mail: Celia.Divino@msnyuhealth.org

S. H. Factor
Department of Medicine, The Mount Sinai Medical Center, New York, NY, USA

medication induced.[4] Whereas earlier case reports tended to focus on the association of ulcers and cancer with PI,[2] more recent reports and reviews have shown an association of immunosuppression and autoimmune disease with PI.[5–7] Whereas the development of PI can be complicated by GI obstruction or bleeding, this is an unusual occurrence, and several studies have described large and persistent cysts that have had no physiologic effect.[8]

The source of gas required for cyst formation is not clear, and pulmonary, intraluminal, and bacterial sources have been proposed. The pulmonary theory proposes that air travels from ruptured alveoli along the vasculature to the bowel wall.[9] The intraluminal theory suggests that air travels across the bowel wall because of pressure and/or mucosal compromise.[10,11] The bacterial theory hypothesizes that bacteria invade a compromised mucosal wall and then release hydrogen.[12,13] A combination of factors is also possible, with pulmonary obstruction, for example, leading to intraabdominal pressure fluctuations that then enhance air movement across the compromised bowel wall.[4]

Although the definitive cause(s) of PI may never be known, review of the clinical histories of patients with PI can at least help guide clinicians in making management decisions. We conducted a large study among adult patients with PI to discover which associated factors favor surgical intervention over medical therapy and to identify risk factors for mortality.

Patients and Methods

We searched the radiology database of the Mount Sinai Medical Center for patients with PI using the keywords "Pneumatosis Intestinalis," "Pneumatosis Cystoides Intestinalis," and "Pneumatosis Coli" for computed tomography (CT) studies done between November 1996–November 2006 in patients ≥15 years of age. We reviewed the charts of all patients identified by the search and obtained information on underlying medical conditions (within the categories of inflammatory, autoimmune, infectious, and pulmonary disorders), concomitant diagnoses (such as sepsis and infection), presenting symptoms (such as emesis, pain, and diarrhea) laboratory results, radiology findings (such as bowel wall thickening, portal venous gas (PVG), free air and PI location), and hospital course. An attending radiologist reviewed all radiologic studies to confirm the findings given on radiologic reports. Sepsis was diagnosed based on chart review and was defined when infection was highly suspected or proven and two or more of the following systemic inflammatory response syndrome (SIRS) criteria were met: heart rate>90 beats per minute, body temperature<36°C or >38°C, respiratory rate>20 breaths per minute or a PaCO2 less than 32 mm Hg, white blood cell count<4,000 cells/mm^3 or >12,000 cells/mm^3, and more than 12% band forms in the white blood cell count differential.[14]

Statistical Analysis

To identify risk factors for mortality among PI patients, univariate analysis using chi-square, Fisher's exact, or *t* test, as appropriate, found associations between death and clinical factors including: underlying medical conditions, concomitant diagnoses, presenting symptoms, laboratory results, radiologic findings, and hospital course. Based on findings in the recent literature, as well as the averages of our variables, we chose to convert continuous variables into dichotomous variables using the following criteria—age≥ 60 years, bicarbonate≤20 mmol/l, WBC≥12 c/mm^3, and lactate≥2 mmol/l.[11,15,16] Only variables approaching statistical significance ($P \leq 0.1$) in univariate analysis that had complete data for at least 90% of all patients were analyzed using multivariable logistic regression to identify factors independently associated with in-hospital mortality. To identify indicators used to choose surgical candidates, similar univariate and multivariate analyses were performed.

To identify patient characteristics associated with mortality and non-surgical therapy, a sub-analysis was done among only those patients who were observed. Univariate analysis identified associations between mortality from surgery and the clinical factors as above. Findings from the sub-analysis were compared to the two previous

Table 1 Summary Data for 40 Patients with PI at the Mount Sinai Hospital (1996–2006)

Category	Number of Patients	Percent
Sex		
Male	23	58
Age Category (years)		
15–29	4	12
30–49	14	35
50–69	14	35
≥70	8	20
Distribution		
Small bowel	8	20
Large bowel	31	78
Both	1	2
Initial management		
Observation	24	60
Surgery	14	35
Crossover		
Observation→surgery	2	5
Outcome		
Survival	32	80
Mortality	8	20

analyses. Statistical calculations were completed using statistical software Statistical Package for the Social Sciences (SPSS) version 11.5 (SPSS, Inc., Chicago, IL). This study was approved by the Institutional Review Board of the Mount Sinai School of Medicine.

Results

The median age of the patients was 60 years old, 60% of the patients were between 40–70 years of age, and 58% were male. The overall surgical rate was 35%, and the mortality rate was 20% (Table 1). Of the two patients whose management changed from observation to surgery, one patient was treated for a week for a severe flare of ulcerative colitis before operative intervention. The other had a diagnosis of primary PI and was treated for 1 month with a combination of antibiotics and oxygen therapy before symptoms worsened and a decision was made to proceed with surgery. With exclusion of the latter two patients, both of whom survived, the mean delay to surgery (CT scan completion to incision in the operating room) was 5.0 h (standard deviation=8.3 h) with six of the seven patients (86%) taken to surgery in less than 6 h surviving. Only four of the seven (57%) taken to surgery in more than 6 h survived. All surgically treated patients had a laparotomy with diversion and/or bowel resection except for one patient who had an exploratory laparoscopy with only lysis of adhesions. Fifty percent of the eight patients who died came from the surgery group.

Many patients had more than one underlying condition and concomitant diagnosis (Table 2). The most common underlying conditions were immunosuppression (42%) and cancer (35%). Three of the 14 patients with cancer were directly obstructed by the cancer (one large bowel obstruction from colon cancer, one small bowel obstruction from lymphoma, and one from metastatic pancreatic cancer). Five of the patients had no significant past medical history and no associated diagnoses and, thus, received the diagnosis of primary PI.

Of presenting signs and symptoms (Table 2), pain was the most common symptom (82%) and emesis was the second most common (48%). The most prevalent radiologic findings were dilated bowel (45%) and bowel thickening (40%). Free air was noted in 22% of patients and PVG in 15% of patients. Two of the patients with free air had

Table 2 Underlying Disorders, Concomitant Diagnoses, Symptoms and Clinical Findings for 40 Patients with PI at the Mount Sinai Hospital (1996–2006)

	Number of Patients	Percent
Medical history		
Immunosuppressed	17	42
Cancer	14	35
Previous bowel resection	9	22
Steroid therapy	7	18
Pulmonary disease	6	15
Liver disease	6	15
History of transplant	5	12
Surgery in past 7 days	5	12
End stage renal (ESRD)	5	12
HIV/AIDS	5	12
Scleroderma	3	8
Lymphoma	2	5
BMT/GVHD	2	5
Inflammatory bowel disease	2	5
Sickle cell	1	2
Trauma in past 7 days	1	2
None	5	12
Concomittant diagnosis		
Bacterial Infection	12	25
Sepsis	7	18
Diverticultis	2	5
Appendicitis	2	5
Clostridium difficile	2	5
Symptoms		
Abdominal pain	33	82
Emesis	19	48
Fever, ≥38.5°C	15	38
Diarrhea	14	35
Guiac positive	12	25
Radiologic findings		
Dilated bowel	18	45
Bowel thickening	16	40
Free air	9	22
Portal venous gas	6	15

Table 3 Symptoms and Primary Indication for Intervention for Patients with PI (n=16) Undergoing Surgery at the Mount Sinai Hospital (1996–2006)

	Number of Patients	Percent
Symptoms		
Abdominal pain	16	100
Emesis	11	69
Diarrhea	7	44
Fever, ≥38.5°C	6	38
Guiac positive	6	38
Rebound	4	25
Guarding	3	19
Primary indication		
Necrotic bowel	6	38
Obstruction	5	31
Primary pneumatosis	2	13
Perforation	1	6
Appendicitis	1	6
Ulcerative colitis	1	6

recently had abdominal surgery and none of the patients with PVG had a history of sphincterotomy, recent bile duct manipulation, or liver transplant. Among the 16 patients who went to surgery, the two most prevalent indications for surgical intervention were necrotic bowel (38%) and obstruction (31%), and the most common physical findings that prompted intervention were abdominal pain (100%) and emesis (69%). The list of remaining diagnoses and findings can be found in Table 3.

Factors associated with death in univariate analysis were immunosuppression, liver disease, sepsis, hypotension, radiologic diagnosis of thickened bowel, and the lab values of bicarbonate≤20 mmol/l and lactate≥2 mmol/l. In multivariable analysis, sepsis was the only characteristic independently associated with death (Table 4).

In univariate analysis, factors significantly associated with surgery were PVG, pain, emesis, and age≥60 years, whereas a WBC>12 c/mm^3 approached statistical significance (p=0.06). In multivariable analysis, age≥60 years, a WBC>12 c/mm^3, and the presence of emesis were independently associated with procession to surgery (Table 5).

Twenty-four patients were included in a sub-analysis of patients who were treated without surgery, of whom four (17%) died. In univariate analysis, both sepsis (2:4 patients died, p=0.02) and liver disease (4:6 patients died, p=0.001) were found to be associated with an increased risk of death. Of the 11 patients with emesis who went to surgery, 9 (80%) had been diagnosed with either a complete or partial bowel obstruction. Only one of these nine patients died. Of the six patients with PVG, four (66%) were diagnosed with

Table 4 Risk Factors for Death and Selected Variables for Patients Diagnosed with PI at the Mount Sinai Hospital (1996–2006)

Risk Factor	Death (n=8)	Percent	Survive (n=32)	Percent	Univariate Analysis (n=40)			Multivariate Analysis		
					OR	95% CI	p Value	OR	95% CI	p Value
Age≥60 years										
Yes	4	50	10	31	2.2	0.5–11	0.28	–	–	–
No	4	50	22	69						
Gender										
Male	4	50	13	40	1.4	0.3–6.9	0.46	–	–	–
Female	4	50	19	60						
Surgical intervention[a]										
Yes	4	50	12	38	1.67	0.4–7.9	0.69	–	–	–
No	4	50	20	62						
Immunosuppressed										
Yes	6	75	11	34	5.73	1.0–33	0.05	–	–	–
No	2	25	21	66						
Liver disease										
Yes	4	50	2	6	15.0	2.0–112	0.01	–	–	–
No	4	50	30	94						
Sepsis										
Yes	5	62	2	6	25.0	3.3–189	0.03	14.0	1.3–146	0.03
No	3	38	30	94						
Emesis										
Yes	2	25	17	53	0.29	0.1–1.7	0.15	–	–	–
No	6	75	15	47						
Hypotension										
Yes	4	50	4	12	7.0	1.2–40	0.04	–	–	–
No	4	50	28	88						
Thickened Bowel[b]										
Yes	6	75	10	31	6.6	1.1–39	0.04	–	–	–
No	2	25	22	69						
Bicarbonate≤20 mmol/l										
Yes	5	63	6	20	6.7	1.2–36	0.03	–	–	–
No	3	37	24	80						
Lactate≥2 mmol/l										
Yes	6	75	3	25	9	1.1–71	0.03	–	–	–
No	2	25	9	75						

[a] Surgical Intervention means that the patient went to surgery after the diagnosis of PI
[b] Detected on CT Scan simultaneous to the diagnosis of PI

Table 5 Factors Associated with Surgery and Selected Variables for Patients Diagnosed with PI at the Mount Sinai Hospital (1996–2006)

Prognostic Factor	Surgery (n=16)	Percent	Observe (n=24)	Percent	Univariate Analysis (n=40)			Multivariate Analysis		
					OR	95% CI	p Value	OR	95% CI	p Value
Age≥60 years										
Yes	10	62	4	17	6.0	1.4–24.7	0.01	18.4	1.3–261	0.03
No	6	38	20	83						
Gender										
Male	7	44	10	42	1.1	0.3–3.9	0.58	–	–	–
Female	9	56	14	58						
Recent bowel operation[a]										
Yes	0	0	5	21	0.79	0.6–0.8	0.07	–	–	–
No	16	100	19	79						
Infection										
Yes	5	31	5	21	1.7	0.4–7.3	0.35	–	–	–
No	11	69	19	79						
Emesis										
Yes	11	69	8	33	4.4	1.1–17	0.05	21.7	2.0–225	0.01
No	5	31	16	67						
Pain										
Yes	16	100	17	71	1.5	1.1–5.4	0.03	–	–	–
No	0	0	7	29						
Portal Venous Gas[b]										
Yes	5	31	1	4	12.4	1.1–121	0.03	–	–	–
No	11	69	23	96						
WBC ≥ 12 c/mm^3										
Yes	9	60	7	29	3.6	0.9–14	0.06	17.8	1.3–238	0.03
No	6	40	17	71						
Bicarbonate≤20 mmol/l										
Yes	7	47	4	17	4.2	0.9–18	0.06	–	–	–
No	8	53	19	83						
Lactate≥2 mmol/l										
Yes	6	50	3	38	1.7	0.2–12	0.67	–	–	–
No	6	50	5	62						

[a] Recent operation=bowel resection within the past 7 days
[b] Detected on CT scan simultaneous to the diagnosis of PI

obstruction before surgery. All four of these patients went to surgery and all four survived.

Discussion

PI is a condition that potentially represents a life-threatening process. Because of its rarity, management has traditionally been driven more by speculation than by statistically verifiable conclusions. Using a large series of consecutive cases of PI in adults, we found that patients who are septic at the time of PI diagnosis are at high risk of poor outcome regardless of surgical status and that the concomitant presence of emesis, a WBC>12 c/mm^3, and/or an age greater than 60 years favored surgical management for PI.

This study confirms and extends the literature on PI in adults. Prior studies on PI in adults consist of numerous case reports, reviews,[2,4,8] and to our knowledge, a total of three series,[15–17] only one of which employed multivariable analysis of its data.[15] Because of high associated mortality rates, sepsis in PI patients has long been considered an indication for surgery. Specifically, case series by both Knechtle et al. and Hawn et al. found that metabolic acidosis associated with elevated lactate was a significant risk factor for death.[15,16] Our study confirms the high mortality risk associated with sepsis, as well as a lactate≥2 mmol/l. While it is possible that this patient group had a poor outcome as their PI had been in existence for a longer duration or was of a more severe form, it is also conceivable that they may have suffered from a fatal disease process unchangeable by surgical intervention. All of these patients had multi-organ decompensation, and their PI may have been secondary to the septic process,[3] rather than to a primary abdominal process. For both of the patients with sepsis who ultimately died, it was clearly

documented that surgery would have been "futile." While we cannot refute the utility of sepsis as an indication for surgery in PI patients, we suggest that the source of sepsis be considered before proceeding with surgery.

The combination of PI and PVG has previously been reported to have high fatality rate and typically; 70% of patients with PI and PVG have bowel ischemia.[17,18] In addition, we found that surgically treated PI patients with PVG (Fig. 1a) had a slightly decreased risk of death ($p>0.05$) compared to other PI patients, and we suggest that these patients should be considered for surgical intervention.

In our study, patients with emesis and a WBC>12 c/mm^3 were significantly more likely to receive surgery. Although the clinical diagnosis of obstruction was not a significant risk factor for mortality or predictor for surgery, its existence is often related to emesis, especially in the patient group that is not being actively decompressed by nasogastric suction. Of the patients with emesis and PI, 80% were diagnosed with either a partial or complete obstruction before surgery (Fig. 1b). Of the patients with obstruction, 75% had emesis, whereas the remainder without emesis were septic and receiving nasogastric decompression in the intensive care unit. Two of five of these patients did not receive surgical intervention and three of five ultimately died. Based on these results, we believe that the presence of obstruction should be considered during the surgical evaluation, but the presence of emesis most likely identifies the more salvageable subset of obstructed patients.

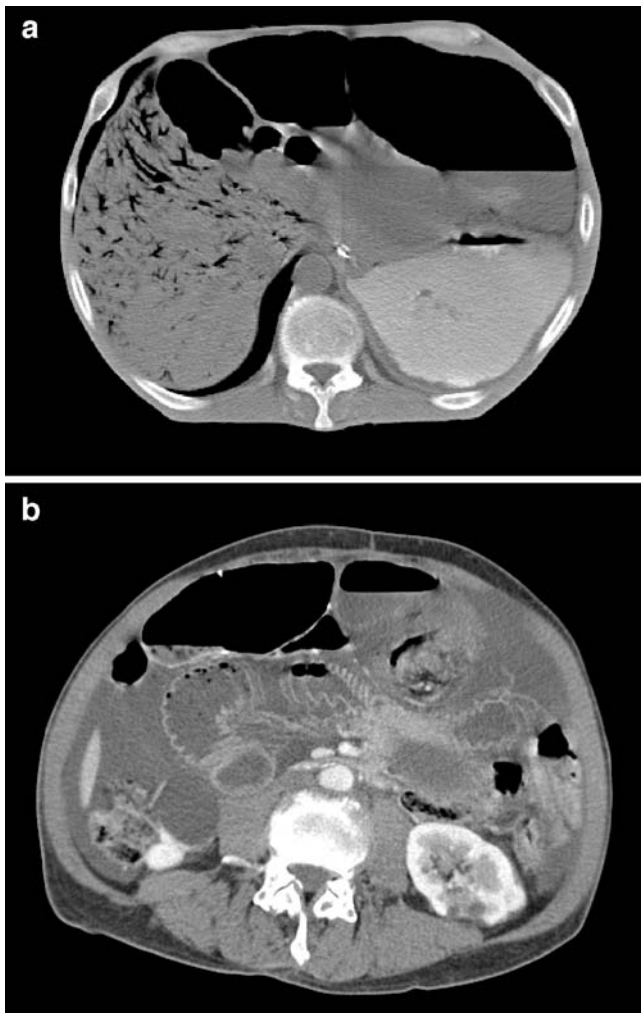

Figure 1 **a** Portal venous gas in a patient with PI. The majority of PI patients with portal venous gas have a surgically treatable condition. **b** PI in the presence of obstruction. PI patients with obstruction and emesis should be taken to surgery.

When presented with the finding of PI, it is important to efficiently categorize patients with PI into three groups—those who can benefit from surgery, those who are critically ill and may be harmed by surgery (or for whom surgery may be futile), and those who have benign pneumatosis. Moreover, it is important that this categorization process occurs rapidly, as is evidenced by the trend that patients who received operations with less of a delay (<6 h) had a lower mortality rate.

Based on our practices, findings from past series discussed above,[15–17] and recent reviews,[4,8] we propose a management algorithm for patients with PI (Fig. 2). Before proceeding, we advise that this algorithm be used as an instrument to assist with clinical decision making, not as an exclusive management guide. The initial assessment should

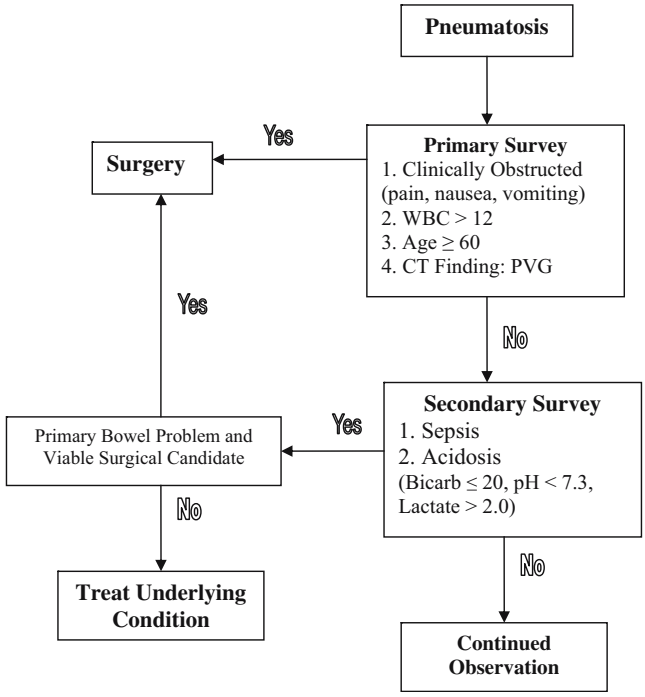

Figure 2 Management algorithm for adult pneumatosis intestinalis (*PI*). Patients with PI should be categorized into three treatment groups—(1) surgical intervention, (2) medical support, and (3) observation.

look for indicators for surgery including symptoms of obstruction (emesis, nausea, pain), a WBC>12 c/mm^3 and PVG on CT. Patients with one or more of these findings, especially those in the age group>60 years, should be considered for surgery. The secondary assessment should evaluate for evidence of sepsis,[16] a lactate level>2.0 mmol/l,[15] and a primary abdominal process. This group of patients will most likely have a high mortality rate, irrespective of the course of action taken, but patients with sepsis of presumed bowel etiology should be treated surgically.

While interpreting the results of our analysis, it is important to consider the strengths and limitations of our study. First, Mount Sinai Hospital is a major non-trauma tertiary care center with a large liver transplant program and many critically ill patients. For these reasons, our patients may not represent an accurate cross-section of all patients with PI. Nevertheless, indicators of a surgically treatable process in our PI patients are likely to work for other PI patients. Second, as autopsy use was inconsistent between patients and many patients were extremely complex, our study used mortality rather than PI-specific mortality as an endpoint. Finally, for the purposes of determining delay to operation and event sequences, we considered the existence of PI to start with its presence on the CT scan. While we could not know exactly when the PI process began, this is the case in the vast majority of clinical scenarios; moreover, this information is much less valuable than the concomitant clinical picture. The strengths of this study are that it is based on the outcomes of decisions made by a variety of clinicians and surgeons and that, in comparison to the available series, [15–17] it represents one of the larger groups of patients with PI.

As imaging techniques improve, it is likely that an increasing number of cases of PI will be detected. Given the potentially fatal consequences of this condition, it is critical to efficiently determine how a patient with PI should be managed. Although it is not possible at this point to definitively establish which patients with PI will benefit most from surgery, we propose that patients with emesis and/or a WBC>12 c/mm^3 concomitant with PI, especially those greater than 60 years of age, should be considered for surgery, and advise that those with sepsis are at high risk for mortality. If validated by larger prospective studies, these conclusions may be incorporated into management guidelines for PI.

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J Gastrointest Surg (2007) 11:1275–1285
DOI 10.1007/s11605-007-0242-8

GASTROINTESTINAL

Experimental Evaluation of Four Biologic Prostheses for Ventral Hernia Repair

Wolfgang B. Gaertner · Margaret E. Bonsack · John P. Delaney

Received: 22 May 2007 / Accepted: 4 July 2007 / Published online: 3 August 2007

Abstract
Purpose To evaluate two bioprostheses derived from bovine pericardium, one cross-linked (Peri-Guard®) and the other non-cross-linked (Veritas®), and to compare them with Alloderm® and Permacol® for abdominal wall repair.
Methods The four prostheses were tested in acute and chronic hernia models. Prostheses were either sutured to the edge of the abdominal wall defect (inlay) or secured as an underlay with surgical tacks. Evaluation at 3 and 6 months included adhesion formation, defect area size and thickness, tensile strength, and histology.
Results Mean adhesion coverage area ranged from 25 to 31%. The two cross-linked materials, Permacol and Peri-Guard, showed greater tensile strength. Significant defect contraction followed repair with Veritas, whereas Alloderm stretched. All prostheses had cellular ingrowth and neovascularization by 3 months. No significant differences were found in prosthesis to abdominal wall breaking strength. Operative site infection occurred in six animals (5 Peri-Guard, 1 Veritas), and overlying skin ulceration in six others (6 Peri-Guard).
Conclusions Permacol provided a strong and durable repair for up to six months. Peri-Guard was equally strong but prone to infection and to skin ulceration. With time, Veritas and Alloderm lost tensile strength associated with marked thinning and with hernia-like bulging in the case of Alloderm.

Keywords Biologic prostheses · Ventral hernia repair · Rat · Experimental

Introduction

Abdominal wall incisional hernia defects developing after laparotomy continue to be a surgical challenge. The incidence of incisional hernia after a primary abdominal operation ranges from 9 to 20%.[1–4] Approximately 200,000 ventral hernia repairs are performed each year in the United States. Subsequent recurrence rates after suture repairs range from 31 to 54%.[5] Hernia recurrence rates with synthetic mesh have been quoted between 1 and 27%.[3,6–8] In general, the use of prosthetic materials has reduced these rates to 10% or less.[9] Obviously, the reported variations depend on operative technique. Prosthetic materials for hernia repair have been associated with complication rates of 5 to 17%,[10] including early surgical site infection, skin erosion, seroma formation, and later, bowel obstruction or fistula formation.[10] These problems seem to be inherent to the use of synthetic meshes. The trade-off is more secure repairs but with more early complications than those found with suture tissue repair.

Implantation of a synthetic prosthesis into a contaminated field leads to a very high rate of infection.[11] Such postoperative infections too often lead to later hernia recurrence.[12] Various strategies have been designed to avoid synthetic repair of an abdominal wall defect in the face of infection or of contamination.[12,13] Component separation and muscle flaps employ native tissue, and although successful in some cases, cannot be applied universally. Postoperative complications (20 to 43%) and reherniation (8 to 32%) are common with this approach.[12,14,15]

Accepted for oral presentation (Quick Shot) at the annual meeting of The Society for Surgery of the Alimentary Tract, Digestive Disease Week, Washington D.C., May 2007.

W. B. Gaertner · M. E. Bonsack · J. P. Delaney (✉)
Department of Surgery, University of Minnesota Medical School, 11-100 Phillips-Wangensteen Bldg., 516 Delaware Street SE, Box 195, Minneapolis, MN 55455, USA
e-mail: delan001@umn.edu

Biologic prostheses are relative newcomers to the field of ventral hernia repair. They are derived from several sources: bovine, porcine, and human. Collagen-rich tissues (skin, pericardium, intestinal submucosa) are harvested and treated to remove cellular elements, leaving the collagen and elastin scaffold intact. An additional manipulation for some prostheses involves collagen cross-linking by chemically treating the tissue with reagents, which react with specific functional groups (primary amines and sulfhydryls). The effect is to retard the degradation of the collagen by blocking collagenase-binding sites. Thereby, the prosthesis remains structurally intact for a longer period of time compared with non-cross-linked materials.[16,17]

The potential benefits of biologic materials include infection tolerance, host tissue ingrowth, vascularization, and lesser adhesion formation than that seen with synthetic prostheses. Downsides include higher cost, and in theory, potential disease transmission. At present, there are no data regarding long-term clinical outcomes, comparing biologic materials to synthetic meshes with respect to the security of the hernia repairs.

Most published experimental studies deal with the three biologic materials that are currently employed in the clinical setting: Permacol® (Tissue Science Laboratories, Andover, MA), a porcine derived cross-linked dermal collagen; Alloderm® (LifeCell Corporation, Branchburg, NJ), a dermal matrix obtained from human cadaveric split-thickness skin; and Surgisis SIS® (Cook Biotech, West Lafayette, IN), an extracellular matrix acquired from the submucosal layer of pig jejunum.

The purpose of the experiments reported here was to evaluate two new bioprostheses derived from bovine pericardium, one cross-linked, Peri-Guard® and the other non-cross-linked, Veritas® (Synovis Surgical Innovations, St. Paul, MN) and to compare them with Permacol and Alloderm for abdominal wall repair in the rat.

Materials and Methods

Animals

Mature, female Sprague–Dawley rats weighing between 300 and 425 g were obtained from Charles River Laboratories, Inc. (River Falls, WI). The animals were acclimated for a minimum of 7 days before initiation of the study and were monitored daily. Rats were individually housed in polycarbonate cages with free access to food and water in a controlled environment with temperatures of 66 to 76°F and a 12-h light–dark cycle. The animals were cared for by the University of Minnesota Research Animal Resources Department in accordance with the principles in the Guide for Care and Use of Laboratory Animals, NIH publication, revised in 1996. They were fed a commercially available rodent diet (TekLad Rodent Diet) and tap water, ad libitum, throughout the duration of the study. This protocol was approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Surgical Technique

Only animals that showed normal appetite and appeared healthy were used. Rats were weighed before operation and through the course of the experiment. Anesthesia was induced with an intraperitoneal injection of sodium pentobarbital, 30 to 40 mg/kg. The abdomen was shaved and prepared with povidone–iodine solution. Procedures were done under antiseptic conditions.

Two ventral hernia models were employed. Model A (acute, immediate repair) consisted of the removal of a 3×3 cm segment of full-thickness abdominal wall from the midline. Alternatively, two 2×2 cm full-thickness defects per animal were made lateral to the midline (double defect model). The defect was replaced with a 3×3 cm piece of biologic prosthesis (Fig. 1). In the double defect model, two 2×2 cm pieces of biologic prostheses were compared in the same animal. Either Veritas vs Alloderm or Peri-Guard vs Permacol were randomly allocated among animals and between the two locations. Prostheses were sewn to the cut edges with a continuous 4–0 polypropylene suture. Care was taken to evert the edges of the mesh toward the subcutaneous tissue. The skin was closed with a running subcuticular 5–0 vicryl (Polyglactin 910) suture.

Model C (chronic, delayed repair) also involved removal of a 3×3 cm segment of full-thickness abdominal wall from the midline, but the defect was left untreated for 21 days to achieve a mature status and was then replaced. In this model, prostheses were either sutured to the edge of the defect (inlay) as in model A, or placed as an underlay using stainless steel surgical tacks (Davol® Salute® Fixation System, Cranston, RI) through a lateral vertical incision (Fig. 2). Procedures were carried out by one surgeon. Biologic prostheses were prepared according to the packaging inserts as to rehydration and/or stretching before implantation.

Animals were randomly assigned to one of the four groups for each model at the time of operation:

1. Peri-Guard	*2. Veritas*
Acute (n=9)	Acute (n=9).
Midline defect (n=6)	Midline defect (n=6)
Lateral defects (n=3)	Lateral defects (n=3)
Chronic (n=16)	Chronic (n=14)
3. Permacol	*4. Alloderm*
Acute (n=3)	Acute (n=5)
Midline defect (n=0)	Midline defect (n=2)
Lateral defects (n=3)	Lateral defects (n=3)
Chronic (n=7)	Chronic (n=5)

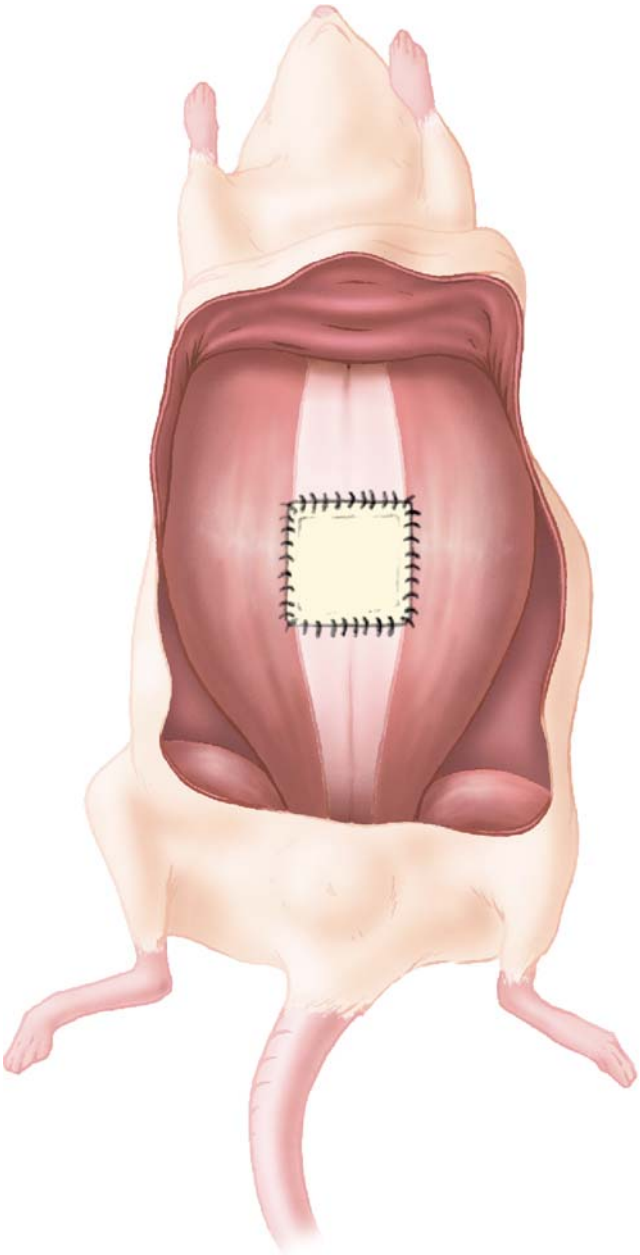

Figure 1 Three by three centimeter midline defect replaced with a 3×3 cm piece of biologic prosthesis.

Results were evaluated at 3 or at 6 months after operation. Approximately half of the animals in each group were killed at each time interval. Evaluation included extent of adhesion formation, defect size, thickness of the defect area, prosthesis tensile strength, abdominal wall-prosthesis breaking strength, bursting strength, and histology.

Animals were euthanized with an intraperitoneal injection of sodium pentobabrbital/phenytoin sodium, 0.2 ml (390 mg/50 mg per ml). The ventral abdominal skin was dissected away from the abdominal wall and a 10× 10 centimeter segment of full-thickness abdominal wall containing the midline or lateral defect sites was excised.

Adhesion Formation

The prosthesis surface was visually divided into four quadrants to help estimate the percent of the surface area involved with adhesions. The location of adhesions, the structures adhered (omentum or intestine), the tenacity of adhesions, and gross appearance of the prosthesis surface, were all recorded. Adhesion tenacity was graded using the system described by Mazuji et al.,[18] which is based on difficulty separating the adherent surfaces (0 to 4). Specimens were photographed for later review and comparisons.

Defect Area and Thickness

The ventral defect dimensions were measured with a conventional metric ruler and expressed as area. Thickness was measured with a dial thickness gauge (Olympus, Center Valley, PA). Defect area is reported in square centimeters (cm^2), and thickness in millimeters (mm).

Tensile and Breaking Strength

Two strips of tissue measuring 6×0.5 cm containing prosthesis, prosthesis–abdominal wall junction, and adjacent abdominal wall were obtained from each defect. Sutures or surgical tacks were carefully removed before tensile testing. One strip was used for measuring the tensile strength of the prosthesis itself and the other for the abdominal wall-prosthesis breaking strength. In five animals, a 6×0.5 cm strip of abdominal wall

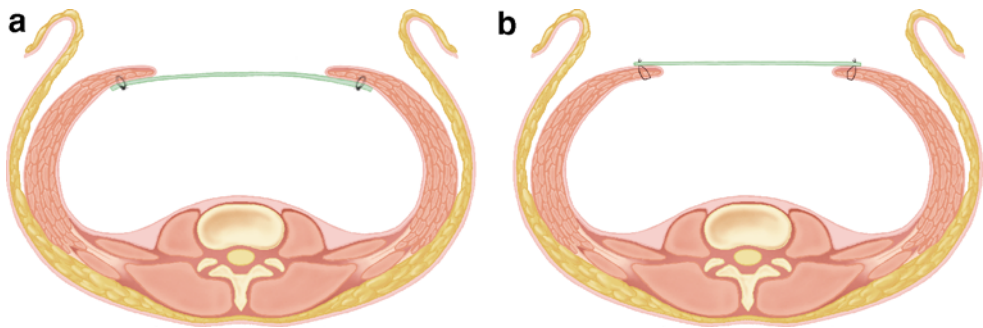

Figure 2 Biologic prostheses placement and fixation method in both ventral hernia models, underlay tacked (**a**) or inlay-sutured (**b**).

was obtained to measure its tensile strength. The original prosthesis breaking strength was measured by testing five 6×0.5 cm strips of each material. These measurements were done with a dynamic tensiometer (QTest-5, MTS Systems, Eden Prairie, MN) and represented in gram force (gf).

Bursting Strength

Bursting strength was evaluated only in the lateral double defect model. Before opening the abdomen, an 18-gauge needle was passed just below the xyphoid and connected to a hand bulb (Fig. 3). Room air was pumped in. Intra-abdominal pressure was measured continuously using an inline pressure gage (VWR, West Chester, PA). At the first sign of air leak, the pressure was recorded, and the location of the defect identified whether initial loss of integrity occurred at the prosthesis or in the abdominal wall.

Histology

A 2-cm wide segment of tissue containing the site of the prosthesis, prosthesis–abdominal wall junction, and normal abdominal wall was excised from all animals. Tissue samples' were fixed in 10% neutral-buffered formalin for 12 h, embedded in paraffin, cut at 7 μm, and stained with hematoxylin and eosin (H&E), and Masson's trichrome, for histologic analysis. A veterinary pathologist assessed characteristics such as cellular infiltration, vascularity, and tissue ingrowth using a light microscope.

Statistical Analysis

Statistical differences were determined using a one-way analysis of variance (ANOVA) and Tukey–Kramer multiple comparison test. $P<0.05$ was considered significant. All calculations were performed using the GraphPad InStat 3 statistics program (GraphPad Software, Inc. San Diego, CA).

Results

Two animals from the Permacol (acute model) group died shortly after the initial procedure because of anesthesia complications. Operative site infection occurred in six animals (five Peri-Guard and one Veritas), and overlying skin ulceration in six others (six Peri-Guard). Subcutaneous infections in the operative site appeared first as a seroma. The first was seen 37 days after operation and others thereafter up to 6 months. Skin ulceration starting as a circular, well-delimited area of discoloration directly overlying the site of the prosthesis first appeared 40 days after operation and thereafter for up to 6 months. In one animal,

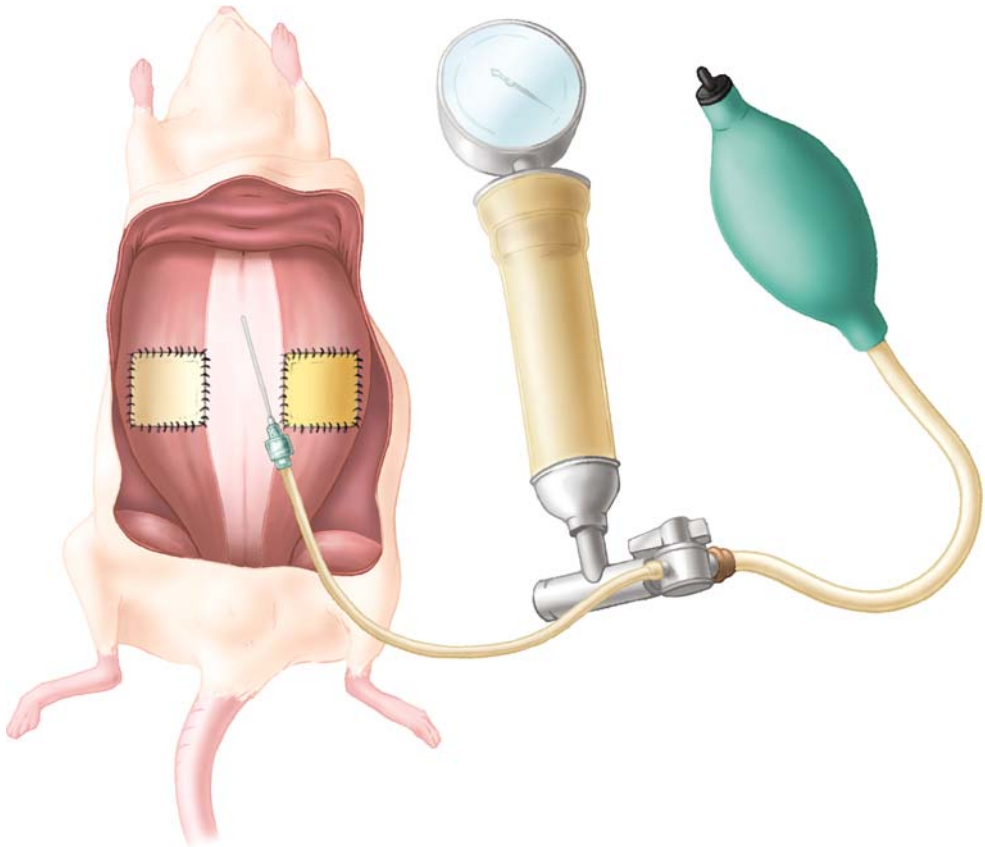

Figure 3 Bursting strength evaluation in the lateral double defect model.

Table 1 Prostheses Findings

Prosthesis (*n*)	Adhesion coverage (mean, %)	Defect Area (mean, cm^2)	Abdominal wall–prosthesis breaking strength (mean, gf)	Bursting pressure (mean, mmHg)
Peri-Guard (25)	31	−1.91	729	100
Permacol (10)	25	−1.17	622	
Veritas (23)	28	−3.43*	870	98
Alloderm (10)	26	+0.79	745	

*$P<0.05$ compared to the other biologic prostheses.

an infected seroma preceded the skin ulceration. Infection and skin ulceration occurred in both delayed and acute ventral hernia models and with either inlay or underlay prosthesis placement.

Tables 1, 2, and 3 summarize the findings in the four groups.

Adhesion Formation

Mean adhesion coverage of the prostheses surface ranged from 25 to 31% ($P>0.05$). Zero adhesions were observed in seven animals (three Veritas, three Peri-Guard, and one Permacol). Twenty rats had intestinal and omental adhesions to the prosthesis surface (seven Veritas, seven Peri-Guard, four Permacol, and two Alloderm). The remaining had only omentum. Infection and/or skin ulceration was associated with larger areas of adhesion coverage (mean, 76%), and adhesion tenacity scores (mean, 4). Absent infection or skin ulceration, adhesion tenacity scores did not differ among models or between prostheses. They all were scored 2 or 3, separable by traction but not requiring sharp dissection (Fig. 4).

Table 2 Defect Area Thickness

Prosthesis (*n*)	Defect area thickness (mean, mm)		
	0 months	3 months	6 months
Peri-Guard (25)	0.50	1.22	0.82
Permacol (10)	1.12	1.55	1.17
Veritas (23)	0.70	0.64	0.40*
Alloderm (10)	1.25	1.10	0.51*

*$P<0.05$ when compared to 0 months.

Table 3 Prostheses Tensile Strength

Prosthesis (*n*)	Prostheses tensile strength (mean, gf)		
	0 months	3 months	6 months
Peri-Guard (25)	2,805	2,912	2,025
Permacol (10)	4,367	2,743	2,596
Veritas (23)	2,655	1,192*	743*
Alloderm (10)	15,231	1,816*	736*

*$P<0.05$ when compared to 0 months.

Defect Area and Thickness

Significant defect contraction followed repair with Veritas (the mean area decreased 3.43 cm^2, $P=0.0006$), while Alloderm stretched (the mean area increased 0.79 cm^2) (Fig. 5). Thickness at the defect area diminished significantly at 6 months with both Veritas and Alloderm ($P<0.05$), so much so that they became translucent (Fig. 6). With Permacol and Peri-Guard, the mean defect area and thickness were virtually identical to when they were originally installed 6months earlier (Table 2).

Tensile and Breaking Strength

Peri-Guard had greater tensile strength than Permacol, Veritas, and Alloderm at 3 months ($P=0.541$). Permacol had greater tensile strength than Peri-Guard, Alloderm, and Veritas at 6 months ($P=0.040$, Table 3). Significant reduction in tensile strength was found for Alloderm and Veritas at 3 and 6 months when compared to the original material ($P<0.001$). Stretching was observed with Alloderm at all time points. No significant differences were found in prosthesis–abdominal wall separation strength nor did inlay vs underlay influence this parameter. Infection and/or ulceration also did not influence tensile strength or junction breaking strength. The mean tensile strength of the abdominal wall muscle itself was 1,020 (range, 897–1182) gf. Only Alloderm and Veritas at 6 months were weaker than the abdominal wall itself ($P=0.408$). Timing of repair (acute vs chronic) or mode of fixation (sutured vs tacked) did not influence tensile strength of the material nor of the junction with the abdominal wall.

Bursting Strength

In all but one animal, air leaked through the abdominal wall at the inguinal area before any break could be observed at the site of the defects. The single prosthesis disruption occurred with Peri-Guard at the prosthesis–abdominal wall interface in an animal with overlying skin ulceration. The mean pressure leading to inguinal disruption was 100 mmHg.

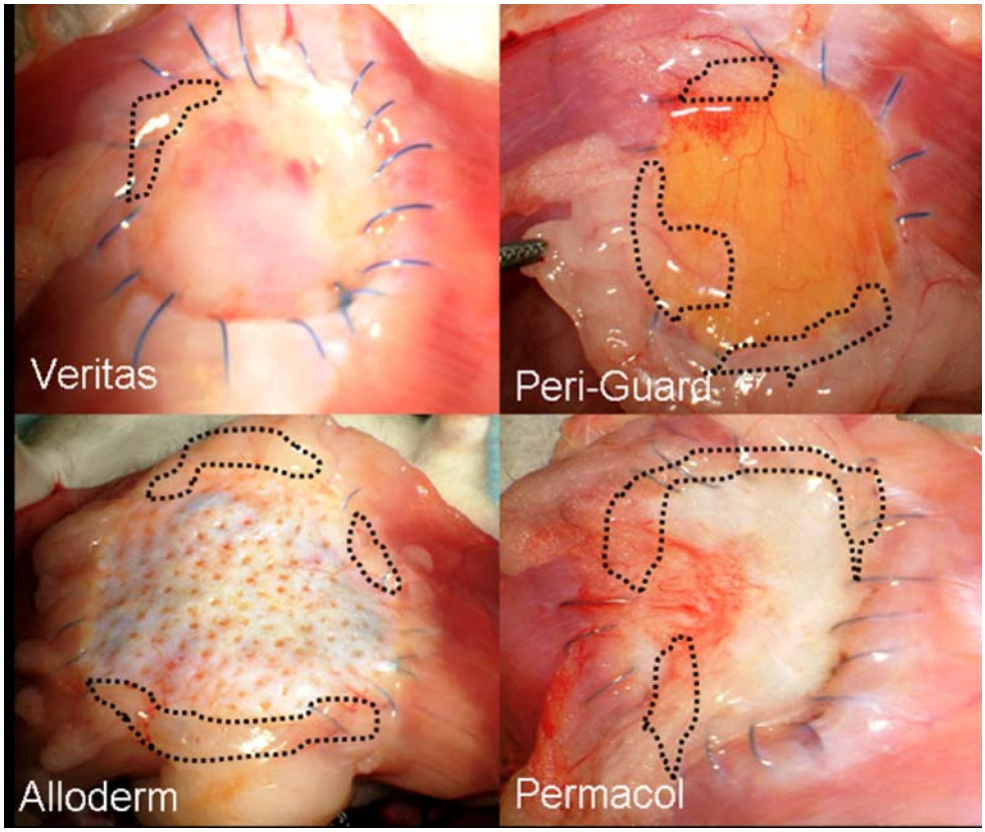

Figure 4 Adhesion coverage at 6 months for all four bioprostheses.

Defect bulging (recurrent hernia) was observed at 6 months with Alloderm (Fig. 5).

Histology

All prostheses had cellular ingrowth and neovascularization by 3 months visible on H&E sections. Alloderm-induced minimal inflammatory response and relatively less ingrowth from the surrounding tissues. Permacol, Peri-Guard and Veritas induced a granulomatous inflammatory response in the surrounding tissues. Veritas could not be identified histologically 3 months after implantation. A layer of collagen was noted at all defect sites, although the source, host, or original prosthesis collagen, could not be distinguished. At 3 and 6 months, skeletal muscle fibers were observed within the collagen layer where Veritas had been placed (Fig. 7). Histology of the defects with infection or skin ulceration showed an extensive inflammatory response in the surrounding tissue. Samples obtained from three of the infected implant sites were positive for Gram-positive Streptococci.

Discussion

Biologic prostheses have been used clinically for replacement of various abdominal wall defects. Reports of inguinal, parastomal, and ventral hernia repair with such materials are recent and have had short follow-up observations. Potential advantages include infection tolerance, native tissue ingrowth, and lesser adhesion formation compared to synthetic meshes.

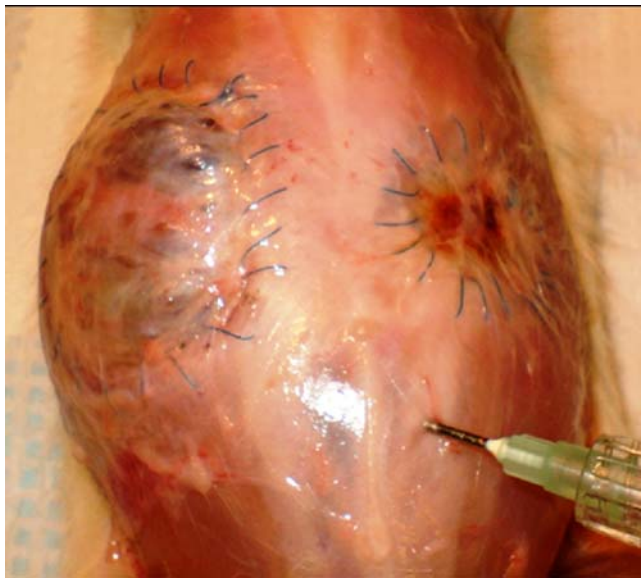

Figure 5 Defect area at 6 months, Alloderm (*left*) vs Veritas (*right*). Defect contraction followed repair with Veritas, while Alloderm stretched.

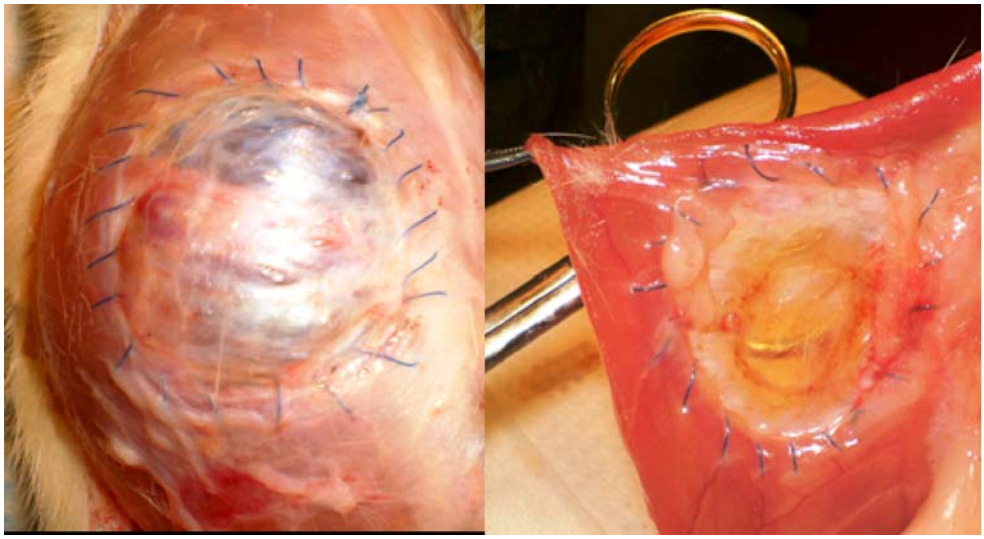

Figure 6 Alloderm at 6 months. Hernia bulging (*left*) and transparency (*right*). The scissors handle can be seen through the defect site.

Ventral hernia repair in the presence of infection or gross contamination presents a dilemma. Infection is one of the consistent risk factors that predict herniation after abdominal wall repair.[3,4,19] Historically, the use of synthetic mesh led to decreased rate of reherniation. However, the incidence of prosthesis-related infection ranged from 5 to 18%.[3,6–8,20,21] Laparoscopic ventral hernia repairs have a 0.7 to 2% incidence of synthetic mesh infection.[22,23] A potential problem with synthetic mesh is its susceptibility to bacterial contamination and chronic infection.[24,25] Bacteria adhere avidly to the polymers and lay down a biofilm, which protects the bacteria from host immunological defenses and from antibiotics. This phenomenon contributes to bacterial survival and consequent chronic infection of the hernia wound.[26]

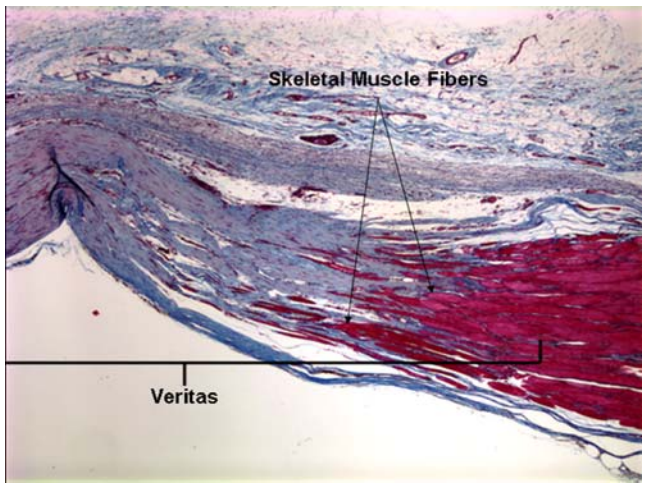

Figure 7 Microscopic view (Masson's trichrome) demonstrating skeletal muscle fibers within the collagen layer where Veritas had been placed.

Infection frequently requires removal of synthetics and attendant morbidity. Expanded polytetrafluoroethylene must always be removed to allow healing. Macroporous mesh can be left in place about half of the time. One of the theoretical advantages of using a biologic prosthesis is that of infection tolerance. While some investigators have studied bacterial proliferation and attachment to the surface of synthetic prostheses,[3,27–31] scant data are available as to the ability of biologic prostheses to resist infection and on the consequences of infection on tensile strength or on prosthesis incorporation. Collagenases are responsible for the breakdown and resorption of implanted collagen materials. Several bacteria have been shown to possess collagenolytic activity.[32,33] Experimental studies have demonstrated that collagen cross-linking with glutaraldehyde imparted resistance against the activity of collagenase.[34,35] Cross-linked biologic prostheses should therefore be relatively resistant to bacterial degradation, and therefore, safe to use in contaminated or infected hernia repair. Recent clinical reports described accelerated degradation of implanted Surgisis (non-cross-linked) when used to reconstruct abdominal wall defects in infected fields.[36,37] Petter-Puchner et al.[38] reported infection in all rats when Surgisis was used in a delayed repair ventral hernia model. The biomaterial was not detectable after 17 days. Carbonell et al.[39] found no significant differences in wash and broth bacterial counts after inoculating various synthetic and biologic prostheses with 10^8 *Staphylococcus aureus* at the time of ventral abdominal wall repair. Of the two biologic prostheses evaluated, Alloderm had lower wash and broth bacterial counts compared to Surgisis ($P>0.05$).

In the present study, we observed six operative site infections (five Peri-Guard and one Veritas). In addition, there were six overlying skin ulcerations without gross pus, all with Peri-Guard. Neither complication depended on the

hernia model or on the fixation technique. Both infection and skin ulceration were associated with increased adhesion coverage and tenacity. While these were unwanted complications, they did provide important information. Infection and/or skin ulceration did not influence tensile strength of the Peri-Guard or abdominal wall-prosthesis breaking strength. Although infected prostheses stimulated an extensive inflammatory response in the surrounding tissue, tissue ingrowth and incorporation was similar to that of the noninfected prostheses.

Implanted biologic prostheses show an early acute inflammatory response consisting mostly of polymorphonucleocyte infiltration that is largely resolved by day 14.[40,41] Simultaneously, the number of mononuclear cells increases.[40,42] After 3 months of implantation, the mononuclear cell infiltrate diminishes, and there appear to be relatively fewer foreign body giant cells with more well-organized collagen and skeletal muscle.[40,41] Angiogenesis also occurs during remodeling. Growth factors present in the host cells that invade the prosthesis elicit early signals for capillary ingrowth.[41] In a rat ventral hernia model, histologic analysis identified newly formed blood vessels in Surgisis as early as 7 days after placement.[40]

Remodeling is a term used by prosthetic device companies to describe native tissue ingrowth into biologic prostheses. It implies the replacement of the implanted or foreign tissue with host tissue. The quality and strength of the tissue that replaces biologic prostheses in humans is not well defined at present. It is unknown when complete replacement of the original prosthesis collagen occurs, if ever. To determine if native tissue truly replaces the prosthetic material, the foreign collagen would have to be marked with a label, which could later be assessed for possible continued presence. Currently, there are no convincing studies proving "remodeling". In animals, it has been shown that Surgisis is histologically absent 28 days after implantation in dog bladder[43] and 56 days in murine subcutaneous pockets.[44] However, when used to repair abdominal wall defects in dogs, Surgisis was histologically absent at 4 months.[45] In company-reported research, ventral hernia repairs with Permacol in a rat model demonstrated the persistence of the implanted Permacol at 9 months,[46] this in accord with our 6-month observations. In a swine model, full-thickness abdominal wall defects were created and repaired with a single layer of Alloderm. At 9 months, Verhoeff's stain demonstrated the presence of elastin, suggesting some persistence of the Alloderm Matrix.[47] Histological analysis of Alloderm biopsied from a single patient 2 years after implantation showed no evidence of elastin.[48]

In the present study, Veritas was histologically absent 3 months after implantation, and Permacol, Peri-Guard, and Alloderm were still present at 6 months. The persistence of these biomaterials was demonstrated by observing histologic characteristics compatible with the original implanted tissues, porcine dermal collagen, bovine pericardium, and human dermal matrix. Biologic prostheses may be replaced by native tissues over time, and if so, serve only as a temporary scaffold for host cells to grow into. The two cross-linked materials appeared as they did at the time of implantation. In fact, at 6 months, Permacol and Peri-Guard appeared grossly intact. The clinical utility of biodegradable materials depends on the balance between the rate of degradation and the rate of native tissue ingrowth. If a biologic prosthesis is absorbed before adequate collagen differentiation, deposition, and neovascularization, the overall quality and strength of the newly formed tissue will likely be insufficient for abdominal wall repair.

In our study, similar tissue ingrowth and neovascularization were found at 3 months in each of the four biologic prostheses. The functional effect of tissue ingrowth was reflected in the observation of similar abdominal wall-prosthesis breaking strength among the four. Tensile strength of the material itself after 6 months was significantly reduced for the non-cross-linked prostheses (Veritas and Alloderm) compared to the cross-linked prostheses (Peri-Guard and Permacol). Stretching, bulging, and translucency were routine with Alloderm. Stretching and bulging have been noted by others with this biomaterial, possibly related to the high elastin content of human cadaveric skin.[49]

The average air leak pressure in the lateral defect model was 100 mmHg. Because all of the leaks occurred at the groin, it is not known how much pressure would have been necessary for the prostheses or the suture line to rupture, only that it would have had to exceed 100 mmHg. Cobb et al.[50] showed that the highest intra-abdominal pressures in healthy humans are generated during coughing and jumping, 107 and 171 mmHg, respectively. Such pressures probably contribute to development of incisional hernias or to recurrence after their repair.

Will a biologic prosthesis provide a lasting hernia cure? The outcome of these repairs in humans is currently unknown. Late follow-up data will be essential to establish the value of biomaterials. Currently, the longest published follow-up for ventral hernia repair with a biologic prosthesis is 2 years.[51] A four- or eight-ply Surgisis mesh was used. No prosthesis-related complications or recurrent hernias were reported. In yet another study, short follow-up observations showed a high rate of seroma formation with eight-layer Surgisis and a 24% hernia recurrence (8 of 33) with Alloderm.[49] Fifteen of the Alloderm patients (15 of 33) developed a diastasis or bulging at the repair site.

Alloderm is extensively used to cover open contaminated abdominal wall defects. Diaz et al.[52] reported a 33% overall repair site infection rate in clean-contaminated or

contaminated-dirty surgical fields when Alloderm was used for abdominal wall reconstruction. Of the 75 patients, 19% required operative management, and 36% removal of the Alloderm. Patton and coworkers[53] used Alloderm in 67 patients undergoing abdominal wall reconstruction in the face of contamination. Indications included incarcerated hernia, infected mesh, or fistulae. Some involved delayed abdominal wall reconstruction after intra-abdominal catastrophe, trauma, dehiscence/evisceration, and spillage of enteric contents. Of the 67, 2 patients required removal of the Alloderm. Eighteen percent developed recurrent hernias at a mean follow-up time of 10.6 months.

In a prospective study, Catena et al.[54] employed Permacol for ventral hernia repair in contaminated fields. No recurrences or wound infections were observed. The mean follow-up was 11.1 (range, 7 to 18) months. In a comparable series, Permacol was used for contaminated abdominal wall reconstructive procedures.[55] Indications included repair after removal of infected mesh, reconstruction after resection of an abdominal wall tumor, repair in the presence of an ostomy and/or an open midline wound, and emergent repair with strangulated bowel and multiple intra-abdominal abscesses. One recurrent hernia developed in nine such patients with a median follow-up of 18.2 months.

The search for an ideal biomaterial for repair of abdominal wall defects is ongoing. Biologic prostheses have favorable characteristics, strength, biocompatibility, native tissue ingrowth, and lesser adhesion formation when compared to synthetic materials. While the tensile strength of the biologic prostheses evaluated in this study is somewhat inferior to that of synthetic materials,[56–58] they were stronger than the abdominal wall itself.

Halm et al.[59] reported a complicated perioperative course in 76% of patients (30 of 39) who underwent re-laparotomy after incisional hernia repair with synthetic mesh placed intraperitoneally. Small bowel resections were necessary in 21% of patients, and enterocutaneous fistula formation occurred in 5%. Enterocutaneous fistula was first described in 1981 as a late complication of intraperitoneal placement of polypropylene mesh.[60] After more reports of such fistulas after mesh repair of incisional hernias with visceral exposure, this technique was no longer recommended.[8,61,62] However, other series of incisional hernia repairs with intraperitoneal polypropylene mesh reported absence of enterocutaneous fistulas as a long-term complication.[63–65] Vrijland et al.[66] reported zero enterocutaneous fistulas in 136 patients who underwent incisional hernia repair with polypropylene mesh after a median follow-up of 34 months. The association between intraperitoneal placement of polypropylene mesh and fistula formation varies from one report to another. To date, there are no reports of intestinal fistulization associated with the use of collagen implants. These bioprostheses also seem to demonstrate considerable tolerance to local septic complications. Biomaterials can serve to avoid placing a synthetic macroporous mesh in contact with the abdominal viscera.

Although larger clinical series and longer follow-up are required, our experimental data suggest that biologic prostheses may play an important role in the management of difficult abdominal wall defects when the use of synthetic foreign material risks infection and subsequent recurrent hernia formation. Indeed, they may prove useful for routine use in intraperitoneal placement via the laparoscopic approach.

Conclusions

Permacol provided a strong and durable repair in two different rat ventral hernia models for up to 6 months. Permacol may, by itself, serve as an adequate substitute for synthetic meshes in primary abdominal defect replacement, and it could be useful for parastomal hernia repair and as a buttress for crural closure in hiatus hernia operations. Peri-Guard was found to be equally strong but prone to frequent infections and separately with overlying skin necrosis. This will be the subject of further study. Veritas and Alloderm lose tensile strength with time, associated with marked thinning. In light of our results and those from the available literature, we would tentatively suggest that Alloderm and Veritas can provide excellent open wound coverage while healing progresses. Neither material is likely to suffice as a permanent bridge for abdominal wall defects, although both might be useful to supplement sutured closures or to separate synthetic meshes from viscera. All four bioprostheses tested became firmly incorporated into the abdominal wall. They induced lesser adhesion coverage than previously found with synthetic prostheses. Longer follow-up observations will be needed to support or refute these conjectures.

Acknowledgement The authors have no financial disclosures to declare. This experimental study was sponsored entirely by: Synovis Surgical Innovations, 2575 University Ave. W., St. Paul, MN 55114.

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J Gastrointest Surg (2007) 11:1286–1293
DOI 10.1007/s11605-007-0238-4

Autologous Versus Allogeneic Transfusions: No Difference in Perioperative Outcome After Partial Hepatectomy

Autologous Transfusion on Hepatectomy Outcome

James O. Park · Mithat Gonen · Michael I. D'Angelica · Ronald P. DeMatteo · Yuman Fong · David Wuest · Leslie H. Blumgart · William R. Jarnagin

Received: 20 June 2007 / Accepted: 30 June 2007 / Published online: 31 July 2007

Abstract Blood transfusion is often necessary in patients undergoing liver resection. Because of the risks associated with allogeneic blood products, preoperative autologous blood donation has been advocated, but its benefit with respect to perioperative outcome remains unclear. This study compares perioperative outcome in patients transfused only with autologous blood to a matched cohort receiving only allogeneic blood. All patients subjected to hepatic resection and given only perioperative autologous red cell transfusions were identified from a prospective database of 2,123 patients and reviewed retrospectively. This group was matched to patients transfused only with a comparable number of allogeneic red cell units and to a control group that received no blood products. All patients in the autologous or allogeneic group received either 1 or 2 U. Matching was based on age, comorbidity, extent of hepatic resection, and estimated blood loss. Matched pair analysis was performed using the paired *t* test, McNemar and Stuart–Maxwell tests. From December 1991 to May 2003, 124 patients undergoing hepatic resection received perioperative autologous blood only, for which optimal matching was possible in 104. The groups were similar with respect to age, comorbidities, and blood loss; the proportions receiving preoperative chemotherapy, requiring a major resection (≥3 segments) or a complex procedure (concomitant major procedure in addition to the principal hepatic resection) were also similar. There were no differences between the autologous and allogeneic groups in length of hospitalization, complications, and operative mortality. In patients undergoing hepatic resection, autologous blood transfusion did not demonstrably improve perioperative outcome when compared to a matched cohort of patients receiving a similar number of allogeneic units.

Keywords Autologous · Transfusion · Hepatectomy · Perioperative · Outcome

To be presented at the 47th Annual Meeting of the Society of Surgery of the Alimentary Tract, May 20–24, 2006, Los Angeles, California.

J. O. Park · M. I. D'Angelica · R. P. DeMatteo · Y. Fong · L. H. Blumgart · W. R. Jarnagin (✉)
Department of Surgery, Memorial Sloan-Kettering Cancer Center,
1275 York Avenue, C897,
New York, NY 10021, USA
e-mail: jarnagiw@mskcc.org

M. Gonen
Departments of Epidemiology and Biostatistics,
Memorial Sloan-Kettering Cancer Center,
New York, NY, USA

D. Wuest
Departments of Clinical Laboratories,
Memorial Sloan-Kettering Cancer Center,
New York, NY, USA

Introduction

The advents of operative technique employing segmental hepatic, anatomic-oriented resections, and anesthetic maneuvers that maintain low central venous pressure have decreased intra-operative blood loss and the subsequent need for transfusion.[1,2] Despite these advances, a significant proportion of patients undergoing liver resection will require transfusion of red cells.[3]

Transfusion of blood products has been implicated as an independent factor associated with adverse perioperative outcome in patients undergoing hepatic resection for colorectal metastases. A prior investigation showed that patients requiring blood transfusion had significantly longer length of hospitalization and higher complication rates compared to those not transfused, and the postoperative mortality rate was proportional to the amount of blood products given.[4]

It has been postulated that immune dysregulation is in part responsible for the detrimental effects of allogeneic transfusion. Intravenous introduction of a large quantity of alloantigens induces downregulation of natural killer cell activity and cytotoxic T-cell function, resulting in a subclinical state of anergy or tolerance.[5] It has been argued that the ensuing immunosuppression results in increased perioperative morbidity, decreased tumor surveillance with increased recurrence, and an overall adverse outcome.[6] The association between perioperative blood transfusion and cancer recurrence remains unclear; however, the potential risk of other transfusion-related complications, such as infectious disease transmission, transfusion reactions, graft-versus-host disease, and stress on the limited allogeneic blood supply have led to the recommended use of preoperative autologous blood donation for high blood loss surgical procedures.[7] Whereas preoperative autologous donation may address some of these issues, much controversy remains regarding its safety, practicality, cost, and overall benefit. These concerns are particularly relevant in a population of cancer patients in whom normalization of hemoglobin levels may be delayed after donation (because of disease or recent chemotherapy) and the luxury of time for multiple donations is often not available.

This study examines the perioperative outcome in liver resection patients transfused only with autologous blood and compares this to a matched cohort of patients not receiving transfusion and those transfused with allogeneic blood only.

Material and Methods

All patients who underwent liver resection from December 1991 to May 2003 were identified from the Memorial Sloan Kettering Cancer Center (MSKCC) hepatobiliary patient database. Patients who received perioperative transfusion of autologous red blood cell products only were identified and matched to patients transfused with a comparable number of allogeneic red cell units and to a control group that received no blood products. Patients were excluded if they received fresh frozen plasma or platelets, or if they received both allogeneic and autologous red cell transfusions.

Optimal cohort matching was then performed based on age (≤60 versus >60), comorbidities (the presence of any of the following conditions: hypertension, ischemic or valvular heart disease, dysrhythmias, cerebrovascular accidents or transient ischemic attacks, peripheral vascular disease, chronic obstructive pulmonary disease, asthma, diabetes mellitus, other endocrine diseases), extent of hepatic resection (<3 segments versus ≥3 segments), and estimated blood loss (EBL, ≤2 l versus >2 l). The matching thresholds for age, extent of resection, and EBL were chosen as the observed median value for the patients who received transfusion. These matching variables were chosen because they were previously shown to correlate strongly with postoperative morbidity.[3]

The autologous donation process is initiated with a discussion between the patient and the surgeon regarding the anticipated perioperative transfusion requirements and the risks and benefits of preoperative autologous donation. If the self-donation option is chosen, up to four appointments for donation are scheduled, with the first donation up to 4 weeks before surgery, the last donation a minimum of 7 days before surgery, and consecutive donations no closer than 4 days together. In practice, the vast majority of patients donated a maximum of 2 U. The criteria for autologous blood donations are as follows: (1) patients must have a hemoglobin ≥11.0 g/dl or hematocrit ≥33%; (2) patients must not have any contraindications to donation (i.e., active infection, unstable angina or angina at rest, aortic stenosis, congestive heart failure, ventricular arrhythmia, myocardial infarction within 3 months, transient ischemic attack), and (3) pass a medical screening examination before each scheduled donation. Iron supplements but not erythropoietin was administered in the post-donation period.

The authors' general approach to patient evaluation, intraoperative anesthetic management, and technical details related to hepatic resection are described elsewhere.[2,3,8] Formal cardiopulmonary evaluation was obtained in patients with co-existing medical conditions suggesting an increased operative risk and in all patients over age 65. A full radiological extent of disease evaluation was performed on all patients, although the nature of the studies obtained varied depending on the diagnosis.

Patients submitted to operation were explored through an extended right subcostal incision or bilateral subcostal incision with vertical midline extension, with a very small fraction requiring a thoracoabdominal incision. In general, for major resections, inflow and outflow vascular control was obtained before parenchymal transection, which was performed using a crushing technique and with an intermittent Pringle maneuver (porta hepatis clamp). Resections were performed with a low central venous pressure (<5 mmHg) and with patients in the Trendelenburg position. After operation, patients were monitored overnight in the recovery room and sent to the regular ward the following day, provided they were clinically stable; transfer to the intensive care unit (ICU) was done only for specific indications and was not routine. The decision to transfuse patients was made by the attending surgeon and was based on the clinical status of each patient; however, guidelines for transfusion of red blood products during the period of study were as follows: hemoglobin, <8 g/dl; hemoglobin, <10 g/dl with coexisting cardiac, pulmonary, or cerebro-

vascular disease and evidence of diminished peripheral oxygen delivery; acute blood loss, >15% of blood volume, or hemoglobin decrease, ≥2 g/dl over 24 h.

Data were obtained from the database and supplemented by review of the medical record where necessary. Preoperative variables analyzed included patient demographics, diagnoses, comorbid medical conditions, and hemoglobin level. Intraoperative data were obtained from the operative note and the anesthesia record and included operating time, portal triad clamp time (Pringle time), EBL, type of hepatic resections, and any other procedures performed. The number of segments resected was based on the Couinaud nomenclature.

A complex resection was defined as the performance of concomitant major extrahepatic procedures (additional organ resection, biliary resection/reconstruction, portal vein and/or vena cava resection/reconstruction, porta hepatic lymphadenectomy, or thoracotomy) and/or additional hepatic resectional or ablative procedures, as previously described.[3] Data regarding the resected specimen (number of tumors, largest tumor size, and hepatic parenchymal abnormalities) were obtained from the pathology record. For all specimens, sections of nontumor-bearing liver were assessed and any parenchymal abnormalities (steatosis, fibrosis, cirrhosis, etc.) were reported according to standard histologic criteria.

The postoperative variables analyzed included complications (any), hospital length of stay (LOS), ICU admission, and mortality. Operative mortality was defined as any death resulting from a complication of the operation, whenever it occurred. Transfusion data were obtained from the computerized record system of the MSKCC blood bank; only transfusions given during the procedure or at some time during the index hospital admission were coded. Complications were graded on a 1–5 scale according to a previously established system to insure standardized reporting.[9] In this classification system, grade 0 represents the absence of complications. Grade 1 complications require no intervention or minor interventions (e.g., oral medications, bowel rest, monitoring). Grade 2 complications require moderate interventions (e.g., intravenous medications, TPN, chest tube insertion). Grade 3 complications require hospital readmission, and surgical or radiologic intervention. Grade 4 complications are those producing chronic disability, or organ loss. Grade 5 complications result in death. Grades 1 and 2 are considered "minor" complications, whereas grades 3 to 5 are grouped as "major" complications.

Biostatistics After identification of patients who were transfused with autologous red cell products only, optimal matching to the control and allogenic groups was performed based on the variables outlined above. Because the data set consisted of matched pairs, comparisons were made using the paired *t* test for continuous variables (such as EBL or BMI), the McNemar's test for dichotomous variables (such as gender or bilobar resections), and the Stuart-Maxwell test for polytomous variables (such as diagnosis). For continuous variables, mean values±standard deviations are given, unless indicated otherwise. In general, the *P* values listed pertain to comparisons between the allogeneic and autologous groups; comparisons among all three groups are specifically indicated in the text. Significant differences are indicated by P values<0.05. This study was reviewed and approved by the Institutional Review Board and found to be Health Insurance Portability and Accountability Act (HIPPA) compliant.

Results

During the period of study, 2,123 patients underwent partial hepatectomy of which 1,020 (48.0%) received a transfusion of any blood product. Of this group, 430 (20.3%) received only red blood cell products (median number of units=2) and 186 (8.8%) received at least one autologous transfusion. One hundred twenty-four patients (5.8%) were transfused with autologous blood only. Optimal cohort matching to patients transfused with a comparable number of allogeneic units and to control patients who received no transfusions, based on the variables described above, was possible in 104 patients. All patients in the autologous and allogeneic groups received no more than 2 U.

The patient characteristics and demographic variables are listed in Table 1. As patient cohort matching was performed based on two of the demographical variables (age and presence of comorbidities), no differences in these variables were observed among the control, allogeneic, or autologous groups. More specifically, the mean age was 56.1 years (median age, 58 years) and 32.7% of patients had underlying comorbidities. Other patient demographics including the patient's gender, body mass index (26.0 kg/m^2), proportion of patients receiving preoperative chemotherapy (within 12 months of resection; 34.3%), and preoperative hemoglobin (12.3 g/dl) were also statistically similar. The distribution of diagnoses demonstrated that the allogeneic group consisted of fewer benign lesions (3.8%; control=16.3%, autologous=11.5%) and more biliary tract cancers (16.3%; control=7.7%, autologous=7.7%) compared to the other two groups. However, this difference did not reach statistical significance.

The intraoperative variables are listed in Table 2. Two of these variables, extent of hepatic resection and EBL, were utilized in the cohort matching of these patients. No difference in the extent of resection was found among the

Table 1 Patient Demographics

Variable	Control (N=104)	Allogeneic* (N=104)	Autologous** (N=104)	Allogeneic versus Autologous (P value)
Age (year)	56.4±12.5	56.2±14.8	55.7±12.5	0.82
Gender (% female)	48.1	70.2	62.5	0.21
Diagnosis (%)				0.92
Benign disease	16.3	3.8	11.5	
Biliary tract cancer	7.7	16.3	7.7	
HCC	8.7	10.6	5.8	
Met CR	59.6	57.7	62.5	
Other Met	5.8	9.6	12.5	
Comorbidities (%)	32.7	32.7	32.7	1.00
BMI (kg/m^2)	26.3±5.0	25.3±5.2	26.3±5.6	0.34
Preoperative chemotherapy (%)	32.7	34.6	35.6	0.10
Preoperative Hgb (g/dl)	13.0±1.9	11.9±1.6	11.9±1.5	0.88

Benign disease Hemangioma 14, FNH 6, adenoma 2, cystadenoma 1, biliary stricture 3, liver abscess 2, hydatid cyst 1, fatty infiltration 1, sclerosing cholangitis 1, polycystic liver 1. *Biliary tract cancer* Gallbladder cancer 12, cholangiocarcinoma–hilar 11, peripheral 10. *Other Met* Neuroendocrine 12, sarcoma 11, breast 3, pancreatic 3, melanoma 1, ovarian 1, paraganglioma 1. *HCC* Hepatocellular carcinoma. *Met CR* Metastatic colorectal cancer. *BMI* Body mass index. *Hgb* Hemoglobin.
*, **P values pertain to comparisons between Allogeneic and Autologous groups

control, allogeneic, or autologous groups, where 65.4% of patients underwent major resections consisting of ≥3 segments, and the average number segments resected was 3.5. The most commonly performed procedure was trisegmentectomy (39.7%), followed by segmentectomy (one or more segments, 25.9%), lobectomy (21.2%), and wedge resection (single or multiple, 4.8%). The proportions of these cases were similar among all three groups. No statistical difference was observed in the fraction of patients requiring bilateral hepatic resections (47.8%), complex resections (35.9%), or repeat hepatic resections (5.8%). The mean EBL was statistically similar between the allogeneic (894 ml) and autologous (899 ml) groups. Although both mean values were greater than the control group (806 ml), the differences were not statistically significant (control versus allogeneic, P=0.26; control versus autologous, P=0.31). The total Pringle time was significantly longer in the allogeneic group (43.0 min) compared to the other two groups (control, 27.6 min; autologous, 29.7 min). Overall, 64.2% of patients had normal hepatic parenchyma (as assessed histologically), and although this proportion was higher in the control

Table 2 Intraoperative Variables

Variable	Control (N=104)	Allogeneic* (N=104)	Autologous** (N=104)	Allogeneic versus Autologous (P value)
Hepatic procedure (%)				0.57
Wedge resection	3.8	6.7	3.8	
Segmentectomy	24.0	26.0	28.8	
Lobectomy	21.2	21.2	21.2	
Trisegmentectomy	39.4	41.3	38.5	
Other	11.5	4.8	7.7	
Segments resected	3.4±1.8	3.6±1.6	3.5±1.7	0.85
≥3 Segments (%)	65.4	65.4	65.4	1.00
Bilobar resections (%)	48.1	46.2	49.0	0.32
Complex resections (%)	34.6	35.6	37.5	0.77
Repeat resection (%)	5.8	6.7	4.8	0.53
EBL (ml)	805.6±495	893.8±570	898.7±707	0.95
Pringle time (min)	27.6±18.7	43.0±24.9	29.7±19.1	0.0001
Normal liver parenchyma (%)	72.7	61.5	58.5	0.10
Largest tumor size (cm)	6.1±4.4	5.9±3.9	4.6±3.2	0.005
Number of tumors removed	2.2±2.5	2.4±2.2	2.4±2.3	0.91

*, **P values pertain to comparisons between allogeneic and autologous groups.

patients, there were no significant differences among the three groups. The mean size of resected tumors was smaller in the autologous group but there was no difference in the number of tumors resected.

The analysis of variables related to perioperative outcome is shown in Table 3. Although EBL was used in cohort matching and the allogeneic and autologous groups received no more than 2 U of red cells, the mean number of units transfused in the allogeneic group was higher compared to the autologous group (1.7 versus 1.4 U). There were no operative deaths in the allogeneic and autologous groups, and only one postoperative death occurred in the control group. Forty-four percent of the entire cohort experienced at least one complication, and complications occurred at a statistically similar rate in all groups. The total number of complications was also similar between all groups. Although the incidence of major complications appeared to be lower in the control group compared to the other two groups, this difference did not reach statistical significance (control versus allogeneic, $P=0.09$; control versus autologous, $P=0.35$). Major complications occurred at a similar rate between the allogeneic and autologous groups. The number of cardiac complications (e.g., arrhythmias, CHF), liver-related complications (e.g., biloma, liver insufficiency), gastrointestinal complications (e.g., ileus, bowel obstruction), and other complications (e.g., urinary retention, deep-vein thrombosis) were identical in the allogeneic and autologous groups. Similarly, the number of infectious complications was also similar in these two groups. The autologous group had a greater number of pulmonary complications (e.g., atelectasis, pneumonia, pleural effusion) compared to the allogeneic group. The fraction of patients requiring postoperative ICU management and the mean length of hospital stay (9.7 days) was statistically similar among all groups.

Discussion

Recent developments in intraoperative maneuvers, such as surgical resection based on segmental hepatic anatomy and anesthetic techniques employing low central venous pressures, in addition to refinements in postoperative management, have contributed to the dramatically improved safety of hepatic resections and the resultant low-operative mortality rates of less than 5%.[1,2] Despite these advances, large contemporary series indicate that a substantial risk of major intraoperative blood loss averaging 600 to 1,100 ml remains in patients undergoing major liver resection, and although the need for blood transfusions has generally declined, the need for blood product support remains high.[3]

Allogeneic blood transfusion has been associated with numerous detrimental sequelae.[10] Despite sophisticated screening tests to minimize the risk, blood-borne diseases

Table 3 Postoperative Variables

Variable	Control ($N=104$)	Allogeneic* ($N=104$)	Autologous** ($N=104$)	Allogeneic versus Autologous (P value)
Units transfused (N)	0	1.7±0.5	1.4±0.5	<0.0001
Operative mortality (%)	1	0	0	1.00
Morbidity (%)	40.4	45.2	48.1	0.65
Total complications (N[a])	62	66	77	0.57
Highest complication grade	5	4	4	1.00
Mean complication score	0.8±1.2	1.0±1.3	1.0±1.2	0.91
Major complications (%)	11.5	24.0	20.2	0.58
Complication type (N, %)				
Cardiac	5 (4.8)	6 (5.8)	7 (6.7)	
Pulmonary	11 (10.6)	10 (9.6)	18 (17.3)	0.78
Gastrointestinal	5 (4.8)	4 (3.8)	4 (3.8)	0.09
Liver related	11 (10.6)	19 (18.3)	17 (16.3)	1.00
Other	11 (10.6)	4 (3.8)	5 (4.8)	0.74
				0.74
Infections (N, %)	19 (18.3)	23 (22.1)	26 (25.0)	0.62
ICU stay (%)	0	2.9	1.9	0.65
Hospital LOS (days)	9.2±4.4	10.2±4.8	9.6±4.2	0.3

Pulmonary Atelectasis, pleural effusion, pneumonia, pneumothorax, pulmonary embolus. *Cardiac* Angina, arrhythmia, CHF, hypotension, MI. *Liver-related* Ascites, bile leak, fluid collection, liver insufficiency. *Gastrointestinal* Bowel obstruction, colitis, delayed gastric emptying, gastritis, ileus. *Other* CVA, TIA, renal failure, urinary retention, pump dysfunction. *Infections* Intra-abdo abscess, pneumonia, line infection, sepsis, *Clostridium difficile* colitis.

[a] Some patients had multiple complications.

*, ***P* values pertain to comparisons between allogeneic and autologous groups.

such as human immunodeficiency virus infection and hepatitis C are transmitted at frequencies of approximately 1 in 2,000,000, whereas contamination by and transmission of various bacterial agents occur at a disturbingly high frequency of 1 in 3,000 and 1 in 500,000 U transfused, respectively, and is the second most frequently reported cause of transfusion-related death after hemolytic reactions.[11–14] A broad spectrum of immune-mediated transfusion reactions ranging from mild, febrile non-hemolytic reactions to full-blown anaphylactic reactions occur in up to 6% of patients receiving allogeneic transfusions. Other donor leukocyte-mediated adverse effects include the inadvertent alloimmunization of a naive recipient to donor erythrocyte, leukocyte, or platelet antigens, or transfusion-related graft-versus-host disease, which is a rare but often fatal condition because of prolonged circulation of donor cells.[15–17] Transfusion of blood products can also be complicated by acute lung injury, which appears to be caused by an interaction between donor anti-granulocyte and/or anti-HLA antibodies and recipient granulocytes. This process known as transfusion-related acute lung injury occurs in 2 per 10,000 U transfused, can progress to acute respiratory distress syndrome, and be a life-threatening event with an estimated mortality of 5%.[18] Furthermore, allogeneic transfusions place an additional stress on the already-limited donor blood supply.[7]

An immunosuppressive effect of allogeneic transfusion has long been known and has been exploited in some clinical situations, such as improved renal allograft survival[19,20] and a reduced rate of recurrent spontaneous abortions.[21] Intense investigation has been focused on the negative impact of transfusion-related immunomodulation on postoperative infection rates, perioperative outcome, and recurrence rates of resected malignancies. The results are conflicting, and opinions remain widely divided. Numerous observational studies have reported an association between perioperative allogeneic transfusion and the increased risk of postoperative infections.[22] However, critics argue that these reports did not adequately adjust for confounding variables such as severity of illness and risk factors for postoperative infections. A review of ten randomized controlled trials comparing leukocyte-reduced to nonleukocyte-reduced allogeneic RBC transfusion on postoperative infection rates are disparate, and a meta-analysis of five randomized controlled trials comparing recipients of allogeneic and autologous RBC or whole blood found no difference in postoperative infection rates.[23] A recent study examining the influence of transfusion on perioperative and long-term outcome in a large cohort of patients undergoing liver resection for colorectal metastases demonstrated that allogeneic transfusions were associated to development of complications, lengthened hospitalization, and increased postoperative mortality.[4]

The effect of transfusion on recurrence and long-term outcome of cancer patients remains just as controversial. There are several studies suggesting a detrimental impact of transfusion on disease-free survival in patients undergoing hepatectomy for colorectal liver metastases and hepatocellular carcinoma.[24–27] Experimental animal models have also demonstrated the tumor-growth-promoting properties of allogeneic transfusions and the ability to lessen these effects by pre-storage leukodepletion.[28] However, three randomized controlled trials comparing the incidence of cancer recurrence in colorectal cancer patients receiving buffy coat-reduced allogeneic red cells to control blood did not demonstrate a statistically significant difference.[29]

With all of the above-mentioned potential risks associated with allogeneic transfusion, the use of autologous transfusion has been promoted. Preoperative autologous donation and transfusion eliminates the risks of transmitting infectious agents, transfusion reactions, alloimmunization, and graft versus host disease. An added benefit is the erythropoiesis stimulated by repeated phlebotomies, which results in accelerated restoration of hemoglobin after intraoperative blood loss.

However, autologous donation and transfusion is not without limitations. The procedure cannot be performed on patients who are anemic, despite anemia being an established preoperative predictor of transfusion requirements. A retrospective study examining the risk factors for increased blood loss and the need for blood transfusion during hepatectomy for hepatocellular carcinoma found that preoperative autologous blood reservation was unnecessary in the majority of cases and found that the only significant independent predictor for transfusion requirement was a preoperative hemoglobin level of less than 11 g/dl, which is a contraindication to autologous blood donation.[30] Several weeks are required for the completion of the collection and processing of the donated blood, time that is usually not available in cancer patients. The cost of preoperative autologous donation and storage is significantly higher than that for allogeneic blood, and unused units are generally stored for 35 days in the blood bank, adding to the clerical burden. Additionally and contrary to popular conception, unused units are usually discarded. Special clerical labels, separate storage space, special handling, extra time and labor, and disposal of approximately 50% of unused blood all contribute to the additional cost and burden on blood bank facility. Furthermore, autologous donation does not necessarily eliminate the risk of mistransfusion as a result of clerical error. For cancer patients, there is an additional concern of viable tumor cells within the donated blood inducing hematogenous spread, although studies suggest that the viability of these cells is negligible with storage of the blood for more than 7 days.[31,32]

In the present study, which attempted to control for several well-known variables associated with perioperative morbidity, there was no difference in length of hospital stay, complication rates, or operative death between the allogeneic and autologous groups. Indeed, despite receiving a somewhat smaller number of transfused units, the autologous group experienced no obvious benefit in a perioperative outcome compared to patients who received allogeneic blood. The data suggest that, in patients receiving a limited number of transfused units, which represents a large majority of patients who require any blood products after hepatectomy in contemporary series, the use of autologous donation does not offer an obvious advantage in terms of reduction in perioperative morbidity.

That is not to say that the use of autologous blood did not offer some benefits. Clearly, there was a reduction in the number of allogeneic units used, thus, sparing that blood for other patients. Furthermore, although the risk of transmitting HIV and HCV virus has been markedly reduced, dissemination of other, as yet unidentified, infectious agents remains a theoretical risk and is averted by preoperative autologous donation. However, the lack of a clear reduction in morbidity in the autologous group, combined with the increased cost and inconvenience to patients, make autologous donation a somewhat less attractive option than other blood conservation techniques, especially in a cancer population where prolonged preoperative delays are not possible. This study is limited, however, because of the relatively small number of patients and number of units transfused, which, in turn, may not allow delineation of more subtle differences in perioperative outcome. Furthermore, such subtle differences, if they exist, may well be masked by the magnitude of the operative procedures performed in the current study. Indeed, this possibility is suggested by the fact that the perioperative outcome in the nontransfused patients was not markedly different from that of the two transfused groups.

Autotransfusion and isovolemic hemodilution are alternative perioperative techniques of blood salvage and conservation that may have a role in patients undergoing major hepatic resection.[33–35] Intraoperative autotransfusion is the collection and transfusion of blood lost during and immediately after the surgical procedure. Up to 50% of the red blood cell volume lost may be retrieved using this technique, and the survival rate of the recovered red cells is comparable to that of allogeneic red cells. In cancer patients, concern has been expressed regarding the potential for dissemination of tumor cells, as they may not be trapped during the filtration process, although the level of risk is uncertain. Intraoperative isovolemic hemodilution or acute normovolemic hemodilution utilizes the simultaneous withdrawal of blood with infusion of cell-free solution to maintain intravascular volume before operative blood loss. This approach reduces red blood cell loss by dilution, and the blood withdrawn is reinfused after hemostasis is achieved. Hemodilution has been shown, in one small study, to reduce the transfusion requirements in liver resection patients,[33] but the technique has not been widely adopted. Additionally, both approaches require specialized training of operating team staff and availability of a blood salvage devices at the time of operation, thereby, increasing the cost and complexity of intraoperative management. To minimize exposure of the patient undergoing major hepatic resection to the potentially harmful effects of allogeneic transfusions and to decrease the burden placed on the limited blood bank resources, a concerted effort in the preoperative identification of patients who will likely require transfusion of blood products based on known risk factors of transfusion and the thoughtful utilization of preoperative autologous donation and intraoperative blood salvage techniques, such as isovolemic hemodilution and autotransfusion, is of paramount importance.

Conclusion

In patients undergoing hepatic resection, autologous blood transfusion does not demonstrably improve perioperative outcome when compared to a matched cohort of patients receiving a similar number of allogeneic units. Although the use of preoperative autologous blood donation spares allogeneic units for use in other patients, the absence of a clear benefit in perioperative outcome using this technique suggests that other blood conservation and salvage maneuvers should be examined.

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J Gastrointest Surg (2007) 11:1294–1297
DOI 10.1007/s11605-006-0078-7

Behavior of the Common Bile Duct Diameter Before and 12 Years after Choledochostomy for Cholecystolithiasis and Choledocholithiasis. A Prospective Study

Attila Csendes · Paula Csendes · Patricio Burdiles · Juan Carlos Diaz · Fernando Maluenda · Ana Maria Burgos

Published online: 8 August 2007

Abstract

Introduction In patients with common bile duct (CBD) stones, the diameter of the CBD is usually dilated. After surgery, the behavior of CBD diameter is not clearly known.

Objective To determine at a late follow-up the width of CBD before and after choledochostomy for CBD stones.

Material and Methods In this prospective study, 39 patients with gallstones and CBD stones were included. They were 30 women and 9 men with a mean age of 52.6 years. In all ultrasound, determination of the CBD caliber before and 12 years after surgery was performed.

Results The mean value of the inner diameter of the CBD before surgery was 11.6 and 12.3 mm in patients below or above 60 years, respectively. Measurement 12 years after surgery showed a mean decrease of nearly 50% of preoperative values, which was highly significant ($p<0.0001$). However, either below or above 60 years, only 75% of the patients showed this decrease, whereas 25% remained unchanged.

Conclusion The dilated preoperative CBD returns to normal or near normal values in 3/4 of the patients after surgical exploration of the CBD and extraction of the stones.

Keywords Common bile duct diameter · Choledochostomy · Ultrasound

The effect of cholecystectomy on the diameter of the common bile duct (CBD) after cholecystolithiasis has been a matter of controversy over many years. There are at least 19 published reports concerning this topic with some conflictive results. On the contrary, there are very few reports dealing with the behavior of the CBD in patients with CBD stones submitted to surgery. We have found, after an extensive review, only four publications[7,8,10,11,15] mentioning very few patients in whom measurements of the diameter of the CBD were performed, before and after choledochostomy for choledocholithiasis.

The purpose of the present prospective study was to determine the changes in the diameter of the CBD before and late after choledochostomy and cholecystectomy for CBD stones.

Material and Methods

Patients studied The present prospective evaluation started on January 1987, when a complete protocol for several histological, manometric bacteriological, and radiological studies was planned in patients with gallstones or CBD stones.[1,3,4] As part of this protocol, the measurement of the CBD diameter before 12 years after surgery was defined.

The protocol was closed on December 1991, when a large number of patients were evaluated. A total of 39 patients submitted to choledochostomy for CBD stones were randomly selected for a late follow-up 12 years after surgery. They were 9 men and 30 women with a mean age

A. Csendes (✉) · P. Csendes · P. Burdiles · J. C. Diaz · F. Maluenda · A. M. Burgos
Department of Surgery, Clinical Hospital University of Chile, Santos Dumont # 999, Santiago, Chile
e-mail: acsendes@machi.med.uchile.cl

A. Csendes · P. Csendes · P. Burdiles · J. C. Diaz · F. Maluenda · A. M. Burgos
Department of Radiology, Clinical Hospital University of Chile, Santos Dumont # 999, Santiago, Chile

Table 1 Mean Diameter of the Common Bile Duct before and 12 Years After Choledochostomy for Choledocholithiasis

	Before surgery (mm)	12 years after surgery (mm)	*p* value
≤60 years of age			
Mean[a]	11.6±4	6.2±2	<0.001
Range	(5–30)	(2–10)	
Increase	1		
Unchanged	6		
Decrease(%)	20(74%)		
≥61 years of age			
Mean[b]	12.3±3	5.75±2	<0.001
Range	(5–19)	(3–11)	
Increase	0		
Unchanged	3		
Decrease(%)	9(75%)		

[a] $n=27$
[b] $n=12$

at the initial operation of 52.6 years (range 22–77). There were 27 patients below 60 years of age and 12 above 61 years. Only patients submitted to cholecystectomy and choledochostomy were included in the present investigation, excluding patients with cholecystectomy or choledochostomy alone, patients with strictures of the CBD, pancreatitis, and biliodigestive fistulas. All of them had an intraoperative cholangiogram that was normal at the end of the surgical procedure.[3]

Ultrasonographic evaluation In all patients 2 to 7 days before surgery, an ultrasound evaluation of the CBD, measuring its internal diameter, was performed by a real time equipment (Aloka, Japan) with a 3.5 MH_2 probe. This test was repeated by a single experienced radiologist 12 years after surgery. After an overnight fast, patients were examined in deep inspiration, lying supine or in a left lateral oblique position with a right subcostal approach.

The extrahepatic bile duct was easily identified at the level of the porta hepatis, where the hepatic artery crosses perpendicularly between them. The CBD diameter was measured at its midportion (suprapancreatic part of the CBD), from its inner border to inner border in an anteroposterior examination.[5,9]

All measurements were expressed in mm and the measurement error for ultrasonography of the CBD was defined as 1± mm.[5,9,10]

Surgical technique The details of our procedure for CBD exploration and T-tube placement have been extensively discussed in detail in a previous publication.[2] Briefly, it starts with a complete Kocher's maneuver. Two lifting sutures are placed transversally at the most distal portion of the choledochus. The anterior wall is opened parallel to the axis of the choledochus. The exploration of the CBD is first done proximally and then distally with a Randall forceps. Then the CBD is irrigated with saline through a soft catheter. This catheter is gently passed into the duodenum. Then, Bakes' dilators up to 6 mm are employed, and the top of the dilator can be easily felt inside the duodenal lumen. A no. 14 T-tube is placed inside the CBD with arms no longer than 3 cm on each side. The choledochal wall is closed with absorbable stitches. An operative cholangiogram is always obtained to exclude the presence of residual stones.

Statistical evaluation For statistical analysis, the exact Fisher test and the chi square test were employed, taking a $p<0.05$ as significant.

Results

The mean values of the internal diameter of CBD before and 12 years after surgery according to age distribution are shown in Table 1. Among patients below 60 years, there was a significant decrease of 46% of mean preoperative values. However, the behavior of each individual patient was not uniform.

Table 2 Diameter of the Common Bile Duct Before and After Choledochostomy for Choledocholithiasis

Author	Year	No. of patients	Diagnostic method	Follow-up (months)	Preoperative (mm)	Postoperative (mm)	Change (mm)	Change (%)	Significance
Le Quesne [7]	1959	18	IC	19.5 (13–33)	15.6 (6–26)	13.6 (6–26)	−2.0	13	No
Longo [8]	1967	8	IC	43–69	13.75 (7–18)	12.7 (8.5–16.2)	−1.0	7	No
Mueller [11]	1981	18	US	10.8	Normal	Normal	–	–	No
Mueller [10]	1982	5	US	7 day	9	5	−4	44	Yes
Wedmann [15]	1988	13	US	36	7.5	5	−2.5	33	<0.05
Csendes	2004	39	US	144	≤60 years, 11.6	6.2	−5.4	46	<0.001
					≥61 years, 12.3	5.7	−6.6	54	<0.001

IC: intravenous cholangiography, *US*: ultrasound

Only one patient showed an increase, whereas six patients remained with an unchanged diameter (variations of 1 mm are included in this group). Twenty patients (74%) demonstrated decrease of the diameter of CBD. Among patients over 61 years, there was also a significant decrease of mean CBD inner diameter, approaching 54% of preoperative value ($p<0.001$). However nine patients (75%) showed this decrease, whereas three patients demonstrated no change. In this group, the decrease of CBD diameter was more pronounced compared to patients below 60 years. One patient from the entire group (2.6%) presented with an asymptomatic residual stone at the CBD. She had an age of 66 years at the first operation with a CBD diameter of 10 mm and an intraoperative cholangiography was interpreted as normal. Twelve years later, she had this single stone with a CBD of 9 mm of diameter. She refused any further endoscopic or surgical removal. Patients who remained with abnormal dilatation of the CBD were asymptomatic, but we did not measure liver function tests among them. There was no simple case with strictures at the T-tube site.

Discussion

The evolution and behavior of the CBD diameter after biliary tract surgery has been a matter of considerable scientific debate in the surgical, anatomical, radiologic, and ultrasonographic literature for several decades. The main focus of interest was directed toward the behavior of the CBD diameter before and after cholecystectomy alone. We have found 19 published reports in English literature concerning this topic, which is extensively analyzed in another study of our group and it is not the purpose to repeat all comments again.

However, it is surprising how few mentions are published concerning the behavior of the CBD diameter in patients will CBD stones submitted to choledochostomy with surgical exploration of the CBD and placement of a T-tube for some weeks. The common surgical belief is that probably the dilated duct diminishes its size, although there is no precise scientific published data on this topic. Table 2 summarizes the reports that we have found after an extensive research in surgical literature that mention the present point of discussion. The first authors performing some measurements of the CBD diameter in patients with choledocholithiasis were Le Quesne et al. who evaluated 18 patients with intravenous cholangiography almost 20 months after surgery.[7] They found no evidence that a dilated CBD diminishes significantly its caliber after cholecystectomy and removal of the stones from the duct. Later, Longo et al. evaluated eight patients almost 4 to 5 years after surgery, finding no significant changes in the diameter of CBD after operation.[8] Mueller et al. in two publications[10,11] evaluated a few patients before and after surgery. In one publication,[11] they only mention "normal values" after surgical clearance of the CBD in 18 patients, mentioning that the 2 cases with dilated ducts could return to normal values, demonstrating for the first time that acutely dilated duct before surgery may return to normal values because of the fact that sparse oblique-oriented muscle fibers and a vast network of elastic fibers throughout its course are present. Therefore, there is a potential for distensibility when an acute obstruction occurs and if the elastic tissue has not been chronically stretched, it may return to normal. In the other publication,[10] they mention five patients with an acute dilatation of the CBD because of stones, which may collapse to normal values if either the obstruction passed or was removed. However, in none of these two reports, a clear measurement of the CBD is mentioned. Finally, Wedmann et al. performed measurements of CBD diameter by ultrasound in 13 patients before and 36 months after surgery.[15] For the first time, a significant decrease was demonstrated. The present report is the largest prospective evaluation with a long-term follow-up, demonstrating a significant decrease of CBD diameter, independent of the age. This decrease is nearly 50% of the preoperative value, reaching almost normal values. It is possible that any patient with abnormal dilatation of the CBD could have some residual or recurrent stone, because the only patient that clearly had a CBD stone was asymptomatic. In our group, the ultrasound detection of CBD stones is 70%. Probably, a closer follow-up with magnetic resonance cholangiopancreatography would answer this question. We have incorporated this radiological technique in our university hospital only since 3 years ago, and therefore none of our patients was evaluated by this technique.

Our preoperative values for CBD diameter are similar to the first report by ultrasound measurements performed by Parulekar.[12] It is important to note that there are discrepancies between the measurements by intravenous cholangiography versus ultrasound determinations, as shown by Mueller et al.[10] The other factor that is very important when determining the diameter of the CBD is the influence of age.[13] This is specially relevant when measurements of the CBD before and after cholecystectomy alone are performed. In these patients with normal CBD diameter in the majority of cases before surgery, it has been shown that some of the "physiological" dilatation seen among these patients is because of the effect of age. It is accepted that above 60 years of age, nearly 1 mm should be added to CBD diameter each decade.[6,13] However, in the present investigation, the age-dependent factor seems to be unimportant because of preoperative CBD dilatation and its decrease after surgery. This decrease seems to be very early and fast

after surgery. Mueller et al. showed that acute dilatation of the CBD returned to normal values 7 days after surgery.[10] Presumably, the stretch potential of the elastic tissue permits dilatation in the presence of obstruction or acute elevations of intraductal pressure. Schein and Beneventano demonstrated that duct caliber could increase up to 2.5 times baseline with increasing intraductal pressure.[14] When obstruction is relieved, the elastic recoil of the duct walls causes prompt return to normal caliber.

In conclusion, the dilatation of the CBD seen in patients with CBD stones returns to normal values after surgery and remains in these values for at least 12 years after surgery. However, this decrease in the width of CBD is seen in only 75% of the patients, whereas the rest show no significant variation.

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J Gastrointest Surg (2007) 11:1298–1308
DOI 10.1007/s11605-007-0173-4

Bacteria Entombed in the Center of Cholesterol Gallstones Induce Fewer Infectious Manifestations than Bacteria in the Matrix of Pigment Stones

Lygia Stewart · J. McLeod Griffiss · Gary A. Jarvis · Lawrence W. Way

Published online: 25 July 2007

Abstract
Purpose The clinical significance of bacteria in the pigment centers of cholesterol stones is unknown. We compared the infectious manifestations and characteristics of bacteria from pigment stones and predominantly cholesterol stones.
Methods Three hundred forty patients were studied. Bile was cultured. Gallstones were cultured and examined with scanning electron microscopy. Level of bacterial immunoglobulin G (bile, serum), complement killing, and tumor necrosis factor-alpha production were determined.
Results Twenty-three percent of cholesterol stones and 68% of pigment stones contained bacteria ($P<0.0001$). Stone culture correlated with scanning electron microscopy results. Pigment stone bacteria were more often present in bile and blood. Cholesterol stone bacteria caused more severe infections (19%) than sterile stones (0%), but less than pigment stone bacteria (57%) ($P<0.0001$). Serum and bile from patients with cholesterol stone bacteria had less bacterial-specific immunoglobulin G. Cholesterol stone bacteria produced more slime. Pigment stone bacteria were more often killed by a patient's serum. Tumor necrosis factor-alpha production of the groups was similar.
Conclusions Bacteria are readily cultured from cholesterol stones with pigment centers, allowing for analysis of their virulence factors. Bacteria sequestered in cholesterol stones cause infectious manifestations, but less than bacteria in pigment stones. Possibly because of their isolation, cholesterol stone bacteria were less often present in bile and blood, induced less immunoglobulin G, were less often killed by a patient's serum, and demonstrated fewer infectious manifestations than pigment stone bacteria. This is the first study to analyze the clinical relevance of bacteria within cholesterol gallstones.

This paper was presented at the American Hepato-Pancreato-Biliary Association meeting last March 2006.

L. Stewart (✉) · L. W. Way
Department of Surgery, University of California San Francisco,
4150 Clement Street,
San Francisco, CA 94121, USA
e-mail: lygia.stewart@med.va.gov

L. Stewart
Surgical Service, San Francisco VA Medical Center,
San Francisco, CA, USA

J. M. Griffiss · G. A. Jarvis
Center for Immunochemistry, San Francisco VA Medical Center,
San Francisco, CA, USA

J. M. Griffiss · G. A. Jarvis
Department of Laboratory Medicine,
University of California San Francisco,
San Francisco, CA, USA

Keywords Biliary bacteria · Gallstones · Pigment stones · Cholesterol stones · Cholangitis · Scanning electron microscopy · Biofilm · Slime

We first reported the presence of bacterial microcolonies in gallstones in 1987.[1] We noted that these bacterial microcolonies were commonly found in pigment stones (PS),[1–4] were often present in mixed stones with exterior pigment,[4] and could also be found in predominantly cholesterol stones (CS) with pigmented centers.[1,2,4] We noted that these gallstone bacteria were associated with infectious manifestations,[2,4] that certain bacterial factors (P1-pili, slime production) facilitated bacterial colonization,[5,6] and that bacterial virulence factors [serum sensitivity, induction of tumor necrosis factor-alpha (TNF-α)] influenced the severity of the associated illness.[4,7,8] Because bacterial microcolonies are much more prevalent in PS, much of the information on

this subject reflects the behavior of bacteria in PS rather than bacteria in the pigment centers of CS.[1–12] Others have reported the presence of bacterial DNA in CS, but not the clinical significance of these bacteria with regard to biliary infections.[13–17] The current study examined the clinical infectious and inflammatory manifestations associated with bacteria in the center of predominantly cholesterol gallstones (CS), and compared them to bacteria in pigment and mixed stones where the bacteria are located throughout the stone matrix and the exterior part of the stone.[1–4] We also studied virulence factors [slime, serum sensitivity, serum and bile immunoglobulin G (IgG), and TNFα production] associated with bacteria from obtained from CS or PS.

Methods

Three hundred forty patients with gallstone disease were studied: 26 from UCSF–Moffitt Hospital and 314 from the San Francisco Veterans Hospital. There were 50 women and 290 men, whose average age was 61 (range 17–104) years. Eighty-four percent were Caucasian, 9% were Hispanic, 4% were African-American, and 3% were Asian. The clinical diagnoses were as follows: biliary colic 31%, acute cholecystitis 34%, pancreatitis or choledocholithiasis (without cholangitis) 16%, cholangitis 14%, and in 5% the gallbladder was removed during other procedures (Whipple, hepatectomy, etc.). Open cholecystectomy was performed in 48% of patients (13% were started laparoscopically), laparoscopic cholecystectomy in 48%, and 4% were treated with endoscopic retrograde cholangiopancreatography (ERCP) or cholecystostomy tube. Eighteen percent of patients underwent preoperative ERCP or percutaneous transhepatic cholangiography (PTC) before cholecystectomy; and 4% were treated with cholecystostomy tube before cholecystectomy.

Severity of illness was categorized using our previously defined criteria[4,7,8], which is similar to the criteria defined by Bone et al.[18] Illness severity, based on preoperative findings, was staged as (1) none: no clinical infection or inflammatory manifestations, included in this group were patients with jaundice but no clinical manifestations of infection or inflammation (fever, leukocytosis, tachycardia, etc.); (2) moderate: fever (T>100°F), leukocytosis (WBC≥11 K/cm^2), focal necrosis of the gallbladder wall; and (3) severe: cholangitis (choledocholithiasis and Charcot's triad—fever/chills, abdominal pain, jaundice), bacteremia/fungemia (recovery of bacteria or fungi from blood culture), hypotension (BP<90/60), organ dysfunction/failure, abdominal or hepatic abscess, empyema/emphysema/gangrenous gallbladder.

Gallstones, bile, and blood (when clinically indicated) were cultured. Gallstones were obtained under sterile conditions at surgery (or ERCP), rinsed with normal saline, crushed, and cultured in tryptic soy broth for 24–48 h. One hundred twenty-four gallstones were also examined under scanning electron microscopy (SEM) for the presence of bacterial microcolonies using our previously described technique.[1] Bile was obtained for culture at surgery, ERCP, or during PTC. Blood cultures were obtained when clinically indicated. Patients whose gallstone cultures were positive for bacteria, or whose gallstones contained bacteria on SEM examination, were considered to have gallstone bacteria. Patients whose gallstones were sterile and devoid of bacterial microcolonies on SEM were considered to have sterile gallstones.

Gallstones were grouped by appearance into two groups: (1) predominantly CS: stones that appeared to be either pure cholesterol (73 stones) (Fig. 1) or predominantly cholesterol with a visible pigmented center (82 stones) (Figs. 2a and 3a); and (2) PS: stones composed entirely of brown or black pigment (136 stones), or mixed stones with pigment on the outside of the stone (32 stones) (Fig. 4a). Pigment and mixed stones with exterior pigment were grouped for this analysis because bacteria (when present) were always found in the pigment portions of the stone,[1–3,7] and we were studying the influence of bacterial location *inside* a CS. When different types of gallstones were present in the same patient, each type of stone was analyzed separately. This was the case in four patients, two with both CS and black PS in their gallbladder, and two with CS in the gallbladder and mixed stones with exterior pigment (classified as PS) in the common bile duct (CBD). Cases with gallbladder and CBD stones of the same type, with identical culture and SEM results, were analyzed as a single entry.

Gallstone composition of 183 representative gallstones (84 CS and 99 PS) was determined using Fourier-transformed infrared spectroscopy (FTIR), as previously described.[2] Stones were air-dried, crushed in an agate mortal and pestle, mixed with potassium bromide in a shaking device, and pressed into pellets at 3,000 psi. They were then examined under FTIR. Standards of varying concentrations were made

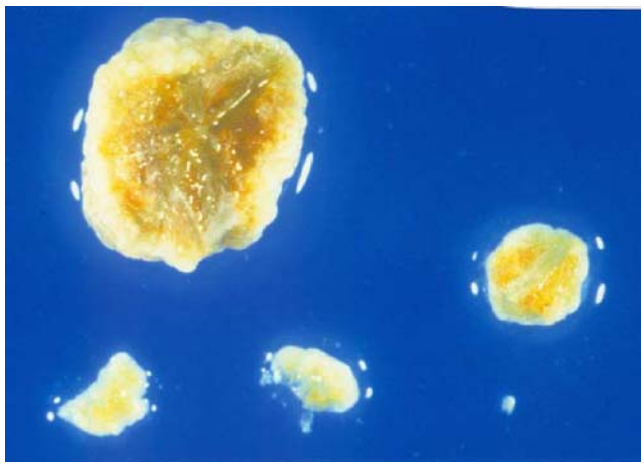

Figure 1 Cholesterol stone without a visible pigmented center. These stones were usually sterile.

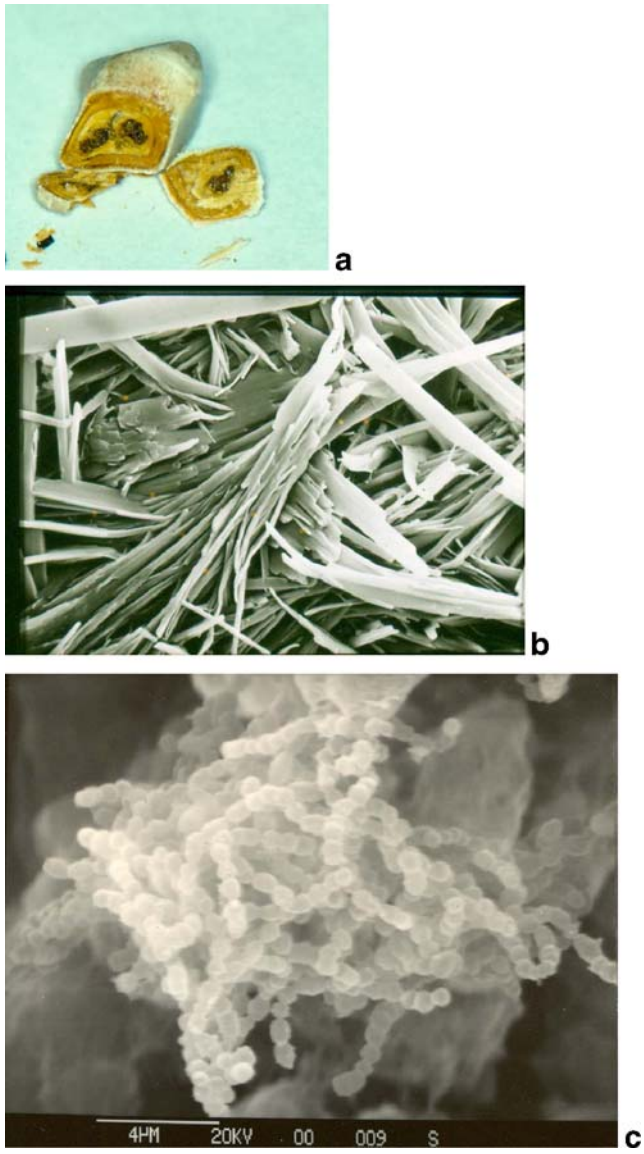

Figure 2 **a** Cholesterol stone with a visible pigmented center. **b** Scanning electron microscopy examination of the stone surface. Cholesterol crystals are seen but no bacterial microcolonies. **c** Scanning electron microscopy examination of the pigmented center demonstrating bacterial microcolonies (cocci). *Streptococcus* species were cultured from this gallstone.

using commercially available cholesterol, conjugated bilirubin (bilirubin isomers, Sigma), Ca-palmitate, Ca-carbonate, and synthesized Ca-bilirubinate. Multiple standards were made to fully bracket the range of compositions of all the gallstones. For the examination of predominantly CS, the standards included compositions composed of cholesterol and Ca-bilirubinate, with cholesterol contents of 70, 80, 90, 95, and 97%.

Bacterial Slime Production

Quantitative bacterial slime production was determined using a previously described assay.[6,19] Glass test tubes (American Scientific Products, McGaw Park, IL, USA) containing 1 ml tryptic soy broth supplemented with 10% (vol/vol) glucose were inoculated with a single colony of bacteria and incubated stationary at 37°C for 48 h. Each tube was decanted and washed two times with 1 ml of H_2O and reacted with Carnoy's solution (absolute ethanol, chloroform, glacial acetic acid 6:3:1). After this, 1 ml of

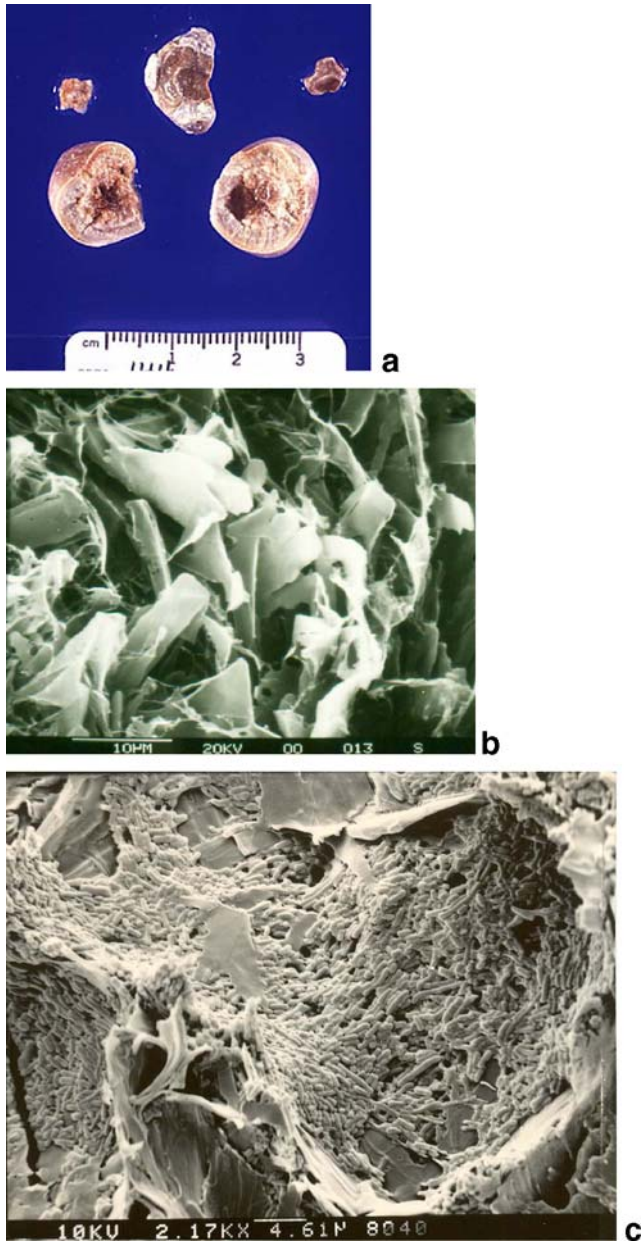

Figure 3 **a**: Cholesterol stone with a visible pigmented center. **b** Scanning electron microscopy examination of the stone surface. Cholesterol crystals are seen but no bacterial microcolonies. **c** Scanning electron microscopy examination of the pigmented center demonstrating bacterial microcolonies (rods). Note that bacteria can be seen in association with cholesterol crystals. Two species of *Escherichia coli* were cultured from this gallstone.

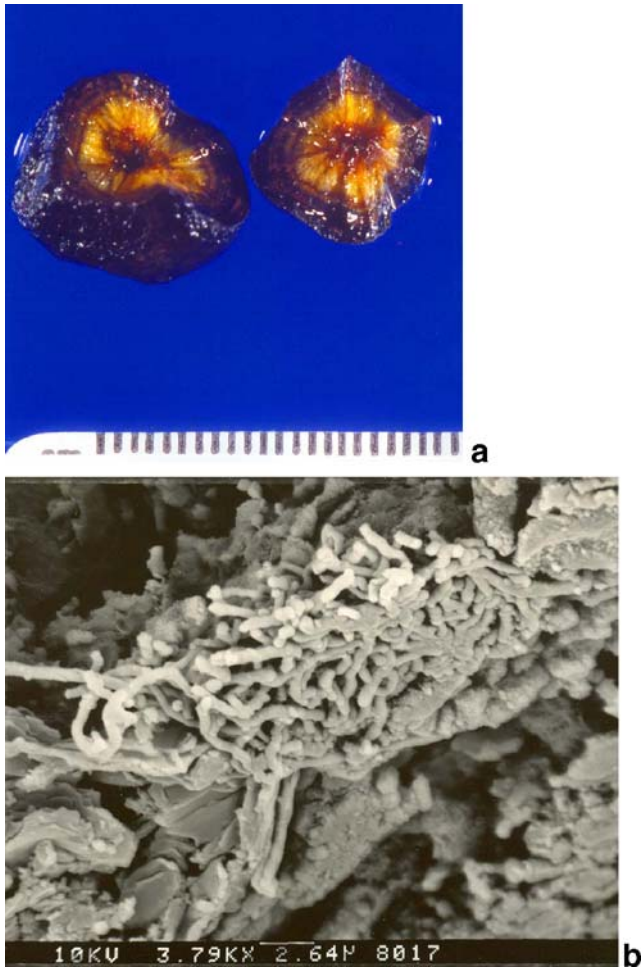

Figure 4 **a** Mixed stone with both cholesterol and pigment portions, but with pigment on the outside of the stone. These types of stones contained bacteria as frequently as stones composed entirely of pigment, and so were grouped as PS. **b** Scanning electron microscopy examination demonstrating bacterial microcolonies (rods) located in the stone's external pigmented layer.

safranin was added to the tubes, and the tubes were gently rotated to uniformly coat the adherent material (slime). Excess stain was removed by washing with 3 ml of H_2O two times. One milliliter of 0.2 M NaOH was added and the sample was heated for 1 h at 85°C. Samples were then vortexed, cooled to room temperature, and the optical density (OD) was determined at 530 nm. Optical density directly correlates with the amount of slime present. Test tubes containing only tryptic soy broth supplemented with 10% (vol/vol) glucose were used as negative controls. Analysis was done in triplicate for each bacterial species.

Serum and Bile Immunoglobulin G Against Biliary Bacteria

A dot blot assay was used to detect specific antibacterial antibodies in bile and serum.[20] Serum from 30 patients (14 with CS, 16 with PS) was analyzed for serum IgG against the 37 gallstone bacterial species from these patient's gallstones and bile. Bile from 19 patients (8 with CS and 11 with PS) was analyzed for bile IgG against the 24 bacterial species recovered from these patients' gallstones and bile. Bacteria, grown on agar were blotted onto nitrocellulose paper, followed by bile or serum (from the patient whose biliary tree harbored the bacterial species). Binding was detected by means of an alkaline-phosphatase-conjugated antibody to human IgG. Blots were scored visually (from 1–4) by two independent observers, and also scored using a densitometer. Agreement between the methods was good. The densitometer values were used for the final analysis. Assays were done in triplicate (for each bacterial species) and the results were averaged.

Complement-mediated Bacterial Killing

Bacteria were obtained as noted above. Serum was obtained from 135 patients at the time of operation. Serum from healthy controls was used for comparison. For standardized comparison of bacterial species a single control serum was used.

Eighty-five gram-negative bacterial isolates were tested for complement-mediated bacterial killing against the serum of the patient that harbored them and control serum using a standard bactericidal assay.[20] Bacteria were suspended in Mueller–Hinton broth to an OD_{530} of 0.1–0.2 and grown to an OD_{530} of 0.6 (about 10^8 bacteria/ml in log phase growth); they were resuspended in veronal-buffered saline (VBS) (OD_{530} of 0.6) and a 1:30,000 dilution in VBS prepared. Twenty-five microliters of bacterial solution, 25 μl of serum, and 75 μl of VBS were combined and incubated for 1 h at 37°C in a shaker bath. This was pour-plated at varying concentrations, incubated overnight in 5% CO_2 at 37°C, and the plates were counted. Growth of bacteria in normal- and heat-inactivated serum (60° for 45 min) was compared with growth in the absence of serum. Lack of bacterial growth in normal serum, coupled with growth in heat-inactivated serum, documented complement-mediated bacterial killing (serum-sensitive bacteria). Bacterial growth in the presence of serum showed resistance to complement-mediated killing (serum-resistant bacteria). More than 50% bacterial killing in serum was classified as serum-sensitive, whereas <50% bacterial growth inhibition in serum was classified as serum-resistant. Most (>93%) bacterial species were either totally (100%) killed in the presence of serum or showed no (0%) growth inhibition in serum.

Tumor Necrosis Factor-alpha Production In Vitro

The human monocytic cell line (ATCC, Rockville, MD, USA) was used in the stimulation bioassay. THP-1 cells

were grown as a suspension in RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal calf serum at 37°C in a 5% CO_2 incubator. The day before, THP-1 cells were transferred to fresh medium without antibiotics. Bacterial samples were plated on Mueller–Hinton agar plates and incubated at 37°C overnight.

Seventy bacterial isolates were chosen for study. For the bioassay, bacteria were suspended in phosphate-buffered saline (PBS) to an OD_{530} of 0.125. Eight hundred microliters of bacterial suspension was placed in duplicate sets of sterile, lipopolysaccharide-free glass tubes. For one set, 200 μl of fresh PBS was added, and for the other set, 200 μl of human serum was added. The two sets of tubes were incubated at 37°C with 5% CO_2 for 1 h, shaking at 75 rpm. Then, 1 ml of a suspension of THP-1 cells (10^6 cells/ml) in antibiotic-free RPMI medium was added to each of the glass tubes and incubated for four more hours. The supernatants were collected by centrifugation of the glass tubes at 3,000 rpm for 20 min at 4°C. Supernatants were transferred to fresh polystyrene, snap-cap tubes and stored at −70°C. The OptEIA ELISA kit for human TNF-α (Pharmingen, San Diego, CA, USA) was used to detect human TNF-α. ELISA TNF-α assay was done in duplicate for each bioassay supernatant (also done in duplicate), giving quadruplicate TNF-α assay results for each individual bacterial species (with and without sera). These results were averaged, and agreement was good.

Statistical Analysis

Statistical analysis was performed using the Student's *t* test or analysis of variance (ANOVA) for continuous variables, and the chi-squared or Fischer exact test for variables on a nominal scale (rates and proportions).

Results

Bacterial culture and analysis results for cases with pigment or CS is shown in Table 1. Bacteria were present in 35 (23%) of 155 CS and 122 (68%) of 179 PS ($P<0.0001$, chi-squared). Bacteria were equally present in the two types of gallstones classified as PS (pure pigment and mixed stones with exterior pigment, 68% in both cases). Among cases with gallstone bacteria, PS harbored more bacterial isolates than CS (average of 2.2 vs 1.3, $P<0.0001$, ANOVA). Among cases with gallstone bacteria, bactibilia was more common in cases with PS than those with CS (88 vs 54%, $P<0.0001$, chi-squared), and more bacterial isolates were present in the bile among cases with PS vs CS (2.1 vs 1, $P<0.0001$, ANOVA). Bacteremia of gallstone bacteria (one or more gallstone isolates also present in blood) was more common in cases with PS bacteria compared to those with CS bacteria (20 vs 3%, $P=0.033$, chi-squared). Among cases with bacteremia, 70% had one blood isolate, 26% had two isolates, and 4% had three isolates. Multiple blood isolates were present only in cases with PS.

Each individual bacterial species isolated from gallstone culture and their presence in bile or blood cultures are shown in Table 2. These data differ from that in Table 1 because many patients had more than one bacterial species in their gallstones, bile, and/or blood. As shown, the distribution of bacterial species present in CS and PS was similar, but individual bacteria in CS were less able to access bile (58 vs 80%, CS bacteria vs PS bacteria, $P=$

Table 1 Incidence and Properties of Bacteria in Cholesterol (CS) and Pigment (PS) stones

	PS	CS	*P* value
Gallstone bacteria (%)	68	23	<0.0001[a]
Gallstone bacteria in bile[b] (%)	88	54	<0.0001[a]
Gallstone bacteria in blood[b] (%)	20	3	0.033[a]
No. of gallstone isolates	2.2 (range 1–6)	1.3 (range 1–4)	<0.0001[c]
No. of bile isolates	2.1 (range 0–6)	1.0 (range 0–4)	<0.0001[c]
Serum IgG (scale 0–4)	3.5	2.9	0.029[c]
Bile IgG (scale 0–4)	2.6	1.7	0.022[c]
Gram-negative bacterial-killing patient sera (%)	62	26	0.013[a]
Gram-negative bacterial-killing control sera (%)	44	32	NS[a]
Slime (OD) (%)			
<10	57	32	
10–100	27	27	0.035[a]
>100	16	41	
In vitro TNF-α initial (pg/ml)	540	520	NS[c]
In vitro TNF-α with sera (pg/ml)	870	830	NS[c]

[a] Chi-squared
[b] Cases with gallstone bacteria and one or more bacterial isolates also present in bile or blood
[c] ANOVA or Student's *t* test

Table 2 Number of Bacterial Isolates recovered from Gallstone, Bile, and Blood by Gallstone Morphology

Bacterial Species	CS			PS		
	Stone	Bile	Blood	Stone	Bile	Blood
Escherichia coli	4	4	1	60	44	8
Enterococcus	3	3	0	45	41	6
Klebsiella species	4	3	0	34	31	12
Enterobacter species	4	3	0	14	12	2
Streptococcus species (non-*Enterococcus*)	3	3	0	12	8	0
Pseudomonas species	5	0	0	11	7	0
Staphylococcus species	3	1	0	10	7	1
Citrobacter freundii	0	0	0	10	9	1
Stenotrophomonas maltophilia	3	0	0	2	1	0
Aeromonas hydrophilia	2	1	0	2	2	1
Yeast	0	0	0	3	2	0
Bacteroides, clostridium, diphtheroids	3	3	0	15	10	1
Others (Heamphilus, Serratia, Proteus, non-speciated GNR/GPC)	4	1	0	17	13	0
Total	38	22 (58%)	1 (2.6%)	235	187** (80%)	32* (14%)

All bacteria species recovered from gallstones and their presence in bile or blood, grouped by gallstone morphology (defined in the text). This data differs from that in Table 1 because each individual bacterial species is tabulated and many patients had more than one bacterial species in their gallstones, bile, and/or blood.
GNR=Gram-negative rods, GPC=Gram-positive cocci.
*P=0.097 (chi-squared), bacteria from PS vs CS that were also present in blood.
**P=0.007 (chi-squared), bacteria from PS vs CS that were also present in bile.

0.007, chi-squared) or blood (2.6 vs 14%, CS bacteria vs PS bacteria, P=0.097, chi-squared).

Gallstone Composition and Bacterial Presence

The average cholesterol content of CS was 94%. There were no differences in the cholesterol content of CS that did or did not have bacteria present (95 vs 93%, P=0.20, ANOVA). Among the 84 CS analyzed for composition, only 7 (8%) were <90% cholesterol [5 were 80–87% cholesterol, two (both sterile) were composed of 60–65% cholesterol, and 30–35% Ca-carbonate]. The average cholesterol content of mixed stones (stones with both cholesterol and pigment portions with the pigment on the stone exterior) was 74% (range 23–96%). Cholesterol content of cholesterol-containing gallstones could not predict the presence of bacteria. Bacterial presence for all cholesterol-containing gallstones (including mixed stones) is shown in Fig. 5. As can be seen, gallstones that were <90% cholesterol usually (75%–88%) contained bacteria. Bacteria were less common in gallstones that were >90% cholesterol, but even in these cases, 39% of gallstones 90–95% cholesterol contained bacteria, and 25% of gallstones >95% cholesterol contained bacteria. Gallstone appearance was a better predictor of bacterial presence, 7% of CS without a visible pigmented center contained bacteria, whereas 38% of CS with a pigmented center contained bacteria (P<0.0001, chi-squared).

Scanning Electron Microscopy

Scanning electron microscopy examination of gallstones from 124 patients correlated well with gallstone culture. Bacterial microcolonies were seen in 56 (97%) of 58 gallstones whose cultures were positive. Scanning electron microscopy examination demonstrated bacteria with the same morphology (rods and/or cocci) as that obtained from culture in 54 (96%) of these 56 cases. In 15 of these 54

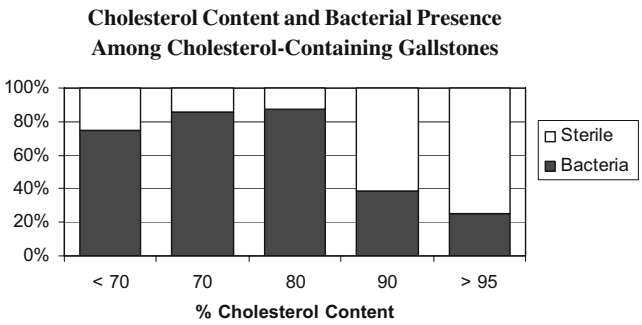

Figure 5 Correlation between gallstone cholesterol content (as determined by FTIR) and the presence of bacteria. All cholesterol-containing gallstones (including mixed stones like that in Fig. 4a) were included in this analysis. Note that cholesterol-containing gallstones composed of <90% cholesterol usually contained bacteria. Bacteria were also present in cholesterol-containing gallstones that were >95% cholesterol.

cases, additional rod or cocci forms were seen in addition to the forms detected in culture. In two cases, with both rod and cocci forms in culture, SEM demonstrated the rods but not the cocci forms. Scanning electron microscopy examination did not show any bacterial microcolonies in 57 (86%) of 66 gallstones with negative cultures, but demonstrated bacterial microcolonies in 9 (14%) of these gallstones, suggesting that SEM examination was more sensitive than culture. Most (8 of 9) of the gallstones with negative culture but bacteria on SEM were PS. Analysis by stone type revealed the following: SEM did not show bacteria in 36 (97%) of 37 sterile CS, and demonstrated bacterial microcolonies in 18 (95%) of 19 CS with positive cultures; and SEM did not demonstrate bacteria in 21 (72%) of 29 sterile PS, and demonstrated bacterial microcolonies in 38 (97%) of 39 PS with positive cultures.

When present, SEM demonstrated the bacterial microcolonies in the pigment portions of the gallstone. Cholesterol stones with a pigmented center are shown in Figs. 2 and 3. The surface of the stones was devoid of bacterial microcolonies (Figs. 2b and 3b), but bacterial microcolonies were seen in the central pigmented portion of the stones (Figs. 2c and 3c). A mixed stone, with external pigment, is shown in Fig. 4. Here the bacterial microcolonies are found in the external pigmented layer (Fig. 4b).

Clinical Diagnosis

As noted above, the clinical diagnoses were biliary colic 31%, acute cholecystitis 33%, pancreatitis or choledocholithiasis (without cholangitis) 16%, cholangitis 14%, and in 5% the gallbladder (and stones) were removed during other procedures (Whipple, hepatectomy, etc.).

Among cases with gallstones confined to the gallbladder, acute cholecystitis was more common in cases with bacterial-laden stones (33 vs 64%, sterile vs bacterial-laden gallbladder stones, $P<0.0001$, chi-squared). But, the incidence of acute cholecystitis was similar in cases with sterile PS or CS (29 vs 40%, $P=0.252$, chi-squared), and cases with bacterial-laden PS or CS (55 vs 68%, $P=0.301$, chi-squared). The severity of the acute cholecystitis illness was greater in cases with bacterial-laden PS compared to CS (see below).

Among cases with CBD stones, 46% presented with cholangitis, 45% with pancreatitis or choledocholithiasis (without cholangitis), and 8% with acute cholecystitis (with associated choledocholithiasis). Cholangitis was never present in cases with sterile PS or CS. Cholangitis was more common in cases with bacterial-laden PS compared to bacterial-laden CS (67 vs 33%, bacterial-laden CBD PS vs CS, $P=0.043$, chi-squared). Severe illnesses were more common in cases with bacterial-laden CBD PS (see below).

Severity of Illness

The severity of the clinical illness correlated with the presence of gallstone bacteria and the type of gallstone. Overall, patients with sterile stones had the least infectious or inflammatory manifestations (severe 0%, moderate 22%, and none 78%); patients with CS containing bacteria followed (severe 16%, moderate 27%, and none 57%); whereas those with PS bacteria had the most severe illnesses (severe 56%, moderate 16%, and none 27%) ($P<0.0001$, all comparisons, chi-squared).

These associations held when cases with gallstones confined to the gallbladder and cases with CBD stones were examined separately. The severity of the associated clinical illness by gallstone type, presence of bacteria in the stones, and gallstone location is shown in Fig. 6, where patients with gallstones confined to the gallbladder and those with CBD stones are graphed separately. As can be seen, irrespective of gallstone location, cases with CS bacteria had significantly more severe illnesses than cases with sterile stones ($P<0.0001$, gallbladder stones only; $P=0.011$, CBD stones; chi-squared). And, cases with CS bacteria had less severe illnesses than cases with PS

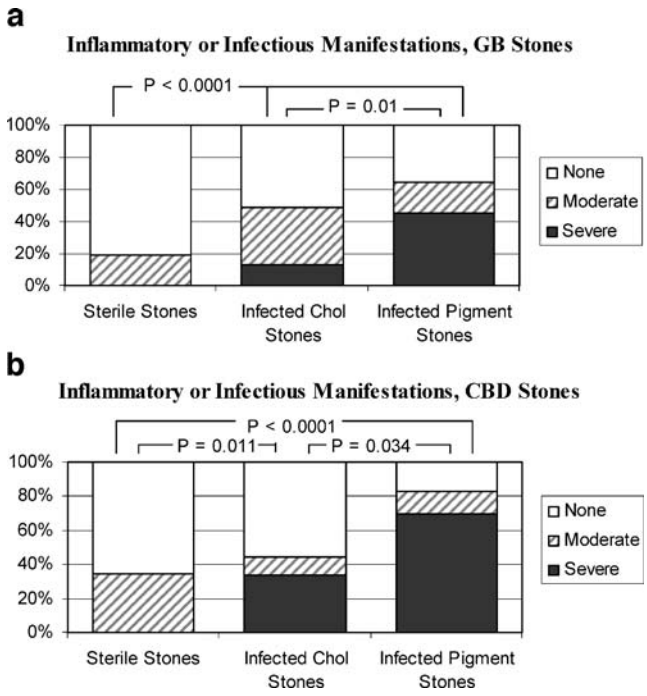

Figure 6 Comparisons between clinical manifestations of cases with sterile gallstones, bacterial-laden predominantly cholesterol (Chol) stones, and bacterial-laden PS. Clinical manifestations are defined in the text. **a** Cases with gallstones confined to the gallbladder, $P<0.0001$ sterile stones vs bacterial-laden Chol or PS (chi-squared), and $P=0.01$ bacterial-laden Chol vs bacterial-laden PS (chi-squared). **b** Cases with CBD stones. $P<0.0001$ sterile stones vs bacterial-laden PS; $P=0.011$ sterile stones vs bacterial-laden Chol stones, and $P=0.034$ bacterial-laden Chol vs bacterial-laden PS (chi-squared, all comparisons).

bacteria (P=0.01, gallbladder stones only; P=0.034, CBD stones; bacterial-laden CS vs PS, chi-squared).

Host Factors and Bacterial Virulence Factors

Because bacteria in the center of CS might be shielded from immune interactions, we assessed the level of IgG present in patient's serum and bile specifically directed against the bacteria in their biliary tree. Level of serum and bile IgG directed against gallstone bacteria for cases with pigment and CS is shown in Fig. 7. As shown, there was more IgG present with activity against PS bacteria than CS bacteria (P=0.029 serum and P=0.022 bile, PS vs CS bacteria, t test). Level of IgG in patient's serum and bile also correlated with bacterial location, that is, whether bacteria were recovered from gallstone culture only or from both gallstone and bile cultures. This is shown in Fig. 8. As shown, there was more IgG activity against bacteria present in both bile and gallstones compared to bacteria present in gallstones only (P=0.001 serum and P=0.008 bile, bacteria in gallstone only vs bile and gallstone, ANOVA).

We also measured the ability of patient's serum to kill gram-negative bacteria present in their biliary tree. This also correlated with stone type. Complement-mediated killing of gram-negative bacteria by patient's serum was more common among PS bacteria than CS bacteria (62 vs 26%, respectively, P=0.013, Fisher exact test), whereas control serum killed both equally (44 vs 32%, PS vs CS bacteria, P=0.21, Fisher exact test) (Table 1).

Quantitative slime production by CS and PS bacteria was compared by grouping bacteria with low (<10), intermediate (10–99), and high (>100) slime production (Table 1). Slime production of CS bacteria and PS bacteria were significantly different (P=0.035, CS bacteria vs PS bacteria, chi-squared). Cholesterol stones bacteria more commonly had high (>100 OD) slime production (41 vs 16%, CS bacteria vs PS bacteria), whereas PS bacteria more commonly had low (<10 OD) slime production (57 vs 32%, PS bacteria vs CS bacteria).

In vitro induction of TNF-α production by bacteria from the two gallstone groups was similar (Table 1). Average in vitro TNF-α production was 540 (±140) pg/ml for CS bacteria and was 520 (±65) pg/ml for PS bacteria (P=NS). In the presence of sera, the average in vitro TNF-α production was 830 (±125) pg/ml for CS bacteria and was 870 (±75) pg/ml for PS bacteria (P=NS).

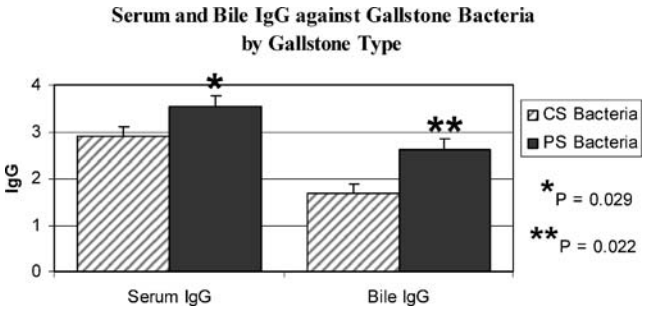

Figure 7 Level of serum and bile IgG directed against biliary bacteria obtained from predominantly CS (CS bacteria) and PS (PS bacteria). Serum and bile from patients was tested for IgG directed against the bacterial present in their biliary tree. The highest value was used in cases with more than one bacterium present in the biliary tree. Error bars indicate standard error of the mean. *P=0.029, serum IgG against CS bacteria vs PS bacteria, t test. **P=0.022, bile IgG against CS bacteria vs PS bacteria, t test.

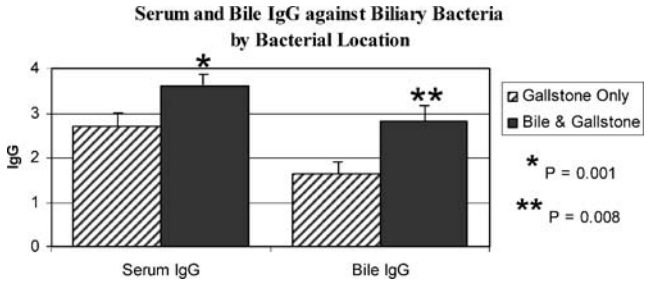

Figure 8 Level of serum and bile IgG directed against biliary bacteria present in the gallstone only compared to bacteria present in both the bile and gallstone. Serum and bile from patients was tested for IgG directed against the bacterial present in their biliary tree. The highest value was used in cases with more than one bacterium present in the biliary tree. Error bars indicate standard error of the mean. *P=0.001, serum IgG against bacteria in gallstone only vs gallstone and bile, ANOVA. **P=0.008, bile IgG against bacteria in gallstone only vs gallstone and bile, ANOVA.

Discussion

This study shows that bacteria are readily cultured from predominantly CS, especially those with a pigmented center, allowing for analysis of their virulence factors. Bacterial microcolonies (when present) are easily seen within the pigmented centers of CS on SEM,[1,3] and there is good agreement between the results of gallstone culture and SEM examination. Gallstone cholesterol content correlated with, but could not *exclude* the possibility of bacterial presence, and so was unreliable as an indicator of bacterial presence in gallstones. Similarly, bile culture, which was negative in 44% of cases with bacterial-laden CS, was also an unreliable indicator of bacterial presence in CS.

We first reported bacterial microcolonies in the center of CS in 1987.[1] More recently, others have used PCR techniques to look for the presence of bacteria in cholesterol and "mixed cholesterol" gallstones.[13–17] These studies examined gallstones that were 70–100% cholesterol, and many used bile cultures as an indicator for gallstone bacteria.[13–15] They defined "mixed CS" as those containing

70–90% cholesterol. The results of the current study show that 44% of CS that harbor bacteria are associated with sterile bile cultures. And, that PCR examination of stones with 70–90% cholesterol should reveal bacterial DNA because bacteria were usually cultured from CS with this composition. We found that the location of stone pigment (in cholesterol-containing stones) was more important than the overall cholesterol content. Mixed stones, containing both cholesterol and pigment portions, with the pigment on the stone exterior, usually harbored bacterial microcolonies in the pigment layer. These mixed stones, which had an average cholesterol content of 74% (some >90%), might be classified as "mixed cholesterol gallstones" if cholesterol composition was used as the sole criteria for stone classification. But mixed stones with exterior pigment contained bacteria as frequently as PS, and were thus grouped as PS in our analysis.

Despite different techniques, the results of the current study agree in many ways with the results using PCR. One group reported no bacterial DNA in gallstones >90% cholesterol,[13,14] whereas others noted bacterial DNA in gallstones containing as much as 97% cholesterol.[16] We found that bacteria were almost always present in cholesterol-containing gallstones that were <90% cholesterol. Furthermore, we found that bacteria were less common, but could be present, in gallstones with cholesterol content as high as 95–98%. We found a similar mix of bacterial species as that reported by most studies utilizing PCR.[13–16] We did not find only cocci bacterial forms as one study reported.[17] Our data suggest that conventional culture techniques and SEM examination can easily identify these bacteria within the pigmented portions of CS, and that PCR may not be necessary for detection of these bacteria.

The most important findings of the current study relates to the clinical significance of bacteria in the pigmented centers of CS. We previously reported that bacterial-laden gallstones were associated with infectious manifestations,[1–7] but not whether bacteria in the center of CS behave differently than bacteria in the matrix of pigment or mixed stones (with exterior pigment). The current study examined this question and noted that CS bacteria do cause infectious manifestations, but less commonly than bacteria in PS. Cases with CS bacteria had more severe illnesses than cases with sterile stones. But CS bacteria were associated with less severe infections than PS bacteria, and this was true even in cases with bacterial-laden CBD stones. Possibly because of their isolation, CS bacteria were less often present in bile and blood cultures, suggesting that the inability of CS bacteria to exit the center of the stone influenced their overall virulence.

We previously reported that gram-negative biliary bacteria killed by complement (serum-sensitive) were associated with more severe illnesses.[7] Because of this observation, we compared serum sensitivity of bacteria from CS and PS. Because only 58% of CS bacteria were present in bile and only 2–3% were present in the blood, we thought that the host (patient) might not mount as great an immune response against many CS organisms. This hypothesis proved consistent. We found that the level of bacterial-specific serum and bile IgG was lower in cases with CS bacteria than those with PS bacteria. Bacterial-specific serum and bile IgG was also lower in cases with gallstone bacteria but not bactibilia, which was more common with CS. Furthermore, because IgG is a component of the classical complement pathway, it was not surprising that CS bacteria were less often killed by patient's serum than PS bacteria. Control serum, in contrast, killed both CS and PS bacteria equally. These findings support the concept that the host immune response against CS bacteria is decreased possibly because of their isolation in the center of the CS. The bacterial-laden gallstone is essentially a biofilm, and others have reported that bacteria in biofilms activate complement less, and induce less macrophage activation, than bacteria in a planktonic state.[21,22] Thus, stable biofilm formation may protect against the induction of inflammatory mediators. Also, bacteria in the center of CS, a bacterial biofilm encrusted by cholesterol crystals, are likely to behave like those in a very stable biofilm.

We also noted previously that serum-sensitive gram-negative bacteria induced more TNF-α than serum-resistant bacteria in an in vitro analysis.[7,8] In the current study, we compared the induction of TNF-α production by CS and PS bacteria and noted no differences. Both groups were equally able to induce TNF-α production. This suggests that once the CS bacteria escape from the stone center, they have the ability to activate inflammatory mediators. Clinically, this was the case, 17% of cases with CS bacteria had severe illnesses, and 33% of cases with bacterial-laden CS in the CBD had severe illnesses, whereas no cases with sterile gallstones had severe illnesses. But given the inability of CS bacteria to exit the gallstone, an in vivo analysis would be more indicative of the inflammatory mediators induced by CS bacteria.

In addition, CS bacteria produced higher levels of slime compared to PS bacteria. Whereas many (57%) PS bacteria were low (0–10) slime formers, CS bacteria more commonly (41%) produced high (>100) levels of slime. This finding is consistent with bacterial facilitation of CS formation. Slime, a complex glycoprotein, is the glue that facilitates macroscopic stone formation.[1,2,6] In Maki's[23] original study of PS formation, β-glucuronidase-producing bacteria only facilitated macroscopic stone formation when Na-alginate (a compound very similar to slime) was added to the mixture. Without the addition of Na-alginate, only Ca-bilirubinate sludge was precipitated. Bacteria with high

slime production likely provide a better nidus for cholesterol crystallization. One study documented shortened cholesterol nucleation time with the addition of *Pseudomonas aeruginosa* or *Enterococcus* spp. to model bile.[24] We noted that most biliary *Pseudomonas* species are high slime (>100) formers.[6] *Pseudomonas* species were also commonly identified with PCR analysis of CS.[16] High slime production might also inhibit bacterial escape from the stone center and reflux into the systemic circulation. This may partly explain why CS bacteria less commonly demonstrated bactibilia and bacteremia.

Finally, our study group contains a large Veteran population, who are mostly men and older than other populations with gallstone disease. The incidence of biliary bacteria is higher in this population, as previously reported,[4] but this makes them an ideal group to study how gallstone bacteria influence the clinical manifestations of biliary disease. We have previously reported the clinical aspects of biliary disease in this population.[3,4] We noted a similar incidence of biliary bacteria in cases with chronic and acute cholecystitis as that reported by other series; but a higher incidence of choledocholithiasis and cholangitis.[4,25–27] The clinical presentation in our patient group was also more complicated, which likely accounts for the higher incidence of open vs laparoscopic procedures in this series. But because CS were present in 48% of cases, patients with predominantly CS were still well represented in the study population.

Conclusion

Bacteria are readily cultured from CS with pigment centers, allowing for analysis of their virulence factors. PCR is unnecessary for detection of these bacteria. Bacteria sequestered in the pigment centers of CS caused infectious manifestations, but less than PS bacteria. Possibly because of their isolation, CS bacteria were less often present in bile and blood, induced less IgG, were less often killed by a patient's serum, and demonstrated fewer infectious manifestations than PS bacteria. In an in vitro setting, CS bacteria and PS bacteria induced similar levels of TNF-α, suggesting that once they escape from the CS, they can induce inflammatory mediators. Cholesterol stones bacteria also produced more slime. Their increased slime production may explain their ability to foster CS nucleation and the decreased infectious manifestations associated with CS bacteria compared to PS bacteria. This is the first study to describe the clinical relevance of bacteria located within the center of predominantly CS.

Acknowledgement This work was supported by a VA Merit Review Grant.

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J Gastrointest Surg (2007) 11:1309–1316
DOI 10.1007/s11605-007-0225-9

Does "Clinical" R0 Have Validity in the Choice of Simple Cholecystectomy for Gallbladder Carcinoma?

Chang Moo Kang · Woo Jung Lee · Gi Hong Choi · Jun Young Kim · Kyung Sik Kim · Jin Sub Choi · Byong Ro Kim

Received: 11 June 2007 / Accepted: 14 June 2007 / Published online: 8 August 2007

Abstract

Background This study was designed to evaluate the survival outcomes of patients undergoing simple cholecystectomy and to investigate which patients would benefit from cholecystectomy alone in treating gallbladder carcinoma.

Methods The available medical records of patients who underwent cholecystectomy alone for gallbladder carcinomas from August 1992 to February 2005 were retrospectively reviewed. Cancer stages were evaluated by clinical meaning based on the AJCC Cancer Staging Manual, 6th edition. "Clinical" R0, defined as gallbladder confined tumor (pT1-3 with negative resection margin) with cN0 and cM0, was tentatively established to evaluate the quality of simple cholecystectomy.

Results Seventy-five patients underwent cholecystectomy alone for gallbladder carcinomas. Twenty-eight patients were male, and forty-seven patients were female, with their mean age 63.5 years (range, 29-80 years). Forty-one patients (54.7%) underwent laparoscopic cholecystectomy, and thirty-four patients (45.3%) underwent open cholecystectomy. T3 lesions were most common (26 patients), followed by T1 (24 patients), T2 (19 patients), and T4 (6 patients). "Clinical R0" could be defined in 48 patients (63%) after simple cholecystectomy. Multivariate analysis showed that incidental gallbladder carcinoma, T stage, and clinical R0 status were independent prognostic factors of long-term survival. When comparing survival outcomes of clinical R0 according to the T stage, no patients with Tis, T1a, and T1b had cancer-related mortality during follow-up. Especially, in patients with T2 gallbladder carcinomas, the mean survival rate was 68.9 months, and the 5-year survival rate was 77.8%. On the contrary, those with T3 lesions had poor prognoses.

Conclusion Cholecystectomy alone could be proper management for well-selected patients with gallbladder carcinomas (incidental gallbladder carcinoma, gallbladder confined carcinoma, clinical R0). More experiences and a proper prospective study must be performed to confirm the meaning of clinical R0 in treating gallbladder carcinoma.

Keywords Cholecystectomy · Gallbladder carcinoma · Clinical R0

The content of this manuscript was presented at the 20th World Congress of the International Society for Digestive Surgery on December 2nd, 2006, in Rome.

C. M. Kang · W. J. Lee · G. H. Choi · J. Y. Kim · K. S. Kim · J. S. Choi (✉) · B. R. Kim
Department of Surgery, Yonsei University College of Medicine, Institute of Gastroenterology, Yonsei University Health System, 250 Seonsanno, Seodaemun-gu, Seoul 120-752, South Korea
e-mail: choi5491@yuhs.ac

Introduction

Gallbladder carcinomas were first described by Maxmillian de Stoll in 1777.[1] Gallbladder carcinoma is the most frequent malignant neoplasm of the biliary tract and the fifth most common cancer of the gastrointestinal tract.[2] There is still controversy about surgical treatment of gallbladder carcinomas. Generally, cholecystectomy alone is an adequate treatment for pathologic stage T1 (pT1) gallbladder carcinoma, providing that the resection margins are not violated by malignant cells.[3–5] On the other hand, radical resection with regional lymph node dissection for T2 or more advanced gallbladder carcinoma is advocated.[6] However, Shirai et al.[7] reported that about 40.5% of patients with T2 gallbladder carcinomas survived more than

5 years after cholecystectomy alone, which implies that radical surgery is too much for patients with such favorable prognoses.

According to anecdotal experiences at our institution, simple cholecystectomy in certain patients is likely to provide an acceptable surgical outcome compared to radical surgery in treating gallbladder carcinoma. This observation prompted us to plan this study. This retrospective study was designed to evaluate the results of simple cholecystectomy in treating gallbladder carcinoma and to investigate which patients would benefit from simple cholecystectomy in treatment of gallbladder carcinoma.

Materials and Methods

The medical records of patients with gallbladder carcinomas who underwent surgical procedures at the Yonsei University Health System, in Seoul, Korea, between August 1992 and February 2005 were retrospectively reviewed. We selected patients who underwent simple cholecystectomies for gallbladder carcinomas. We investigated the survival results and clinical predictive factors for favorable survival outcomes after simple cholecystectomy in treating gallbladder carcinomas. Incidental gallbladder carcinoma was defined as carcinoma of the gallbladder first diagnosed at the histological examination of the resected gallbladder or gallbladder mass detected during a medical checkup without any symptoms. The clinical TNM (cTNM) stage was evaluated based on the AJCC Cancer Staging Manual; 6th edition.[8] cTNM classification is based on evidence acquired before treatment, including physical examination, image study, endoscopy, biopsy, and surgical exploration. Nodal status was determined by preoperative image studies or histological examination of incidentally sampled lymph nodes during simple cholecystectomy. We tentatively developed an additional criterion, "clinical" R0, to evaluate the quality simple cholecystectomy cases, which was defined as no malignant cells on gallbladder resection margins in the pathologic report, no grossly residual tumors, no lymph node enlargement, and no distant metastasis based on image studies and operative findings [that is, gallbladder confined tumor (pT1-3 with negative resection margin), cN0 and cM0]. R1 was defined as the presence of a microscopic residual tumor (positive resection margin), and R2 as the presence of a macroscopic residual tumor. To delineate the characteristics of gallbladder carcinoma treated by cholecystectomy alone, we compared cases of patients who underwent radical cholecystectomies during the same period to those of patients who underwent simple cholecystectomies by T stage. The standard radical operation in our department is composed of at least wedge resection of the gallbladder bed including about 2 cm thickness of liver parenchyma, resection of soft tissue along the hepatoduodenal ligament with or without bile duct resection, and dissection of regional nodes from the hepatic artery to the extent of the right side of the celiac axis with retroperitoneal soft tissue clearance (16A2, 16B1). However, slight modifications of the extent of surgery according to the patient's general conditions, tumor factors, and the surgeon's preference were made. Statistical analysis was performed using SPSS software (Statistical Package for the Social Sciences, version 10.0). The categorical variables are expressed as frequencies (%), whereas continuous variables are presented as a mean with their range or ± standard deviation. Follow-up data were obtained from medical records and patient or family member telephone interviews. Survival was calculated from the date of diagnosis to the date of death or last follow-up. Cumulative survival rates and plots were estimated using the Kaplan-Meier method. The

Table 1 Characteristics of Gallbladder Carcinoma Treated by Simple Cholecystectomy

	Simple Cholecystectomy Alone (N=75)
Tumor size (cm)	2.7±1.9
Tumor location	
Distal (fundus+body)	40 (61.5)
Proximal (neck+cystic duct)	9 (13.8)
Diffuse	16 (24.6)
Tumor morphology	
Polypoid	42 (61.8)
Flat (focal wall thickening)	8 (11.8)
Diffuse wall thickening	13 (19.1)
Diffuse infiltrative	5 (7.2)
T stage	
Tis	14 (18.7)
T1a	2 (2.7)
T1b	8 (10.7)
T2	19 (25.3)
T3	26 (34.7)
T4	6 (8)
N stage	
NX	35 (47.3)
N0	23 (31.1)
N1	16 (21.6)
Distant metastasis	6 (8)
Grade	
Well	20 (37)
Moderate	24 (44)
Poor	9 (16)
Undifferentiated	1 (1.9)
Residual tumor	
R2	19 (26.3)
R1	8 (10.5)
Clinical R0	48 (63.2)
Pathologic R0	–

Table 2 Analysis for Predicting Favorable Survival After Simple Cholecystectomy in Gallbladder Carcinoma

Variables	*N*	Mean Survival (Month)	*p* Value	
			Univariate	Multivariate
Gender				
Male	27	70.7	0.8462	
Female	47	81.5		
Symptoms				
Symptomatic	27	32.9	0.0012[a]	0.011
Incidental	46	100.6		
Cholecystitis				
No	42	85.0	0.210	
Yes	25	43.4		
APBDU				
No	71	78.7	0.5241	
Yes	3	101.3		
Tumor morphology				
Elevated (polypoid+flat)	49	93.7	0.0312[a]	
Diffuse	18	30.9		
Tumor location				
Distal (fundus+body)	40	97.8	0.1848[a]	
Proximal (neck+cystic duct)	8	65.3		
Diffuse	18	27.7		
Ca 19-9				
≤37	22	82.8	0.402[a]	
>37	12	24.1		
T stage				
Tis	14	NA	<0.0001[a]	0.004
T1a	2	NA		
T1b	7	117.9		
T2	19	54.3		
T3	25	26.3		
T4	6	6.5		
N stage				
N0	22	130.3	<0.0001[a]	
NX	35	81.0		
N1	15	18.8		
M stage				
M0	68	85.4	0.024[a]	
M1	6	11.3		
R status				
R2	19	8.9	<0.0001[a]	0.041
R1	8	46.5		
Clinical R0	48	113.7		
Grade				
Well	20	88.6	0.0132[a]	
Moderate	22	35.1		
Poor	9	15.4		
Undifferentiated	1	7.2		

[a] The Bonferroni correction was made.

log-rank test was applied to check for statistical significance. Cox multivariate analyses were performed to determine the independent prognostic factors for survival. Differences in *p* value less than 0.05 were considered significant.

Results

General Characteristics of Patients

Between August 1992 and February 2005, a total of 217 patients with gallbladder carcinomas underwent surgical procedures at our institution. Among them, 75 patients underwent cholecystectomy alone for gallbladder carcinomas. Twenty-eight patients were male, and forty-seven patients were female. The mean age at time of surgery was 63.5 years (range, 29–80 years). Forty-six patients (62.2%) had incidental gallbladder carcinomas. Abdominal pain and discomfort were the most frequent symptoms, found in 48 patients (63.2%). Gallstone disease was associated in 34 patients (48.6%), and GB empyema was noted in 10 patients (14.5%). Forty-one patients (54.7%) underwent laparoscopic cholecystectomy, and thirty-four patients (45.3%) underwent conventional open cholecystectomy. Five operation-related complications (6.6%) occurred. Bile leak was found in three patients, bleeding in one, and wound infection in one patient; however, there were no mortalities (<30 days after cholecystectomy alone).

Characteristics of Gallbladder Carcinoma

All gallbladder carcinomas were adenocarcinomas. Polypoid tumors were mainly located in the distal part of the gallbladder (fundus and body, more than 60%). T3 lesions were the most common (26 patients), followed by T1 (24 patients) and T2 (19 patients). Left supraclavicular lymph nodes (two patients), liver metastasis (three patients), and peritoneal seeding (positive rectal shelf, one patient) were present in six cases of distant metastasis at the time of surgery. Clinical R0 could be defined in 48 patients (63.2%) after simple cholecystectomy (Table 1).

Table 3 R Status According to pT Stage

	R2	R1	Clinical R0	Total
Tis		1	13	14
T1a			2	2
T1b		1	7	8
T2	3	2	14	19
T3	10	4	12	26
T4	6			6
Total	19	8	48	75

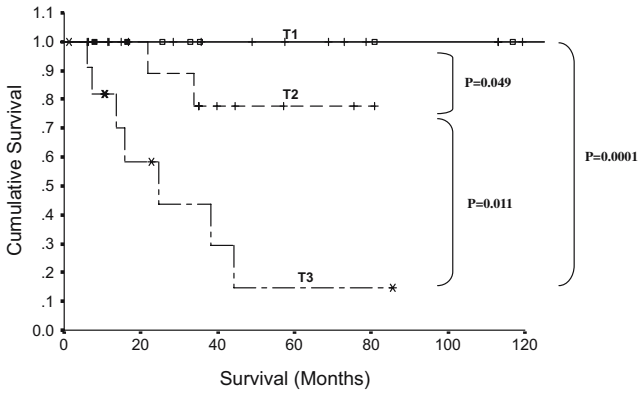

Figure 1 Survival outcomes of clinical R0 according to T stage after simple cholecystectomy.

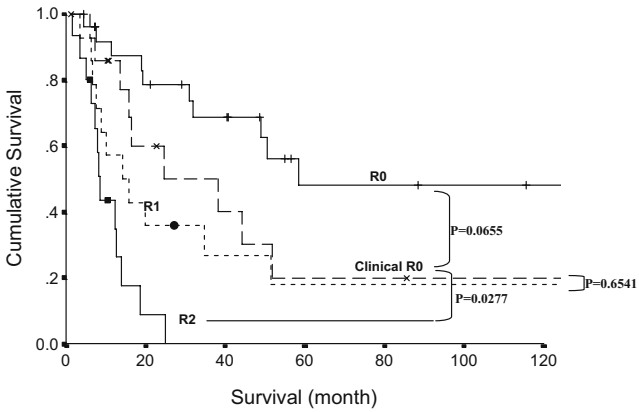

Figure 3 Survival differences between clinical R0 and R0 in T3 gallbladder carcinoma.

Prognostic Factors for Favorable Survival Outcomes: Univariate Analysis

Overall survival of patients with gallbladder carcinomas who underwent simple cholecystectomy was mean 80.2 months with a 5-year survival rate of 47.9%. The survival differences according to T stage were statistically significant, as shown in Table 2 ($p<0.0001$). The 5-year survival rate of patients with gallbladder carcinomas with Tis, T1b, and T2 lesions was 100, 75, and 56.2%, respectively. Residual tumor status after simple cholecystectomy alone significantly influenced survival differences. That is, considerable survival differences between clinical R0 and R1 or R2 were noted (clinical R0 vs R2, $p<0.0001$, and clinical R0 vs R1 $p=0.0045$). In addition, clinical lymph node metastasis and distant metastasis, tumor grade, tumor morphology, and incidental gallbladder carcinoma were all significant prognostic factors in univariate analysis ($p<0.05$, Table 2).

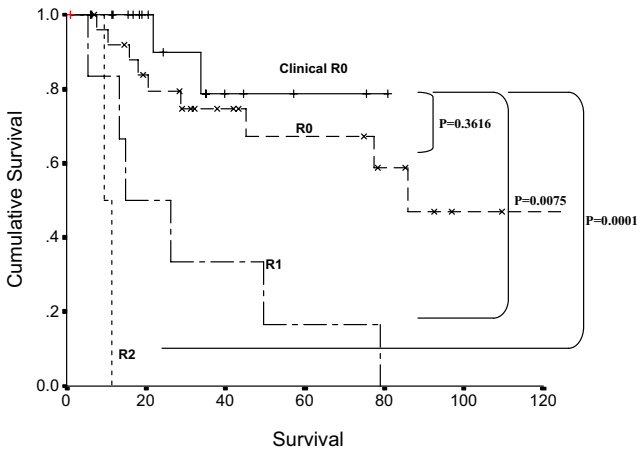

Figure 2 Survival differences between clinical R0 and R0 in T2 gallbladder carcinoma.

Prognostic Factors for Favorable Survival Outcomes: Multivariate Analysis

When multivariate analysis (Cox regression) is performed using variables with significant survival differences in univariate analysis as covariates, incidental gallbladder carcinoma ($p=0.011$), T-stage ($p=0.004$), and R status ($p=0.041$) were independent prognostic factors for good survival outcomes after simple cholecystectomy in patients with gallbladder carcinomas (Table 2).

Analysis of Clinical R0 Status

Forty-eight patients (61.2%) were defined as having clinical R0 after cholecystectomy alone. With the advancement of T stage, the frequency of R1 and R2 increases (Table 3). Almost all early gallbladder carcinomas (T1) were defined as clinical R0 after simple cholecystectomy alone; however, frequently, R1 and R2 resulted from simple cholecystectomy in patients with gallbladder carcinomas more advanced than T2 lesions.

When comparing survival outcomes of clinical R0 according to T stage, significant differences were noted (Fig. 1). No patients with Tis, T1a, and T1b lesions had cancer-related mortality after simple cholecystectomy. Especially, patients with T2 gallbladder carcinomas showed acceptable survival outcomes after cholecystectomy alone. Their mean survival was 68.9 months, and the 5-year survival rate was 77.8%. To the contrary, those with T3 lesions had relatively poor prognoses. Their mean survival was 32.8 months with a 5-year survival rate of 14.6%, which were considerably different from patients with T1 and T2 gallbladder carcinomas with clinical R0 (Fig. 1).

When comparing the survival outcomes according to R status after surgery in patients with T2 and T3 gallbladder carcinomas, no survival differences were noted between patients whose carcinomas were defined as clinical R0 and R0 resulting from radical cholecystectomy (Figs. 2 and 3).

Table 4 Comparison of Simple Cholecystectomy and Radical Cholecystectomy at our Institution (Yonsei Medical Center, Seoul, Korea)

Variables	T1b		T2		T3		T4	
	SC (*N*=8)	RC (*N*=19)	SC (*N*=19)	RC (*N*=33)	SC (*N*=26)	RC (*N*=43)	SC (*N*=6)	RC (*N*=12)
Gender								
Male	2	2	6	16	9	17	4	8
Female	6	4	13	17	17	26	2	4
Age (mean±SD, years)	63.4±10.4	65.3±8.5	67.1±7.3	62.9±6.7	65.9±12.9	56.4±8.9	63.2±5.1	62.5±10.6
GB stone								
(−)	4	2	10	21	12	25	4	11
(+)	3	4	7	9	14	15	2	1
Cholecystitis[a]								
(−)	4	5	9	26	13	34	6	12
(+)	2	1	8	4	13	5	–	–
Incidental								
(−)	2	2	8	11	10	29	5	12
(+)	5	4	10	19	16	10	1	–
Jaundice								
(−)	5	6	15	28	26	35	4	8–
(+)	–	–	1	2	–	4	2	4
Morphology								
Flat	1	–	1	4	5	7	–	–
Polypoid	5	6	13	19	9	17	2	5
Diffuse wall thickening	1	–	4	5	7	3	1	1
Infiltrative	–	–	–	1	3	10	2	5
Tumor size (mean±SD, cm)	2.9±2.4	2.3±1.9	3.0±2.4	3.2±1.9	2.8±1.6	3.4±1.6	3.0±0.5	5.4±3.5
Tumor site								
Fundus	2	3	7	9	3	10	2	2
Body	2	3	2	7	8	4	–	3
Neck	1	–	2	7	3	9	–	2
Cystic duct	–	–	–	–	2	3	–	1
Diffuse	1	–	5	4	7	8	3	3
Neural Invasion								
(−)	6	6	15	28	23	33	4	12
(+)	–	–	–	–	2	5	1	–
Lymphatic invasion								
(−)	5	6	12	27	22	31	5	10
(+)	1	–	3	1	3	7	–	2
Vascular invasion								
(−)	5	6	12	26	23	31	5	10
(+)	1	–	3	2	2	7	–	2
Grade								
Well	6	2	7	12	5	8	–	1
Moderate	1	2	9	9	10	19	3	5
Poorly	–	–	1	4	6	8	2	1
Undifferentiated	–	–	–	1	1	1	–	–
Operative time (mean±SD, min)	70±33.4	192±55.4*	88±36.2	236±120.5*	113±68.9	266±105.4*	123±39.9	272±155.4***
Bleeding amount (mean±SD, ml)	NA	NA	132.5±144.9	664.0±408.8	NA	871.4±606.5	NA	NA
Transfusion								
(−)	6	5	16	21	23	15	5	3
(+)	–	–	–	9*	2	22*	1	8*
Complications	–	2	1	9**	3	10	1	2

Both Tis and T1a lesions were excluded because the number of radical cholecystectomies in that lesion is limited

[a] Including gallbladder empyema

*$p<0.05$

**$p=0.72$

***$p=0.058$

Comparison of Simple Cholecystectomy and Radical Cholecystectomy at our Institution (Yonsei University Health System, Seoul, Korea)

One hundred and seven cases of radical cholecystectomy during the same period were compared with current simple cholecystectomized cases. A high incidence of cholecystitis including empyema, incidental diagnosis, shorter operative time, less bleeding, lower incidence of blood transfusion, and complications were noted in patients who underwent simple cholecystectomies compared to the patients who underwent radical cholecystectomies (Table 4).

Discussion

Gallbladder carcinoma is known as a highly fatal disease with a poor prognosis. The 5-year survival rate for patients with cancers of the gallbladder ranges from 0% to 10% in most reported series.[9] The poor prognosis of this disease may be caused by the anatomical position of the gallbladder and the high proportion of tumors that are advanced at the time of presentation. Gallbladder carcinoma is difficult to diagnose clinically because the symptoms and signs are vague and nonspecific. However, early diagnosis of gallbladder carcinoma has increased because of the recent improvement of preoperative imaging[10–12] and increased concerns about people's own health status in accordance with improving personal economic status. In addition, clinical application of extensive radical surgery promises an improvement in survival.[13, 14]

Generally, simple cholecystectomy is accepted as the proper treatment for Tis and T1a gallbladder carcinomas. In the case of T1b lesions, despite the controversy, simple cholecystectomy is likely to be recommended. Wakai et al.[3] recently suggested that cholecystectomy alone could be a standard treatment for T1b gallbladder carcinoma as cholecystectomy provides patients with a 10-year survival rate of more than 85%. Whereas on the other hand, radical cholecystectomy is strongly recommended for patients whose gallbladder carcinomas are staged as more severe than T2 lesions.[15] Shirai et al.[7] reported a 5-year survival rate of 90% after patients underwent radical resection for T2 and T3 tumors compared with a 5-year survival rate of only 40% for patients who underwent simple cholecystectomies. De Aretxabala et al.[16] showed a 50% improved 5-year survival rate (70 vs 20%) for patients who underwent radical cholecystectomies versus patients who underwent simple cholecystectomies. Fong et al.[17] reported a 5-year survival rate of 61% for patients with T2 tumors who underwent radical cholecystectomies compared with a 5-year survival rate of only 19% for patients with T2 tumors who underwent simple cholecystectomies. Furthermore, support for radical resection of locally advanced disease also has been accumulated during the past decade. Five-year survival rates of 15 to 63% and 7 to 25% have been reported for T3 and T4 gallbladder carcinomas, respectively.[6]

Although the number of patients with gallbladder carcinomas is limited and our clinical data are based on retrospective observation, which might have had unavoidable selection bias, the results of simple cholecystectomy were not disappointing and seemed to be comparable to previous reports. The 10-year survival rate of T1b tumors was 75%, and the 5-year survival rate of patients with T2 lesions was 56.2%. Especially, the result of patients with T2 lesions is superior to the results of patients who underwent simple cholecystectomies in the abovementioned literature.[7,15,17] Then, we hypothesized that this selection bias could be, in other words, a sort of selection criterion for simple cholecystectomy in the treatment of gallbladder carcinoma. Therefore, the purpose of our clinical observation is to reveal possible selection criterion for simple cholecystectomy in gallbladder carcinoma. In fact, this study was performed to evaluate the results of simple cholecystectomies, and we would like to find which patients could benefit from simple cholecystectomy as a treatment for gallbladder carcinoma.

According to our results (Table 4), an overall comparison of patients who underwent simple cholecystectomies and radical cholecystectomies according to T stage shows that less substantial morbidity, shorter operative times, smaller bleeding amount, and lower incidence of transfusion were noted in patients who underwent simple cholecystectomies, which means that limited surgery in patients with gallbladder carcinomas is beneficial. If the oncologic outcomes were comparable in patients who underwent simple cholecystectomies in well-selected cases of patients with gallbladder carcinomas, more extended surgery, which always brings a risk of postoperative morbidity and mortality, could be avoided in the selected population.

The highlight of this study might determine the validity of clinical R0 as a selection criterion for the choice of simple cholecystectomy. To some extent, clinical R0 apparently seems to reflect the status of the TNM stage. As mentioned above, we defined clinical R0 as when the resected gallbladder showed a negative margin on histopathologic examination, preoperative radiologic images revealed neither distant metastasis nor metastatic lymph node, and intraoperative findings showed no evidence of grossly residual tumors. From the viewpoint of this definition, advanced cases of T3, T4, N1, and M1 tumors have a high possibility of being excluded from clinical R0. On the other hand, not only early lesions, T1, but also some portions of T2 tumors have a high chance of being included as clinical R0. As a matter of fact, our results show that most gallbladder carcinomas actually fall into clinical R0 in

cases of early lesions, Tis, T1a, and T1b. In the case of T2 lesions, about 74% of these lesions (14 out of 19 patients) fall into clinical R0. However, no T4 lesions could be considered clinical R0, and 48% of the T3 lesions (12 out of 25 patients) fall into clinical R0 (Table 3).

The survival outcomes of patients with clinical R0 gallbladder carcinomas were acceptable for patients with T1 and T2 tumors (Fig. 1). No cancer-related mortality was observed in any patients with Tis, T1a, and T1b tumors with clinical R0. Especially, with respect to 14 patients with T2 gallbladder carcinomas with clinical R0 (73.7%), the 5-year survival rate was 77.8%. Interestingly, this result is definitively superior to recent survival outcomes of patients who underwent simple cholecystectomies. Fong et al.[17] reported that patients with radiographically resectable disease but not subjected to repeat resection after undergoing simple cholecystectomies had a 5-year survival rate of 19%, whereas those treated with radical resection had a 5-year survival rate of 61%, which emphasizes that radical resection is not only safe but reasonable cancer therapy for those with T2 gallbladder cancers. Suzuki et al.[18] reported an overall 5-year survival rate in those 20 patients with T2 gallbladder carcinomas of 77% and also concluded that a radical second operation enhanced the chance for cure in patients with T2 gallbladder carcinomas. However, according to the present results of survival comparison in T2 gallbladder carcinomas, clinical R0 and R0 from radical surgery have no considerable survival differences (Fig. 2). To the contrary, the prognosis for patients with T3 lesions with clinical R0 was relatively poor, compared to patients with either T1 or T2 lesions with clinical R0. According to our results, the 5-year survival rate of patients with pT3 gallbladder carcinomas with clinical R0 was 14.6%, which is significantly different from that of patients with pT2 lesions (Fig. 1, $p=0.011$). In addition, when comparing the survival rates according to R status after surgery in patients with T3 lesions, the survival outcome of patients with clinical R0 was apparently inferior to that of patients with R0 from radical surgery (Fig. 3). Although the differences between the two groups have no statistic meaning, the statistical power seems to be too weak to support the survival comparativeness ($p=0.0655$). Consequently, we would like to emphasize that, unlike gallbladder carcinoma with T2 lesions, the value of clinical R0 in T3 gallbladder carcinoma cannot be guaranteed; therefore, additional or initial radical surgery should be considered for patients with T3 lesions to secure the negative resection margin and to control microscopic extension to the soft tissues, which could not be detected by macroscopic and radiological findings.

The concept of clinical R0 was tentatively used in this study to evaluate the surgical results after simple cholecystectomy. The critical drawback of this concept is that we cannot truly evaluate the microscopic lymph node metastasis, which could be definitively investigated in extended radical surgery. For example, about 30 to 40% of patients with T2 gallbladder carcinomas are known to have lymph node metastasis. In turn, these patients can lose the chance for long-term survival provided by radical surgery. Therefore, for the purposes of clinical application of this concept, we might need to design another clinical study that compares preoperatively predictive clinical R0 and pathologically provided residual tumor status in the same patients with gallbladder carcinomas.

The current study has unavoidable weak points in the analysis of the data. First, this study is based on retrospective available medical records, which means it is difficult to find the proper reasons why surgeons did not perform additional surgery with curative intent after simple cholecystectomies in patients with T2 and T3 lesions. Second, more than four surgeons were involved in treating gallbladder carcinomas according to the history of our department. We must take surgeon factors into account when comparing the results of radical surgery and simple cholecystectomy. Different extents of lymph node dissection and liver resection must also be considered in data analysis. However, our retrospective observations suggest that the survival results of patients who underwent simple cholecystectomies were acceptable for patients with T1 and T2 lesions with "clinical" R0 and provoke the possible role of simple cholecystectomy in well-selected patients with T2 lesions.

Conclusion

We carefully concluded that patients with incidentally detected gallbladder carcinomas could be expected to have good prognoses after undergoing simple cholecystectomy alone without additional radical surgery, as long as clinical R0 is ensured. However, we could not guarantee the validity of clinical R0 in patients with T3 lesions; therefore, radical cholecystectomy is warranted in such cases. More experiences and proper prospective study must be performed to validate surgical meaning of clinical R0 in treating gallbladder carcinomas.

Acknowledgement We give special thanks to Seo Won. Youl (Biostatistical Supporting Laboratory Yonsei University College of Medicine) for so much advice in analyzing biostatics data.

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J Gastrointest Surg (2007) 11:1317–1321
DOI 10.1007/s11605-007-0237-5

Pancreatic Pseudocysts: Is Delayed Surgical Intervention Associated with Adverse Outcomes?

Kaori Ito · Alexander Perez · Hiromichi Ito · Edward E. Whang

Received: 8 June 2007 / Accepted: 30 June 2007 / Published online: 3 August 2007

Abstract

Background Nonsurgical interventions are increasingly applied for pancreatic pseudocysts. We hypothesized that surgical therapy applied after failure of percutaneous or endoscopic therapies for pseudocysts is associated with poorer outcomes than cases in which surgery is the initial intervention.

Materials and methods Medical records of all 284 patients admitted with pancreatic pseudocysts at our institution (1/1990–9/2005) were analyzed. Forty-six patients underwent surgery as the initial intervention (group A). Among 162 patients who underwent percutaneous or endoscopic drainage as the initial intervention, 75 patients required subsequent surgery after failure of nonsurgical intervention (group B).

Results Groups were comparable in demographic variables and in location, number, and size of pseudocysts. Forty-two percent of group B patients developed infection within their pseudocysts after their nonsurgical interventions. Compared to group A patients, group B patients had higher rates of overall perioperative morbidity (47.8% vs 73.3%, $p=0.01$) and postoperative readmission (24.0% vs 44.7%, $p=0.04$). Five (6.7%) group B patients died in the perioperative period; there were no perioperative deaths among group A patients.

Conclusion Delayed surgical intervention for pancreatic pseudocysts is associated with higher incidences of postoperative complications, readmission, morbidity, and mortality. The increasing application of nonsurgical interventions needs to be reevaluated.

Keywords Pancreatic pseudocyst · Pancreatitis · Pancreatic surgery

Abbreviations

CT	Computed tomography
ERCP	Endosccopic retrograde cholangiopancreatography
MRCP	Magnetic resonance cholangiopancreatography

This study was presented at the American Hepato-Pancreato-Biliary Association 2007 Annual Meeting on April 21, 2007 at Las Vegas, Nevada.

K. Ito · A. Perez · H. Ito · E. E. Whang (✉)
Department of Surgery, Brigham and Women's Hospital,
Harvard Medical School,
75 Francis Street,
Boston, MA 02115, USA
e-mail: ewhang1@partners.org

Introduction

Pancreatic pseudocysts develop in 5 to 15% of patients diagnosed with acute pancreatitis and in 20 to 40% of patients diagnosed with chronic pancreatitis.[1–10] Well-accepted indications for interventions directed at pseudocysts include the presence of symptoms attributable to them and pseudocyst growth during a period of observation.[1,11,12]

Historically, surgical drainage and/or resection were the predominant therapeutic modalities for pseudocysts that required intervention. More recently, endoscopic and percutaneous pseudocyst drainage have been increasingly applied because of their perceived lower associated morbidity and mortality rates.[13–15] As a result, the role of surgery for pseudocysts is increasingly limited to salvage therapy for patients who fail endoscopic or percutaneous drainage.[13–17] Indeed, it has been our observation that many patients who fail these nonsurgical therapies or suffer complications induced by these procedures were, in

Table 1 Pancreatitis and Pseudocyst Characteristics

	Group A (n=46) Initial Surgery		Group B (n=75) Delayed Surgery		p Value
	n	%	n	%	
Etiology of pancreatitis					
Chronic	19	41.3	19	25.3	0.07
Alcohol	14	30.4	13	17.3	0.12
Others	5	10.9	6	8.0	0.74
Acute	27	58.7	56	74.7	0.07
Biliary	11	23.9	19	25.3	>0.99
Alcohol	11	23.9	17	22.7	>0.99
Trauma	1	2.2	3	4.0	>0.99
Others	4	8.7	17	22.7	0.05
Characteristics of pseudocysts					
Location					
Head	18	39.1	20	26.7	0.16
Body	13	28.3	35	46.7	0.06
Tail	15	32.6	20	26.7	0.54
Number					
1	31	67.4	42	56.0	0.25
≥2	15	32.6	33	44.0	0.25
Diameter					
≥6 cm	32	69.6	52	69.3	>0.99
Main pancreatic duct morphology					
Patients with ERCP/MRCP	21	39.1	47	62.7	0.04
Stricture	4	8.7	15	19.7	0.13
Disruption	3	6.5	13	17.3	0.10
Stricture and disruption	1	2.2	1	1.3	>0.99
Calculus	2	4.3	0	0	0.14
Dilatation	1	2.2	3	4.0	>0.99
Others	10	21.7	14	18.7	0.81
Disconnected pancreatic duct syndrome	0	0	2	2.7	0.52

retrospect, excellent operative candidates who might have had favorable outcomes had they undergone surgery initially.

Therefore, we performed this study to test the hypothesis that surgical therapy applied after failure of percutaneous or endoscopic therapies for pseudocysts is associated with poorer outcomes than cases in which surgery is the initial intervention.

Materials and Methods

This study was conducted with the approval of the Brigham and Women's Hospital Institutional Review Board. Patients were identified using the ICD 9 code for "cyst and pseudocyst of pancreas" (577.2). Patients with simple cysts or cystic neoplasms were excluded.

We also excluded patients whose cysts failed to meet the following Atlanta Symposium criteria for pseudocysts: (1) they are collections of pancreatic juice enclosed by a wall of fibrous or granulation tissue, which arise as a consequence of acute pancreatitis, pancreatic trauma, or chronic pancreatitis; (2) their formation requires 4 or more weeks from onset of pancreatitis; and (3) pseudocyst contents should consist of clear pancreatic fluid (no pus or necrotic debris).[18]

For patients who underwent aspiration and/or drainage of putative pseudocysts, procedure reports were reviewed. Patients in whom pus or necrotic debris was identified during these procedures were excluded. Further, all study patients underwent computed tomographic (CT) scanning. In each case, images were reviewed by an attending radiologist to confirm that identified cysts met the following imaging criteria for pseudocysts: uniform, low-attenuation fluid collections with a thin uniform wall that enhance after the administration of intravenous contrast.[19–21]

Based on these inclusion and exclusion criteria, we identified 284 consecutive patients admitted to our institution with pancreatic pseudocysts from January 1990 through September 2005. Forty-six of these patients underwent surgery as the initial interventional therapy

(group A). Among the 162 patients who underwent percutaneous (89 patients) or endoscopic drainage (73 patients) as the initial intervention, 75 patients (42 patients after percutaneous drainage, 33 patients after endoscopic drainage) required subsequent surgery after failure of nonsurgical intervention (group B). The choice of initial treatment was based on physician preference. We compared groups A and B with respect to demographic variables, comorbidity rates, etiology of pancreatitis, pseudocyst and pancreatic duct morphology, and operative procedures and outcomes.

To identify factors associated with failure of nonsurgical intervention, we compared group B patients with the 87 patients treated with nonsurgical intervention who did not require subsequent surgery directed at their pseudocysts. We evaluated the following factors in univariate and multivariate analyses: patient age and gender, etiology of pancreatitis, presence or absence of infection within the pseudocyst, pseudocyst location, number of pseudocysts, pseudocyst diameter, main pancreatic duct morphology (disruption, stricture, disruption plus stricture, calculus, and dilatation), and type (initial percutaneous drainage or initial endoscopic drainage) and number of interventions.

Data were evaluated using two-tailed Student's *t* test and Fisher's exact test, as appropriate. Multivariate analysis was performed using logistic regression. Criterion for statistical significance was $p<0.05$.

Results

Patients

Groups A and B were similar with respect to demographic variables and comorbidities. The percentages of patients in groups A and B who were male were 65.2 and 60.0%, respectively ($p=0.70$).. Median ages of patients in groups A and B were 46 years (range, 21–74 years) and 52 years (range, 20–86 years), respectively ($p=0.12$) No differences were evident between the two groups in percentages of patients with preexisting comorbidity (17.4% vs 26.7%, $p=0.27$).

Groups A and B were similar with respect to the proportion of patients having acute vs chronic pancreatitis and to the distribution of the major etiologies of pancreatitis (Table 1). Likewise, the location, number, and diameter of pseudocysts were similar in the two groups (Table 1). A greater percentage of group B patients than group A patients underwent preoperative endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance cholangiopancreatography (MRCP). However, the morphological features of the main pancreatic duct as characterized in these studies were statistically indistinguishable in the two groups (Table 1).

Table 2 Indication for Surgery and Interval Between Diagnosis and Surgery

	Group A ($n=46$) Initial Surgery		Group B ($n=75$) Delayed Surgery		*p* Value
	n	%	*n*	%	
Indication for surgery					
Pain	29	63.0	30	40.0	0.02
Infection	6	13.0	32	42.7	<0.01
Rupture	1	2.2	0	0	0.38
Hemorrhage	0	0	0	0	–
Others	10	21.7	13	17.3	0.63
Median interval from diagnosis to surgery (days)	31 (range, 20–140)		91 (range, 21–608)		<0.001

Table 3 Operative Procedures

	Group A ($n=46$) Initial Surgery		Group B ($n=75$) Delayed Surgery		*p* Value
	n	%	*n*	%	
Cystogastrostomy	13	28.3	12	16.0	0.11
Cystojejunostomy	12	26.1	13	17.3	0.28
Distal pancreatectomy	5	10.9	5	6.7	0.65
Whipple procedure	1	2.2	2	2.7	>0.99
Drainage	2	4.3	5	6.7	0.71
Peustow and drainage	4	8.7	6	8.0	>0.99
Others	9	19.5	32	42.7	0.01

Surgery

The median interval from pseudocyst diagnosis to surgery was three times greater for group B than for group A patients (Table 2, $p<0.001$). Specific indications for surgery are shown in Table 2. Infection within the pseudocyst (defined as positive bacterial cultures of nonpurulent pseudocyst fluid obtained on aspiration before surgery) was present in more than 40% of group B patients, in whom it was the most common indication for surgery. There were no differences between the two groups in the distribution of operative procedures applied (Table 3).

Postoperative outcomes are shown in Table 4. Group B patients had higher incidences of pancreatic complications and infectious complications. Further, Group B patients had higher rates of overall perioperative morbidity and of postoperative readmission than Group A patients. Five (6.7%) group B patients died in the perioperative period. Three of these deaths resulted from organ failure in the setting of postoperative necrotizing pancreatitis. Two deaths resulted from sepsis in patients with infected pseudocysts.

Table 4 Postoperative Outcomes

	Group A (n=46) Initial Surgery		Group B (n=75) Delayed Surgery		p Value
	n	%	n	%	
Pancreatic complications	12	26.1	40	53.3	0.004
Recurrent pancreatitis	8	17.4	27	36.0	
Recurrence of pseudocysts	2	4.3	7	9.3	
Pancreatic fistula	1	2.2	5	6.7	
Pancreatic duct stricture	1	2.2	1	1.3	
Infectious complications	4	8.7	19	25.3	0.03
Wound infection	1	2.2	8	10.7	
Sepsis	2	4.3	6	8.0	
Abscess	1	2.2	4	5.3	
Cholangitis	0	0	1	1.3	
Reoperation	6	13.0	9	12.0	>0.99
Readmission	12	26.1	34	45.3	0.04
Morbidity	22	47.8	55	73.3	0.01
Mortality	0	0	5	6.7	0.16
Median total length of hospital stay (days)	12 (range, 4–105)		17 (range, 1–164)		0.10
Median post-op length of hospital stay (days)	9 (range, 4–59)		10 (range, 1–151)		0.11

In contrast, there were no perioperative deaths among group A patients.

Factors Associated with Failure of Nonsurgical Intervention

Factors identified to be associated with failure of nonsurgical intervention on univariate analysis were as follows: number of nonsurgical interventional procedures ≥2 (100% vs 81.6%, $p<0.01$), infection within pseudocysts (as defined above, 50.7% vs 20.7%, $p<0.01$), pseudocyst diameter ≥6 cm (76.0% vs 55.1%, $p=0.01$), and main pancreatic duct stricture (47.6% vs 19.5%, $p=0.07$).

Among these four factors, those identified on multivariate analysis to be independently associated with failure of nonsurgical intervention are summarized in Table 5.

Discussion

Percutaneous and endoscopic drainage have been increasingly applied as first-line therapies for pancreatic pseudocysts, as these procedures are widely believed to be safe and efficacious.[13–15] However, these procedures are neither innocuous nor universally efficacious. For example, percutaneous pseudocyst drainage is associated with infectious complications in up to 50% of cases and failure rates as high as 58%.[17,22] Further, endoscopic pseudocyst drainage is associated with complications (such as bleeding, perforation, infection, and pancreatic duct stricture) in up to 36% of cases and failure rates as high as 40%.[23–26] Although surgery as first-line therapy for pseudocysts is undergoing extinction, it is increasingly applied as salvage therapy for failures and complications of percutaneous and endoscopic drainage.

Table 5 Factors Associated with Failure of Nonsurgical Interventions

Variable	p Value	Odds Ratio (95% CI)
Number of nonsurgical interventions ≥2	<0.001	1.3 (0.14–0.43)
Infection of pseudocysts at diagnosis	<0.01	1.3 (0.11–0.41)
Diameter of pseudocyst ≥6 cm	0.02	1.2 (0.01–0.32)

CI Confidence interval

In our study, surgery, when applied after failure of nonsurgical intervention, was associated with higher perioperative morbidity and readmission rates than cases in which surgery was the initial intervention. Five patients who underwent delayed surgery died in the preoperative period, whereas no patient who underwent surgery as the initial intervention died.

Our findings are broadly consistent with those of a smaller series reported by Rao et al.[26] In their study, the authors compared 52 patients who underwent surgery as the initial intervention for pseudocysts with 18 patients who underwent surgery after failure of nonsurgical intervention. Delayed surgery was associated with higher perioperative morbidity rates and delayed pseudocyst resolution.[26]

Surgery performed in the context of failed nonsurgical intervention for pseudocysts has also been studied by Nealon and Walser.[16] Although they did not specifically report comparisons of outcomes associated with initial vs delayed surgery, they made the important observation that main pancreatic duct disruption is predictive of failure of nonsurgical therapies for pseudocysts.

In our analysis, we identified need for ≥2 interventional procedures, infection within pseudocysts, and pseudocyst diameter ≥6 cm as additional factors associated with failure of nonsurgical intervention. The presence of one or more of these factors warrants consideration of surgery as the initial intervention in patients able to tolerate surgery.

Limitations of our study include sample size considerations and the potential for selection bias, a pitfall inherent to the retrospective study design. However, ours is among the largest cohorts of patients with pseudocysts treated with surgery yet reported. Further, our study groups were comparable with respect to demographic variables, comorbidity rates, and disease severity. To date, no prospective randomized trials comparing therapeutic options for pseudocysts has been reported. Until data from such studies are available, we must base clinical decision making on available evidence. Our data, taken together with those reported by others,[16,26] suggest that even in the current era, some patients with pancreatic pseudocysts are best served by surgery as the initial intervention.

Conclusion

Delayed surgical intervention for pancreatic pseudocyst is associated with higher incidences of preoperative infection, postoperative pancreatic and infectious complications, readmission, morbidity, and mortality. The increasing application of nonsurgical interventions needs to be reevaluated.

Acknowledgment The authors thank Jan Rounds for her excellent secretarial assistance.

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J Gastrointest Surg (2007) 11:1322–1327
DOI 10.1007/s11605-007-0240-x

Retroduodenal Resection of Ampullary Carcinoid Tumor in a Patient with Cavernous Transformation of the Portal Vein

Shin Hwang · Ki-Myung Moon · Jeong-Ik Park · Myung-Hwan Kim · Sung-Gyu Lee

Received: 2 July 2007 / Accepted: 2 July 2007 / Published online: 3 August 2007

Abstract Although pancreatoduodenectomy is the standard treatment for periampullary neoplasms, limited pancreas-preserving resections are sometimes performed. This report describes a carcinoid tumor of the ampulla of Vater for which pancreatoduodenectomy was not feasible because of diffuse cavernous transformation of the portal vein (PV) secondary to main PV obliteration of unknown cause. We performed retroduodenal resection of the ampullary carcinoid with total preservation of the pancreas. The duodenal wall defect was primarily repaired, and the pancreatic and bile ducts were separately reconstructed using Roux-en-Y pancreaticojejunostomy and choledochojejunostomy. The patient recovered uneventfully and is currently progressing well at 10 months postoperatively, with no tumor recurrence or complications. The surgical procedures are described, and the literature pertaining to this limited surgery is reviewed.

Keywords Carcinoid tumor · Ampullectomy · Pancreatoduodenectomy

Abbreviations

CT	computed tomography
PV	portal vein
CBD	common bile duct

Introduction

Although pancreatoduodenectomy is often performed to treat various neoplastic diseases of the ampulla of Vater, less invasive treatments such as transduodenal ampullectomy or segmental resection of the duodenum have sometimes been used. These latter local control procedures have definite limits on the extent of resection and are associated with higher recurrence rates and, as such, are not recommended for malignant tumors. For patients with premalignant or benign tumors, such limited resection procedures have been used to preserve organ function or reduce operative risk compared with conventional pancreatoduodenectomy. However, limited resection for periampullary lesions often can be an unattractive choice, as the surgical morbidity is comparable to that of pancreatoduodenectomy, and there are risks associated with an insufficient resection margin. As a result, such limited resections have been reported in only a small number of patients worldwide to date. Indeed, local or limited resection is generally recommended only when patients have particular conditions that prevent pancreatoduodenectomy.

The present report describes a very rare case of carcinoid tumor of the ampulla of Vater associated with diffuse cavernous transformation of the portal vein (PV) secondary to the main PV obliteration of unknown origin. Because the carcinoid tumor appeared too large to perform transduodenal ampullectomy, we searched the literature for feasible surgical options. We decided that the best surgical option was retroduodenal resection of the ampullary carcinoid tumor, followed by reconstruction using Roux-en-Y pancreaticojejunostomy and choledochojejunostomy. The procedures were

S. Hwang (✉) · K.-M. Moon · J.-I. Park · S.-G. Lee
Division of Hepatobiliary Surgery and Liver Transplantation,
Department of Surgery, Asan Medical Center,
University of Ulsan College of Medicine,
Seoul, South Korea
e-mail: shwang@amc.seoul.kr

M.-H. Kim
Department of Internal Medicine, Asan Medical Center,
University of Ulsan College of Medicine,
Seoul, South Korea

successful, and the patient recovered uneventfully. The present report describes the surgical procedures and reviews the literature pertaining to this limited resection surgery.

Case Report

A 66-year-old man was transferred to our institution with suspected ampulla of Vater cancer (Fig. 1). Initially, the patient had visited a local clinic because of vague abdominal pain, without any evidence of jaundice or pancreatitis. Magnetic resonance cholangiopancreatography indicated that the tumor was approximately 2 cm in diameter and that the intrapancreatic distal common bile duct (CBD) and pancreatic duct were displaced (Fig. 2). Confirmation of a carcinoid tumor was achieved after an endoscopic biopsy that revealed a well-differentiated neuroendocrine tumor.[1–4]

However, imaging studies revealed that the main PV and superior mesenteric vein were obliterated, and there was diffuse cavernous transformation between the pancreas and hepatic hilum. The patient stated this vascular abnormality had not previously been diagnosed, and he had no specific medical or social history. Although the entire portal flow was diverted through collaterals, there was no abnormality in liver function or hepatic parenchymal perfusion according to liver dynamic computed tomography (CT) and Doppler ultrasonography. The major pathways of the splanchnic blood flow were delineated using a three-dimensional reconstruction of dynamic CT scan (Fig. 3). The CBD was undulated by engorged collateral veins, and most of the head of the pancreas was also filled with collaterals. The superior mesenteric vein was obliterated, and the inferior mesenteric vein became a major collateral branch.

This case was our first in which major hepatobiliary surgery was required under such rare circumstances. We searched the literature to assist in designing a surgical plan. We carefully inspected the anatomy around the head of the pancreas during five other pancreatoduodenectomies for periampullary cancers, with special intention to determine the extent of safe resection and feasible reconstruction methods for this patient. No additional surgical procedure other than standard pancreatoduodenectomy was given during such inspection process. It appeared that removing the head of the pancreas was probably not feasible without extensive destruction of the collateral veins. Similarly, a transduodenal ampullectomy appeared inadequate because the ampullary mass was too large to perform a conventional ampullectomy

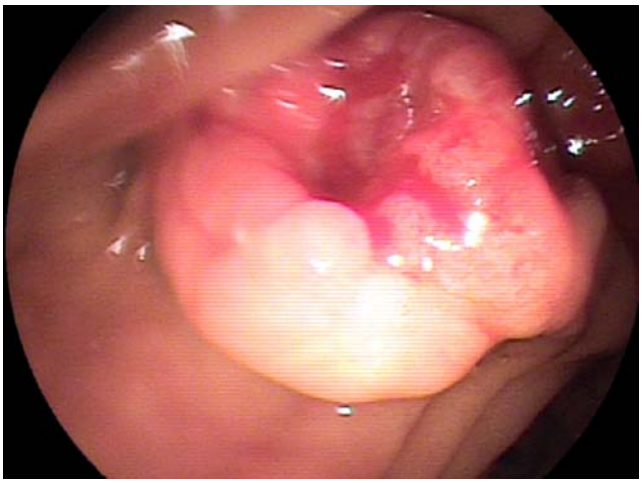

Figure 1 Gross photograph of the endoscopic finding of the polypoid mass at the ampulla of Vater.

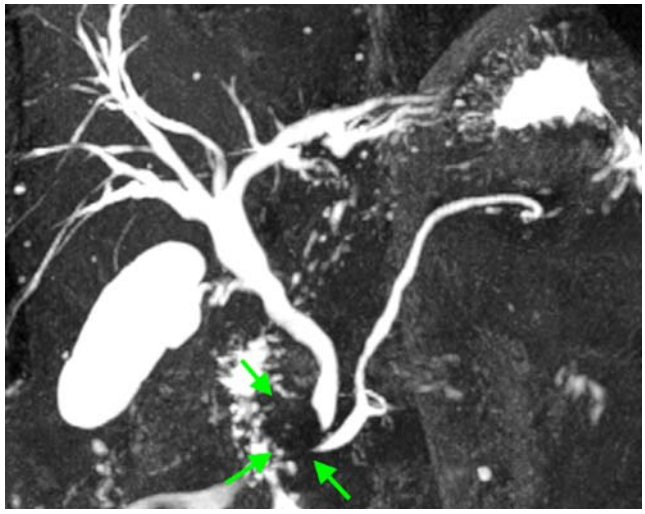

Figure 2 Magnetic resonance cholangiopancreatography delineating the ampullary mass involving the distal CBD and pancreatic duct. *Arrows* indicate the boundary of a 2-cm-sized round mass. The outline of the CBD appeared undulated because of engorged PV collaterals.

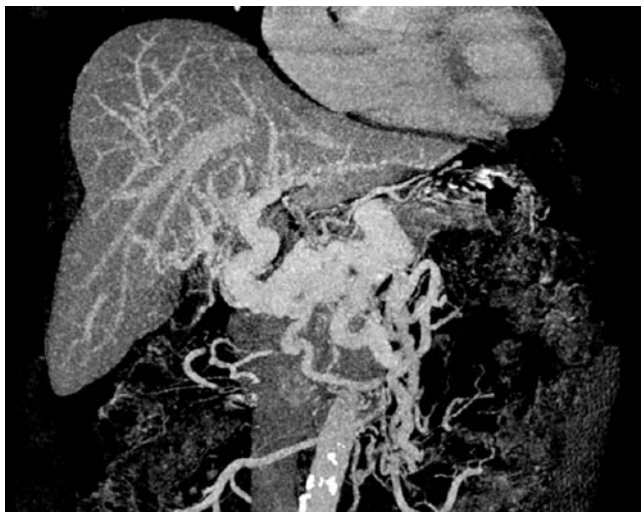

Figure 3 Three-dimensional reconstruction image of multidetector dynamic CT scan revealing diffuse cavernous transformation between the pancreas and hepatic hilum. The main PV and superior mesenteric vein were not visible.

and there was no alternative to cope with the possibility of not obtaining a deep tumor-free resection margin. Ultimately, a decision was made to take a retroduodenal approach to obtain a sufficient resection margin. The patient and family agreed to undergo this unique ampullary carcinoid resection procedure. The above process to determine the optimal surgical plan took 10 days.

The operation was performed through a mirrored L-shaped incision. After a time-consuming meticulous dissection of the hepatoduodenal ligament and pancreas head, we confirmed that the PV collaterals replaced most of the head of the pancreas (Fig. 4a). Manual palpation and intraoperative ultrasonography indicated that the tumor mass did not invade deep into the pancreas. Cholecystectomy was carefully performed after secure dissection of the collateral veins. No lymph nodes were found to be tumor-positive after frozen-section biopsies.

The second portion of the duodenum was mobilized from the pancreatic parenchyma, and after that, the distal CBD and pancreatic duct could be encircled separately. These duct structures were further excavated into the pancreatic parenchyma and cut separately within the pancreatic parenchyma (Fig. 4b). Frozen-section biopsies showed that both deep resection margins of these ducts were tumor-free. The dorsal duodenal wall containing the ampulla of Vater was elliptically excised with 5-mm long resection margins (Fig. 4c). This wedge-shaped duodenal wall defect was securely repaired using two-layer sutures immediately after excision (Fig. 4d). A jejunal Roux limb was prepared for the pancreatic and biliary reconstructions.

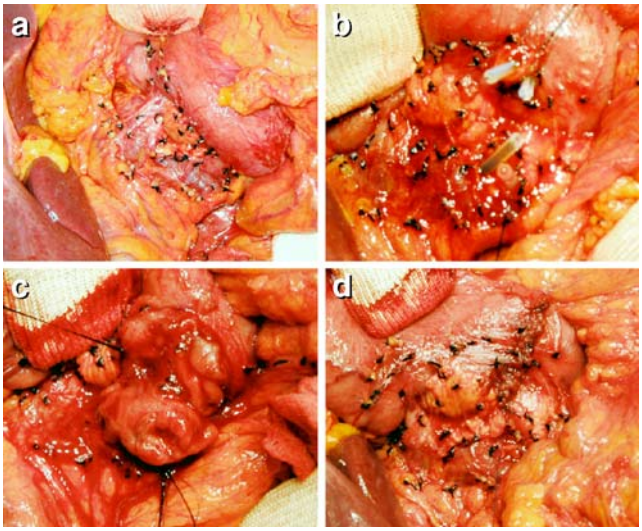

Figure 4 The surgical procedure sequences. **a** Meticulous dissection reveals the boundary of collateral formation at the hepatoduodenal ligament and pancreas head. **b** The distal CBD and pancreatic duct are cut within the pancreatic parenchyma. Four short silastic tubes are inserted to show their orifices at both sides. Some of the duodenal feeding vessels are preserved to prevent ischemia-induced bowel stenosis. **c** The dorsal duodenal wall containing the ampulla of Vater was elliptically excised leaving 5-mm-long margins. **d** This duodenal wall defect was repaired using two-layer sutures. A 1-cm-sized side-hole was made at the mid-CBD for separate side-to-side choledocho-jejunostomy. After that, the distal CBD orifice was closed to use as a buttress wall for pancreaticojejunostomy.

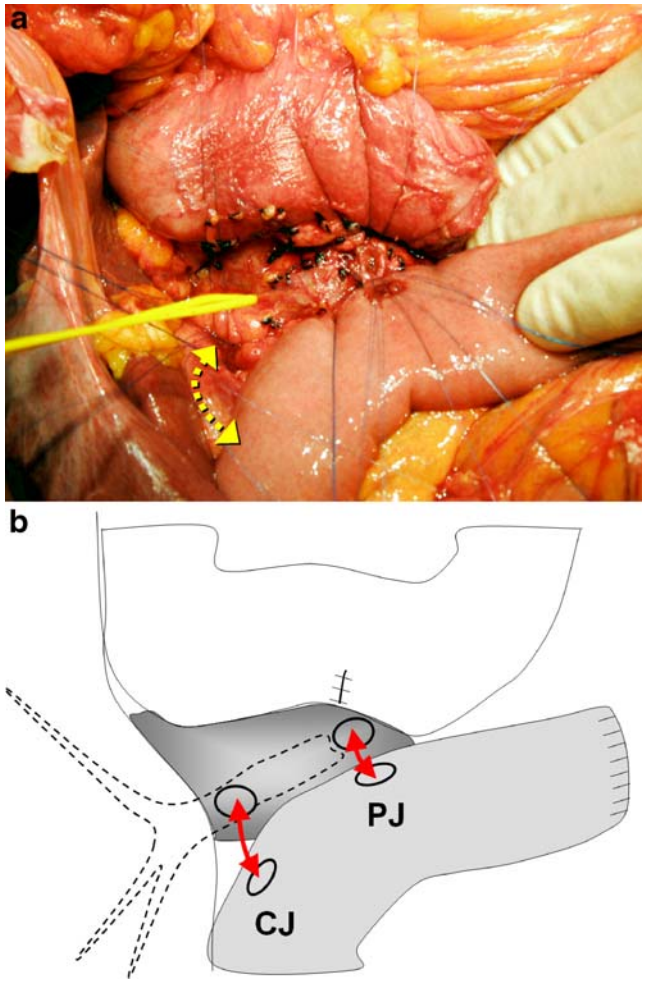

Figure 5 Intraoperative photograph (**a**) and illustration (**b**) of the pancreatic and biliary reconstruction. Single-layer duct-to-mucosa pancreaticojejunostomy (*PJ*) and choledochojejunostomy (*CJ*) were separately performed. *Dotted lines* indicate the outline of the biliary tree. Some blind stump of the distal CBD is left within the head of the pancreas.

The stump wall of the pancreatic duct orifice was thicker than would be expected after conventional pancreatoduodenectomy. Single bilio-pancreato-enteric anastomosis after unification plasty of the distal CBD and pancreatic duct openings appeared feasible at a glance, but we thought such reconstruction might have higher risk of anastomotic leak than separate reconstructions of the bile duct and pancreatic duct. Thus, we chose to perform separate reconstructions to minimize the operative risk. The distal CBD stump was first closed and used as a buttress wall for an end-to-side duct-to-mucosa pancreaticojejunostomy (Fig. 5). A drain tube was inserted into the main pancreatic duct for external drainage, and this tube was pulled out through a 3-cm-long Witzel tunnel.

Before closure of the distal CBD opening, a 1-cm-sized side-hole was made at the mid-CBD wall for side-to-side choledochojejunostomy (Fig. 5). As the portal collaterals were dispersed over the whole CBD wall, the total circumference of this side-hole was sutured for complete hemostasis. After completion of the side-to-side choledochojejunostomy, another external drainage tube was inserted into the jejunal limb to decompress the bile juice. A Roux-en-Y jejunojejunostomy was then performed. For effective drainage to cope with any potential pancreatic leak, two sets of suction-type cigarette drains were inserted in which Jackson–Pratt-type suction drain bags were attached.

Pathologic assessment of the resected specimen revealed that the final diagnosis was a 1.8-cm-sized well-differentiated neuroendocrine tumor (carcinoid tumor) of the duodenal papilla. The tumor cells were positive for chromogranin, cytokeratin 7, cluster of differentiation 56, and synaptophysin, and Ki-67 labeling index is 2–3%, supporting this diagnosis. There were no vascular or perineural invasions. All bile duct, pancreatic duct, and duodenal and deep resection margins were tumor-free. All of four sampled lymph nodes proved tumor-negative.

The patient fasted for 1 week postoperatively, after which oral food intake commenced. There were no surgical complications. Follow-up CT scans confirmed that the portal collateral pathways remained intact. Up to 700–800 ml/day of pancreatic juice drained through the external drainage tube despite daily administration of octreotide. We had to keep this tube in place for 4 weeks in fear of pancreaticojejunostomy leak. The jejunal loop drainage tube was removed at 5 weeks postoperatively, and the patient was discharged shortly thereafter. To date, the patient is progressing well 10 months postoperatively, and there are no signs of tumor recurrence or complications.

Table 1 Literature Reports on Pancreas-preserving Duodenal Resection Requiring Pancreatic and Biliary Reconstruction for Periampullary Lesions

Author	Publication year	Diagnosis	Patient no.	Extent of duodenectomy and pancreatic/biliary reconstruction	Complication rate (%)	Outcome
Imamura et al. [12]	2005	Bleeding, gastrinoma, FAP	3	Total duodenectomy Conjoined reimplantation to jejunal Roux limb	0	All alive for mean 11 months
Mackey et al. [5]	2005	Duodenal FAP	21	Total duodenectomy Conjoined reimplantation to jejunal Roux limb	38	No local recurrence for mean 79 months
Kimura et al. [13]	2005	Retroperitoneal liposarcoma	1	Total duodenectomy Conjoined reimplantation to jejunal Roux limb	100	Survived, but no follow-up data
Eisenberger et al. [14]	2004	Benign to carcinoma	4	Total duodenectomy Conjoined reimplantation to jejunal Roux limb	50	No recurrence for mean 24 months
Lundell et al. [15]	2002	Adenoma, lipoma, FAP	4	Total duodenectomy Conjoined reimplantation to jejunal Roux limb	25	No patient loss, but no follow-up data
Sarmiento et al. [16]	2002	Adenoma, FAP	8	Total duodenectomy Conjoined reimplantatio to jejunal Roux limb	62.5	All alive for mean 23 months
Kalady et al. [7]	2002	Duodenal FAP	3	Total duodenectomy Conjoined reimplantation to jejunal Roux limb	67	No local recurrence for mean 2.2 years
Nagai et al. [17]	1999	Necrosis, bleeding, lymphoma	3	Total duodenectomy Button or conjoined reimplantation to jejunal Roux limb	67	Two dead within 2 months, one alive for 7 months
Tsiotos et al. [18]	1998	Adenoma, FAP	4	Total duodenectomy Ampullojejunostomy to jejunal Roux limb	25	All alive for mean 7 months
Ryu et al. [19]	1996	Adenoma	2	Segmental duodenectomy Reimplantation to the remnant duodenum	0	All alive for mean 9 months

FAP Familial adenomatous polyposis

Discussion

Historically, the main reasons for performing limited resection surgery for neoplasms involving the ampulla of Vater are to reduce surgical morbidity and to preserve organ functions. However, ongoing improvements in pancreatoduodenectomy procedures and perioperative care have reduced morbidity and mortality rates significantly, although they can be still high in low-volume institutions.[5,6] There does not appear to be a consensus regarding the technical safety and affordability of the tumor-free resection margin when performing limited resection of ampulla of Vater tumors. We guess that some surgeons continue to perform such a limited resection as a technical challenge in the situation that they can convert the limited surgery to pancreatoduodenectomy during operation. However, in the present case, there was no option other than limited resection, as the PV collaterals did not permit resection of the head of the pancreas. Ampullary carcinoid tumor is a very rare tumor, and only in 100 and some more cases were reported to date. Previous reports indicate excellent outcomes after the removal of well-differentiated neuroendocrine tumors.[1–4] In the present case, we would have performed pylorus-preserving pancreatoduodenectomy had the patient not had such a rare PV abnormality.

There are three types of limited surgical procedures that can be used as substitutes for pancreatoduodenectomy for periampullary tumors, namely local resection, pancreas preservation, and duodenum preservation. The extent and location of the primary tumor dictate which procedure is to be used.[5,7,8]

For ampullary and duodenal tumors, the least aggressive of these procedures is a transduodenal ampullectomy.[9–11] It requires in situ reconstruction of the resected duct stumps to the duodenal wall. It can be applicable to the benign and premalignant lesions deeply located within the ampulla, with concurrent removal of some pancreatic parenchyma.[11] However, in this patient, we considered two limitations of transduodenal ampullectomy: whether a sufficient resection margin can be obtained and deep excision does not guarantee secure reconstruction. In our experience, most initial transduodenal ampullectomies were converted to pancreatoduodenectomies for these reasons.

The intermediately aggressive procedure is a retroduodenal resection of the ampulla, which was undertaken in the present case. Although the duodenal wall may require excision as a wedge or short segment, duodenal continuity can be maintained after primary repair. The distal CBD and pancreatic duct orifices can be simultaneously reimplanted into the duodenal wall or jejunal Roux limb. Separate implantation of the distal CBD orifice into the duodenal wall combined with Roux-en-Y pancreaticojejunostomy or separate enteric reconstruction of the pancreatic duct and CBD orifice can be used on a case-by-case basis. In this patient, some blind distal CBD was left within the head of the pancreas (Fig. 5). There may be some risk of complication from leaving a blind CBD stump, but it may not be serious considering that side-to-side choledochojejunostomy in patients with benign distal CBD stenosis has been well tolerated during long-term follow-up.

The most aggressive procedure is a major or total resection of the duodenum in which the jejunal Roux limb is used for anastomosis to the stomach and for reconstruction of the distal CBD and pancreatic duct openings. Button preservation of the ampulla and subsequent reimplantation to the jejunal loop is feasible only when the ampulla and surrounding margin is not involved. A literature review on pancreas-preserving duodenal resection procedures is summarized in Table 1.[5,7,12–19]

It appears that there are theoretically both technical and functional advantages to the pancreas-preserving procedure. As the periductal tissue at the head of the pancreas is more fibrous than in the body portion, performing pancreaticojejunostomy appears to be more secure than in pancreatoduodenectomy (Fig. 5). Preservation of the entire pancreatic parenchyma provides the advantage of uncompromised endocrine and exocrine functions. Far more pancreatic juice was drained in the present patient than we observed after pancreatoduodenectomy in patients with normal pancreatic parenchyma (average of 314±222 ml/day for the latter patients).[20]

In conclusion, this unique case and the literature review suggest that retroduodenal resection of the ampullary tumor combined with pancreatic-biliary reconstruction using jejunal Roux limb is a feasible alternative to pancreatoduodenectomy for limited resection of the ampulla of Vater neoplasms. However, the technical dexterity required and the risk of insufficient resection margin indicate that this procedure should be regarded as an option in only a small number of highly selected patients.

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DOI 10.1007/s11605-007-0211-2

A Decision Analysis Model Identifies the Interval of Efficacy for Transarterial Chemoembolization (TACE) in Cirrhotic Patients with Hepatocellular Carcinoma Awaiting Liver Transplantation

Thomas A. Aloia · René Adam · Didier Samuel · Daniel Azoulay · Denis Castaing

Received: 15 May 2007 / Accepted: 10 June 2007 / Published online: 8 August 2007

Abstract

Introduction For liver transplant candidates with hepatocellular carcinoma (HCC), the ability of neoadjuvant transarterial chemoembolization (TACE) to improve outcomes remains unproven. The objective of our study was to determine if there was a specific time interval where neoadjuvant TACE would decrease the number of HCC patients removed from the pretransplant waitlist.

Materials and Methods A decision model was developed to simulate a randomized trial of neoadjuvant treatment with TACE vs. no TACE in 600 virtual patients with HCC and cirrhosis. Transition probabilities for TACE morbidity (1±1%), TACE response rates (30±20%), and disease progression (7±7% per month) were assigned by systematic review of the literature (18 reports). Sensitivity analyses were performed to determine time thresholds where TACE would decrease the number of delisted patients.

Results TACE treatment had statistical benefit at waitlist time breakpoints of 4 and 9 months ($P<0.05$). When waitlist times were less than 4 months, waitlist attrition was similar (20% vs. 34%, $P=0.08$). When waitlist times exceed 9 months, waitlist dropout rates re-equilibrated (33% vs. 46%, $P=0.06$). Review of the current literature determined that only those studies reporting on patients with waitlist times between 4 and 9 months found a benefit to neoadjuvant TACE.

Conclusions This analysis indicates that the benefit of neoadjuvant TACE may be limited to those patients transplanted from 4 to 9 months from first TACE. These data may help transplant programs to tailor TACE treatments based on predicted waitlist times to achieve optimal resource utilization and improved organ allocation efficiency.

Keywords Primary liver cancer · Adjuvant therapy · Outcomes analysis

Presented at the 7th World Congress of the International Hepato Pancreato Biliary Association Meeting, September 6, 2006, Edinburgh, Scotland.

T. A. Aloia (✉)
Division of Abdominal Transplantation and Hepatobiliary Surgery, Department of Surgery, Baylor College of Medicine, 1709 Dryden, Suite 15.37,
Houston, TX 77030, USA
e-mail: taaloia@tmhs.org

T. A. Aloia · R. Adam · D. Samuel · D. Azoulay · D. Castaing
Centre Hépatobiliaire, Paul Brousse Hospital, Université Paris XI, Villejuif, France

Introduction

The ideal treatment for patients with hepatocellular carcinoma (HCC) and cirrhosis is orthotopic liver transplantation (OLT). In current clinical practice, however, imbalance in the availability of matched donor organs mandates that most of the patients with HCC and cirrhosis will spend a variable amount of time on a waitlist before OLT. During this volatile waitlist period, HCC patients can experience progression of malignant disease and/or complications of cirrhosis, which may reduce or even remove the indication for liver transplantation.

To address the possibility of malignant disease progression while on the waitlist, many transplant programs have offered (and continue to offer) local treatment modalities to

HCC patients, including resection, ablation, and transarterial chemoembolisation (TACE). As TACE appears to have the highest safety margin of these three options, it has become a popular initial treatment modality for patients with HCC awaiting OLT.

Although palliative TACE has been shown to prolong survivals in patients with unresectable HCC who are not transplant candidates,[1] data on the efficacy of TACE as a neoadjuvant treatment for HCC patients awaiting OLT is conflicting. Several studies have shown improvement in the transplant waitlist dropout rate after TACE.[2–5] However, other studies have found no impact on this endpoint.[6,7] Despite early promising results from well-designed single center studies,[8–10] no study has proven that neoadjuvant TACE improves post transplant disease-free survivals.[11–15]

The variability in response rates to TACE suggest that there are multiple factors which influence outcome after this treatment. To better understand these factors, we designed a decision analysis model based on a systematic review of the available literature to determine the time thresholds where neoadjuvant TACE is predicted to significantly decrease the number of delisted patients. These data were then compared to previously published outcomes to determine if the conflicting data in the literature could be explained.

Materials and Methods

Using commercially available software (TreeAge Pro Healthcare Edition, TreeAge, Williamstown, MA), a decision analysis model was developed to simulate a randomized trial of neoadjuvant treatment with TACE vs. no TACE in 600 virtual patients (300 in each group) with HCC and cirrhosis awaiting OLT. This sample size allowed for reliable detection of a 10% difference in dropout rates with an alpha of 0.05 and a beta of 0.10. Transition probabilities for decision points were assigned by systematic review of the English language literature (18 relevant reports), including TACE morbidity (1±1%), TACE response rates (30±20%), and progression of malignant disease (7±7% per month). Median waitlist times were varied from 1 to 18 months at 1 month intervals. An assumption was built into the model that TACE treatments would be repeated every 2.5 months up to a maximum of three total treatments. Sensitivity analyses were performed to determine the impact of study factor variance on the time thresholds where TACE would significantly decrease the number of delisted patients. Chi-squared tests were used to assess differences in dropout rates at each waitlist time interval.

The model was validated by comparing the median waitlist times and dropout rates for previously reported clinical studies that found a benefit for TACE before OLT to those studies that found no benefit for neoadjuvant TACE.

Results

Analysis of Decision Model Output

Analysis of the decision model output comparing waitlist dropout rates in virtual patients treated with and without neoadjuvant TACE while varying the waitlist time determined that TACE treatment had statistical benefit at waitlist time breakpoints of 4 and 9 months ($P<0.05$, Fig. 1). When waitlist times were less than 4 months, waitlist attrition was similar (20% vs. 34%, $P=0.08$). When waitlist times exceed 9 months, cumulative morbidity from repeated TACE treatments and disease progression outweighed the benefits of TACE and led to re-equilibration of waitlist dropout rates (33% vs. 46%, $P=0.06$).

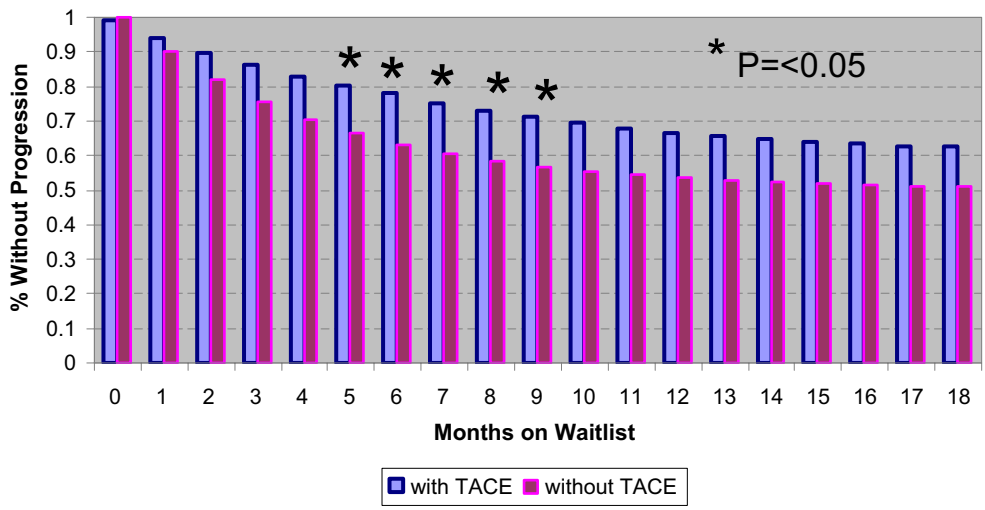

Figure 1 Differences in transplant waitlist dropout because of disease progression between 300 virtual patients treated with neoadjuvant TACE and 300 virtual patients with no TACE treatments before liver transplantation.

Sensitivity Analyses

To determine the impact on differences in waitlist dropout rates and significant waitlist time intervals, sensitivity analyses were performed for each study variable. Variance of the disease progression rate had no impact on the waitlist time interval. Likewise, variance of the TACE complication rate had no influence on the waitlist time interval. In contrast, variance of the TACE response rate did impact the duration of the significant time interval. When the TACE response rate was greater than 50%, the waitlist time interval where differences in the waitlist dropout rates remained statistically significant was extended from 4 to 11 months after initial TACE treatment.

Validation of the Decision Model

The six studies (all single center), which report waitlist dropout rates for patients undergoing TACE while awaiting liver transplantation, were examined to determine the median number of TACE treatments delivered, the waitlist dropout rate, and the median times to dropout or transplant (Table 1).[2–7] Four of the six studies were positive, concluding that TACE was associated with reduced waitlist dropout (range 0–18%) and a benefit to patients awaiting liver transplant.[2–5] In contrast, the remaining two studies were negative, concluding that TACE had no impact on waitlist dropout (range 35–46%).[6,7] The median time to liver transplant for each of these studies was plotted (Fig. 2). This plot reveals that each of the positive studies were from programs with median waitlist times between 4 and 9 months. All of the studies reporting negative results were from programs with a median waitlist time ≤4 or >9 months.

In addition, studies comparing long-term post OLT outcomes (disease-free and overall survivals) in patients treated with and without neoadjuvant TACE were examined (Table 2). Three of these studies include data on waitlist times.[12,15,16] Both Oldhafer et al. and Decaens et al. reported on patients with short waitlist times (range 118–128 days) and found no impact of neoadjuvant TACE on survivals. The third study, by Porrett et al., reporting data on waitlist times, bridges the MELD scoring era. In the preMELD era, the median waitlist time was 574 days and in the postMELD era, the median waitlist time was 54 days. As predicted by the decision analysis model, neither cohort reported in this study was found to have a survival benefit from TACE (13 of 31 treatment group patients in this study received TACE).

Discussion

A recent systematic literature review has concluded that TACE has no benefit in patients awaiting liver transplantation.[17] This conclusion is contrary to the experience at many transplant centers, which continue to use TACE as a primary therapeutic modality in patients with HCC and cirrhosis. To resolve these discrepancies, we developed a decision analysis model that took the form of a randomized trial comparing TACE treatment to no TACE treatment with 300 virtual patients in each arm. The aim of the study was to identify a time threshold where TACE would have the greatest benefit. Furthermore, we validated the model's findings by analyzing reported waitlist dropout rates in negative and positive TACE studies.

The significant finding from the analysis of the decision model was that TACE was predicted to only benefit patients

Table 1 Studies assessing the impact of neoadjuvant TACE on waitlist dropout rates

Author, year	No of patients	Stage	Mean waitlist time (OLT/dropout)	Dropout (%)	Factors associated with dropout
Roayaie et al., 2002[7]	80	III, IV	142 d/207 d	46	Tumor size
Graziadei et al., 2003[2]	48	I, II	178 d/–	0	–
	15	III, IV (downstaged)	254 d/189 d	20	Stage IVa
Hayashi et al., 2004[6]	20	I, II	343 d/na	35	None
Maddala et al., 2004[5]	54	I, II	211 d/83 d	15	None
Fisher et al., 2004[4]	22	I, II, III	276 d[a]/225 d	18	AFP>400 ng/ml T-stage=3 Bilobar HCC
Yao et al., 2003[3]	58	I–II	180 d/280 d	11	Tumor no >2
		I–III	186 d/323 d	31	Solitary HCC>3 cm Lack of NAT

No number, *d* days, *NAT* neoadjuvant treatment, *na* not available.
[a] PreMELD mean waitlist time 410 days, PostMELD mean waitlist time 66 days.

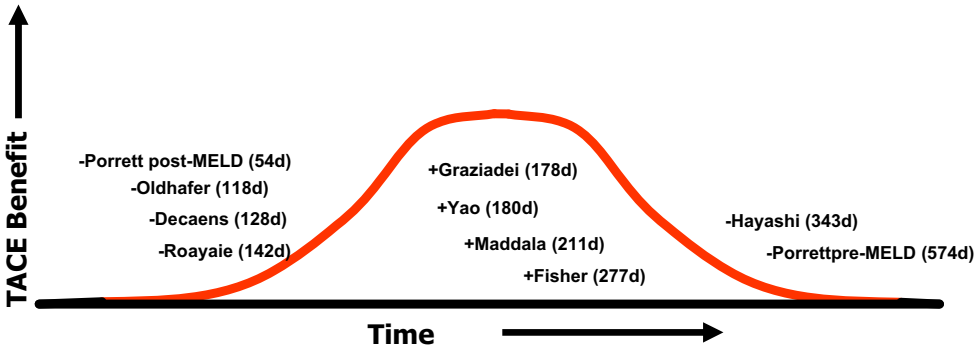

Figure 2 Plot of positive (+) and negative (−) studies for neoadjuvant TACE in transplant waitlisted patients comparing average waitlist times to the predicted interval of peak TACE efficacy.

with HCC who were transplanted between 4 and 9 months from initiation of TACE treatments. This finding was supported by the six available reports on this topic. All clinical studies that found a benefit to neoadjuvant TACE reported median waitlist times from 178 to 277 days.[2–5] In contrast, the remaining studies, which found no benefit to TACE, were all from centers with median waitlist times ≤4 or >9 months.[6,7] Furthermore, three studies, which report no benefit to neoadjuvant TACE in terms of long-term outcomes, were also found to have median waitlist times ≤4 or >9 months.[12,15,16]

Several studies have shown that, after various local treatments, dropout increases with time on the transplant waitlist.[3,18,19] It is interesting to note that Yao et al. have demonstrated that the highest frequency of dropout occurs between 6 and 12 months on the waitlist.[3] In 58 patients who initially met the UNOS transplant criteria, they found that the cumulative waitlist dropout rate at 6 months was 11%, rising steeply to 57% at 12 months, and plateauing thereafter, with a cumulative 24-month dropout rate of 75%. These findings suggest that local therapies that reduce disease progression during this time interval of peak waitlist dropout could significantly impact outcomes for waitlisted patients.

Conclusion

We conclude from these analyses that the question of whether or not TACE is beneficial to patients with HCC who are awaiting liver transplantation does not have an absolute yes or no answer. TACE is likely to benefit certain patients, specifically those with a predicted waitlist time from 4 to 9 months. Given these findings, individual centers should be able to tailor the use of TACE therapy based on historical waitlist times, blood group, HCC stage, severity of cirrhosis, and waitlist rank (i.e. MELD score). This approach would be anticipated to maximize TACE

Table 2 Recent studies comparing outcomes for patients with hepatocellular carcinoma treated with neoadjuvant TACE and untreated before liver transplant

Author	Year	No of pts treated/untreated	Stage	Waitlist time	Dropout	Survivals (year)		
						1	3	5
Majno et al.[13a]	1997	54	na	na	na	74%	62%	57%
		57	na	na	na	77%	66%	59%
Oldhafer et al.[12]	1998	21	I–IV	118d	na	61%	48%	na
		21	I–IV	na	na	62%	54%	na
Perez Saborido et al.[20]	2005	18	I–IVa	na	na	83%	61%	61%
		28	I–IVa	na	na	77%	59%	38%
Decaens et al.[15]	2005	100	I–IV	128 d	na	na	na	59%
		100	I–IV	131 d	na	na	na	59%
Yao et al.[21a]	2005	85	T2/3	na	na	96%	na	94%
		41	T2/3	na	na	92%	na	81%
Porrett et al.[16]	2006	31[b]	T1–2	574 d/54 d[c]	na	na	84%	na
		33	T1–2	543 d/199 d[c]	na	na	91%	na

No number, *pts* patients, *d* days, *na* not available.
[a] Survivals are disease/recurrence-free.
[b] Only 13 patients were treated with TACE.
[c] Waitlist times preMELD/postMELD.

benefit, reduce exposure to TACE related morbidity, reduce unnecessary resource utilization, and improve organ allocation efficiency.

Acknowledgement Thomas Aloia, M.D. received grant support for this project from the 2005–2006 International Union Against Cancer/ American Cancer Society-Beginning Investigator Award.

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J Gastrointest Surg (2007) 11:1333–1338
DOI 10.1007/s11605-007-0214-z

Histological, CT, and Intraoperative Ultrasound Appearance of Hepatic Tumors Previously Treated by Laparoscopic Radiofrequency Ablation

T. Mason · E. Berber · J. C. Graybill · A. Siperstein

Received: 10 June 2007 / Accepted: 10 June 2007 / Published online: 25 July 2007

Abstract

Purpose The purpose of this paper is to compare intraoperative biopsy results of previously ablated liver tumors with their preoperative computed tomography (CT) and intraoperative laparoscopic ultrasound (LUS) appearances in patients undergoing repeat radiofrequency ablation (RFA).

Methods Seventy repeat RFA procedures were performed in 59 (13%) patients. Laparoscopically, suspected recurrent and stable appearing foci were biopsied using an 18 G biopsy gun. Preoperative CT and LUS appearances of the previously ablated lesions were compared with core biopsy results.

Results There were 33 patients with colorectal cancer, 11 with hepatocellular cancer, 8 with neuroendocrine tumors, and 7 with other tumor types. Two hundred lesions were treated by RFA in these 70 repeat ablations. Suspected recurrent tumor foci were enhanced on CT and produced a more finely stippled echo pattern on LUS. Biopsy confirmed recurrent tumor in 72 of 84 such lesions. Previously ablated foci had a CT appearance of a hypodense, nonenhancing lesion without evidence of adjacent enhancing foci. Laparoscopic ultrasound appearance was of a hypoechoic lesion with a coarse internal pattern with the tracks of the ablation catheter probes often still visible. Biopsy found necrotic tissue in 21 of 22 such lesions appearing radiologically to be without recurrence. Biopsy of an ablated focus adjacent to an area of suspected recurrence showed necrotic tissue in 17 of 22 lesions and viable cancer in 5.

Conclusion CT and LUS appearance of previously ablated foci showed good correlation with core biopsies. CT scan is reliable in following RFA lesions, without the need for routine biopsy. LUS reliably distinguished recurrent from ablated lesions in patients undergoing repeat ablation.

Keywords Radiofrequency thermal ablation · Laparoscopic · Local recurrence

Introduction

Primary and metastatic liver cancer poses a therapeutic challenge to both surgical and medical clinicians. Despite multimodal therapy, patients often succumb to extrahepatic disease or intrinsic parenchymal disease[1]. In the United States, the most common type of tumor that presents with metastatic disease to the liver is colorectal adenocarcinoma. Of the 160,000 new cases every year, 25% will present with liver metastasis[2]. Hepatocellular carcinoma is the most common liver tumor worldwide and is often unresponsive to surgical and medical treatment[3]. Metastatic neuroendocrine tumors, although less common, are often multifocal and symptomatic because of hormone secretion[4–6].

Surgical resection is currently the best treatment for both primary and metastatic liver tumors. Unfortunately, only 10–20% of patients with colorectal metastasis[7,8], 12–39% of patients with hepatocellular carcinoma[9], and less than 10% of patients with hepatic neuroendocrine metastases are candidates for curative resection[10]. This has led to the development of a number of alternatives to resection in

Presented at the AHPBA 2005 Congress on 4/14–17/2005 in Ft. Lauderdale, Florida as a poster

T. Mason · E. Berber (✉) · J. C. Graybill · A. Siperstein
The Cleveland Clinic Foundation, Department of General Surgery,
9500 Euclid Avenue, A80,
Cleveland, OH 44195, USA
e-mail: berbere@ccf.org

treatment of these tumors. Alternative therapies include cryotherapy, alcohol injection, chemoembolization, and radiofrequency ablation (RFA)[11].

In the past decade, RFA has gained wide acceptance as a minimally invasive modality for the local destruction of hepatic tumors that are not amenable to surgical resection. Initial studies focused on percutaneous ultrasound-guided RF ablation of liver tumors[12–14]. In our practice, we have been applying RF ablation laparoscopically and have reported data on a variety of liver tumors[15–17]. The patients undergoing RFA of their liver tumors are at risk for developing new liver disease or local recurrence at the site of the ablation in follow-up, which occurs in approximately 12% of lesions[18]. These patients have traditionally been followed-up by serial CT or MR scans to assess for local tumor control after radiofrequency ablation[19]. Biopsy of the ablated foci is an objective means of determining whether there is a recurrence of the disease at the ablated site. Currently, there is little data in the literature regarding the role of biopsy of previously ablated foci to rule out recurrent tumor in routine follow-up after RFA. The first goal of this paper is to compare biopsy results of previously ablated lesions with their preoperative CT and intraoperative laparoscopic ultrasound (US) appearances in patients undergoing repeat RFA. This information could then be used to define certain CT and laparoscopic US appearances that would correlate with either viable or necrotic tumor in follow-up.

Another goal of this paper is to describe the surgical strategy for repeat ablation procedures. Although there is abundant data in the literature about the local recurrence rate after RFA of liver tumors, there are no guidelines about the technical conduct of such operations regarding how recurrent areas should be ablated and stable-appearing lesions managed.

Materials and Methods

Between 1996 and 2004, 461 patients underwent laparoscopic radiofrequency ablation of 1,428 unresectable primary and metastatic liver tumors. Fifty-nine of these patients (13%) required 70 repeat ablations for suspicion of locally recurrent and/or new liver tumors at serial CT scans done at follow-up.

Laparoscopic radiofrequency ablation was performed under general anesthesia with endotracheal intubation. Patients were placed on the operating table in the supine position. One gram of cefazolin was administered as a surgical prophylactic antibiotic. Two 12 mm ports were placed under the right costal margin, one for the laparoscope and one for the laparoscopic ultrasound probe. After diagnostic laparoscopy, liver ultrasound was performed using a linear 7.5 mHz Aloka side-viewing laparoscopic ultrasound transducer (Aloka, Wallingford, CT, USA). Once a lesion was localized by ultrasound, a core biopsy was performed using an 18-gauge spring-loaded biopsy gun with an echogenic needle tip to improve ultrasound visibility.

RFA was performed using the first and second generation ablation systems, which have been described in detail elsewhere[20,21]. The system was connected to a laptop computer, which provided continuous monitoring of the ablation parameters. Various algorithms were used to perform RFA ablation[20,22]. Patients were admitted to the hospital postoperatively and observed overnight. The patients were followed-up by liver CT scans obtained 1 week post ablation and then quarterly to evaluate for liver tumor recurrence.

During the repeat ablation procedures, biopsies were taken from previously ablated lesions that appeared to be free of recurrence and from areas that were suspicious for local recurrence based on preoperative triphasic CT scan and intraoperative laparoscopic ultrasound. The RFA sites that appeared to be free of recurrence were selected for biopsy based on size: the dominant lesions were biopsied as there is evidence in the literature that lesion size is a predictor of local recurrence[18]. All biopsies were done using an 18G biopsy gun. Preoperative CT and laparoscopic ultrasound appearances of the previously ablated lesions were then compared with the core biopsy results.

Results

Of the 59 patients requiring repeat ablation of liver tumors, 33 had colorectal cancer, 11 hepatocellular carcinoma, 8 neuroendocrine tumors, and 7 had other tumor types. Forty-eight patients underwent repeat ablation once and 11 patients underwent repeat ablation twice in their follow-up. Mean± SEM time to repeat radiofrequency ablation was 12±1 month. Two hundred lesions were treated by RFA in the 70 repeat ablation procedures. Out of these 200 lesions, 84 were locally recurrent (recurrence at the site of a previous ablation) and 116 were non-ablation site intrahepatic new lesions. Three different groups were identified on biopsies.

Previously ablated foci had a CT appearance of a hypodense, non-enhancing lesion without evidence of adjacent enhancing foci. Laparoscopic ultrasound appearance in these cases was of a hypoechoic lesion with a coarse internal pattern with the tracks of the ablation catheter probes often still present. Biopsy found necrotic tissue in 21 (96%) and viable tumor in 1 (4%) of such 22 lesions appearing radiographically to be without recurrence (Fig. 1). These 21 lesions with negative biopsies were followed-up with quarterly triphasic CT scans for a mean of 15 months (median 12, range 1–60 months) and all, but 1 lesion, have remained

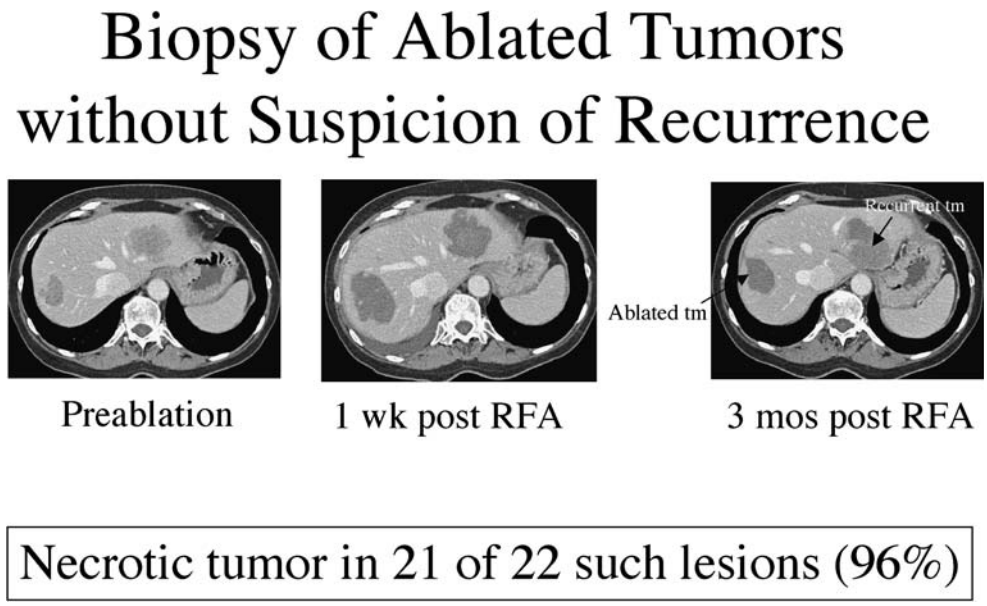

Figure 1 CT appearance of a stable and a locally recurrent lesion in the right and left lobe of the liver, respectively. Stable lesions on CT scans appear as punctate, well-circumscribed ablation zones that decrease in size in follow-up (*right lobe lesion*) (**a** and **b**). Locally recurrent lesions frequently appear as a new enhancing area developing at the periphery of the original ablation zone (*left lobe lesion*) (**c**). In this series, intraoperative biopsy confirmed necrotic tumor in 21 of 22 ablated foci without suspicion of recurrence on CT and laparoscopic ultrasound.

stable without any evidence of recurrence. One lesion showed evidence of recurrence at 3 months on CT scan.

Suspected recurrent tumor foci were enhancing on CT and produced a more finely stippled echo pattern on laparoscopic ultrasound. Biopsy confirmed viable tumor in 72 (86%) of 84 lesions suspected to have recurrent disease at the site of previous ablation by imaging (Figs. 1 and 2).

Biopsies were also taken from the center of previously ablated foci adjacent to an area of suspected recurrence. These core regions seemed to be stable on preoperative CT scans and laparoscopic ultrasound without any evidence of enhancement or a finely stippled sonomorphology, respectively. Seventeen (77%) of such 22 biopsies showed necrotic tissue at the core of the previous ablation zone while 5 (23%) of 22 demonstrated viable cancer (Fig. 3).

The biopsies were evaluated by frozen section and permanent section. The mean number of biopsies performed per lesion was 1.1 with 5 lesions requiring 2 biopsies and 3 lesions requiring 3 biopsies because of the pathologist needing more tissue at frozen section to make a decision.

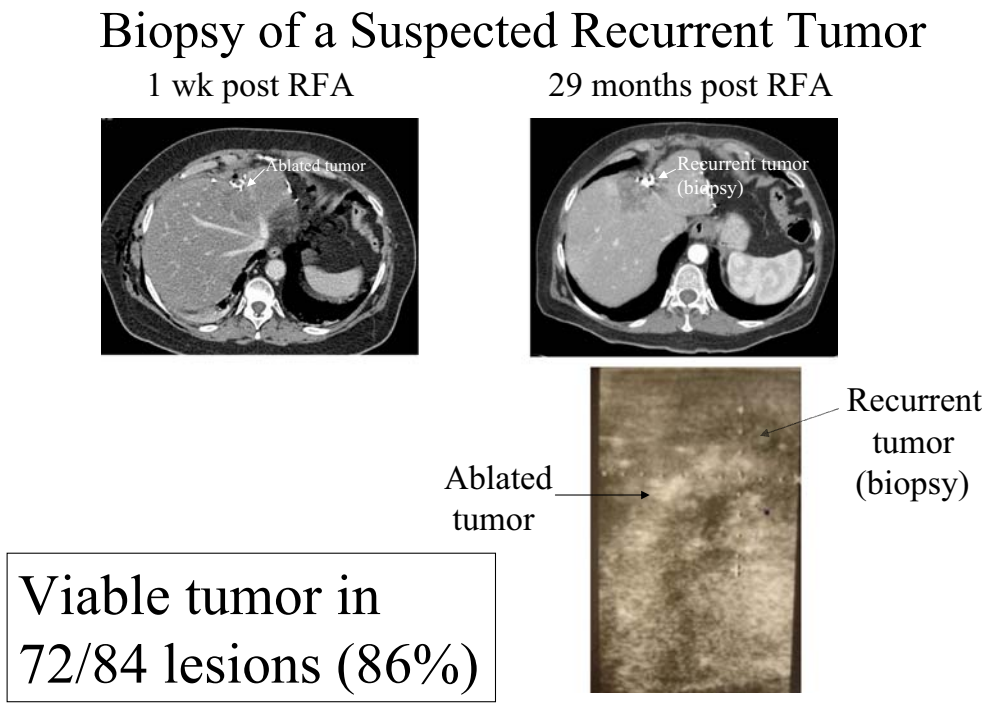

Figure 2 CT and ultrasound appearance of locally recurrent tumors after RFA (**a**). In this case, at 29 months, CT (**b**) showed an enhancing area at the periphery of the original ablation zone, which appeared as a more finely stippled echo pattern on laparoscopic ultrasound (**c**). Biopsy confirmed viable tumor in 72 (86%) of 84 such lesions suspected to have recurrent disease by imaging.

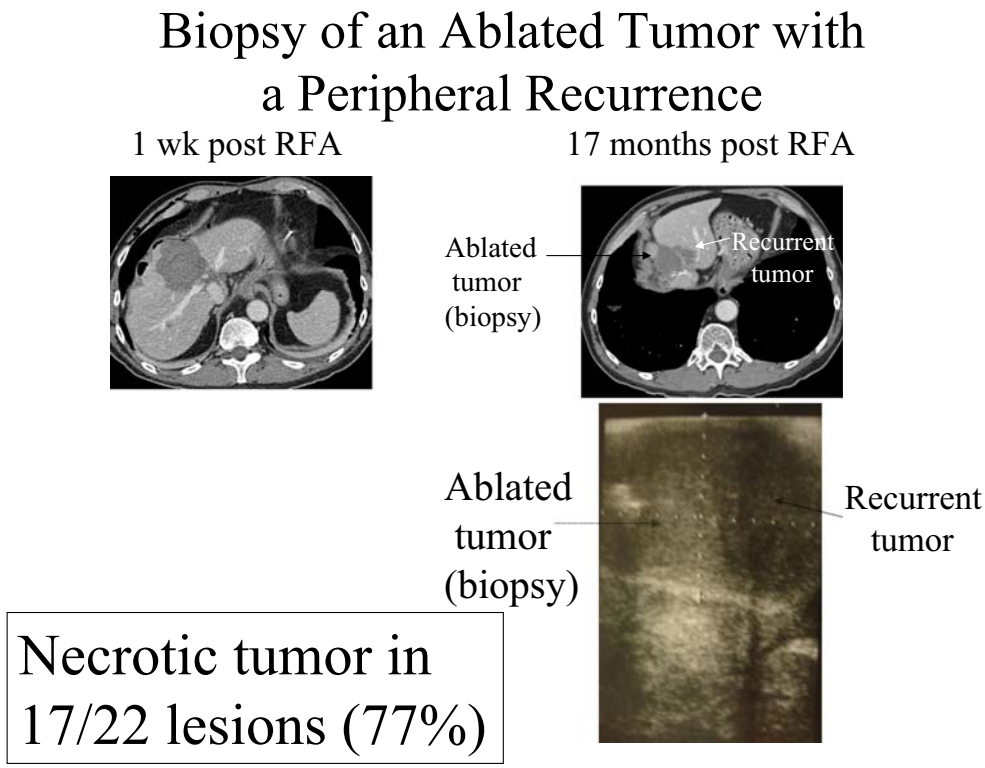

Figure 3 Local recurrence after laparoscopic RFA of a liver tumor (**a**). At 17 months, a recurrence is seen on the CT scan as a discrete enhancing focus lateral to the original ablation zone (**b**). This recurrent area has a distinct appearance on laparoscopic ultrasound as a more finely stippled image (**c**). Biopsies taken from the center of previously ablated foci adjacent to an area of suspected recurrence based on preoperative CT and intraoperative US showed viable tumor in 5 (23%) of 22 such lesions and necrotic tissue in 17 (77%) lesions.

Overall preoperative triphasic CT had a sensitivity of 97%, specificity of 63%, positive predictive value of 86%, and a negative predictive value of 91% when compared to biopsy results and follow-up data for assessing liver recurrence after a prior RFA procedure.

Discussion

Radiofrequency ablation is being increasingly applied for the treatment of unresectable liver tumors. As the patient numbers increase and the length of patient follow-up increases, the issue of recognizing local recurrence becomes very important. When a liver recurrence is detected in follow-up and the patient is taken to the operating room for a re-ablation procedure, the surgeon is faced with the dilemma of what to do about the previously ablated lesions if they seem to be stable on preoperative imaging studies. Another question is whether a local recurrence at the periphery of a previous ablation should be ablated alone or in combination with previous ablation zone if imaging studies suggest viable tumor only at the periphery.

There is very little data in the literature that correlates post ablation CT findings with a tissue biopsy result. Current data with cyrotherapy, chemoembolization, and ethanol injection all use post procedure CTs alone to monitor for recurrence[23,24]. This has led to the question of how well these surrogate radiologic studies correlate with recurrence. Although the indication to take the patient to the operating room for a repeat ablation procedure was made based on preoperative CT scans, for the purposes of this study, lesions that were determined to be recurrent or stable on imaging were also biopsied intraoperatively. Our data demonstrated a good correlation between preoperative triphasic liver CT scans and intraoperative ultrasound guided biopsy for evaluating the liver tumors for recurrence. Based on our data, triphasic liver CT scan had a positive predictive value of 86% and a negative predictive value of 91% when compared to intraoperative biopsy results and follow-up data. The high positive predictive and negative predictive values are indicative of a reliable system for surveillance for recurrence after RFA of liver tumors.

Our data showed that when a focal recurrence is suspected at the periphery of a previously ablated lesion that appears not to have recurrence at the core, there is a 23% chance that there is also viable tumor in the core lesion. This finding justifies an argument for a more aggressive treatment of the lesions during repeat ablation. This treatment would need to involve a re-ablation of the original core lesion and the new peripheral occurrence. The decision to ablate these 2 areas separately or en bloc should be made based on the size of these areas versus the ablation catheter. We like to obtain at least a centimeter of margin around the tumor with the ablation. If this is achieved with a single deployment of the catheter, that is acceptable,

otherwise, multiple overlapping ablations are necessary. Large tumors with irregular borders might also need multiple overlapping ablations.

The local recurrence rate after RFA of liver tumor ranges between 1.8% and 39%[18,25,26]. Factors that effect recurrence rate include the mode of RFA delivery, large size of a lesion, and proximity of a lesion to a major blood vessel. Percutaneous RFA has the greatest recurrence rate, as high as 39%[26]. Local recurrence after open RFA has been reported to range between 8% and 9.3%[27,28]. Laparoscopic RFA is in between these two modalities at 12%[18]. There is no established methodology in the literature about the concept of re-ablation of the necrotic core of a lesion along with a recurrence in the periphery.

Tumor surveillance is a significant issue after RFA of liver tumors. Triphasic CT has become the standard of care for assessing local recurrence after RFA. In the immediate post ablation CT scans, there is an initial rim of hyperenhancement around an ablated lesion that is reactive and because of the ablation of a rim of normal liver parenchyma around the tumor. This will fade after 1 month. After this time, any type of enhancement at the periphery of an ablated lesion, particularly a nodular enhancement, is suggestive of a local recurrence. According to the literature, triphasic CT is 44% sensitive for detecting recurrence at 2 months after RFA. After 4 months, sensitivity approaches 100%. MRI may be useful in surveillance for recurrence as well. There is no difference in sensitivity at 4 months, however, MRI has a sensitivity of 89% for detecting recurrence at 2 months[29].

There is also scant data in the literature about the use of laparoscopic ultrasound in re-ablation to assess for recurrent tumor. Our results show that laparoscopic ultrasound complements the information provided by preoperative CT scans when evaluating a lesion for tumor recurrence in the operating room. Recurrent and stable ablation defects have distinguishably different appearances on laparoscopic ultrasound, which can guide the core biopsy procedure. Based on our data, CT and laparoscopic ultrasound appearance of previously ablated foci showed good correlation with core biopsies. We therefore feel that CT scan is reliable in following-up RFA lesions without the need for routine biopsy. When a patient develops a recurrent liver disease on CT scan in follow-up after a RFA procedure, laparoscopic US can supplement the CT scan by allowing the surgeon to distinguish recurrent from stable lesions intraoperatively and will facilitate the repeat ablation procedure. This is especially useful in patients with multiple liver lesions (i.e., neuroendocrine liver metastases), where finding the exact lesion suggested by the CT scan could be problematic. In these cases, the different laparoscopic sonomorphology of a recurrent versus a stable lesion will help the surgeon with the conduct of the procedure.

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J Gastrointest Surg (2007) 11:1339–1350
DOI 10.1007/s11605-007-0212-1

Long-Term Effects of Extrinsic Denervation on VIP and Substance P Innervation in Circular Muscle of Rat Jejunum

Michael S. Kasparek · Javairiah Fatima · Corey W. Iqbal · Judith A. Duenes · Michael G. Sarr

Received: 17 May 2007 / Accepted: 10 June 2007 / Published online: 17 July 2007

Abstract
Intestinal denervation contributes to enteric motor dysfunction after small bowel transplantation (SBT). Our aim was to determine long-term effects of extrinsic denervation on function of nonadrenergic, noncholinergic innervation with substance P and vasoactive intestinal polypeptide (VIP). Contractile activity of jejunal circular muscle strips from six age-matched, naive control rats (NC) and eight rats 1 year after syngeneic SBT was studied in tissue chambers. Spontaneous contractile activity did not differ between groups. Exogenous VIP inhibited contractile activity dose-dependently to a comparable degree in both groups. The VIP antagonist ([D-*p*-Cl-Phe^{6},Leu^{17}]-VIP) and the nitric oxide synthase inhibitor L-N^{G}-nitro-arginine did not affect VIP-induced inhibition but increased contractile activity during electrical field stimulation (EFS) in both groups. Exogenous substance P increased contractile activity dose-dependently, greater in NC than SBT. The substance P antagonist ([D-Pro^{2},D-Trp^{7,9}]-substance P) inhibited effects of exogenous substance P and decreased the excitatory EFS response. Immunohistofluorescence showed tyrosine hydroxylase staining after SBT indicating sympathetic reinnervation. In jejunal circular muscle after chronic denervation, response to exogenous substance P, but not VIP, is decreased, whereas endogenous release of both neurotransmitters is preserved. Alterations in balance of excitatory and inhibitory pathways occur despite extrinsic reinnervation and might contribute to enteric motor dysfunction after SBT.

Keywords Extrinsic denervation · Motility · Small intestine · Substance P · Vasoactive intestinal polypeptide

Parts of this work were presented at the annual meeting of the Society for Surgery of the Alimentary Tract in Washington, DC, on May 21, 2007 and published in abstract form in Gastroenterology 2007;132: A890.

M. S. Kasparek · J. Fatima · C. W. Iqbal · J. A. Duenes · M. G. Sarr
Department of Surgery and Gastrointestinal Research Unit,
Mayo Clinic,
200 First Street SW,
Rochester, MN 55905, USA

M. S. Kasparek
Department of Surgery, Eberhard-Karls-University,
Hoppe-Seyler-Strasse 6,
72076 Tuebingen, Germany

M. G. Sarr (✉)
Gastroenterology Research Unit (AL 2-435),
Mayo Clinic College of Medicine,
200 First Street SW,
Rochester, MN 55905, USA
e-mail: sarr.michael@mayo.edu

Introduction

Over the last decade, small bowel transplantation (SBT) has become a clinical reality. The major initial problems of SBT were overcame partially with new immunosuppressive medications and technical improvements, such that currently, SBT is considered an alternative to chronic total parenteral nutrition, can be lifesaving, and has the potential to improve quality of life in patients with intestinal failure.[1–3] Many challenges in postoperative management of patients after SBT remain, however, and enteric motor dysfunction with high stomal output/diarrhea can be a major problem.[1,4,5] The fact that dogs develop a similar enteric dysfunction after autotransplantation of the small bowel, in which no immunosuppressants are necessary and immune phenomena are avoided, has lead us to hypothesize that the extrinsic denervation inherent to SBT contributes to enteric motor dysfunction after SBT6.

In a model of syngeneic SBT in rats, our group has studied previously the effects of SBT on cholinergic, adrenergic, and nitrergic innervation of longitudinal and

circular smooth muscle of the transplanted jejunum and ileum.[7–14] Alterations in other nonadrenergic, noncholinergic (NANC) neurotransmitters, such as the inhibitory neurotransmitter vasoactive intestinal polypeptide (VIP) and the excitatory neurotransmitter substance P, are less well studied after SBT. Both neuropeptides play an important role in enteric motor innervation, such as the peristaltic reflex,[15] and might therefore contribute to the enteric motor dysfunction after SBT. Previous studies from our laboratory showed a decrease in sensitivity to exogenous substance P and a diminished endogenous release of VIP 8 weeks after SBT in the *longitudinal* muscle of rat jejunum;[16] in addition, a decreased sensitivity to substance P persisted 1 year after SBT despite extrinsic reinnervation.[17]

Coordinated enteric motor function is the result of the interplay of the longitudinal and circular muscle layers. The innervation with VIP and substance P differs considerably between these two muscle layers;[18–22] however, we are aware of no studies examining the long-term changes in the innervation of *circular* muscle of rat jejunum with VIP and substance P after SBT.

Therefore, the aim of the current study was to determine long-term changes in functional enteric innervation of *circular* muscle of jejunum with the two NANC neuropeptides VIP and substance P 1 year after SBT. We studied the effect of exogenous application as well as the effect of endogenous release of these neurotransmitters triggered by electrical field stimulation (EFS). By using age-matched control rats, we controlled for any effect of aging on our findings. In addition, we evaluated sympathetic reinnervation by immunohistofluorescence. Our hypothesis was that SBT is associated with long-term changes in the functional innervation with both VIP and substance P, which contribute to the enteric motor dysfunction after SBT.

Materials and Methods

Preparation of Animals

The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Mayo Foundation. Procedures and animal care were performed according to the guidelines of the IACUC of the Mayo Foundation in accordance with the guidelines of the National Institutes of Health and the Public Health Service Policy of the Humane Use and Care of Laboratory Animals.

Experimental Groups

We used syngeneic, male Lewis rats (Harlan, Indianapolis, IN) in all experiments; syngeneic, inbred rats were especially important to avoid confounding effects of immune phenomena or need for pharmacologic immunosuppression. Anesthesia was induced by inhalation of isoflurane 2% (Abbott Laboratories, North Chicago, IL) and maintained by subsequent intraperitoneal sodium pentobarbital (30–50 mg/kg; AmproPharmacy, Arcadia, CA). To carry out complete extrinsic denervation of the jejunoileum, orthotopic small bowel *iso*transplantation (SBT) was performed in 3-month-old rats (n=8) using standard microvascular techniques as described previously.[8] In brief, the jejunoileum was removed with a short segment of aorta from the donor rat after flushing the intestinal lumen and the graft vasculature with chilled 154 mM NaCl. The donor aorta was anastomosed to recipient aorta (end-to-side) and the donor portal vein to recipient inferior vena cava (end-to-side). After resecting the recipient's jejunoileum, intestinal continuity was re-established by end-to-end jejunojejunostomy and ileoileostomy. Rats were allowed free access to water immediately postoperatively and to food 24 h postoperatively. Rats were maintained with free access to water immediately and food after 24 h and studied about 1 year after SBT.

To eliminate effects of aging on our results, age-matched, non-operated rats were used as naive controls (NC; n=6). These rats were fully 15 months of age because SBT was performed in 3-month-old rats in our study. At the time of study, the weight of rats [median (range)] did not differ between groups (NC, 500 g (475–550 g) vs SBT, 500 g [475–600 g]; p=NS).

Recording of Contractile Activity

A segment of jejunum about 10 cm distal to either the ligament of Treitz or the prior jejunojejunostomy (after SBT) was harvested and immersed in chilled, modified Krebs–Ringer's bicarbonate solution (concentrations in mmol/l: NaCl 116.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 23.8, calcium disodium edetate 0.26, and glucose 11.1) preoxygenated with 95% oxygen/5% carbon dioxide (Puritan-Bennett Corp., Lenexa, Canada). The jejunal segment was then opened along its mesenteric border. Eight, full-thickness muscle strips (8 mm long and 2 mm wide) were cut in the direction of the circular muscle layer and suspended vertically in 10-ml tissue chambers filled with modified Krebs–Ringer's bicarbonate solution, which was bubbled continuously with 95% oxygen/5% carbon dioxide and maintained at 37.5°C. One end of the muscle strip was attached to a fixed hook, and the other end was connected to a noncompliant force transducer (Kulite Semiconductors Products, Inc., Leonia, NJ) to measure isometric force. Contractile activity was monitored in real time on a chart recorder (Grass 7D polygraph; Grass Instrument Co., Quincy, MA) and saved digitally on a personal computer using dedicated software (MP-100A-CE and AcqKnowledge; Biopac Systems, Inc., Goleta, CA) to allow detailed computer analysis later.

Experimental Design

After a 90-min equilibration period with intervening washouts of the bath solution every 15 min, each strip was stretched incrementally at 10-min intervals to its optimal length (L_0) beyond which further stretching did not increase amplitude or frequency of contractions.[8] Atropine (10^{-7} M), phentolamine (10^{-5} M), and propranolol (5×10^{-6} M) were then added to the bath solution for establishment of NANC conditions. All experiments were performed subsequently at L_0 under NANC conditions. Strips without spontaneous contractile activity (<2%) were excluded from the study. At least two muscle strips per rat were studied under each experimental condition.

Two muscle strips per rat were exposed to stepwise, increasing concentrations of VIP in a cumulative fashion (3×10^{-9} to 10^{-6} M). After washout of the bath solution, the effect of the VIP antagonist ([D-*p*-Cl-Phe^6,Leu^{17}]-VIP; 10^{-6} M) on baseline contractile activity was studied before the cumulative dose–response to VIP was repeated in presence of the VIP antagonist. Thereafter, effects of the VIP antagonist, the NO synthase inhibitor L-N^G-nitro arginine (L-NNA; 10^{-4} M), and the combination of VIP antagonist and L-NNA on the response to exogenous VIP (10^{-7} M) were also studied.

Cumulative concentrations of substance P (3×10^{-9} to 10^{-6} M) were evaluated in two other strips per rat. After evaluating the effect of the substance P antagonist ([D-Pro^2, D-Trp^{7,9}]-substance P; 10^{-5} M) on baseline contractile activity, the effect of the substance P antagonist on the cumulative dose–response to exogenous substance P was studied. Afterwards, the effect of VIP (10^{-7} M) was studied with and without the VIP antagonist (10^{-6} M) and L-NNA (10^{-4} M) after each muscle strip was first precontracted with substance P (10^{-7} M) for 90 s.

Four strips from each rat were exposed to EFS with a constant voltage (20 V), pulse width (4 ms), and duration of stimulation (10 s) at 3, 6, and 20 Hz. Between each EFS, 10 min were allowed for recovery of spontaneous contractile activity before the next EFS was applied. The bath solution was exchanged after each series of stimulations. Two of the four strips were exposed to EFS with and without the VIP antagonist and L-NNA alone and in combination. The other two strips were studied in the presence and absence of the substance P antagonist. At the end of each experiment, muscle strips were blotted on filter paper and weighed to normalize motility data per milligram tissue weight.

Immunohistofluorescence Microscopy

Immunofluorescence microscopy for tyrosine hydroxylase (TH), an enzyme which is found only in postganglionic, extrinsic sympathetic neurons in the gut, was performed in both groups to look for sympathetic reinnervation after SBT; NC rats served as controls. Whole mounts of duodenum (serving as nondenervated control tissue) and jejunum (each 1×1 cm) were prepared by removing mucosa, pinning tissue onto a piece of Sylgard (Dow Corning Corporate, Midland, MI), and fixing tissue in 4% paraformaldehyde overnight. The tissue was rinsed with 0.1 M phosphate-buffered saline (PBS) and incubated for 1 h in 10% normal donkey serum (NDS) containing PBS and 0.3% Triton X-100 (blocking solution). The tissue was then incubated for 48 h at 4°C in the primary antibody solution containing 5% NDS and the affinity-purified polyclonal antibody to sheep anti-TH (dilution 1:400; both antibodies from Chemicon International Inc., Temecula, CA); the tissue was washed with 0.1 M PBS and incubated for 36 h at 4°C in the secondary antibody solution containing 2.5% NDS and Cy3-conjugated rabbit antisheep antibody (1:200; Chemicon). The tissue was washed again with 0.1 M PBS, and confocal microscopy was performed using a laser scanning microscope (Carl Zeiss LSM 5 Pascal, Version 3.2, Heidelberg, Germany) with digital pictures taken at appropriate levels.

Data Analysis

Baseline contractile activity was measured as area under the contractile curve for 5 min (g×5 min/mg tissue) under NANC conditions for each strip at L_0. For cumulative dose–responses, the effect of stepwise increasing concentrations of VIP and substance P on contractile activity was measured for a 5-min interval after each increase in concentration of the peptide and was compared to a 5-min interval directly before the first dose was administered (baseline contractile activity). When the response to a single dose of VIP, substance P, or their antagonists was evaluated, the effect of the drug on contractile activity in a 5-min interval was compared to baseline contractile activity during an equally long interval immediately before the drug was given. When experiments were conducted with precontraction of muscle strips with substance P, the effect of VIP for the next 5-min interval was compared to the last 60 s of the 90-s precontraction interval directly before VIP was administered. The drug responses are presented as "percent change from baseline contractile activity" (defined as 0%), which represents the contractile activity 5 min or 60 s before drug administration. Positive values represent increases, and negative values represent decreases in contractile activity.

For the EFS experiments, we determined the response to EFS only during the 10-s period of stimulation and did not evaluate the so-called "off contraction" immediately after termination of EFS. In control rat jejunum, EFS at low

frequencies (1–7 Hz) is associated with a dominant, net inhibitory effect, whereas higher frequencies (>10 Hz) cause a net excitatory response.[7,11] In a previous study in longitudinal muscle of rat jejunum, EFS at low frequencies inhibited typically contractile activity during the first 4–6 s of EFS after which some contractile activity recurred.[16] Because of this pattern, we evaluated the effect of EFS separately not only for the entire 10 s of EFS but also for the first 4 s and the last 6 s of EFS. Contractile activity measured as area under the contractile curve was expressed as the percent of baseline contractile activity for an equally long interval measured during the 40 s immediately before EFS. Additionally, the duration of complete inhibition of contractile activity during EFS was analyzed visually for each stimulation and is given in seconds of inhibition. The response to EFS at 20 Hz was evaluated accordingly.

Data are summarized as mean±SEM. Analysis of variance (ANOVA) was used to determine the effect of different drugs or EFS on each response variable (contractile activity or duration of inhibition during EFS). Repeated measures ANOVA was used for repeated measurements within the same rat and were treated as repeated factors (e.g., for dose responses to VIP or substance P or for EFS responses under different conditions). The two groups of rats were independent factors. When the overall main effect was statistically significant when analyzed by ANOVA, post-hoc pairwise comparisons were performed using paired Student's *t* tests (for repeated factors) or two-sample *t* tests (for independent group factors). Additional *t* tests were used for single comparisons (e.g. vs baseline contractile activity). A Bonferroni correction was applied when evaluating the statistical significance of multiple *t* tests. ANOVA on ranks was used when data were not normally distributed.

Drugs

Atropine sulfate, phentolamine hydrochloride, DL-propranolol hydrochloride, L-N^G-nitro arginine, substance P, [D-Pro^2,D-$Trp^{7,9}$]-substance P, VIP, and [D-*p*-Cl-Phe^6,Leu^{17}]-VIP were purchased from Sigma-Aldrich, St. Louis, MO, and Triton X-100 from Fisher Scientific, Fair Lawn, NJ.

Results

Spontaneous Contractile Activity

After reaching L_0, the area under the contractile curve was measured during a 5-min interval under NANC conditions and normalized by the wet tissue weight. Spontaneous contractile activity did not differ between NC and SBT (7.5±1.3 vs 6.7±1.0 g×5 min/mg tissue, respectively; p=NS).

Response to Exogenous VIP

VIP inhibited spontaneous contractile activity dose-dependently in both groups (Fig. 1a). Inhibition of contractile activity (% change from baseline) by VIP was not different between NC and SBT. The VIP antagonist (10^{-6} M) had no apparent effect on spontaneous contractile activity in either group (NC, −3±1%; SBT, 6±1%; p=NS) and did not prevent the VIP-induced inhibition of contractile activity (Figs. 1b and 2). Neither the VIP antagonist (10^{-6} M), L-NNA (10^{-4} M), or the combination of VIP antagonist and L-NNA prevented the inhibition caused by 10^{-7} M VIP (Fig. 2).

Precontraction of muscle strips with a nonmaximal dose of substance P (10^{-7} M) did not increase the inhibitory effect of VIP (10^{-7} M; Table 1). Although the inhibitory effect of VIP (10^{-7} M) on spontaneous contractile activity was not different between groups, after precontraction, the inhibitory effect of VIP (10^{-7} M) was much more pronounced in NC compared to SBT. The inhibitory effect of VIP (10^{-7} M) on substance P-stimulated contractile activity was unaffected by the VIP antagonist alone and in combination with L-NNA.

Response to Exogenous Substance P

Substance P increased contractile activity dose-dependently in both groups (Fig. 3a), but the response to substance P was greater in NC compared to SBT (p<0.002; ANOVA). The substance P antagonist (10^{-5} M) had a mild procontractile effect and increased spontaneous contractile activity to a similar extent in both groups [NC, 17±10%; SBT, 19±9%; both p<0.05 (paired *t* test)] and prevented the substance P effect completely in SBT and partially in NC [both p<0.02 vs without substance P antagonist (ANOVA); Fig. 3b]. Although less-pronounced, a procontractile effect of exogenous substance P remained in the presence of the substance P antagonist but only in NC [p<0.002 (ANOVA)].

Response to EFS

In SBT, EFS at 3 Hz caused a net inhibition when measured over the entire 10 s of EFS, the first 4 s, and the last 6 s of stimulation (each p<0.05; Fig. 4a–c). The VIP antagonist had no effect on contractile activity during EFS at 3 Hz in either group. In contrast, in the NC group, L-NNA caused an increase in contractile activity when quantitated over the entire 10 s (Fig. 4a) and during the last 6 s of EFS compared to the control response (Fig. 4c); when the VIP antagonist and L-NNA were combined, the EFS response was further increased. In SBT, L-NNA reversed the inhibition caused by EFS both for the entire 10 s and the

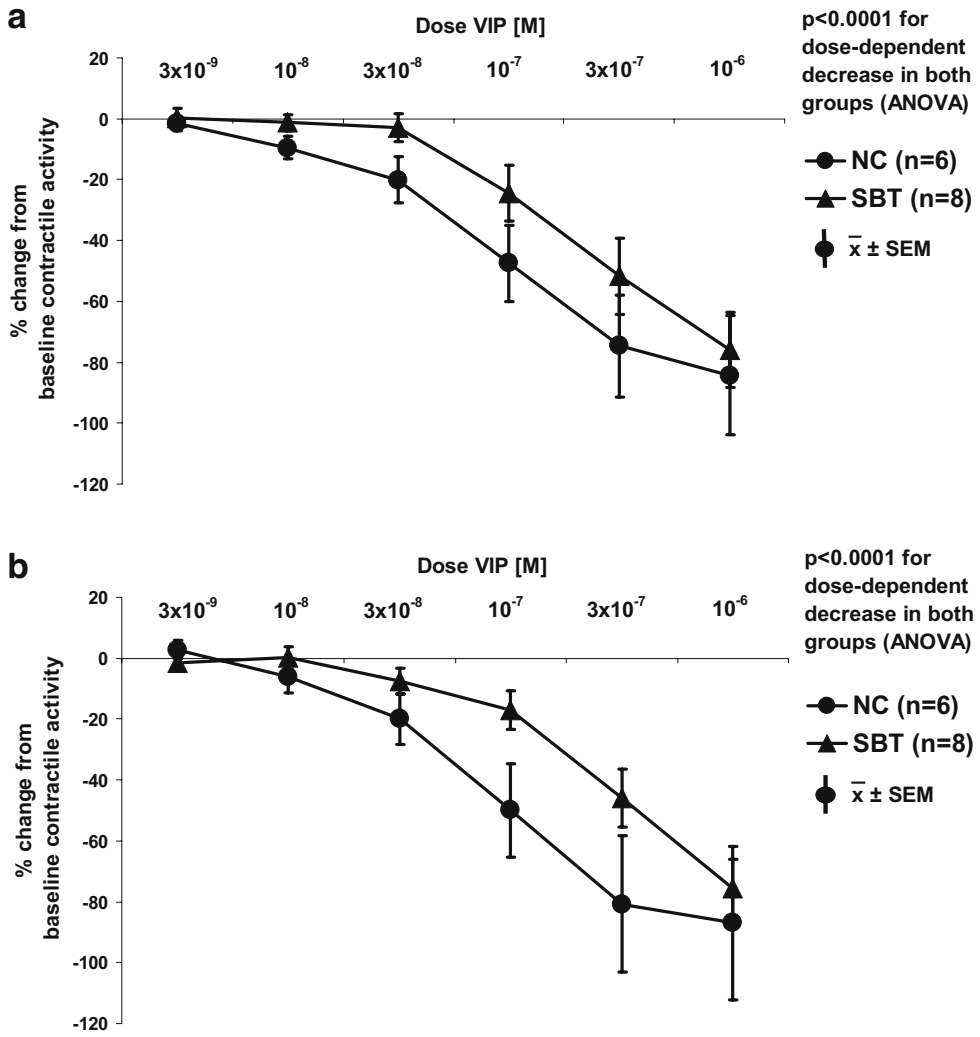

Figure 1 Response of spontaneous contractile activity to increasing concentrations of VIP (3×10^{-9} to 10^{-6} M) without (**a**) and with VIP antagonist (10^{-6} M; **b**).

last 6 s of EFS, but unlike in NC, L-NNA did not convert the EFS to a net excitatory contractile response over baseline; the combination of L-NNA and VIP antagonist had no additive response as it did in NC. The responses during the first 4 s of EFS also differed between groups. EFS caused a net inhibition in both groups. Addition of the VIP antagonist, L-NNA, and their combination had no effect on this net inhibition of EFS in NC (Fig. 4b). In contrast, in SBT, the combination of L-NNA and VIP antagonist increased net contractile activity over that of the response with the VIP antagonist alone [−65±13% vs 4±25%; $p<0.05$ (ANOVA)], although either alone had no effect.

Under control conditions in both groups, EFS at 6 Hz did not alter contractile activity during any interval (Fig. 5a–c). The VIP antagonist and L-NNA alone had no effect on contractile activity during EFS at 6 Hz; however, their combination caused an increase in contractile activity

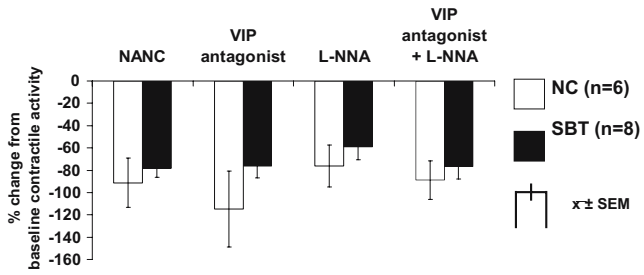

Figure 2 Response of spontaneous contractile activity to VIP (10^{-7} M) without and with VIP antagonist (10^{-6} M), L-NNA (10^{-4} M), and the combination of VIP antagonist and L-NNA.

Table 1 Effect of VIP Antagonist (10^{-6} M) and L-NNA (10^{-4} M) on the Response to VIP (10^{-7} M) After Precontraction with Substance P (10^{-7} M)

Group	Contractile Activity (% Change from Baseline)		
	Control Response	VIP Antagonist	VIP Antagonist + L-NNA
NC	−50±7*	−60±3[a]	−60±6[a]
SBT	−24±8	−29±8	−17±9

Data are $\bar{x}$ ±SEM; $n\geq6$ rats

[a] Differs from response in SBT under the same condition (ANOVA)

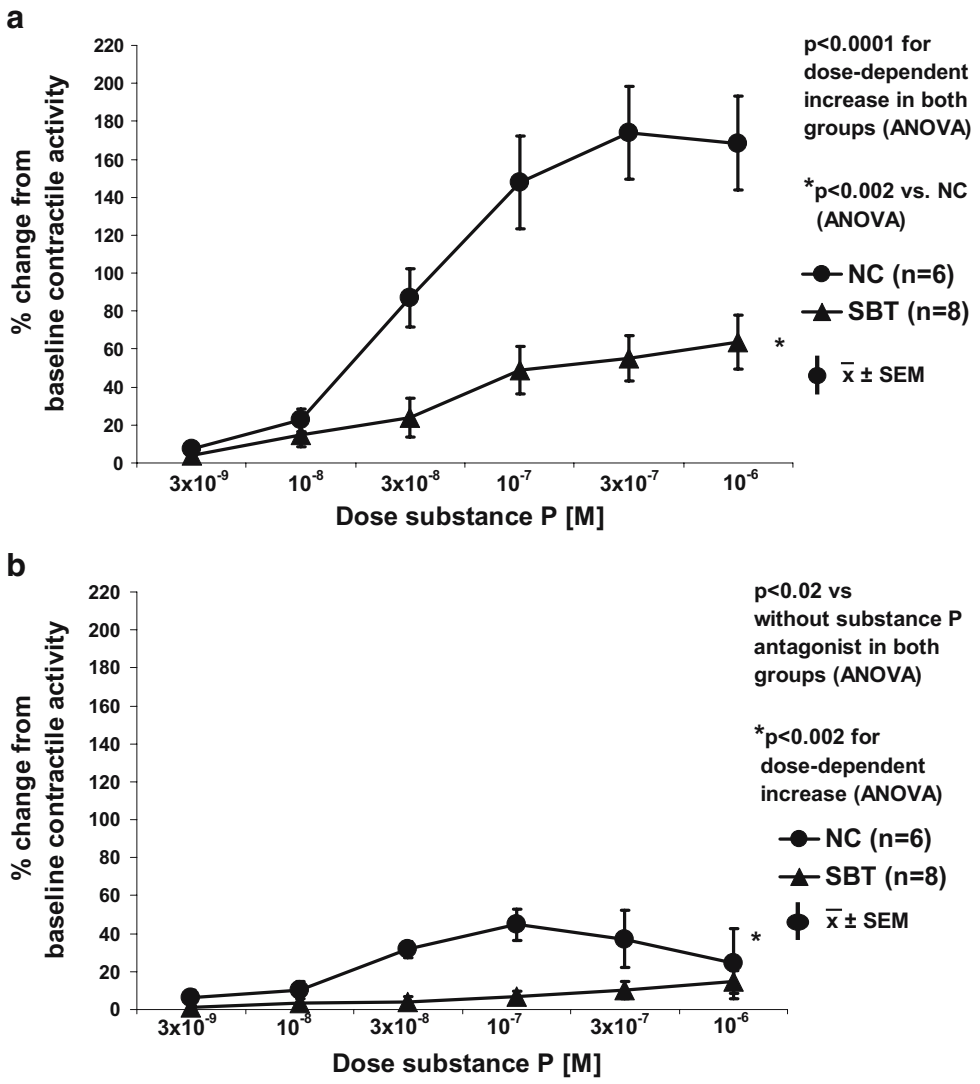

Figure 3 Response of spontaneous contractile activity to increasing concentrations of substance P (3×10^{-9} to 10^{-6} M) without (**a**) and with substance P antagonist (10^{-5} M; **b**).

compared to all other conditions converting the response to net excitation when quantitated over the entire 10 s and the last 6 s of EFS (each $p<0.05$ vs baseline contractile activity). The VIP antagonist, L-NNA, and the combination of both drugs had no effect on the EFS response during the first 4 s nor on the duration of inhibition (data not shown).

EFS at 20 Hz caused a net excitation in both groups when quantitated over the entire 10 s, the first 4 s, and the last 6 s of EFS (all $p<0.002$; Fig. 6a–c). This excitatory EFS response was reversed by the substance P antagonist when quantitated over the entire 10 s and over the last 6 s of EFS in both groups. In contrast, the substance P antagonist also prevented EFS-induced excitation during the first 4 s of EFS but only in NC [164±32% vs 33±22%; $p<0.05$ (ANOVA)].

Immunohistofluorescence Microscopy

Immunohistofluorescence staining for TH was performed in the duodenum and jejunum in both groups (Fig. 7). Staining for TH-positive neurons was present in duodenum and jejunum of NC and SBT with comparable immunopositivity, although we have shown previously that TH staining is absent in the jejunoileum early (≤2 months) after SBT.[16] No subjective differences were evident in the jejunum between NC and SBT.

Discussion

The aim of our study was to determine the long-term effects of extrinsic denervation (1 year after SBT) on intrinsic neural control of contractile activity mediated by both inhibitory (VIP) and excitatory (substance P) NANC neurotransmitters in the circular smooth muscle of rat jejunum. Our hypothesis was that the extrinsic denervation obligated by SBT leads to long-term changes in enteric neural modulation of contractile activity mediated by these specific neurotransmitters. By using age-matched, naive

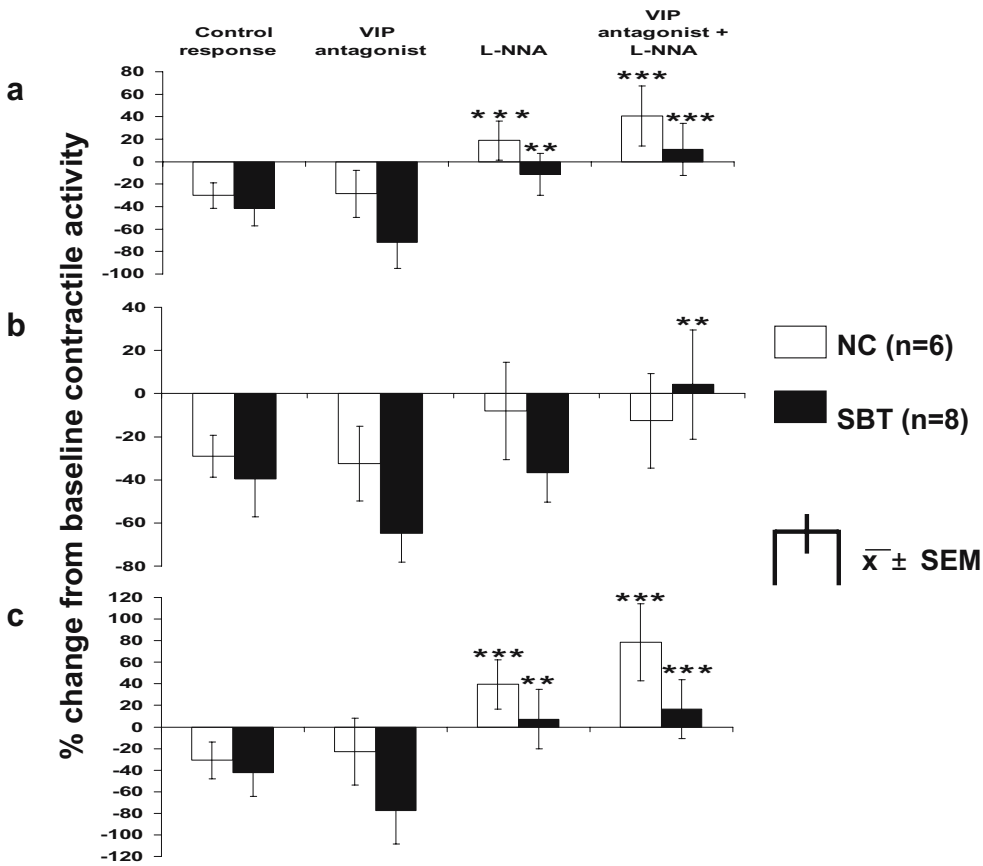

Figure 4 Effect of VIP antagonist (10^{-6} M) and L-NNA (10^{-4} M) on the EFS response at 3 Hz for the entire 10-s interval (**a**), the first 4 s (**b**), and the last 6 s (**c**) of EFS. *$p<0.05$ (ANOVA) compared to control response. **$p<0.05$ (ANOVA) compared to response with VIP antagonist alone.

control rats, we avoided potentially confounding changes secondary to aging. We evaluated responses to exogenously applied neuropeptides and to EFS to explore indirectly any changes of receptor expression, signaling pathways, or endogenous release of VIP and substance P by EFS which might contribute to enteric motor dysfunction after SBT.[1,4–6] Exogenous VIP evoked a dose-dependent, profound inhibition of contractile activity, which was not affected by SBT and was not blocked by either the VIP antagonist, inhibition of NO production, or the combination of both. In contrast, the procontractile effect of exogenous substance P was diminished in animals 1 year after SBT and was blocked efficiently by the substance P antagonist in both groups. Endogenous release of VIP, NO, and substance P during EFS was preserved 1 year after SBT.

We studied VIP to explore one aspect of NANC-mediated inhibition of smooth muscle contractile activity. The role of VIP (and NO) in the control of enteric contractile activity is complex, incompletely understood, and varies sometimes markedly between species and across anatomic regions.[17] In jejunal longitudinal muscle of control rats, exogenous VIP has no apparent effect on spontaneous contractile activity;[16,21,22] in contrast, others have shown that VIP has an inhibitory effect on circular muscle of rat jejunum,[19,20] which is consistent with our current study showing a profound, dose-dependent inhibition of contractile activity by exogenous VIP 1 year after SBT. This result, however, is in conflict with a study from Tomita et al.,[19] which suggested that the sensitivity to exogenous VIP was decreased 18 week after SBT in circular muscle of rat jejunum. In addition to methodologic differences, Tomita et al. studied changes in VIP and substance P-mediated neurotransmission not only earlier after SBT than in our study but also in younger rats; indeed, several studies and work of our own (unpublished observations) have suggested functional effects of aging on neurotransmission in the enteric nervous system. Although the density of NO, VIP, and substance P-containing neurons is unaffected visually by aging,[23,24] alterations in their release, receptor expression, and activity with age cannot be excluded. For instance, an age-related decrease in nitrergic NANC inhibition was demonstrated in jejunal longitudinal muscle and circular muscle of the rectum in the rat.[25] It is for these reasons that we used age-matched control rats to avoid any potentially confounding effects of aging.

In contrast to our previous study in longitudinal muscle of rat jejunum,[16] precontraction of muscle strips with substance P did not increase the relative inhibitory effect of VIP in circular muscle, which might be caused by the more pronounced inhibitory effect of VIP in circular muscle. We had hypothesized that the increased inhibitory effect of VIP after precontraction found in longitudinal

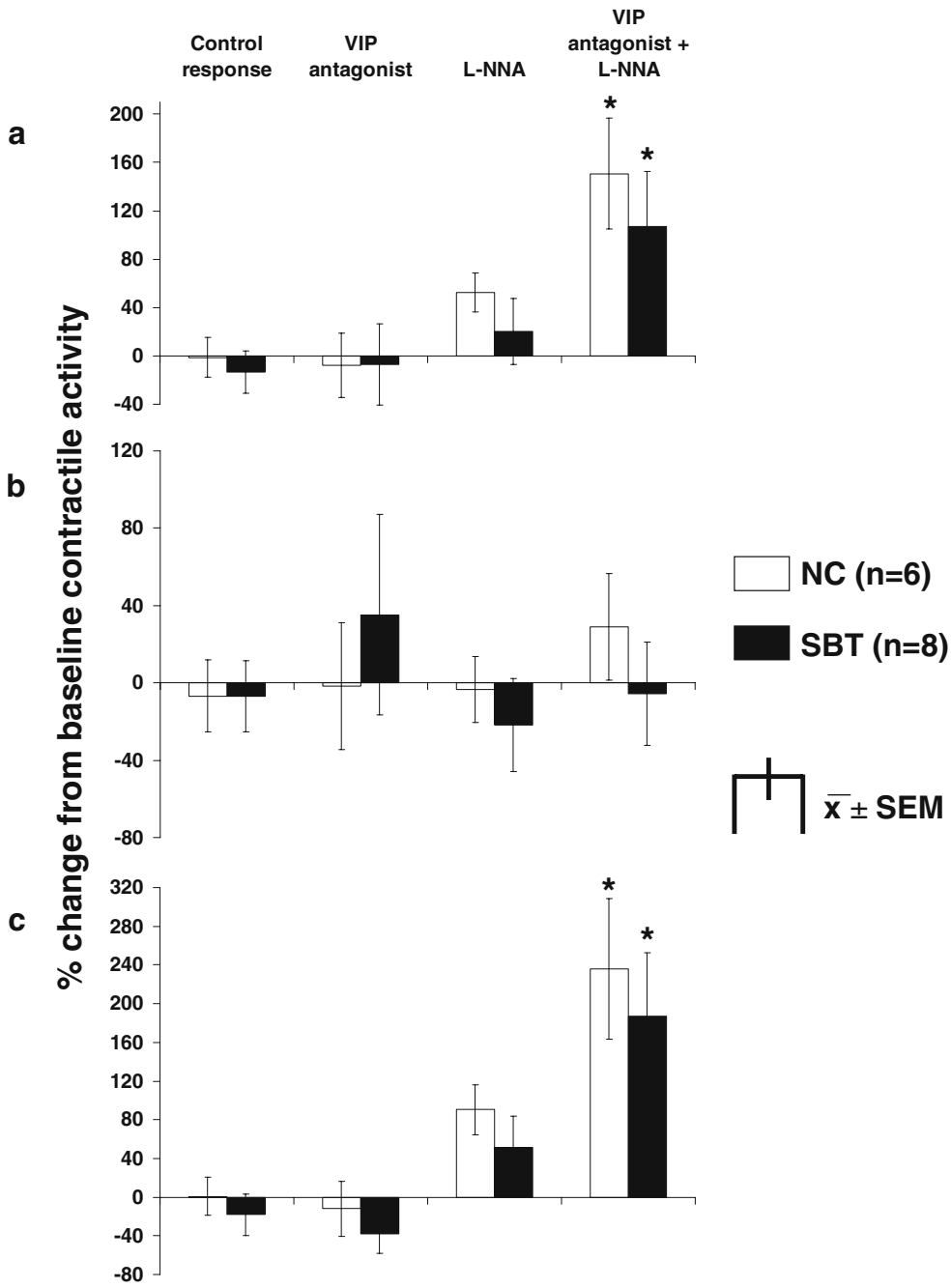

Figure 5 Effect of VIP antagonist (10^{-6} M) and L-NNA (10^{-4} M) on the EFS response at 6 Hz for the entire 10-s interval (**a**), the fist 4 s (**b**), and the last 6 s (**c**) of EFS. *$p<0.05$ (ANOVA) compared to all other conditions (control response, VIP antagonist alone, L-NNA alone) and baseline contractile activity.

muscle might reflect involvement of VIP in the descending inhibitory arm of stimulated contractile activity (e.g., during the peristaltic reflex[15]); the lack of a more pronounced effect of VIP after precontraction in circular muscle might imply a different functional role of VIP in the local reflex coordination of contractile function in the circular muscle. Interestingly, after precontraction, the inhibitory effect of VIP was greater in NC than in SBT, which is due likely to the more pronounced procontractile effect of substance P in NC (see below).

VIP and NO are believed to be the dominant mediators of NANC inhibition of enteric contractile activity; they are often released together, and they appear to interact.[18] VIP induces its receptor-mediated inhibitory effect on the smooth muscle cell by activating adenylate cyclase and stimulating cAMP production through VPAC1 and VPAC2 receptors, both of which are blocked by the competitive VIP antagonist we used.[26,27] However, VIP also induces production and release of NO from the presynaptic neuron; in addition, VIP has been reported to induce NO production in the smooth muscle cell by activation of the natriuretic peptide clearance receptor.[28] In contrast to our previous work in longitudinal muscle in younger rats,[16] the prominent VIP-induced inhibition was not affected by the VIP

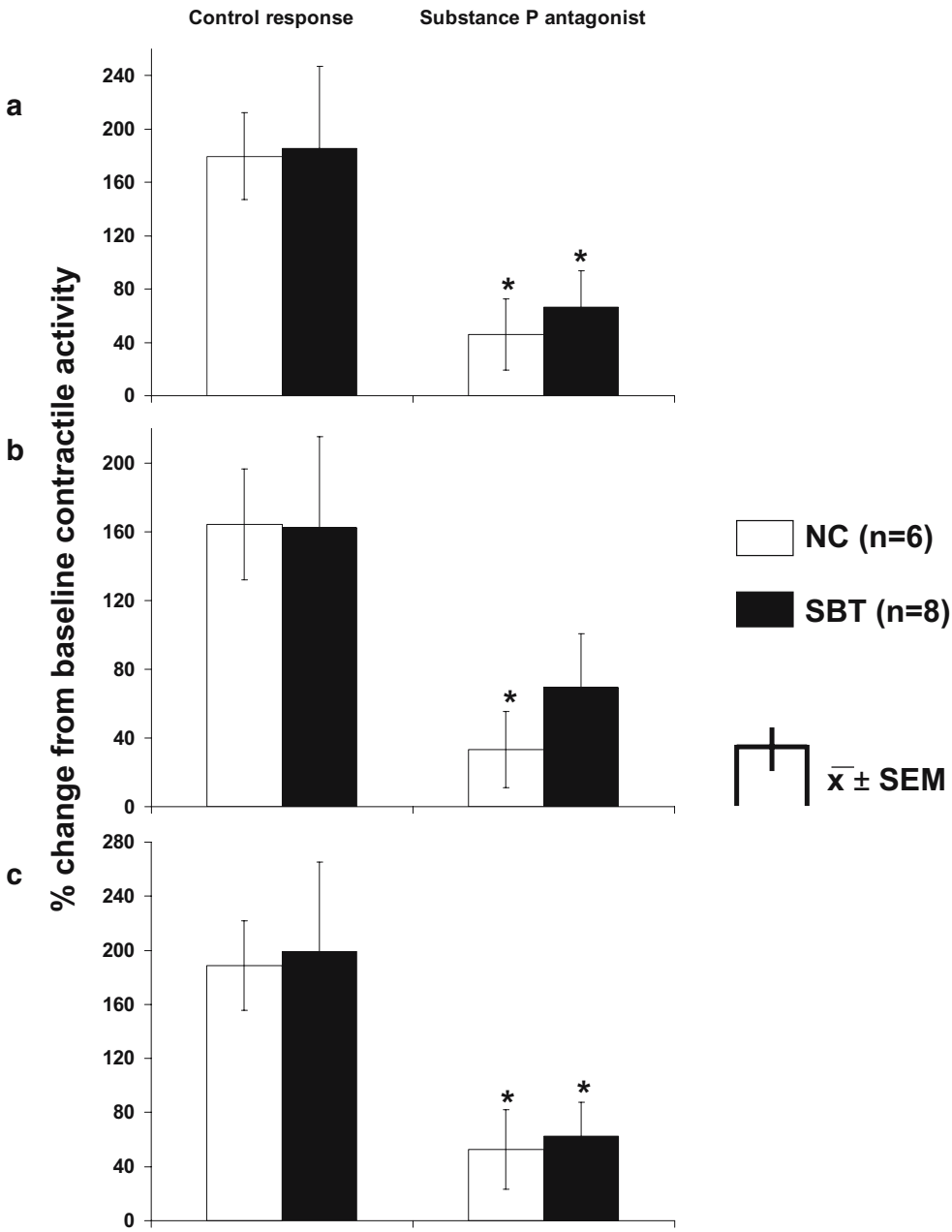

Figure 6 Effect of substance P antagonist (10^{-5} M) on the excitatory EFS response at 20 Hz for the entire 10-s interval (**a**) as well as the first 4 s (**b**) and the last 6 s (**c**) of EFS. *$p<0.05$ (ANOVA) compared to control response.

antagonist nor by L-NNA in the current study. Whether the lack of effect of blocking VIP receptors or NO production is related to the inability of these antagonists to diffuse into the circular muscle, changes in receptor affinity for these antagonists, or alterations in intracellular signaling pathways, or to other differences in regulation of contractile activity is unknown.[7,14,20,29] The former two possibilities are unlikely because the antagonists had notable effects on the response to EFS.

We used EFS at low frequencies (<6 Hz) to induce inhibitory effects on contractile activity via endogenous release of inhibitory neurotransmitters, such as NO and presumably VIP.[14,20] L-NNA increased the net contractile response to low-frequency EFS (3 Hz); when the dominant inhibitory effect of endogenously released NO was blocked by L-NNA, the more subtle inhibitory effect of endogenously released VIP was blocked by the VIP antagonist, causing a further increase in contractile activity. Because L-NNA had no effect on the inhibitory response to exogenous VIP, the inhibitory effect of exogenous VIP is not mediated by NO either via a presynaptic neuron or in the circular muscle. This observation is at variance with our previous study in which NO did not mediate the EFS-induced inhibition in rat jejunal circular muscle in 3- to 5-month-old rats.[14] These differences might be caused by age-related changes in enteric neural control. The ability of the VIP

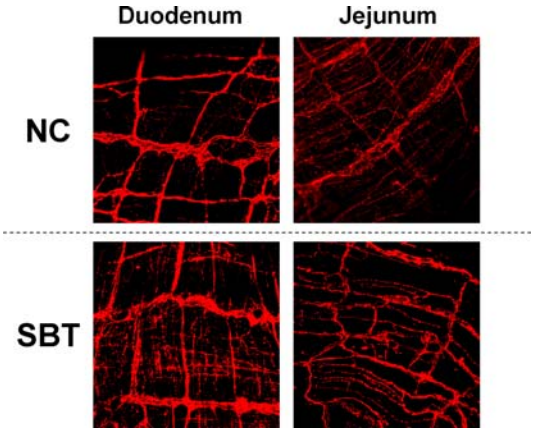

Figure 7 Immunofluorescence for tyrosine hydroxylase (TH) in the duodenum and jejunum of NC and SBT.

antagonist to reverse the inhibitory response to endogenously released VIP despite the lack of effect on exogenous VIP is difficult to explain, but might be caused by differences in the interaction between VIP and its antagonist when VIP is released locally compared to the effect of exogenously applied VIP. Our study cannot shed further insight into this problem.

The effect of L-NNA and the VIP antagonist on contractile activity during EFS at 3 and 6 Hz was comparable between groups, demonstrating that release of NO and VIP is maintained 1 year after SBT. This finding is consistent with several morphologic studies in rats, piglets, dogs, and human tissue that showed preservation of immunoreactivity for NO and VIP after SBT.[30–37] In contrast, in the longitudinal muscle of rat jejunum 1 year after SBT, endogenous release of VIP during EFS was increased,[17] again highlighting the functional differences between the different muscle layers.

We also studied neurally mediated mechanisms of substance P-mediated NANC procontractile activity. Similar to our findings in rat jejunal longitudinal muscle 8 week[16] and 1 year after SBT,[17] our study showed a decrease in the sensitivity to substance P in the circular muscle 1 year after SBT. Our findings do not agree with those of Tomita et al.[19] who described an increase in sensitivity to exogenous substance P 18 week after SBT; although there are methodologic differences in our studies and the groups of rats were studied at different time points after SBT, we cannot explain these differences. The substance P antagonist itself evoked a slight but definite excitatory effect, a property of the antagonist known from other studies.[16,38] Unlike the inability of the VIP antagonist to block the effect of exogenous VIP, the substance P antagonist inhibited the effect of exogenous substance P in both groups, but the effect was less complete in NC, possibly because of the more pronounced effect of substance P in this group or the recruitment of other procontractile mechanisms by the effect of substance P. In addition, the substance P antagonist decreased the net excitatory response to EFS at 20 Hz to a similar extent in both groups. Maintenance of endogenous release of substance P after SBT is in accordance with immunohistologic studies after SBT.[30–36] Although substance P appears to play an important role as a dominant excitatory NANC neurotransmitter in rat circular muscle in the jejunum, other procontractile NANC transmitters, such as neurokinine A, enkephaline, galanin,[39] and others are released potentially as well with EFS at 20 Hz and might explain the persistent net excitatory response during EFS after blocking substance P. Indeed, the differences in the early (first 4 s) and later (last 6 s) EFS response and the EFS response over the entire 10 s of the stimulation are consistent with findings in rat longitudinal muscle and implies involvement of other important NANC inhibitory and excitatory neurotransmitters especially during the early EFS response [e.g., adenosine triphosphate (ATP), carbon monoxide, gamma aminobutyearic acid, hydrogen sulfide, neuropeptide Y, or pituitary adenylate cyclase activating peptide].[39] Our experiments, however, do not allow us to further differentiate their participation.

Finally, we were also interested in intestinal adaptation to extrinsic denervation as well as reinnervation of the jejunal muscularis 1 year after SBT. Previous studies in the rat have shown that sympathetic reinnervation occurs 3–4 week after SBT, initially along the mesenteric vessels,[40] after 15 week on the mesenteric side of the transplanted small bowel, and by 27 week after SBT to the antimesenteric side.[34,41] Our study supports and extends these findings by exploring the functional effects of reinnervation with an ostensibly normal pattern of TH immunoreactivity on subjective appearance. The changes we showed in substance P-mediated neurotransmission occurred despite the extrinsic (sympathetic) reinnervation, which suggests that extrinsic reinnervation after SBT does not necessarily reverse denervation-related alterations in enteric neural control.

Conclusion

We have demonstrated a decrease in sensitivity to exogenous substance P 1 year after SBT while the endogenous release of substance P during EFS is maintained. In contrast, the sensitivity for exogenous VIP and its endogenous release is not altered 1 year after SBT. These functional changes in enteric neural control occur despite sympathetic reinnervation. Because VIP and substance P are important NANC neurotransmitters involved in enteric reflexes and enteric neural control of contractile activity, alterations in the balance of inhibitory and excitatory

neurotransmission within the enteric nervous system might contribute to the enteric motor dysfunction after SBT.

Acknowledgment We want to thank Deborah I. Frank for her expert assistance in the preparation of this manuscript. This study was supported by grant DK 39337 from the National Institutes of Health, United States Public Health Services (M.G.S.) and grant KA 2329/1-1 from the Deutsche Forschungsgemeinschaft, Germany (M.S.K.).

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J Gastrointest Surg (2007) 11:1351–1354
DOI 10.1007/s11605-007-0217-9

Gangliocytic Paraganglioma: Case Report and Review of the Literature

Agnes Witkiewicz · Avi Galler · Charles J. Yeo · Samuel D. Gross

Received: 15 May 2007 / Accepted: 12 June 2007 / Published online: 25 July 2007

Abstract Gangliocytic paraganglioma is a rare tumor, which occurs nearly exclusively in the second portion of the duodenum. Generally, this tumor has a benign clinical course, although rarely, it may recur or metastasize to regional lymph nodes. Only one case with distant metastasis has been reported. We present a case of duodenal gangliocytic paraganglioma treated first by local resection followed by pylorus-preserving pancreaticoduodenectomy. Examination of the first specimen revealed focal nuclear pleomorphism and mitotic activity, in addition to the presence of three characteristic histologic components: epithelioid, ganglion, and spindle cell. In the subsequent pancreaticoduodenectomy specimen, there was no residual tumor identified in the periampullary area, but metastatic gangliocytic paraganglioma was present in two of seven lymph nodes. This case report confirms the malignant potential of this tumor. We review the published literature on gangliocytic paragangliomas pursuing a malignant course. We conclude that surgical therapy of these neoplasms should not be limited to local resection, as disease recurrence, lymph node involvement, and rarely distant metastasis may occur.

Keywords Gangliocytic paraganglioma · Epithelioid cells · Ganglion cells · Spindle cell

Introduction

Gangliocytic paraganglioma (GP) is a rare tumor, which occurs nearly exclusively in the second portion of the duodenum[1,2]. The lesion was first described by Dahl et al.[3] in 1957 and further characterized as a benign nonchromaffin paraganglioma by Taylor and Helwig[4] in 1961. Kepes and Zacharias[5] coined the term "gangliocytic paraganglioma" in 1971, recognizing the features in common with both paraganglioma and ganglioneuroma. Generally, this tumor has a benign clinical course, although rarely, it may recur or metastasize to regional lymph nodes[6–9]. There has been one report of distant metastases[10]. We report a case of a 38-year-old female with a periampullary gangliocytic paraganglioma with lymph node metastases and review the published literature on GPs pursuing a malignant course.

Case Presentation

A 38-year-old woman with thalassemia trait presented to a local hospital with right upper quadrant pain. A presumptive diagnosis of hiatal hernia with ulcers was made, and the patient was started on a proton pump inhibitor. Upon further workup, an upper endoscopy revealed a mass in the duodenum, near the ampulla of Vater. After several negative biopsies, she underwent endoscopic excision of the mass, which proved to be a gangliocytic paraganglioma extending to the margin. Due to the positive margin status, the patient was referred for surgical consultation and regional resection was recommended. Three months following endoscopic resection, the patient underwent pylorus-preserving pancreaticoduodenectomy with standard reconstruction. Fol-

A. Witkiewicz
Department of Pathology,
Thomas Jefferson University,
Philadelphia, PA, USA

A. Galler · C. J. Yeo · S. D. Gross
Department of Surgery,
Thomas Jefferson University,
Philadelphia, PA, USA

C. J. Yeo (✉)
Jefferson Medical College, Thomas Jefferson University,
1015 Walnut Street, Suite 620,
Philadelphia, PA 19107, USA
e-mail: charles.yeo@jefferson.edu

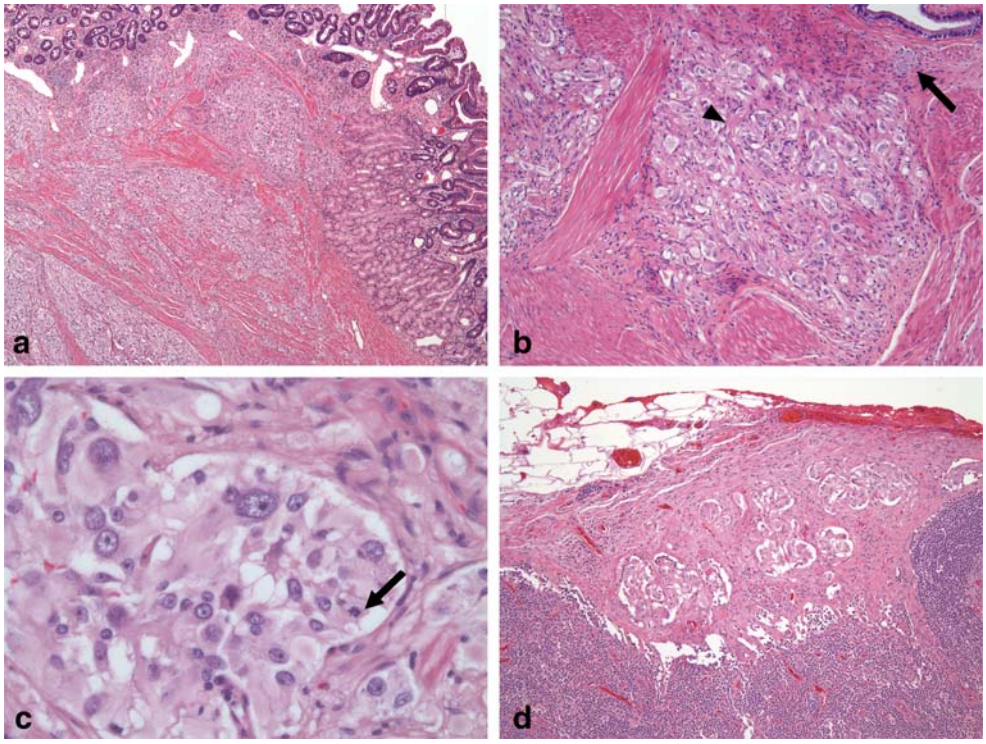

Figure 1 **a** Submucosal location of the tumor in the periampullary region (H&E, 10X). **b** The tumor consists of epithelioid cells forming nests, fascicles of spindle cells (*arrow*) and ganglion-like cells (*arrowhead*; H&E, 20X). **c** Nuclear pleomorphism and mitosis (*arrowhead*) are present (H&E, 40X) **d** Metastatic tumor in a lymph node composed of epithelioid and spindle cell (H&E, 20X).

lowing an uneventful recovery, she was discharged from the hospital on the eighth postoperative day.

Pathologic evaluation of the endoscopic ampullectomy specimen showed a 1.5-cm polypoid tumor in the submucosa with extension to the muscularis propria (Fig. 1a). The lesion was nonencapsulated and had an infiltrative margin. The tumor was composed of three morphologically distinct cell populations: epithelioid cells, spindle cells, and scattered ganglion cells (Fig. 1b). The epithelioid cells, arranged in nests and trabeculae, had granular eosinophilic cytoplasm and nuclei showing mild to moderate atypia (Fig. 1c). The spindle cells formed slender fascicles wrapping around nests of epithelioid cells. The ganglion cells had round nuclei with prominent nucleoli and abundant eosinophilic cytoplasm. The mitotic count was two per ten high-power fields, and necrosis was not present. In the subsequent pancreaticoduodenectomy specimen, there was no residual tumor identified in the periampullary area, but metastatic gangliocytic paraganglioma was present in two of seven lymph nodes (Fig. 1d). The metastatic foci show the presence of the three cellular components identified in the primary tumor. Immunohistochemical stains were performed on the metastatic tumor. The chromogranin stain showed positivity in the epithelioid cells, while synaptophysin

Table 1 Gangliocytic Paragangliomas with Lymph Node or Distant Metastasis

Author	Treatment	Tumor Site	Nodal Spread	Distant Spread	Follow-up
Burke et al.[1]	Whipple procedure	Duodenum	Yes	No	NED 91 mo
Buchler et al.[21]	Local resection	Ampulla of Vater	Yes	No	NED 20 mo
Korbi et al.[22]	Whipple procedure	Duodenum	Yes	No	Not known
Tomic et al.[23]	Whipple procedure	Head of pancreas	Yes	No	NED 19 mo
Bucher et al.[24]	Whipple procedure	Ampulla of Vater	Yes	No	NED 40 mo
Dookhan et al.[8]	Local resection	Duodenum	Yes	No	Not known
Inai et al.[7]	Local resection[a]	Ampulla of Vater	Yes	No	NED 30 mo
Hashimoto et al.[9]	Whipple procedure	Ampulla of Vater	Yes	No	NED 14 mo
Ljungberg et al.[25]	Local resection	Ampulla of Vater	Yes	No	Not known
Sundararajan[6]	Whipple procedure	Duodenum	Yes	No	NED 9 mo
Wong[26]	Whipple procedure	Ampulla of Vater	Yes	No	Not known
Henry et al.[10]	Whipple procedure	Not known	No	Liver, Bone	Not known

[a] Whipple procedure after recurrence

NED, indicates alive without evidence of disease; *Mo*, months.

highlighted epithelioid and ganglion cells. The spindle cells stained with S100 and neurofilament. Cytokeratin and glial fibrillary acidic protein were negative in all three cell populations.

Discussion

GP is usually seen in the periampullary region of duodenum, with rare cases reported in the jejunum, pylorus[1,2], esophagus[11], pancreas[10], and appendix[12]. Recently, three cases of pulmonary GPs have been reported[13–15]. The age at presentation ranges from 15 to 82 years (mean 54), and there is slight male predominance.

Clinically, GPs arising in the gastrointestinal tract present with bleeding, abdominal pain or obstruction; some cases are found incidentally at endoscopy or autopsy[2]. In the largest reported series of 51 GPs, abdominal pain was the most common presenting symptom and was usually attributed (like in our case) to ulcer disease[1]. The three cases of pulmonary GP reported to date presented with chest pain, pneumonia, and Cushing's syndrome, respectively[13–15]. The tumor is usually unrelated to other diseases although an association with von Recklinghausen disease has been reported[2,16].

GP has three characteristic histologic components: epithelioid, ganglion, and spindle cell. Recognition of this triad aids diagnosis on routine hematoxylin and eosin sections. The proportion of the three cell types varies in each tumor, but each component shows characteristic immunohistochemical staining similar to those observed in our case[17].

Theories on the origin of GPs are widely divergent. Studies have not been able to reconcile the combination of endocrine, ganglion, and spindle cells observed in a single tumor. The tumor components are of different embryologic origins, the first being of endodermal origin and the other two originating from neural crest tissue. Initially, it was suggested that these tumors were of ectodermal origin, from pluripotent stem cells derived from the neural crest, which were found in Lieberkühn's glands or the celiac ganglion during fetal development[3]. Given the presentation of GPs in various sites in the duodenum and its variable histology, some authors have proposed that it originated from an endodermal pluripotent progenitor or stem cell that has the potential for divergent differentiation[16]. It was also proposed that GPs were hamartomas of endodermal (epithelial cells) and neuroectodermal (ganglion and spindle cells) origin[17]. However, evidence conflicting with the hamartoma theory includes cases such as ours of lymph node and distant metastasis. Most authors consider GPs to be variants of gastrointestinal tract paragangliomas[18]. Paragangliomas may differentiate to other neuroectodermal elements, including neurons and Schwann cells[19]. Further evidence of potential for divergent differentiation of neoplastic neuroendocrine cells includes the observation that, when stimulated by nerve growth factor, cell cultures from carcinoid tumor or small cell carcinoma differentiate toward neurons[20].

Most GPs are benign and are amenable to local resection[2]. However, rare instances of recurrence, lymph node involvement, and distant metastases have been previously reported[6–10,21–26]. Ten cases of GPs with malignant features are summarized in Table 1. In nine cases, lymph node metastases were identified. One patient treated initially by local resection developed bone and liver metastasis 3 months later[10]. Outcomes of these ten cases clearly indicate the rare malignant potential of GP. Furthermore the incidence of malignant cases could be underestimated since majority of the reported as benign GPs cases underwent local resection only, which does not allow examination of lymph nodes. In addition most cases have been published as single case reports, without long term follow up data.

To date, histologic features predicting malignant potential has not been defined, although the presence of nuclear pleomorphism, mitotic activity, and infiltrative margin, as seen in our case, raises the concern for aggressive behavior. Since GP may recur or metastasize, pancreaticoduodenectomy with lymph node dissection may be indicated for large lesions with infiltrative margin or lesions with pleomorphism and mitoses.

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J Gastrointest Surg (2007) 11:1355–1360
DOI 10.1007/s11605-007-0223-y

Association of Hypoalbuminemia on the First Postoperative Day and Complications Following Esophagectomy

Aoife M. Ryan · Aine Hearty · Ruth S. Prichard · Aileen Cunningham · Suzanne P. Rowley · John V. Reynolds

Published online: 8 August 2007

Abstract
Objective Changes in serum albumin may reflect systemic immunoinflammation and hypermetabolism in response to insults such as trauma and sepsis. Esophagectomy is associated with a major metabolic stress, and the aim of this study was to determine if the absolute albumin level on the first postoperative day was of value in predicting in-hospital complications.
Methods A retrospective study of 200 patients undergoing esophagectomy for malignant disease at St. James Hospital between 1999 and 2005 was performed. Patients who had pre and postoperative (days 1, 3, and 7) serum albumin levels measured were included in the study. Patients were subdivided into three postoperative albumin categories <20 g/l, 20–25 g/l, >25 g/l. Logistic regression analysis was performed to calculate the odds of morbidity and mortality according to the day 1 albumin level.
Results Patients with an albumin of less than 20 g/l on the first postoperative day were twice as likely to develop postoperative complications than those with an albumin of greater than 20 g/l (54 vs 28% respectively, $p<0.011$). Correspondingly, these patients also had a significantly higher rate of Adult Respiratory Distress Syndrome (22 vs 5%, $p<0.001$), respiratory failure (27 vs 8%, $p<0.01$) and in-hospital mortality (27 vs 6% ($p<0.001$). On multivariate logistic regression analysis, day 1 albumin level was independently related to postoperative complications (odds ratios, 0.89: 95%; confidence intervals, 0.83–0.96; $p<0.005$). In addition, albumin <20 g/l on the first postoperative day was associated with the need for further surgery and a return to ICU.
Conclusion Serum albumin concentration on the first postoperative day is a better predictor of surgical outcome than many other preoperative risk factors. It is a low cost test that may be used as a prognostic tool to detect the risk of adverse surgical outcomes.

Keywords Albumin · Esophagectomy · Morbidity · Mortality · Complications

A. M. Ryan · R. S. Prichard · A. Cunningham · S. P. Rowley · J. V. Reynolds (✉)
University Department of Clinical Surgery,
Trinity Center for Health Sciences, St. James's Hospital,
Dublin, Ireland
e-mail: reynoljv@tcd.ie

A. Hearty
Department of Clinical Medicine,
Trinity Center for Health Sciences, St. James's Hospital,
Dublin, Ireland

Introduction

Esophagectomy is associated with a significant risk of morbidity and mortality rate, the largest prospective outcome cohort in the literature reported a morbidity rate of 50% and mortality rate of 10%[1]. Several preoperative risk factors have been identified, including advancing age, comorbid disease, preoperative chemoradiotherapy, low body mass index (BMI) and decreased functional status[2–4].

In addition to preoperative factors, the early course postoperatively may help predict short-term outcomes. Numerous studies have looked at immune perturbations postoperatively as a predictor of systemic inflammatory response syndrome (SIRS) and sepsis[5]. Esophagectomy induces profound changes in the endocrine, neuroendocrine and immune system as well as significant changes in organ

function[6]. The release of inflammatory mediators causes endothelial dysfunction with severe capillary leakage, loss of protein, and fluid shift from the intravascular space into the interstitium. In a subset of patients overwhelming stress response leads to SIRS, which may be associated with organ dysfunction and failure.

The acute phase response to surgery, measured by C-reactive protein (CRP) and IL-6, has been studied after major surgery and an exaggerated response may be associated with adverse outcomes[7–9]. These markers may inversely relate to serum albumin, yet to date no studies have addressed early postoperative hypoalbuminemia as a marker of risk. In this study, the value of early postoperative hypoalbuminemia as a marker of outcome after esophagectomy was studied, the hypothesis being that if postoperative day one serum albumin is an early marker of the magnitude of the systemic imunoinflammatory response to major trauma, then this may have prognostic implications for the further clinical course. We report herein confirmatory evidence that a low albumin on the first postoperative day is by multivariate analysis a predictor of adverse outcomes.

Patients and Methods

A retrospective study of 200 esophagectomy patients in whom the relevant data was collected prospectively was performed. All patients had undergone an esophagectomy at St. James Hospital, Dublin between 1999 and 2005. One hundred ninety-five patients had a thoracotomy as a component of their surgical management, either combined with an abdominal and neck exploration (three-stage) for mid and upperesophageal cancers, or cancer arising in long-segment Barrett's esophagus, or with an abdominal exploration (two-stage) for most lower third and junctional tumors, or combined with a total gastrectomy for junctional tumors with significant gastric extension (Type III). A two-field lymphadenectomy (abdominal and thoracic) was performed in all cases. All patients were extubated immediately after surgery and managed in a high dependency unit (HDU). All patients with a gastric remnant had a pyloroplasty, and patients were fed enterally from 12 h postoperatively via a needle catheter jejunostomy. Only two senior anesthesiologists look after esophagectomy cases at this institution, and four intensivists run the HDU/ICU. All patients have a thoracic epidural, and the unit policy is to limit i.v. fluid administration intraoperatively and in the first 24 h. Exact details on the fluid volumes administered and on fluid balance were not available for this study.

The preoperative medical comorbidities were recorded, as well as BMI and percentage weight loss at presentation. The patients' age, pulmonary function, and cigarette consumption were also noted. Intraoperative blood loss, length of operation, intent of surgery (palliative or curative), blood products given, and the type of operation were all recorded. Assessment of the length of stay in both the intensive care and the HDU were noted, as was the length of inpatient stay postoperatively.

Serum albumin concentrations were measured both preoperatively and on days 1, 3, and 7 postoperatively. Additionally, patients had full blood counts (with hematocrit quantification) and renal profile analysis for urea and creatinine levels; these were performed daily. CRP and immune cell parameters were not routinely measured at that time.

Postoperative Complications

All complications from surgery to discharge from the hospital were prospectively documented. Major postoperative complications, including, Adult Respiratory Distress Syndrome (ARDS), sepsis, multiorgan failure (MOF), renal failure, heart failure, respiratory failure, pneumonia/respiratory tract infection, empyema, major thromboembolic event, wound infection, anastamotic leak, pancreatic fistula, and in-hospital mortality were documented. Respiratory failure was defined as the requirement for mechanical ventilation beyond 24 h after surgery. ARDS and MOF were defined as per Bone et al.[10], sepsis required evidence of SIRS with microbiological evidence of infection, and the diagnosis of pneumonia required either positive sputum cultures of clear clinical and radiographic evidence of consolidation.

Statistical Analysis

Data manipulation and statistical analyses were conducted using SPSS® Version 11.0 for Windows™ (SPSS® Inc., Chicago, IL). Mean (±standard deviation) values for albumin taken at different stages preop and postoperatively were compared with each other using paired samples t tests. *The normal reference range for albumin was 35–50 g/l.* Three albumin categories were also created based on day 1 albumin (<20, 20–25, and >25 g/l). Cross-tabulation was used to compare albumin categories, postoperative complications and patient status with other categorical variables. Significant differences were tested using Pearson Chi-square analysis. Differences in mean laboratory data across categories of day 1 albumin status were evaluated using one-way analysis of variance. Where statistically significant effects were encountered ($p<0.05$), comparisons of means were made using Scheffe post hoc multiple comparisons test. For values that did not comply with Levene's test for homogeneity of variance, the Tamhane post hoc multiple comparisons test was used.

Binary logistic regression analysis was used to determine whether certain variables could predict postoperative complications (no/yes) and patient status (alive/dead). These predictor variables included sex, age, smoking status, presence of comorbid disease, postoperative complications, >10% weight loss, preoperative, and postoperative laboratory test results. Initially, all predictor variables were assessed independently. The models were used to generate odds ratios (OR) with their respective 95% confidence intervals (CI) to quantify the likelihood of having a postoperative complication or the likelihood of death.

Separate models were created for predicting postoperative complications and patient status. The set of variables remaining statistically significant at $p<0.200$ in these initial models were incorporated to produce multiple logistic regression models. This approach identifies important variables for the final models that may not be identified using the traditional statistical cut-off point of $p<0.05$. In each of the final multiple regression models, significance was taken at $p<0.05$.

Results

Two hundred patients who underwent upper gastrointestinal surgery for malignancy were studied—143 males and 57 females. Patient demographics are described in Table 1. The median age at diagnosis was 61 years (range, 29–77). One hundred and thirty five patients had a two-stage esophagectomy, 60 had a three stage and five patients had a transhiatial esophagectomy. The median operative time was 5 h (range, 2–9.5). The surgical intent was curative in 78% of cases. The median length of stay in the HDU was 3 days (range, 0–32 days), and the median length of stay in hospital postsurgery was 18 days .

In total, 62 patients (31%) developed a major postoperative complication (Table 2). The most common postoperative complications after esophagectomy were pneumonia (16%), sepsis (12.5%), respiratory failure (10%), and ARDS (7.5%). The in-hospital mortality rate was 8 per cent. The mean preoperative albumin level in esophagectomy patients was 39 g/l (3.9). This fell significantly to 25.8 g/l (4.4) on the first postoperative day ($p<0.0001$).

When the patients were grouped into three albumin categories on postoperative day 1 (POD1, <20, 20–24.9, and >25 g/l) significant associations were observed with postoperative complications (Table 3). Patients with an albumin <20 g/l on the first postoperative day were twice as likely to develop postoperative complications than those with an albumin >20 g/l (54 vs 28% respectively, $p<0.011$). When compared to patients with an albumin level >20 g/l on postoperative day 1 (Table 3), patients with an albumin <20 g/l had significantly higher rate of ARDS (22 vs 5%, $p<0.001$), respiratory failure (27 vs 8%, $p<0.01$) and were also more than four times more likely to die in the hospital (27 vs 6% ($p<0.001$). Sixteen patients died in hospital postoperatively, six of whom (30%) were in the albumin <20 g/l group on postoperative day 1, and all died from multiple organ dysfunction that was not evident on the first postoperative day.

The mean (+SD) percentage decrease in serum albumin in the group with POD1 albumin <20 g/l was 53.6%

Table 1 Comparison of Patient Demographics, Blood Loss, and Operative Time, Average Length of Stay, Postoperative Complications and Mortality Postesophagectomy Data Shown as Median (range)

Esophagectomy	
Number of Patients	200
Male: Female	143:57
Age	61(29–77)
Median BMI on Diagnosis	25.5
BMI <20 kg/m2	9%
>10% wt loss in 6 months	33%
Comorbidities (yes: no)	125:75
ASA Grade	
One	81
Two	95
Three	19
Four	4
Surgery only: Multimodal	95:105
Blood transfusion in first 48 h	46%
Operative Time (hrs)	5 (2–9.5)
Stay in ICU (days)	0(0–39)
Stay in HDU (days)	3 (0–32)
Length of hospital stay post surgery (days)	18 (12–106)
Postoperative Complication (yes: no)	82:118
In-hospital mortality (yes: no)	17:183

ASA American Society of Anesthesiologists; *BMI* Body Mass Index, *HDU* High Dependency Unit; *ICU* Intensive Care Unit

Table 2 In-Hospital Complications in Order of Frequency (n=200)

In-Hospital Complications	Frequency
Pneumonia/RTI	32 (16%)
Sepsis	25 (12%)
Respiratory Failure	21 (10%)
Mortality	16 (8%)
ARDS	15 (7%)
Renal failure	14 (7%)
Multiorgan failure	10 (5%)
Chyle fistula	6 (3%)
Anastomotic leak	9 (4%)
Major thromboembolic event	7 (3%)
Heart failure	3 (1.5%)
Empyema	2 (1%)
Wound Infection	2 (1%)

ARDS Adult respiratory distress syndrome; *RTI* Respiratory Tract Infection

Table 3 Postoperative Complications According to Albumin Level on First Postoperative Day Postesophagectomy

	<20 g/l n=22	20–24.9 g/l n=53	>25 g/l n=125	P Value
Complication				
Major Complications	12 (54%)	13 (24%)	37 (29%)	0.01
ARDS	5 (22%)	0 (0%)	10 (8%)	0.001
Respiratory Failure	6 (27%)	5 (9%)	10 (8%)	0.01
Mortality	6 (27%)	1 (2%)	9 (7%)	0.001
Anastamotic Leak	1 (5%)	1 (2%)	7 (6%)	ns
Pneumonia/RTI	6 (27%)	8 (15%)	18 (14%)	ns
Sepsis	4 (18%)	4 (7.5%)	17 (13%)	ns
MOF	3 (14%)	1 (2%)	6 (5%)	ns
Renal Failure	3 (14%)	2 (4%)	9 (7%)	ns
Chyle leak	1 (4%)	3 (6%)	2 (1%)	ns

ARDS Adult Respiratory Distress Syndrome, *MOF* Multiple Organ Failure; *RTI* Respiratory Tract Infection; *ns* Nonsignificant.

(+10.37) compared with 30.7% (+9.45) in the group with an albumin >20 g/l (p=0.00001). However, the risk of mortality, ARDS, anastomotic leak, sepsis, or pneumonia was not significantly associated with percentage change from preoperative levels to the first postoperative day.

The demographics of each albumin group were analyzed for association with preoperative albumin, sex, American Society of Anesthesiologists grade, duration of surgery, type of regimen (multimodal or surgery alone), type of surgery, pathological stage, and blood loss and blood transfusion within the first 24 h (Table 4). The median albumin preoperatively was similar in all groups. There was a significant association between albumin <20 g/l and a three-stage esophagectomy, squamous subtype, and median blood loss and the requirement for blood transfusions.

On multivariate logistic regression analysis (Table 5) day 1 albumin level was independently related to postoperative complications (OR, 0.85: 95%; CI, 0.85–0.77; p<0.001), as was female sex and current or previous history of tobacco use. This albumin assessment was controlled for urea and

Table 4 Albumin Categories

	Albumin			P Value
	<20 g/l	20–24.9 g/l	>25 g/l	
Age	62.5(44–70)	61(37–76)	60(29–77)	ns
Male/Female	14:8	34:19	94:31	
Preoperative albumin	40 (30–47)	38 (28–45)	40 (30–48)	ns
ASA Grade				
1	5 (23%)	24 (45%)	52 (42%)	
2	12 (54%)	21 (40%)	62 (50%)	
3	4 (18%)	7 (13%)	8 (6%)	ns
4	1 (4%)	1(2%)	2 (2%)	
Length of Operation	6(3 – 9.5)	5.5 (3.75–8)	5.5(3.75–8)	ns
Surgery Only: Multimodal	11:11	22:30	62:62	ns
Operation Type				
Transhiatial	1(5%)	1(2%)	3(3%)	
2-Stage	10 (45%)	28(53%)	96(77%)	0.001
3-Stage	11 (50%)	24(45%)	26(20%)	
Surgical Intent				
Curative: Palliative	15:8	38:15	87:38	ns
Morphology				
Adenocarcinoma	10(45%)	30(57%)	93(73%)	
SCC	12(55%)	22(42%)	27(21%)	0.006
Other	0	1(2%)	5(5%)	
Pathologic Stage				
0–2	10 (45%)	33 (62%)	74 (59%)	0.065
3–4	9 (41%)	19 (36%)	45 (36%)	
Unknown	3 (14%)	1 (2%)	8 (6%)	
Blood Loss	2000(200–4000)	820(180–2875)	960(100 –3300)	0.005
Median Units Transfused	2	0.5	0	0.045

Table 5 Prediction of Post operative Complications After Esophagectomy—Multivariate Logistic Regression Analysis Controlled for Day 1 Haematocrit Level, Blood Loss, Length Of Operation, Treatment Intent (Palliative or Curative) and Blood Products Given

Factor	Odds Ratio	95% C.I.	*P* Value
Female	3.3	1.34–8.1	0.009
Current Smoker	2.43	0.95–6.2	0.06
Ex Smoker	2.53	1.08–5.9	0.03
Day 1 Albumin	0.85	0.85–0.77	0.001

hematocrit; median (range) urea on the first postoperative day was 4.8 (3.3–113), 5 (2.5–11.7), and 5.8 (2.9–13.5) respectively in the < 20, 20–25, and > 25 subgroups. The median associated hematocrit was 0.3 (0.2–0.4) in all three albumin groups on the first postoperative day.

Discussion

Despite recent advances in the treatment of patients with esophageal carcinoma, the overall morbidity after esophageal resection remains high, with major morbidity of approximately 50% and in-hospital mortality rate of 5–10%[11,12]. Neoadjuvant therapies including chemotherapy alone or combined with radiation have been widely applied in recent years[13] and may be associated with increased postoperative complication rates[14]. The most common and serious postoperative morbidity arises from pulmonary complications, including pneumonia, ARDS, and respiratory failure[15–17].

Hypoalbuminemia preoperatively or pretrauma is independently associated with the development postoperative complications, especially the development of infective complications[10,18–22]. In upper gastrointestinal cancer surgery, low preoperative serum albumin levels have significantly correlated with anastomotic leak as well as major morbidity and in-hospital mortality[23–25]. Serum albumin levels decrease in acute illness and injury, as the liver reprioritizes protein synthesis from visceral proteins to acute phase reactant proteins, and hypoalbuminemia as a negative acute phase protein may act as a marker of underlying systemic immunoinflammation.[26–28] The decrease in albumin is a result of a combination of factors including hemodilution during fluid resuscitation, and capillary leakage into the interstitial space. The degree of capillary permeability is proportional to the inflammatory response mounted by the patient, and therefore those with the greatest rate of vascular permeability are associated with the highest mortality. The development and degree of hypoalbuminemia thus relates to the severity of the underlying traumatic insult and therefore to the ultimate outcome.

This hypothesis, that albumin may reflect immunoinflammation and may be a marker of the magnitude of this response, was the primary focus of this study, which to our knowledge, is the first report on early postoperative hypoalbuminemia and short-term outcome after major upper gastrointestinal surgery. The study revealed that a serum albumin level of <20 g/l on the first postoperative day was an independent predictor of complications—it was associated with a doubling of the in-hospital complication rate, a 3.5-fold increase the rate of respiratory failure and a five-fold increase in the incidence of ARDS and in-hospital mortality. Importantly, the absolute level of albumin on the first postoperative day rather than a percentage change from preoperative levels was the significant measurement, suggesting that profound hypoalbuminemia is a serum marker of a heightened systemic response with associated adverse risks. The equivalent outcomes in the 20–25 g/l group and the 25–30 g/l group are also consistent with this thesis.

In this study, there was no association of the low postoperative albumin level with preoperative neoadjuvant therapy or pathological stage, nor with preoperative serum albumin levels, but it was significantly associated with the three-stage resection, squamous pathology, and blood loss and requirement for blood transfusions. We acknowledge that exact details on intraoperative and early postoperative fluid balance would be helpful, and this is now automated, but this was not fully recorded during the study period. The albumin effect by multivariate analysis was significant when factors including hematocrit and urea were taken into account, thus the possibility that this represents a dilution effect on serum albumin concentrations from excessive fluid administration, although possible, is unlikely. Moreover, the consistency of anesthesiology involved in these cases, and the integrated care pathway in the early postoperative period established in this unit make it improbable that this represents solely an effect of fluid administration.

The association between a low serum albumin on the first postoperative day and the development of complications and overall mortality may be a sign of systemic immunoinflammation and hypermetabolism, a marker of the host response to a severe operative insult. The operative insult may have been somewhat greater in this cohort, as reflected by blood loss and transfusion, and the higher number of three-stage resections. The data at minimum suggests that an albumin less than 20 g/l on the first postoperative day may identify a cohort postoperatively that should continue to be monitored closely in HDU or ICU. Further research on the relationship of hypoalbuminemia to the early systemic immunoinflammatory response after major surgery is required, as well as a better understanding of the therapeutic implications.

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J Gastrointest Surg (2007) 11:1361–1364
DOI 10.1007/s11605-007-0233-9

Effects of Surgery on Peripheral N-Terminal Propeptide of Type III Procollagen in Patients with Crohn's Disease

Matilde De Simone · Michele M. Ciulla · Ugo Cioffi · Luca Poggi · Barbara Oreggia · Roberta Paliotti · Fiorenzo Botti · Alberto Carrara · Fiorenza Agosti · Alessandro Sartorio · Ettore Contessini-Avesani

Received: 7 May 2007 / Accepted: 30 June 2007 / Published online: 9 August 2007

Abstract
Aim This study investigates the effects of surgery on collagen turnover in patients affected by Crohn's disease (CD).
Methods Fifteen patients affected by active CD, assessed according to the Crohn's disease activity index, and confirmed by histology, with different pharmacological treatments, were enrolled in the study. N-Terminal propeptide of type III collagen was assessed on peripheral blood before and 6 months after surgery, as an index of collagen turnover. A control group of 15 healthy age- and sex-matched subjects was also studied.
Results In CD patients peripheral N-terminal propeptide of type III collagen serum levels were significantly higher than in controls before surgery (5.0±1.8 vs 2.7±0.7 μg/l, respectively; p=0.0001). Six months after these values were significantly reduced (from 5.0±1.8 to 3.1±0.8 μg/l; p=0.003). Independently on the pretreatment regimen and the duration of the disease, an improvement in the patients' symptoms was observed.
Conclusions The surgical resection of the affected intestinal segment in CD patients seems to be able to break down the collagen synthesis processes. Peripheral N-terminal propeptide of type III collagen could be seen as an additive marker to clinical and endoscopic observations after surgery.

M. De Simone (✉) · U. Cioffi · L. Poggi · B. Oreggia · F. Botti · A. Carrara · E. Contessini-Avesani
Department of Surgery, Fondazione IRCCS,
Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena,
University of Milan,
Milan, Italy
e-mail: matilde.desimone@unimi.it

M. M. Ciulla · R. Paliotti
Istituto di Medicina Cardiovascolare,
Centro Interuniversitario di Fisiologia Clinica e Ipertensione,
Fondazione IRCCS Ospedale Maggiore Policlinico,
Mangiagalli e Regina Elena,
University of Milan,
Milan, Italy

F. Agosti · A. Sartorio
Divisione Malattie Metaboliche III,
Istituto Auxologico Italiano, IRCCS,
Milano e Piancavallo (VB),
Milan, Italy

F. Agosti · A. Sartorio
Laboratorio Sperimentale di Ricerche Auxo-Endocrinologiche (LSRE), Istituto Auxologico Italiano, IRCCS,
Milano e Piancavallo (VB),
Milan, Italy

Keywords Procollagen type III peptide · Crohn's disease · Inflammatory bowel disease

Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) of unknown origin with a bimodal distribution of the peak incidence,[1,2] where genetic factors,[3–5] abnormal inflammation pathways activation,[6] and environmental influences[7] including smoking, use of oral contraceptives, appendectomy, and various infections,[8,9] seem to concur in the pathogenesis and progression of the disease at different levels. This pathologic condition is characterized by focal or diffuse inflammation of the alimentary tract, mucosal damage, and epithelial destruction. CD may be associated with an inability of the intestinal mucosa to protect itself from luminal challenges and inappropriate repair after intestinal injury.[10–12] An increased synthesis of collagen types I, III, and V plays an important role in the pathophysiological mechanism leading to intestinal fibrosis.[13–17] This event is also well documented in

fibrotic processes involving other organs such as liver, pancreas, lung, and heart.[18–21]

The biochemical evaluation of collagen synthesis has been hampered by the lack of specific markers capable of revealing the subtle modifications occurring at the level of collagen turnover. In recent years, new specific and reliable markers of collagen synthesis have been reported to be useful in monitoring the course of several metabolic and endocrine disorders.[22]

In a previous study, we have demonstrated in IBD patients that N-terminal propeptide of type III collagen levels (PIIINP) are significantly increased in splanchnic and peripheral circulation.[23] Until now it is not known if the surgical approach could modify the evolution of the disease in terms of collagen metabolism.

In the hypothesis that the disease is sustained by local inflammation processes, the aim of this study is to ascertain if surgery is able to normalize collagen metabolism by removing the affected intestinal segment.

Methods

Fifteen patients affected by active CD (age 36.6±10.2, years from diagnosis 9.8±5.1), submitted to surgery, were enrolled in the study. Three patients had CD in small bowel only, two in large bowel only, ten had ileocolonic disease. Disease activity was assessed according to the Crohn's disease activity index (CDAI).[24] According to CDAI, 2 patients were classified to have a moderate form of the disease whereas 13 patients as having a severe form of the disease. The clinical diagnosis was confirmed by histology; all cases under study fulfilled the histological criteria, i.e., deep ulcers, marked proliferation of small lymphoid nodules involving all layers of intestinal wall, sometimes with sarcoid-type granulomas and serosal inflammation.

The clinical profile of the studied patients is reported in Table 1. One patient did not receive any medication, whereas other patients received two or three drugs for the treatment of CD (Table 2). A control group of 15 healthy age- and sex-matched subjects was also studied.

Table 1 Clinical Aspects of the Studied Patients Before and After Surgery

Patient No.	Age	Sex	Years from Diagnosis	CDAI	
				Before Surgery	After Surgery
1	34	F	12	Severe	Inactive
2	35	M	8	Moderate	Inactive
3	38	M	15	Severe	Moderate
4	32	F	8	Severe	Moderate
5	43	F	14	Severe	Moderate
6	61	F	7	Severe	Inactive
7	33	M	8	Severe	Inactive
8	35	F	19	Severe	Severe
9	21	F	4	Severe	Moderate
10	42	M	7	Severe	Moderate
11	25	F	6	Severe	Inactive
12	51	F	18	Severe	Moderate
13	34	M	1	Severe	Moderate
14	24	F	7	Moderate	Inactive
15	41	F	13	Severe	Moderate

CDAI = Crohn's disease activity index

Table 2 Pharmacological Treatment of the Patients Affected by CD Before and After Surgery

Type of Drug	No. of Patients Before Surgery	No. of Patients After Surgery
None	1	1
5-ASA	3	9
Corticosteroids	1	–
5-ASA + corticosteroids	4	2
5-ASA + corticosteroids + AZT	6	–
5-ASA + AZT	–	3
Total patients	15	15

5-ASA = 5-Aminosalicylic acid, AZT = azathioprine

Collagen Metabolism

PIIINP levels were assessed according to a previously described procedure[23] by commercial kits (Orion Diagnostica, Finland). The intra- and interassay coefficients of variation were 4.3 and 5.3%, respectively, whereas the sensitivity was 0.2 μg/l. All the samples were run in the same session to avoid any changes in interassay variability.

Statistical Analysis

Data were analyzed using a computer statistical software (SPSS Rel 10; SPSS Inc., Chicago, IL, USA). All the quantitative variables were tested for Gaussian distribution with the Kolmogorov–Smirnov test; all of them that followed this distribution were presented as mean±standard deviation.

Differences in basal PIIINP levels between CD patients and controls were tested for significance using the analysis of variance with the Bonferroni correction. The effect of surgery was tested by ANOVA, comparing the PIIINP values in each patient before and 6 months after surgery. Data were adjusted for PIIINP basal values, with sex and age as covariates. In all cases, a p value of less than 0.05 was considered significant.

The procedures were conducted in accord with the ethical standards of the Helsinki Declaration of 1975, revised in 1983.

Results

Before surgery, serum PIIINP levels in CD patients were significantly higher than in healthy controls (5.0±1.8 vs 2.7± 0.7 μg/l, respectively; $p=0.0001$) (Fig. 1).

Six months after surgery a significant decrease in PIIINP values was observed in CD patients (from 5.0±1.8 to 3.1± 0.8 μg/l; $p=0.003$) (Fig. 1). This effect was not related to the pretreatment regimen and was not dependent on the duration of the disease. No sex differences were observed in PIIINP values before and after surgery. After surgery the patients' classification made on the basis of the CDAI changed markedly. In fact, only one case was considered still severe, eight cases were classified as moderate, and six patients were considered to have inactive disease. This finding corresponded to a reduction of 46.7% of the active forms of the disease (moderate or severe) during the 6 months of follow-up. Consequently, the pharmacological treatment was simplified in 9/15 patients with a significant reduction of the combined therapy (Table 2).

Discussion

CD is a chronic pathology characterized by an early onset followed by sporadic episodes of acute symptoms during lifetime, debilitating the patient's capacity to perform one's daily functions.[25] This disease is predominantly seen in developed countries, and the risk is highest in first-degree relatives.[26,27] The diagnosis and the recurrences are currently established on the basis of clinical, endoscopic, radiological imaging (x-rays, ultrasound, computed tomography scan, magnetic resonance imaging), and histological findings. Characteristic pathological appearances include the formation of skip lesions (discrete regions of inflamed bowel separated by uninvolved mucosa), aphthous ulceration, and fistulation; these signs relate to the presence of an underlying granulomatous transmural inflammation.[28,29]

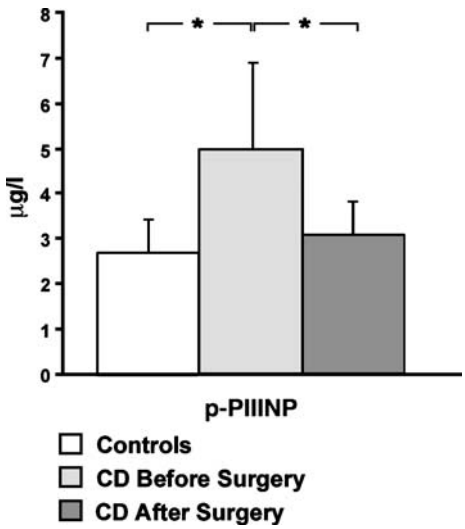

Figure 1 N terminal propeptide of Type III collagen (PIIINP) in controls and in patients with Crohn's disease (CD).*$p<0.005$.

Several markers such as erythrocyte sedimentation rate, C-reactive protein, serum interleukin-6 are now used to predict the recurrence and to control the clinical activity of the disease.[30,31]

The challenge for clinicians is in finding a new serologic marker or tests that have the specificity and the sensitivity criteria to replace the more expensive and invasive methods now used in screening patients with IBD.[30] Above all, it would be important for clinicians to have a single noninvasive marker in predicting relapses and in monitoring the disease activity or the effects of the therapy.

In a previous study, we have found that peripheral collagen metabolism in IBD patients was increased compared with healthy subjects.[23]

In the present study, we have found that serum PIIINP levels dropped significantly after surgery (Fig. 1). These changes were related to an improvement of patient's symptoms and to CDAI. In fact, after surgery, only one case had a severe form of the disease, whereas eight cases had a moderate form of disease, and in six cases the CD was inactive. Consequently the therapeutic regimen was simplified in 9/15 patients (Table 2).

The transmural inflammation involving all layers of the bowel wall in CD patients is accompanied by deposition of fibrotic tissue containing large amounts of collagen types I, III, and V, inducing repair processes that may cause structuring lesions.[14] We hypothesize that the chronic inflammation processes in CD is supported locally by the affected intestinal segment and, consequently, its surgical resection is able to break down the collagen synthesis processes, therefore reducing peripheral PIIINP levels, and possibly the risk of collagen deposition far from the main target organs.[2,32]

Conclusion CD represents a challenging pathology for both patients and physicians and its impact on everyday lives of patients may be enormous.[2] In this present study, we provide evidence that at 6-month follow-up peripheral serum PIIINP levels decrease significantly after surgery, and is followed by an improvement in the patients symptoms, the CDAI, and the maintenance therapy. Peripheral PIIINP could be seen as an additive marker to our clinical and endoscopic observation after surgery. Furthermore, the serial determination of this peripheral marker of collagen turnover in the postsurgical follow-up of patients affected by CD might be useful for the early detection of disease recurrence, as reported by Sartorio et al.[33] in active and preclinical Cushing's syndrome.

Additional data obtained with more prolonged follow-up study (1 and 2 years) are needed to elucidate whether the earlier surgical option could arrest the evolution of the disease locally and far from the main target organs.

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J Gastrointest Surg (2007) 11:1365–1367
DOI 10.1007/s11605-007-0230-z

A Novel Technique to Relieve a Closed-Loop Obstruction Secondary to a Competent Ileocecal Valve and an Unresectable Mid-Colon Tumor

Andrew McKay · Oliver F. Bathe

Received: 6 June 2007 / Accepted: 17 June 2007 / Published online: 17 July 2007

Abstract A competent ileocecal valve complicates the surgical treatment of an unresectable obstructing mid-colon tumor. Specifically, it may not be feasible to bypass with a colocolotomy, especially when the sigmoid colon has limited mobility or if the ascending colon is severely distended and edematous. A technique is described in which the closedloop obstruction is relieved at its proximal extent by an ileocecal valvuloplasty. A circular stapling device is fired across the ileocecal valve. Once the ileocecal valve is rendered competent, a loop ileostomy or a colocolotomy can be constructed, providing effective palliation for this difficult situation.

Keywords Ileocecal valve · Valvuloplasty · Bowel obstruction · Closed loop · Unresectable

Introduction

An impending large bowel obstruction caused by colorectal carcinoma is an urgent surgical situation that must be addressed before complete obstruction arises. A competent ileocecal (IC) valve complicates the situation because it will lead to a closed loop between the valve and the tumor. Curative resection of the tumor is the treatment of choice. However, in some cases, local invasion into critical structures will preclude this possibility. This is a particularly difficult problem in the case of an extensive transverse colon cancer, which encompasses the root of the small bowel mesentery including the superior mesenteric vessels (Fig. 1). In such a situation, it may not be feasible to bypass with a colocolotomy, especially when the sigmoid colon has limited mobility or if the ascending colon is severely distended and edematous. Rather, an ileostomy or a bypass from terminal ileum to sigmoid colon may be considered. If such options are chosen, then the closed-loop obstruction secondary to the competent IC valve must also be addressed.

Here, we describe a novel technique to overcome a closed-loop obstruction caused by a competent IC valve and an unresectable distal colonic lesion. The closed loop obstruction of the right colon is alleviated by opening the competent IC valve to decompress the right colon using a circular end-to-end stapling device. Whereas clinical scenarios where this technique could be used are unusual, this technique is another useful tool available to the general surgeon if such a situation does arise.

Methods

After each of the options is considered and it is elected to construct an ileostomy or an enterocolotomy, the closed-loop obstruction must be decompressed. An IC valvuloplasty is an effective method of decompression.

A small longitudinal enterotomy is fashioned in the terminal ileum approximately 10–20 cm proximal to the IC valve. This enterotomy is made to be 3–4 cm in length, or

A. McKay · O. F. Bathe
Department of Surgery, University of Calgary,
Calgary, AB, Canada

O. F. Bathe
Department of Oncology, University of Calgary,
Calgary, AB, Canada

O. F. Bathe (✉)
Division of Surgical Oncology, Tom Baker Cancer Centre,
1331–29 Street NW,
Calgary, AB T2N 4N2, Canada
e-mail: bathe@ucalgary.ca

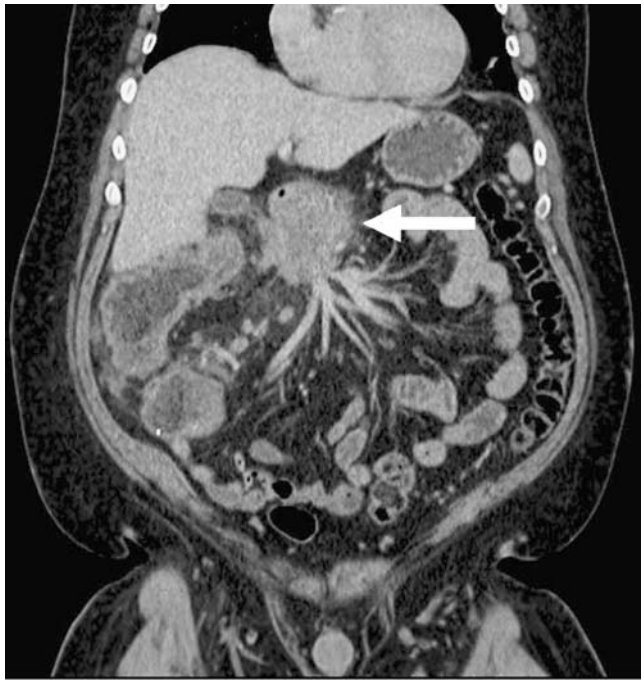

Figure 1 A closed loop obstruction between a competent ileocecal valve and an unresectable distal colon tumor which encases the superior mesenteric vessels (*white arrow*).

just sufficient to allow entry of the head of a 31-mm circular stapling device (e.g., Premium Plus CEEA™, Autosuture™) into the lumen of the small bowel. Stay sutures are placed on either side of the enterotomy to facilitate manipulation of the stapler within in the bowel and to facilitate later closure (Fig. 2). Alternatively, the enterotomy can later be used as the site of an ileostomy or an enterocolotomy.

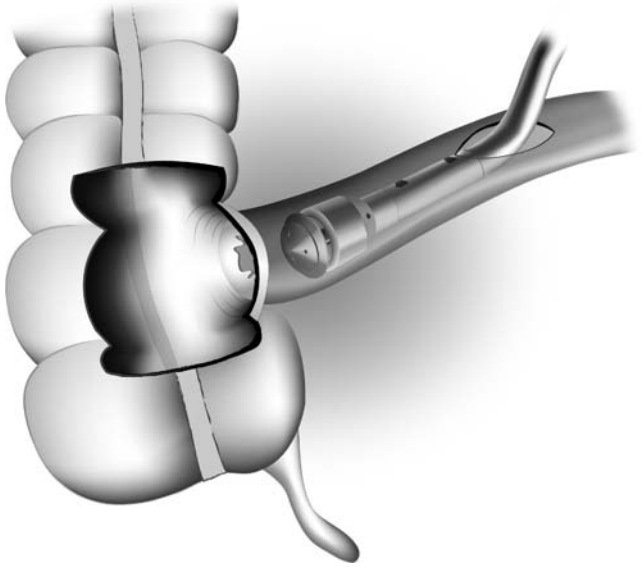

Figure 2 An enterotomy is fashioned in the terminal ileum 6–8 cm proximal to the ileocecal valve and the stapler is introduced into the small bowel lumen. Stay sutures are placed on either side of the enterotomy to facilitate manipulation of the stapler within in the bowel and to facilitate later closure.

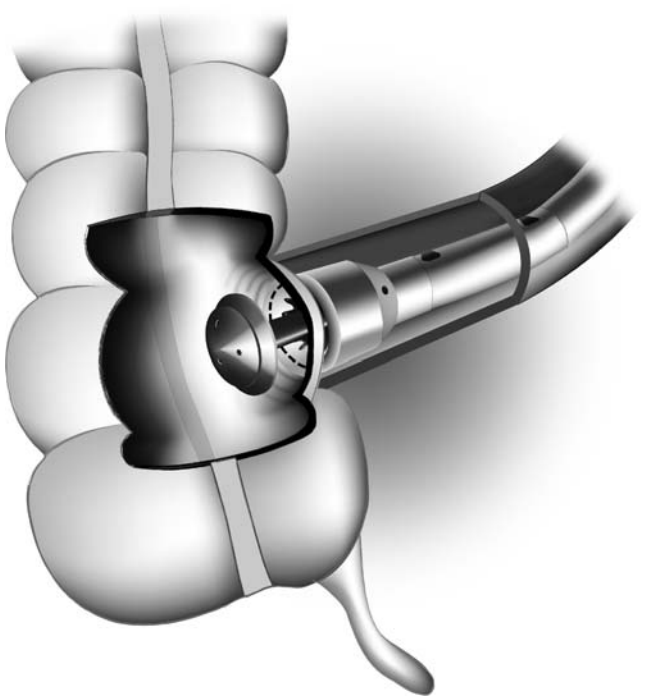

Figure 3 The circular stapling device is gently manipulated through the enterotomy to lie across the ileocecal valve. Care is taken to ensure that the full thickness of small bowel wall or cecal wall are not incorporated into the staple line and the stapler is fired. The "doughnuts" of the stapler include only the ileocecal valve and some mucosa.

The stapling device is gently manipulated into proper position across the valve. Care is taken to ensure that the stapler will incorporate the full thickness of the IC valve, including the terminal ileum and the cecum. Once it is ensured that the valve is included in the stapling device, the stapler is fired (Fig. 3). The muscularis propria and serosa of the bowel

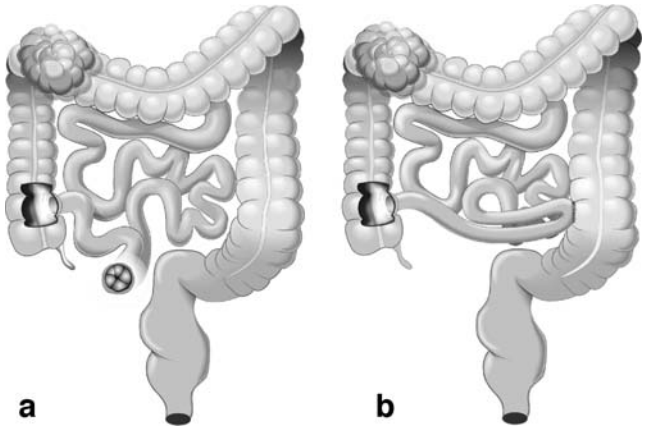

Figure 4 The enterotomy can be fashioned to create an ileostomy (a). Alternatively, it may be the site of an anastomosis to the left colon (b).

are not included in the "doughnuts" of the stapler. Rather, the "doughnuts" should include only the valve and some mucosa. The firing of the stapler should immediately result in decompression of the distended right colon. The widened valve can be visually inspected through the enterotomy.

The enterotomy can be fashioned to create an ileostomy. Alternatively, it can be anstomosed to sigmoid colon, bypassing the unresectable mid-colonic lesion (Fig. 4).

Discussion

Palliation in the case of an unresectable transverse colon tumor presents some unique challenges, particularly in the presence of a closed loop obstruction secondary to a competent IC valve. An obstructing colon tumor with a competent IC valve also creates a closed loop. Other options to relieve the closed loop obstruction are to create a proximal colostomy or cecostomy and to perform an anastomosis between the terminal ileum and the cecum or right colon. An IC valvuloplasty may be preferred over the former alternative because it avoids an unwanted permanent stoma in a palliative situation. The valvuloplasty may be preferred over the latter alternative because it avoids a suture or staple line in distended abnormal colon lessening the risk of anastomotic leak. The obstruction could be addressed through a single enterotomy in the relatively normal terminal ileum.

To our knowledge, this technique has not been previously described. A plasty of the IC valve has been reported in the setting of Crohn's strictures[1], which was performed in a different fashion. A circular stapling device would not have been practical in such an instance since the Crohn's disease would have involved a small portion of terminal ileum. This would have been too much tissue to staple in this manner.

In summary, while clinical scenarios where this technique could be used are unusual, this technique represents another useful tool available to the General Surgeon if such a situation does arise.

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J Gastrointest Surg (2007) 11:1368–1372
DOI 10.1007/s11605-007-0160-9

Review Article: Appendicitis In Groin Hernias

Alan K. Meinke

Received: 21 April 2006 / Accepted: 20 November 2006 / Published online: 30 March 2007

Abstract To review the clinical presentation, outcome and causes of acute appendicitis presenting within a groin hernia. A comprehensive review of the past 70 years of English language surgical literature was conducted pertaining to acute appendicitis presenting within an inguinal or femoral hernia. Thirty-four reports describing 45 patients were reviewed to determine age, position, gender, pathologic stage at presentation, causal suppositions, and clinical outcomes. Hernial appendicitis presented as an inguinal abscess or a tender inguinal mass, often in the femoral position, and most commonly at the extremes of age. It was almost never recognized preoperatively, and, because of the sequestered nature of the inflammatory process, presented with few classic systemic signs or symptoms suggestive of acute appendicitis. Advanced pathologic stage and death correlated with the patient's age, delay in presentation, and delay in recognition. Evaluation of an inguinal abscess or a nonreducible tender groin hernia presenting in a patient at the extremes of age, should include computed tomography to rule out an occult acute appendicitis within the hernia, as systemic signs and symptoms of appendicitis are rarely evident. The condition appears to be caused by inflammatory adhesions caused by appendicitis occurring within an enlarged hernial orifice rather than appendicitis caused by external compression of the appendix base. Early recognition of this unique presentation of appendicitis allows trans-hernial appendectomy and immediate herniorraphy. Delayed diagnosis requires drainage of abscess with appendectomy and interval hernia repair.

Keywords Hernia · Appendicitis · Amyand's hernia · Inguinal appendicitis · Hernial appendicitis

The first successful drainage of an acutely inflamed appendiceal abscess presenting in the groin was reported by DeGarengrot in 1731,[1] followed by the first successful appendiceal resection for appendicitis in this position by Amyand in 1735.[2,3] In Amyand's patient, the inflamed appendix presented in the hernial sac of an 11-year-old boy in whom the appendix had been perforated by a pin. The first successful resection of an inflamed appendix presenting in a groin hernia in the United States was in a patient in whom the appendicitis was also caused by a pin. The appendix was removed through the hernial sac by Hall in 1886.[2,4,5] Since then, predominantly, case reports of hernial appendicitis (HA) have been published, emphasizing that this presentation of appendicitis is rarely diagnosed preoperatively and commonly presents with a high incidence of gangrene and perforation.

This paper is based on a comprehensive review of recent surgical literature to further identify and hopefully reduce the incidence of clinically advanced hernial appendicitis (Amyand's Hernia).

Methods

A retrospective review of 70 years of English language surgical literature identified 45 cases of hernial appendix reported in 34 articles from 1937 to 2006. Thorough evaluation and comparison of case reports was limited by the lack of completeness of many of the reports. Some articles omitted the position of presentation, the age and sex of the patient, the pathologic stage, or the duration of symptoms. Only reports from Carey, Sjouji, and Thomas reported more than two cases of this condition.[6–8]

A. K. Meinke (✉)
Department of Surgery, Norwalk Hospital,
Norwalk, CT 06880, USA
e-mail: ameinke@pol.net

Ryan's 1937 review of *Hernia of the Vermiform Appendix*, including case reports from both Hesselbach and Morgagni, identified 537 cases of mostly pathologically normal appendices presenting in a hernial position.[1] A review from Lyass emphasized that most cases of Hernial Appendicitis in the pre-antibiotic era, presented as a groin abscess requiring incision and drainage with resulting mortality rates of 14–30%.[2]

Results

Age of Patients

The age at presentation of hernial appendicitis ranges from 5 weeks premature[7,9] to 89 years,[10] with clusters at the extremes of age in a bimodal pattern demonstrating median peaks at 37 days and 69 years of age (Fig. 1). This peak in the older age group is consistent with that of a series of seven adult patients with hernial appendicitis reported by Thomas. He identified a median peak at age 69 years.[8] This bimodal pattern of age distribution is similar to perforated abdominal appendicitis[11] and curiously is the inverse of the age distributions of routine appendicitis[11–14] and incarcerated inguinal hernia.[15]

Whereas acute appendicitis is rare in children under 2 years of age,[16] 21% (7/33) of patients with hernial appendicitis are found in this age group with more than half of these young patients (12%, 4/33) presenting as neonates (less than 2 weeks of age) (Fig. 1). Srouji noted six cases of hernial appendicitis in a review of 106 cases of acute appendicitis in children under the age of 30 days.[7] The *hernial orifice* is large at birth, narrows at midlife[17] and then enlarges again with age.[18] A large neonatal hernial orifice and a significantly large incidence of inguinal hernia is commonly observed at birth (1–5%).[19] This widened hernial orifice may also explain the low incidence of symptomatic incarcerated hernia in neonates.[15] A persistent processus vaginalis that fails to close after descent of the testis at the 28th week of gestation explains the high incidence of inguinal hernia in premature infants. The processus vaginalis can often remain open for a year or longer in full-term infants.[17,20]

The *appendiceal orifice* is also larger in diameter at birth[7,20] and steadily narrows in caliber throughout life.[21,22] Neonatal appendicitis is rare because the orifice of the neonatal appendix has the configuration of an open "fetal funnel,"[7,13,23] making it difficult to obstruct by the most common causes of appendicitis (lymphoid hyperplasia 60%, fecaliths 35%).[24] It has been postulated that the high incidence of hernial appendicitis in neonates is caused by external compression of the appendiceal lumen by the inguinal ring[7,23] or because the hernial appendix is in an exposed position of increased susceptibility to trauma exacerbated by increasing abdominal pressure of the crying infant.[25] Whereas the adult femoral ring is "tight and unyielding"[15] and can explain the higher incidence of appendiceal incarceration in a femoral position (Table 2), the neonatal inguinal ring is widely patent,[17] making external compression of the appendiceal lumen by the inguinal ring a less plausible explanation of appendicitis in this age group.

As with children, the incidence of abdominal appendicitis is low in the older age group most likely because of an age-related reduction in incidence of peri-appendiceal lymphoid hyperplasia[24] and fecalith diameter to appendiceal orifice disproportion. The low incidence of incarcerated inguinal hernia in the older age group[15] is likely caused by a progressive enlargement of the inguinal canal with age.[18] It is curious that although the incidence of both abdominal appendicitis and incarcerated inguinal hernia is low in the older age group, the incidence of incarcerated inguinal appendicitis in this same age group is high. We

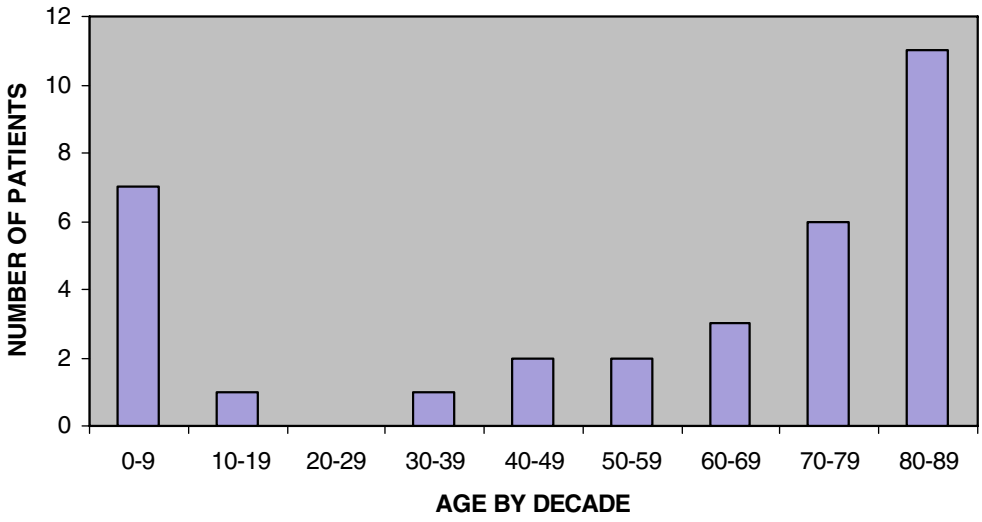

Figure 1 Age at presentation of acute hernial appendicitis by decade (N=33).

suggest that this high incidence of hernial appendicitis in both the very young and the very old is simply a consequence of an anatomic opportunity of the acutely inflamed appendix to lie within an enlarged hernial orifice characteristic of patients at the extremes of age.

Location of Appendicitis

A *non-inflamed* appendix is found in the hernia sac at the time of herniorraphy in 0.19% to 0.6% of adults,[1,26] and in 1.15% of infants.[7] A hernial position of an *inflamed* appendix is identified in only 0.08 to 0.13% of cases of appendicitis.[1,2,26] Although acute appendicitis within the hernia sac presents primarily on the right side (95% [37/39]; Table 1),[27–29] both normal and inflamed appendices have also been reported in the left inguinal position (2/39; Table 1),[30–34] the obturator orifice[1,35], within an umbilical hernia[1], an incisional hernia[36], within a trocar port site hernia[37], and as the precipitant of necrotizing fascitis of the right thigh.[38]

In this review of hernial appendicitis presenting in the groin position (Table 2), 71% (22/31) were male (gender was not reported in two patients) and 97% (32/33) right-sided. Only a single patient presented with appendicitis in the left groin hernia. Twenty-four percent (8/33) of hernial appendicitis presented in a femoral position (Table 2), three to seven times higher than both incarcerated and non-incarcerated intestinal femoral hernias (3.4 to 7.0%).[15,24,39] Two-thirds (6/9) of hernial appendicitis in adult females presented in the femoral position (Table 3). In the pediatric group, 88% (7/8) presented on the right side, with no pediatric patients presenting in the femoral position (Table 2).

Symptoms

Hernia appendicitis usually presents with symptoms similar to those of an incarcerated hernia, viz., a tender, painful scrotal mass.[6,40] Often the condition presents as a primary or recurrent groin abscess requiring incision and drainage.[2] Delay or lack of diagnosis is common (Fig. 2),[6,41,42] because the right lower quadrant abdominal pain is often absent and systemic manifestations of classic appendicitis, such as a fever and leukocytosis, are not often present because of the sequestration of the infectious process within the hernial sac.[4,6,7,30] This isolation of the infectious process has been credited with the superior survival rate of *neonatal* hernial appendicitis in the pre-computer tomographic (CT) era, when the preoperative diagnosis of neonatal intra-abdominal appendicitis had "virtually never been made."[7] Before 1970, only 10 of 33 patients with neonatal appendicitis survived, seven of which presented within a hernial sac.[9]

Hernial appendicitis is rarely diagnosed before surgery and is often misdiagnosed as testicular torsion, epididymitis, or acute hydrocele, and less commonly as acute diverticulitis with abscess when presenting in the left groin.[10,41,43] Although hernial appendicitis has been reported most commonly within the peritoneal sac, it has also been reported within the groin but outside of the sac[44] and within the abdominal cavity but still incarcerated within the hernial sac after hernial reduction "en masse".[45]

Diagnosis

The first use of CT radiography to make the diagnosis of an acutely inflamed hernial appendicitis was reported in 2000.[4,46,47] CT remains integral to preoperative diagnosis (Fig. 3). Conventional abdominal roentgenographs are of little diagnostic help.[6]

Pathology

The pathologic stage of appendicitis was reported in 39 cases of inguinal appendicitis. Advanced pathology was

Table 1 Position of Appendicitis within an Inguinal Hernia, *N*=39

Site	Total	Sex and Age Identified	Sex and Age not Identified
Right inguinal	27 (69%)	24	3
Right femoral	10 (26%)	8	2
Left inguinal	2 (5%)	1	1
All	39	33	6

Table 2 Position of Appendicitis within an Inguinal Hernia by Sex and Age, *N*=33

	Right Inguinal	Right Femoral	Left Inguinal	Left Femoral	Total
Adult male	14	2	0	0	16/33
Adult female	3	6	0	0	9/33
Child male	5	0	1	0	6/33
Child unknown gender	2	0	0	0	2/33
	24	8	1	0	33

Table 3 Position of Appendicitis within an Inguinal Hernia in Adults by Sex, *N*=25

Gender	Right Inguinal	Right Femoral	Left Inguinal	Left Femoral
Male	88% (14/16)	12% (2/16)	0 (0/16)	0 (0/16)
Female	33% (3/9)	66% (6/9)	0 (0/9)	0 (0/9)
Both	68% (17/25)	32% (8/25)	0 (0/25)	0 (0/25)

Numbers in parentheses = no. of patients with appendicitis/total no. of patients

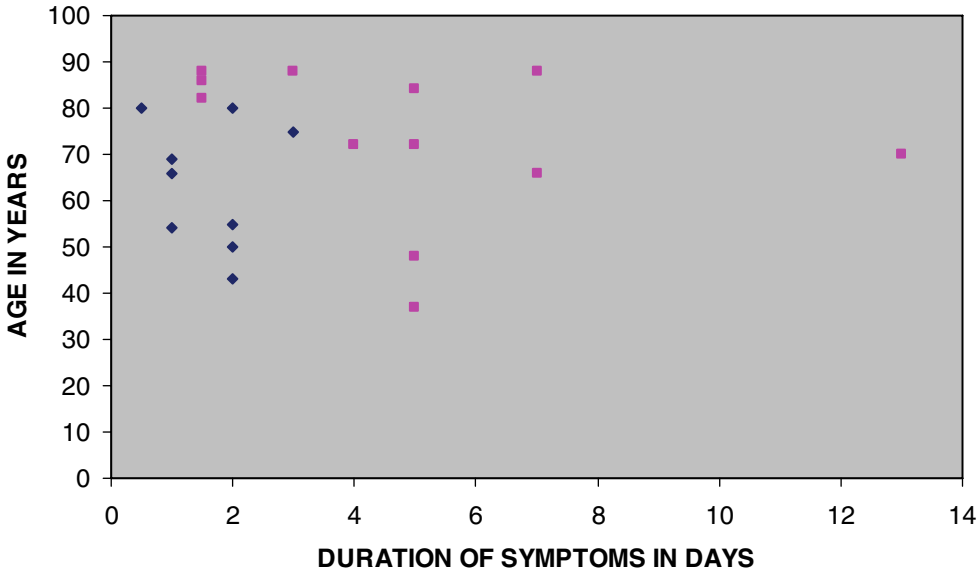

Figure 2 Pathology of appendix versus age of patient and duration of symptoms. Boxes: (N=13) gangrenous or perforated appendicitis, symptoms >3 days (N=7) ***or*** age >80 years (off graph: patient age 89 presented at 6 months). Diamonds: (N=9) acute appendicitis, symptoms ≤3 days and age ≤80 years.

noted in 67% (26/39) of patients (perforation 41% [16/39], gangrene 26% [10/39]) (Table 4). Clinical presentations and outcomes were reported in only 22 cases (Fig. 2). When adult cases were compared with age of presentation and clinical outcomes, it is significant to note that all deaths and advanced presentations occurred in patients either over the age of 80 *or* when diagnosis was delayed more than 3 days after onset of symptoms This delayed presentation of advanced pathology is very similar to the clinical presentation of abdominal appendicitis in the elderly.[21,48] All patients presenting with simple acute hernial appendicitis presented with both a duration of symptoms of 3 or less days and at an age of 80 years or less.

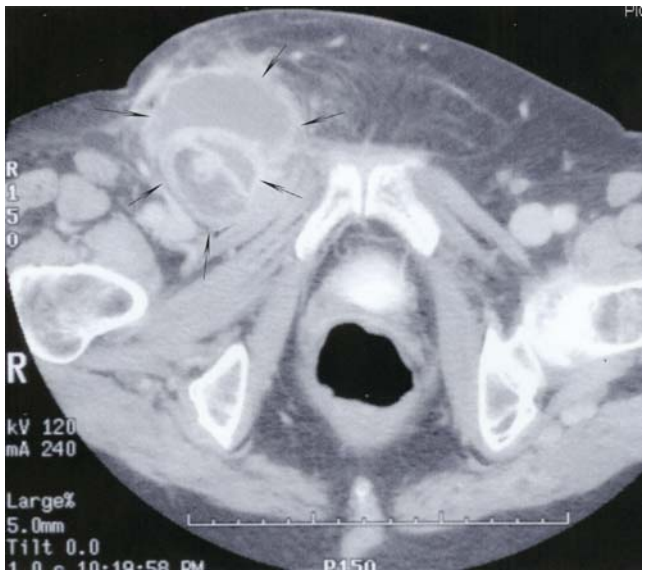

Figure 3 Computed tomogram of an incarcerated, perforated, acute appendicitis in a right-sided inguinal hernia (arrows).

Conclusions

Acute appendicitis occurring within a groin hernia, in distinct contrast to routine abdominal appendicitis, presents in a bimodal pattern, at the extremes of age, most commonly in males, as either an inguinal abscess or as a tender inguinal mass, often in the femoral position, frequently unrecognized, with few classic systemic signs or symptoms of appendicitis. Advanced pathologic stage and death correlates with patient age and delayed diagnosis. In both children and adults, the incidence of appendicitis presenting in a hernial position correlates with the size of hernial orifice. We postulate that the higher incidence of incarcerated hernial appendicitis is caused by inflammatory adhesions of routine appendicitis coincidently found within an enlarged hernial orifice typical of patients at the extremes of age. The advanced clinical presentation of hernial appendicitis is caused by a low index of suspicion and a lack of systemic signs and symptoms of appendicitis sequestered within a groin hernia. When identified at an early pathologic stage, appendectomy can usually be completed through the hernial sac accompanied by an immediate nonmesh herniorraphy to reduce the possibility of postoperative infection. When identified at an advanced

Table 4 Pathology of Appendicitis within an Inguinal Hernia, N=39 (%)

Type of Pathology	Adults	Children	All
Acute inflammation	8 (26%)	5 (63%)	13 (33%)
Perforated	14 (45%)	2 (25%)	16 (41%)
Gangrenous	9 (29%)	1 (12%)	10 (26%)
All	31 (100%)	8 (100%)	39 (100%)

pathologic stage, simple abscess drainage and interval herniorraphy is advised.

Acknowledgments The author would like to thank Horace Laffaye, MD for editorial assistance and Chris Meinke for proofreading in the preparation of this paper.

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J Gastrointest Surg (2007) 11:1373–1375
DOI 10.1007/s11605-006-0055-1

Eosinophilic Colitis

V. R. Velchuru · M. A. B. Khan · H. B. Hellquist · J. G. N. Studley

Published online: 20 January 2007

Abstract Eosinophilic colitis is a rare chronic inflammatory bowel condition of unknown etiology. We report a case of cecal volvulus causing obstruction in a patient with eosinophilic colitis. A 48-year-old lady presented with abdominal pain, constipation, and abdominal distension. Clinically and radiologically, she was diagnosed to have cecal volvulus. Preoperative colonoscopic reduction failed. At laparotomy, a right hemicolectomy with primary anastomosis was undertaken. Histology of the resected specimen showed diffuse eosinophilic infiltration suggesting eosinophilic colitis. To the best of our knowledge, this association has been never reported.

Keywords Eosinophilic colitis · Cecal volvulus

Case History

A 48-year-old lady presented as an emergency with colicky abdominal pain, constipation, and progressive abdominal distension of 3 days duration. She has had similar nonspecific episodes, which resolved spontaneously. Two years previously, she had presented with rectal bleeding, mucus, and change in bowel habit and was noted to have iron-deficiency anemia. Colonoscopy was unremarkable.

On examination, there was distension in the upper and left half of the abdomen with minimal tenderness. Abdominal x-ray suggested cecal volvulus (Fig. 1). Hematological and biochemistry investigations were normal. As there was no evidence of peritonitis, colonoscopic derotation was initially attempted with no success. At laparotomy, cecal volvulus was noted with a grossly distended cecum and ascending colon. Right hemicolectomy and primary anastomosis was performed from which she made an uneventful recovery. Gross examination of the resected specimen, 15 cm of the ileum and 32 cm of the colon, showed a dilated cecum up to 6 cm in diameter. Sections from the ileum showed normal mucosa without any prominent inflammation. Microscopy of the cecum showed pronounced edema in the submucosa and a striking eosinophilic infiltrate in the submucosa and muscularis propria. The mucosa was minimally affected. The infiltrate was particularly dense (>80 eosinophils per high power field) in the muscularis propria. There was a paucity of any other inflammatory cell types (Fig. 2a and b). Postoperative stool examination did not reveal any evidence of parasitic infestations.

Discussion

Primary eosinophilic enterocolitis is a rare chronic inflammatory bowel disease characterized by nonspecific gastrointestinal symptoms, bowel wall infiltration by eosinophils in a diffuse or segmentary manner, and absence of parasite infestation.[1,2] This condition was first described by Kaijser in 1937. It can affect any part of the gastrointestinal tract.[1,2] The stomach and small bowel are the commonest sites. The esophagus and the colon are less frequently involved.

V. R. Velchuru (✉) · M. A. B. Khan · J. G. N. Studley
Department of Surgery, James Paget Hospital NHS Trust,
Lowestoft Road, Gorleston,
Great Yarmouth NR31 6LA, UK
e-mail: velchuru@hotmail.com

H. B. Hellquist
Department of Histopathology, James Paget Hospital NHS Trust,
Lowestoft Road, Gorleston,
Great Yarmouth NR31 6LA, UK

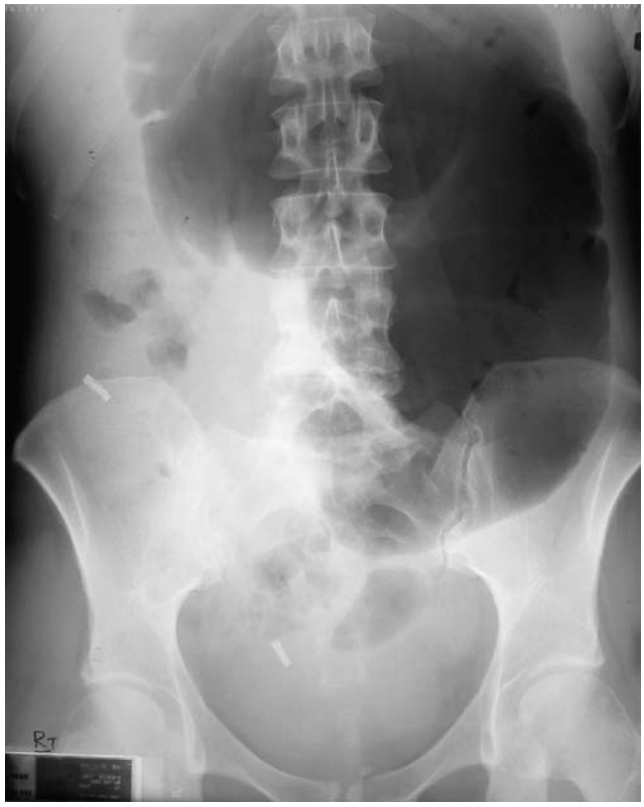

Figure 1 Plain abdominal x-ray showing cecal volvulus.

Secondary eosinophilic infiltration is noted in a variety of diseases, malignancy, and parasite infestation.

Etiology is unknown.[3] However, 50% are known to have a history of allergy. It has been associated with rheumatoid arthritis and connective tissue diseases including scleroderma and dermatomyositis. It has been reported as been masquerading as colonic cancer and has been associated with colonic cancer.

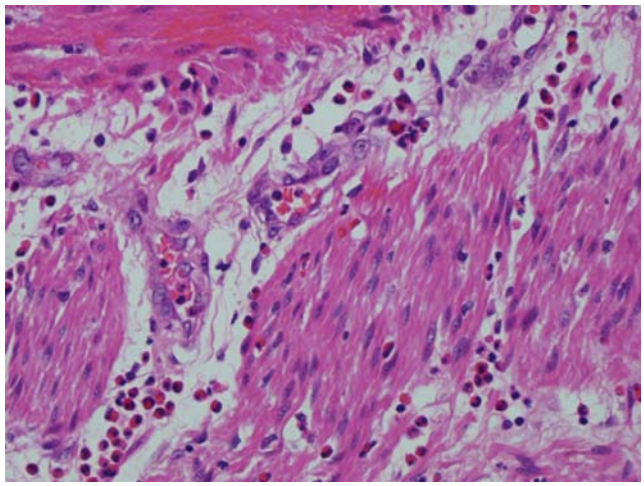

Figure 2 Higher magnification illustrating the diffuse infiltration of numerous eosinophils in the muscularis propria and submucosa (H&E, ×40).

Clinical manifestations of eosinophilic colitis are related to the location and depth of eosinophilic infiltration of the bowel wall. Klein described three specific patterns[2] of this condition.

1. Mucosal disease associated with protein-losing enteropathy, diarrhea, bleeding (iron deficiency anemia)
2. Subserosal disease with symptoms of bloating and ascites
3. Transmural disease presenting as colicky abdominal pain and intestinal obstruction.[1]

Endoscopy and radiology can be helpful in diagnosis. On endoscopy, a nodular pattern with erosions of the mucosa is seen. Patchy distribution of the disease is reported (as evident in this case), and as a result, diagnosis can be missed, and so, more than one panendoscopic examination and biopsies are suggested.[1] Radiological appearances typically show strictures, thickening of the bowel wall and mucosal folds,[1] a rigid ileocecal valve open to reflux, and ulcerative and polypoidal lesions. Twenty to ninety percent of patients have peripheral eosinophilia, and hence, this finding is not a satisfactory diagnostic tool. On histology, heavy eosinophilic infiltrate throughout the layers is seen (Fig. 2) with areas of submucosal edema leading to polypoidal elevations throwing overlying mucosa into folds.[1,3] Once diagnosed, the treatment is steroids,[1,2] with a good response. However, a laparotomy provided the diagnosis in most cases in a literature review.[3] A cecal volvulus in association with eosinophilic colitis has never been reported.

Twenty-two percent of volvulus of the large bowel occurs in the cecum. Plain abdominal x-ray is diagnostic in 62% of cases (coffee bean deformity).[4] Preoperative colonoscopic derotation can be achieved in the absence of peritonitis, and the success rate has been reported as 12.5%.[4] If unsuccessful, definitive treatment with colonic resection and primary anastomosis is the treatment of choice. Colonic volvulus has been reported in celiac sprue,[5] and *Clostridium difficile* infection,[6] suggesting acute mucosal inflammation being the contributory factor in the development of a volvulus.[6]

Eosinophilic colitis has been known to cause obstruction in the gut. However, cecal volvulus in eosinophilic colitis has not been reported. Transmural eosinophilic infiltration of the bowel wall and acute mucosal inflammation may play a role in causation of volvulus.

Conclusion

A case of cecal volvulus in eosinophilic colitis has been presented. In our case, colonoscopic reduction of the cecal

volvulus failed, and subsequently, the patient underwent a laparotomy and right hemicolectomy with an uneventful recovery. The diagnosis of eosinophilic colitis was made on the resection specimen. Eosinophilic colitis has been known to cause obstruction in the gut. However, a case of cecal volvulus has not been previously reported.

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J Gastrointest Surg (2007) 11:1376–1378
DOI 10.1007/s11605-007-0178-z

Letter to the Editor

Surgical Treatment of Iatrogenic Biliary Tract Injuries: An Old Technique Revisited

To the Editor:

We read with great interest the articles by Mercado et al.[1]: in the "How I Do It" session of September–October, 2006 Journal of Gastrointestinal Surgery issue, titled: "Bile Duct Growing Factor: An Alternative Technique for Reconstruction of Thin Bile Ducts After Iatrogenic Injury" and in the January, 2006 Journal of Gastrointestinal Surgery issue, titled: "Long-term Evaluation of Biliary Reconstruction After Partial resection of Segments IV and V in Iatrogenic Injuries"[2].

The description of alternative techniques for repair of biliary strictures is of utmost importance as they still represent the major complication of cholecystectomy[3]. Although the laparoscopic modality represents a significant technical advance in performing cholecystectomy, injuries of the biliary tract during laparoscopic cholecystectomy are at least twofold higher after the laparoscopic procedure[4].

Our interest in reading the technique description and its results in the papers now published by Mercado et al. relates to the fact that we had the opportunity to propose and publish this very same modality of surgical repair of bile duct strictures 20 years ago[5]. The preliminary results obtained in 54 of 57 patients operated upon using this technique, followed for an average period of 2.9 years (1–10 years), were reported by our group 12 years later[6]. There was no postoperative mortality in this series, and good late results were obtained in 20 of 26 (76.9%) patients with injuries classified as Bismuth III or IV and in 96.4% of patients with Bismuth I and II type lesions[7]. None of the patients received a transanastomotic tube, including those with small diameter ducts (less than 4 mm). After this publication, another 159 patients with benign biliary strictures were operated according to this technique with similar results.

Some technical aspects of the technique deserve further consideration. The main feature of this technique, which should be strongly stressed, is that by dissecting just the anterior aspect of the biliary duct above the stenotic site, one does not jeopardize its blood supply and prevents ductal ischemia that may eventually occur when a thorough dissection of this structure is attempted. Furthermore, placement of separate stitches and tying knots in small diameter ducts may represent a hazardous procedure. Because of this, it is safer to construct the posterior row of the anastomosis by means of a continuous suture with absorbable material. In fact, more recently, we have been using a continuous suture even for the anterior row. The point raised by Dr. Mercado about the difficulty in the placement of stitches in the upper angle of small intrahepatic bile ducts is very well taken. To minimize this problem, we always use a parachute technique, which was proposed for fine cardiovascular anastomosis[8]. This procedure has been recently reported for small diameter bile duct hepaticojejunostomy[9].

All the injuries reported in Mercado's[1] publication were classified as Strasberg E1 or E2. However, the major problem occurs in the Bismuth type IV and Strasberg E4 lesions, when the right and the left hepatic ducts are separated by the stricture, which occurred in 49% of our patients[6]. In this situation, a double anastomosis of each duct to the same jejunal loop must be performed, as adequate biliary drainage of both liver lobes is essential for correction of biliary stasis, which is essential for the prevention of postoperative cholangitis. Nevertheless, because discontinuity of the right and left ducts is a major factor influencing failure of the stricture repair in long-term follow-up, the most appropriate approach to this situation is represented by hepatic resection[10].

The achievement of good results in the correction of bile duct injuries depends upon the fulfillment of important technical principles, such as the maintenance of bile duct vascular supply during the maneuvers to expose its healthy portion above the stricture site and the construction of a wide watertight mucomucosal hepaticojejunostomy. These principles are fully attainable by the technique described herein.

José Eduardo M. Cunha, Marcel C.C. Machado, T. Bacchella and J. Jukemura
Department of Gastroenterology-Surgical Division, São Paulo University School of Medicine, São Paulo, Brazil
e-mail: jemcunha@yahoo.com

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Reply from Dr. Mercado:

We want to thank Dr. Monteiro da Cunha, Dr. Machado, Dr. Bacchella, and Dr. Jukemura for their interest in our work about bile duct reconstruction. Indeed, by reviewing the literature referenced in their letter, I realized that in a very important journal, the former "Surgery Gynecology and Obstetrics,"[1] they published a paper in which they described a bile duct reconstruction technique with similar characteristics to the procedure that our group described in the Journal of Gastrointestinal Surgery.

We apologize for this omission, indeed it is a very important work that needs to be recognized. We were not aware of its existence. The results reported in the late follow-up in a large series of patients have to be applauded because they have a remarkable long-term outcome.

On the technical aspects of the technique, I would like to make some observations. We recommend the "bile duct growing factor technique" for those patients who have very thin and extrahepatic preserved bile ducts. This is why our series only includes patients with Strasberg El and E2 injuries. We agree that, for the injuries in which the confluence is not preserved (either by the injury itself or as a late consequence of ischemic damage), a double anastomosis is needed. In some infrequent cases (11 out of 432 in our series), a hepatectomy of the affected lobe is needed as well.

The technique described by Dr. Machado et al., according to their publication, refers to an anastomosis done above a scarred stenosis, very similar to the technique described by Hepp[2] and Jarnaign[3] and Gutgemann[4]. When a scarred stenosis is found, usually the ducts above it are dilated (although very rare cases do not dilate), giving us the opportunity to do a wide anastomosis just above it. When a stenosis is developed, usually the injury is stable, with an ischemic level reached, so a high bilioenteric anastomosis (nonischemic, wide, and in "healthy" ducts) can be done. We also agree that by doing a cephalic dissection of the duct directed to the left one achieves better results because a higher quality duct is used in almost all instances.

The anterior section of the main ducts prevents damage to the duct circulation in the lateral aspects. Nevertheless, at the level of the lobar ducts, the distribution of the vessels becomes a net that surrounds the whole circumference of the duct.

Our technique's main target are those patients with thin ducts, identified at the index surgery, in which the ischemic damage cannot be completely assessed or in those cases with early referral that have an external fistula and/or bilioperitoneum without multiorganic failure, making them suitable for early repair. As we stated, we did a bile duct reconstruction using also this technique in two patients after liver transplant with sclerosing cholangitis who had very thin ducts.

We use 5–0 hydrolizable absorbable monofilament sutures with everted knots. We do not favor the use of running sutures because of the incidence of dehiscence (if at one point the edges suffer ischemia, the whole suture is jeopardized); however, we agree, that if it is what the surgeon prefers, it can be done. The technique leaves the distal edge of the duct open, favoring the "slide effect" of bile flow, promoting in this way the drainage. We also do not favor routine placement of stents. Most of our repairs, nowadays, are done without a stent; although at the beginning of our experience, we used a stent in the majority of cases. We place them now in selected cases[5].

As Dr. Monteiro da Cunha states, there are several techniques available to reconstruct the bile ducts. Each patient has to be individualized to select the best technique.

Miguel Angel Mercado MD

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