J Gastrointest Surg (2010) 14:1477–1482
DOI 10.1007/s11605-010-1311-y

PRESIDENTIAL ADDRESS

Zen and the Art of Surgery: How to Make Johnny a Surgeon

David Mahvi

Received: 7 May 2010 / Accepted: 9 August 2010 / Published online: 14 September 2010

Keywords Zen · Surgical Training · Intraoperative teaching

Thank you for that very generous and kind introduction.

I first want to thank a few people. My first real brush with leadership was in a parking lot in Orlando. My former boss, Bing Rikkers, was walking out of the Specialist Schools and Academies Trust (SSAT) reception in the wild animal park with my oldest son and I. Bing told me that he had a job he thought that I would enjoy. He thrust me into developing the program for this organization. He has done that for several other equally difficult, time consuming, but incredibly rewarding jobs (all of which seemed like a bit of a stretch for me). He also asked me to run our residency program which has been the source of the greatest rewards in my academic life. I will forever be grateful that he chose to come to me in Wisconsin. I hope everyone, especially the residents in the audience, can find someone like Bing that pushes you out of your comfort zone.

Second, I would like to thank Barbara Bass. Barbara, for no reason, took me under her wing. I really have no idea why she "tapped" me. There was no particular benefit to her. She introduced me to some of my closest friends and included me in events she certainly did not need to. She has been a source of advice and inspiration since the day I met her.

Last, my wife Chris who asked me not to mention that she is here. She has been there in good times and in bad, and she constantly inspires me to follow her lead and do the right thing.

So what do I talk about in a completely undefined talk? I thought initially that I would talk about research advances in gastrointestinal (GI) cancer. I then thought, maybe, I would talk about medical device development. This has both been a great scientific interest and a fun way to train students.

Bing has given some spectacular talks on leadership, which I have found really interesting. Health care reform and how to finance a surgical practice has been the focus of my academic life the last couple of years. Bing and one of my best friends, Tom Zdeblick, suggested that this should come from the heart.[1] When I thought about it, the thing that I am most passionate about is training surgeons. The thing that has consistently given me the most academic pleasure is watching a resident or a new faculty member "get it", and then succeed. The system seems to be working. Every year the people we see get smarter, but our residents do not feel competent when they finish. Five years and they are not comfortable.

Frank Lewis, the director of the American Board of Surgery (ABS), recently outlined the issues facing American surgery in the clearest terms I have seen. He acknowledged that the majority of surgical residents are seeking further training. They are doing this for many reasons, but most relevant for this discussion, many do not feel like they have the skills to independently treat surgical patients. This is reflected both in the increase in fellowship training and in the worrisome rise in the failure rate of the ABS certifying exam.[2]

Dr. Lewis concluded that we should consider earlier specialization in general surgery. This has already been done

D. Mahvi (✉)
Feinberg School of Medicine, Northwestern University,
Chicago, IL, USA
e-mail: dmahvi@nmh.org

with great success in vascular surgery. Early specialization means that specialty training is interspersed with general surgery training, with the last 18 months or so devoted to the specialty. In vascular surgery, the first 3 years are typically devoted to general surgery, the last two to vascular. The middle year is split about evenly. There are, of course, lots of details that will need to be worked out. These issues should not stop us from pursuing this course. The other option is to leave our system alone and let the market set the training. This, to me at least, means we are evading our responsibility. I think we can do better, but we need to change the way we do business. We need to do it for our trainees and for our patients. First, why is this important?

This is Gonzalo Gasca (Fig. 1). Mr. Gasca is a patient of mine. He, unfortunately, had pancreatic cancer. Mr. Gasca was in his 70s and retired after he raised his family. He did not sit around the house after he retired, but began living to work. He was one of the guys at Wrigley Field that points you to your seats (per the HIPPA guys, I cleared this with his wife). I have trouble imagining a better job.

He was treated preoperatively with chemoradiation, and after a long stretch in the OR we discovered that we were going to leave the tumor behind and had to abandon the procedure. The next morning, with some trepidation, I went to talk to him. I sat down next to his bed and told him that we left the tumor and that there was not much more that we could do. His response was, "You look like this is bothering you a lot. You did your best. I know it. That's how life goes. You need to stop thinking about this or you won't be able to focus on the next guy." He then sent me on my way. He died a couple of months later. His wife sent me a thank you note. One of the interesting things about speeches like this is that you can intersperse little parables to illustrate points like this story of Mr. Gasca.

Fig. 1 Gonzalo Gasca

A small boy was walking along a beach at low tide, where countless thousands of small sea creatures, having been washed up, were stranded and doomed to perish. A man watched as the boy picked up individual creatures and took them back into the water. "I can see you're being very kind," said the watching man, "But there must be a million of them; it can't possibly make any difference." Returning from the water's edge, the boy said, "It will for that one."

Sometimes we lose sight that, ultimately, we are training surgeons to take care of people. Individual people like Mr. Gasca. Now, we are initially training surgeons to be good at hundreds of diseases and masters of none. We should be training them to walk out of that patient's room and know that they did their best and that their best was exactly what that patient needed.

So why did this guy trust me? I suggest it is because he thought I was an expert, not that I was the best surgeon in the world or the US or Chicago, but an expert. The first question every patient asks me is: How many of these have you done? Patients know intuitively that, though volume does not equal quality, it matters, but we are not training experts in general surgery. We are not training experts in GI surgery except in a few fellowships. We are training good and competent surgeons and hoping that these really smart people will become experts.

I would like to suggest that we need to change our focus to training experts, not good surgeons, but experts in the management of GI diseases. We need to develop experts who will get better and better over their career. As other parts of *general surgery* have developed their own focus, we have retained what I consider the best part and the core of surgery—the GI tract. This core of GI surgery is markedly different than GI surgery 20 years ago. The knowledge base has expanded, most GI diseases are not approached with a big incision and most of the diseases we treat are done as teams, not as individuals. We have trained really good surgeons more and more broadly. As GI surgery has become more focused on minimal access, we have had to train for twice as many procedures. We can keep doing this and let others "finish them", or we can try to change the way we do this. So how do we deliver quality to our customer—the patient?

We are now finishing residents, competent to do a few procedures—laparoscopic cholecystectomy, hernia repair, perhaps right colectomy—but what else are they really trained to do? The world shifted while we were not watching. I know you have all heard the phrase "just a general surgeon". We have always answered the "just a general surgeon" question with an eye roll, those guys do not understand what we do. My thought is: Those guys may be right. I am not sure, but they might be right. I propose

that we need to train experts and focus on quality. I propose that we need to train experts, not just good surgeons.

Before we develop experts, we need to develop competence. Dick Bell gave a great talk at the Central Surgical last year entitled: *Why Johnny Cannot Operate*. I stole part of his title. He showed sobering data that of the 121 essential operations only ten were performed more than 20 times by the average resident. Eighty-three were performed less than five times. Nothing obscure here, these are essential procedures.[3] Dr. Bell suggested that we rethink what it means to be competent. Many essential procedures are being performed once or not at all. How can we be competent at something we have never done?

Let me highlight the average number of cases performed by our US graduating chief residents for three GI procedures: trans-anal excision of a rectal tumor, zero; bile duct exploration, one; vagotomy, zero. The surgeons we are finishing are not competent to do these operations, but maybe performing 80 laparoscopic cholecystectomies translates into competence in other GI diseases? Overwhelmingly, the evidence is no. This is clear not just in surgery but in almost any technical field. An expert pianist is not also an expert violinist.

Maybe a bowel anastomosis, though, is a bowel anastomosis, but a colectomy and an esophagectomy are not just anastomoses. If you have never done a sigmoid colectomy, no number of esophagectomies is going to teach you how to avoid injury to the ureter.

Experts tend to see patterns that become more complex as they develop expertise. They recognize when something does not fit this pattern. An expert surgeon knows when something is wrong. They, perhaps, cannot verbalize it, but the pattern is wrong. They recognize that they need to slow down when something is not right.[4] The answer, thus, is NO. Eighty laparoscopic cholecystectomies do generate expertise for laparoscopic cholecystectomies but not for colon resection. So, we are not really training surgeons for competence in the breath of GI diseases.

Can I take this argument a step further and suggest we develop experts in a smaller piece of surgery? *But*, and this is a big but, to become an expert requires 10,000 h of deliberate practice over an extended period of time.[5] It does not matter what task you pick—sports, music, chess. Ten thousand hours in one thing. Ten thousand hours of violin practice, not music practice. Does 10,000 h managing pancreatic cancer make one an expert in the management of rectal cancer? Our "general surgery" paradigm says yes. The public, the residents, and the data on survival of patients with GI cancer and surgical groups and academic departments say no.

So what is deliberate practice? It has three components—it must be beyond your current level of performance, there must be feedback, and you must be doing it not because you are required, but for its own reward.

It is easy to imagine feedback in surgery—anastomoses leak, patients die, during our training, the experts critique our performance. In chess, a grand master typically spends 4 h a day evaluating the moves of experts in other matches. An expert pianist practices alone, 4 h per day. An athlete competes with other elite athletes, but how does all this apply to the development of expert surgeons? Can we train an expert or even a competent general surgeon in our current 5-year training programs? Do our residents really spend 4 h a day in deliberate practice? At that rate, it will take 10 years for them to become an expert, but, and this is another big but, they need to continue deliberate practice for longer than we are training them. Anders Ericsson has written extensively on this topic. He presents many examples from medicine, all with basically the same outcome. Residents are better than medical students at almost any task tested, for instance, detecting an abnormality in heart sounds. Cardiologists are better still. After 10 years in practice, cardiologists remain just as good. The general practitioner, however, is not as good as the medical student (Fig. 2).

I propose that, to develop expertise, the focus has to be narrower than general surgery and maybe more narrow than GI surgery. This can obviously be taken to an extreme, experts in only right colon diverticulitis or something equally ridiculous. Broadly trained GI surgeons able to deal with GI emergencies and trauma are essential. We also need broadly trained surgeons to deal with access to care in the rural parts of the US. There will clearly be other paths to competence in general surgery—critical care, rural surgery, trauma, surgical oncology, etc.

I serve on the GI surgery advisory committee of the American Board of Surgery led by Ken Sharp which has taken on the task of restructuring GI surgery. Though I do not speak for the board, what I propose has broad support, and a consensus for GI surgery has developed.

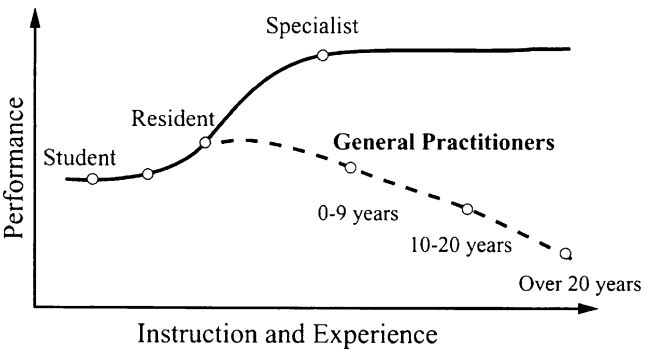

Fig. 2 Ericsson, K.A. (2004) Deliberate Practice and the Acquisition and Maintenance of Expert Performance in Medicine and Related Domains. Academic Medicine 79(10): S70-S81

If we took this broadly trained GI surgeon after 3 or 4 years and then focused their training in either colorectal disease, pancreatic/biliary/hepatic disease or foregut disease, these expert surgeons could deal with almost any GI emergency and be an expert in colorectal, pancreatic/biliary/hepatic, or foregut surgery.

I suggest that we change the way we train GI surgeons. Dick Bell suggested that we change the standards for case experience, improve operating room (OR) teaching and make operative skill a required competency. That is obviously a great start. I would move a bit beyond that and make three other recommendations for training GI surgeons.

1. Focus the first 4 years of surgical training on the development of broad competency in GI surgery. Then, focus 2 years of training on either HBP, foregut, or colorectal surgery. This will change residency training. Not every program could do everything, and some residents would need to move to obtain these last 2 years of training, but they are already moving to do fellowships, so this just makes it more formal.
2. Expand training to 6 years. The last year must be one of independence. Our residents are already training 6 or 7 years. This would assure both expertise and independence.
3. Forget about being "just a general surgeon", and instead, become an advanced GI surgeon. Advanced GI surgery must become synonymous with quality, not basic competence.

So why go there? Our trainees have adapted to our current system and are almost all selecting more training. The SCORE project of the ABS has defined a terrific curriculum for our residents. Nothing is broken. Our outcomes are the envy of the world. Our training attracts the best of the world. I suggest that we need to alter the training paradigm, mostly for the patients, but also for our trainees.

The Zen in my title: "Zen and the art of surgical training" is why we need to train expert surgeons. So that they are effortless experts and that effortless expertise generates happiness and contentment in our surgical workforce. Effortlessness is the essence of Zen.[6]

Another parable (from David Foster Wallace): There are these two young fish swimming along and they happen to meet an older fish swimming the other way who nods at them and says "Morning, boys. How's the water?", and the two young fish swim on for a bit, and then eventually one of them looks over at the other and says "What the hell is water?"[6]

I am not claiming to be the wise older fish mostly because I am not wise, but I also have this illusion that I am not getting older. The point is that, sometimes, what is the most obvious is the hardest to see. I could be speaking about the obvious conclusion that if you need 10,000 h of deliberate practice to be an expert, we are not even close to delivering it or maybe that everyone does not need to be an expert. I suspect, though, that the people in this room are either experts or want to be or they would not waste their time traveling to DDW. I am suggesting that sometimes the obvious realities are the hardest to see and for sure the hardest to discuss. So that is what I would like to finish with.

I want to speak directly to the residents and fellows and those just starting down this path. The real value of your surgical training (I am paraphrasing David Foster Wallace again here) is how to avoid becoming a comfortable, respectable, well-compensated, two-house, three-car, unhappy surgeon working endlessly at their job. The older people in the audience perhaps understand that there is a reason they call it work.

You get in your car and start driving to work, some moron in a Hummer cuts you off while talking on a cell phone, the attending anesthesiologist has refused to see your patient because the midline incision is not marked, the OR takes 2 h to turn over because the cleaning crew is short, this means you miss the soccer game for the fourth straight time. You guys are not there yet, but you will be.

Your default is: I cannot believe these overweight lazy brain-dead people are keeping me from doing what I want to do. Your default is—it is about *me*. It is easy to treat a nurse as if she (still overwhelmingly) is your servant. It is easy to treat the guys that clean the OR rooms like they do not exist *or* I can force—and I really mean force—myself to think that the cleaning crew is understaffed because one of the crew's kids was sick and the day care would not take him or the Hummer driver is taking his kid to the emergency room (ER) or the nurse has something to offer in the care of your patient.

Maybe you should listen. Listen to scrub technicians and nurses, listen to the residents, listen to your friends, and listen to yourself. Perhaps their lives are more tedious and boring than yours. It is possible, maybe not true, but possible. It takes effort to consider this, but you get to decide. You get to decide if you will pay attention, you get to decide who you will listen to, and you get to decide what has meaning. Let me illustrate with another parable from Kamala Masters.

A Buddhist practitioner went to visit her teacher. He was 84 and she was taking him to visit Buddhist sacred sites. At one point, they were in a train station. It was blazing hot. The train was 5 h late. There were no restrooms. They had no food. The station agents kept changing the track, so they had to keep getting up and moving. The student started to worry about how her teacher was holding up since she and her friends were barely coping, and he looked so frail. Finally, she decided to ask him if he was all right and he

replied, "There is heat here, but I am not hot. There is hunger here, but I am not hungry. There is irritation here, but I am not irritated."[7]

Let us go back to surgery. There is confusion here, but I am not confused. There is anger here, but I am not angry. There is irritation here, but I am not irritated. Only the surgeon can bring order from chaos and confusion in the OR. When a surgeon becomes confused, unsure what to do, irritated or angry, the system tends to fail and the danger of a bad outcome rises.

The real value of surgical education is the freedom that comes with self awareness and the flow that happens during a great surgical procedure. This means focusing completely and totally on the patient.

The alternative is a focus on something that will fade—beauty, intellect, wealth, even personal freedom. None of this has anything to do with how smart you are, though we have already selected you for that. It has to do with staying completely in the moment. This is the essence of Zen. If your mind is ready and focused, it is open to everything. If you are thinking about what happened yesterday or what you need to do tonight, there are few possibilities.

The beauty of surgery is that we have the means to get to that place every day. We just need to be in the OR; we need to be with our patients. "It's the water", but being in the OR does not mean standing around checking text messages or answering pages, it means being totally engaged.

A great book called *Flow* proposed that there were three professions in which flow was an intrinsic part of the job, professional dancing, rock climbing, and surgery. Not medicine in general, but surgery. The author Mihaly Csikszentmihalyi interviewed many creative people, and they all described the same experience (Fig. 3).[8] A reality that was different from everyday life. A pianist described it as a state of ecstasy in which the music seemed to flow out of his hands as if his hands moved by themselves. Ecstasy is a Greek word that actually means standing to the side. A poet described it as opening a door and floating through. It is not something he could force, and most of the forces were trying to keep him from opening the door.

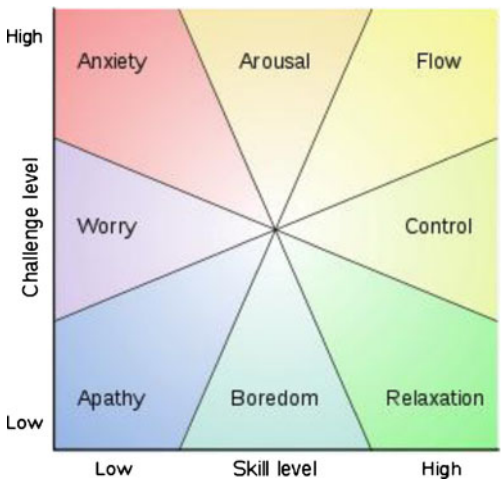

Fig. 3 Mihaly Csikszentmihalyi Flow: The psychology of optimal experience Harper 1991

The mystical part of this is that the author concludes that this is the path to true happiness. Furthermore, that this is not something that is confined to smart people or artists, but that it is available to everyone, *but*—and of course there is no free lunch—that is why they call it work. It takes 10,000 h of deliberate practice over 10 years to get there. Let me try for a minute to describe what he considered flow and apply it to surgery:

> First, there must be total concentration with clarity of the task before you.
> You must have the ability to look at a surgical problem and know what to do, the complex pattern recognition needed to know where everything is or might be.
> There must be a feeling of being totally present and totally in the moment.
> Even though it is a stretch of your ability, you must have the feeling that you have the skills to do the task. We are back to that 10,000 h.
> As things become more complex, a feeling of serenity, effortlessness, and clarity should become more noticeable, and finally
> Timelessness: that star wars coming out of hyperspace feeling after a particularly complex procedure, realizing that hours passed in what seemed to be a moment.

I suggest that you have all felt it doing something. You have felt total engagement in which you are not aware of time passing. The slowing down of an operation or a sport when it is the most dangerous. You have a feeling that your hands are just doing something and when you think about how your hands do it you lose that feeling. The feeling that your mind is not concerned with anything but what you are doing right now.

We have the ability to achieve this every day in the OR. The trick is to get there, to stay there, and to apply it to the rest of what we do; apply it to the real world of traffic and Hummers.

So this is all very esoteric and interesting, but what does it have to do with GI surgery? The system we have set up is to broadly train a minimally competent surgeon who can hopefully get more training by others and become experts.

It comes back to the fish. Why did every one of us pick surgery—not because it is easy, not because it makes us rich, but because we thought it was cool. I vividly remember watching R. Scott Jones removing a bile duct cancer when I had no real idea what a bile duct was. It was effortless without a wasted motion. That was why I wanted to be a surgeon.

We need to focus completely on developing the skills that made us want to do this in the first place. The problem is that a trainee does not typically have the adequate skills to develop this ecstasy of stepping off to the side. If you are worried about the 200 steps of a Whipple procedure you cannot develop flow, same deal with a colostomy or anything.

You get back to that pesky 10,000 h and the need for deliberate practice for its own reward because clearly you can do a procedure without flow and do a good job. The commitment to get to this level needs to be that of the violinist practicing 4 h a day for 10 years. My proposal is that it is worth it and that we should focus within our training programs to help our trainees become experts. That is how to make Johnny a surgeon. Not through curriculum, not by decreasing what we expect of them. We can make Johnny or Joannie (sorry) a surgeon by narrowing their GI focus and giving them the means to develop flow. That means deliberate practice outside their comfort zone. It means independence in the OR and in the ER. It means getting back to attracting people that want to do surgery because it is just the coolest thing in the entire world. We want those guys and gals, and we must not ever turn them off.

So my advice to the younger surgeons: Commit yourself to becoming an expert in something. Prioritize your time, and do what makes you happy. Please do not think of this as an assault on work hours. I totally support them. The first thing I tell new young faculty is to take up golf, and never ever skip a family vacation. How do you not miss that soccer game? By not planning something that you know deep down will conflict with it. Multitasking does not work.

When you are "at work" and training is not school, it is work. Watch, without distraction, someone that seems to have flow. You know who they are. Get out of the library or the cafeteria and watch people do surgery, watch them talk to patients. You did not dedicate all this time to training to not drink from it at every opportunity.

Know a disease so well that you do not have to think to know what to do. Know an operation so well that you anticipate what will happen. Know a patient so well that you know what they would want, and when you are not in the OR, focus on true happiness. Find people that make you happy and hang with them.

My mother died last October. I do not bring this up for sympathy. She developed unresectable lung cancer and was 83. She focused her life on having a gin and tonic with my wife Chris every night. Only I could make it appropriately. That hour every day with the two women of my life was a special gift. You will never know where that gift will be lurking unless you stop a bit and listen.

I am convinced that we need to change the way we make Johnny a surgeon. Our focus must shift to developing expert GI surgeons focused on specific GI diseases. We need to focus on the Zen of this wonderful thing we call surgery. It is the water that makes this all worthwhile. It is why we all chose to do this. It will bring us nothing but happiness and satisfaction. That is how to make Johnny a surgeon.

This is truly the pinnacle of my career. It is a pleasure to share this day with my closest friends. I feel like I have grown up as a surgeon in this society and thank the SSAT for this privilege.

A final parable:

A lecturer at a university is giving a pre-exam lecture on time management. On his desk is a bag of sand, a bag of pebbles, some big rocks and a bucket. He asks for a volunteer to put all three grades of stone into the bucket, and a keen student duly steps up to carry out the task, starting with the sand, then the pebbles, then the rocks, which do not all fit in the bucket.

"The is an analogy of poor time management," trills the lecturer, "If you'd have put the rocks in first, then the pebbles, then the sand, all three would have fit. This is much like time management, in that by completing your biggest tasks first, you leave room to complete your medium tasks, then your smaller ones. By completing your smallest tasks first you spend so much time on them you leave yourself unable to complete either medium or large tasks satisfactorily. Let me show you.", and the lecturer re-fills the bucket, big rocks first, then pebbles, then sand, shaking the bucket between each so that everything fits.

"But Sir," says one student, slouched at the back of the theater, "You've forgotten one thing." at which the student approaches the bucket, produces a can of beer, opens it and pours into the bucket. "No matter how busy you are," quips the student with a smile, "There's always time for a quick beer."

Thank you again for this great honor.

References

1. Zdeblick TA (2009)The second half. Spine 34(26):2835-7
2. Yeo H, Viola K, Berg D, Bell RH, et al Attitudes, training experiences, and professional expectations of US general surgery residents. JAMA 2009; 302:1301-8
3. Bell RH. Why Johnny cannot operate. Surgery 2009 146: 533-42
4. Moulton CA, Regehr G, Mylopoulos M, MacRae HM. Slowing down when you should: a new model of expert judgment. Acad Med. 2007 82(10 Suppl):S109-16
5. Ericsson, K.A. Deliberate Practice and the Acquisition and Maintenance of Expert Performance in Medicine and Related Domains. Academic Medicine 2004 79(10): S70-S81
6. David Foster Wallace This Is Water: Some Thoughts, Delivered on a Significant Occasion, about Living a Compassionate Life (Hardcover) Little Brown and company 2009
7. Toni Bernhard. How to be sick: Wisdom Publications 2010
8. Mihaly Csikszentmihalyi- Flow: The psychology of optimal experience Harper 1991

J Gastrointest Surg (2010) 14:1483–1491
DOI 10.1007/s11605-010-1322-8

2010 SSAT PLENARY PRESENTATION

Long-term Follow-up After Anti-reflux Surgery in Patients with Barrett's Esophagus

Joerg Zehetner · Steven R. DeMeester · Shahin Ayazi · Jesse L. Costales · Florian Augustin · Arzu Oezcelik · John C. Lipham · Helen J. Sohn · Jeffrey A. Hagen · Tom R. DeMeester

Received: 2 May 2010 / Accepted: 9 August 2010 / Published online: 8 September 2010

Abstract
Background Factors associated with the risk of progression of Barrett's esophagus remain unclear, and the impact of therapy on this risk remains uncertain. The aim of this study was to assess patients followed long-term after anti-reflux surgery for Barrett's esophagus.
Methods A retrospective review was performed of all patients with Barrett's who underwent anti-reflux surgery from 1989 to 2009 and had ≥5 years of follow-up.
Results There were 303 patients and 75 had follow-up ≥5 years. Median follow-up time for the 75 patients was 8.9 years (range 5–18). Regression was seen in 31%. Progression occurred in 8%, and these patients were significantly more likely to have a failed fundoplication (67% vs. 16%, $p=0.0129$). The rate of progression from non-dysplastic Barrett's to high-grade dysplasia or cancer was 0.8% per patient year, and was seven times higher in patients with a failed fundoplication.
Conclusion Compared to the accepted rate of progression of non-dysplastic Barrett's to high-grade dysplasia or cancer of 1.0% per patient year, anti-reflux surgery reduces this rate during long-term follow-up. The rate of progression was significantly lower in patients with an intact compared to a disrupted fundoplication, further suggesting that anti-reflux surgery can alter the natural history of Barrett's esophagus.

Keywords Barrett's esophagus · Anti-reflux surgery · Long-term follow-up

This paper was presented as an oral presentation at the SSAT Annual Meeting scientific session as well as in the SSAT Residents and Fellows Research Conference at the DDW 2010 in New Orleans.

J. Zehetner · S. R. DeMeester · S. Ayazi · J. L. Costales · F. Augustin · A. Oezcelik · J. C. Lipham · H. J. Sohn · J. A. Hagen · T. R. DeMeester
Department of Surgery, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

S. R. DeMeester (✉)
1510 San Pablo St, Suite 514, Los Angeles, CA 90033, USA
e-mail: sdemeester@surgery.usc.edu

Introduction

Esophageal adenocarcinoma is the fastest increasing cancer in the USA.[1] The relationship between chronic gastroesophageal reflux disease and the development of Barrett's esophagus, the precursor to esophageal cancer, is well established. Further, it is widely accepted that in patients with non-dysplastic Barrett's esophagus, the rate of progression to adenocarcinoma is 0.6% per patient year, and for progression to high-grade dysplasia or cancer 1.0% per patient year.[2,3] The impact of medical or surgical therapy for reflux disease on this rate of progression in patients with Barrett's is unclear. To date, there are no large randomized controlled trials comparing medical versus surgical therapy for Barrett's esophagus. Such trials are unlikely to be performed due to the low overall frequency of progression and the requirement for large numbers of patients to be studied for many years. From an epidemiologic standpoint,

the observation that the incidence of esophageal adenocarcinoma continues to increase despite the widespread availability and use of proton-pump inhibitors would argue against the efficacy of medical therapy for preventing progression. Likewise, population-based studies have not demonstrated a convincing reduction in the risk of progression in patients that have had anti-reflux surgery.[4] The aim of this study was to assess the rate of progression in patients with Barrett's esophagus followed long-term after anti-reflux surgery at a single institution focused on the surgical treatment of reflux disease and Barrett's esophagus for more than 20 years.

Methods

A retrospective chart review was performed of patients with Barrett's esophagus who underwent anti-reflux surgery from 1989 to 2009. Only patients with a visible segment of columnar-lined esophagus with biopsies showing goblet cells on histology were included in the study. The study was approved by the Keck School of Medicine and the University of Southern California Institutional Review Board.

Preoperative Studies

All patients underwent a pre-operative work-up including video esophagram, endoscopy with biopsies, and esophageal manometry. The length of the columnar-lined esophagus was defined as the distance between the top of the gastric rugal folds (the endoscopic gastroesophageal junction) and the highest point of the proximal extent of the columnar mucosa. The presence and size of a hiatal hernia was assessed by endoscopy and recorded in centimeters based on the distance from the gastroesophageal junction to the crural impression. Biopsies in all patients were routinely taken from the antrum and body of the stomach and from four quadrants at the gastroesophageal junction, and every 2 cm in a columnar-lined esophagus up to the squamocolumnar junction, with additional biopsies of any visible abnormalities. Biopsies were analyzed by a single expert pathologist. Esophageal motility was performed with a water-perfused system and the resting characteristics of the lower esophageal sphincter were assessed as previously described.[5] Esophageal pH monitoring was performed selectively using either a standard trans-nasal probe or the Bravo pH capsule (Given Imaging, Israel) placed 5 cm above the upper border of the manometrically defined lower esophageal sphincter as previously described.[6] Esophageal acid exposure was expressed as a composite pH score, with the upper limits of normal being 14.7 for the standard pH probe (for 24-h period) and 16.0 for the Bravo pH capsule (for 48-h period).[6] Prior to pH testing proton pump inhibitors were discontinued for 2 weeks.

Postoperative Studies

After surgery, all patients were enrolled in an annual endoscopic surveillance program using the same biopsy protocol performed during the pre-operative evaluation. During each endoscopy, the fundoplication was assessed in the retroflexed view and classified as either intact or disrupted, based on the integrity of the fundoplication and crural repair. Manometry and ambulatory esophageal pH studies were obtained selectively.

Statistical Analysis

Data are expressed as median and interquartile range (IQR). Comparisons of proportions were performed using chi-square or Fisher's exact test. Continuous variables were compared using the Mann–Whitney test or Kruskal–Wallis test. A p value of less than 0.05 was considered as significant.

Results

There were 303 patients with Barrett's esophagus who underwent anti-reflux surgery during the study period. There was no mortality and there were no major complications. Minor complications occurred in 9.6% of patients (Table 1).

Among the 303 patients, there were 245 patients that had their procedure performed prior to 2004, and were eligible for inclusion in the long-term follow-up group. However, only 75 of these patients had consistent follow-up at our center beyond 5 years. In these 75 patients, the median follow-up after fundoplication was 8.9 years (range 5–18, IQR 7–12), for a total of 705 patient years of follow-up. Demographic data, patient characteristics, and length of Barrett's esophagus in these patients are shown in Table 2.

Table 1 Minor complications

All patients (n=303)

	n	Percentage
Delayed gastric emptying	9	3
Atrial fibrillation	5	1.7
Pneumonia	3	1
Wound infection	3	1
Urinary tract infection	3	1
Anemia	2	0.7
Diarrhea	2	0.7
Chylothorax	1	0.3
Esophageal hematoma	1	0.3
TOTAL	29	9.6

In 67 patients, there was no dysplasia; while in eight patients, low-grade dysplasia was present on pre-operative biopsies. The majority of patients had a Nissen fundoplication (Table 3). Reoperation was required in 18 patients (24%) for wrap disruption±recurrent hernia or progression.

Status of the Fundoplication

Postoperative pH monitoring was performed in 30/75 patients (40%) at a median of 47 months (IQR 31–87) after the anti-reflux surgery. Most commonly, the pH study was obtained electively in asymptomatic patients to confirm function of the fundoplication, and in this group an abnormal test occurred in 13% (Table 4). When the pH test was performed because a failed fundoplication was suspected on endoscopy it was positive in every patient, suggesting that endoscopic evaluation of a fundoplication is a reliable method to assess for a failed procedure.

Surveillance endoscopy was performed annually, and post-operative endoscopy reports were available in all 75 of the long-term follow-up patients. At the time of the most recent endoscopy, 60 patients (80%) had an intact fundoplication. There was no significant difference in the prevalence of a failed fundoplication comparing the laparoscopic, transthoracic, and transabdominal approach (17% vs. 23% vs. 29%, p=0.6815). The median time to identify a failed fundoplication was 112 months (IQR 98–141), and was similar for complete and partial fundoplications (122 vs. 112 months, p=0.4818).

Status of the Barrett's

Regression

After fundoplication, regression occurred in two forms, loss of intestinal metaplasia and loss of dysplasia. A decreased length of columnar mucosa was not considered regression, and complete loss of intestinal metaplasia was not considered present until two endoscopies with biopsies confirmed the absence of goblet cells.

Regression occurred in 23 of the 75 patients (31%). In the 67 patients with non-dysplastic Barrett's pre-operatively, 17 patients (25%) had complete loss of intestinal metaplasia. In the eight patients with preoperative low-grade dysplasia, regression was seen in six (75%); in five patients there was loss of dysplasia and one there was loss of intestinal metaplasia (Table 5).

Progression

After fundoplication, progression consisted of the development of dysplasia or adenocarcinoma from non-dysplastic Barrett's, or development of high-grade dysplasia or cancer from low-grade dysplasia. In total, progression occurred in 6/75 patients (8%) at a median of 94 months (Table 6). Progression occurred in 5% of the 67 patients with non-dysplastic Barrett's, and was to high-grade dysplasia in 4 patients and cancer in 1 patient (Fig. 1). In the eight patients with pre-operative low-grade dysplasia, the only progression was to cancer in one patient (Fig. 2). Thus, in this series only 2 patients progressed to cancer.

All six patients were treated for their progressive disease. Treatment in the four patients that developed high-grade dysplasia was endoscopic resection and ablation in two patients and esophagectomy in two patients. Treatment in the two patients that progressed to cancer was endoscopic resection and ablation in one and esophagectomy in the other patient. To date, no patient with progression has died from esophageal cancer.

Rate of Progression

The rate of progression from non-dysplastic Barrett's esophagus to adenocarcinoma in this cohort was 0.16% per patient year. However, four patients were treated for progression to high-grade dysplasia, confounding this analysis. The rate of progression from non-dysplastic Barrett's to high-grade dysplasia was 0.64% per patient year, and to either high-grade dysplasia or cancer was 0.8% per patient year. The rate of progression to cancer in

Table 2 Demographics, patient characteristics, and length of Barrett's esophagus in the long-term follow-up patients (n=75)

	Barrett's, no dysplasia n=67	Low-grade dysplasia n=8	TOTAL n=75
Age in years	53.2 (44–59)	58.6 (55–65)	54.5 (47–61)
Gender: male/female	45/22	6/2	51/24
FU time in years	8.9 (7–11)	9.3 (8–13)	8.9 (7–12)
Long-segment Barrett's esophagus	39%	63%	41%
Length of Barrett's esophagus in cm	2 (1–5)	3 (2–9)	2 (1–5)
Hiatal hernia	82%	100%	84%
Size of hernia in cm	4 (3–5)	5 (3–6)	4 (3–5)

Values are medians (IQR)

Table 3 Type of operation in the long-term follow-up patients (n=75)

	n	Percentage
Laparoscopic Nissen fundoplication	41	54.7
Transthoracic Nissen fundoplication	18	24
Transabdominal Nissen fundoplication	5	6.7
Transabdominal Collis Nissen fundoplication	2	2.7
Transthoracic Collis–Belsey fundoplication	6	8
Transthoracic Belsey fundoplication	2	2.7
Laparoscopic Toupet fundoplication	1	1.3

patients with preoperative low-grade dysplasia was 1.2% per patient year.

Patients with progression were significantly more likely to have a failed fundoplication (Fig. 3). In patients with a failed fundoplication, the rate of progression from non-dysplastic Barrett's esophagus to high-grade dysplasia or cancer was 2.6% per patient year, compared to 0.36% per patient year for those with an intact fundoplication. The rate of progression in patients with long versus short segment Barrett's was not significantly different (1% with long segment versus 0.68% with short segment, p=0.2).

Discussion

The management of Barrett's esophagus continues to be controversial over 50 years after its description by Dr. Norman Barrett.[7] Given the risk of progression to cancer, most physicians recommend surveillance endoscopy once a diagnosis of Barrett's has been made. However, the cost/benefit ratio of surveillance depends significantly on the frequency of progression, and there are some that dispute the recommendation for surveillance in patients with non-dysplastic Barrett's.[8–10] Others are promoting ablation of the Barrett's to reduce the risk of progression, and perhaps eliminate the need for surveillance.[11]

Probably the most controversial issue regarding management of Barrett's esophagus pertains to therapy for the accompanying reflux disease. Barrett's esophagus develops as a consequence of long-standing gastroesophageal reflux, and most patients with reflux are managed with acid suppression medications, typically proton pump inhibitors. In patients with Barrett's, it is unclear what the end-point of medical therapy should be: control of symptoms or normalization of acid exposure by pH testing. Control of symptoms is certainly the easiest approach; but in Barrett's patients often heartburn symptoms are minimal or absent since the columnar mucosa is less sensitive to acid. Further, control of symptoms may have little or no impact on the natural history of the disease. An alternative approach is to escalate the medical therapy until acid exposure in the distal esophagus has been normalized by pH testing. This approach is challenging since suppression of gastric acid production 24 h a day, 7 days a week is difficult.[12] Further, even with effective acid suppression patients with Barrett's may have continued regurgitation symptoms as a consequence of a hiatal hernia and incompetent lower esophageal sphincter, and impedance studies have shown that the number of reflux events is unchanged.[13] Most concerning, though, is that reflux of weakly acidic material may be more injurious than reflux of strongly acid material since pH 3–5 is the range where bile salts are most able to enter cells and promote injury.[14–18] Clinically, there is speculation that prolonged and perhaps inadequate acid suppression therapy may actually promote the development and progression of Barrett's.[19]

Anti-reflux surgery abolishes reflux and eliminates the concern regarding continued reflux of gastric contents in patients with Barrett's. Logically, elimination of reflux should promote a quiescent state in the Barrett's, and potentially reduce the risk of progression. This concept is supported at the molecular level by studies showing that anti-reflux surgery reduced or normalized the expression of genes potentially involved in the progression of Barrett's to cancer.[20–22] Clinically, a number of studies have shown that anti-reflux surgery alters the natural history of Barrett's.[23–26] In the only randomized trial comparing medical therapy to anti-reflux surgery for patients with Barrett's esophagus, Parrilla et al. [27] reported that patients with a functioning fundoplication had a significantly reduced incidence of developing dysplasia compared to medically treated patients. Contrasting these findings, two large population studies from Sweden showed that anti-reflux surgery was not protective against progression to cancer. However, the serious flaw in both these studies is that the prevalence of Barrett's was not known, and it is quite likely that far more patients in the anti-reflux surgery group had Barrett's

Table 4 Results of pH testing in the long-term follow-up patients (n=30)

Indication	n	Abnormal pH testing
Evaluation of reflux control (asymptomatic)	15	2 (13%)
Failed fundoplication on endoscopy	11	11 (100%)
Recurrent reflux symptoms	4	2 (50%)

Table 5 Patients with preoperative Barrett's esophagus with LGD (n=8)

Pt	Length (cm)	Regression	Progression	Last biopsy	FU-time (months)
1	10	No	No	LGD	85/130[a]
2	1	Yes	No	No IM	161
3	8	Yes	No	IM	117
4	3	Yes	No	IM	149
5	11	Yes	No	IM	98
6	2	No	Yes	IMC[b]	85
7	2	Yes	No	IM	98
8	3	Yes	No	IM	150

IM intestinal metaplasia, *LGD* low-grade dysplasia, *IMC* intramucosal cancer

[a] Last biopsy after 85 months, Patient died unrelated to LGD after 130 months

[b] Transhiatal esophagectomy (final pathology IMC)

compared to the control group.[4,28] Since the presence of Barrett's is the leading known risk factor for subsequent development of esophageal adenocarcinoma, both studies only add to the controversy rather than providing any reliable answer to this important issue.

In this study of patients with follow-up beyond 5 years after anti-reflux surgery, we showed that progression of Barrett's to cancer was uncommon, occurring in only two patients. In one of these patients, the cancer was detected at 3 years after anti-reflux surgery. In this patient, the molecular mechanisms driving progression likely were already in motion prior to the anti-reflux procedure. This patient had an intact fundoplication, and as has been suggested in our previous review of the literature this is the case for most cancers that occur within 5 years of antireflux surgery, while cancers developing later tend to be in patients with a failed fundoplication.[29]

The overall rate of progression to cancer from non-dysplastic Barrett's in our study was 0.16% per patient year, but our analysis for progression to cancer was confounded by the fact that we treated four patients when high-grade dysplasia was identified. Whether any of these patients would have progressed to cancer had they not been treated is unknown, but the literature would suggest that the rate of progression of high-grade dysplasia to cancer is approximately 6.6% per patient year.[2] When we evaluated progression from non-dysplastic Barrett's to either high-grade dysplasia or cancer in our patients, the rate was 0.8% per patient year. These rates are lower than expected based on published rates of progression for non-dysplastic Barrett's to high-grade dysplasia or cancer in 1.0%.[3] Further, progression to cancer in our patients with low-grade dysplasia pre-operatively was 1.2%, also lower than the published rate of 1.7%.[2] This decreased rate of progression after anti-reflux surgery for both non-dysplastic Barrett's and for patients with low-grade dysplasia suggests that the fundoplication is impacting the natural history of this disease. Further evidence that the fundoplication is altering the risk of progression is our finding that the risk of progression is significantly increased in patients with a failed fundoplication. We calculated the risk of progression to be increased sevenfold in those with a failed fundoplication compared to those with a functioning fundoplication. Logically, if the fundoplication was not altering the risk of Barrett's progression then whether it was intact or failed should not influence the rate of progression. Lagergren et al.[30] also noted that patients with persistent reflux after a fundoplication were more likely to develop cancer than control patients. Consequently, the important question at this point is not whether antireflux surgery reduces the risk of Barrett's progression, but whether the altered risk is similar or greater than that with medical therapy in these patients.

We found that at the time of the latest endoscopy 20% of our fundoplications appeared to have failed. While we strive for a 0% failure rate, that is unrealistic in any patient,

Table 6 Characteristics of patients with progression (n=6)

Pt	Age (years)	Barrett's length (cm)	Initial histology	Final histology	Time (months) to progression	Failed fundoplication
1	55	10	IM	HGD	115	Yes
2	51	6	IM	HGD	89	Yes
3	51	8	IM	HGD	98	Yes
4	61	1	IM	HGD	135	Yes
5	61	1	IM	IMC	36	No
6	62	8	LGD	IMC	85	No

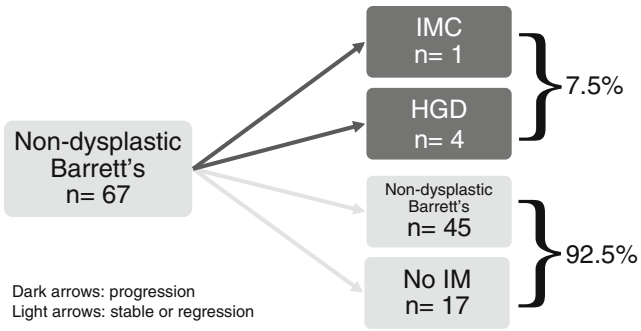

Fig. 1 Progression occurred in 7.5% of the 67 patients with non-dysplastic Barrett's, and was to low-grade dysplasia in zero, high-grade dysplasia in 6%, and cancer in 1.5%

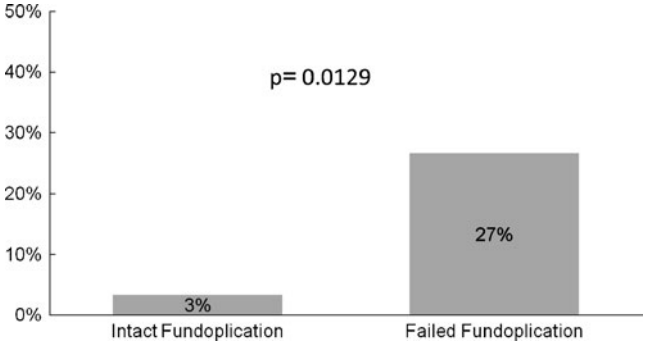

Fig. 3 The prevalence of progression was significantly increased in patients with a failed fundoplication

and certainly in patients with advanced reflux disease. Likewise, we would like for no patient to develop cancer after a fundoplication, but that again is unrealistic and should not be the bar set for anti-reflux surgery. It is not standard to require that a medical or surgical therapy reduce the risk of a disease down to baseline for the general population for it to be considered beneficial. For example, no matter how aggressive, medical therapy for coronary artery disease will not reduce the risk of a myocardial infarction back to the baseline risk for a population without coronary artery disease. Instead, the accepted standard is that the therapy reduces the risk compared to those that are untreated or treated with an alternative therapy. With this concept in mind, we believe that anti-reflux surgery is being held to an unfair standard in regards to prevention of progression of Barrett's, as evidenced by the recent study published by Lagergren et al.[28] where the risk of cancer in patients that had anti-reflux surgery was compared to the general Swedish population, and the conclusion was that anti-reflux surgery did not prevent cancer. The same study could be done for medical therapy of heart disease, and certainly the findings would be that the medical therapy does not prevent myocardial infarction. Similarly, it is clear that proton pump inhibitor therapy does not prevent esophageal adenocarcinoma. The control population for these comparisons needs to be those with the disease, in the case of esophageal adenocarcinoma those with reflux disease and Barrett's esophagus, not the general population. Had Lagergren et al. compared the risk of cancer in those with reflux and Barrett's esophagus treated with either PPI or anti-reflux surgery the findings would have been meaningful.

In addition to a failed fundoplication representing a risk factor for progression, we found that progression tended to occur more commonly in long segments of Barrett's. In four of the six patients with progression the length of Barrett's was 6 cm or longer. Importantly, though, progression occurred in two patients with only 1 cm tongues of columnar mucosa, confirming that any Barrett's has to be taken seriously as a risk for cancer. This emphasizes the importance of complete eradication of Barrett's if ablation therapy is adopted, and the importance of continued surveillance in any patient with Barrett's esophagus. In our patients with progression, three were treated with endoscopic resection and ablation. Endoscopic therapy was successful in all 3 patients, with no recurrence of high-grade dysplasia or cancer to date.

Although most of the focus in patients with Barrett's is on progression, it is important to recognize that regression after antireflux surgery was common, occurring in 31% of our 75 long-term follow-up patients. Further, loss of intestinal metaplasia on two consecutive endoscopies occurred in 24% of patients. In these patients, surveillance endoscopy can be stretched out for many years or perhaps eliminated provided their fundoplication remains intact.

We recognize that there are shortcomings to our study, including the fact that of the 245 patients that could have been evaluated beyond 5 years after anti-reflux surgery we were only able to report on 75, but that is the reality in many centers in the USA where patients move and change insurance carriers. It is possible that some cancers developed in these patients, but there is no reason to think that our 75 patients were biased against the development of cancer compared to the other 245 patients. Instead, patients

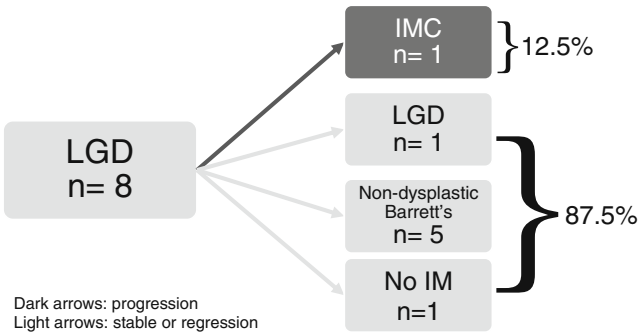

Fig. 2 In the eight patients with pre-operative low-grade dysplasia, progression to high-grade dysplasia occurred in none of the patients and to cancer in one (12.5%) patient

that return to us for all their follow-up tend to be the more difficult or complex patients, and are likely to have a higher rather than lower risk for failure of their fundoplication or progression of their Barrett's. We also would have preferred to have pH monitoring on all patients after antireflux surgery, but were only able to get 15 asymptomatic patients to agree to a routine post-operative pH study. Lastly, our calculation for the rate of progression to cancer was confounded by the treatment of high-grade dysplasia in four patients, but by combining the rates of progression to high-grade dysplasia and cancer this issue was largely addressed.

Conclusion

In conclusion, we have shown that anti-reflux surgery for patients with non-dysplastic Barrett's and low-grade dysplasia is safe, and likely reduces the rate of progression. Whether it reduces the risk of progression to a similar or greater extent than medical therapy for reflux disease and Barrett's remains an important and unanswered question. Compared to patients with an intact fundoplication, those with failure of their fundoplication were at increased risk for progression. Either a reoperation or aggressive medical therapy is recommended in these patients. Annual surveillance endoscopy allowed timely detection of progression, and endoscopic or surgical therapy was successful in these patients, with no cancer deaths to date.

References

1. Pohl H, Welch HG: **The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence**. *J Natl Cancer Inst* 2005, **97**(2):142–146.
2. Wani S, Puli SR, Shaheen NJ, Westhoff B, Slehria S, Bansal A, Rastogi A, Sayana H, Sharma P: **Esophageal adenocarcinoma in Barrett's esophagus after endoscopic ablative therapy: a meta-analysis and systematic review**. *Am J Gastroenterol* 2009, **104**(2):502–513.
3. Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ: **Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis**. *Clin Gastroenterol Hepatol*, **8**(3):235–244; quiz e232.
4. Ye W, Chow WH, Lagergren J, Yin L, Nyren O: **Risk of adenocarcinomas of the esophagus and gastric cardia in patients with gastroesophageal reflux diseases and after antireflux surgery**. *Gastroenterology* 2001, **121**(6):1286–1293
5. Zaninotto G, DeMeester TR, Schwizer W, Johansson KE, Cheng SC: **The lower esophageal sphincter in health and disease**. *Am J Surg* 1988, **155**(1):104–111.
6. Ayazi S, Lipham JC, Portale G, Peyre CG, Streets CG, Leers JM, Demeester SR, Banki F, Chan LS, Hagen JA *et al*: **Bravo catheter-free pH monitoring: normal values, concordance, optimal diagnostic thresholds, and accuracy**. *Clin Gastroenterol Hepatol* 2009, **7**(1):60–67.
7. Barrett NR: **The lower esophagus lined by columnar epithelium**. *Surgery* 1957, **41**(6):881–894.
8. Somerville M, Garside R, Pitt M, Stein K: **Surveillance of Barrett's oesophagus: is it worthwhile?** *Eur J Cancer* 2008, **44**(4):588–599.
9. Sharma P, Sidorenko EI: **Are screening and surveillance for Barrett's oesophagus really worthwhile?** *Gut* 2005, **54 Suppl 1**: i27–i32.
10. Inadomi JM, Sampliner R, Lagergren J, Lieberman D, Fendrick AM, Vakil N: **Screening and surveillance for Barrett esophagus in high-risk groups: a cost-utility analysis**. *Ann Intern Med* 2003, **138**(3):176–186.
11. Das A, Wells C, Kim HJ, Fleischer DE, Crowell MD, Sharma VK: **An economic analysis of endoscopic ablative therapy for management of nondysplastic Barrett's esophagus**. *Endoscopy* 2009, **41**(5):400–408.
12. Mainie I, Tutuian R, Castell DO: **Addition of a H2 receptor antagonist to PPI improves acid control and decreases nocturnal acid breakthrough**. *J Clin Gastroenterol* 2008, **42**(6):676–679.
13. Vela MF, Camacho-Lobato L, Srinivasan R, Tutuian R, Katz PO, Castell DO: **Simultaneous intraesophageal impedance and pH measurement of acid and nonacid gastroesophageal reflux: effect of omeprazole**. *Gastroenterology* 2001, **120**(7):1599–1606.
14. Schweitzer EJ, Bass BL, Batzri S, Harmon JW: **Bile acid accumulation by rabbit esophageal mucosa**. *Dig Dis Sci* 1986, **31**(10):1105–1113.
15. Armstrong D, Rytina ER, Murphy GM, Dowling RH: **Gastric mucosal toxicity of duodenal juice constituents in the rat. Acute studies using ex vivo rat gastric chamber model**. *Dig Dis Sci* 1994, **39**(2):327–339.
16. Chiu PW, Ayazi S, Hagen JA, Lipham JC, Zehetner J, Abate E, Oezcelik A, Hsieh CC, DeMeester SR, Banki F *et al*: **Esophageal pH exposure and epithelial cell differentiation**. *Dis Esophagus* 2009, **22**(7):596–599.
17. DeMeester TR, Peters JH, Bremner CG, Chandrasoma P: **Biology of gastroesophageal reflux disease: pathophysiology relating to medical and surgical treatment**. *Annu Rev Med* 1999, **50**:469–506.
18. Theisen J, Peters JH, Fein M, Hughes M, Hagen JA, Demeester SR, Demeester TR, Laird PW: **The mutagenic potential of duodenoesophageal reflux**. *Ann Surg* 2005, **241**(1):63–68.
19. Kauer WK, Peters JH, DeMeester TR, Ireland AP, Bremner CG, Hagen JA: **Mixed reflux of gastric and duodenal juices is more harmful to the esophagus than gastric juice alone. The need for surgical therapy re-emphasized**. *Ann Surg* 1995, **222**(4):525–531; discussion 531–523.
20. Oh DS, DeMeester SR, Vallbohmer D, Mori R, Kuramochi H, Hagen JA, Lipham J, Danenberg KD, Danenberg PV, Chandrasoma P *et al*: **Reduction of interleukin 8 gene expression in reflux esophagitis and Barrett's esophagus with antireflux surgery**. *Arch Surg* 2007, **142**(6):554–559; discussion 559–560.
21. Vallbohmer D, DeMeester SR, Oh DS, Banki F, Kuramochi H, Shimizu D, Hagen JA, Danenberg KD, Danenberg PV, Chandrasoma PT *et al*: **Antireflux surgery normalizes cyclooxygenase-2 expression in squamous epithelium of the distal esophagus**. *Am J Gastroenterol* 2006, **101**(7):1458–1466.
22. Babar M, Ennis D, Abdel-Latif M, Byrne PJ, Ravi N, Reynolds JV: **Differential molecular changes in patients with asymptomatic long-segment Barrett's esophagus treated by antireflux surgery or medical therapy**. *Am J Surg*, **199**(2):137–143.
23. Katz D, Rothstein R, Schned A, Dunn J, Seaver K, Antonioli D: **The development of dysplasia and adenocarcinoma during endoscopic surveillance of Barrett's esophagus**. *Am J Gastroenterol* 1998, **93**(4):536–541.

24. Oelschlager BK, Barreca M, Chang L, Oleynikov D, Pellegrini CA: **Clinical and pathologic response of Barrett's esophagus to laparoscopic antireflux surgery**. *Ann Surg* 2003, **238**(4):458-464; discussion 464–456.
25. Gurski RR, Peters JH, Hagen JA, DeMeester SR, Bremner CG, Chandrasoma PT, DeMeester TR: **Barrett's esophagus can and does regress after antireflux surgery: a study of prevalence and predictive features**. *J Am Coll Surg* 2003, **196**(5):706–712; discussion 712–703.
26. Hofstetter WL, Peters JH, DeMeester TR, Hagen JA, DeMeester SR, Crookes PF, Tsai P, Banki F, Bremner CG: **Long-term outcome of antireflux surgery in patients with Barrett's esophagus**. *Ann Surg* 2001, **234**(4):532–538; discussion 538–539.
27. Parrilla P, Martinez de Haro LF, Ortiz A, Munitiz V, Molina J, Bermejo J, Canteras M: **Long-term results of a randomized prospective study comparing medical and surgical treatment of Barrett's esophagus**. *Ann Surg* 2003, **237**(3):291–298.
28. Lagergren J, Ye W, Lagergren P, Lu Y: **The risk of esophageal adenocarcinoma after antireflux surgery**. *Gastroenterology* 2010: in press.
29. DeMeester SR, Peters JH, Demeester TR: **Barrett's esophagus**. *Curr Probl Surg* 2001, **38**(8):558–640.31.
30. Lagergren J, Viklund P: **Is esophageal adenocarcinoma occurring late after antireflux surgery due to persistent postoperative reflux?** *World J Surg* 2007, **31**(3):465–469.

Discussant

Dr. Jeffrey l. Ponsky (Cleveland, OH, USA): I am delighted and proud to review this paper. This group always presents provocative and insightful assessments of current therapies for what we do in surgery, and this paper is no exception. And it took great work and courage to go back and muster these patients.

This disease has changed in our careers. This disease has changed so that we don't see squamous carcinoma anymore, we see Barrett's adenocarcinoma. And it's happening quickly.

The logic in doing an antireflux procedure to relieve the insult to the esophagus is clear. This paper doesn't answer all the questions because it's a retrospective study, but it's a good beginning.

I question the relationship between your visual inspection of a failed Nissen and the pH study, which was not done in every patient that had a visual inspection showing a failed wrap. That would be important to do in any future study.

The question of whether the degree of disruption was related to the degree in pH failure would also help us know some things. Furthermore, and finally, I would like to ask you a couple of questions about future work.

Number one, does an intact wrap—and many endoscopists ask this—interfere with the good surveillance of the lower EG junction in biopsying the Barrett's in these patients?

Number two, now that we have ways to effectively ablate the mucosa, wouldn't it make sense, in conjunction with the antireflux surgery, even in the nondysplastic Barrett's, where we see a 7.5% conversion to either intramucosal cancer or high-grade dysplasia, to ablate all Barrett's, dysplastic or nondysplastic, prior to doing a wrap, and really give it the full go?

Closing Discussant

Dr. Joerg Zehetner: The first question was if an intact wrap impairs the surveillance biopsies. I don't think so. I don't think there's a problem in surveillance biopsies if carefully done when the wrap is intact.

The second question, that's a good point. There are a lot of papers coming out or studies, and we will hear about them during the next few days, of ablation therapy for low grade or nondysplastic Barrett's esophagus. It has been shown that in patients with low grade dysplasia regression after antireflux surgery occurs in 75%. So for low-grade dysplasia the best initial option is an antireflux procedure.

Whether an ablation for any Barrett's is useful or not has to be shown in long term studies. And as we know from our patients who progressed, four of them had long segment Barrett's esophagus but two of them had a short segment of 1 cm. So if ablation is performed or if you think of doing ablation for nondysplastic Barrett's esophagus, you would have to remove 100% of the Barrett's esophagus to make sense. And surveillance is still necessary.

To come back to your comment in the beginning, that's one of the limitations in our study that we don't have 100% pH data postoperatively. So when we calculated our risk and rate of progressions, we tried to somehow group these patients into those with evidence of an intact versus failed fundoplication.

Accepting that the definitions are not 100% precise, we still believe that the rate of progression is much higher if you have a failed fundoplication.

Discussant

Dr Carlos A. Pellegrini (Seattle, WA, USA): About 8 years ago or so, your group reported here the rate of regression for Barrett's esophagus following an antireflux operation. My recollection is that you were around 20 or 30% regression.

As you know, we reported a regression rate of 33% among a group of 106 patients with Barrett's esophagus followed for 5 years. In your presentation you have primarily focused on the progression of Barrett's. Can you tell us whether you observed regression? And in particular, how many patients with short-segment Barrett's had no Barrett's at the end of the 5-year period?

Closing Discussant

Dr. Joerg Zehetner: We had 31% with regression. These are 23 patients, 17 of these patients were patients who regressed from nondysplastic Barrett's esophagus to no IM. We had it on the slides with the flow chart. And from the low grade dysplasia patients, we had four patients who regressed to Barrett's esophagus, nondysplastic Barrett's esophagus, and we had one patient who regressed to squamous mucosa.

Discussant

Dr. Dan Smith (Jacksonville, FL, USA): I'm curious about the timing from fundoplication to when progression took place. You had followup out to 8 or 9 years. How many of these progressed within the first year after fundoplication versus much later?

Closing Discussant

Dr. Joerg Zehetner: We had one patient who progressed after 3 years. So in this patient—and this was the patient who had an intact fundoplication—

we assumed that the cascade from Barrett's esophagus, metaplasia, dysplasia, high grade dysplasia, cancer, was already on its way.

In a review about 10 years ago, it was suggested that progression that occurred during the first 5 years after the fundoplication, most of the time, this sequence was already on the way. The other five patients that progressed in our study were at about 90 to 120 months after the initial operation, so nearly an average of 10 years after the initial operation. In these patients, we consider that they progressed due to failure of the fundoplication.

Discussant

Dr. Jeff Peters (Rochester, NY, USA): A fascinating observation buried in there if I read your data right is that you had a 500% decrease in the progression in nondysplastic, about a 40% or 50% decrease in low-grade and a very small decrease in high-grade. The question then is, do you think there's a real difference in whether we can impact progression between those three states?

Is it harder to impact progression depending on whether it's nondysplastic, low-grade, or high-grade?

Closing Discussant

Dr. Joerg Zehetner: So I think high grade is a different topic, because we didn't start out with any high grade dysplasia in this population. All the patients had preoperatively either nondysplastic Barrett's or low grade dysplasia. And I think we can impact low grade dysplasia very good with an antireflux surgery, as 75% of them regressed. One patient went to cancer. He was operated with a transhiatal esophagectomy. It was intramucosal cancer, the patient is still alive. And one patient was stable at low grade dysplasia, and died last year after 15 years of follow-up, unrelated to the low grade dysplasia.

So I think we can impact low grade dysplasia very good with an antireflux surgery.

J Gastrointest Surg (2010) 14:1492–1501
DOI 10.1007/s11605-010-1328-2

2010 SSAT PLENARY PRESENTATION

Patient and Peri-operative Predictors of Morbidity and Mortality After Esophagectomy: American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP), 2005–2008

Birat Dhungel · Brian S. Diggs · John G. Hunter · Brett C. Sheppard · John T. Vetto · James P. Dolan

Received: 5 May 2010 / Accepted: 9 August 2010 / Published online: 8 September 2010

Abstract
Purpose Our aim was to determine what specific patient and peri-operative factors contribute to major complications after esophagectomy.
Methods Using the American College of Surgeons National Surgical Quality Improvement Program database, data for esophagectomies between the years 2005 and 2008 were extracted and analyzed. Thirty-day post-operative complications were classified into seven major groups: (1) wound infections, (2) respiratory complications (pneumonia, intubation), (3) cardiac complications, (4) deep venous thrombosis, (5) sepsis/septic shock, (6) re-operation, and (7) death. Univariate analysis and logistic regression modeling were performed to determine if a significant association existed between patient factors or peri-operative factors and these post-operative complications.
Results One thousand thirty-two patients who underwent esophagectomy were identified. Diabetes was the strongest pre-operative independent predictor of death (odds ratio (OR) 10.98; 95% confidence interval (CI) 1.37–1.15, $p<0.1$) or respiratory (OR 1.86; 95% CI 1.03–3.29, $p=0.04$) or cardiac (OR 5.14; 95% CI 1.93–13.20, $p<0.01$) complications following esophagectomy. Thoracotomy performed during the operation was not associated with an increased risk of respiratory or cardiac complications.
Conclusions The major predictors of morbidity after an esophagectomy are the patient factors of diabetes, dyspnea, peripheral vascular disease, and cerebrovascular accident while the peri-operative factors are pre-operative international normalized ratio, contaminated wound classification, and American Society of Anesthesiologists class. Similarly, the major predictors of mortality are diabetes, dyspnea, and age for patient factors and contaminated wound classification for peri-operative factors.

Keywords Esophagectomy · Peri-operative factors · Esophageal morbidity · Esophageal mortality · ACS-NSQIP

Presented at the 51st annual meeting of the Society for Surgery of the Alimentary Tract, New Orleans, LA, USA, 1 May–5 May, 2010.

B. Dhungel · B. S. Diggs · J. G. Hunter · B. C. Sheppard · J. T. Vetto · J. P. Dolan (✉)
Division of General Surgery, Department of Surgery,
Oregon Health and Science University,
3181 SW Sam Jackson Park Road, L223A,
Portland, OR 97239, USA
e-mail: dolanj@ohsu.edu

Introduction

Esophagectomy remains the standard surgical treatment of high-grade dysplasia and invasive esophageal cancer in medically fit patients. However, despite technical advances and improvements in peri-operative care, the procedure is still associated with high morbidity, high mortality, and a protracted recovery period.[1–3] Because of these observations, there continues to be considerable interest in identifying specific factors that contribute to complications or death after esophagectomy in order to attempt to mitigate the risks associated with this procedure.

A number of prior studies have attempted to identify predictors of morbidity and mortality after esophagectomy,[1–8] but results have been mixed. Two recent multi-institutional studies[1,2] have investigated these issues in different patient groups using the Department of Veterans Affairs National Surgical Quality Improvement Program (VA-NSQIP) database and the Society of Thoracic Surgeons General Thoracic Database (STS-GTB). Both these studies identified predictors of major morbidity and mortality after esophagectomy and advocated risk stratification of patients before the procedure.

In an attempt to further clarify these issues, our aim was to determine what specific patient and peri-operative factors contribute to major complications after esophagectomy by using the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) database. For the purpose of this study, we classified complications into seven major groups: wound infections, respiratory complications, cardiac complications, deep venous thrombosis (DVT), sepsis/septic shock, need for re-operation, and death. We then investigated the association between specific patient and peri-operative variables as collected by ACS-NSQIP and these post-operative complication groups.

Materials and Methods

Study Population

For this retrospective study, the population was drawn from the ACS-NSQIP database between the years 2005 and 2008. The ACS-NSQIP is a nationally validated, risk-adjusted, outcomes-based program that attempts to quantify the quality of contemporary surgical care. The program collects data on 136 pre-operative and intra-operative variables and also reports 30-day post-operative morbidity and mortality. ACS-NSQIP samples the first 40 cases performed within consecutive 8-day cycles from general surgery, vascular surgery, and specific subspecialty procedures, and the data are collected, validated, and submitted by a trained surgical clinical reviewer at each site. The program limits the sampling volume related to herniorrhaphies, breast lumpectomies, and laparoscopic cholecystectomies because of the low incidence of morbidity and mortality associated with these procedures. It contains 152,490 cases submitted from 121 sites between 2005 and 2006 and 211,407 cases submitted from 186 sites in 2007. In 2008, 271,368 cases were submitted from 211 hospitals. Approximately half of the participating sites are community hospitals as defined by ACS-NSQIP.[9]

Data Extraction and Factors Impacting Analysis and Modeling

For each year under review, patients who had undergone esophagectomy were identified and extracted from the overall dataset using the appropriate procedure codes as defined by the *Current Procedural Terminology* (*CPT*), 2008 edition. Procedure-specific CPT codes are listed in the Appendix.

Based on clinical relevance and frequency of occurrence within NSQIP, complications from these esophagectomy procedures were identified and divided into seven major groups: wound infections, respiratory complications (pneumonia, intubation), cardiac complications (myocardial infarction, cardiac arrest requiring cardiopulmonary resuscitation), DVT, sepsis/septic shock, re-operation, and death. Specific patient and peri-operative variables were then selected for analysis for their contribution to these complications based on our determination of their importance to peri-operative and post-operative complication.

For the purpose of our analysis, patients with dyspnea on exertion and at rest were combined into one group (N=110, 11%) and compared with patients without dyspnea (N=922, 89%). Diabetic patients on oral agents (N=120, 12%) and those on insulin therapy (N=35, 3%) were each separately compared with non-diabetics (N=877, 85%). Similarly, patients with contaminated wounds (N=32, 3%) and dirty/infected wounds (N=16, 2%) were each separately compared with clean contaminated wounds (N=984, 95%). There were no clean wounds. For the American Society of Anesthesiologists' (ASA) physical status classification, patients in ASA I (N=15, 1%), ASA III (N=691, 67%), and ASA IV and V (N=85, 8%) were each separately compared with patients classified as ASA II (N=241, 23%). Race was initially evaluated as a variable in our analysis but was not found to be significantly associated with complications and was subsequently excluded from review.

Data Analysis

Data extraction for the primary (esophagectomy) procedures and statistical analysis was performed using R Software (R Foundation for Statistical Computing, Vienna, Austria; Version 2.10.0, 2009). For univariate analysis, continuous variables were compared using the Mann–Whitney U test to compare medians given the nonparametric distribution of the data. Categorical variables were compared using the chi-square test to compare proportions. Univariate models were then generated, for each complication group. Patient and peri-operative variables found to be have $p<0.2$ under univariate analysis

were included in a multivariate logistic regression model in order to examine the relationship between these variables and the outcomes of interest (post-operative complications). Separate multivariate models were created for the seven complication groups.

Results

A total of 1,032 patients who had undergone esophagectomy for various reasons were identified. The ten most common indications for esophagectomy are shown in Table 1. There were 818 males and 241 females with a mean age of 62.8 years. The majority of the patients were Caucasians (N=850, 82%), followed by African Americans (N=33, 3%), Hispanics (N=31, 3%), Asians (N=26, 3%), and American Indians (N=4, 0%). The distribution of patient and peri-operative risk factors for our study population is shown in Table 2.

The total in-hospital and 30-day mortality was 3.0% (30 out of 1,032 patients), and morbidity was 50% (518 out of 1,032 patients). Respiratory complications were the most frequently reported events (27%), and these included patients who had pneumonia, unplanned intubation, or were ventilator dependent >48 h. Sepsis and septic shock was the second most common morbidity (23%), followed by wound complications (21%), re-operation (14%), DVT (5%), and cardiac complications (3%).

Univariate analysis of patient and peri-operative factors is summarized in Tables 3 and 4, respectively. Patient factors, including age, diabetes (diabetes mellitus), basal mass index (BMI), smoking, alcohol consumption (ETOH), dyspnea, history of chronic obstructive pulmonary disease (HxCOPD), steroid use, hypertension, history of cardiac surgery (HxCS), peripheral vascular disease (PVD), history of cerebrovascular accident (HxCVA), and weight loss were all significantly associated with adverse post-operative outcomes (p<0.05 in all cases). Similarly, peri-operative factors such as radiation within 90 days, advanced ASA class, pre-operative white blood cell count (WBC), international normalized ratio (INR), serum albumin level as well as wound class, emergency case, operation time, and intra-operative blood transfusion were also significantly associated (p<0.05) with one or more complication group.

Patient and peri-operative factors found to be significant in univariate analysis (p<0.2) were then subjected to multivariate regression modeling. A contaminated wound was the strongest predictor of mortality (odds ratio (OR) 18.52; 95% confidence interval (CI) 2.94–108.76, p<0.01). Diabetes was also a strong independent predictor for death (OR 10.98; 95% CI 1.37–64.79, p<0.01) or respiratory (OR 1.86; 95% CI 1.03–3.29, p=0.04) or cardiac (OR 5.14; 95% CI 1.93–13.20, p<0.01) complications following esophagectomy (Table 5). Dyspnea was found to be the third largest risk factor for death (OR 6.248; 95% CI 1.85–20.53, p<0.01). Thoracotomy performed during the operation was not associated with an increased risk of respiratory or cardiac complications.

Discussion

The incidence of esophageal adenocarcinoma is rising at an alarming rate in Western populations.[10] Esophagectomy plays an important role in the treatment of this disease but continues to be a high-risk procedure with significant morbidity and mortality.[11–13] Previous analyses of large national databases have reported in-hospital or 30-day mortality rates between 2.7% and 9.8%.[1,2,4] Other studies have suggested that outcomes are influenced by case volume,[5,12] while others have argued that patient-based factors have the greater influence on inpatient mortality than case volume alone.[6] Taken together, these reports suggest that combination of different factors may have either direct or indirect effects on patient outcomes and mortality after esophageal surgery.

In this study, we have examined the contribution of both patient and peri-operative factors to mortality and morbidity after esophagectomy using the ACS-NSQIP. We found 30-day mortality of 3.0% which is within the range reported in prior studies.[1,2,4] We also found a 50% morbidity which is also similar to previous reports. [1,2,7]

We identified a number of patient and peri-operative variables that are associated with mortality and morbidity after esophagectomy. Three of these factors (age, diabetes, and contaminated wound) had an impact on both morbidity and mortality, while one variable (dyspnea) was associated with mortality alone and nine (smoking within a year pre-operatively, ETOH, PVD, history of cerebrovascular accident with neurological deficit, steroid use, pre-operative WBC count, pre-operative INR, ASA class III, operation time, thoracotomy, and intra-operative blood transfusion) were significantly associated with morbidity only. Some of the patient factors (BMI, history of severe COPD, steroid, hypertension, history of cardiac surgery, >10% loss body weight in last 6 months) and some peri-operative factors (chemotherapy within 30 days pre-operatively, decreased pre-operative hematocrit and platelet levels, increased pre-operative sodium levels, emergency cases) were statistically significant in the univariate analysis but were found to be insignificant in the multivariate models. These findings may be attributable to the low frequency of occurrence of these factors reported in the ACS-NSQIP database.

Table 1 Ten most common indications for esophagectomy: ACS-NSQIP, 2005–2008

ICD-9 codes	Indication	*N* (%)
151.0	Malignant neoplasm of cardia	264 (26)
150.9	Malignant neoplasm of esophagus unspecified site	238 (23)
150.5	Malignant neoplasm of lower third of esophagus	195 (19)
150.8	Malignant neoplasm of other specified part of esophagus	48 (4.6)
530.85	Barrett's esophagus	39 (3.8)
151	Malignant neoplasm of stomach	31 (3.0)
530.4	Stricture and stenosis of esophagus	22 (2.13)
530.0	Achalasia and cardiospasm	19 (1.8)
150.4	Malignant neoplasm of middle third of esophagus	16 (1.6)
150	Malignant neoplasm of esophagus	15 (1.5)

ACS-NSQIP American College of Surgeons National Surgical Quality Improvement Program, *ICD-9 codes* International Classification of Diseases, 9th Revision

Table 2 Distribution of patient and peri-operative risk factors of patients undergoing esophagectomy

	N (% or mean)
Patient factors	
DM (insulin); diabetes mellitus requiring insulin	35 (3%)
DM (oral); diabetes mellitus requiring oral agents	120 (12%)
Smoke; current smoker <1 year pre-operatively	159 (25%)
ETOH; alcohol >2 drinks/day in 2 weeks before admission	54 (5%)
HxPVD; history of revascularization/amputation for peripheral vascular d/s	28 (3%)
BMI; body mass index (missing in 13 patients)	1,019 (27 kg/m^2)
Dyspnea	110 (11%)
HxCOPD; history of severe COPD	69 (7%)
HTN; hypertension requiring medication	525 (51%)
HxCS; previous cardiac surgery	72 (7%)
HxCVA; history of cerebrovascular accident with neurological deficit	16 (2%)
Weight loss; >10% loss body weight in last 6 months	215 (21%)
Steroid; chronic oral or intravenous corticosteroids <30 days pre-operatively	20 (2%)
Peri-operative factors	
Chemo; chemotherapy for malignancy <30 days pre-operatively	77 (7%)
Rad; radiotherapy for malignancy <90 days pre-operatively	249 (24%)
ASA I; American Society of Anesthesiologists Physical Status class I	15 (1%)
ASA III; American Society of Anesthesiologists Physical Status class III	691 (67%)
ASA IV/V; American Society of Anesthesiologists Physical Status class IV/V	85 (8%)
PrWBC; pre-operative WBC (missing in 30 patients)	1,002 (6.7 k/ml)
PrHct; pre-operative hematrocrit (missing in 28 patients)	1,004 (38.1%)
PrPlatelet; pre-operative platelet count (missing in 31 patients)	1,001 (250.5 k/ml)
PrINR; pre-operative international normalized ratio (missing in 259 patients)	773 (1.05)
PrNa; pre-operative sodium (missing in 45 patients)	987 (138.8 mEq/l)
PrBUN; pre-operative blood urea nitrogen (missing in 61 patients)	971 (15.8 mg/dl)
PrBilirubin; pre-operative total bilirubin (missing in 244 patients)	788 (0.57 mg/dl)
PrAlkPhos; pre-operative alkaline phosphatase (missing in 246 patients)	786 (85.0 IU/l)
PrAlbumin; pre-operative serum albumin (missing in 249 patients)	783 (3.9 mg/dl)
Emergency case	19 (2%)
Thoracotomy	599 (58%)
Contaminated case	32 (3%)
Dirty/infected case	16 (2%)
OR time; total operation time	1,032 (338.1 min)
RBC; intra-operative blood transfusion	288 (27.9%)

Table 3 Univariate analysis of patient factors in relationship to post-operative complications following esophagectomy ($p<0.2$)

	Complications (p value)*						
	Wound	Resp.	Cardiac	DVT	Sepsis	Re-op.	Death
Patient factors							
Age	0.06	**0.01**	0.09	**<0.01**	NS	NS	**<0.01**
DM (insulin)	NS	0.11	0.13	NS	0.48	NS	**0.03**
DM (oral)	NS	**<0.01**	**<0.01**	NS	**<0.01**	NS	**0.03**
BMI	0.09	NS	NS	NS	NS	**0.04**	NS
Smoking	NS	0.14	NS	0.18	**0.03**	NS	NS
ETOH	NS	**0.15**	NS	NS	0.06	NS	NS
Dyspnea	NS	**<0.01**	NS	NS	**0.05**	0.11	**<0.01**
HxCOPD	*0.19*	**<0.01**	NS	NS	**<0.01**	NS	NS
Steroid use	NS	**0.02**	NS	NS	0.20	NS	NS
HTN	NS	**0.03**	**0.03**	NS	0.14	0.17	NS
HxCS	NS	0.11	NS	NS	**<0.01**	NS	NS
HxPVD	**0.02**	**<0.01**	NS	**0.03**	NS	NS	**0.02**
HxCVA	NS	NS	NS	**<0.01**	NS	NS	NS
Weight loss	NS	NS	NS	NS	0.19	0.08	**0.01**

NS not significant, *Resp.* respiratory, *DVT* deep venous thrombosis, *Re-op.* re-operation, *DM* (*oral*) diabetes mellitus requiring oral agents, *BMI* body mass index, *ETOH* alcohol greater than two drinks per day in 2 weeks before admission, *HxCOPD* history of severe COPD, *HTN* hypertension requiring medication, *HxMI* history of myocardial infarction 6 months pre-operatively, *HxCS* previous cardiac surgery, *HxPVD* history of peripheral vascular disease, *HxCVA* history of cerebrovascular accident with neurological deficit

*$p<0.05$ in bold

We found that increasing patient age significantly increases the risk of death after esophagectomy although determination of an age cutoff for this increased risk was beyond the scope of our current study. Age has been associated with mortality after esophagectomy in numerous other studies investigating different patient populations.[1,2,14] Recently, Pultrum and colleagues concluded that advanced age (>70 years) had a minor influence on

Table 4 Univariate analysis of peri-operative factors in relationship to post-operative complications following esophagectomy ($p<0.2$)

	Complications (p value)*						
	Wound	Resp.	Cardiac	DVT	Sepsis	Re-op.	Death
Peri-op. factors							
Chemotherapy	NS	0.08	NS	NS	NS	0.17	NS
Radiation	**0.03**	NS	NS	0.14	NS	NS	NS
ASA I	NS	0.97	0.99	NS	0.97	0.31	NS
ASA III	NS	**0.05**	0.17	NS	**<0.01**	0.19	NS
ASA IV/V	NS	**0.02**	**0.03**	NS	**<0.01**	**0.02**	NS
prWBC	NS	**<0.01**	**<0.01**	0.10	NS	NS	0.09
prHct	0.06	NS	NS	NS	NS	NS	NS
prPlatelet	0.19	0.08	NS	NS	NS	NS	0.19
prINR	NS	**0.03**	NS	NS	0.09	NS	0.14
prNa	NS	NS	NS	NS	NS	NS	0.14
prBUN	NS	NS	0.12	NS	NS	NS	NS
prBili	NS	NS	NS	NS	NS	0.11	0.12
prAlkPhos	NS	NS	NS	0.11	0.07	0.15	NS
prAlbumin	NS	**<0.01**	NS	NS	NS	NS	0.19
Emergency	NS	NS	**0.05**	NS	NS	**<0.01**	NS
Thoracotomy	0.14	NS	NS	NS	NS	0.06	NS
Contaminated	0.08	**0.04**	NS	NS	**0.05**	0.78	**0.04**
Dirty/Infected	0.18	**<0.01**	NS	NS	0.36	**<0.01**	0.99
Operation time	0.19	**<0.01**	**0.01**	**0.02**	**<0.01**	**<0.01**	NS
RBC	0.11	**0.02**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**0.02**

NS not significant, *Resp.* respiratory, *DVT* deep venous thrombosis, *Re-op.* re-operation, *ASA* American Society of Anesthesiologists Physical Status class, *PrWBC* pre-operative WBC, *PrHct* pre-operative hematrocrit, *PrPlatelet* pre-operative platelet count, *PrINR* pre-operative international normalized ratio, *PrNa* pre-operative sodium, *PrBUN* pre-operative blood urea nitrogen, *PrBili* pre-operative total bilirubin, *PrAlkPhos* pre-operative alkaline phosphatase, *PrAlbumin* pre-operative serum albumin, *RBC* intra-operative blood transfusion

*$p<0.05$ in bold

Table 5 Multivariate analysis of patient and peri-operative factors associated with significant morbidity and mortality following esophagectomy

	Complication	Odds ratio (95% CI)	*p* value
Patient factors			
Age	Wound infection	0.986 (0.972–0.999)	0.03
	DVT	1.034 (1.003–1.069)	0.04
	Death	1.077 (1.020–1.145)	0.01
Diabetes	Sepsis/septic shock	1.991 (1.087–3.570)	0.02
	Respiratory	1.856 (1.029–3.294)	0.04
	Cardiac	5.137 (1.934–13.196)	<0.01
	Death	10.978 (1.366–64.787)	0.01
Smoking	Respiratory	1.615 (1.010–2.571)	0.04
Alcohol	Respiratory	2.678 (1.168–6.053)	0.02
Dyspnea	Death	6.248 (1.846–20.528)	<0.01
Peripheral vascular disease	Wound infection	2.431 (1.015–5.562)	0.04
	DVT	6.259 (1.640–19.561)	<0.01
CVA	DVT	7.063 (1.360–28.593)	<0.01
Peri-operative factors			
Pre-operative WBC count (k/ml)	Cardiac	1.122 (1.015–1.247)	0.03
Pre-operative INR	Sepsis/septic shock	2.279 (1.204–6.329)	0.04
Contaminated wound	Wound infection	2.766 (1.230–5.961)	0.01
	Sepsis/septic shock	3.196 (1.115–8.899)	0.03
	Death	18.518 (2.936–108.775)	<0.01
ASA class III	Sepsis/septic shock	1.913 (1.036–3.777)	0.05
Operation time	Sepsis/septic shock	1.002 (1.000–1.003)	0.05
	Respiratory	1.002 (1.001–1.004)	<0.01
	Cardiac	1.004 (1.002–1.007)	<0.01
Thoracotomy	Wound infection	0.712 (0.513–0.988)	0.04
Intra-operative blood transfusion	DVT	1.135 (1.043–1.252)	<0.01
	Sepsis/septic shock	1.094 (1.019–1.192)	0.02
	Re-operation	1.098 (1.025–1.188)	0.01

post-operative course, recurrent disease, and survival in patients who underwent an extended esophagectomy.[15] All patients in their study were operated on by an experienced team of surgeons at a high-volume university medical center. It is not surprising that age has a direct influence on outcomes after major operations as elderly patients generally have more major comorbidities that may complicate operations including decreased capacity to adapt to stress, greater functional impairment, cardiovascular disease, cerebrovascular disease, and diminished cognitive function.[16]

Diabetes was the strongest independent patient factor predictive for death and respiratory or cardiac complications following esophagectomy. Diabetic patients in our cohort were taking oral hypoglycemic agents more frequently than they were using insulin (12% and 3%, respectively). Overall, diabetes is a chronic debilitating disease that has been linked to poor outcomes after a large number of surgical procedures. Its primary means of contribution to these outcomes appears to be principally due to end organ damage, which also may explain the increased risk of cardiac events in these patients (OR 5.14; 95% CI 1.93–13.20, $p<0.01$). Our current findings of diabetes as the single largest independent patient risk factor for death after esophagectomy (OR 10.98; 95% CI 1.37–64.79, $p<0.01$) adds further evidence to that already reported. Bailey and colleagues looked at 1,777 patients in the VA-NSQIP database and found almost twice the increased risk of dying associated with this diagnosis.[1] Likewise, Wright and colleagues reported increased risk of complications (OR 1.19; 95% CI 1.05–1.36, $p<0.01$) related to diabetes when reporting on their series of 2,315 patients from the STS-GTB database.[2]

Dyspnea was the second leading patient risk factor for death (OR 6.25; 95% CI 1.85–20.53, $p<0.01$). Dyspnea remains a strong indicator of cardiopulmonary disease, including congestive heart failure (CHF) and chronic obstructive pulmonary disease (COPD). Bailey and colleagues reported dyspnea and COPD to be major preoperative factors impacting morbidity.[1] Wright and col-

leagues also found that their patients with a forced expiratory volume at 1 second (FEV1) <60% of predicted or those with CHF had significantly increased adverse outcomes post-operatively. Surprisingly, dyspnea was not directly associated with respiratory complications in our study population, but this may again be related to sample size. We found that diabetes, smoking, ETOH, and operation time were associated with respiratory complications. Respiratory complications such as pneumonia and intubation were the most commonly reported complications in this study. Bailey and colleagues have also reported these same variables as impacting morbidity after esophagectomy.[1]

Although a number of other reports have implicated the thoracotomy portion of an esophageal resection as the leading contributor to post-operative pulmonary complications, this is not a significant association based on this study. We have found that thoracotomy performed as part of the procedure imparts a decrease in risk for wound infection (OR 0.71; 95% CI 0.51–0.99, $p=0.04$), and the reason for this particularly "counterintuitive" finding remains unclear.

Similar to our current study, Wright and colleagues examined the association between patient factors such as BMI, history of PVD, history of COPD, hypertension (HTN) and chronic steroid use, and adverse outcome. They found that history of PVD, HTN, and steroid use significantly affected outcome.[2] In the current study, even though we found significant association between most of these factors and adverse outcome in the univariate analysis, only PVD was found to be significant in the multivariate model. Patients with history of PVD were found to be greatly susceptible to DVT (OR 6.26; 95% CI 1.64–19.56, $p<0.01$) and have more than doubled the risk for wound infection (OR 2.43; 95% CI 1.02–5.56, $p=0.04$). In addition, we were also able to associate CVA with DVT (OR 7.06; 95% CI 1.36–28.59, $p<0.01$). An association between CVA and DVT has been shown in several other reports[17,18] and is not an original finding of our investigation.

Peri-operative factors such as pre-operative leukocytosis and decreased INR were associated with increased risk of cardiac complications and sepsis or septic shock, respectively. ASA class, which has been used as a surrogate for comorbidities, has been associated with morbidity in a number of studies.[1,2] In adding to these findings, we associated ASA class III patients with increased risk of sepsis and septic shock (OR 1.91; 95% CI 1.04–3.78, $p=0.05$).

Prolonged operation time and intra-operative blood transfusion were also found to be associated with significant complications after an esophagectomy. Presence of these variables may represent advanced disease or a patient with numerous other morbidities. This association has been reported by various studies in the past.[1,19] Similarly, contaminated wound class and its association with poor outcomes are not a new finding,[20] but the magnitude of its association with mortality in our study (OR 18.52; 95% CI 2.94–108.78, $p<0.01$) is somewhat surprising. The 95% CI reported with this variable is very broad, and this, in turn, suggests that a number of other factors may be confounding the association between wound class and death.

There are several limitations associated with the use of ACS-NSQIP database. In some cases, our sample size for this relatively uncommon operation may have caused difficulty in obtaining statistically significant associations between some known patient and peri-operative variables and post-operative outcomes. Although the ACS-NSQIP reliably and prospectively collects pertinent historical, laboratory, intra-operative, and patient data for surgical procedures covering a number of specialties, the program does not allow for procedure-specific data collection. Some information such as pre-operative variables (pulmonary function tests), operative data (tumor histology and stage), and procedure-specific complications (anastomotic leaks) that could contribute more detail to our analysis are not available. In the current database format, anastomotic leaks are not reported directly and are most likely recorded as deep wound infections which may function as a rough surrogate for this occurrence. In this study, total wound complication rate was 21% (220 out of 1,032) which included both superficial and deep wound infections. A final limitation is our decision to examine a limited number of variables available from the ACS-NSQIP database which appeared to have the greatest affect on post-operative morbidity and mortality instead of analyzing the impact of all available NSQIP variables. The possibility of false-positive statistical findings is always a concern when working with a large number of statistical comparisons. However, the likelihood of such a random event becomes less likely when the same factor is found to be associated with multiple complication groups. Despite these limitations, our study is one of the largest current reviews of patient and peri-operative variables impacting morbidity and mortality after esophagectomy as derived from multiple institutions across the USA.

Conclusion

Our findings show that the major predictors of morbidity after an esophagectomy are the patient factors of diabetes, dyspnea, peripheral vascular disease, and CVA while the peri-operative factors are pre-operative INR, contaminated wound classifica-

tion, and ASA class. Similarly, the major predictors of mortality are diabetes, dyspnea, and age for patient factors and contaminated wound classification for peri-operative factors.

Appendix

Procedure-specific CPT codes

CPT code	Procedure	Cases
43107	Total or near total esophagectomy, without thoracotomy; with pharyngogastrostomy or cervical esophagogastrostomy, with or without pyloroplasty (transhiatal)	422
43108	Total or near total esophagectomy, without thoracotomy; with colon interposition or small intestine reconstruction, including intestine mobilization, preparation and anastomosis(es)	11
43112	Total or near total esophagectomy, with thoracotomy; with pharyngogastrostomy or cervical esophagogastrostomy, with or without pyloroplasty	179
43113	Total or near total esophagectomy, with thoracotomy; with colon interposition or small intestine reconstruction, including intestine mobilization, preparation, and anastomosis(es)	15
43116	Partial esophagectomy, cervical, with free intestinal graft, including microvascular anastomosis, obtaining the graft and intestinal reconstruction	4
43117	Partial esophagectomy, distal two thirds, with thoracotomy and separate abdominal incision, with or without proximal gastrectomy; with thoracic esophagogastrostomy, with or without pyloroplasty (Ivor Lewis)	204
43118	Partial esophagectomy, distal two thirds, with thoracotomy and separate abdominal incision, with or without proximal gastrectomy; with colon interposition or small intestine reconstruction, including intestine mobilization, preparation, and anastomosis(es)	12
43121	Partial esophagectomy, distal two thirds, with thoracotomy only, with or without proximal gastrectomy, with thoracic esophagogastrostomy, with or without pyloroplasty	22
43122	Partial esophagectomy, thoracoabdominal or abdominal approach, with or without proximal gastrectomy; with esophagogastrostomy, with or without pyloroplasty	135
43123	Partial esophagectomy, thoracoabdominal or abdominal approach, with or without proximal gastrectomy; with colon interposition or small intestine reconstruction, including intestine mobilization, preparation, and anastomosis(es)	12
43124	Total or partial esophagectomy, without reconstruction (any approach), with cervical esophagostomy	16

CPT codes Current Procedural Terminology procedure codes as derived from *Current Procedural Terminology*, 2007 edition

References

1. Bailey SH, Bull DA, Harpole DH, Rentz JJ, Neumayer LA, Pappas TN, Daley J, Henderson WG, Krasnicka B, Khuri SF. Outcomes after esophagectomy: a ten-year prospective cohort. Ann Thorac Surg. 2003; 75: 217–22.
2. Wright CD, Kucharczuk JC, O'Brien SM, Grab JD, Allen MS. Predictors of major morbidity and mortality after esophagectomy for esophageal cancer: a Society of Thoracic Surgeons General Thoracic Surgery Database risk adjustment model. J Thorac Cardiovasc Surg. 2009; 137: 587–95.
3. Bartels H, Stein HJ, Siewert JR. Preoperative risk analysis and postoperative mortality of esophagectomy for resectable esophageal cancer. Br J Surg 1998; 85: 840–4.
4. Kohn GP, Galanko JA, Meyers MO, Feins HR, Farrell TM. National trends in esophageal surgery—are outcomes as good as we believe? J Gastrointestinal Surg. 2009; 13: 1900–12.
5. Chang AC, Birkmeyer JD. The volume performance relationship in esophagectomy. Thorac Surg Clin. 2006; 18: 87–94.
6. Rodgers M, Jobe BA, O'Rourke RW, Sheppard B, Diggs B, Hunter JG. Case volume as a predictor of inpatient mortality after esophagectomy. Arch Surg. 2007; 142: 829–39.
7. Atkins BZ, Shah AS, Hutcheson KA, Mangum JH, Pappas TN, Harpole DH, D'Amico TA. Reducing hospital morbidity and mortality following esophagectomy. Ann Thorac Surg. 2004; 78: 1170–6.
8. Karl RC, Schreiber R, Boulware D, Baker S, Coppola D. Factors affecting morbidity, mortality, and survival in patients undergoing Ivor Lewis esophagogastrectomy. Ann Surg. 2000; 231: 635–43.
9. ACS-NSQIP Program Specifics, Surgical Case Inclusion/Exclusion Overview. Available at: http://acsnsqip.org/main/program_case_inclusion_exclusion.asp Accessed April 15, 2010.
10. Trivers KF, Sabatino SA, Stewart SL. Trends in esophageal cancer incidence by histology, United States, 1998–2003. Int J Cancer 2008; 123: 1422–1428.
11. Muller JM, Erasmi H, Stelzner M, Zieren U, Pichlmaier H. Surgical therapy for esophageal carcinoma. Br J Surg 1990; 77: 845–57.
12. Birkmeyer JD, Siewers AE, Finlayson EVA, Stukel TA, Lucas FL, Batista I, Welch HG, Wennberg DE. Hospital volume and surgical mortality in the United States. N Eng J Med 2002; 346: 1128–37.
13. Dimick JB, Pronovost PJ, Cowan JA, Lipsett PA. Complications and costs after high-risk surgery: where should we focus quality improvement initiatives. J Am Coll Surg 2003; 196: 671–78.
14. Ferguson MK, Martin TR, Reeder LB, Olak J. Mortality after esophagectomy: risk factor analysis. World J Surg. 1997; 21: 599–603.
15. Pultrum BB, Bosch DJ, Nijsten MWN, Rodgers MGG, Groen H, Slaets JPJ, Plukker JTM. Extended esophagectomy in elderly patients with esophageal cancer: minor effect of age alone in determining the postoperative course and survival. Ann Surg Oncol. 2010; 17(6):1572–80.
16. Orringer MB. Age does not preclude an esophagectomy...if only it were that simple. Ann Surg Oncol. 2010; 17(6):1487–9.
17. Kelly J, Rudd A, Lewis RR, Coshall C, Moody A, Hunt BJ. Venous thromboembolism after acute ischemic stroke: a prospective study using magnetic resonance direct thrombus imaging, Stroke 2004; 35: 2320–355.
18. CLOTS Trials Collaboration, Dennis M, Sandercock PA, Reid J, Graham C, Murray G, Venables G, Rudd A, Bowler G. Effectiveness of thigh-length graduated compression stockings to reduce the risk of deep vein thrombosis after stroke (CLOTS trial 1): a multicentre, randomised controlled trial. Lancet 2009; 373:1958–965.
19. Mangran AJ, Horan TC, Pearlson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, Infect Control Hosp Epidemiol 1999; 20:247–278.

20. Culver DH, Horan TC, Gaynes RP, Martone WJ, Jarvism WR, Emori G, Banerjee S, Edwards JR, Tolson JS, Henderson TA, Hughes JM. Surgical wound infection rates by wound class, operative procedure, and patient risk index. Am J Med 1991; 91:152–157.

Discussant

Dr. Henry Pitt (Indianapolis, IN): I would like to congratulate the authors on using the ACS-NSQIP database to determine which risk factors lead to bad outcomes after esophagectomy.

The NSQIP database is robust, certainly compared to many single-institution studies, but it may not be mature enough yet to determine exactly which risk factors result in a bad outcome after esophagectomy.

My group has been working with the statisticians at the college over the last year with respect to pancreatectomy. In that analysis, we have over 7,000 operations to analyze. However, the College statisticians are concerned that enough data are not yet available to properly analyze esophagectomy.

I have three questions.

The first question has to do with your choice to use only the 2005, 2006, and 2007 data which include 600-plus operations. In fact, as of last summer, the 2008 data were available, and there were another 400-plus esophagectomies in the database. Including the 2008 data would have given you more than a thousand rather than 600 cases to analyze. Why didn't you use the most recent Participant Use File data?

The second question has to do with your statistical analysis. You did a univariate analysis of 36 factors, looking at seven different outcomes, which gave you 252 separate univariate analyses. Again, in working with statisticians at the college, they have lumped the analyses into mortality, serious morbidity, and overall morbidity, or just three outcomes. Among the seven outcomes that you chose to analyze, three of them had a very low incidence. DVT was only 5%, mortality only 4%, and cardiac only 2%. Therefore, you were not likely to see differences when the percentage of the complication was so low.

Thirdly, when you went from the univariate to the multivariable analysis, you came up with 14 variables that were significant. In the manuscript, in your conclusion, you only picked three of those 14 as being important on the basis of their hazard ratios. However, the others were just as statistically significant. You chose diabetes, peripheral vascular disease, and dyspnea, which are hard to alter. Why not choose parameters such as low hematocrit or radiation therapy or blood transfusions that you might be able to affect.

In summary, I applaud your efforts, but I think that this report is a little premature and needs better statistical support.

Closing Discussant

Dr. Birat Dhungel: For the first question regarding the use of data from 2005 to 2007 only, when we started this project, ACS-NSQIP had not published their 2008 data. But now since it is available, we will definitely look into it to yield a more statistically robust analysis.

For your second question, where we used only 36 variables for univariate analysis, actually we had used more than 36. I think we had about 58 variables that we looked into but the ones I showed in those two tables in the PowerPoint are the ones with a p value less than 0.05, that is, significant ones in univariate analysis. I think this is also related to low number of patients with those factors reported in the NSQIP database.

For example, we also looked at something like race, which other studies have found to be significant. But in our review, it was not significant. So we dropped that off, too.

And for your third question about focusing on those three factors, dyspnea, diabetes, and peripheral vascular disease, you're right; I focused on those because of their strong association with increased risk for morbidity and mortality. These factors that I listed, I think, are chronic issues and can also be addressed and more so at the primary care level.

Discussant

Dr. Steven Demeester (Los Angeles, CA): One of the leading causes of morbidity and mortality after esophagectomy is anastomotic complications and graft ischemia. We previously looked at that and found that factors that correlated with anastomotic complications were diabetes, peripheral vascular disease, and neoadjuvant therapy.

Did you look at what caused the morbidity and mortality in your analysis here of global morbidity? Because I suspect that what you are finding then is those same factors are causing anastomotic complications, leaks, graft ischemia, and subsequent complications.

Secondly, did you analyze it based on squamous versus adenocarcinoma to see differences in the different tumor histologies?

Closing Discussant

Dr. Birat Dhungel: For your first question about anastomotic leaks, one of the limitations with the use of NSQIP database is that it does not report on procedure-specific complications. So I think anastomotic leaks here are likely included in the deep wound infections. But they do not list it separately, so we could not analyze it.

No, we did not analyze cases for adenocarcinoma versus squamous cell carcinoma separately. That's definitely something that can be done using this database.

Discussion of Paper #1044 (40)

Title of Paper: Patient and Peri-operative Predictors of Morbidity and Mortality After Esophagectomy: American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP), 2005–2007.

Discussant

Dr. Robert Rout (Gainesville, FL): In my experience, patients with cancer have severe problems with nutrition. And did you look at the albumin and the prealbumin in these patients?

Closing Discussant

Dr. Birat Dhungel: We did and we did not find it to be significant in this study. This can be looked at again in the future when we have more patients added to the data set.

Discussant

Dr. Henry Pitt (Indianapolis, IN): Additional work has been done to develop procedure-specific outcomes for esophagectomy, pancreatectomy, hepatectomy, and other procedures. Going forward, your

hospitals will be able to keep track of new preoperative, intra-operative, and post-operative variables that are procedure specific. Therefore, when the new "procedure targeted" module becomes available, I would recommend that your hospitals switch to this option.

Discussant

Dr. Margo Shoup (Maywood, IL): I noticed that you included patients who had ASA classes 4 and 5 undergoing esophagectomy. Those had to have been emergent cases. I would encourage you to exclude those patients and just look at the patients that are undergoing elective esophagectomy.

Closing Discussant

Dr. Birat Dhungel: That's a very good suggestion. I think we had less than 10% of patients with ASA classes IV and V combined. Thank you.

J Gastrointest Surg (2010) 14:1502–1510
DOI 10.1007/s11605-010-1315-7

2010 SSAT PLENARY PRESENTATION

Eating Behavior in Rats Subjected to Vagotomy, Sleeve Gastrectomy, and Duodenal Switch

Yosuke Kodama · Chun-Mei Zhao · Bård Kulseng · Duan Chen

Received: 26 April 2010 / Accepted: 9 August 2010 / Published online: 8 September 2010

Abstract
Background/Aim Food intake, eating behavior, and metabolic parameters in rats that underwent bilateral truncal vagotomy, sleeve gastrectomy, and duodenal switch procedures were examined.
Methods Rats were subjected to bilateral truncal vagotomy plus pyloroplasty (VTPP), pyloroplasty (PP), laparotomy, sleeve gastrectomy (SG), or duodenal switch (DS; with and without SG).
Results VTPP, but neither PP nor laparotomy, reduced body weight (BW; 10%) transiently (1 week postoperatively). SG reduced BW (10%) for 6 weeks, while DS alone or SG followed by DS led to a continuous BW loss from 15% at 1 week to 50% at 8 weeks postoperatively. Food intake was higher and the satiety ratio was lower during the night than the day for all groups of surgeries. Neither VTPP nor SG had measurable effect on food intake, eating behavior and metabolic parameters. DS reduced daily food intake by more than 50%, which was associated with hypercholecystokinin(CCK)emia, reduced meal size and increased satiety ratio, and increased fecal energy content (measured at 8 weeks).
Conclusions Weight loss after VTPP, SG, or DS differed in terms of degree, duration, and underlying mechanisms. DS without SG was most effective in the long-term, probably due to hyperCCKemia-induced reduction in food intake and long-limb intestinal bypass-induced malabsorption.

Keywords Body weight · Food intake · Obesity surgery

Introduction

During the evolution of surgery for morbid obesity, many different surgical procedures have been developed in order to reduce food intake and/or nutrition absorption. For instance, gastric bypass surgery is designed to create a small pouch in the stomach to produce early satiety and a consequent reduction in food intake, and moreover to induce malabsorption by creating a short gut syndrome and/or by accomplishing distal mixing of bile acid and pancreatic juice with ingested nutrients, thereby reducing absorption. It has also been demonstrated that weight loss surgery, including gastric bypass, changes the perception of food and thus eating behavior, leading to the concept of "behavior surgery".[1] We recently reported that rats developed an altered eating behavior for the short term, but not the long term after gastric bypass. Gastric-bypassed rats ate more during the daytime than sham-operated control rats and were unable to keep up with the control rats with respect to meal size and eating rate during the night. More

Y. Kodama · C.-M. Zhao · B. Kulseng · D. Chen
Department of Cancer Research and Molecular Medicine,
Norwegian University of Science and Technology,
Trondheim, Norway

B. Kulseng
Departments of Surgery and Endocrinology,
St. Olavs University Hospital,
Trondheim, Norway

D. Chen (✉)
Department of Cancer Research and Molecular Medicine,
Faculty of Medicine,
Norwegian University of Science and Technology,
Laboratory Centre (3rd Floor-East Side),
Erling Skjalgssons Gate 1,
7006 Trondheim, Norway
e-mail: duan.chen@ntnu.no

interestingly, neither their food intake nor absorption was reduced, despite the fact that the rats had a loss in body weight following the gastric bypass.[2]

Based on the hypothesis that "common obesity" has hypothalamic origins, truncal vagotomy was used for treatment of severe obesity.[3] Based on the understanding that the vagus nerve controls satiety/hunger and energy homeostasis, an alternative minimally invasive treatment, the so-called "Vagal BLocking for Obesity Control" (VBLOC), has been developed to intermittently block vagal nerve trunks with high frequency and low power electrical signals through the laparoscopically implanted device.[4] Hence, the first aim of the present study was to analyze the eating behavior and energy expenditure in rats subjected to bilateral truncal vagotomy. The measurements were performed by utilizing a state-of-the-art method known as a comprehensive laboratory animal metabolic monitoring system (CLAMS), as performed in our previous studies.[2,5,6]

Laparoscopically assisted vertical gastrectomy using a Dexterity Pneumo Sleeve device, the so-called sleeve gastrectomy, was originally proposed as the first stage followed by Roux-en-Y gastric bypass or duodenal switch as the second stage.[7,8] This procedure has been recently considered as an independent weight loss surgery, based on clinical outcomes and presumably underlying mechanism in which the ghrelin-rich gastric fundus is eliminated and the volume of the stomach is reduced.[9–12] Previously, we compared the eating behavior in rats that underwent a total gastrectomy vs. gastric bypass (i.e., end-to-end anastomosis of esophagus–proximal jejunum) and found that the food intake and meal size were reduced after gastrectomy but not gastric bypass, thus suggesting that the control of food intake was independent of the food reservoir function of the stomach.[5] Therefore, the second aim of the present study was to analyze the eating behavior and energy expenditure in rats subjected to sleeve gastrectomy.

The duodenal switch procedure was originally created as a surgical solution for primary bile reflux gastritis and/or to decrease post-gastrectomy symptoms after distal gastrectomy and gastroduodenostomy.[13] Currently, a combined procedure of sleeve gastrectomy and duodenal switch has been applied to the treatment of morbid obesity based on the rationale that the sleeve gastrectomy preserves the pylorus and first portion of the duodenum which negates the possibility of dumping symptoms and reduces the risk of marginal ulcers.[8] The duodenal switch procedure achieves complete pancreaticobiliary diversion. As a result, postprandial biliary and pancreatic secretion will be reduced or eliminated, and the negative feedback effect of the bile acid and pancreatic juice on cholecystokinin (CCK)-producing cells in the duodenum and jejunum will be deprived, thereby leading to an increase in circulating CCK levels. Since CCK is well known as a satiety hormone, we hypothesized that the duodenal switch procedure could be an independent weight loss surgery because this procedure would reduce the food intake due to hyperCCKemia and induce malabsorption due to long-limb intestinal bypass. Hence, the third aim of the present study was to evaluate the effects of a duodenal switch with and without a sleeve gastrectomy on body weight, eating behavior, serum CCK levels, fecal energy content, and energy expenditure.

Materials and Methods

Animals

Male rats (Sprague–Dawley, 3 months old) were purchased from Taconic M&B, Skensved, Denmark. The males were preferred because females change their food intake during ovulation and males grow faster than females, making it easier to detect body weight change. The rats were housed in individually ventilated Makrolon cages with 12 h light/dark cycle, room temperature of 22°C and 40–60% relative humidity. They were allowed free access to tap water and standard rat pellet food (RM1 801002, Scanbur BK AS, Sweden). The study was approved by the Norwegian National Animal Research Authority (Forsøksdyrutvalget, FDU).

Experimental Design

The animals were divided into the following groups: laparotomy (LAP), pyloroplasty (PP), bilateral truncal vagotomy plus pyloroplasty (VTPP), sleeve gastrectomy (SG), duodenal switch alone (DS), SG as the first stage and then DS as the second stage (SG_1+DS_2), and SG and DS simultaneously (SG+DS). In consideration of the "3Rs" for the human use of animals (e.g., reduction of animal numbers to the minimum consistent with achieving the scientific purposes of the experiment),[14] rats in control groups have been re-used after a 9-week recovery from previous operation and revealed an unchanged eating behavior and metabolic parameters. The rats were first subjected to LAP ($n=7$), PP ($n=7$), or VTPP ($n=7$), respectively. After 9 weeks, LAP and PP rats were subjected to SG and DS, respectively. After an additional 11 weeks, SG rats were subjected to DS, and VTPP rats were simultaneously subjected to both SG and DS. An additional group of age-matched rats were subjected to LAP ($n=7$).

Each rat was monitored weekly with respect to the body weight development throughout the study period. Each rat was placed in the CLAMS cage three times for 48 h, i.e., 1 week before surgery, 1–2 and 8–11 weeks after surgery for measurements of the eating and metabolic parameters.

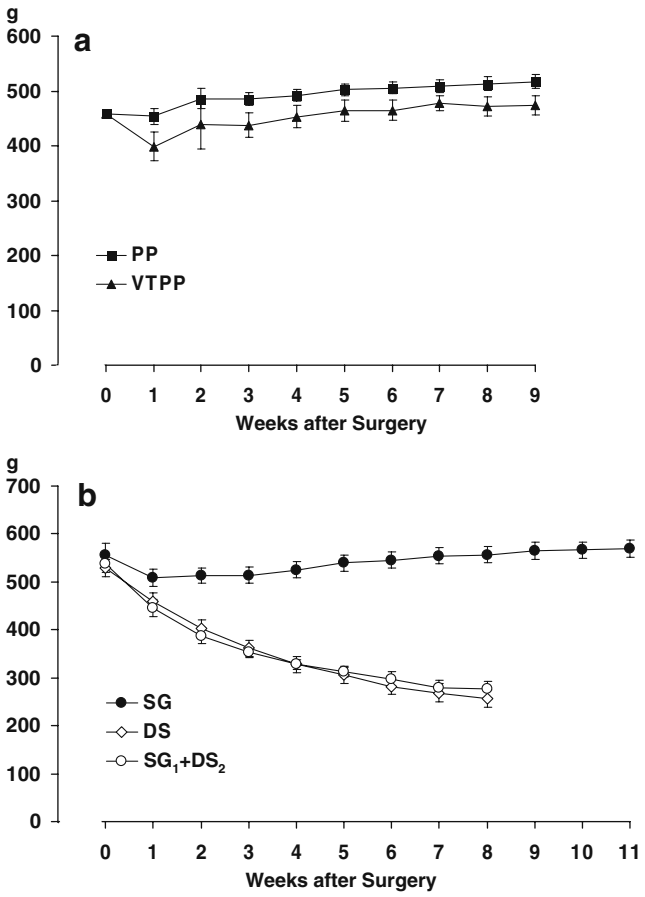

Fig. 1 Body weight developments after pyloroplasty (PP) and bilateral truncal vagotomy plus pyloroplasty (VTPP) (**a**), and after sleeve gastrectomy (SG), duodenal switch alone (DS) and SG as the first stage and then DS as the second stage ($SG_{1+}DS_2$) (**b**). Data are expressed as mean ± SEM

Surgery

Rats were deprived of food but not water for 12 h pre- and 24 h post-operation. All operations were performed under general anesthesia with isofluran (4% for induction and 2% for maintenance). Buprenorphine (0.05 mg/kg) was administrated as a pain reliever subcutaneously immediately after surgery. LAP was performed through middle-line incision. PP was performed by cutting off the pyloric sphincter (2 mm) and suturing it vertically against the incision. VTPP was achieved by cutting both the anterior and posterior vagal trunks immediately below the diaphragm and, while at the same time performing a PP to prevent gastroparesis-induced food retention and gastric dilation. SG was performed by resecting 70% of the glandular stomach along the greater curvature. DS was constructed by transecting the duodenum 1 cm to the pylorus, and a common channel was created by dividing the ileum 5 cm proximal to the ileocecal junction (rats have a much longer jejunum than humans). The distal limb of the ileum was anastomosed to the post-pyloric duodenum in an end-to-end manner, and the stump of the duodenum was closed with cross-suture. The distal anastomosis was performed by joining the distal biliopancreatic limb at 1 cm to the ileocecal junction in an end-to-side manner.

Eating and Metabolic Parameters

Eating and metabolic parameters were automatically recorded by the comprehensive laboratory animal monitoring system (CLAMS; Columbus Instruments International, Columbus, OH, USA). This system is composed of a four-chamber indirect calorimeter designed for the continuous monitoring of individual rats from each chamber. An air sample was withdrawn every 5 min. The energy expenditure (kcal/h) was calculated according to equation: $(3.815 + 1.232\ \mathrm{RER}) \times VO_2$, where the respiratory exchange ratio (RER) was the volume of CO_2 produced per volume of O_2 consumed. VO_2 was the volume of O_2 consumed per hour per kilogram of mass of the animal. The energy expenditure is expressed as kcal/h/100 g body weight. Urine production was automatically recorded by weight. In order for rats to acclimate to this system, they were placed in these metabolic cages for 24 h before the first CLAMS monitoring. The high-resolution eating data was generated by monitoring all eating balances every 0.5 s, providing accumulated food intake, meal size, and meal duration. The end of an eating event (meal) was when the balance was stable for more than 10 s and a minimum of 0.05 g was

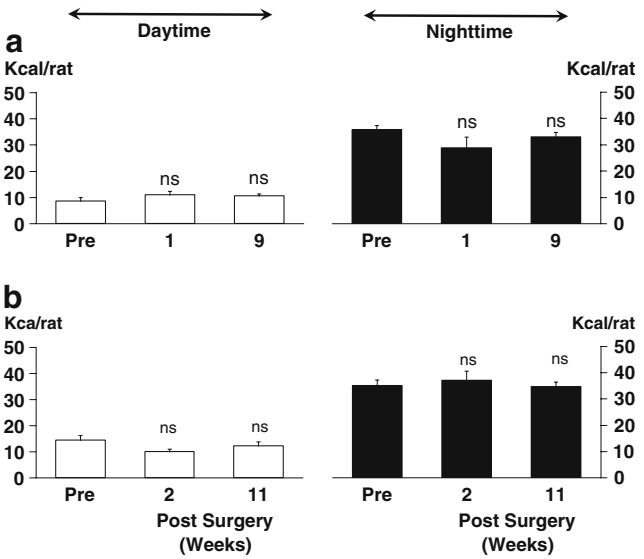

Fig. 2 Calories intake (kcal/rat) during the day and the night at 1 week before (Pre), 1 and 9 weeks after bilateral truncal vagotomy plus pyloroplasty (VTPP) (**a**), 1 week before (Pre), 2 and 11 weeks after sleeve gastrectomy (SG) (**b**). Data are expressed as mean ± SEM. *ns* not significant between pre- vs. post-operation

eaten. Parameters during daytime and nighttime for each rat included: meal size, meal duration, accumulated food intake, intermeal interval, rate of eating, and satiety ratio. The intermeal interval was defined as the interval in minutes between two meals. The rate of eating was calculated by dividing meal size by meal duration. The satiety ratio, an index of non-eating time produced by each gram of food consumed, was calculated as intermeal interval divided by meal size. The rats were placed in the CLAMS chambers for 48 h (data from the first 24 h were not used in the analysis) with free access to standard rat powder food (RM1 811004, Scanbur BK AS, Sweden) and tap water. The total metabolizable energy was 2.57 kcal/g for both RM1 801002 and 811004.

Determination of Energy Content in Feces

Feces were collected when the rats were placed in CLAMS cages 8 weeks after DS or age-matched control LAP, and dried for 72 h at 60°C. The energy content was determined by means of an adiabatic bomb calorimeter (IKA-Calorimeter C 5000, IKA-Werke GmbH & Co. KG, Staufen, Germany).

Determination of Serum CCK Levels

CCK levels in serum were analyzed by radioimmunoassay with sulfated CCK-8 as standard, using a CCK kit (Eurodiagnostica AB, Malmö, Sweden).

Statistical Analysis

The data were expressed as mean ± SEM. Comparisons between surgical groups and between three time points (1 week before, 1–2 and 8–11 weeks after surgery) were performed using an independent sample *t* test or ANOVA followed by a Tukey's test when applicable. $p<0.05$ was considered statistically significant.

Table 1 Eating and metabolic parameters at 1 week before VTPP, 1 and 9 weeks after VTPP

	Parameters	1 W before VTPP	1 W after VTPP	9 W after VTPP
Daytime	Food intake (g)	3.37±0.51	4.27±0.53 ns	4.08±0.31 ns
	Food intake (g/100 g body weight)	0.74±0.11	1.11±0.18 ns	0.86±0.07 ns
	Calories intake (kcal)	8.66±1.30	10.97±1.37 ns	10.49±0.79 ns
	Calories intake (kcal/100 g body weight)	1.91±0.29	2.86±0.46 ns	2.20±0.18 ns
	Number of meals	13.86±1.77	12.43±1.73 ns	15.57±1.95 ns
	Meal size (g/meal)	0.25±0.03	0.39±0.07 ns	0.28±0.03 ns
	Meal duration (min/meal)	1.00±0.20	1.81±0.52 ns	1.12±0.14 ns
	Intermeal interval (min)	51.86±6.11	59.91±10.49 ns	46.82±6.47 ns
	Satiety ratio (min/g)	230.76±43.10	165.10±20.64 ns	166.71±13.47 ns
	Rate of eating (g/min)	0.27±0.02	0.27±0.04 ns	0.26±0.02 ns
Nighttime	Food intake (g)	13.90±0.63	11.18±1.61 ns	12.80±0.70 ns
	Food intake (g/100 g body weight)	3.06±0.15	2.72±0.26 ns	2.68±0.14 ns
	Calories intake (kcal)	35.72±1.62	28.73±4.14 ns	32.89±1.80 ns
	Calories intake (kcal/100 g body weight)	7.87±0.39	6.99±0.66 ns	6.88±0.36 ns
	Number of meals	31.00±3.04	24.29±3.02 ns	31.00±5.32 ns
	Meal size (g/meal)	0.46±0.03	0.48±0.06 ns	0.50±0.10 ns
	Meal duration (min/meal)	1.96±0.29	2.64±0.46 ns	2.12±0.32 ns
	Intermeal interval (min)	21.78±1.92	29.97±5.48 ns	25.66±5.36 ns
	Satiety ratio (min/g)	46.70±2.27	65.33±10.13 ns	50.59±2.40 ns
	Rate of eating (g/min)	0.25±0.02	0.20±0.03 ns	0.24±0.02 ns
24 h	Energy expenditure (kcal/h/100 g body weight)	0.39±0.01	0.43±0.01 ns	0.36±0.01*
	RER	0.95±0.01	0.93±0.01 ns	0.95±0.01 ns
	Ambulatory activity	7,494.14±1,240.97	6,902.00±923.12 ns	6,504.00±999.08 ns

Data are expressed as mean ± SEM

ns not significant

*$p<0.01$ (1 W vs. 9 W)

Results

Mortality

There was no mortality in rats that underwent LAP, PP, or VTPP. The mortality rate was one of seven in rats subjected to SG, two of seven to DS alone, one of six to SG_1+DS_2, and six of seven to SG+DS simultaneously.

Body Weight

Both LAP and PP had no effect on body weight development. VTPP transiently reduced the body weight (about 10% at 1 week postoperatively; Fig. 1a). SG reduced the body weight (approximately 10%) for about 6 weeks (Fig. 1b). DS alone or SG followed by DS reduced the body weight in a similar manner: a rapid and continuous weight loss of about 10% at 1 week and 50% at 8 weeks postoperatively (Fig. 1b).

Food Intake, Eating Behavior, Energy Expenditure, and Fecal Energy Content

Food intake was higher and the satiety ratio was lower during the night than the day for each rat.

There were no differences between LAP and PP in terms of food intake and eating behavior parameters at either 1 or 9 weeks postoperatively (data not shown).

VTPP was without any measurable effects on food intake, eating behavior, and metabolic parameters measured at either 1 or 9 weeks postoperatively (Fig. 2a; Table 1).

SG had no effects on food intake and eating behavior parameters, except for meal duration during the night measured at 2 weeks (Fig. 2b; Table 2). In addition, SG reduced the water intake during one interval (0.91±0.07 vs. 0.47±0.07 mL at 2 weeks and vs. 0.45±0.08 mL at 11 weeks, both $p<0.01$). Energy expenditure was increased at 2 weeks and RER was increased at 11 weeks postoperatively (Table 2).

Table 2 Eating and metabolic parameters at 1 week before SG, 2 and 11 weeks after SG

	Parameters	1 W before SG	2 W after SG	11 W after SG
Daytime	Food intake (g)	5.62±0.70	3.92±0.38 ns	4.76±0.63 ns
	Food intake (g/100 g body weight)	1.01±0.15	0.77±0.07 ns	0.83±0.10 ns
	Calories intake (kcal)	14.43±1.79	10.08±0.97 ns	12.24±1.62 ns
	Calories intake (kcal/100 g body weight)	2.60±0.38	1.97±0.18 ns	2.13±0.27 ns
	Number of meals	13.83±1.83	9.83±1.42 ns	11.17±1.54 ns
	Meal size (g/meal)	0.41±0.01	0.44±0.08 ns	0.43±0.04 ns
	Meal duration (min/meal)	1.52±0.13	1.72±0.20 ns	1.29±0.07 ns
	Intermeal interval (min)	51.45±7.09	70.24±8.34 ns	62.28±7.13 ns
	Satiety ratio (min/g)	124.13±14.28	170.13±18.28 ns	149.69±23.39 ns
	Rate of eating (g/min)	0.28±0.02	0.25±0.03 ns	0.33±0.02 ns
Nighttime	Food intake (g)	13.61±0.89	14.41±1.39 ns	13.47±0.69 ns
	Food intake (g/100 g body weight)	2.43±0.17	2.80±0.22 ns	2.34±0.09 ns
	Calories intake (kcal)	34.97±2.28	37.04±3.57 ns	34.63±1.78 ns
	Calories intake (kcal/100 g body weight)	6.23±0.43	7.19±0.56 ns	6.01±0.22 ns
	Number of meals	28.50±3.40	23.17±3.59 ns	28.00±4.27 ns
	Meal size (g/meal)	0.50±0.06	0.73±0.15 ns	0.53±0.07 ns
	Meal duration (min/meal)	1.69±0.19	2.88±0.47*	1.77±0.18 ns
	Intermeal interval (min)	24.46±2.86	31.58±5.83 ns	26.76±5.05 ns
	Satiety ratio (min/g)	48.93±3.63	45.93±4.93 ns	48.68±2.50 ns
	Rate of eating (g/min)	0.30±0.00	0.25±0.02 ns	0.30±0.01 ns
24 h	Energy expenditure (kcal/h/100 g body weight)	0.34±0.01	0.38±0.00*	0.37±0.01 ns
	RER	0.96±0.01	0.94±0.01 ns	1.02±0.01**, ***
	Ambulatory activity	7,014.50±1,001.57	6,721.17±807.00 ns	5,094.67±549.75 ns

Data are expressed as mean ± SEM

ns not significant

*$p<0.05$

**$p<0.01$ (pre vs. 2 W or 11 W)

***$p<0.01$ (2 W vs.11 W)

DS regardless of whether it was accompanied by SG reduced the daily food/calories intake by approximately 50% when measured at 2 as well as 8 weeks postoperatively (Fig. 3a; Table 3). The reduced food intake was associated with a reduced meal size and an increased satiety ratio, but not with the number of meals (Fig. 3b–d; Table 3). The fecal energy content of DS rats was higher than that of control LAP rats (20411.15±177.86 J/g vs. 18756.36±51.61 J/g, $p<0.001$) at 8 weeks. It was difficult to collect the feces at 2 weeks after DS due to a severe diarrhea. There were no differences between DS and SG_1+DS_2 in terms of food intake and eating behavior parameters, except RER and energy expenditure at 2 weeks and water intake at 8 weeks postoperatively.

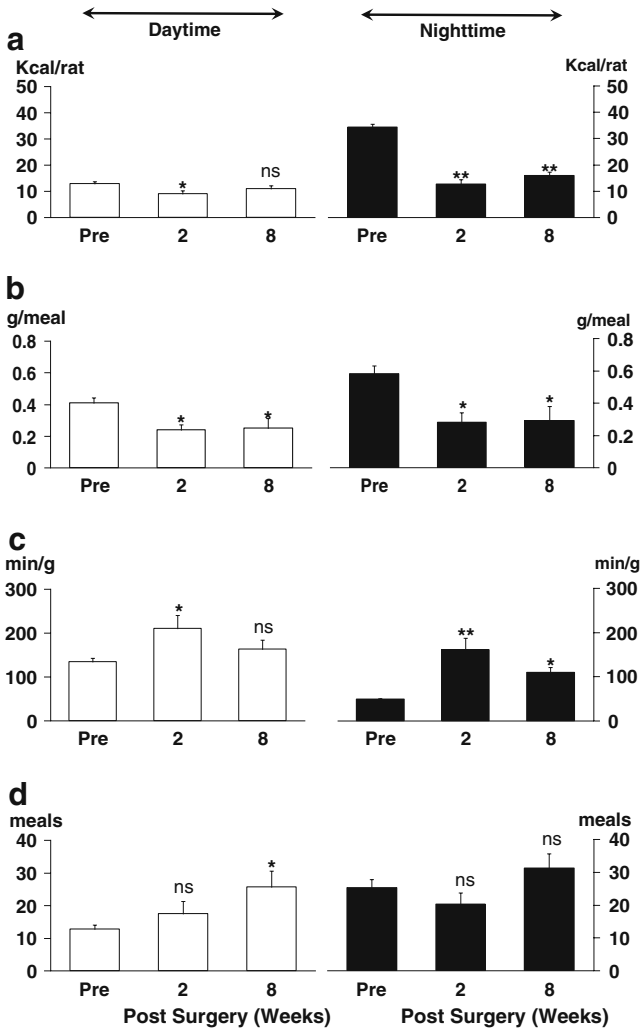

Fig. 3 Calories intake (kcal/rat) (**a**), meal size (g/meal) (**b**), satiety ratio(min/g) (**c**), number of meals (meals) (**d**) during the day and the night at 1 week before (Pre), 2 and 8 weeks after duodenal switch (DS)(regardless of whether it was accompanied by SG). Data are expressed as mean ± SEM. *$p<0.05$, **$p<0.01$, *ns* not significant between pre- vs. 2 W or 8 W postoperatively

One surviving rat that was subjected to SG+DS had reduced food intake and altered eating behavior, much like the rats subjected to SG_1+DS_2. However, this was not analyzed statistically.

Circulating CCK Levels

Serum CCK levels were 12.6±3.0 pmol/L in SG_1+DS_2 rats. This was more than ten times higher than the value of rats subjected to the sham operation in our previous report (plasma CCK levels were 1.1±0.5 pmol/L).[15] Unfortunately, there was a technical error in the determination of the CCK levels in age-matched control LAP and DS rats in the present study.

Discussion

The role of vagus in physiologically controlling eating behavior has been studied during the past decades. It is believed that food interacts with the gut to provide the brain via vagal afferents with information regarding food composition, amount of ingested food and energy content. The brain determines the rate of nutrient absorption, partitioning, storage, and mobilization through vagal efferents as well as the sympathetic nervous system and hormonal mechanisms.[16] This food–gut-brain axis is considered as an autonomic neurohumoral pathway regulating energy homeostasis. In the present study, disruption of the gut-brain axis by VTPP was without any measureable effect on the energy homeostasis. The body weight loss was slight (about 15%) and transient (1 week postoperatively). This may explain why vagotomy as treatment for obesity has received little attention since it was used 30 years ago.[3] However, with the substantial need for effective treatment of obesity at younger ages and the improved safety of laparoscopic procedures, it has been suggested that surgical treatment can be justified at lower levels of BMI, before the eating disorder has become intractable and requires malabsorptive operations. The possibility for utilizing a laparoscopic abdominal vagotomy has been well discussed elsewhere.[17]

SG weight loss surgery is believed to be restrictive as well as a neurohormone-mediated procedure. The early clinical results seem promising, but long-term data is still needed to define the place of LSG within the bariatric surgery armamentarium.[18,19] In the present study, SG reduced the body weight by about 10% in the short-term (1–6 weeks) but not in the long-term (after 7 weeks). The reduction was not associated with reduced food intake but possibly with increased energy expenditure, which is in line with previous observations in rats subjected to total gastrectomy or gastric bypass.[5] The underlying physiological mechanisms are still unknown. It should also be

Table 3 Eating and metabolic parameters at 1 week before DS both with and without SG, 2 and 8 weeks after DS both with and without SG

	Parameters	1 W before operation	2 W after operation	8 W after operation
Daytime	Food intake (g)	4.99±0.31	3.54±0.44*	4.30±0.44 ns
	Food intake (g/100 g body weight)	0.92±0.05	0.87±0.11 ns	1.60±0.20**, ****
	Calories intake (kcal)	12.83±0.79	9.11±1.14*	11.05±1.14 ns
	Calories intake (kcal/100 g body weight)	2.36±0.13	2.25±0.28 ns	4.10±0.51**, ****
	Number of meals	12.80±1.18	17.50±3.79 ns	25.70±4.86*
	Meal size (g/meal)	0.41±0.03	0.24±0.03*	0.25±0.06*
	Meal duration (min/meal)	1.33±0.10	2.35±0.41 ns	3.74±1.82 ns
	Intermeal interval (min)	54.01±4.00	54.86±14.48 ns	42.19±12.04 ns
	Satiety ratio (min/g)	134.37±8.03	210.35±29.60*	163.30±20.66 ns
	Rate of eating (g/min)	0.31±0.01	0.13±0.03**	0.10±0.01**
Nighttime	Food intake (g)	13.34±0.43	4.89±0.73**	6.16±0.54**
	Food intake (g/100 g body weight)	2.47±0.12	1.19±0.17**	2.20±0.15****
	Calories intake (kcal)	34.29±1.10	12.56±1.86**	15.82±1.38**
	Calories intake (kcal/100 g body weight)	6.35±0.30	3.07±0.44**	5.65±0.39****
	Number of meals	25.20±2.62	20.30±3.43 ns	31.20±4.48 ns
	Meal size (g/meal)	0.58±0.05	0.28±0.06*	0.29±0.09*
	Meal duration (min/meal)	1.96±0.19	3.64±1.28 ns	3.84±1.58 ns
	Intermeal interval (min)	28.19±2.96	40.29±6.97 ns	27.13±7.01 ns
	Satiety ratio (min/g)	48.90±1.66	161.24±26.37**	109.27±11.90*
	Rate of eating (g/min)	0.30±0.01	0.10±0.01**	0.09±0.01**
24 h	Energy expenditure (kcal/h/100 g body weight)	0.37±0.01	0.38±0.02 ns	0.43±0.01*, ***
	RER	0.99±0.02	0.90±0.03 ns	1.11±0.07****
	Ambulatory activity	5,410.30±696.92	5,773.60±891.92 ns	3,533.70±618.31 ns

Data are expressed as mean ± SEM

ns not significant

*$p<0.05$

**$p<0.01$ (pre vs. 2 W or 8 W)

***$p<0.05$

****$p<0.01$ (2 W vs. 8 W)

mentioned that SG rats, like totally gastrectomized rats, seemed to drink frequently postoperatively, probably owing to lower ghrelin and obestatin levels.[5,20,21]

In the present study, DS alone and SG+DS exhibited well-matched postoperative effects on body weight, metabolic parameters and eating behavior, leading to a long lasting and effective body weight loss. More interestingly, the results of the present study support our hypothesis that the DS procedure per se could be considered as an independent weight loss surgery because this procedure reduces the food intake due to hyperCCKemia and induces malabsorption due to intestinal bypass. As expected after pancreaticobiliary diversion,[15,22–24] circulating CCK levels were elevated after SG_1+DS_2, which in turn increased the satiety ratio and reduced meal size. Malabsorption is believed to be due to long-limb intestinal bypass after DS. In fact, DS patients also showed a decreased appetite and continuously body weight loss.[25] In addition to CCK, glucagon-like peptide-1 (GLP-1) levels in the circulation have been reported to be elevated in rats subjected to pancreaticobiliary diversion, which could have a beneficial effect on ß cells in the pancreas.[26,27] Unfortunately, GLP-1 was not measured in the present study, thus it will be of interest for future study.

In conclusion, VTPP, SG, and DS, like gastric bypass, reduced the body weight, though the effectiveness and underlying mechanisms appear different. Since obesity is believed to a multifactorial disease, the options for the treatment, including various surgical procedures, should be individualized.

Acknowledgments The authors thank Dr. Catia Martins for determination of CCK levels, Jostein Johansen for assistance of data analysis at Department of Cancer Research and Molecular Medicine, and Morten Grønli, and Erik Langørgen for assistance of bomb calorimeter at Department of Energy and Process Engineering, Norwegian University of Science and Technology.

References

1. Miras AD, le Roux CW. Bariatric surgery and taste: novel mechanisms of weight loss. Curr Opin Gastroenterol. 2010;26(2):140–5.
2. Furnes MW, Tømmerås K, Arum CJ, Zhao CM, Chen D. Gastric bypass surgery causes body weight loss without reducing food intake in rats. Obes Surg. 2008;18(4):415–22.
3. Kral JG. Vagotomy for treatment of severe obesity. Lancet. 1978;1 (8059):307–8.
4. Camilleri M, Toouli J, Herrera MF, Kulseng B, Kow L, Pantoja JP, Marvik R, Johnsen G, Billington CJ, Moody FG, Knudson MB, Tweden KS, Vollmer M, Wilson RR, Anvari M. Intra-abdominal vagal blocking (VBLOC therapy): clinical results with a new implantable medical device. Surgery. 2008;143(6):723–31.
5. Furnes MW, Stenstrom B, Tømmerås K, Skoglund T, Dickson SL, Kulseng B, Zhao CM, Chen D. Feeding behavior in rats subjected to gastrectomy or gastric bypass surgery. Eur Surg Res. 2008;40 (3):279–88.
6. Furnes MW, Zhao CM, Chen D. Development of obesity is associated with increased calories per meal rather than per day. A study of high-fat diet-induced obesity in young rats. Obes Surg. 2009;19(10):1430–8.
7. Naitoh T, Gagner M. Laparoscopically assisted gastric surgery using dexterity pneumo sleeve. Surg Endosc. 1997;11(8):830–3.
8. Anthone GJ. The duodenal switch operation for morbid obesity. Surg Clin North Am. 2005;85(4):819–33
9. Rosen DJ, Dakin GF, Pomp A. Sleeve gastrectomy. Minerva Chir. 2009;64(3):285–95.
10. Rubino F, Schauer PR, Kaplan LM, Cummings DE. Metabolic surgery to treat type 2 diabetes: clinical outcomes and mechanisms of action. Annu Rev Med. 2010;61:393–411.
11. Bohdjalian A, Langer FB, Shakeri-Leidenmühler S, Gfrerer L, Ludvik B, Zacherl J, Prager G. Sleeve gastrectomy as sole and definitive bariatric procedure: 5-year results for weight loss and ghrelin. Obes Surg. 2010;20(5):535–40.
12. Abu-Jaish W, Rosenthal RJ. Sleeve gastrectomy: a new surgical approach for morbid obesity. Expert Rev Gastroenterol Hepatol. 2010;4(1):101–19.
13. DeMeester TR, Fuchs KH, Ball CS, Albertucci M, Smyrk TC, Marcus JN. Experimental and clinical results with proximal end-to-end duodenojejunostomy for pathologic duodenogastric reflux. Ann Surg. 1987;206(4):414–26.
14. Russell WMS, Burch RL. The Principles of Humane Experimental Technique. London: Methuen & Co. LTD. 1959.
15. Chen D, Nylander A-G, Rehfeld JF, Axelson J, Ihse I, Hakanson R. Does vagotomy affect the growth of the pancreas in the rat? Scand J Gastroenterol 1992;27:606–608.
16. Berthoud HR. The vagus nerve, food intake and obesity. Regul Pept. 2008;149(1–3):15–25.
17. Kral JG. Psychosurgery for obesity. Obes Facts. 2009;2(6):339–41.
18. Ward M, Prachand V. Surgical treatment of obesity. Gastrointest Endosc. 2009;70(5):985–90.
19. Frezza EE, Chiriva-Internati M, Wachtel MS. Analysis of the results of sleeve gastrectomy for morbid obesity and the role of ghrelin. Surg Today. 2008;38(6):481–3.
20. Samson WK, White MM, Price C, Ferguson AV. Obestatin acts in brain to inhibit thirst. Am J Physiol Regul Integr Comp Physiol. 2007;292(1):R637–43.
21. Hashimoto H, Fujihara H, Kawasaki M, Saito T, Shibata M, Otsubo H, Takei Y, Ueta Y. Centrally and peripherally administered ghrelin potently inhibits water intake in rats. Endocrinology. 2007;148(4):1638–47.
22. Chen D, Nylander AG, Rehfeld JF, Sundler F, Håkanson R. Hypercholecystokininemia produced by pancreaticobiliary diversion causes gastrin-like effects on enterochromaffin-like cells in the stomach of rats subjected to portacaval shunting or antrectomy. Scand J Gastroenterol. 1993;28(11):988–92.
23. Chen D, Andersson K, Iovanna JL, Dagorn JC, Håkanson R. Effects of hypercholecystokininemia produced by pancreaticobiliary diversion on pancreatic growth and enzyme mRNA levels in starved rats. Scand J Gastroenterol. 1993;28(4):311–4.
24. Chen D, Nylander AG, Norlén P, Håkanson R. Gastrin does not stimulate growth of the rat pancreas. Scand J Gastroenterol. 1996;31(4):404–10.
25. Marceau P, Hould FS, Simard S, Lebel S, Bourque RA, Potvin M, Biron S. Biliopancreatic diversion with duodenal switch. World J Surg. 1998;22(9):947–54.
26. Borg CM, le Roux CW, Ghatei MA, Bloom SR, Patel AG. Biliopancreatic diversion in rats is associated with intestinal hypertrophy and with increased GLP-1, GLP-2 and PYY levels. Obes Surg. 2007;17(9):1193–8.
27. Miazza BM, Widgren S, Chayvialle JA, Nicolet T, Loizeau E. Exocrine pancreatic nodules after long-term pancreaticobiliary diversion in rats. An effect of raised CCK plasma concentrations. Gut. 1987;28 Suppl:269–73.

Discussant

DR. THOMAS H. MAGNUSON (Baltimore, MD): I would like to thank Dr. Kodama and congratulate him on an excellent presentation, and he and his coauthors on an excellent manuscript. They have given us some important insights into how some these bariatric operations work from a metabolic perspective. This is an important topic, with the ultimate goal of better selecting the right operation for each individual patient.

They found, interestingly, that vagotomy alone or sleeve gastrectomy alone seemed to have little impact on weight loss or eating behavior, but the duodenal switch operation did have a dramatic impact on weight loss and also altered energy expenditure.

I have a couple quick questions.

First, with regards to your sleeve gastrectomy model, it looks like this didn't work very well, but yet there's other animal models, obese rats and mice for example, as well as our human clinical experience, showing that the sleeve works pretty well as an operation for weight loss. I wonder if you could comment briefly on why your results differ from that of others. Did you measure circulating levels of ghrelin, which has been implicated as being important in the function of the sleeve gastrectomy as an appetite suppressant?

The second question involves your duodenal switch model. It looks like these animals lost dramatic weight, but was this really a physiologic model? It seems like most of these animals had severe diarrhea and over 30 or 40% of your animals actually died in the study. Was this due to severe malnutrition? Did you measure nutritional parameters, such as serum albumin levels, to make sure this wasn't a model of severe protein calorie malnutrition contributing to the deaths and the severe diarrhea?

In addition, you measured CCK levels and postulated that that might be an effect of the duodenal switch and its impact on satiety, but did you think about measuring other GI peptides. GLP-1, adiponectin, NPY and PYY have all been implicated as being important to weight loss in animal models that bypass variable lengths of intestine.

Once again, I enjoyed your presentation.

Closing discussant

DR. YOSUKE KODAMA: Thank you very much for your comments. In this study with normal rats, duodenal switch, but not vagotomy or sleeve gastrectomy, resulted in the dramatic weight loss. It will be of interest to repeat this study but with obese rats to see whether the effect differs. We did not measure ghrelin levels in this study, because we did not see a significant weight loss after the sleeve gastrectomy.

As reported in our manuscript, 2 of 7 rats died after duodenal switch procedure alone, and 1 of 6 rats died after sleeve gastrectomy as the first stage and duodenal switch as the second stage. Such mortality is generally acceptable in the experimental surgery with small animals. All survived animals from duodenal switch had severe diarrhea but it lasted only for a short time period (e.g. 2 weeks). We did not measure the serum albumin levels. In the case that the two procedures were performed at the same time, 6 of 7 rats died, which was most likely due to surgical trauma.

This was our first experimental study suggesting that duodenal switch alone might be used as an independent weight loss surgery. The underlying mechanism should be further investigated, for example, by measuring not only CCK but also other GI hormones as you have suggested.

J Gastrointest Surg (2010) 14:1511–1520
DOI 10.1007/s11605-010-1333-5

2010 SSAT PLENARY PRESENTATION

A Systematic Review of POSSUM and its Related Models as Predictors of Post-operative Mortality and Morbidity in Patients Undergoing Surgery for Colorectal Cancer

Colin Hewitt Richards · Fiona E. Leitch · Paul G. Horgan · Donald C. McMillan

Received: 28 April 2010 / Accepted: 12 August 2010 / Published online: 8 September 2010

Abstract
Introduction The Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM) model and its Portsmouth (P-POSSUM) and colorectal (CR-POSSUM) modifications are used extensively to predict and audit post-operative mortality and morbidity. This aim of this systematic review was to assess the predictive value of the POSSUM models in colorectal cancer surgery.
Methods Major electronic databases, including Medline, Embase, Cochrane Library and Pubmed were searched for original studies published between 1991 and 2010. Two independent reviewers assessed each study against inclusion and exclusion criteria. All data was specific to colorectal cancer surgery. Predictive value was assessed by calculating observed to expected (O/E) ratios.
Results Nineteen studies were included in final review. The mortality analysis included ten studies (4,799 patients) on POSSUM, 17 studies (6,576 patients) on P-POSSUM and 14 studies (5,230 patients) on CR-POSSUM. Weighted O/E ratios for mortality were 0.31 (CI 0.31–0.32) for POSSUM, 0.90 (CI 0.88–0.92) for P-POSSUM and 0.64 (CI 0.63–0.65) for CR-POSSUM. The morbidity analysis included four studies (768 patients) on POSSUM with a weighted O/E ratio of 0.96 (CI 0.94–0.98).
Conclusions P-POSSUM was the most accurate model for predicting post-operative mortality after colorectal cancer surgery. The original POSSUM model was accurate in predicting post-operative complications.

Keywords Mortality · Colorectal cancer · Surgical scoring systems · Systematic review · POSSUM · P-POSSUM · CR-POSSUM

Introduction

Colorectal cancer is the second most common cause of cancer death in the UK. Each year, the disease accounts for approximately 35,000 new cases and over 16,000 deaths.[1] The majority of patients undergo potentially curative surgery.

With centres performing increasing numbers of colorectal cancer resections each year, scoring systems that accurately predict post-operative mortality and morbidity are needed. Accurate prediction of outcome can increase the precision of individual prognosis and allow improved treatment planning and resource allocation. In addition, the application of a scoring system that adjusts for the confounding effect of case mix can allow fair and comparative audit.[2]

A number of such scoring systems exist[3–5] but the Physiologic and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM) model has been recommended as the most appropriate for surgical practice.[6] This model, utilising scores relating to twelve physiological and six operative variables, was developed to predict 30-day mortality and morbidity after general surgical operations.[7]

Joint contribution: Colin H. Richards and Fiona E. Leitch

C. H. Richards (✉) · F. E. Leitch · P. G. Horgan · D. C. McMillan
University Department of Surgery,
Faculty of Medicine-University of Glasgow, Royal Infirmary,
Glasgow G4 0SF, UK
e-mail: colinrichards@nhs.net

POSSUM has since been used to allow comparison of performance between individual surgeons[8,9] and hospitals.[2]

The POSSUM model was subsequently applied to a large number of general surgical patients but was found to consistently over-predict mortality, especially in low risk patients. This led to the development of the Portsmouth modification (P-POSSUM)[10,11] which used the same physiological and operative variables but a different regression equation to predict mortality. More recently, in a further attempt to improve accuracy, specialty-specific models have been developed and applied to vascular (V-POSSUM and RAAA-POSSUM),[12,13] gastro-oesophageal (O-POSSUM)[14] and colorectal surgery (CR-POSSUM).[15] It should be noted that P-POSSUM and the specialty-specific models, including CR-POSSUM, relate only to the prediction of post-operative mortality and are not designed to predict post-operative complications. Tables 1 and 2 summarise the variables and risk equations used in the POSSUM, P-POSSUM and CR-POSSUM scoring systems.

The application of such predictive models to colorectal cancer surgery has generated conflicting results. The aim of the present study was to undertake a systematic review of the value of POSSUM, P-POSSUM and CR-POSSUM in predicting post-operative mortality and morbidity in patients undergoing surgery for colorectal cancer.

Methods

Search Strategy

This systematic review of published data was undertaken according to a pre-defined protocol. A search, using appropriate key terms was made of the following online databases: Medline, Embase, Pubmed, Cochrane Controlled Trials Register, the Cochrane Database of Systematic Reviews, CancerLit, and the Database of Abstracts and Reviews. The following sources were also searched for continuing or recently completed studies: National Research Register archive, Medical Research Council trials directory, www.ClinicalTrials.gov and www.who.int/trial search. Eligibility was restricted to studies published between 1991 (publication of original POSSUM model) and January 2010 with full text available in English language. The electronic search was supplemented by hand searching the reference lists of all relevant review and original articles. The literature search was repeated immediately prior to the final analysis.

Criteria for Review

Only studies relating exclusively to colorectal cancer surgery were included; this was defined as any operation for confirmed colorectal malignancy and was predominantly, but not exclusively, major colon or rectal resection with curative intent. Studies with no extractable cancer-specific data were excluded. These exclusion criteria were applicable to the large studies used in the original construction of all three POSSUM models.[7,10,11,15] If a study included colorectal cancer patients but did not report cancer-specific data the lead author was contacted and asked to provide raw data on the cancer subgroup. Data quoted as unpublished or derived from abstracts were not used.

Review Procedure

Titles and abstract were studied to assess relevance. Full text was obtained for selected studies and information entered into an electronic database using a standardised data extraction tool. Two authors (CHR, EFL) independently assessed selected studies against the above criteria. Disagreements regarding inclusion were resolved by discussion with a third party (DCM).

Statistical Analysis

Post-operative mortality and morbidity were assessed by 30-day or in-hospital rates. The accuracy of each POSSUM model was assessed by the ratio of observed to expected events (O/E ratio). To account for sample size, a weighted mean O/E ratio with 95% confidence intervals was calculated for each model. An O/E ratio equal to 1 confers 100% predictive accuracy, a ratio <1 implied model over-prediction of events and a ratio >1 implied model under-prediction of events. Analysis was carried out using SPSS software (version 15.0, SPSS. Inc, Chicago, IL, USA).

Results

The titles and abstracts of 345 published articles were examined before full text was obtained for 48 studies. Thirty of these studies did not meet the inclusion criteria; 24 did not contain data specific to colorectal cancer surgery,[2,5,7–11,15–31] four were review articles,[6,32–34] one study referred to a duplicate dataset[35] and one article was not available in English language.[36] One further study was included after the author provided colorectal cancer-specific data on request.[37] Nineteen independent studies were thus included in the final review. The selection process and exclusion criteria applied are summarised in Fig. 1.

Studies Included in the Mortality Analysis

Nineteen studies, comprising data on 6,929 patients, reported the accuracy of POSSUM, P-POSSUM or CR-

Table 1 The physiological and operative variables used in the calculation of POSSUM and P-POSSUM scores

	Score			
	1	2	4	8
Physiological variables				
Age	<60	61–70	>70	
Cardiac	Normal	Cardiac drugs	Oedema	JVP
			Warfarin	Cardiomegaly
Respiratory	Normal	SOB exertion	SOB stairs	SOB rest
		Mild COPD	Mod COPD	Fibrosis
ECG	Normal		AF (60–90)	Other abnormality
Systolic BP (mmHg)	110–130	131–170 100–109	≥ 171 90–99	≤ 89
Pulse (beats/min)	50–80	81–100	101–120	≥120
		40–49		≤39
Haemoglobin (g/dL)	13–16	11.5–12.9	10–11.4	≤9.9
		16.1–17	17.1–18	≥18.1
White cell count ($\times 10^{12}$/L)	4–10	10.1–20	≥ 20.1	
		3.1–3.9	≤ 3	
Sodium (mmol/L)	≥136	131–135	126–130	≤125
Potassium (mmol/L)	3.5–5	3.2–3.4	2.9–3.1	≤2.8
		5.1–5.3	5.4–5.9	≥6
Urea (mmol/L)	≤7.5	7.6–10	10.1–15	≥15.1
GCS	15	12–14	9–11	≤8
Operative variables				
Operation category	Minor	Intermediate	Major	Major+
No. of procedures	1	2	>2	
Total blood loss (ml)	<100	101–500	501–999	>1,000
Peritoneal soiling	None	Serous fluid	Local pus	Free pus
Malignancy	None	Primary only	Nodal mets	Distant mets
Timing of operation	Elective		Urgent	Emergency

The regression equations used to calculate the risk (R) of mortality and morbidity are as follows: POSSUM mortality equation : $\text{Log}[R/(R-1)] = -7.04 + (0.13\text{xphysiological score}) + (0.16\text{xoperative score})$P − POSSUM mortality equation : $\text{Log}[R/(R-1)] = -9.065 + (0.16\text{xphysiological score}) + (0.15\text{xoperative score})$POSSUM morbidity equation : $\text{Log}[R/(R-1)] = -5.91 + (0.16\text{xphysiological score}) + (0.19\text{xoperative score})$

POSSUM in predicting post-operative mortality after colorectal cancer surgery. These studies are summarised in Table 3. Several studies applied more than one POSSUM model to their population. Nine studies collected and applied model data prospectively while ten collected at least part of their data retrospectively. The majority of studies (14/19) reported death rates as 30-day mortality while four studies reported in-hospital rates. In the study[38] that reported both, the 30-day figure was used in the current review. (Table 3)

The operative and pathological details of the study populations are summarised in Table 4. The operations performed were predominantly major open colorectal cancer resections. Less than 5% of operations for colorectal cancer were carried out laparoscopically. Approximately three quarters (76%) of all operations were performed on an elective basis. Only one study[39] reported data exclusive to emergency colorectal cancer operations. The majority of surgery was undertaken with a curative intent with 54% of patients graded as Dukes A or Dukes B cancers. The operative characteristics were similar in the POSSUM, P-POSSUM and CR-POSSUM study populations.

Studies Included in the Morbidity Analysis

Four studies, comprising data on 768 patients, reported the accuracy of POSSUM in predicting post-operative

Table 2 The physiological and operative variables used in the calculation of CR-POSSUM score

	Score				
	1	2	3	4	8
Physiological variables					
Age	≤60		61–70	71–80	≥81
Cardiac failure	None/mild	Moderate	Severe		
Systolic BP (mmHg)	100–170	>170 90–99	<90		
Pulse (beats/min)	40–100	101–120	>120 <40		
Urea (mmol/l)	≤10	10.1–15	>15		
Haemoglobin (g/dl)	13–16	10–12.9 16.1–18	<10 >18		
Operative variables					
Operative severity	Minor		Intermediate	Major	Major+
Peritoneal soiling	None/Serous	Local pus	Free pus or faeces		
Operative urgency	Elective		Urgent		Emergency
Cancer staging	None Dukes A/B	Dukes C	Dukes D		

The regression equation used to calculate the risk (R) of mortality is as follows: CR − POSSUM mortality equation : $\mathrm{Log}[R/(R-1)] = -9.167 + (0.33 \times \text{physiological score}) + (0.30 \times \text{operative score})$

morbidity after colorectal cancer surgery. The patient and operative characteristics of these studies are shown in Table 5. Two studies reported morbidity based on the original POSSUM definitions[40,41] while two studies[42,43] used an arbitrary list of complications. Two studies[41,43] assessed morbidity rates retrospectively. There were also considerable differences in the operations performed. One study[43] reported data relating to a predominantly elderly population with a high proportion of emergency surgery (56%) while another[42] reported exclusively on elective rectal cancer resections with a high proportion of neoadjuvant chemo-radiotherapy (62%).

Observed to Expected Mortality

The ratios of observed to expected post-operative mortality in the POSSUM, P-POSSUM and CR-POSSUM studies are shown in Table 6. Ten studies (4,799 patients) reported data

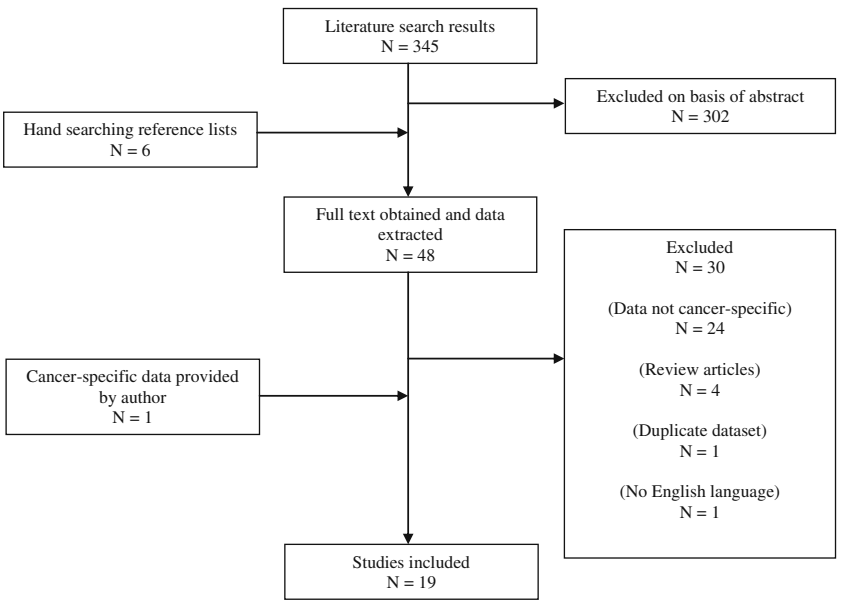

Fig. 1 QUOROM flow chart depicting the study selection process and application of exclusion criteria. Nineteen independent studies were included in the final review (mortality analysis=19/19 studies; morbidity analysis=4/19 studies). *QUOROM* quality of reporting of meta-analyses

Table 3 Summary characteristics of 19 studies of POSSUM, P-POSSUM and CR-POSSUM and prediction of post-operative mortality in patients undergoing surgery for colorectal cancer

Author	Year	Data analysis	CRC patients	Mortality reporting	Observed mortality (%)	POSSUM predicted mortality (%)	POSSUM O/E ratio	P-POSSUM predicted mortality (%)	P-POSSUM O/E ratio	CR-POSSUM predicted mortality (%)	CR-POSSUM O/E ratio
Menon[40]	2002	Prospective	173	30 day	8.7	–	–	15.6	0.6	–	–
Isbister[41]	2002	Retrospective	145	30 day	1.4	6.7	0.2	3.5	0.4	–	–
Senagore[47]	2004	Prospective	890	30 day	2.2	10.7	0.2	11.2	0.2	4.9	0.5
Poon[39]	2005	Both	160	30 day	11.3	–	–	15	0.8	–	–
Slim[48]	2006	Prospective	997	In-hospital	3.7	13.3	0.3	5.5	0.7	–	–
Vather[37a]	2006	Retrospective	325	In-hospital	3.7	8.3	0.4	3	1.2	7.2	0.5
Tez[49]	2006	Retrospective	321	30 day	6.6	–	–	9	0.7	7.8	0.9
Bromage[50]	2007	Both	304	In-hospital	6.5	21.9	0.3	10.3	0.6	8.1	0.8
Oomen[51]	2007	Retrospective	120	30 day	1.7	10.6	0.2	3.8	0.5	3.8	0.5
Horzic[52]	2007	Retrospective	120	Both	8.3	–	–	6.7	1.3	7.5	1.1
Ferjani[53]	2007	Prospective	618	30 day	10.2	12.7	0.8	4.4	2.3	9.6	1.1
Can[54]	2008	Prospective	224	30 day	3.6	13.4	0.3	5.2	0.7	–	–
Ugolini[43]	2008	Prospective	177	30 day	10.7	–	–	11.2	1	13.1	0.8
Valenti[42]	2009	Both	273	30 day	0.7	6.6	0.1	2	0.4	2.1	0.3
Tan[55]	2009	Both	121	30 day	1.6	–	–	–	–	11.2	0.1
Ren[56]	2009	Prospective	903	In-hospital	1.0	5.6	0.2	2.8	0.4	4.8	0.2
Ugolini[57]	2009	Prospective	208	30 day	6.3	–	–	7.9	0.8	9.1	0.7
Leung[58]	2009	Prospective	618	30 day	10.0	–	–	4.4	2.4	9.5	1
Hariharan[17]	2009	Retrospective	232	30 day	6.9	–	–	–	–	7.7	0.9

CRC colorectal cancer

[a] Data presented is colorectal cancer specific and was supplied by the author on request

Table 4 Clinicopathological characteristics of 19 studies of POSSUM, P-POSSUM and CR-POSSUM and prediction of post-operative mortality in patients undergoing surgery for colorectal cancer

Operative details	POSSUM patients n=4799 (%)	P-POSSUM patients n=6576 (%)	CR-POSSUM patients n=5230 (%)	All patients n=6929 (%)
No. of studies	10	17	14	19
Major operation.	99	99	100	99
Minor operation.	1	1	0	1
Open operation	100	96	96	96
Laparoscopic operation	0	4	4	4
Dukes A/B	57[a]	54[a]	57[a]	54[a]
Dukes C/D	40[a]	43[a]	40[a]	43[a]
Elective presentation	87	74	80	76
Emergency presentation	13	26	20	24

Data are presented as mean percentages

CRC colorectal cancer

[a] Values do not equal 100% because not every study reported all characteristics

on the original POSSUM model. The observed mortality in these patients ranged from 1.4% to 10.2% while the predicted mortality ranged from 5.6% to 21.9%. Seventeen studies (6,576 patients) reported on P-POSSUM. The observed mortality ranged from 0.7% to 11.3% while the predicted mortality ranged from 2% to 15.6%. Fourteen studies (5,230 patients) reported on CR-POSSUM. The observed mortality ranged from 0.7% to 10.7% while the predicted mortality ranged from 2.1% to 13.1%. The weighted observed to expected ratio for mortality for POSSUM, P-POSSUM and CR-POSSUM was 0.31, 0.90 and 0.64, respectively (Table 6).

Observed to Expected Morbidity

There was considerable variation in the observed and predicted rates of morbidity between the four studies. The observed morbidity rate ranged from 26.4% to 54.5% while

Table 5 Summary characteristics of four studies of POSSUM and prediction of post-operative morbidity in patients undergoing surgery for colorectal cancer

Author	Year	Patients with CRC	Study characteristics	Morbidity recording	Definitions used	Morbidity observed (%)	Morbidity expected (%)	O/E ratio
Menon[40]	2001	173	Elective 79% Dukes A/B 50% Dukes C/D 50%	Prospective	POSSUM definitions	28.9	32.1	0.90
Isbister[41]	2002	145	Single surgeon Rectal cancer 100% Pre-op DXT 5%	Retrospective	POSSUM definitions	54.5	35.4	1.54
Valenti[42]	2008	273	Elective 100% Rectal cancer 100% Pre-op CTx/DXT 62%	Prospective	Arbitrary list	26.4	31.2	0.84
Ugolini[43]	2008	177	Elderly population Elective 44% Emergency 56%	Retrospective	Arbitrary list	42.7	59.3	0.72

CRC colorectal cancer, *CTx* chemotherapy, *DXT* radiotherapy

Table 6 Summary data of all studies of POSSUM, P-POSSUM and CR-POSSUM and prediction of post-operative mortality and morbidity in patients undergoing surgery for colorectal cancer

Model	Studies	CRC patients	O/E ratio range	Weighted O/E ratio	95% confidence interval
Mortality prediction					
POSSUM	10	4,799	0.11–0.80	0.31	0.31–0.32
P-POSSUM	17	6,576	0.20–2.36	0.90	0.88–0.92
CR-POSSUM	14	5,230	0.14–1.11	0.64	0.63–0.65
Morbidity prediction					
POSSUM	4	768	0.72–1.54	0.96	0.94–0.98

Data is presented as the mean observed to expected (O/E) ratio, weighted according to study sample size

CRC colorectal cancer

the predicted morbidity ranged from 31.2% to 59.3%. The weighted observed to expected ratio for morbidity for POSSUM was 0.96 (Table 5).

Discussion

The present study is, to our knowledge, the first systematic review of POSSUM and its related models as predictors of post-operative mortality in patients undergoing surgery for colorectal cancer. The results of the present review of 6,929 colorectal cancer operations, indicate that, compared with the original POSSUM model, both P-POSSUM and CR-POSSUM are better predictors of post-operative mortality. Furthermore, compared with P-POSSUM, CR-POSSUM offers no additional predictive value.

The reasons for the lack of additional value of CR-POSSUM in patients undergoing surgery for colorectal cancer are not clear. P-POSSUM was originally developed by analysing over 10,000 operative procedures[10,11] and, although these were not described in detail, all procedures required hospital admission. In the development of CR-POSSUM, Tekkis and coworkers (2004), in almost 7,000 colorectal surgical cases, included a large proportion of minor and non-cancer operations (approximately half). Therefore, it may be that P-POSSUM, developed on a population undergoing inpatient surgical operations (i.e. greater surgical severity), better predicts post-operative mortality in patients with colorectal cancer than the CR-POSSUM model, based solely on surgical site. This heterogeneity of patients and procedures even within a colorectal specialty highlights the inherent problem of developing specialty-specific models to predict post-operative mortality. In addition, previous reports may simply have lacked power as very large populations are needed to assess the predictive value of any model given the low mortality rates associated with the majority of colorectal procedures.

The present study is also, to our knowledge, the first systematic review of POSSUM as a predictor of post-operative morbidity in patients undergoing surgery for colorectal cancer. The results of the present review of 768 colorectal cancer operations, suggest that POSSUM performs well (O/E ratio 0.96) as a predictor of post-operative morbidity. Given that post-operative complications are considerably more common than post-operative death in patients undergoing surgery for colorectal cancer, these results would suggest an important future role for POSSUM as an audit tool. In the past, complications after major colorectal resection have not been well defined or uniformly recorded. More recently, in attempt to address this, the complications following colorectal cancer surgery have been defined as infectious (wound infection, intra-abdominal abscess, anastomotic leak, pneumonia and septicaemia) and non-infectious (cardiac events encompassing acute coronary syndrome and acute myocardial infarction and pulmonary embolism).[44,45] This will enable reliable recording of post-operative complications in such patients and a more detailed examination of the predictive value of POSSUM and other indices in future large scale studies.

There are a number of limitations to the present study. We restricted the review to the examination of the POSSUM models. Other models have been used to predict post-operative mortality in colorectal cancer surgery including the American Society of Anaesthesiologists (ASA) grade,[4] the Simplified Acute Physiology Score (SAPS II)[46] and the Acute Physiology and Chronic Health Evaluation (APACHE II) score.[3] More recently, the Association of Coloproctology of Great Britain and Ireland (ACPGBI) have developed a model to predict post-operative mortality after colorectal cancer surgery[5] although as yet there are few reports of its use in the literature. In the present study the POSSUM models alone were examined since these models were developed specifically to predict mortality and morbidity after operative surgery and have been extensively applied to general surgical populations.

In summary, in the present systematic review, both P-POSSUM and CR-POSSUM, compared with the original POSSUM, are better predictors of post-operative mortality after colorectal cancer surgery. Furthermore, CR-POSSUM offers no additional predictive value over P-POSSUM.

References

1. Cancerstats. Available at: www.cancerresearchuk.org. Accessed March 2010.
2. Sagar PM, Hartley MN, Mancey-Jones B, Sedman PC, May J, Macfie J. Comparative audit of colorectal resection with the POSSUM scoring system. Br J Surg. 1994 Oct;81(10):1492–4.
3. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985 Oct;13(10):818–29.
4. Dripps RD, Lamont A, Eckenhoff JE. The role of anesthesia in surgical mortality. JAMA. 1961 Oct 21;178:261–6.
5. Tekkis PP, Poloniecki JD, Thompson MR, Stamatakis JD. Operative mortality in colorectal cancer: prospective national study. BMJ. 2003 Nov 22;327(7425):1196–201.
6. Jones HJ, de Cossart L. Risk scoring in surgical patients. Br J Surg. 1999 Feb;86(2):149–57.
7. Copeland GP, Jones D, Walters M. POSSUM: a scoring system for surgical audit. Br J Surg. 1991 Mar;78(3):355–60.
8. Copeland GP, Sagar P, Brennan J, Roberts G, Ward J, Cornford P, Millar A, Harris C. Risk-adjusted analysis of surgeon performance: a 1-year study. Br J Surg. 1995 Mar;82(3):408–11.
9. Sagar PM, Hartley MN, MacFie J, Taylor BA, Copeland GP. Comparison of individual surgeon's performance. Risk-adjusted analysis with POSSUM scoring system. Dis Colon Rectum. 1996 Jun;39(6):654–8.
10. Whiteley MS, Prytherch DR, Higgins B, Weaver PC, Prout WG. An evaluation of the POSSUM surgical scoring system. Br J Surg. 1996 Jun;83(6):812–5.
11. Prytherch DR, Whiteley MS, Higgins B, Weaver PC, Prout WG, Powell SJ. POSSUM and Portsmouth POSSUM for predicting mortality. Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity. Br J Surg. 1998 Sep;85(9):1217–20.
12. Prytherch DR, Ridler BM, Beard JD, Earnshaw JJ; Audit and Research Committee, The Vascular Surgical Society of Great Britian and Ireland. A model for national outcome audit in vascular surgery. Eur J Vasc Endovasc Surg. 2001 Jun;21(6):477–83.
13. Prytherch DR, Sutton GL, Boyle JR. Portsmouth POSSUM models for abdominal aortic aneurysm surgery. Br J Surg. 2001 Jul;88(7):958–63.
14. Tekkis PP, McCulloch P, Poloniecki JD, Prytherch DR, Kessaris N, Steger AC. Risk-adjusted prediction of operative mortality in oesophagogastric surgery with O-POSSUM. Br J Surg. 2004 Mar;91(3):288–95.
15. Tekkis PP, Prytherch DR, Kocher HM, Senapati A, Poloniecki JD, Stamatakis JD, Windsor AC. Development of a dedicated risk-adjustment scoring system for colorectal surgery (colorectal POSSUM). Br J Surg. 2004 Sep;91(9):1174–82.
16. Wakabayashi H, Sano T, Yachida S, Okano K, Izuishi K, Suzuki Y. Validation of risk assessment scoring systems for an audit of elective surgery for gastrointestinal cancer in elderly patients: an audit. Int J Surg. 2007 Oct;5(5):323–7.
17. Anwar MA, D'Souza F, Coulter R, Memon B, Khan IM, Memon MA. Outcome of acutely perforated colorectal cancers: experience of a single district general hospital.Surg Oncol. 2006 Aug;15(2):91–6.
18. Bennett-Guerrero E, Hyam JA, Shaefi S, Prytherch DR, Sutton GL, Weaver PC, Mythen MG, Grocott MP, Parides MK. Comparison of P-POSSUM risk-adjusted mortality rates after surgery between patients in the USA and the UK. Br J Surg. 2003 Dec;90(12):1593–8.
19. Brooks MJ, Sutton R, Sarin S. Comparison of Surgical Risk Score, POSSUM and P-POSSUM in higher-risk surgical patients. Br J Surg. 2005 Oct;92(10):1288–92.
20. Ertan T, Yoldas O, Kilic YA, Kilic M, Göcmen E, Koc M, Tez M. External validation of prognostic models among cancer patients undergoing emergency colorectal surgery. Am J Surg. 2008 Apr;195(4):439–41.
21. Ishizuka M, Nagata H, Takagi K, Horie T, Kubota K. POSSUM is an optimal system for predicting mortality due to colorectal perforation. Hepatogastroenterology. 2008 Mar-Apr;55(82–83):430–3.
22. Jones DR, Copeland GP, de Cossart L. Comparison of POSSUM with APACHE II for prediction of outcome from a surgical high-dependency unit. Br J Surg. 1992 Dec;79(12):1293–6.
23. Kumar P, Rodrigues GS. Comparison of POSSUM and P-POSSUM for risk-adjusted audit of patients undergoing emergency laparotomy. Ulus Travma Acil Cerrahi Derg. 2009 Jan;15(1):19–22.
24. Law WL, Lam CM, Lee YM. Evaluation of outcome of laparoscopic colorectal resection with POSSUM, Portsmouth POSSUM and colorectal POSSUM. Br J Surg. 2006 Jan;93(1):94–9.
25. Metcalfe MS, Norwood MG, Miller AS, Hemingway D. Unreasonable expectations in emergency colorectal cancer surgery. Colorectal Dis. 2005 May;7(3):275–8.
26. Nicole O, Thomas M et al. Evaluation of the P−POSSUM mortality prediction algorithm in Australian surgical intensive care unit patients. ANZ J Surgery.2002;72(10):735–38
27. Ramkumar T, Ng V, Fowler L, Farouk R. A comparison of POSSUM, P-POSSUM and colorectal POSSUM for the prediction of postoperative mortality in patients undergoing colorectal resection. Dis Colon Rectum. 2006 Mar;49(3):330–5.
28. Senagore AJ, Delaney CP, Duepree HJ, Brady KM, Fazio VW. Evaluation of POSSUM and P-POSSUM scoring systems in assessing outcome after laparoscopic colectomy. Br J Surg. 2003 Oct;90(10):1280–4.
29. Yii MK, Ng KJ. Risk-adjusted surgical audit with the POSSUM scoring system in a developing country. Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity. Br J Surg. 2002 Jan;89(1):110–3.
30. Tekkis PP, Kessaris N, Kocher HM, Poloniecki JD, Lyttle J, Windsor AC. Evaluation of POSSUM and P-POSSUM scoring systems in patients undergoing colorectal surgery. Br J Surg. 2003 Mar;90(3):340–5.
31. Tekkis PP, Kocher HM, Bentley AJ, Cullen PT, South LM, Trotter GA, Ellul JP. Operative mortality rates among surgeons: comparison of POSSUM and p-POSSUM scoring systems in gastrointestinal surgery. Dis Colon Rectum. 2000 Nov;43(11):1528–32.
32. Neary WD, Heather BP, Earnshaw JJ. The Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM). Br J Surg. 2003 Feb;90(2):157–65.
33. Copeland GP. The POSSUM system of surgical audit. Arch Surg. 2002 Jan;137(1):15–9.
34. Colorectal Cancer Collaborative Group. Surgery for colorectal cancer in elderly patients: a systematic review. Lancet. 2000 Sep 16;356(9234):968–74.
35. Al-Homoud S, Purkayastha S, Aziz O, Smith JJ, Thompson MD, Darzi AW, Stamatakis JD, Tekkis PP. Evaluating operative risk in

colorectal cancer surgery: ASA and POSSUM-based predictive models. Surg Oncol. 2004 Aug-Nov;13(2–3):83–92.
36. Makoto W, Naokuni Y, Tomokazu K et al. Estimation of mortality and morbidity risk in colorectal surgery using POSSUM predictor equation. Japanese Journal of Gastroenterological surgery. 2004; 37 (11) 1714–1720
37. Vather R, Zargar-Shoshtari K, Adegbola S, Hill AG. Comparison of the possum, P-POSSUM and Cr-POSSUM scoring systems as predictors of postoperative mortality in patients undergoing major colorectal surgery. ANZ J Surg. 2006 Sep;76(9):812–6.
38. Horzic M, Kopljar M, Cupurdija K, Bielen DV, Vergles D, Lackovic Z. Comparison of P-POSSUM and Cr-POSSUM scores in patients undergoing colorectal cancer resection. Arch Surg. 2007 Nov;142(11):1043–8.
39. Poon JT, Chan B, Law WL. Evaluation of P-POSSUM in surgery for obstructing colorectal cancer and correlation of the predicted mortality with different surgical options. Dis Colon Rectum. 2005 Mar;48(3):493–8.
40. Menon KV, Farouk R. An analysis of the accuracy of P-POSSUM scoring for mortality risk assessment after surgery for colorectal cancer. Colorectal Dis. 2002 May;4(3):197–200.
41. Isbister WH, Al-Sanea N. POSSUM: a re-evaluation in patients undergoing surgery for rectal cancer. The Physiological and Operative Severity Score for Enumeration of Mortality and Morbidity. ANZ J Surg. 2002 Jun;72(6):421–5.
42. Valenti V, Hernandez-Lizoain JL, Baixauli J, Pastor C, Martinez-Regueira F, Beunza JJ, Aristu JJ, Alvarez Cienfuegos J. Analysis of POSSUM score and postoperative morbidity in patients with rectal cancer undergoing surgery. Langenbecks Arch Surg. 2009 Jan;394(1):55–63.
43. Ugolini G, Rosati G, Montroni I, Zanotti S, Alessio M, Giampaolo L, Blume JF, Taffurelli M. Can elderly patients with colorectal cancer tolerate planned surgical treatment? A practical approach to a common dilemma. Colorectal Dis.2008(11):750–755
44. Ytting H, Christensen IJ, Jensenius JC, Thiel S, Nielsen HJ. Preoperative mannan-binding lectin pathway and prognosis in colorectal cancer. Cancer Immunol Immunother. 2005 Mar;54 (3):265–72.
45. Kørner H, Nielsen HJ, Søreide JA, Nedrebø BS, Søreide K, Knapp JC. Diagnostic accuracy of C-reactive protein for intra-abdominal infections after colorectal resections. J Gastrointest Surg. 2009 Sep;13(9):1599–606.
46. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score(SAPS II) based on a European/North American multicenter study. JAMA. 1993 Dec 22–29;270(24):2957–63.
47. Senagore AJ, Warmuth AJ, Delaney CP, Tekkis PP, Fazio VW. POSSUM, P-POSSUM, and CR-POSSUM: implementation issues in a United States health care system for prediction of outcome for colon cancer resection. Dis Colon Rectum. 2004 Sep;47(9):1435–41.
48. Slim K, Panis Y, Alves A, Kwiatkowski F, Mathieu P, Mantion G; Association Française de Chirurgie. Predicting postoperative mortality in patients undergoing colorectal surgery. World J Surg. 2006 Jan;30(1):100–6.
49. Tez M, Yoldaş O, Gocmen E, Külah B, Koc M. Evaluation of P-POSSUM and CR-POSSUM scores in patients with colorectal cancer undergoing resection. World J Surg. 2006 Dec;30 (12):2266–9.
50. Bromage SJ, Cunliffe WJ. Validation of the CR-POSSUM risk-adjusted scoring system for major colorectal cancer surgery in a single center. Dis Colon Rectum. 2007 Feb;50(2):192–6.
51. Oomen JL, Cuesta MA, Engel AF. Comparison of outcome of POSSUM, p-POSSUM, and cr-POSSUM scoring after elective resection of the sigmoid colon for carcinoma or complicated diverticular disease. Scand J Gastroenterol. 2007 Jul;42(7):841–7.
52. Ferjani AM, Griffin D, Stallard N, Wong LS. A newly devised scoring system for prediction of mortality in patients with colorectal cancer: a prospective study. Lancet Oncol. 2007 Apr;8 (4):317–22.
53. Can MF, Yagci G, Tufan T, Ozturk E, Zeybek N, Cetiner S. Can SAPS II predict operative mortality more accurately than POSSUM and P-POSSUM in patients with colorectal carcinoma undergoing resection? World J Surg. 2008 Apr;32 (4):589–95.
54. Ren L, Upadhyay AM, Wang L, Li L, Lu J, Fu W. Mortality rate prediction by Physiological and Operative Severity Score for the Enumeration of Mortality and Morbidity (POSSUM), Portsmouth POSSUM and Colorectal POSSUM and the developmentof new scoring systems in Chinese colorectal cancer patients. Am J Surg. 2009 Jul;198(1):31–8.
55. Tan KY, Kawamura Y, Mizokami K, Sasaki J, Tsujinaka S, Maeda T, Konishi F. Colorectal surgery in octogenarian patients—outcomes and predictors of morbidity. Int J Colorectal Dis. 2009 Feb;24(2):185–9.
56. Ugolini G, Rosati G, Montroni I, Zanotti S, Manaresi A, Giampaolo L,Taffurelli M, Pricolo V. An easy-to-use solution for clinical audit in colorectal cancer surgery. Surgery. 2009 Jan;145(1):86–92.
57. Leung E, Ferjani AM, Stellard N, Wong LS. Predicting post-operative mortality in patients undergoing colorectal surgery using P-POSSUM and CR-POSSUM scores: a prospective study. Int J Colorectal Dis. 2009 Dec;24(12):1459–64.
58. Hariharan S, Chen D, Ramkissoon A, Taklalsingh N, Bodkyn C, Cupidore R, Ramdin A, Ramsaroop A, Sinanan V, Teelucksingh S, Verma S. Perioperative outcome of colorectal cancer and validation of CR-POSSUM in a Caribbean country. Int J Surg.2009 Dec;7(6):534–8.

Discussion

Discussant

Dr. Matthew M. Hutter (Boston, MA): Thank you, Dr. Richards. Congratulations on an excellent presentation of a very interesting study.

Judging by the multiple studies you examined with conflicting results comparing these three scoring systems, POSSUM, P-POSSUM, and colorectal POSSUM, it seems Logical to proceed with the meta-analysis or systematic review to make order out of the chaos. However, my underlying concern is that the reason the individual studies disagree is that none of the scoring systems are ultimately very good in assessing the quality of care.

For the P-POSSUM, the supposed winner of the three, there's a wide range in the O/E ratios from .2 to 2.4. That means that there's either a tremendous range in the quality of care provided to the patients in these studies or that even the P-POSSUM actually does a very bad job of predicting mortality. The problem is we don't know which one is true.

So my first question then is given what you have learned about these scores, would you want your performance as a surgeon, or that of your authors, your partners, your hospital,

hospital system, to be measured or graded According to such scores?

My second question is, if so or if not, what would you do, what important characteristics would you use to create a system that would be better?

Closing Discussant

Dr. Colin Richards: I think you're right and that, ultimately, no scoring system is perfect. Although the POSSUM models are far from perfect I believe they have some good points.

Number one, the majority of data required is routinely collected, so it's possible, even retrospectively, to calculate an accurate P-POSSUM score for patients. Secondly, although there is variation in the reported OE ratios I believe that in large populations with rigorous data collection P-POSSUM will prove accurate.

The fact that there is a wide variation in reported OE ratios was one of the reasons we undertook the review. Some studies are reporting an OE ratio of 0.2 while some studies are saying over 2.0.

Having undertaken the review I think some of this variation is certainly because the number of events or the number of deaths in these studies is low. The actual mortality, especially in elective colorectal cancer surgery, is so low that you need a population of at least 400 or 500 patients over an appropriate time period before you can get any sense of it. In smaller populations one or two deaths in a row make a big difference and change the O/E ratio significantly. So I think many of the original studies were underpowered and that's one of the reasons we are getting such a disparity.

I also believe that is one of the advantages to pooling the data like this; when you take the patient numbers up to 6,000 or 7,000, you get less disparity and the model appears more accurate.

In terms of whether we would be prepared to use it in our hospital, I think the short answer is yes. I think if you're going to compare mortality between institutions or between surgeons, you're much better doing it in a risk-adjusted rather than a crude fashion. In terms of risk-adjustment scoring for colorectal cancer surgery, this is the best we currently have.

If we were to create a better system, what would change? I think, ideally, you would want a system which relies on a smaller number of variables, is simple to construct and one which can grade patients pre-operatively instead of having to go back and add things such as pathology results post-operatively. Having a predicted risk of death before you undertake the operation would be advantageous in surgical planning and patient counseling. I would also place more emphasis on whether the surgery was elective or emergency side because that appears to have a greater impact on mortality than say the 'complexity' of the operation. Currently in the colorectal POSSUM score, for example.

A complex major operation, such as a high anterior resection adds a significant amount of points, compared to a left hemicolectomy. I think that nowadays, most surgeons would not expect or indeed observe a post-operative mortality difference between such similar elective operations.

J Gastrointest Surg (2010) 14:1521–1528
DOI 10.1007/s11605-010-1340-6

2010 SSAT PLENARY PRESENTATION

Relationship of EMAST and Microsatellite Instability Among Patients with Rectal Cancer

Bikash Devaraj · Aaron Lee · Betty L. Cabrera · Katsumi Miyai · Linda Luo · Sonia Ramamoorthy · Temitope Keku · Robert S. Sandler · Kathleen L. McGuire · John M. Carethers

Received: 30 April 2010 / Accepted: 18 August 2010 / Published online: 16 September 2010

Abstract
Background Elevated microsatellite instability at selected tetranucleotide repeats (EMAST) is a genetic signature identified in 60% of sporadic colon cancers and may be linked with heterogeneous expression of the DNA mismatch repair (MMR) protein hMSH3. Unlike microsatellite instability-high (MSI-H) in which hypermethylation of hMLH1 occurs followed by multiple susceptible gene mutations, EMAST may be associated with inflammation and subsequent relaxation of MMR function with the biological consequences not known. We evaluated the prevalence of EMAST and MSI in a population-based cohort of rectal cancers, as EMAST has not been previously determined in rectal cancers.
Methods We analyzed 147 sporadic cases of rectal cancer using five tetranucleotide microsatellite markers and National-Cancer-Institute-recommended MSI (mononucleotide and dinucleotide) markers. EMAST and MSI determinations were made on analysis of DNA sequences of the polymerase chain reaction products and determined positive if at least two loci were found to have frame-shifted repeats upon comparison between normal and cancer samples from the same patient. We correlated EMAST data with race, gender, and tumor stage and examined the samples for lymphocyte infiltration.
Results Among this cohort of patients with rectal cancer (mean age 62.2±10.3 years, 36% female, 24% African American), 3/147 (2%) showed MSI (three males, two African American) and 49/147 (33%) demonstrated EMAST. Rectal tumors from African Americans were more likely to show EMAST than Caucasians (18/37, 49% vs. 27/104, 26%, p=0.014) and were associated with advanced stage (18/29, 62% EMAST vs. 18/53, 37%, non-EMAST p=0.02). There was no association between EMAST and gender. EMAST was more prevalent in rectal tumors that showed peri-tumoral infiltration compared to those without (30/49, 60% EMAST vs. 24/98, 25% non-EMAST, p=0.0001).
Conclusions EMAST in rectal cancer is common and MSI is rare. EMAST is associated with African-American race and may be more commonly seen with metastatic disease. The etiology and consequences of EMAST are under investigation, but its association with immune cell infiltration suggests that inflammation may play a role for its development.

BD and JMC designed the research. BD, AL, BLC, LL, SR performed the research. K Miyai provided pathological expertise. BD, AL and JMC analyzed data. TK and RSS provided specimens. KL McGuire provided inflammatory cell expertise. BD and JMC wrote the paper. All authors have approved the final version of this manuscript.

B. Devaraj · L. Luo · S. Ramamoorthy
Department of Surgery, University of California, San Diego, CA, USA

K. Miyai
Department of Pathology, University of California, San Diego, CA, USA

A. Lee · B. L. Cabrera · J. M. Carethers
Department of Medicine, University of California, San Diego, CA, USA

S. Ramamoorthy · J. M. Carethers
Moores Comprehensive Cancer Center, University of California, San Diego, CA, USA

Keywords EMAST · Microsatellite instability · Rectal cancer · DNA mismatch repair · African American

Abbreviations

EMAST	Elevated microsatellite instability at selected tetranucleotide repeats
MSI	Microsatellite instability
MSI-H	Microsatellite instability-high
MMR	Mismatch repair

Introduction

Microsatellite instability (MSI) is a hallmark of mismatch repair (MMR) dysfunction and is detected by instability at mononucleotide or dinucleotide microsatellite DNA sequences. MSI is seen in patients with Lynch syndrome and in approximately 15% of patients with sporadic colorectal cancer. The other 85% of sporadic colorectal cancers do not demonstrate this MSI pattern and have not been associated with MMR deficiency.[1,2] Alterations involving specific tetranucleotide microsatellite DNA sequences, termed "elevated microsatellite alterations at selected tetranucleotide repeats," or EMAST, have not been linked to MMR dysfunction. EMAST has been previously observed in non-small-cell lung,[3,4] skin,[5] ovarian,[6] and bladder cancers.[5,7] The etiology for EMAST is not known, but EMAST has been used as a biomarker for some of these tumors.

Most recently, EMAST has been shown to have a prevalence of ~60% among a cohort of sporadic colon cancers.[8] Although the underlying mechanism behind EMAST remains unknown, the authors suggests that MSH3, an MMR gene involved in repair of longer repeat sequences such as those greater than dinucleotide repeats, may be linked to EMAST due to its "heterogeneous" immunohistochemical expression in some colon cancers.[8] This type of pattern suggests an acquired defect, as no germ line mutation in MSH3 has ever been demonstrated.[2] Some MMR genes, in particular MSH6 and PMS2, can be downregulated in the setting of inflammation,[9] suggesting a potential mechanism for "relaxation" of DNA MMR function. An association between inflammation and EMAST has not been previously demonstrated.

Rectal cancers have a number of differences from colon cancers, but both disease processes are often lumped together in studies as colorectal cancer. Differences include (a) its embryonic origin, (b) its gender differences in incidence, (c) its molecular profile of genes, and (d) its approach to treatment.[10–13] Classic MSI prevalence has been described among cohorts ranging from 0% to 20%,[14–16] but EMAST has never been evaluated among rectal cancers.

In this study, we evaluated the prevalence of MSI and EMAST in sporadic rectal cancer. We also analyzed clinicopathological features including race, gender, and disease stage and correlated these with EMAST prevalence. We also correlated the presence of inflammatory cells histologically with EMAST as a means to assess linkage of inflammation to EMAST. We observed that sporadic rectal tumors demonstrate rare MSI but commonly demonstrate EMAST. In addition, EMAST in rectal tumors is associated with the African-American race, advanced stage, and the presence of chronic inflammation.

Methods

Patient Tissue and DNA Extraction

Formalin-fixed, paraffin-embedded tissues from 147 unselected sporadic rectal cancer patients that had linked epidemiological data were used for this study. Data included gender, race, age, and tumor stage. Corresponding normal tissue was microdissected for comparison against tumor tissue from the same patient. All tissues were obtained from the North Carolina Rectal Cancer Study cohort. The project was a population-based cohort assessing rectal cancer and epidemiological data from 33 counties in North Carolina.[17]

Paraffin-embedded normal and tumor tissues were cut into 5-μm sections, and microdissection was performed under microscopy. Genomic DNA was isolated using GeneReleaser (Bioventure, Inc.) and then treated with proteinase K.[18]

DNA Amplification

Each matched pair of tumor and normal tissue was subjected to 35–40 cycles of polymerase chain reaction

T. Keku · R. S. Sandler
University of North Carolina,
Chapel Hill, NC, USA

K. L. McGuire
San Diego State University,
San Diego, CA, USA

J. M. Carethers
VA Research Service,
San Diego, CA, USA

J. M. Carethers (✉)
Department of Internal Medicine, University of Michigan,
3101 Taubman Center, 1500 E. Medical Center Drive,
Ann Arbor, MI 48109, USA
e-mail: jcarethe@umich.edu

(PCR). PCR was performed in a total volume of 25 μl inclusive of 10–20 ng of genomic DNA, 0.2 μmol of each primer, and 20 μl of PCR Supermix (Invitrogen, Inc.). PCR parameters were as follows for 35–40 cycles: 92°C for 1 min, 58°C for 40 s, and 72°C for 1 min for most primer sets.

Mononucleotide, Dinucleotide, and Tetranucleotide Microsatellite Analysis for Rectal Cancer Tissues

Primers for each of the tetranucleotide microsatellite loci were designed and are listed in Table 1. A total of five EMAST markers (MYCL1, D20S85, D8S321, D20S82, and D9S242) and five National Cancer Institute (NCI)-recommended microsatellite markers (BAT25, BAT26, D5S346, D2S123, and D17S250)[1] were used. All PCR products were sequenced at the UCSD DNA Sequencing Facility in order to determine frameshifts (or instability) at each locus. Classification of microsatellite instability was performed in accordance with previously established protocols: tumors were classified as MSI-H if two or more loci showed instability compared to normal controls, MSI-L if only one locus demonstrated instability.[1] MSS tumors were classified when no instability occurred at any locus. We determined EMAST in tumors demonstrating instability at tetranucleotide loci in at least two or more of the loci studied when compared to normal controls from the same patient. Non-EMAST tumors were classified if only one or no instability in tetranucleotide loci was observed. A locus was considered unstable if there was a frameshift difference in the number of repeats between the tumor and normal samples.

Inflammatory Cell Infiltrate Analysis

Hematoxylin–eosin staining was performed on all 147 samples. Each of the samples was then analyzed by a single board-certified clinical pathologist to ascertain the presence of inflammatory cell infiltration within or around the tumors. The pathologist was blinded to the MSI and EMAST data.

Table 1 Tetranucleotide microsatellite PCR primer sequences

Primer	Sequence
MYCL1	Fwd: TGG CGA GAC TCC ATC AAA G
	Rev: CCT TTT AAG CTG CAA CAA TTT C
D20S85	Fwd: GAG TAT CCA GAG AGC TAT TA
	Rev: ATT ACA GTG TGA GAC CCT G
D8S321	Fwd: GAT GAA AGA ATG ATA GAT TAC AG
	Rev: ATC TTC TCA TGC CAT ATC TGC
D20S82	Fwd: GCC TTG ATC ACA CCA CTA CA
	Rev: GTG GTC ACT AAA GTT TCT GCT
D9S242	Fwd: GTG AGA GTT CCT TCT GGC
	Rev: ACT CCA GTA CAA GAC TCT G

Statistical Analysis

We performed statistical analysis using the Fisher exact test between clinicopathologic statuses or degree of inflammatory cell infiltration and the MSS, MSI, and EMAST groups. All p values represent two-sided statistical tests with statistical significance at $p<0.05$.

Results

EMAST Is Common and MSI Is Rare in Rectal Cancers

We utilized 147 rectal cancers that had linked epidemiological data from the North Carolina Rectal Cancer Study.[17] We utilized five tetranucleotide markers that have been traditionally used to define EMAST, and we considered rectal tumors as EMAST if at least two markers demonstrated instability. Based on this definition, 49/147 (33%) demonstrated EMAST. All EMAST tumors were MSS, as MSI was rare as described below. A representative sequence demonstrating EMAST loci instability is shown in Fig. 1 and the frequency of total positive markers is shown in Table 2. The highest frequency of MSI in EMAST tumors was demonstrated at the D20S82 locus (32/49, 65%), followed by the D8S321 locus (30/49, 61%). The frequency of positive markers for EMAST tumors is shown in Table 2. The data suggest that EMAST is common in rectal tumors.

Using the NCI-recommended markers for MSI, only 3/147 (2%) demonstrated MSI-H. All other tumors were MSS (Table 3). Thus, in our population-based cohort of rectal cancer, MSI-H is rare.

EMAST Is Correlated with Patient Race and Tumor Stage

Among the 147 patients in this cohort, the mean age was 62 ±10 years (range 43–79). Sixty-two percent of the patients were male and 26% were African American. Tumor stage data were available for 82 of the 147 patients in our cohort. Correlations between age, stage, and gender with MSI and EMAST and non-EMAST tumors are shown in Tables 3 and 4, respectively. A stage classification of *local* was ascribed to stages 1 and 2 while *regional* classification was limited to stage 3 and *distant* to stage 4 rectal cancers.

Rectal tumors in African Americans were more likely to demonstrate EMAST compared to those in Caucasians (18/37, 49% vs. 27/104, 26%, $p=0.014$). EMAST tumors in

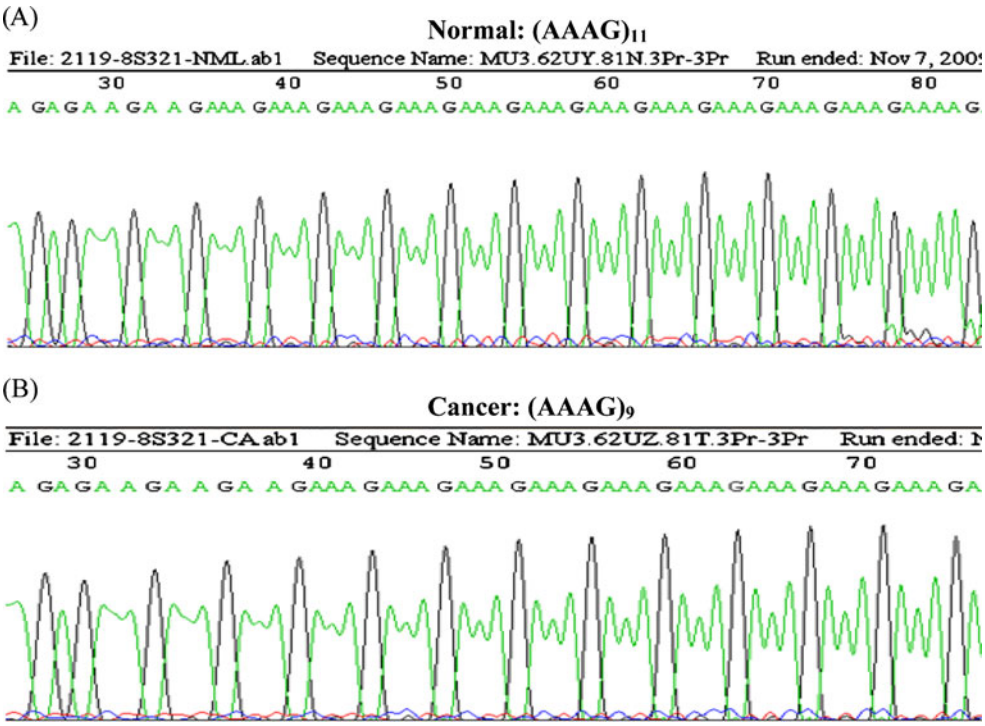

Fig. 1 D8S321 locus instability. Comparison between the normal (**b**) and cancer (**b**) sequences from the same patient reveals a deletion of two tetranucleotide repeats

our cohort were associated with a more advanced stage (stage 3 and above) (18/29, 62% vs. 18/53, 37% advanced for non-EMAST, $p=0.02$). There was no gender association for EMAST tumors (female 20/54, 37% vs. male 29/87, 33%, $p=0.717$).

EMAST Is Correlated with Chronic Inflammation

We assessed all 147 rectal tumors for the pattern and degree of chronic inflammation. Invasive margin, intratumoral, and cancer nests stromal patterns describe inflammatory cells that surround or are at the peripheral edge of the tumor, are within the tumor, or surround the epithelial components of the tumor, respectively. We considered a tumor positive for inflammation if >50% of an average of five high-power fields have mononuclear cell infiltrates. With this definition, 54 tumors were positive for chronic inflammation. EMAST tumors were associated with chronic inflammation when compared to non-EMAST tumors (Fig. 2a, Table 4). Although both EMAST and non-EMAST tumors demonstrated an invasive margin or leading edge pattern of inflammation, EMAST tumors showed in addition a predominant amount of chronic inflammation in the stroma surrounding tumor nests (Fig. 2ab, Table 5). Neither EMAST nor non-EMAST tumors demonstrated any intratumoral pattern of inflammatory cell infiltration. The proximity of inflammatory cells to the epithelia components of the tumor might influence EMAST formation.

Discussion

This study evaluated the prevalence of MSI and EMAST in rectal adenocarcinomas and assessed these with available epidemiological parameters. Rectal cancer, often lumped together with colon cancer, has unique features from colon cancer that include its embryologic origin, its gender differences in incidence, its lower 5-year survivability, its increased local recurrence, and its treatment algorithms. We observed that MSI is rare while EMAST is common among rectal cancers. In this first assessment of clinical parameters with EMAST, EMAST is associated with African-American

Table 2 Frequency of tetranucleotide marker mutations in rectal cancers

Loci	MYCL1	D20S85	D8S321	D20S82	D9S242
All rectal tumors					
# Times mutated ($N=147$)	35 (25%)	9 (8%)	43 (30%)	43 (30%)	35 (24%)
EMAST tumors					
# Times mutated ($N=49$)	23 (47%)	5 (10%)	30 (61%)	32 (65%)	24 (49%)

Table 3 MSI and clinicopathological associations among rectal cancers

	MSI-H (*N*=3)	MSS (*N*=144)	*p* value	Total (*N*=147)
% In cohort population	2%	98%	NA	100%
Mean age (SD)	63.7	62.2	NA	62(10)
Gender (M/F)	3/0 (100%/0%)	84/54 (61%/39%)	0.286	87/54 (62%/38%)
Race (black/white)	2/1 (66%/34%)	35/103 (22%/78%)	0.168	37/104 (26%/74%)
Stage				
Local (stages 1 and 2)	–	46 (56%)	NA	46 (56%)
Regional (stages 3 and 4)	–	36 (44%)		36 (44%)
Inflammatory cell infiltration (%)	–	54(37.5%)	NA	54 (37%)

race and more advanced disease. Additionally, in the assessment of chronic inflammation, we found that EMAST was associated with its presence.

The finding shows that only 2% MSI cases among these rectal cancers is consistent with other studies.[15,16] MSI has been consistently associated more with right-sided sporadic colon cancer and less with left-sided tumors.[2,20–22]

EMAST has not been previously defined in rectal cancers. Based on our criteria, approximately one third of rectal tumors demonstrate EMAST. This is about half the prevalence reported in the two studies of EMAST in colon cancer.[8,23] A potential reason for this discrepancy is our stringent criteria for at least two tetranucleotide markers positive for its definition, while other studies indicate that one positive tetranucleotide marker can define EMAST. Unlike that for the definition of MSI, there is no consensus on the definition of EMAST in tumors. To date, there is also no consensus tetranucleotide marker panel for EMAST, although most investigators have used similar markers to our study. Yamada et al. have proposed a panel of 10 tetranucleotide in addition to the five loci tested in our study, including L17835, D19S394, L17686, UT5320, and D11S488.[23] Because of the polymorphic nature of tetranucleotide repeats and the ability of microsatellites to have varying mutation rates based on their sequence context,[24] we used two or more positive markers to define EMAST. In addition, the majority of the limited studies on EMAST have used two or more tetranucleotide loci instability as criteria for determination of EMAST. To our knowledge, Yamada et al. are the first authors to use one or more tetranucleotide loci instability as criteria for the determination of EMAST.

EMAST is a biomarker for several tumors including endometrial, ovarian, brain, breast, bladder, lung, and soft-tissue sarcoma.[3–7, 25–27] Several studies could find no link between EMAST and DNA MMR deficiency, the cause of MSI. While the etiology of EMAST is still not clear, general clues point toward some epigenetic relaxation of DNA MMR as one possibility for its cause. It has been shown that (a) colon cancers have heterogeneous expression of the DNA MMR protein MSH3,[8] suggesting an acquired loss and (b) oxidative stress has been shown to reduce the expression of MSH6 and PMS2, causing faulty DNA MMR that can be corrected when the stress is removed.[9,28] Additionally, we show in the present study a linkage between EMAST and chronic inflammation, further suggesting that inflammation may fuel the occurrence of EMAST in rectal and possibly other tumors. This hypothesis will need to be tested with appropriate experiments to definitively link EMAST to inflammation. There is a well-established association between tumorigenesis and inflammation as suggested in numerous studies.[29–31] Given this, it would be of great interest to ascertain if the association with

Table 4 EMAST and non-EMAST tumor and clinicopathological associations among rectal cancers

	EMAST (*N*=49)	Non-EMAST (*N*=98)	*p* value	Total (*N*=147)
% In cohort population	33%	67%	NA	100%
Mean age (Std dev)	65.2	61.2	NA	62 (10)
Gender (M/F)	29/20 (60%/40%)	58/34 (63%/37%)	0.717	87/54 (62%/38%)
Race (black/white)	18/27 (40%/60%)	19/77 (25%/75%)	0.014	37/104 (26%/74%)
Stage				
Local (stages 1 and 2)	11 (38%)	35 (63%)	0.02	46 (56%)
Regional/distant (stages 3 and 4)	18 (62%)	18 (37%)		36 (44%)
Inflammatory cell infiltration (%)	30 (60%)	24 (25%)	0.0001	54 (37%)

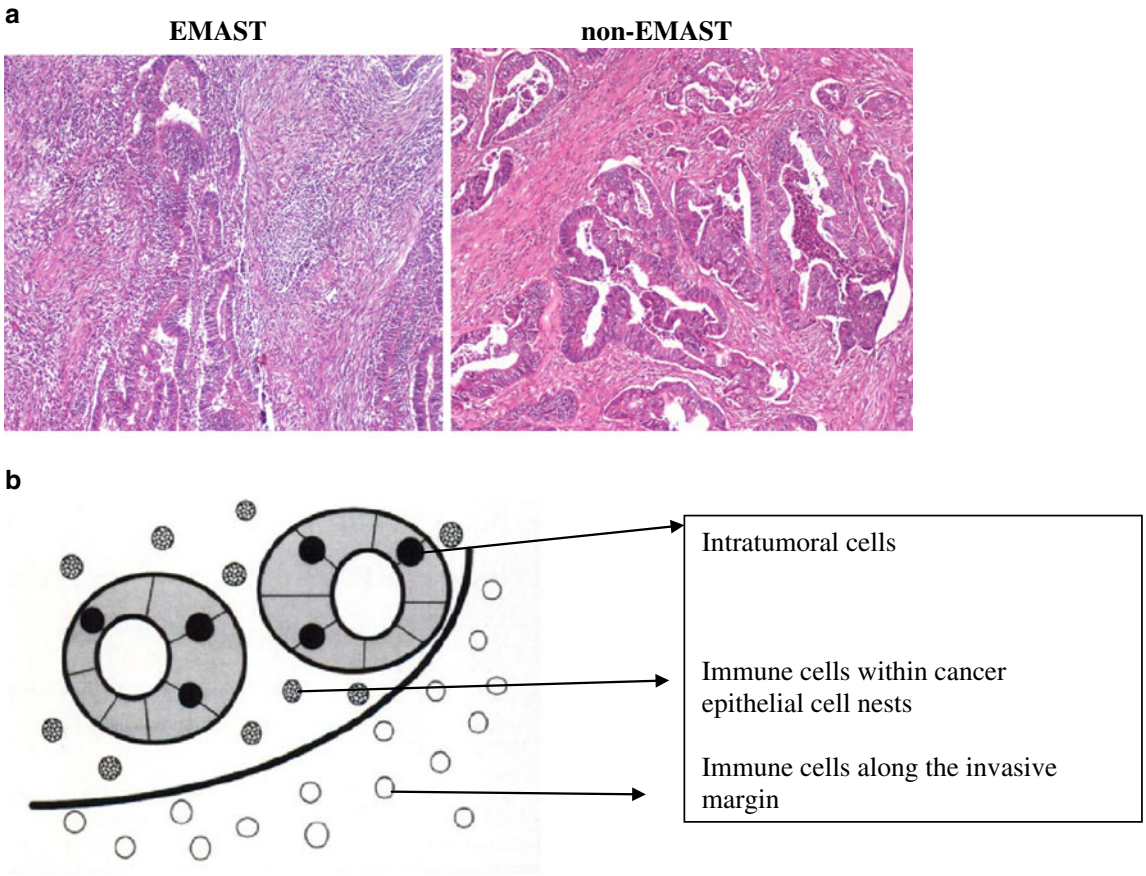

Fig. 2 **a** Inflammatory cell infiltration in EMAST versus non-EMAST tumor. A significant increase in inflammatory cell infiltrate is observed in the stroma of EMAST tumors compared to non-EMAST tumors. **b** Pattern of inflammatory cell infiltration. The vast majority of EMAST tumors demonstrated inflammation within the cancer cell nests and along the invasive margin. Inflammation in non-EMAST tumors had a much lower incidence and occurred only along the invasive margin. Adapted from Naito et al.[19]

inflammation demonstrated here with EMAST does also lead to tumorigenesis, either primarily through down-regulation of specific mismatch repair genes as shown previously in our lab[9] or secondarily via frameshift mutations of specific genes containing such tetranucleotide repeats in their sequences. It is our hope that this preliminary study demonstrating the high prevalence of EMAST would foster increased research that might help clarify the biological significance of EMAST.

In our study, EMAST was associated with more advanced disease compared to non-EMAST tumors. We also noted a higher incidence of EMAST among rectal tumors from African Americans compared to Caucasians. This observation suggests the possibility that EMAST might be predictive of a reduced survival compared to non-EMAST tumors, although this has not been evaluated. In contrast to our results, Yamada et al. did not find any significant correlation between EMAST and disease stage.[23] One possible reason that could account for the observed differences between the two studies is the varying ethnicity in each study (Caucasians and African Americans vs. Japanese) that makes up our respective cohorts.

In summary, among our rectal cancer cohort, we rarely found MSI but EMAST has a common prevalence. EMAST in rectal tumors was associated with tumors from African Americans and with patients with advanced stage. EMAST was also associated with the presence of chronic inflammatory cells. We suggest that EMAST may confer a poorer prognosis among rectal cancer patients, and its etiology is caused by inflammation.

Table 5 Lymphocyte infiltration patterns in EMAST and non-EMAST rectal tumors

	EMAST (*N*=49)	Non-EMAST (98)	*p* value	Total
Lymphocyte infiltration	30 (60%)	24 (25%)	0.0001	54
Stromal nests	24 (83%)	4 (17%)	<0.0001	28
Margin	30 (100%)	24 (100%)	NA	54

Acknowledgements Supported by the US Public Health Service (DK067287 to JMC), the UCSD Digestive Diseases Research Development Center (DK080506), and the San Diego State University/UCSD Comprehensive Cancer Center Partnership (CA132379 and CA13238).

Disclosure All authors declare no conflict or competing interest for this manuscript.

References

1. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998;58:5248–57.
2. Grady WM and Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 2008;135:1079–1099.
3. Ahrendt SA, Chow JT, Xu LH et al. Molecular detection of tumor cells in bronchoalveolar lavage fluid from patients with early stage lung cancer. J Natl Cancer Inst 1999;91:332–9.
4. Xu L, Chow J, Bonacum J et al. Microsatellite instability at AAAG repeat sequences in respiratory tract cancers. Intl J Cancer 2001;91:200–4.
5. Danaee H, Nelson HH, Karagas MR et al. Microsatellite instability at tetranucleotide repeats in skin and bladder cancer. Oncogene 2002;21:4894–9.
6. Singer G, Kallinowski T, Hartmann A et al. Different types of microsatellite instability in ovarian carcinoma. Intl J Cancer 2004;112:643–6.
7. Catto JW, Azzouzi AR, Amira N et al. Distinct patterns of microsatellite instability are seen in tumors of the urinary tract. Oncogene 2003;22:8699–706.
8. Haugen AC, Goel A, Yamada K, Boland CR et al. Genetic instability caused by loss of MutS homologue 3 in human colorectal cancer. *Cancer Res.* 2008;68:8465–8472.
9. Chang CL, Marra G, Carethers JM et al. Oxidative stress inactivates the human DNA mismatch repair system. Am J Physiol Cell Physiol 2002;283:C148–154.
10. Julien LA, Thorsen AG. Current neoadjuvant strategies in rectal cancer. J Surg Oncol. 2010 Mar 15; 101(4):321–6
11. Park J, Neuman HB, Wong WD et al. Randomized control trials in rectal and anal cancer. Surg Oncol Clin N Am. 2010 Jan; 19(1):205–23
12. Berardi R, Cascinnus S et al. Locally advanced rectal cancer: from molecular profiling to clinical practice. A literature review. Exp Opin Pharmacother. 2009 Oct; 10(15):2467–78
13. Kuremsky JG, McLeod HL et al. Biomarkers for response to neoadjuvant chemoradiation for rectal cancer. Int J Radiat Oncol Biol Phys. 2009 Jul 1; 74(3):673–88
14. Choi MY, Chung DC et al. Microsatellite instability is frequently observed in rectal cancer and is influenced by neoadjuvant chemoradiation. Int J Radiat Oncol Biol Phys. 2007 Aug 1; 68(5):1584
15. Kalady MF, Sanchez JA, Manilich E, Hammel J, Casey G, Church JM. Divergent oncogenic changes influence survival differences between colon and rectal adenocarcinomas. Dis Colon Rectum. 2009 Jun;52(6):1039–45.
16. Slattery ML, Curtin K, Wolff RK, Boucher KM, Sweeney C, Edwards S, Caan BJ, Samowitz W. A comparison of colon and rectal somatic DNA alterations. Dis Colon Rectum. 2009 Jul;52(7):1304–11.
17. Samowitz et al. Microsatellite instability and survival in rectal cancer. Cancer Causes Control. 2009 Nov;20(9):1763–8.
18. Satia JA, Keku T, Galanko JA, Martin C, Doctolero RT, Tajima A, Sandler RS, Carethers JM. Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study. Cancer Epidemiol Biomarkers Prev, 14:429–436, 2005.
19. Naito Y et al. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 1998;58:3491–4
20. Huang S, Lee JK, Smith EJ, Doctolero RT, Tajima A, Beck SE, Weidner N, Carethers JM. Evidence for an hMSH3 defect in familial hamartomatous polyps. Cancer 2010 (in press).
21. Urso E, Pucciarelli S, Agostini M, Maretto I, Mescoli C, Bertorelle R, Viel A, Rugge M, Nitti D. Proximal colon cancer in patients aged 51–60 years of age should be tested for microsatellites instability. A comment on the Revised Bethesda Guidelines. Int J Colorectal Dis. 2008 Aug;23(8):801–6.
22. Cho YK, Kim HC, Kim SH, Park JH, Yun HR, Cho YB, Yun SH, Lee WY, Chun HK. Location-related differences in sporadic microsatellite unstable colorectal cancer. Dig Liver Dis. 2010;42:611–615
23. Yamada K, Kanazawa S, Koike J, Sugiyama H, Xu C, Funahashi K, Boland CR, Koi M, Hemmi H. Microsatellite instability at tetranucleotide repeats in sporadic colorectal cancer in Japan. Oncol Rep. 2010 Feb;23(2):551–61.
24. Chung H, Lopez CG, Holmstrom J, Young DJ, Lai JF, Ream-Robinson D, Carethers JM. Both microsatellite length and sequence context determine frameshift mutation rates in defective DNA mismatch repair. Hum Mol Genet. 2010 Apr; 19:2638–2647
25. Yoo Duk Choi, Jin Choi, Jo Heon Kim et al. Microsatellite instability at a tetranucleotide repeat in type I endometrial carcinoma. J Exp Clin Cancer Res. 2008; 27(1): 88
26. Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BA, von Deimling A, Wiestler OD. Instability of short tandem repeats (microsatellites) in human cancers. Nat Genet. 1994;6:152–6. doi:10.1038/ng0294-152.
27. Ahrendt SA, Decker PA, Doffek K, Wang B, Xu L, Demeure MJ, Jen J, Sidransky D. Microsatellite instability at selected tetranucleotide repeats is associated with p53 mutations in non-small cell lung cancer. Cancer Res. 2000 May 1;60(9):2488–91.
28. Edwards RA, Witherspoon M, Wang K, Afrasiabi K, Pham T, Birnbaumer L, Lipkin SM. Epigenetic repression of DNA mismatch repair by inflammation and hypoxia in inflammatory bowel disease-associated colorectal cancer. Cancer Res. 2009 Aug 15;69(16):6423–9.
29. Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010 Jun;138(6):2101-2114.e5
30. Erdman SE, Poutahidis T. Roles for inflammation and regulatory T cells in colon cancer. Toxicol Pathol. 2010;38(1):76–87.
31. Wang S, Liu Z, Wang L, Zhang X. NF-kappaB signaling pathway, inflammation and colorectal cancer. Cell Mol Immunol. 2009 Oct;6(5):327–34.

Discussant

Dr. David Shibata (Tampa, FL): I would like to congratulate the authors on a very interesting paper and particularly to give us a greater awareness of what may be a new subset of mismatch repair deficient colorectal cancer.

One of my major questions about your study is with respect to the actual patients that were included in this study. There are a fair amount of locally advanced rectal cancers here. So my question is, were any of your tissues pretreated with chemotherapy and/or radiation?

Closing discussant

Dr. Bikash Devaraj: Most of the stage II and above cancer patients were treated with preoperative chemo XRT. We did a subanalysis trying to determine just that, and we found that there was no relation between advanced stage and inflammation at this point.

Discussant

Dr. David Shibata (Tampa, FL): I think it's interesting, and this brings up an important point, that MSH3 is a very quirky mismatch repair gene and, in fact, it's very heterogenous in terms of its expression, even between individual tumor cells. In fact, it may be—as you stated—that the expression is impacted by cellular stress. So I would be very curious to see, and I think it would be interesting for your study, to do MSH-3 immunohistochemistry on your specimens, and particularly for those patients that were treated with radiation. It would be very interesting to see what the correlation would be between EMAST and MSH3 in those tissues.

The other question then is, given that this seems to be a state-based registry, why is there such a lack of data on tumor stage on almost half of the patients?

Closing discussant

Dr. Bikash Devaraj: I don't have a good answer to that question at this point.

Discussant

Dr. David Shibata (Tampa, FL): Because I think in terms of making a statement on prognosis and stage, to have half of your patients not having stage data, I think if you can try to get that, I think that would strengthen things, certainly.

Closing discussant

Dr. Bikash Devaraj: But, again, if you look at the overall correlation and as you know, about half of them are not there, them we did not have stage data for—it's about equivalent in terms of the stage that we have for EMAST tumors versus non-EMAST tumors. Thus, so I still think you can draw a correlation between the two.

Discussant

Dr. David Shibata (Tampa, FL): And finally, I think the other studies that you had cited invariably show that MSI-high tumors were all EMAST positive. And I'm just wondering whether you did genetic testing on these patients. Were they HNPCC perhaps and not just sporadic MLH1 deficient tumors?

Closing discussant

Dr. Bisash Devaraj: As far as we know, none of these patients were HNPCC patients. And you are correct, the majority of the papers out there do show that EMAST tumors tend to be MSI-high tumors, too. But again, we had such low numbers of traditional MSI-high tumors that this may be one of the reasons why we did not see that correlation.

Discussant

Dr. David Shibata (Tampa, FL): Did you categorize any as MSI-low?

Closing discussant

Dr. Bikash Devaraj: No, we did not.

J Gastrointest Surg (2010) 14:1529–1535
DOI 10.1007/s11605-010-1314-8

2010 SSAT PLENARY PRESENTATION

Radiation Dose from Computed Tomography in Patients with Necrotizing Pancreatitis: How Much Is Too Much?

Chad G. Ball · Camilo Correa-Gallego · Thomas J. Howard · Nicholas J. Zyromski · Michael G. House · Henry A. Pitt · Atilla Nakeeb · Christian M. Schmidt · Fatih Akisik · Keith D. Lillemoe

Received: 26 April 2010 / Accepted: 9 August 2010 / Published online: 8 September 2010

Abstract
Objectives Low-dose ionizing radiation from medical imaging has been indirectly linked with subsequent cancer. Computed tomography (CT) is the gold standard for defining pancreatic necrosis. The primary goal was to identify the frequency and effective radiation dose of CT imaging for patients with necrotizing pancreatitis.
Methods All patients with necrotizing pancreatitis (2003–2007) were retrospectively analyzed for CT-related radiation exposure.
Results Necrosis was identified in 18% (238/1290) of patients with acute pancreatitis (mean age=53 years; hospital/ICU length of stay=23/7 days; mortality=9%). A median of five CTs/patient [interquartile range (IQR)=4] were performed during a median 2.6-month interval. The average effective dose was 40 mSv per patient (equivalent to 2,000 chest X-rays; 13.2 years of background radiation; one out of 250 increased risk of fatal cancer). The actual effective dose was 63 mSv considering various scanner technologies. CTs were infrequently (20%) followed by direct intervention (199 interventional radiology, 118 operative, 12 endoscopic) (median=1; IQR=2). Magnetic resonance imaging did not have a CT-sparing effect. Mean direct hospital costs increased linearly with CT number (R=0.7).
Conclusions The effective radiation dose received by patients with necrotizing pancreatitis is significant. Management changes infrequently follow CT imaging. The ubiquitous use of CT in necrotizing pancreatitis raises substantial public health concerns and mandates a careful reassessment of its utility.

Keywords Necrotizing pancreatitis · Radiation · Computed tomography

Introduction

Acute pancreatitis represents a continuum of disease that challenges our clinical, social, and financial management.[1–6] Necrotizing pancreatitis is particularly virulent because it involves degradation of the pancreatic gland and/or surrounding peripancreatic tissues.[4–6] It also increases the risk of developing acute organ dysfunction associated with severe acute pancreatitis.[7] More specifically, the extent of necrosis can predict both local complications as well as the degree of overall organ degredation.[8,9]

The current non-invasive, gold standard modality for identifying the initial extent as well as the evolution of pancreatic necrosis is computed tomography (CT) with intravenous contrast medium. As a result, multiple CT-based classification schemes have been developed in an attempt to better prognosticate the clinical course of this disease.[10,11] In addition to the inherent risk of contrast-

This manuscript was presented in oral format at the Presidential Plenary Session at the Society for Surgery of the Alimentary Tract/ Digestive Diseases Week on May 3, 2010.

C. G. Ball (✉) · C. Correa-Gallego · T. J. Howard · N. J. Zyromski · M. G. House · H. A. Pitt · A. Nakeeb · C. M. Schmidt · K. D. Lillemoe
Departments of Surgery, Indiana University, 545 Barnhill Drive, Emerson Hall 203, Indianapolis, IN 46202, USA
e-mail: ball.chad@gmail.com

F. Akisik
Departments of Radiology, Indiana University, Indianapolis, IN, USA

associated nephropathy, CT imaging also exposes patients to a measurable dose of ionizing radiation.[12–15] Considering the frequent need for multiple CT scans during the course of necrotizing pancreatitis, a patient's potential risk from repeated radiation exposure is substantial. The increasing use of CT imaging[16–21] coupled to the growing incidence of acute pancreatitis,[3,22–25] makes this public health issue especially topical.

Given indirect evidence suggesting that low-dose ionizing radiation is associated with the subsequent development of both solid cancers and leukemia,[26] the primary goal of this study was to identify the frequency and effective radiation dose of CT imaging for patients with necrotizing pancreatitis at a high-volume pancreas referral center. A secondary goal was to identify the proportion of CT examinations that resulted in a subsequent therapeutic intervention or change in management.

Materials and Methods

The study population consisted of all patients with necrotizing pancreatitis treated at Indiana University Hospital (IUH) between January 1, 2003 and December 31, 2007. IUH is a tertiary care hospital with a high-volume referral pattern for both pancreatitis and malignant pancreatic disease. The pancreatitis database, computer-based charts, and picture archiving and communication system supplied all data in this retrospective study. CT scans were reviewed to identify all patients with pancreatic and peripancreatic necrosis (both sterile and infected necrosis were included). Operative reports were also reviewed. Patients with equivocal radiologic findings of pancreatic necrosis on CT imaging who did not undergo surgical debridement were excluded. Therapeutic interventions following CT imaging were defined as: (1) operative pancreatic debridements/necrosectomies (with or without a cholecystectomy); (2) percutaneous drainage, feeding access placement, and/or angioembolization by interventional radiologists; and (3) ductal imaging, sphincterotomy, and/or pancreatic duct stent placement using endoscopic retrograde cholangiopancreatography (ERCP). Interventions occurring within 96 h of imaging were considered to have been influenced by that CT scan.

IUH utilized four different CT scanners during the study interval. From January 1, 2003 to June 17, 2003 a Phillips four channel (Mx8000 CT Twin 7180 Gantry) was employed. Between June 17, 2003 and March 5, 2004, a Phillips 16 channel (Mx8000 IDT Gantry) was utilized. Between March 5, 2004 and September 2, 2008 both a Phillips 40 channel (Brilliance 40 Gantry with DMS) and a Phillips 64 channel (Brilliance 64 Gantry with DMS) were employed. After September 2, 2008, all CT imaging was performed on a Phillips 64 channel (Brilliance 64 Gantry with DMS). Newer CT technologies (40- and 64-channel detectors) included automatic exposure control software. Pancreatic CT studies at IUH typically included a very generous torso profile that included cross-sectional images of the majority of the pelvis.

All radiation dosing is discussed using "effective doses." This entity is reported as Sieverts (Sv) in standard SI units [1 Sievert (effective dose equivalent)=1 Roentgen equivalent man]. The effective dose accounts for the absorption of radiation dose and estimates the whole-body dose that is actually delivered during a radiologic procedure. As a result, this measure allows comparisons to other types of non-medical radiation exposure. Natural background radiation dose is defined as 3 mSv per year. Chest radiographs (posteroanterior) deliver an individual effective dose of 0.02 mSv.[18] An increased risk of fatal cancer is calculated by multiplying the effective dose (Sv) by the risk coefficient of fatal cancer in adults.

Data analysis was performed using Stata version 8.0 (Stata Corp, College Station, TX). Normally or near-normally distributed variables were reported as means and non-normally distributed variables as medians. Means were compared using the Student's t test and medians using the Mann–Whitney U test. Differences in proportions among categorical data were assessed using Fischer's exact test. A p value less than 0.05 was considered to represent statistical significance for all comparisons.

Results

Over the 5-year study period, 1,290 patients were diagnosed with acute pancreatitis. Of these, 255 (20%) displayed a necrotizing variant (38, 47, 50, 60 and 60 patients for 2003 to 2007, respectively). Seventy percent (178) of these patients required operative pancreatic or peripancreatic debridement. Seventeen patients were excluded from the analysis because of missing patient data. The mean age of the remaining 238 patients was 53 years [standard deviation (SD)=16.1] (Table 1). Sixty-two percent were male. The cohort displayed a mean hospital and intensive care unit (ICU) length of stay of 23 and 7 days, respectively (SD=7.8 and 12.1, respectively). The overall mortality rate was 8.8%. Patients surviving to discharge were released to home with self care only (41%), home with home-care assistance (30%), long-term care hospitalization (10%), skilled nursing facility (9%), rehabilitation facility (7%), or other destinations (3%). Patients had a median imaging follow-up interval of 2.6 months [interquartile range (IQR)=7.2) (mean=8.3 months].

A median of five abdominal CT examinations per patient (IQR=4) were completed during their initial hospitalization

Table 1 Patient demographics

Total no. of patients	238
Mean age (years)	53
Male gender (%)	62
Mean hospital length of stay (days)	23
Mean intensive care length of stay (days)	7
Overall mortality (%)	8.8

and outpatient follow-up interval. The average effective dose of this imaging load is 40 mSv (Table 2).[18] By isolating the effective doses delivered by each specific CT scanner over the course of the study period (four-, 16-, 40- and 64-channel machines), the average effective dose among the study cohorts at IUH was actually 47 mSv per patient. Thirty-five percent of all CT scans also used two phases (arterial and venous). This increased the delivered average effective dose to 63 mSv per patient (Table 2). The mean ICU length of stay increased concurrent to the number of CT examinations in a stepwise manner (R=0.69).

The median number of post imaging interventions was one (IQR=2) with a resultant CT/intervention rate of 20%. Of the 1,202 total CT examinations performed, 531 (44%) were completed in patients considered to be physiologically ill at the time of imaging (ICU admission with sepsis and/or organ failure). This patient subset displayed a higher (31%) CT/intervention rate compared to patients without acute physiologic illness (p<0.001). Postimaging interventions included 189 (57%) interventional (non-angiography), 118 (36%) operative, 12 (4%) ERCP, and ten (3%) angioembolization procedures. MRI scans (78 patients) did not have a CT-sparing effect as these patients still underwent a median of five CT scans. The median number of CT scans for patients who underwent an initial operative intervention was similar to patients initially managed with non-operative [interventional radiology (IR) or gastroenterology] techniques (p=0.19). The time interval between CT scans for patients who underwent a post-imaging intervention was similar to those who underwent no procedures (IR or ERCP) (p=0.11). CT examinations performed after discharge from the hospital were indicated for evaluation of a known pancreatic fistula (72%), interval follow-up (19%), or for unclear reasons (9%).

Table 2 Comparison of radiation equivalents for a median of five abdominal CT scans

Scanner-specific effective dose (mSv)	Total effective dose (mSv)	Chest radiograph equivalents	Equivalent background radiation time (years)	Increased risk of fatal cancer
8[a]	63	3,150	20.75	1/160
8[b]	40	2,000	13.2	1/250
5.3[c]	26.5	1,325	8.75	1/377
1.5[d]	7.6	380	2.5	1/1315

[a] Doses for study patients when dual-phase and scanner technologies are included

[b] United States mean abdominal CT scan effective dose

[c] United Kingdom mean abdominal CT scan effective dose

[d] Doses using the lowest reported effective dose possible for an abdominal CT scan

The mean direct hospital cost increased in a stepwise manner with the number of CT examinations obtained (R=0.72). These increased from a mean of US $14,831 with one CT scan to US $67,470 with ten scans. The cost of performing as well as interpreting a CT scan for pancreatitis at IUH is US $600–1,200 (charge to insurer). The mean hospital (variable direct) costs for patients with necrotizing pancreatitis are approximately threefold higher than for non-necrotizing acute pancreatitis. Radiology costs account for 5% of the total hospital cost in all cases of pancreatitis.

Discussion

Although CT technology was invented in 1971,[27] recent improvements in scanner speed, image resolution, and ease of use have created a veritable explosion in both applications and indications.[28,29] In 1980, approximately 3 million CT scans were performed in the United States, compared to 62 million in 2006.[17] This change has led to a nearly sixfold increase in the per capita radiation exposure from medical imaging.[13] The revolution in spiral CT technology is also evident in terms of the absolute number of scanners. As of 1996, the United States and Japan had 26 and 64 machines per 1 million people, respectively.[16] Based on its utility for a broad range of screening endeavors, from evaluating seasoned astronauts for cardiovascular disease[30] to identifying occult injuries in severely injured blunt trauma patients,[31] CT use is again primed to increase. More specifically, interest in CT colonography,[32,33] CT lung screening for smokers,[34,35] coronary artery CT screening,[36] and whole-body health screening examinations[37,38] is significant.

While the majority (80% to 85%) of human radiation exposure arises from equal amounts of solar and radon sources (background dose=1 to 3 mSv per year), medical imaging creates most of the remaining 15% to 20%.[12,28,39,40] Of all CT imaging, 75% is obtained in a hospital setting, with up to half being scans of the torso.[41] Furthermore, abdominal CT imaging accounted for up to 31% of the annual cumulative effective dose from medical imaging procedures in a study of nearly 1 million non-elderly adults.[13]

The stochastic risk of DNA mutations and therefore carcinogenesis (solid organ, thyroid, leukemia) following

exposure to medical imaging currently assumes a linear, no-threshold extrapolation model based on data from the Japanese atomic bomb survivors (organ doses compared to organ-specific cancer incidence).[12–15,42] This dose-biologic effect relationship is the subject of significant controversy given its reliance on risks extrapolated from high doses as well as the possibility that it overestimates risk by ignoring the human body's natural defense mechanisms against radiocarcinogenesis at low doses.[43–46] Unfortunately, no large-scale epidemiologic data exist to confirm the potential cancer risks associated with CT imaging using this conservative approach.[47]

In addition to the unclear oncologic risks of medical imaging exposure, the delivered effective dose can vary significantly based on the individual CT scanner (i.e., number of "slices"). The reported effective dose for a standard single-phase abdominal CT scan ranges from 1.5 to 10 mSv depending on the number of channels.[12,20] If the generally recognized average effective dose of 8 mSv is utilized, our patients would have been exposed to a mean of 40 mSv. This exposure is classified as a high annual dose, with less than 1% of the United States population being exposed to greater than 20 cumulative mSv per year.[13] In comparison, exposure for both pilots/flight crews (1,000 flight hours per year) and occupational radiation workers approximate 5 mSv per year. It also far exceeds goal occupational radiation exposure levels defined by the International Commission on Radiological Protection guidelines.[48,49] Recent estimates of the lifetime risk of radiation-induced cancer approximate one person in 100 for those exposed to 100 mSv (relative risk= 1.024)(Table 1).[50] The lifetime risk of cancer from all other causes is 42 in 100, and the risk of dying from a motor vehicle crash in the United States is one in 77.[26,50–53]

The need for repeat CT imaging in the same patient extends beyond pancreatitis. Mettler and colleagues reported that among all patients in the literature undergoing CT imaging, 30% underwent at least three scans (7% underwent more than five and 4% more than eight scans).[20] Given evidence that radiation exposure is more harmful in younger patients,[13] the best studied adult population is the trauma cohort. The number of CT examinations in a subset of severely injured patients who spent at least 30 days in the ICU (mean injury severity score (ISS)=32) was 7.8.[40] Similarly, a study of 172 trauma patients with a mean ISS of 23 used multi-site dosimeters to identify a mean effective dose of 22.7 mSv.[53] This led to an extrapolation of 190 cancer deaths per 100,000 patients exposed to imaging studies following major trauma.[53]

Given the calculated effective dose of 40 mSv in our necrotizing pancreatitis patients (assuming a similar life expectancy), we predict significantly more deaths from radiation-induced cancer. Although this estimate accounts for the fact that medical radiation exposure tends to accumulate in ill patients with an inherently reduced life expectancy (less time to manifest radiation-induced cancers), the precise relationship between trauma patients and those with severe acute pancreatitis is unknown (mean age =53 vs. 43% of all injured U.S. patients >45 years of age).[3,54] Interestingly, the effective dose of 40 mSv is identical to that reported for patients with pancreatic cancer during their first year (40.1 mSv).[15] Unfortunately, patients with pancreatic cancer have significantly shorter life expectancies than those with necrotizing pancreatitis as evidenced by a 5-year exposure of only 68.8 mSv per patient.[15] It should also be noted that our estimated effective dose does not include adjunctive radiologic investigations. These procedures most commonly include fluoroscopy and angiography as well as other occasional CT studies (pulmonary emboli protocols=15 mSv). Unfortunately, despite our center's generally aggressive use of MRI for pancreas disease, this philosophy did not have a CT-sparing effect in this patient population. More specifically, although we have found great utility for MRI in evaluating the integrity of the pancreatic duct (i.e., diagnosing disconnected left pancreatic remnants), its utility was limited elsewhere.

The actual effective dose of our cohort was 47 mSv because of the progression from four- to 64-channel scanners over the study interval. With the application of recent dose optimization strategies such as automatic exposure control available for 64-channel scanners, the effective dose per scan has been reported to be as low as 1.52 mSv.[12] Had this technology been available for each of our patients with necrotizing pancreatitis, the actual delivered effective dose could have been reduced by over 80%. Put into perspective, this would lower the lifetime risk of cancer to less than one in 15,000 per individual 64-channel CT examination. Although we used single-phase arterial-enhanced CT scans in the majority of patients (65%) to determine the extent of disease, some authors have proposed routine use of tri-phasic CT to improve the delineation between pancreatitis and cancer.[55] The total effective dose would therefore need to be multiplied by the number of phases. More specifically, the effective dose in our patients was actually 63 mSv when dual-phase CTs were accounted for (Table 1). This compares to a mean of 31 mSv in a recent study of patients with acute pancreatitis.[56] As a result of this variance, each center must assess its own CT technology and clinical practice in an effort to quantify the associated risk to patients with pancreatitis.

As noted by Fazel and colleagues,[13] unlike the exposure of workers in health care and nuclear industries, the exposure of patients to radiation cannot be restricted. As a result, the potential stochastic risks of CT imaging must be carefully weighed against the clinical importance of each individual procedure.[57,58] Although the precise real-time

thought process of the ordering clinicians was unavailable for this retrospective study, the rate of post-imaging therapeutic interventions was employed as a surrogate for a change in clinical management. Only 20% of all CT scans in the cohort were followed by subsequent interventions (interventional radiology, operative, or ERCP procedures). This value echoes the 20% rate of subsequent alterations in management among trauma patients who undergo a CT scan of their chest.[59] This rate increased to 31% in patients with necrotizing pancreatitis who displayed more severe physiologic illness (sepsis, organ failure with ICU admission). While the importance of an individual CT study cannot be understated, the likelihood of altering a patient's clinical pathway based on the subsequent findings must be considered before exposing them to radiation. This includes not only adding an intervention, but also the ability to *avoid* a planned procedure. Although the contribution of a CT scan to the overall cost of a hospital stay for patients with necrotizing pancreatitis is relatively small, the observation of increasing direct costs concurrent to the number of CT scans in our patient cohort is notable. It not only reflects an increasing severity of illness, but also highlights the importance of cost containment by utilizing carefully planned diagnostic modalities and evidence-based therapies.

In coupling the frequency of CT imaging for patients with necrotizing pancreatitis to the increasing population incidence of acute pancreatitis, the potential risk of radiation exposure will continue to be a significant public health issue. This issue is especially important in younger patients undergoing repeated CT examinations. Attempts at increasing the awareness of this issue are ongoing.[60–62] Although the widespread adoption of CT imaging represents the most important advancement in the history of diagnostic imaging, strategies to reduce radiation exposure remain crucial. These include ensuring the use of automatic exposure-control software,[63,64] replacing outdated scanners, and a simple reduction in the overall number of CT examinations when possible. This may be achieved, in part, by using alternative modalities such as MRI as well as limiting surveillance to single-phase studies. Equally important, a careful assessment of the likelihood of altering a patient's clinical management based on the results of a given CT scan is essential.

Acknowledgements We would like to acknowledge Karl Mockler for the technical assistance.

References

1. Neoptolemos JP, Raraty M, Finch M, Sutton R. Acute pancreatitis: the substantial human and financial costs. Gut 1998;42:886–891.
2. Uhl W, Warshaw A, Imrie C, Bassi C, McKay CJ, Lankisch PG, Carter R, Di Magno E, Banks PA, Whitcomb DC, Dervenis C, Ulrich CD, Satake K, Ghaneh P, Hartwig W, Werner J, McEntee G, Neoptolemos JP, Buchler MW. IAP guidelines for the surgical management of acute pancreatitis. Pancreatology 2002;2:565–573.
3. Frey CF, Zhou H, Harvey DJ, White RH. The incidence and case-fatality rates of acute biliary, alcoholic, and idiopathic pancreatitis in California, 1994–2001. Pancreas 2006;33:336–344.
4. Bradley EL 3rd, Howard TJ, van Sonnenberg E, Fotoohi M. Intervention in necrotizing pancreatitis: an evidence-based review of surgical and percutaneous alternatives. J Gastrointest Surg 2008;12:634–639.
5. Howard TJ, Patel JB, Zyromski NJ, Sandrasegaran K, Yu J, Nakeeb A, Pitt HA, Lillemoe KD. Declining morbidity and mortality rates in the surgical management of pancreatic necrosis. J Gastrointest Surg 2007;11:43–49.
6. Parikh PY, Pitt HA, Kilbane M, Howard TJ, Nakeeb A, Schmidt CM, Lillemoe KD, Zyromski NJ. Pancreatic necrosectomy: North American mortality is much lower than expected. J Am Coll Surg 2009;209:712–719.
7. Bradley EL 3rd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, GA, September 11–13, 1992. Arch Surg 1993;128:586–590.
8. Karimgani I, Porter KA, Langevin RE, Banks PA. Prognostic factors in sterile pancreatic necrosis. Gastroenterology 1992;103:1636–1640.
9. Charbonney E, Nathens AB. Severe acute pancreatitis: A review. Surg Infect 2008;9:573–578.
10. Balthazar EJ, Ranson JH, Naidich DP, Megibow AJ, Caccavale R, Cooper MM. Acute pancreatitis: Prognostic value of CT. Radiology 1985;156:767–772.
11. De Waele JJ, Delrue L, Hoste EA, De Vos M, Duyck P, Colardyn FA. Extrapancreatic inflammation on abdominal computed tomography as an early predictor of disease severity in acute pancreatitis: evaluation of a new scoring system. Pancreas 2007;34:185–190.
12. Hui CM, MacGregor JH, Tien HC, Kortbeek JB. Radiation dose from initial trauma assessment and resuscitation: review of the literature. Can J Surg 2009;52:147–152.
13. Fazel R, Krumholz HM, Wang Y, Ross JS, Chen J, Ting HH, Shah ND, Nasir K, Einstein AJ, Nallamothu BK. Exposure from low-dose ionizing radiation from medical imaging procedures. NEJM 2009;361:849–857.
14. Brenner DJ, Hall EJ. Computed tomography—An increasing source of radiation exposure. NEJM 2009;357:2277–2284.
15. Brix G, Nissen-Meyer S, Lechel U, Nissen-Meyer J, Griebel J, Nekolaa AE, Becker C, Reiser M. Radiation exposures of cancer patients from medical x-rays: How relevant are they for individual patients and population exposure? Eur J Rad 2009; 72(2):342–347
16. Sources and effects of ionizing radiation: United Nations Scientific Committee on the effects of atomic radiation: UNESCEAR 2000 report to the General Assembly. New York: United Nations, 2000.
17. IMV 2006 CT Market Summary Report. Des Plains, IL: IMV Medical Information Division, 2006.
18. Amis Jr ES, Butler PF, Applegate KE, Birnbaum SB, Brateman LF, Hevezi JM, Mettler FA, Morin RL, Pentecost MJ, Smith GC, Strauss KJ, Zeman RK. American College of Radiology. American College of Radiology White paper on radiation dose in medicine. J Am Coll Radiol 2007;4:272–284.
19. Nekolla E, Veit R, Griebel J, Brix G. Frequency and effective dose of diagnostic x-ray procedures in Germany. Biomed Tech 2005;5-:1334–1335.
20. Mettler FA. Huda W, Yoshizumi TT, Mahesh M. Effective doses in radiology and diagnostic nuclear medicine: A catalog. Radiology 2008;248:254–263.

21. Nishizawa K, Matsumoto M, Iwai K, Maruyama T. Survey of CT practice in Japan and collective effective dose estimation. Nippon Acta Radiol 2004;64:151–158.
22. Goldacer MJ, Roberts SE. Hospital admission for acute pancreatitis in an English population, 1963–98: database study of incidence and mortality. BMJ 2004;328:1466–1469.
23. Eland IA, Sturkenboom MJ, Wilson JH, Sticker BH. Incidence and mortality of acute pancreatitis between 1985 and 1995. Scand J Gastroenterol 2000;35:1110–1116.
24. Floyd A, Pederson L, Nielsen GL, Thorladcius-Ussing O, Sorensen HT. Secular trends in incidence and 30-day case fatality of acute pancreatitis in North Jutland County, Denmark: a register-based study from 1981–2000. Scand J Gastroeterol 2002;37:1461–1465.
25. Appelros S, Borgstrom A. Incidence, etiology and mortality rate of acute pancreatitis over 10 years in a defined urban population in Sweden. Br J Surg 1999;86:465–470.
26. National Research Council. Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2. Washington, DC: National Academic Press, 2006.
27. Ambrose J, Hounsfield G. Computerized transverse axial tomography. Br J Radiol 1973;46:148–149.
28. Frush DP. Review of radiation issues for computed tomography. Semin Ultrasound CT MR 2004;25:17–24.
29. Mayo JR, Aldrich J, Muller NL. Radiation exposure at chest CT: a statement of the Fleischner Society. Radiology 2003;228:15–21.
30. Hamilton DR, Murray JD, Ball CG. Cardiac health for astronauts: coronary calcification scores and CRP as criteria for selection and retention. Aviat Space Environ Med 2006;77:377–387.
31. Ball CG, Kirkpatrick AW, Feliciano DV. Can J Surg. The occult pneumothorax: what have we learned? Can J Surg 2009;52:E173–E179.
32. Heiken JP, Peterson CM, Menias CO. Virtual colonoscopy for colorectal cancer screening: current status. Cancer Imaging 2005;5:S133–S139.
33. Brenner DJ, Georgsson MA. Mass screening with CT colonography: should the radiation exposure be of concern? Gastroenterology 2005;129:328–337.
34. Henschke CL, Yankelevitz DF, Libby DM, Pasmantier MW, Smith JP, Miettinen OS. Survival of patients with stage I lung cancer detected on CT screening. NEJM 2006;355:1763–1771.
35. Bach PB, Jett JR, Pastorino U, Tockman MS, Swensen SJ, Begg CB. Computed tomography screening and lung cancer outcomes. JAMA 2007;297:953–961.
36. Einstein AJ, Henzlova MJ, Rajagopalan S. Estimating risk of cancer associates with radiation exposure from 64-slice computed tomography coronary angiography. JAMA 2007;18:317–323.
37. Brenner DJ, Elliston CD. Estimated radiation risks potentially associated with full-body CT screening. Radiology 2004;232:735–738.
38. Beinfeld MT, Wittenberg E, Gazella GS. Cost-effectiveness of whole-body CT screening. Radiology 2005;234: 415–422.
39. UNSCEAR 2000. The United Nations Scientific Committee on the effects of atomic radiation. Health Phys 2000;79:314.
40. Kim PK, Gracias VH, Maidment AD, O'Shea M, Reilly PM, Schwab CW. Cumulative radiation dose caused by radiologic studies in critically ill trauma patients. J Trauma 2004;57:510–514.
41. What's NEXT? Nationwide Evaluation of X-ray Trends: 2000 computed tomography. Conference of Radiation Control Program Directors, Department of Health and Human Services. 2006. http://www.crcpd.org/Pubs/NexTrifolds/NEXT2000CT_T.pdf. Accessed on November 2, 2009.
42. Ron E. Ionizing radiation and cancer risk: evidence from epidemiology. Pediatr Radiol 2002;32:232–237.
43. Tubiana M. Computed tomography and radiation exposure. NEJM 2009;358:850–853.
44. Breckow J. Linear-no-threshold is a radiation-protection standard rather than a mechanistic effect model. Radiat Environ Biophys 2006;44:257–260.
45. Tubiana M, Aurengo A, Averbeck D, Masse R. The debate on the use of linear no threshold for assessing the effects of low doses. J Radiol Prot 2006;26:317–324.
46. Tubiana M, Aurengo A, Averbeck D, Masse R. Low-dose risk assessment: comments on the summary of the International Workshop. Radiat Res 2007;167:742–744.
47. Giles J. Study warns of 'avoidable' risks of CT scans. Nature 2004;431:391.
48. Casarett G, Bair WJ, Meinhold CB. NCRP Report No. 91. Recommendations on limited exposure to ionizing radiation. Bethesda (MD) National Council on Radiation Protection and Measurements. 1980; p. 3–10.
49. International Commission on Radiation Protection. Development of the Draft 2005 recommendations of the ICRP: a collection of papers. A report of ICRP supporting guidance 4. Ann ICRP 2004;34 Suppl:1–44.
50. American College of Surgeons: Committee on Trauma. National Trauma Data Bank 2009 Annual Report. http://www.facs.org/trauma/ntdb/ntdbannualreport2009.pdf. Accessed on November 17, 2009.
51. Garfinkel L. Probability of developing or dying of cancer. United States, 1991. Sta Bull Metrop Insur Co 1995;76:1–201.
52. Slovis T. CT and computed radiography: the pictures are great, but is the radiation dose greater than required? AJR Am J Roentgenol 2002;179:39–41.
53. Tien HC, Tremblay LN, Rizoli SB, Gelberg J, Spencer F, Caldwell C, Brenneman FD. Radiation exposure from diagnostic imaging in severely injured trauma patients. J Trauma 2007;176:289–296.
54. Strate T, Yekebas E, Knoefel WT, Bloechle C, Izbicki JR. Pathogenesis and the natural course of chronic pancreatitis. Eur J Gastroenterol Hepatol 2002;14:929–934.
55. Yamada Y, Mori H, Matsumoto S, Kiyosue H, Hori Y, Hongo N. Pancreatic adenocarcinoma versus pancreatitis: differentiation with triple-phase helical CT. Abdom Imaging 2010. 35(2):163–171.
56. Morgan DE, Ragheb CM, Lockhart ME, Cary B, Fineberg NS, Berland LL. Acute pancreatitis: Computed tomography utilization and radiation exposure are related to severity but not patient age. Clin Gastroenterol Hepatol 2010;8:303–308.
57. United States Nuclear Regulatory Commission. Regulation (10 CFR), subpart B. Washington, DC: Nuclear Regulatory Commission. http://www.nrc.gov/reading-rm/doc-collections/cfr/part02/part020-1101.html. Accessed on November 2, 2009.
58. Prasad KN, Cole WC, Haase GM. Radiation protection in humans: extending the concept of low as reasonably achievable (ALARA) from dose to biological damage. Br J Radiol 2003;327:371–372.
59. Plurad D, Green D, Demetriades D, Rhee P. The increasing use of chest computed tomography for trauma: is it being overutilized? J Trauma 2007;62:631–5.
60. Lee CI, Haims AH, Monico EP, Forman HP. Diagnostic CT scans: assessment of patient, physician, and radiologist awareness of radiation dose and possible risk. Radiology 2004; 231:393–398.
61. Goske MJ, Applegate KE, Boylan J, Butler PR, Callahan MJ, Coley BD, Farley S, Frush DP, Hernanz-Schulman M, Jaramillo D, Johnson ND, Kaste SC, Morrison G, Strauss KJ. Image Gently (SM): a national education and communication campaign in radiology using the science of social marketing. J Am Coll Radiol 2008;5:1200–1205.
62. Gerber TC, Carr JJ, Arai AE, Dixon RL, Ferrari VA, Gomes AS, Heller GV, McCollough CH, McNitt-Gray MF, Mettler FA, Mieres JH, Morin RL, Yester MV. Ionizing radiation in cardiac imaging: a science advisory from the American Heart Association Committee on Cardiovascular Imaging and Intervention of the Council on Cardiovascular Radiology and Intervention 2009;119:1056–1065.
63. McCollough CH. CT dose: How to measure, how to reduce. Health Phys 2008;95:508–517.

64. Lee CH, Goo JM, Ye HJ, Ye SJ, Park CM, Chun EJ, Im JG. Radiation dose modulation techniques in the multidetector CT era: from basics to practice. Radiographics 2008;28:1451–1459.

Discussant

Dr. Jeffrey B. Matthews (Chicago, IL): You have highlighted a very important issue that transcends this disease, which is the issue of reducing radiation exposure among hospitalized patients who are undergoing treatment for various disorders. It is obviously particularly a problem for patients with necrotizing pancreatitis because of the frequent interest in getting follow-up imaging studies. Your study poses the larger question of diagnostic restraint, trying to optimize that ratio between the number of times we get an imaging study and the times we are going to intervene on the basis of the findings. When we are dealing with necrotizing pancreatitis, I think sometimes we have to remind ourselves that this is not a disease that responds to radiation therapy, but it is the nature of the disease that repeat imaging is going to be needed because so many of these people are going to require repeat intervention.

In many institutions, including our own, we image pancreatic disease with a tri-phasic CT scan. You have used bi-phasic in 35% of your patients, and while these thin-slice studies are very useful as an initial way to define the extent of disease in a variety of pancreatic conditions, it may or may not be necessary for the follow-up studies (and one can question whether it is really needed even at the initial presentation of acute pancreatitis). So I think there is certainly an opportunity to reduce the exposure. In our institution, our radiologists push back very hard on our almost reflexive ordering of pancreatic protocol CTs. Have you started to put in place tighter protocols in your institution to reduce the use of multiphase studies as the frequency of these studies?

Secondly, you made the point that, in your retrospective study, the use of MR did not alter the number of total CT images. I think that may also reflect the fact that we, as surgeons, simply find it easier to read CTs rather than MR images. While it is difficult to obtain MR imaging in critically ill ICU patients, there is probably also an opportunity to substitute MR for CT to follow the progression of collections during convalescence. Going forward, are you doing anything to increase the use of MR as your routine follow-up studies to reducing the number of times that you would be using multiphasic CT studies?

Closing Discussant

Dr. Chad G. Ball: Whether you are talking about young trauma patients and injury screening technologies, or about surveillance in necrotizing pancreatitis, I think many of these issues are fundamentally the same. As a result, I divide this topic into three separate areas.

First, is the test going to change your management? This is clearly a physician-driven factor. It is also different for everybody within their individual practice. As a result, it relies on personal vigilance.

The second component is hardware. With recent improvements in scanner hardware, the effective dose is going down almost exponentially. This refers to the number of channels or the number of slices. So a 128 scanner is not just twice as good as a 64-slice scanner; it is substantially more than that in terms of reducing the effective dose.

The third concept that is important to this issue is the wizards who write the scanner software. Techniques such as progressive modulation and automatic exposure control are but two examples. There is a whole host of very neat trickery. With every iteration, a new version of their software is substantially better. Although some of these tricks are specific to trauma patient screening, most are still relevant to all patients. You also want a radiology group that is going to be active and be willing to absorb the financial cost of updating software and hardware because outside of the individual ordering physician, that is the only way to limit the effective dose.

Shielding non-scanned body parts is also a helpful tool. Unfortunately, it is something we tend to ignore and therefore the practical reality is it does not happen very often.

The MR question is a very intriguing one. Indiana University is one of the most aggressive MR pancreas institutions in the world. They have done somewhere between 3,500 and 4,000 pancreas-specific MRs. The radiologists are particularly proud of this practice. The truth, however, is that as surgeons, we use it most commonly in a clinical setting to evaluate ductal integrity and therefore to avoid getting into unplanned skullduggery within the operating room in scenarios such as disconnected left pancreatic remnants.

In terms of the CT-sparing effect, sure, if you were going to get five CTs plus an MR, then theoretically it spared a CT in that given patient. When we looked at it retrospectively, however, those patients, at the end of the day, were getting the same number of CT scans as the non-MRI folks. Do I think that is something that has to be a significant focus moving forward at our institution, as well as elsewhere? Absolutely. All non-radiation technologies must be explored as potential options. The last point I will make is that MR imaging is limited somewhat if you have a large fluid collection associated with necrotizing pancreatitis. It makes it tough to delineate some of the typical markers that we all look for.

Discussant

Dr. Charles Vollmer, Jr. (Boston, MA): This is a fantastic job. Great work.

Two quick questions: Were you able to break down the proportion of these scans that were done in the diagnostic mode before the definitive intervention and then those obtained thereafter, after a definitive intervention? I know that could be a little hard to ascertain because there are multiple interventions in some of these cases, but I am just wondering how much of this is because we are worried about when to act, how to act and when to pull the trigger, versus thereafter; Did we do a good job? Are we surveilling and following up the effect of the intervention?

The second question is along the lines of this MRI discussion by Dr Matthews. Could we even help and simplify this even better and save costs by using ultrasounds, since by and large most of us are worried about fluid collection development and management?

Closing Discussant

Dr. Chad G. Ball: The simple answer to your first question is that about one fifth of the radiation exposure is up-front. The reality is that at IU, there are arguably eight very busy pancreatic surgeons, and the individual practice variance is substantial. I suspect that if you looked at other institutions across the country, the way people use CT, MRI, and ERCP in their clinical management of this disease probably varies dramatically. As a result, it is a tough question to answer by just saying 20%. I think it is more complicated.

Your second question is a very interesting idea. I use a significant amount of ultrasonography in the care of critically injured patients. Although I do not necessarily know too much data about using it in the context you mention, it seems like a great thought.

J Gastrointest Surg (2010) 14:1536–1546
DOI 10.1007/s11605-010-1319-3

2010 SSAT PLENARY PRESENTATION

One Hundred Thirty Resections for Pancreatic Neuroendocrine Tumor: Evaluating the Impact of Minimally Invasive and Parenchyma-Sparing Techniques

Joseph DiNorcia · Minna K. Lee · Patrick L. Reavey · Jeanine M. Genkinger · James A. Lee · Beth A. Schrope · John A. Chabot · John D. Allendorf

Received: 29 April 2010 / Accepted: 9 August 2010 / Published online: 8 September 2010

Abstract
Background Increasingly, surgeons apply minimally invasive and parenchyma-sparing techniques to the management of pancreatic neuroendocrine tumor (PNET). The aim of this study was to evaluate the impact of these approaches on patient outcomes.
Methods We retrospectively collected data on patients with PNET and compared perioperative and pathologic variables. Survival was analyzed using the Kaplan–Meier method. Factors influencing survival were evaluated using a Cox proportional hazards model.
Results One hundred thirty patients underwent resection for PNET. Traditional resections included 43 pancreaticoduodenectomies (PD), 38 open distal pancreatectomies (DP), and four total pancreatectomies. Minimally invasive and parenchyma-sparing resections included 25 laparoscopic DP, 11 central pancreatectomies, five enucleations, three partial pancreatectomies, and one laparoscopic-assisted PD. Compared to traditional resections, the minimally invasive and parenchyma-sparing resections had shorter hospital stays. By univariate analysis of neuroendocrine carcinoma, liver metastases and positive resection margins correlated with poor survival. There was an increase in minimally invasive or parenchyma-sparing resections over the study period with no differences in morbidity, mortality, or survival.
Conclusion In this series, there has been a significant increase in minimally invasive and parenchyma-sparing techniques for PNET. This shift did not increase morbidity or compromise survival. In addition, minimally invasive and parenchyma-sparing operations yielded shorter hospital stays.

Keywords Pancreatic neuroendocrine tumor · Pancreatectomy · Minimally invasive · Laparoscopic · Parenchyma-sparing resection

This manuscript was presented at the SSAT Annual Meeting in New Orleans, May 2010.

J. DiNorcia · M. K. Lee · P. L. Reavey · J. A. Lee · B. A. Schrope · J. A. Chabot · J. D. Allendorf (✉)
College of Physicians and Surgeons, Department of Surgery, Columbia University,
161 Fort Washington Avenue, Suite 820,
New York, NY 10032, USA
e-mail: jda13@columbia.edu

J. M. Genkinger
Mailman School of Public Health, Department of Epidemiology, Columbia University,
722 West 168th Street, Room 803,
New York, NY 10032, USA

Introduction

Pancreatic neuroendocrine tumors (PNETs) are a rare subset that accounts for less than 3% of all pancreatic tumors.[1,2] While most are commonly sporadic, PNETs also can be associated with genetic syndromes such as multiple endocrine neoplasia type I or von Hippel-Lindau disease.[3] PNETs exhibit a wide spectrum of clinical behavior that has made classification and staging difficult. The majority of PNETs are associated with prolonged survival, yet there can be significant variability in outcomes because of their biological heterogeneity.[4–6]

A clinical classification system divides PNETs into functional and nonfunctional tumors. Patients with functional tumors usually present with syndromes of gastrointestinal hormone overproduction, whereas patients with nonfunctional tumors usually present with mass effect or symptoms of

metastatic disease.[3,7] In 2000, the World Health Organization (WHO) introduced a classification system based on clinical and histopathologic features that divides PNETs into well-differentiated endocrine tumor with either benign or uncertain behavior (WDT), well-differentiated endocrine carcinoma (WDCa), or poorly differentiated endocrine carcinoma (PDCa).[8] In 2006, the European Neuroendocrine Tumor Society (ENETS) applied a TNM staging system to PNETs.[9] Both the WHO and ENETS-TNM systems recently have been validated for prognostic stratification of PNET patients.[7,10]

Surgery is the only curative modality for PNETs. Recent studies have demonstrated improved survival across all stages of disease, advocating resection of the primary tumor in localized, regional, and metastatic disease.[3,11–16] With the advent of laparoscopy and advances in surgical technique, minimally invasive and parenchyma-sparing operations are gaining acceptance in the management of various pancreatic diseases, including PNETs. The purpose of this study was twofold: to evaluate our institution's surgical experience with PNETs and to compare outcomes between patients who underwent traditional versus minimally invasive/parenchyma-sparing resections.

Materials and Methods

We performed a retrospective review of a prospectively maintained database of patients with pancreatic disease. The database is maintained by The Pancreas Center of Columbia University Medical Center (CUMC) and includes the patients of four surgeons (J.A., J.C., J.L., B.S.). After approval from the institutional review board and in compliance with the Health Insurance Portability and Accountability Act regulations, we queried our database to identify all patients who underwent pancreatic resection for PNET at CUMC from 1994 to 2009. We excluded patients with multiple endocrine neoplasia type I and von Hippel-Lindau disease because they can be associated with multiple other neoplasms that require more than pancreatic resection for cure.

Descriptive data were collected by review of patients' medical records. Preoperative variables included age, gender, and race. Patients with signs, symptoms, and biochemical evidence of hormonal excess were considered to have functional tumors. Patients with no recognizable clinical syndrome and normal serum hormone levels were considered to have nonfunctional tumors, regardless of immunohistochemical results on final pathology. Operations were grouped as traditional or minimally invasive/parenchyma sparing. Traditional operations included pancreaticoduodenectomy (PD), open distal pancreatectomy (ODP), and total pancreatectomy (TP). Minimally invasive operations included all laparoscopic and laparoscopic-assisted operations. Parenchyma-sparing operations included central pancreatectomy (CP), partial pancreatectomy, and enucleation.

Intraoperative variables were obtained from nurse, anesthesiologist, and surgeon reports. Operating room (OR) time was defined as the time between patient entry into and exit from the OR. Anesthesia time was defined as the time between start of anesthesia care in the OR and patient exit from the OR. Incision time was defined as the time between incision start and incision close. Pathologic variables including tumor grade, tumor diameter, lymphovascular invasion, regional lymph node status (N), and margin status were determined from final pathology reports. Tumors were classified according to the WHO system[8] and staged according to the ENETS-TNM system.[9] Because Ki-67 was not available on early pathology reports, we did not include it in our analysis.

Perioperative complications were gathered from daily progress notes and discharge summaries and graded using the system proposed by DeOliveira and colleagues.[17] Overall morbidity was defined as any complication, and major morbidity was defined as complications grade III and greater. Pancreatic fistula was assessed and graded according to the International Study Group on Pancreatic Fistula recommendations.[18] Length of stay (LOS) was calculated from date of operation to date of hospital discharge. Readmission rate was defined as readmission within 30 days of hospital discharge. Perioperative mortality was defined as death within 30 days of the operation or within the same hospital admission as the operation.

Survival was calculated from the date of operation through the date of last follow-up. Survival probability was estimated using the Kaplan–Meier method. Survival durations were determined from the Kaplan–Meier curves and compared using the log-rank test. For a subset analysis of the impact of minimally invasive and parenchyma-sparing techniques, we divided the entire cohort at the median date of operation into our early and recent experience.

Continuous variables were compared using Student's t test or Wilcoxon rank-sum test. Categorical variables were compared using Pearson's chi-squared test or Fisher's exact test as appropriate. Univariate and multivariate analyses were performed using Cox proportional hazards models. Continuous variables are reported as mean±standard deviation or median and interquartile range (IQR). Categorical variables are presented as number and percentage (%). A p value of less than 0.05 was considered statistically

significant. Statistical analyses were conducted using the R statistical software program (version 2.8).

Results

Demographics, Entire Cohort

A total of 130 patients underwent pancreatectomy for PNETs between October 12, 1994, and December 31, 2009. The mean age was 61.0 years (12.8 years) with 76 (58.5%) female patients. Ninety-seven (74.6%) patients were Caucasian. Nineteen (14.6%) patients had functional tumors, including 16 insulinomas, two gastrinomas, and one vasoactive intestinal polypeptide-secreting tumor. One hundred eleven (85.4%) patients had nonfunctional tumors (Table 1).

Operations, Entire Cohort

Eighty-five (65.4%) patients underwent traditional pancreatic resection, which included 43 (33.1%) pancreaticoduodenectomies, 38 (29.2%) open distal pancreatectomies, and four (3.1%) total pancreatectomies. Forty-five (34.6%) patients underwent minimally invasive/parenchyma-sparing pancreatic resection, which included 25 (19.2%) laparoscopic distal pancreatectomies, 11 (8.5%) central pancreatectomies, five (3.8%) enucleations, three (2.3%) partial pancreatectomies, and one (0.8%) laparoscopic-assisted pancreaticoduodenectomy (Table 2).

Table 1 Demographics and tumor types for patients with PNET undergoing pancreatic resection

Variable	PNET (n=130)
Demographics	
Age, year, mean (SD)	61.0 (12.8)
Gender, M/F	54/76
Race (%)	
Caucasian	97 (74.6)
Hispanic	10 (7.7)
Black	8 (6.2)
Asian	6 (4.6)
Other	9 (6.9)
Tumor type	
Functional (%)	19 (14.6)
Insulinoma	16
Gastrinoma	2
VIPoma	1
Nonfunctional (%)	111 (85.4)

SD standard deviation, *VIPoma* vasoactive intestinal polypeptide-secreting tumor

Pathologic Characteristics, Entire Cohort

Seventy-six (58.5%) tumors were low grade, 22 (16.9%) intermediate grade, and nine (6.9%) high grade. Median tumor size for the entire cohort was 2.5 cm (IQR 1.5–5 cm) with 51 (39.2%) less than 2 cm and 79 (60.8%) greater than or equal to 2 cm. Fifty-five (42.3%) tumors had microscopic evidence of lymphovascular invasion. Twenty-eight (21.5%) patients had positive regional lymph nodes, and seven (5.4%) patients had distant metastases. Three (2.3%) of the seven patients with metastatic disease underwent resection of their primary PNET only, three (2.3%) underwent synchronous liver resection (one) or cryoablation (two), and one (0.8%) underwent en bloc splenectomy and retroperitoneal debulking. One hundred fourteen (87.7%) patients had complete resection (R0), and 16 (12.3%) patients had evidence of microscopic disease on the pancreatic margin (R1). According to WHO classification, 78 (60.0%) patients had WDT (43 benign, 35 uncertain behavior), 42 (32.3%) had WDCa, and 10 (7.7%) had PDCa. In terms of TNM stage, 48 (36.9%) patients were stage 1, 51 (39.2%) stage 2, 24 (18.5%) stage 3, and seven (5.4%) stage 4 (Table 3).

Outcomes, Entire Cohort

The overall surgical morbidity was 54.6% with a major morbidity rate of 22.3%. Seven (5.4%) patients required reoperation, and 17 (13.1%) required readmission. There were three (2.3%) perioperative deaths. Of the three deaths, two occurred after pancreaticoduodenectomy for WDT and

Table 2 Operations performed for PNETs

Variable	PNET (n=130)
Traditional resection (%)	85 (65.4)
Pancreaticoduodenectomy	43 (33.1)
Open distal pancreatectomy	38 (29.2)
Total pancreatectomy	4 (3.1)
Minimally invasive/parenchyma-sparing resection (%)	45 (34.6)
Laparoscopic distal pancreatectomy	25 (19.2)
Central pancreatectomy	11 (8.5)
Enucleation	5 (3.8)
Partial pancreatectomy	3 (2.3)
Laparoscopic-assisted pancreaticoduodenectomy	1 (0.8)

Table 3 Pathologic characteristics for all PNETs

Variable	PNET (n=130)
Grade (%)[a]	
Low	76 (58.5)
Intermediate	22 (16.9)
High	9 (6.9)
Lesion size (%)	
<2 cm	51 (39.2)
≥2 cm	79 (60.8)
Lymphovascular invasion (%)[a]	
Present	55 (42.3)
Absent	66 (50.8)
Lymph nodes (%)	
Positive	28 (21.5)
Negative	102 (78.5)
Distant metastases (%)	
Present	7 (5.4)
Absent	123 (94.6)
Resection margins (%)	
Positive	16 (12.3)
Negative	114 (87.7)
WHO classification	
WDT (%)	78 (60.0)
Benign	43
Uncertain	35
WDCa (%)	42 (32.3)
PDCa (%)	10 (7.7)
TNM stage	
Stage 1 (%)	48 (36.9)
Stage 2 (%)	51 (39.2)
Stage 3 (%)	24 (18.5)
Stage 4 (%)	7 (5.4)

WDT well-differentiated tumor, *WDCa* well-differentiated carcinoma, *PDCa* poorly differentiated carcinoma

[a] Data were not available for all patients

WDCa, and one after total pancreatectomy for WDCa. Two of these operations involved concomitant portomesenteric venous resection and reconstruction. Median LOS was 7.5 days (IQR 5–12 days; Table 4).

The median follow-up for the entire cohort was 36.3 months (IQR 11.6–59.8 months) during which time 15 patients developed recurrences. Seven patients had recurrences in the liver, three had recurrences in the liver and pancreatic bed, four had recurrences in the pancreatic bed only, and one had recurrence in the duodenum. The 5-year survival for the entire cohort was 82.5%. The 5-year survival for patients with WDT, WDCa, and PDCa was 93.9%, 75.8%, and 29.2%, respectively (p<0.01). When WDT was separated into benign and uncertain behavior tumors, the 5-year survival rates were 100% and 89.0%, respectively (Fig. 1a). The 5-year survival for patients with TNM stages 1, 2, 3, and 4 was 92.3%, 81.2%, 89.4%, and 23.8%, respectively (p<0.01; Fig. 1b).

Minimally Invasive and Parenchyma-Sparing Versus Traditional Pancreatic Resection

We compared PNET patients who underwent minimally invasive/parenchyma-sparing pancreatic resection (n=45) to patients who underwent traditional pancreatic resection (n=85). The two groups were similar with respect to age, gender, and race. The groups differed significantly in pathologic characteristics of their tumors. The minimally invasive/parenchyma-sparing group had smaller, lower-grade tumors with less lymphovascular invasion and fewer regional nodal metastases. There were no differences in incidence of positive margins between the two groups. The minimally invasive/parenchyma-sparing group had more well-differentiated tumors and lower stage of disease (Table 5).

There were no differences in overall morbidity (48.9% vs. 57.6%, p=0.34), reoperation (0% vs. 8.2%, p=0.10), and readmission (11.1% vs. 14.1%, p=0.79) between the two groups. Fewer patients who underwent minimally invasive/parenchyma-sparing resections had major complications (11.1% vs. 28.2%, p=0.03). There were no perioperative deaths in the minimally invasive/parenchyma-sparing group versus three (3.5%) in the traditional resection group (p=0.55). Patients who underwent minimally invasive/parenchyma-sparing operations had significantly shorter median LOS (6 days, IQR 5–7 days vs. 9 days, IQR 6–15 days; p<0.01).

With a median follow-up of 25.4 months (IQR 14.7–42.5 months), two (4.4%) patients in the minimally invasive/parenchyma-sparing group developed recurrences. Both recurrences occurred after laparoscopic distal pancreatectomy. With a median follow-up of 42.7 months (IQR 6.7–66.7 months), 13 (15.3%) patients in the traditional resection group developed recurrences (p=0.09). During the follow-up, no patient died in the minimally invasive/parenchyma-sparing group, and the

Table 4 Outcomes after resection for PNET

Variable	PNET (n=130)
Morbidity, any (%)	71 (54.6)
Morbidity, major (%)	29 (22.3)
Reoperation (%)	7 (5.4)
Readmission (%)	17 (13.1)
Mortality (%)	3 (2.3)
LOS, days, median (IQR)	7.5 (5–12)

LOS length of stay, *IQR* interquartile range

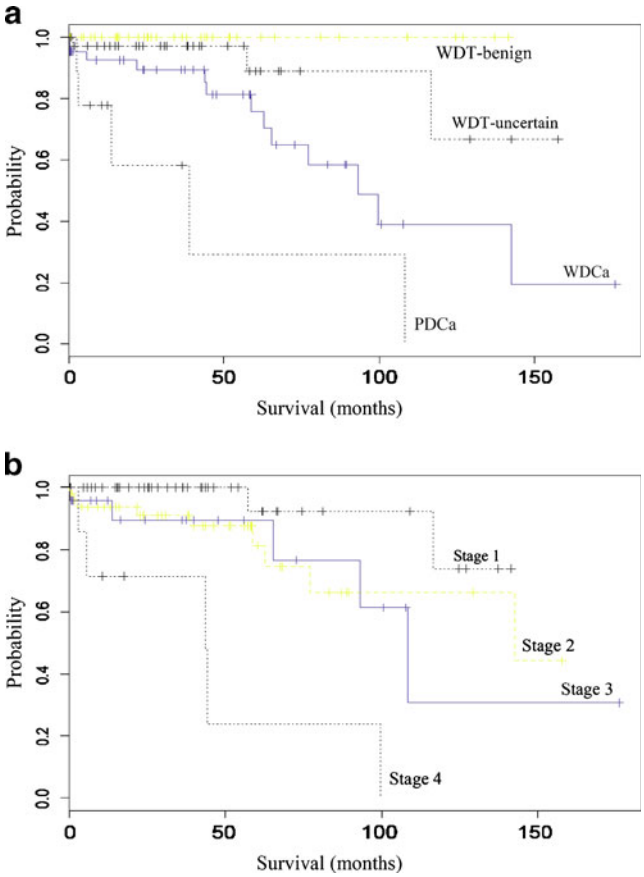

Fig. 1 Kaplan–Meier estimates of survival for patients who underwent R0/R1 resection for PNETs. **a** Five-year survival estimates stratified by WHO classification for patients with WDT-benign, WDT-uncertain, WDCa, and PDCa were 100%, 89%, 76%, and 29%, respectively (log-rank test, $p<0.01$). **b** Five-year survival estimates stratified by ENETS-TNM stage for patients with stage 1, 2, 3, and 4 disease were 92%, 81%, 89%, and 24%, respectively (log-rank test, $p<0.01$)

5-year survival in the traditional resection group was 77.4% ($p=0.16$; Table 6).

Potential Predictors of Poor Survival in Patients with Pancreatic Endocrine Carcinoma

We performed an analysis of potential prognostic factors impacting survival after pancreatic resection in a subset of patients with pancreatic endocrine carcinoma (WDCa and PDCa). On univariate analysis, positive resection margins ($p<0.01$) and presence of distant metastatic disease ($p=0.02$) were associated with poor survival. Well versus poor differentiation by WHO classification closely approached but did not reach statistical significance ($p=0.054$). Tumor size ($p=0.83$) and TNM stage ($p=0.27$) did not correlate with poor survival (Table 7). When controlling for differentiation and resection margins on multivariate analysis, distant metastasis was no longer associated with poor survival ($p=0.12$; Table 8).

Recent Versus Early Experience with Minimally Invasive and Parenchyma-Sparing Techniques

After dividing the entire cohort at the median date of operation (June 2006), 63 patients were in the early group and 67 patients were in the recent group. There was a significant increase in the use of minimally invasive and parenchyma-sparing techniques, with 31 (46.3%) cases in the recent group and 14 (22.2%) in the early group ($p<0.01$). Despite this increase, there were no significant differences in demographics, pathologic characteristics, outcomes, or survival between the early and recent groups (Tables 9 and 10).

Discussion

PNETs are uncommon tumors with an incidence of one to two cases per 1,000,000 people.[3] Their natural history is incompletely understood because their often indolent course can delay diagnosis and treatment. In addition, PNETs exhibit a spectrum of biological behavior ranging from benign to highly malignant.[19] This heterogeneity has made it challenging both to devise clinically effective stratification systems as well as to understand the extent of resection needed for cure. In this series, we reviewed our institution's experience with the surgical management of 130 PNETs. We examined pathologic characteristics according to current classification and staging systems and used multivariable regression models to evaluate potential factors that affect survival. Finally, we analyzed the impact of minimally invasive and parenchyma-sparing techniques on patient outcomes.

The majority of PNETs in our series were nonfunctional, consistent with other reports in the literature. In a recent review of 9,821 patients identified from the National Cancer Database, Bilimoria and colleagues[4] found that 85% of the PNETs were nonfunctional. The distinction between functional and nonfunctional, however, does not provide adequate prognostic information. For this reason, several recent studies in the literature have evaluated the WHO classification and ENETS-TNM staging systems. Ito and colleagues[10] presented their experience with 73 consecutive PNET patients stratified according to these systems. The authors reported 5-year survival rates of 100%, 57%, and 8% for WDT, WDCa, and PDCa, respectively, and 100%, 90%, 57%, and 8% for stages 1, 2, 3, and 4, respectively. They concluded that both systems were useful prognostic tools for classifying and staging patients with PNETs.

Survival rates from our data showed similar results using the two systems. The 5-year survival rates for patients with WDT, WDCa, and PDCa were 93.9%, 75.8%, and 29.2%,

Table 5 Demographic and pathologic characteristics: minimally invasive and parenchyma-sparing vs. traditional pancreatic resection

Variable	Minimally invasive/ parenchyma-sparing (n=45)	Traditional resection (n=85)	p value
Demographics			
Age, year, mean (SD)	60.9 (12.6)	61.0 (13.0)	0.96
Gender, M/F	19/26	35/50	0.91
Race (%)			
Caucasian	35 (77.8)	62 (72.9)	0.55[a]
Hispanic	4 (8.9)	6 (7.1)	
Black	3 (6.7)	5 (5.9)	
Asian	0 (0)	0 (0)	
Other	3 (6.7)	6 (7.1)	
Pathology			
Grade (%)[b]			
Low	34 (75.6)	42 (49.4)	0.02[c]
Intermediate	4 (8.9)	18 (21.2)	
High	2 (4.4)	7 (8.2)	
Lesion size (%)			
<2 cm	25 (55.6)	26 (30.6)	<0.01
≥2 cm	20 (44.4)	59 (69.4)	
Lymphovascular invasion (%)[b]			
Present	8 (17.8)	47 (55.3)	<0.01
Absent	34 (75.6)	32 (37.6)	
Lymph nodes (%)			
Positive	3 (6.7)	25 (29.4)	<0.01
Negative	42 (93.3)	60 (70.6)	
Distant metastases (%)			
Present	0 (0)	7 (8.2)	0.10
Absent	45 (100)	78 (91.8)	
Resection margins (%)			
Positive	3 (6.7)	13 (15.3)	0.26
Negative	42 (93.3)	72 (84.7)	
WHO Classification			
WDT (%)	39 (86.7)	39 (45.9)	<0.01[d]
Benign	27	16	
Uncertain	12	23	
WDCa (%)	4 (8.9)	38 (44.7)	
PDCa (%)	2 (4.4)	8 (9.4)	
TNM stage			
Stage 1 (%)[c]	25 (55.5)	23 (27.1)	<0.01[e]
Stage 2 (%)	17 (37.8)	34 (40.0)	
Stage 3 (%)	3 (6.7)	21 (24.7)	
Stage 4 (%)	0 (0)	7 (8.2)	

SD standard deviation, *WDT* well-differentiated tumor, *WDCa* well-differentiated carcinoma, *PDCa* poorly differentiated carcinoma

[a] Statistical analysis performed on Caucasian versus all other races

[b] Data were not available on all patients

[c] Statistical analysis performed on low-grade versus all others (intermediate- and high-grade)

[d] Statistical analysis performed on WDT versus carcinomas (WDCa and PDCa)

[e] Statistical analysis performed on early stage (stages 1 and 2) versus late stage (stages 3 and 4)

respectively; the 5-year survival rates for patients with TNM stages 1, 2, 3, and 4 were 92.3%, 81.2%, 89.4%, and 23.8%, respectively. Although 5-year survival estimates for stage 2 and 3 disease were incongruous, stage 3 PNETs ultimately exhibited worse survival at later time points on the Kaplan–Meier curve (Fig. 1b). Interestingly, Scarpa and colleagues[7] recently evaluated the ENETS-TNM system and found limited prognostic value for intermediate stages of disease. The authors proposed modifications to improve stratification, though the system has yet to be adopted universally.

Along with classification and staging of PNETs, recent series have examined pathologic characteristics that predict poor survival. Ito and colleagues, for example, found that tumor grade, nodal metastasis, and distant metastasis predicted poor survival. Bilimoria and colleagues[4] identified tumor grade and distant metastasis as the most significant predictors of survival. In contrast, Kazanjian and colleagues[20] found that lymphovascular invasion, not resection margins or distant metastasis, predicted poor survival. In our study, we examined factors influencing

Table 6 Outcomes: minimally invasive and parenchyma-sparing vs. traditional pancreatic resection

Variable	Minimally invasive/ parenchyma-sparing (n=45)	Traditional resection (n=85)	p value
Morbidity, any (%)	22 (48.9)	49 (57.6)	0.34
Morbidity, major (%)	5 (11.1)	24 (28.2)	0.03
Reoperation (%)	0 (0)	7 (8.2)	0.10
Readmission (%)	5 (11.1)	12 (14.1)	0.79
Mortality (%)	0 (0)	3 (3.5)	0.55
Length of stay, days, median (IQR)	6 (5–7)	9 (6–15)	<0.01
Follow-up, months, median (IQR)	25.4 (14.7–42.5)	42.7 (6.7–66.7)	0.16
Recurrence (%)	2 (4.4)	13 (15.3)	0.09
5-year survival	100%	77.4%	0.16

IQR interquartile range

survival in carcinoma patients only. Positive resection margins and distant metastasis were significantly associated with poor survival, and well-differentiated versus poorly differentiated carcinoma by WHO classification very closely approached significance. When controlling for differentiation and resection margins, distant metastasis no longer correlated with poor survival. We were unable to report the impact of tumor grade and lymphovascular invasion because incomplete data on early pathology reports did not fit the hazards model. Pathology reports continue to evolve with changes in classification and staging systems, and future prospective studies that consistently evaluate newly identified pathologic factors such as Ki-67 will help clarify the current systems.

Surgery with curative intent is the mainstay of treatment for all stages of PNETs. Since the first report of laparoscopic pancreatic resection for insulinoma in the 1990s, minimally invasive techniques are being applied with increasing frequency to the management of pancreatic diseases.[21–23] Similarly, after studies emerged demonstrating the safety and feasibility of enucleation and central pancreatectomy, parenchyma-sparing operations are being performed more commonly for pancreatic lesions, including PNETs.[24–28] Our institution's experience mirrors this shift as almost 50% of pancreatic resections performed for PNETs since 2006 have involved minimally invasive or parenchyma-sparing techniques.

Recent series in the literature document the safety and feasibility of these modern surgical approaches to PNETs. Pitt and colleagues[29] evaluated patients who underwent surgery for small (≤3 cm) PNETs and found comparable outcomes between enucleation and traditional resection. Although there was a higher incidence of pancreatic fistula after enucleation, these fistulae were less severe than after traditional resection. Of note, the patients with pancreatic head PNETs treated by enucleation had decreased blood loss, operative time, and length of stay compared to those who underwent pancreaticoduodenectomy. In a recent series of 49 consecutive patients, Fernández-Cruz and colleagues[30] evaluated laparoscopic distal pancreatectomy and laparoscopic enucleation for select cases of benign and malignant PNETs. They achieved R0 resection in all cases of malignant PNETs and reported no recurrences at the time of follow-up. Although they lacked an open-surgery control group, they noted no mortality, reduced morbidity, and short hospital stays. Our data demonstrate similar benefits and oncologic outcomes. There was no difference in overall morbidity, though minimally invasive/parenchyma-sparing resection patients had significantly decreased major morbidity than those who underwent traditional resections. Moreover, the minimally invasive/parenchyma-sparing resection patients had significantly shorter hospital stays compared to traditional resection patients. Together, these data suggest that minimally

Table 7 Univariate analysis of pancreatic neuroendocrine carcinoma: pathologic factors and survival

Variable	HR	95% CI for HR	p value
Age (>62 years)	1.03	0.39–2.70	0.96
Gender (female)	0.90	0.34–2.37	0.83
Tumor type (functional)	1.24	0.27–5.68	0.78
Lesion (≥2 cm)	1.18	0.27–5.24	0.83
Metastasis (present)	3.47	1.19–10.14	0.02
Resection margins (positive)	4.66	1.62–13.40	<0.01
Lymph nodes (positive)	0.78	0.30–2.02	0.61
WHO classification (PDCa)	0.34	0.12–1.02	0.05
TNM stage (stage increase by 1)	1.45	0.75–2.80	0.27

Table 8 Multivariate analysis of pancreatic neuroendocrine carcinoma: pathologic factors and survival

Variable	HR	95% CI for HR	*p* value
Metastasis (present)	2.52	0.78–8.11	0.12
WHO classification (PDCa)	0.27	0.09–0.88	0.03
Resection margins (positive)	4.10	1.31–12.80	0.02

invasive and parenchyma-sparing techniques for PNETs are feasible, safe, and beneficial.

Concerns over oncologic outcomes have limited the role of minimally invasive and parenchyma-sparing operations for malignant pancreatic disease. A recent study by Kooby and colleagues[31] demonstrated comparable oncologic outcomes between laparoscopic and open distal pancreatectomy for adenocarcinoma, though no study has evaluated similar outcomes for malignant PNET. In our series, two patients with high-grade carcinoma (PDCa) underwent R0 laparoscopic distal pancreatectomy without morbidity or mortality. Both patients were alive and recurrence-free at the conclusion of study follow-up. Two other patients in the minimally invasive/parenchyma-sparing group developed recurrences after R0 laparoscopic distal pancreatectomy. One patient who had WDT of uncertain behavior developed liver metastases and recurrence in the surgical bed that were treated with chemotherapy. The other patient had WDCa and developed liver metastases that were treated with chemotherapy. Both patients were alive at the conclusion of study follow-up. Another concern with laparoscopic resection of malignant disease is the potential to seed port sites with tumor. No patient in this study developed port site disease during the follow-up period.

It is important to note differences in pathology between the minimally invasive/parenchyma-sparing group and the traditional resection group. We acknowledge that, in general, small low-grade PNETs without locoregional or distant metastases

Table 9 Patients and pathology: early vs. recent experience

Variable	Early (*n*=63)	Recent (*n*=67)	*p* value
Demographics			
Age, year, mean (SD)	59.4 (12.2)	62.4 (13.3)	0.19
Gender, M/F	23/40	31/36	0.26
Race (%)			
Caucasian	46 (73.0)	51 (76.1)	0.68[a]
Hispanic	6 (9.5)	4 (6.0)	
Black	4 (6.4)	4 (6.0)	
Asian	4 (6.4)	2 (3.0)	
Other	3 (4.7)	6 (8.9)	
Type of resection (%)			
Traditional resection	49 (77.8)	36 (53.7)	<0.01
Minimally invasive/ parenchyma-sparing resection	14 (22.2)	31 (46.3)	
Pathology			
Grade (%)[b]			
Low	31 (49.2)	45 (67.2)	0.51[c]
Intermediate	7 (11.1)	15 (22.4)	
High	3 (4.8)	6 (9.0)	
Lesion size (%)			
<2 cm	24 (38.1)	27 (40.3)	0.80
≥2 cm	39 (61.9)	40 (59.7)	
Lymphovascular invasion (%)[b]			
Present	33 (52.4)	22 (32.8)	<0.01
Absent	22 (34.9)	44 (65.7)	
Lymph nodes (%)			
Positive	13 (20.6)	15 (22.4)	0.81
Negative	50 (79.4)	52 (77.6)	
Distant metastases (%)			
Present	4 (6.4)	3 (4.5)	0.71
Absent	59 (93.6)	64 (95.5)	
Resection margins (%)			
Positive	6 (9.5)	10 (14.9)	0.43
Negative	57 (90.5)	57 (85.1)	
WHO Classification			
WDT (%)	35 (55.6)	43 (64.2)	0.32[d]
Benign	19	24	
Uncertain	16	19	
WDCa (%)	24 (38.1)	18 (26.9)	
PDCa (%)	4 (6.3)	6 (8.9)	
TNM Stage			
Stage 1 (%)[c]	21(33.3)	27 (40.3)	0.67[e]
Stage 2 (%)	28 (44.4)	23 (34.3)	
Stage 3 (%)	10 (15.9)	14 (20.9)	
Stage 4 (%)	4 (6.4)	3 (4.5)	

SD standard deviation, *WDT* well-differentiated tumor, *WDCa* well-differentiated carcinoma, *PDCa* poorly differentiated carcinoma

[a] Statistical analysis performed on Caucasian versus all other races

[b] Data were not available on all patients

[c] Statistical analysis performed on low-grade versus all others (intermediate and high grade)

[d] Statistical analysis performed on WDT versus carcinomas (WDCa and PDCa)

[e] Statistical analysis performed on early stage (stages 1 and 2) versus late stage (stages 3 and 4)

Table 10 Outcomes: early vs. recent experience

Variable	Early (*n*=63)	Recent (*n*=67)	*p* value
Morbidity, any (%)	31 (49.2)	40 (59.7)	0.23
Morbidity, major (%)	10 (15.9)	19 (28.4)	0.09
Reoperation (%)	1 (1.6)	6 (9.0)	0.12
Readmission (%)	7 (11.1)	10 (14.9)	0.61
Mortality (%)	0 (0)	3 (4.5)	0.25
Length of stay, days, median (IQR)	7 (5–10)	8 (5–15)	0.41

IQR interquartile range

were selected for the minimally invasive and parenchyma-sparing approaches, introducing selection bias. Other limitations include those of any single-institution retrospective review. Incomplete pathologic data may have biased classification and staging, though our data concur with similar studies in the literature. Lastly, the study population is defined by the demographics seen at our institution, a high-volume academic center with experienced pancreatic surgeons, and results may not be universally applicable. Nonetheless, our data suggest that patients who undergo surgical management of PNETs have prolonged survival and that judicious use of minimally invasive and parenchyma-sparing techniques is safe and effective for patients with these tumors.

Conclusion

In this series, the WHO classification and ENETS-TNM staging systems provided useful stratification of patients who underwent pancreatic resection for PNETs. Positive resection margins and distant metastasis were associated with poor survival in patients with pancreatic endocrine carcinoma. In recent years, there has been a significant increase in minimally invasive and parenchyma-sparing techniques for PNET patients at our institution. These techniques have been applied safely in select patients, achieving shorter hospital stays without compromising oncologic outcomes.

Acknowledgements This work was supported by the I.W. Foundation, The Doris Duke Charitable Foundation, and an institutional Ruth L. Kirschstein National Research Service Award (T32 HL 007854-14).

References

1. Halfdanarson TR, Rabe KG, Rubin J, Petersen GM. Pancreatic neuroendocrine tumor (PNETs): incidence, prognosis and recent trend toward improved survival. Ann Oncol 2008;19:1727–1733.
2. Halfdanarson TR, Rubin J, Farnell MB, Grant CS, Petersen GM. Pancreatic endocrine neoplasms: epidemiology and prognosis of pancreatic endocrine tumors. Endocr Relat Cancer 2008;15:409–427.
3. Hill JS, McPhee JT, McDade TP, Zhou Z, Sullivan ME, Whalen GF, Tseng JF. Pancreatic neuroendocrine tumors: the impact of surgical resection on survival. Cancer 2009;115:741–751.
4. Bilimoria KY, Tomlinson JS, Merkow RP, Stewart AK, Ko CY, Talamonti MS, Bentrem DJ. Clinicopathologic features and treatment trends of pancreatic neuroendocrine tumors: analysis of 9,821 patients. J Gastrointest Surg 2007;11:1460–1469.
5. Bilimoria KY, Talamonti MS, Tomlinson JS, Stewart AK, Winchester DP, Ko CY, Bentrem DJ. Prognostic score predicting survival after resection of pancreatic neuroendocrine tumors: analysis of 3851 patients. Ann Surg 2008;247:490–500.
6. Nissen NN, Kim AS, Yu R, Wolin EM, Friedman ML, Lo SK, Wachsman AM, Colquhoun SD. Pancreatic neuroendocrine tumors: presentation, management, and outcomes. Am Surg 2009;10:1025–1029.
7. Scarpa A, Mantovani W, Capelli P, Beghelli S, Boninsegna L, Bettini R, Panzuto F, Pederzoli P, delle Fave G, Falconi M. Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients. Mod Pathol 2010;doi:10.1038/modpathol.2010.58
8. Kloppel G, Perren A, Heitz PU. The gastroenteropancreatic neuroendocrine cell system and its tumors: the WHO classification. Ann NY Acad Sci 2004;1014:13–27.
9. Rindi G, Klöppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Eriksson B, Falchetti A, Falconi A, Komminoth P, Körner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B. TNM staging of foregut (neuro) endocrine tumors: a consensus proposal including a grading system. Virchows Arch 2006;449:395–401.
10. Ito H, Abramson M, Ito K, Swanson E, Cho N, Ruan DT, Swanson RS, Whang EE. Surgery and staging of pancreatic neuroendocrine tumors: a 14-year experience. J Gastrointest Surg 2010;14:891–898.
11. Norton JA, Kivlen M, Li M, Scheider D, Chuter T, Jensen RT. Morbidity and mortality of aggressive resection in patients with advanced neuroendocrine tumors. Arch Surg 2003;138:859–865.
12. Schurr PG, Strate T, Rese K, Kaifi JT, Reichelt U, Petri S, Kleinhans H, Yekebas EF, Izbicki JR. Aggressive surgery improves long-term survival in neuroendocrine pancreatic tumors: an institutional experience. Ann Surg 2007;245:271–281.
13. Nguyen SQ, Angel LP, Divino CM, Schluender S, Warner RRP. Surgery in malignant pancreatic neuroendocrine tumors. J Surg Onc 2007;96:397–403.
14. Nomura N, Fujii T, Kanazumi N, Takeda S, Nomoto S, Kasuya H, Sugimoto H, Yamada S, Nakao A. Nonfunctioning neuroendocrine pancreatic tumors: our experience and management. J Hepatobiliary Pancreat Surg 2009;16:639:647.
15. Hilal MA, McPhail MJW, Zeidan BA, Jones CE, Johnson CD, Pearce NW. Aggressive multi-visceral pancreatic resections for locally advanced neuroendocrine tumours. Is it worth it? JOP 2009:10:276–279.
16. Franko J, Feng W, Yip L, Genovese E, Moser AJ. Non-functional neuroendocrine carcinoma of the pancreas: incidence, tumor biology, and outcomes in 2,158 patients. J Gastrointest Surg 2010;14:541–548.
17. DeOliveira ML, Winter JM, Schafer M, Cunningham SC, Cameron JL, Yeo CJ, Clavien PA. Assessment of complications after pancreatic surgery: a novel grading system applied to 633 patients undergoing pancreaticoduodenectomy. Ann Surg 2006;244:931–9.
18. Bassi C, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J, Neoptolemos J, Sarr M, Traverso W, Buchler M. Postoperative pancreatic fistula: an international study group (ISGPF) definition. Surgery 2005;138:8–13.
19. Dimou AT, Syrigos KN, Saif MW. Neuroendocrine tumors of the pancreas: what's new. Highlights from the "2010 ASCO gastrointestinal cancers symposium." JOP 2010;11:135–138.
20. Kazanjian KK, Reber HA, Hines OJ. Resection of pancreatic neuroendocrine tumors: results of 70 cases. Arch Surg 2006;141:765–770.
21. Gagner M, Pomp A, Herrera MF. Early experience with laparoscopic resections of islet cell tumors. Surgery 1996;120:1051–1054.
22. Briggs CD, Mann CD, Irving GRB, Neal CP, Peterson M, Cameron IC, Berry DP. Systematic review of minimally invasive pancreatic resection. J Gastrointest Surg 2009;13:1129–1137.
23. Gumbs AA, Grès P, Madureira FA, Gayet B. Laparoscopic vs. open resection of noninvasive intraductal pancreatic mucinous neoplasms. J Gastrointest Surg 2008;12:707–712.
24. Crippa S, Bassi C, Warshaw AL, Falconi M, Partelli S, Thayer SP, et al. Middle pancreatectomy: indications, short- and long-term operative outcomes. Ann Surg 2007;246:69–76.
25. Allendorf JD, Schrope BA, Lauerman MH, Inabnet WB, Chabot JA. Postoperative glycemic control after central pancreatectomy for mid-gland lesions. World J Surg 2007;31:164–8.

26. Adham M, Giunippero A, Hervieu V, Courbiere M, Partensky C. Central pancreatectomy: a single-center experience of 50 cases. Arch Surg 2008;143:175–80.
27. Crippa S, Boninsegna L, Partelli S, Falconi M. Parenchyma-sparing resections for pancreatic neoplasms. J Hepatobiliary Pancreat Surg 2009;doi:10.1007/s00534-009-0224-1.
28. Falconi M, Zerbi A, Crippa S, Balzano G, Boninsegna L, Capitanio V, Bassi C, DiCarlo V, Pederzoli P. Parenchyma-preserving resections for small nonfunctioning pancreatic endocrine tumors. Ann Surg Oncol 2010;doi:10.1245/s10434-010-0949-8.
29. Pitt SC, Pitt HA, Baker MS, Christians K, Touzios JG, Kiely JM, Weber SM, Wilson SD, Howard TJ, Talamonti MS, Rikkers LF. Small pancreatic and periampullary neuroendocrine tumors: resect or enucleate? J Gastrointest Surg 2009;13:1692–1698.
30. Fernández-Cruz L, Blanco L, Cosa R, Rendón H. Is laparoscopic resection adequate in patients with neuroendocrine pancreatic tumors? World J Surg 2008;32:904–917.
31. Kooby DA, Hawkins WG, Schmidt CM, Weber SM, Bentrem DJ, Gillespie TW, Sellers JB, Merchant NB, Scoggins CR, Martin RC 3rd, Kim HJ, Ahmad S, Cho CS, Parikh AA, Chu CK, Hamilton NA, Doyle CJ, Pinchot S, Hayman A, McClaine R, Nakeeb A, Staley CA, McMasters KM, Lillemoe KD. A multicenter analysis of distal pancreatectomy for adenocarcinoma: is laparoscopic resection appropriate? J Am Coll Surg 2010;210:779–785.

Discussant

Dr. Charles m. Vollmer (Boston, MA): Congratulations. I have really been impressed by the full breadth of your group's work here this week. It's been amazing. This is going to be one of the largest if not the largest and, may I say, a bodacious series on pancreatic neuroendocrine surgery. The primary point to take away after reading your paper on this, and you pointed this out, is that there is good fidelity with the newly developed staging systems. You also properly acknowledge that there are different biologies of the groups that you assessed and there probably is a bias in how the biology affects the outcome.

A few questions for you:

Are there temporal process effects that can be affecting these outcomes? So many of these minimal-access operations and parenchymal-sparing operations are clustered in the recent time period from 2006 onward. In other words, is it an effect of the operation or the time frame in which this is going on? And can you compare head to head with the traditional operations in that time frame?

The second question would be, are you actually mixing disparate concepts by clustering minimally invasive operations with parenchymal-sparing operations? I think these are two different theoretical paradigms conceptually. And I wonder if there are still outcome differences if you segregate those into three different groups in the entirety.

The third question would be, for your parenchymal-sparing group, are you actually achieving the objective of endocrine and exocrine maintenance for that?

The last question would be, what would you and your group do with a 0.8-cm, incidentally identified neuroendocrine tumor in the pancreatic head, which is totally asymptomatic, given the fact that these biologies are largely going to be on the low-grade end of the spectrum? What is your approach to this kind of scenario?

Closing Discussant

Dr. John Allendorf: In terms of whether or not outcomes were a function of time, we have done a comparison of the early and late outcomes, and we found really no differences in those outcomes. But, as you point out, many more of the minimally invasive and parenchymal-sparing operations were done in our later experience.

It's a bit jarring when you first look at the presentation of the data to include these two very different surgical techniques. And that's why, in the background, I took you through some of our previous work, where we looked specifically at distal pancreatectomies, open versus laparoscopic, and found a shorter length of stay and a lower morbidity. And we looked specifically at our central pancreatectomy cohort and compared them to the alternative, which would be distal pancreatectomy, and found a lower incidence of diabetes. However, these analyses also include patients with cystic neoplasms. So I can't tell you for the specific histology of pancreatic neuroendocrine tumors that that holds true, but the best I can do is to extrapolate from the procedure-specific studies.

It's been our approach to resect small neuroendocrine neoplasms. I don't know if you were asking what type of resection we would do, enucleation or Whipple. I think it would depend on the anatomy, how close it was to the pancreatic duct, whether I was concerned about a fistula or maybe disconnecting the pancreas. But if it was well away from the pancreatic duct, I think I would offer the patient an enucleation. If it was near the pancreatic duct, I probably would offer the patient a pancreaticoduodenectomy. One could argue that something as small as that and as indolent as that could be watched. But I don't think we have ample evidence to support observation, and it's a hard sell to the patient to tell them that they have a pancreatic neoplasm and we're going to leave it alone and watch it. We are gaining a knowledge about the natural history of these, but I don't think we are at the point of being comfortable with observation, so at this point I would resect.

Discussant

Dr. Henry Pitt (Indianapolis, IN): Historically, when the morbidity and mortality of Whipple and open distal pancreatectomy were significant, most experts recommended enucleation of neuroendocrine tumors. As mortality decreased, the pendulum swung toward resection. Currently, I believe that the pendulum should swing back toward more enucleations of small neuroendocrine tumors.

You had 51 tumors that were 2 cm or less, but only five were enucleated. For lesions in the head and neck of the pancreas, enucleation may be more appropriate because of decreased morbidity and comparable survival. An analysis published this past year by Susan Pitt came to this conclusion. What are your current policies with respect to enucleation?

Closing Discussant

Dr. John Allendorf: I'm familiar with your paper. Our institutional bias has been to try to avoid pancreatic fistulas. However, based on the work that you have done and the Barcelona group, even though with

enucleation there's a higher fistula rate, the fistulas tend to be more easily controlled and of a lower grade.

And so, going forward, it is important to consider the long-term effects that we are perpetrating upon our patients as far as endocrine and exocrine insufficiency. Many of these patients are going to live for a long time, and very few of these patients die from these operations, so it's not just the perioperative complication rate but the long-term complications or sequelae of doing pancreatic resections that become important.

So although our bias has been to resect small neuroendocrine neoplasms and to avoid pancreatic fistula, I think going ahead in the future we are going to give more consideration to enucleation.

J Gastrointest Surg (2010) 14:1547–1559
DOI 10.1007/s11605-010-1323-7

2010 SSAT PLENARY PRESENTATION

Image-Guided Stereotactic Radiosurgery for Locally Advanced Pancreatic Adenocarcinoma Results of First 85 Patients

Mukund S. Didolkar · Cardella W. Coleman · Mark J. Brenner · Kyo U. Chu · Nicole Olexa · Elizabeth Stanwyck · Airong Yu · Nagaraj Neerchal · Stuart Rabinowitz

Received: 3 May 2010 / Accepted: 9 August 2010 / Published online: 14 September 2010

Abstract
Background Locally advanced unresectable pancreatic adenocarcinoma is characterized by poor survival despite chemotherapy and conventional radiation therapy (RT). Recent advances in real-time image-guided stereotactic radiosurgery (SRS) have made it possible to treat these cancers in two to four fractions followed by systemic chemotherapy.
Aims The aims of this study includes the following: (1) obtain local control of the disease; (2) improve the survival of these unresectable patients; (3) evaluate the toxicity of SRS; and (4) report results of the largest series from a single center.
Methods Pancreatic SRS involves delivery of high doses of accurately targeted radiation given non-invasively in two to four fractions. We treated 85 consecutive patients with locally advanced and recurrent pancreatic adenocarcinoma from February 2004 to November 2009. Age range: 36–88 years, median 66 years; sex: 50 males, 35 females; race: 79 Caucasian, five African American, one Asian; histology: 80 adenocarcinoma, three islet cell, two other. Pre-SRS staging: T_{3-4} 85; N_+ 16, N_x 57, N_0 12; M_0 64, M_1 21. All patients were unresectable at the time of SRS. Seventy-one had no prior surgical resection, and 14 had local recurrence after prior surgical resection. Twenty-nine patients had progression of disease after prior conventional RT. Location of the tumor: head, 57; body and tail, 28. Pre-SRS chemotherapy was given in 48 patients. All patients received gemcitabine-based chemotherapy regimen after SRS. Median tumor volume was 60 cm^3. PET/CT scans done in 55 patients were positive in 52 and negative in three patients. Average maximum standard uptake value was 6.9. Pain score on a scale of 1–10 was: 0–3 in 54, 4–7 in 18, and 8–10 in 13 patients. SRS doses ranged from 15 to 30 Gy with a mean dose of 25.5 Gy delivered in 3 days divided in equal fractions. Mean conformality index was 1.6, and mean isodose line was 80%.
Results Tumor control: complete, partial, and stable disease were observed in 78 patients for the duration of 3–36 months with median of 8 months. Pain relief was noted in majority of patients lasting for 18–24 weeks. Most of the patients died of distant disease progression while their primary tumor was controlled. Overall median survival from diagnosis was 18.6 months and from SRS it was 8.65 months. For the group of 35 patients with adenocarcinoma without prior surgical

Presented at the 51st Annual Meeting of the Society for Surgery of the Alimentary Tract in New Orleans, 7 May 2010.

M. S. Didolkar (✉) · K. U. Chu · N. Olexa
Division of Surgical Oncology, Department of Surgery,
Sinai Hospital of Baltimore,
2401 W. Belvedere Avenue,
Baltimore, MD 21225, USA
e-mail: mdidolka@lifebridgehealth.org

C. W. Coleman · M. J. Brenner
Department of Radiation Oncology, Sinai Hospital of Baltimore,
Baltimore, USA

E. Stanwyck · A. Yu · N. Neerchal
Department of Mathematics and Statistics,
University of Maryland at Baltimore County Campus,
Baltimore, MD, USA

S. Rabinowitz
Department of Radiology, Sinai Hospital of Baltimore,
Baltimore, USA

resection or RT and no distant metastases, the average and 1-year survival from diagnosis was 15 months and 50%, respectively, and from SRS it was 11.15 months and 30.5%, respectively.
Toxicity A total of 19 (22.37%) patients developed grades III/IV GI toxicity including duodenitis, 12 (14.1%); gastritis, 11 (12.9%); diarrhea, three (3.5%); and renal failure was noted in one (1.2%). Three patient had both gastritis and duodenitis. Toxicity was significantly more prevalent in the first 40 patients compared with the last 45 patients (32.5 vs 13.9%).
Conclusions SRS for unresectable pancreatic carcinoma can be delivered in three fractions with minimal morbidity and a local tumor control rate of 91.7%. The survival is comparable or better than the reported results for advanced pancreatic cancer, specifically for the group of previously untreated patients with unresectable tumors. Development of distant metastases remains a significant factor.

Keywords Locally advanced pancreatic carcinoma · Stereotactic radiosurgery

Introduction

Pancreatic cancer is the second most common gastrointestinal malignancy and although it is the ninth most common cancer amongst all sites, it is the fourth leading cause of cancer deaths in the USA. In 2009, it is estimated that 42,470 people developed pancreatic cancer and 35,240 died from it.[1] Pancreatic cancer carries a grave prognosis with overall 1- and 5-year survival rates of 24% and 5%, respectively. Moreover, only 7% of cases are diagnosed at an early stage and only 15% to 20% of patients have resectable disease at diagnosis. Approximately 30–40% have locally advanced unresectable tumor and 40% have metastatic disease.[2,3]

The median survival of locally advanced pancreatic cancer remains 6–11 months in the majority of prospective clinical trials despite advances in chemotherapy, radiation therapy (RT) and chemo-radiation therapy (CRT) in the last two decades.[4–11] Improvement in relief of pain and quality of life remains a great problem.

In the last two decades, a few noteworthy improvements in chemotherapy, RT and a combination of CRT have made only a very modest impact on the overall prognosis. Gemcitabine-based chemotherapy has improved response rate and survival.[12] The addition of erlotinib to gemcitabine made a very mild improvement in response rate and survival.[13] Many clinical trials of concomitant CRT showed improvement over RT or chemotherapy alone.[4,5,7] Few studies showed adverse or no beneficial effect of CRT versus chemotherapy alone.[8,14]

All previous trials used conventional RT along with either 5-FU or gemcitabine-based chemotherapy. Improvements in conventional RT were possible because of advances in computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). Now, 3D conformal radiation therapy is the standard way of delivery for RT. Intensity-modulated radiation therapy (IMRT) has also impacted the delivery of megavoltage photon-based therapy by concentrating on the tumor target and sparing surrounding normal tissues.

In the last 5 years, further improvement in the precise delivery of high dose RT to the tumor was made possible with the development of real-time image-guided stereotactic radiosurgery (SRS). It enables a biologically larger dosage of radiation in one to three fractions as opposed to 30 to 40 fractions used in conventional methods of delivery.[15–18] With sub-millimeter accuracy of delivery of RT, the maximum dose could be delivered at the target with minimal dose to adjacent critical structures thus achieving the best therapeutic ratio.

We treated 85 patients with locally advanced or recurrent unresectable pancreatic cancer by SRS and chemotherapy with the following aims: (1) To obtain local control of the disease. (2) To improve the survival of the unresectable pancreatic cancer patients. (3) To evaluate the toxicity of SRS. (4) To compare our results with the results of other prospective studies with conventional CRT.

Materials and Methods

From 2 March 2004 to 11 May 2009, a total of 85 patients with biopsy proven locally advanced pancreatic cancer were treated with SRS at our center. Pre-SRS evaluation in all patients included complete history and physical, Karnofsky performance score, complete metabolic panel (CMP), CA19-9, and pain score recorded on severity of pain from 0 to 10. Pre-SRS tumor staging was done by triphasic or biphasic CT in all patients and by PET/CT in the latter 55 patients. All primary or recurrent tumors were unresectable by conventional criteria: (a) visceral arterial encasement, (b) extrapancreatic retroperitoneal tumor extension near aorta or vena cava, and/or (c) complete obliteration of portal or superior mesenteric vein. Age range of patients was from 36 to 88 years with the median age of 66 years. Fifty patients (58.8%) were males and 35 (41.2%) were females. Racial distribution was: Caucasian, 79; African American, five; and Asian, one. Tumor location was in the head 57 (67%) and body/tail of the pancreas in 28 (33%). Histology of tumor was adenocarcinoma in 80 (94.12%) neuroendocrine/islet cell carcinoma in three (3.53%) and other histologies in two (2.35%) patients.

Fourteen (16.5%) patients had locally recurrent (unresectable) tumor after previous surgical resection (Whipple procedure or distal pancreatectomy). Seventy-one patients (83.5%) had no prior surgical resection. Fifty-six patients (65.9%) had no prior radiation therapy. Prior conventional RT was given in 29 patients (34.1%) and they had local progression of tumor at the time of SRS. Fourteen of this group had locally recurrent disease after surgical resection and adjuvant CRT; and remaining 15 had local progression after prior conventional CRT. The range of conventional RT dose delivered prior to SRS was 36–60 Gy (median 50 Gy). Forty-eight patients (56.5%) received prior chemotherapy for their disease and they had local progression of disease prior to SRS. None of the patients received pre-SRS chemotherapy for radiosensitizing purposes.

Tumor staging at diagnosis and pre-SRS time is given in Table 1. For pre-SRS T category, all patients were surgically unresectable. The largest single tumor diameter measured by CT ranged from 1.2 to 10 cm with a median diameter of 4 cm and mean of 4.3 cm. The majority of the patients were staged N_x as CT, PET/CT or endoscopic ultrasound (EUS) could not identify nodal metastasis with certainty. Twenty-one patients who had distant metastasis were given SRS for large symptomatic pancreatic tumors. Most of these patients had severe pain and their distant metastatic disease was controlled by systemic chemotherapy.

Pain was evaluated on the scale of 0 to 10. Pre-SRS evaluation of pain showed no pain to mild pain (pain score 0–3) in 54, moderate pain (pain score 4–7) in 18, and severe pain (pain score 8–10) in 13 patients. Pre-SRS score of general performance as measured by Karnofsky method was less than 80% in 14 patients and more than 80% in 71 patients.

Pre-SRS PET/CT was positive in 52 patients and negative in three. Thirty patients in the study, mostly in the initial period did not get a PET/CT scan. Pre-SRS maximum standard uptake value (SUV) ranged from 2 to 21 with a median of 6.0 and mean of 6.9. Pre-SRS values of CA19-9 in 65 patients with adenocarcinoma ranged from two to 38,975 units (median, 234 units).

Table 1 Stage of the disease

	At diagnosis TNM	Pre-SRS TNM or rTNM
T_1	1	0
T_2	2	0
T_3	22	18
T_4	60	67
N_0	13	12
N_1	20	16
N_x	52	57
M_0	66	64
M_1	19	21

Post-SRS follow-up was done in all patients every 8–12 weeks with complete physical examination, CMP and CA19-9. CT scans were obtained every 8–12 weeks and in the latter 42 patients, PET/CT scans were obtained every 12–18 weeks. Of the 55 patients who had pre-SRS PET/CT for planning purposes, we could obtain post-SRS PET/CT in only 42 patients because either they had distant progression or we were unable to obtain studies because of insurance limitation.

All patients had post-SRS chemotherapy within 3–4 weeks after SRS. The chemotherapy regimen included gemcitabine alone or gemcitabine with erlotinib, taxol, xeloda, and bevacizumab. Post-SRS chemotherapy decisions were made by their medical oncologists. Toxicity was recorded as per NCI guidelines.[19] Grades III and IV toxicity was correlated to tumor volume, prior RT, surgery, or chemotherapy and to early or late time periods of when the SRS was administered.

Response Evaluation

Response to SRS was recorded after every evaluation by CT in all and PET/CT in the latter 42 patients. Response Evaluation Criteria in Solid Tumors (RECIST) criteria were used for response evaluation.[20] We modified RECIST criteria of response by utilizing PET/CT scans in evaluation. Tissue reaction producing fibrosis at the tumor site frequently made it impossible to measure complete or partial disappearance of the tumor on CT while PET/CT has been shown to be able to differentiate fibrosis from residual viable malignancy with 18F-FDG (fluorodeoxyglucose). A complete response (CR) was the disappearance of the primary tumor by CT scan and in the patients who had PET/CT, no significant uptake in the tumor bed. A partial response (PR) was defined as at least 30% decrease in the largest diameter of the tumor and reduction in maximum SUV value. Stable disease (SD) was defined as less than 30% decrease in the largest diameter of the tumor or less than 20% increase in largest tumor diameter and no increase in the maximum SUV on PET/CT. Progression of disease was defined as more than 20% increase in the largest diameter of the tumor and increase in the maximum SUV. Local progression free response (local tumor control) included all patients with CR, PR, and SD.

SRS Technical Consideration

The SRS system (CyberKnife®) is a frameless, image-guided RT system that has a 6-megavolt linear accelerator mounted on a robotic arm with 6° of freedom. The imaging system is composed of two diagnostic orthogonal X-ray

sources on the ceiling paired with amorphous silicon detectors that capture digital radiographic images of the patient in real-time. It is capable of delivering a high dose of radiation with 0.12 mm accuracy. It delivers unhindered non-coplanar treatment to pancreatic tumors through 150–200 uniquely angled beams per fraction. It requires gold fiducials implanted in the tumor to track the delivery of these beams.

One to 2 weeks before the SRS, five gold fiducials were implanted in and around the pancreatic tumor 2–5 cm apart and in three different planes. For fiducial placement, in addition to the tumor site, other preferred sites were the psoas muscle, crus of the diaphragm, periosteum of the vertebral body, and the laminae. Dilated distal pancreatic duct and vessels were avoided. The fiducial placement procedure was performed by the interventional radiologist either under CT guidance or by the surgeon during laparatomy for attempted resection or biliary bypass. In cases where no extra tumoral (spine) fiducials were placed, we used XSight™ (Accuray Incorporated, Sunnyvale, CA), a spine tracking algorithm to establish 3D rotational orientation. The accuracy of XSight™ System is comparable to that of the fiducial tracking method for precision SRS delivery.[21]

After allowing the implanted fiducials to settle, each patient was imaged using a CT with 1.5-mm slice thickness with the patient in an immobilized position accomplished by a custom-made Vac-Loc device (Bionix Radiation Therapy, Toledo, Ohio); oral and IV contrast were always used for delineation of surrounding critical structures, except in patients allergic to IV contrast. In the latter 55 patients, PET/CT scans were done at the same time. Fusion images of CT and PET/CT scan were used for 3D reconstruction and planning. The resulting CT volume was used in the treatment planning and creation of the normal tissue constraints through contouring the tumor and adjacent critical structures. The critical structures contoured were the duodenum, stomach, liver, kidneys and spinal cord. The gross tumor volume (GTV) and the surrounding organs including the liver, stomach, spinal cord and both kidneys were contoured jointly by the surgical and radiation oncologists. The GTV included the volume that was identifiable on the planning CT and PET/CT, unless additional information was available through intraoperative or EUS sources. The size of the GTV ranged from 9.8 – 223.3 cm^3 with a median of 59.7 cm^3 and mean of 70.74 cm^3. The planning treatment volume (PTV) included the GTV and a 3 mm margin around the tumor margin. The dose to critical structures was limited to known tolerance levels for at least 90% of the volume of the respective organs (Figs. 1, 2, 3, and 4).

A total dose of 15–30 Gy (median, 25.5 Gy) was prescribed to a median 80% isodose line (range, 75% to 88%) in one to four fractions (mean, three fractions). During the treatment, the patient was allowed to breathe freely and the motion of the target volume was tracked by Synchrony® Respiratory Tracking System (Accuray Incorporated Sunnyvale CA), in most of the patients. Synchrony® uses a correlation algorithm to generate a model of the motion of the internal fiducials and external light emitting diodes placed on the patient's chest.[22] This model algorithm was generated right before the initiation of the treatment and updated throughout the treatment each time an X-ray image was acquired.

Statistical Methods

Patient data was entered in Microsoft Access® data base retrospectively and prospectively. SAS 9.2 program was used for computing. The Kaplan–Meier Estimate (product-limit estimate) method was used for survival data.[23] For calculating the p values, non-parametric methods used were log-rank test and Wilcoxon test. Chi-square test was used to detect the association between categorical variables. Survival graphs were created by software R (2.10.1) program developed by Bell Labs.

Results

Tumor Control and Duration

Local tumor control (CR+PR+SD) was obtained in 78 (91.7%) patients. Of these 78 patients, ten (11.8%) had CR, 27 (31.7%) had PR, and 41 (48.2%) had SD. The duration of response was from 3 to 36 months with the median of 8 months. Amongst the local progression free group of patients, most developed distant metastases while their local disease was under control. Five patients had progression of local disease at 1, 8, 12 16, and 25.8 months. Two patients did not get follow-up imaging studies or they were lost to follow-up.

Of the 42 patients who had both pre- and post-SRS PET/CT, 10 showed no appreciable uptake on post treatment scans and 32 demonstrated mild uptake in the tumor. They had a minimal decrease in their SUV values at post-SRS evaluation. Mean and median pre- versus post-SRS SUV values were: 6.9 and 6 (SD±4.3) versus 4.5 and 4 (SD±2.92), respectively, p= NS. Those patients who had CR by PET/CT never showed complete disappearance of the tumor by CT evaluation, suggesting the residual density on the CT represented fibrotic reaction (Figs. 5, 6, 7). The 46 patients with adenocarcinoma and M_0 disease who had both pre- and post-SRS, CA19-9 evaluation showed improvement in post-SRS CA19-9 value. Median and mean values for pre- versus post-SRS were: 245 and 2,172 (SD−6,459) versus 138 and 1,124 (SD±2,191.6), respectively, p=NS.

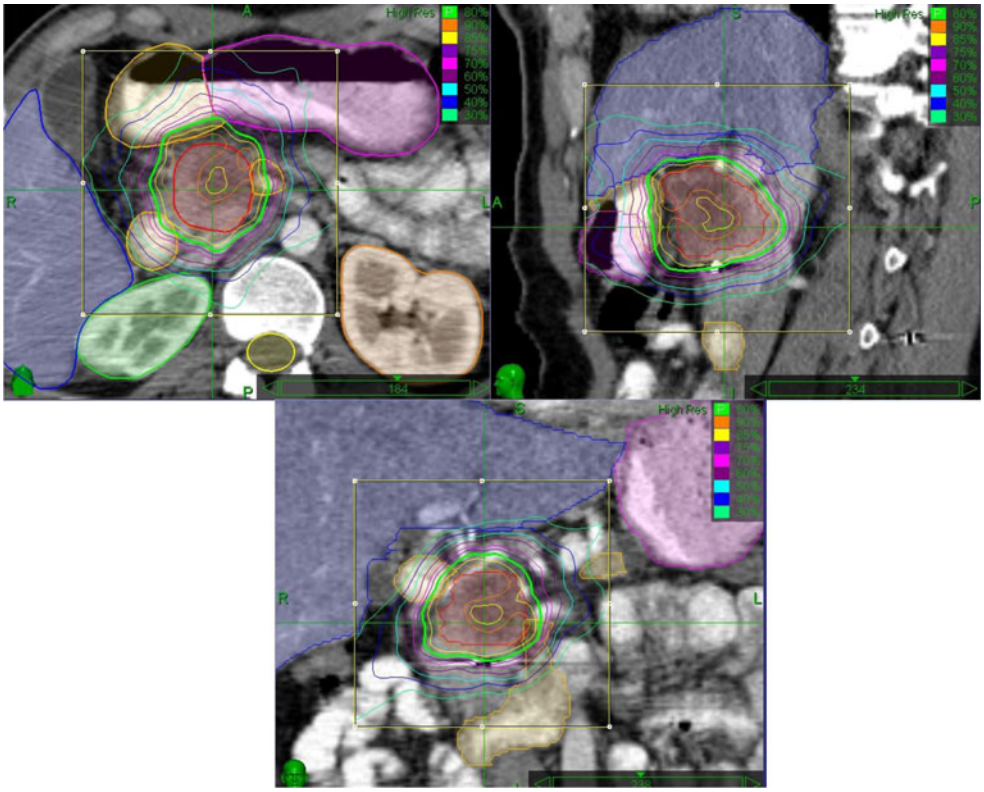

Fig. 1 Contouring of pancreatic head carcinoma and adjacent critical organs viz. stomach, duodenum, kidneys, liver and spinal cord. (Axial, sagittal, and coronal views)

Distant Disease Progression

Distant progression of disease was seen in 65 patients including those who had distant metastases prior to SRS. Distant progression of disease occurred from 1 to 41 months with the median time interval of 91 days. Distant disease progression occurred at multiple sites. Most common sites were peritoneum, liver, lymph nodes and lung.

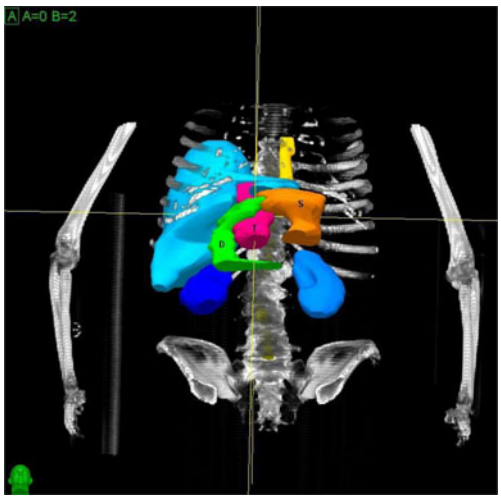

Fig. 2 3D construction of contoured tumor and critical organs tumor (*T*), stomach (*S*), and duodenum (*D*)

Pain Control

Patients who had severe pain (score of 8–10) had relief of pain to a much lower scale and the duration of relief was up to 24 weeks from SRS. Patients who had moderate pain (score 4–7) had relief of pain lasting for 18-week period (Fig. 8).

Of the 31 patients who had pain score of more than 4, 15 had complete relief of pain lasting for more than 6 months. The remaining 16 patients had relief of pain to lower scores after SRS compared with pre-SRS pain scores.

Toxicity

A total of 19 (22.3%) patients developed multiple grades III or IV gastrointestinal toxicities. Duodenitis was seen in 12 (14.1%), gastritis in 11 (12.9%), and diarrhea in three (3.5%) patients. Of the total 19 patients who had upper GI tract toxicity, three had both gastritis and duodenitis. Furthermore, of the 12 patients who had duodenitis within 6 weeks of SRS, seven had late duodenitis as well. It resulted frequently in upper GI hemorrhage or duodenal obstruction. Tumor recurrence was seen in two patients with late duodenal toxicity.

Diarrhea was more related to post-SRS chemotherapy started within 3–4 weeks of SRS. Renal toxicity was not related to radiation to the kidneys but to deteriorating general condition with peritoneal implants and ascitis.

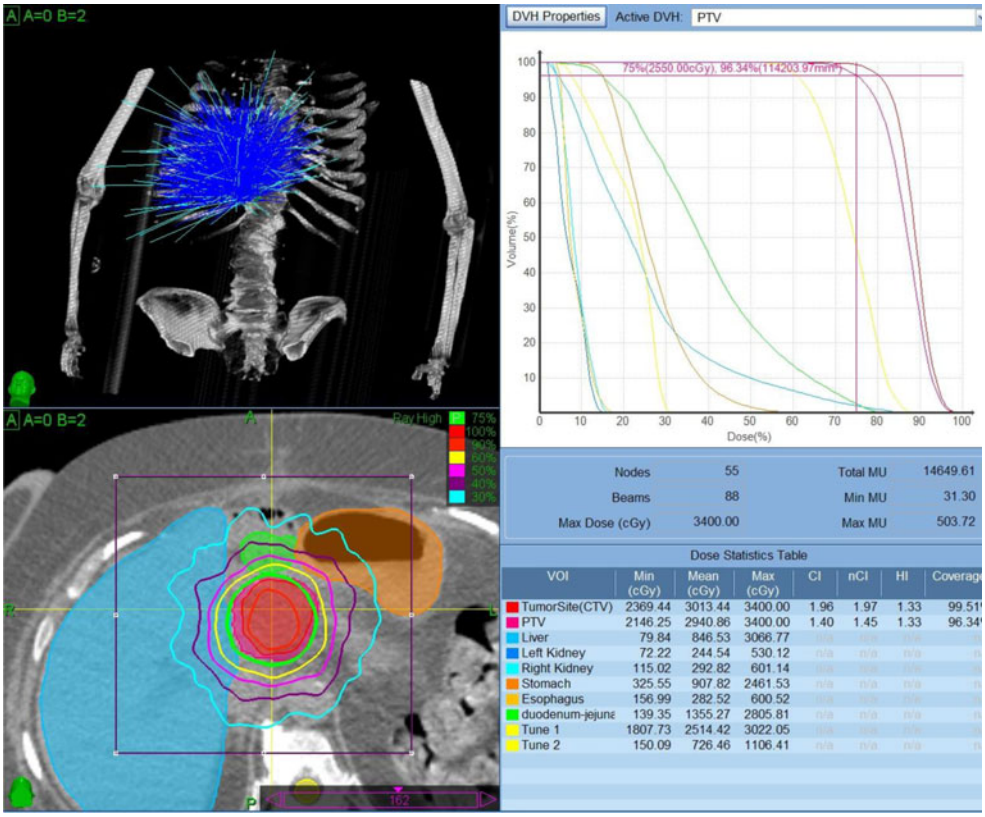

Fig. 3 *Left panel*, SRS treatment plan; *right upper panel*, dose volume histogram (*DVH*); *right lower panel*, dose distribution to critical structures

GI toxicity was correlated to prior RT, prior surgical resection, GTV and first 40 patients versus last 43 patients. Two patients could not be evaluated for toxicity because of noncompliance in follow-up. Statistically significant correlation of GI toxicity was noted in patients treated in early years versus latter years of the study period (Table 2).

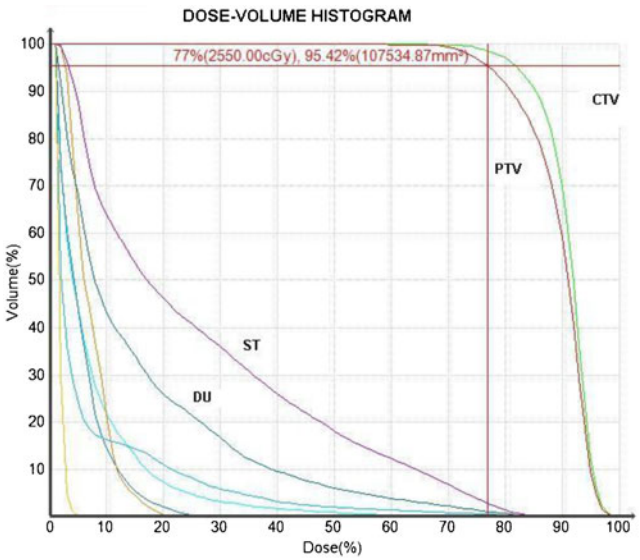

Fig. 4 Dose volume histogram. *CTV* clinical target volume, *PTV* planning target volume, *ST* stomach, *DU* duodenum

One patient died 3 weeks after SRS treatment. The cause of death was sepsis and ascitis. The patient was on chemotherapy after SRS. We do not think the cause of death was from SRS treatment.

Surgical intervention was not needed in these patients when they developed GI toxicity. Most of these patients were treated with conservative medical management. Few patients needed duodenal stent for obstruction from progression of tumor 5–6 months after SRS.

Survival

At the end of the study period 13 patients are alive with disease, two of these having no disease progression. Sixty-one patients died of disease, nine died of other causes (sepsis, neutropenia, cardiac, or lung problems), and two patients were lost to follow-up.

Overall survival from the diagnosis in all 85 patients ranged from 6 weeks to 48 months with the mean of 22.9 months and median of 18.6 months. Mean and median overall survival from the first treatment of SRS was 13.24 and 8.65 months (Fig. 9). The survival was correlated to many factors. Median survival for patients with carcinoma in the body and tail was slightly higher than for the head of pancreas 13 versus 11.2 months but p value was not significant.

Mean survival of patients without distant disease progression was statistically better than those who had

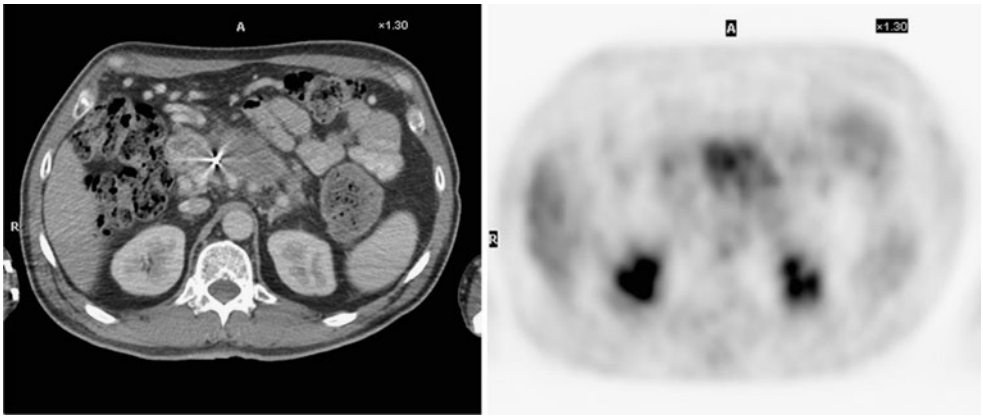

Fig. 5 Pre-SRS CT and PET/CT showing tumor at the proximal body of pancreas

distant disease progression. Median survival for patients without distant disease progression although not reached is more than 18 versus 11.56 months in patients with distant disease progression (Fig. 10).

Post-SRS mean survival for patients who had no prior RT was better but not statistically significant than those who had prior RT (15 vs 9.21 months, Fig. 11). Prior RT did not affect survival either from diagnosis or from SRS. From the time of diagnosis, a trend of better survival was seen in the first 18–20 months in RT group because 14 patients in that group had surgical resection followed by adjuvant CRT (Fig. 11).

Most importantly, the estimated survival for the group of patients with adenocarcinoma only but without prior surgical resection, or RT or presence of distant metastases at the time of diagnosis and SRS is shown in (Fig. 12). The median, mean and 1 year survival from diagnosis was 13.4, 15.04 months (range, 2.2–30 months), and 50%; while the survival figures from the first SRS treatment were: 8.65, 11.15 months (range, 1–28.2 months), and 30.5%.

Characteristics of 49 patients who survived less than 1 year after SRS were compared with those 28 patients who survived for more than 1 year. Patients who died of other causes were excluded from the analysis.

These two groups were analyzed for tumor volume, age, gender, percentage of isodose, prior RT, histology, and PET CT scan results. No statistically significant difference was found in these two groups. A trend was seen for larger tumor volume in short survivor group compared with long survivor group (62 vs 46 cm^3).

Survival was further analyzed for those patients who became PET/CT negative. Median survival for the 15 patients who became PET/CT negative was 17 months. This compares well with the overall median survival of the 8.6 months for the entire group.

Discussion

Pancreatic adenocarcinoma carries a grave prognosis. It ranks at or near the bottom of the list of all cancers in relation to patient survival from diagnosis. Resection of the tumor by pancreatico-duodenectomy or distal pancreatectomy is the only proven method to achieve improved survival. Very few patients are resectable at diagnosis. From 1985 to 1995 in the report from the National Cancer database, only 9% patients at the time of diagnosis out of 100,313 patients had surgical resection.[24]

In the last 25 years, even in resected pancreatic cancers, the survival reported in 5 large prospective randomized trials has not improved much.[25–29] In these trials median survival for the patients receiving adjuvant CRT or chemotherapy only ranged from 16.9 to 22.1 months. In fact, from 1985 to 2008 despite better RT methods and use

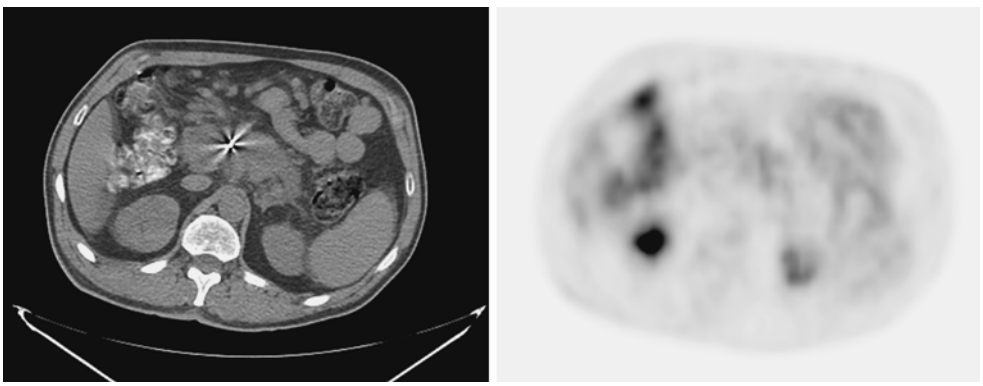

Fig. 6 Post-SRS CT and PET/CT showing complete response by PET/CT (*right panel*) but no complete disappearance of tumor by CT (*left panel*)

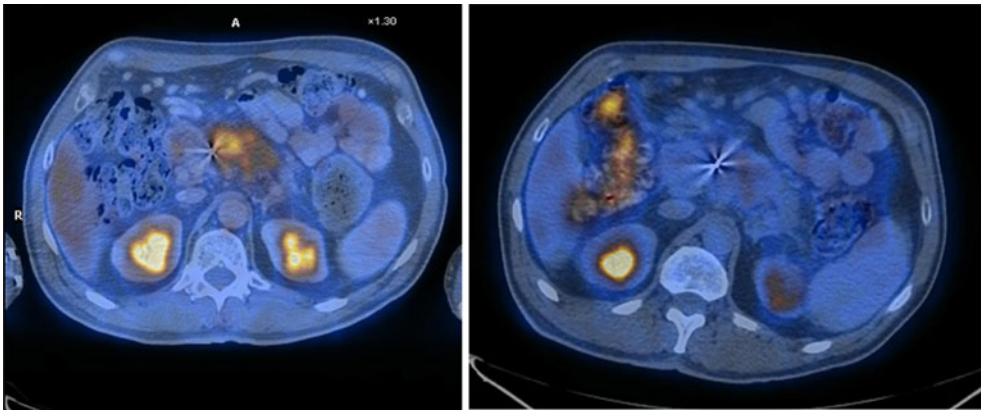

Fig. 7 Pre-SRS PET/CT fusion images showing active tumor at the proximal body of pancreas (*left panel*); post-SRS PET/CT fusion images showing no activity of ^{18} F-FDG in tumor but no disappearance of tumor on CT images

of gemcitabine in the last decade, the median survival remained essentially unchanged from 20–22 months. Local recurrence rates were high, from 23% to 51% after resection and distant metastasis rate was also very high 50% to 77%.

If such is the prognosis in resected patients, then the prognosis for unresectable pancreatic cancer is more discouraging. Almost 30% to 40% patients have locally advanced pancreatic cancer.[30,31] They are not only unresectable, but frequently a majority of these patients will have micrometastases undetected by present available imaging techniques including PET/CT. Furthermore, many of these patients are symptomatic because of invasion of the visceral nerves and adjacent viscera. Median and 1 year survival for these patients is reported to be 7.2 months and 27%.[2,3]

Before 1981, these patients were treated either by chemotherapy or conventional RT and palliative procedures without any impact on survival. Since the initial reports in 1981 and 1985 by the Gastrointestinal Tumor Study Group of improved results by using of combined modality of treatment, chemotherapy and radiation therapy followed by chemotherapy, this approach has become standard not only in pancreatic but practically in all GI cancers.[4,6] The standard chemotherapeutic agents used were 5-FU mainly and for radiation therapy conventional super voltage radiation of 1.8 Gy given daily 5 days per week for 30–40 days with total dose of 40 to 60 Gy.

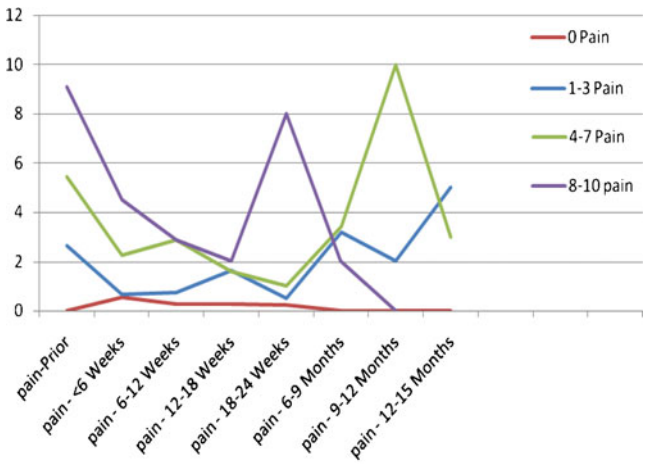

Fig. 8 Pre- and post-SRS mean pain score

Improvement in systemic chemotherapy with the use of gemcitabine over 5-FU made a positive impact in progression free and overall survival, and betterment of disease related symptoms.[12] The addition of other chemotherapy drugs, platinum agents (cisplatin, oxaliplatin), irinotecan, capcitabine, and anitfoliates (pemetrexed) to gemcitabine has made little improvement over gemcitabine alone.[32–34] Lastly, for systemic chemotherapy, the addition of erlotinib, an epidermal growth factor receptor inhibitor, has shown a very small but statistically significant survival advantage over gemcitabine alone.[13]

In the last 15 years, parallel to the progress in systemic chemo/molecular therapy in advanced pancreatic cancer, radiation therapy has made tremendous progress in achieving maximum therapeutic ratio with minimal dose to adjacent normal structures. 3D conformal RT has become standard. Innovations in imaging techniques, CT, MRI, and PET/CT made it easy to plan and deliver IMRT.

With the recent advances in stereotactic image-guided technology, including real-time image guidance, now it is possible to deliver high doses of radiation therapy with sub-millimeter accuracy in non-CNS body tumors. Although retroperitoneally located, the movements of the pancreas with each respiration cycle are considerable, ranging from 1.1 to 2 cm in different direction.[35] Synchrony®, which utilizes respiratory gating technology, can account for such movements thereby delivering the high dose to the target without much radiation exposure to adjacent viscera. SRS can deliver 25.5 Gy dose in 1 day. This will be a biologically equivalent dose of 85.5 Gy. To deliver 87.5 Gy by conventional RT, it would take 41 days at a daily dose of 1.8 Gy. Similarly, in our study, a 25.5 Gy dose given in three fractions is biologically equivalent to 47.2 Gy. Delivery of a dose of 47.2 Gy dose by conventional method would require 22 fractions of a 1.8 Gy daily dose given over 4 to 5 weeks.[36,37]

Table 2 Toxicity table

Factor		No.	GI toxicity	%	*p* value
GTV in cm^3	<80	54	14	25.9	NS
	>81	29	5	17.2	NS
Prior surgical resection	Yes	14	2	14.3	NS
	No	69	17	24.6	
Prior RT	Yes	29	6	20.7	NS
	No	54	13	24	
Prior Chemo	Yes	47	10	21.3	NS
	No	36	9	25	
Year of Treatment	2004–2005	40	13	32.5	0.04
	2006–2009	43	6	13.9	
Total		83	19	22.3	–

GTV gross tumor volume

Intra-operative radiation therapy (IORT) for unresectable and resected patients with pancreatic cancer did not confer any survival benefit in both randomized trials.[38,39] Compared with IORT, SRS can deliver more dose in one fraction without elaborate operating room RT set up.

For any modality of therapy to succeed in locally advanced unresectable pancreatic cancer, it must address two major points:

a. Since most patients develop metastatic disease an effective systemic treatment of micrometastases is most important.

b. Since uncontrolled local disease causes excruciating pain, deterioration of quality of life, duodenal obstruction and bleeding, an effective local modality of treatment delivered in a short time period with minimal toxicity is equally important.

This study cannot address the first major point but it can address the second point. Local progression of disease after chemo-radiation therapy has been reported from 42% to

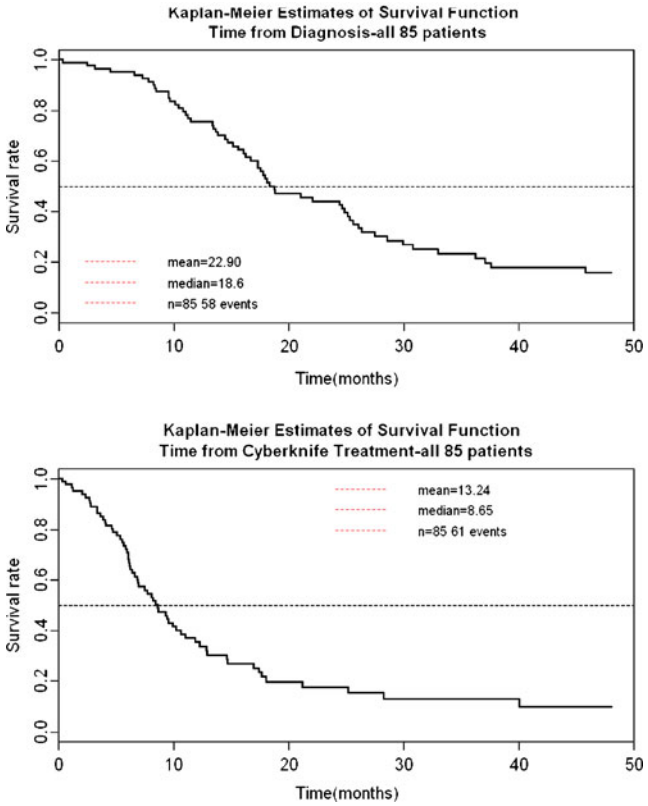

Fig. 9 Estimated overall survival of 85 patients from diagnosis and SRS

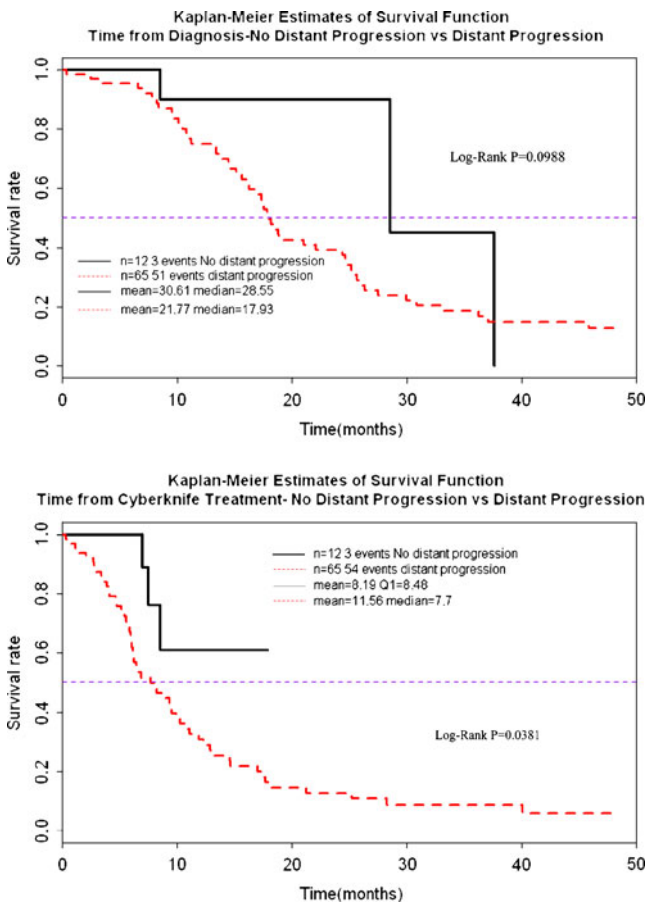

Fig. 10 Estimated survival of patients with and without distant disease progression Q1=median has not reached for patients with no distant progression

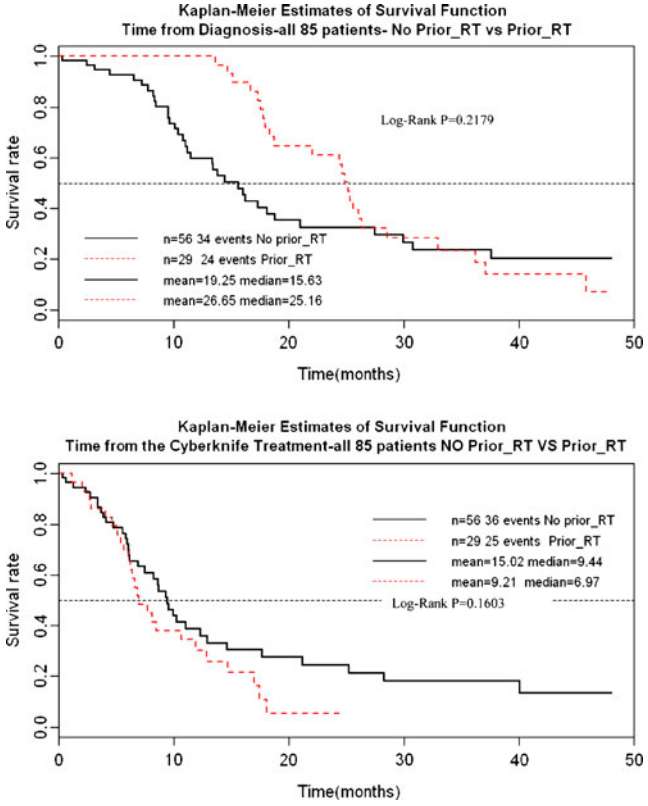

Fig. 11 Estimated survival of patients with prior RT versus no prior RT

62% in many prospective studies utilizing modern 3D conformal RT.[10,40–42] At the time of local progression, almost equal number of these patients will also have metastatic disease as well. SRS has low rate of local progression. Previous studies at other institutions and one at our institution showed local progression rate to be very low from 7–22%.[15,18,43,44]

Although no complete responses were observed in the Stanford series and they have not reported on partial response after SRS, we observed complete response by PET/CT and CT in ten (11.8%) patients and partial response in 27 (31.7%) patients. Local tumor control (CR+PR+SD) was observed in 91.7% patients, which is comparable to the recent reports from other centers. The duration of local progression free response was comparable to that reported by others.[15,43,44]

Frequently, utilization of PET/CT added a new dimension to evaluate the response to cancer therapy. The most commonly used criteria for response evaluation is RECIST.[20] For intra-abdominal malignancy, CT scan is the most common imaging technology used for accurate measurement of tumor. On several occasions, we observed no disease on PET/CT imaging or considerable decrease in SUV after SRS, but CT showing no corresponding disappearance of tumor. CT showed either fibrosis or inflammatory changes making measurement of the tumor almost impossible. PET/CT has been shown to be an accurate means of assessing treatment response in many cancers, particularly lymphomas, where PET/CT can predict tumor response after one or two cycles of chemotherapy. It has been shown to be more predictive than CT. Our results suggest the same may be true for assessing treatment response following SRS. Additional studies would be helpful to quantify and confirm this.

Lasting control of the local disease can be obtained by SRS. In our series, the majority of patients developed distant metastatic disease, usually at multiple sites, while the primary tumor had no progression. Median time for duration of local response was 8 months in our series. Similar results have been reported by Stanford & Harvard Groups.[15,43]

Can we increase the response rate by increasing the dose of SRS to more than 25.5 Gy as administered in our series? Phase I study by Koong and associates on escalation dose of radiation therapy from 15 to 20 Gy and ultimately 25 Gy showed tolerable GI toxicity and excellent tumor control by the latter dose in the first 15 patients.[17] Since the progression free local control of disease is much higher than conventional RT, we doubt that an increase in RT doses by SRS will achieve additional responses, free of toxicity.

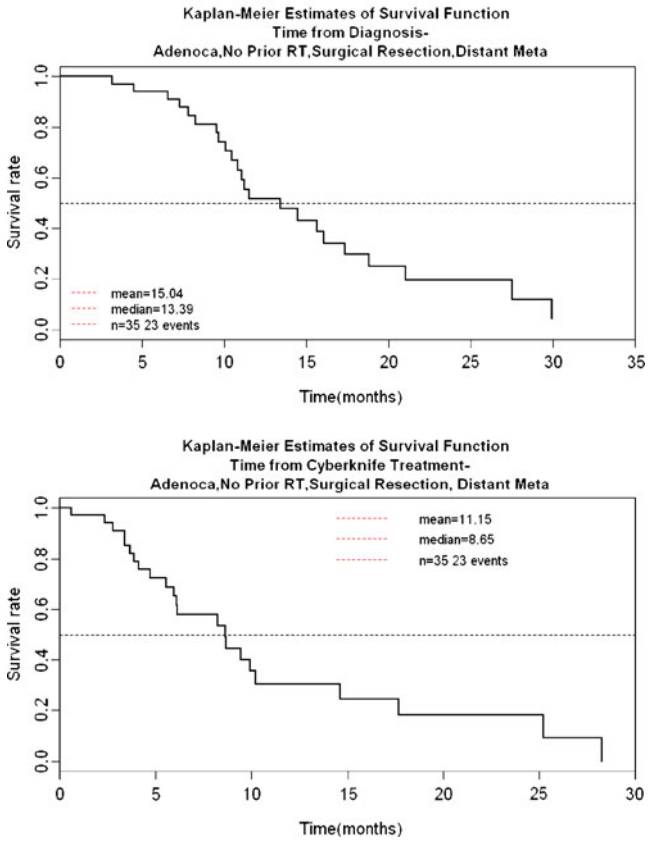

Fig. 12 Estimated survival of patients with adenocarcinoma only but who had no prior surgical resection, RT or distant metastasis

For the last 20 years, the vast majority of patients routinely received concomitant CRT. Gastrointestinal toxicity is the most common and the most debilitating side effect after CRT. Grades III and IV GI toxicity in the form of nausea, vomiting, gastritis, duodenitis and diarrhea ranged from 20 to 48.7% in recent prospective studies.[8–11,41] The toxicity increased when full dose of chemotherapy and multiple chemotherapeutic drugs were used concomitantly with RT. Additionally, local tumor response and survival remained unchanged compared with conventional CRT doses.[8,9,11]

In the present series, the most common toxicity was that of the gastrointestinal tract (22.3%). We correlated toxicity to multiple factors (Table 2). To our surprise, prior RT did not correlate to post-SRS GI toxicity. We thought that altered anatomic structures by prior surgery will affect proper contouring of the bowel resulting in increased GI toxicity, but in our series, prior surgery did not correlate with more GI toxicity. Similarly, volume of tumor which can affect exposure of adjacent GI structures did not correlate with more GI toxicity. The most important factor correlating the GI toxicity was the initial first 2 years period versus the latter 3 years of SRS delivery. The toxicity was statistically significant in patients treated in the first 2 years of the study period. We think two factors may have contributed to this difference. A better delineation of duodenum, stomach and small bowel in preplanning imaging studies in the latter period may have played the role. We did further analysis of duodenal exposure to RT, however, we found that the exposure of the duodenum to radiation was well below the range of the toxicity dose.

We think the other important factor in reducing GI toxicity in the latter period was the technical development in tracking respiratory motion by Synchrony® which was not used in first few patients. Although not perfect in tracking pancreatic tumor motion with respiratory movements, Synchrony® can help in tracking tumor motion in super-inferior (SI) and antero-posterior (AP) and left to right (L–R) direction. The mean pancreatic motion in one study was: SI direction 20.8 mm, L–R direction 11.3 mm, and in AP direction 13 mm.[35] Along with many other improvements, this single technical improvement distinguishes SRS from 3D conformal RT, IMRT, and IORT.

Up to 85% patients with advanced pancreatic cancer have severe pain.[45] Two randomized studies, one from Hopkins and the other from Mayo Clinic, showed beneficial effect of neurolytic celiac plexus block over narcotic administration only.[46,47] In our study, post-SRS symptomatic pain relief was uniformly seen in all patients having moderate and severe pain. Usually, relief of pain was noted in the first week of treatment and lasted for 18–24 weeks (Fig. 8).

Our study of the patients with locally advanced pancreatic cancer has a mixture of patients, some with distant metastasis and some with recurrence after prior surgical excision and some with prior RT, which makes inferences regarding survival difficult. However, we have isolated the group of patients with adenocarcinoma only without prior RT, surgical resection and distant metastasis before SRS. Their median and 1-year survival from diagnosis is better than the reported survival from the National Cancer database of 12,981 patients with stage III cancer.[3] Most of the patients in our study died of disease from distant metastasis. Despite improvements in chemotherapy and molecular based therapy, we cannot prevent or control distant micrometastases. In fact, in our study the group of patients with no progression of distant disease showed the highest survival rate compared with the group with progression of distant disease.

Since the local control cannot translate to the development of distant metastasis, the best strategy to improve the survival with any form of RT including SRS is to avoid patients who develop metastatic disease during induction chemotherapy courses prior to the delivery of SRS. SRS should be followed by systemic chemotherapy. This approach is suggested by studies from MD Anderson, and GERCOR (Groupe Coopérateur Multidisciplinaire en Oncologie) in Europe.[7,42] SRS has the advantage over conventional 3D-RT because it can be administered in 1 to 3 days, rather than the typical 5–6-week course of conventional RT. It has much less grades III and IV GI toxicity compared with concurrent CRT treatment. SRS delivers much larger biologically equivalent doses in the fewest fractions. Improvements are urgently needed in treatment of micrometastases which are often present in these patients. Local disease control in these unresectable locally advanced pancreatic cancer is much better with SRS than that of conventional CRT.

Acknowledgements Many thanks to the Mangione Family Foundation that provided some of the financial support with this study. We thank Philip Mone, Radiation Physicist, for technical data.

Discussant DR. MARK P. CALLERY (Boston, MA): Twenty-five of your 85 patients in your study were M1. What was the indication for CyberKnife radiotherapy in patients with metastases? Was it for pain control? And did these patients dilute your overall results?

Closing discussant DR. MUKUND S. DIDOLKAR: The main indication for SRS was pain in these patients. The other indication was the progression of the primary tumor in the presence of stable or responded (CR or PR) metastatic disease on chemotherapy. To answer the second question, it did affect the overall results because the survival for the group of patients without distant metastases was much higher than the survival of the patients with distant metastases.

References

1. American Cancer Society. Cancer Facts & Figures 2009 Atlanta: American Cancer Society; 2009.

2. Ries LAG, Young JL, Keel GE, Eisner MP, Lin YD, Horner M-J (eds). SEER Survival Monograph; Cancer Survival Among Adults: US SEER Program, 1988–2001, Patient and tumor characteristics. NCI, SEER Program, NIH Pub. No. 07-6215, Bethesda, MD, 2007.
3. Bilimoria KY, Bentrem DJ, Ko CY, et al. Validation of the 6th Edition AJCC Pancreatic Cancer Staging System Report from National Cancer Database. Cancer 2007;110:738–744.
4. Moertel CG. Frytak S. Hahn RG. O'Connell MJ. Reitemeier RJ. Rubin J. Schutt AJ. Weiland LH. Childs DS. Holbrook MA. Lavin PT. Livstone E. Spiro H. Knowlton A. Kalser M. Barkin J. Lessner H. Mann-Kaplan R. Ramming K. Douglas HO Jr. Thomas P. Nave H. Bateman J. Lokich J. Brooks J. Chaffey J. Corson JM. Zamcheck N. Novak JW. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads+5-fluorouracil), and high dose radiation+5-fluorouracil: The Gastrointestinal Tumor Study Group. Cancer 1981;48(8):1705–1710.
5. Gastrointestinal Tumor Study Group. Treatment of locally unresectable carcinoma of the pancreas: comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. Gastrointestinal Tumor Study Group. Journal of the National Cancer Institute 1988;80(10):751–755.
6. Gastrointestinal Tumor Study Group. Radiation therapy combined with Adriamycin or 5-fluorouracil for the treatment of locally unresectable pancreatic carcinoma. Gastrointestinal Tumor Study Group. Cancer 1985;56(11):2563–2568.
7. Huguet F. Andre T. Hammel P. Artru P. Balosso J. Selle F. Deniaud-Alexandre E. Ruszniewski P. Touboul E. Labianca R. de Gramont A. Louvet C. Impact of chemoradiotherapy after disease control with chemotherapy in locally advanced pancreatic adenocarcinoma in GERCOR phase II and III studies. Journal of Clinical Oncology 2007;25(3):326–331.
8. Chauffert B. Mornex F. Bonnetain F. Rougier P. Mariette C. Bouche O. Bosset JF. Aparicio T. Mineur L. Azzedine A. Hammel P. Butel J. Stremsdoerfer N. Maingon P. Bedenne L. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. Annals of Oncology 2008;19(9):1592–1599.
9. Wilkowski R. Boeck S. Ostermaier S. et al. Chemoradiotherapy with concurrent gemcitabine and cisplatin with or without sequential chemotherapy with gemcitabine/cisplatin vs chemoradiotherapy with concurrent 5-fluorouracil in patients with locally advanced pancreatic cancer-a multi-center randomized phase II study. Brit J of Cancer 2009;101:1853–1859.
10. Haddock MG. Swaminathan R. Foster NR. Hauge MD. Martenson JA. Camoriano JK. Stella PJ. Tenglin RC. Schaefer PL. Moore DF Jr. Alberts SR. Gemcitabine, cisplatin, and radiotherapy for patients with locally advanced pancreatic adenocarcinoma: results of the North Central Cancer Treatment Group Phase II Study N9942. Journal of Clinical Oncology 2007;25(18):2567–2572.
11. Small W Jr. Berlin J. Freedman GM. Lawrence T. Talamonti MS. Mulcahy MF. Chakravarthy AB. Konski AA. Zalupski MM. Philip PA. Kinsella TJ. Merchant NB. Hoffman JP. Benson AB. Nicol S. Xu RM. Gill JF. McGinn CJ. Full-dose gemcitabine with concurrent radiation therapy in patients with nonmetastatic pancreatic cancer: a multicenter phase II trial. Journal of Clinical Oncology 2008;26(6):942–947.
12. Burris HA. Moore MJ. Andersen J. Green MR. Rothenberg ML. Modiano MR. Cripps MC. Portenoy RK. Storniolo AM. Tarassoff P. Nelson R. Dorr FA. Stephens CD. Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. Journal of Clinical Oncology 1997;15(6):2403–2413.
13. Moore MJ. Goldtein D. Hamm J. et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer. A phase III trial of National Cancer Institute of Canada Clinical Trial Group. J. Clin Oncol 2007;25:1960–1966.
14. Klaassen DJ. MacIntyre JM. Catton GE. Engstrom PF. Moertel CG. Treatment of locally unresectable cancer of the stomach and pancreas: a randomized comparison of 5-fluorouracil alone with radiation plus concurrent and maintenance 5-fluorouracil-an Eastern Cooperative Oncology Group study. Journal of Clinical Oncology 1985;3(3):373–378.
15. Chang DT. Schlenberg D. Shen J. et al. Stereotactic Radiotherapy for unresectable Adenocarcinoma of the pancreas. Cancer 2009;115:665–672.
16. Koong AC. Christofferson E. Le QT. Goodman KA. Ho A. Kuo T. Ford JM. Fisher GA. Greco R. Norton J. Yang GP. Phase II study to assess the efficacy of conventionally fractionated radiotherapy followed by a stereotactic radiosurgery boost in patients with locally advanced pancreatic cancer. International Journal of Radiation Oncology, Biology, Physics. 2005;63(2):320–323.
17. Koong AC. Le QT. Ho A. Fong B. Fisher G. Cho C. Ford J. Poen J. Gibbs IC. Mehta VK. Kee S. Trueblood W. Yang G. Bastidas JA. Phase I study of stereotactic radiosurgery in patients with locally advanced pancreatic cancer. International Journal of Radiation Oncology, Biology, Physics 2004;58(4):1017–1021.
18. Hoffelt C. & Didolkar M. Stereotactic radiosurgery for unresectable adenocarcinoma of pancreas. Initial experience at Sinai Hospital of Baltimore. In: Urschel HC (ed) Robotic radiosurgery: treating tumors that move with respiration. Springer, Berlin 2007. pp. 177–192.
19. National Cancer Institute common toxicity criteria version 2.0. Available at: http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf. Accessed 18 Jan 2010.
20. Therasse P. Arbuck SG. Eisenhauer EA. Wanders J. Kaplan RS. Rubinstein L. Verweij J. Van Glabbeke M. Van Oosterom AT. Christian MC. Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. Journal of the National Cancer Institute 2000;92(3):205–216.
21. Fu D. Kahn R. Wang B. et al. Xsight Lung tracking system: a fiducial-less method for respiratory motion tracking. In: Urschel HC (ed) Treating tumors that move with respiration. Springer, Berlin 2007. pp. 265–282.
22. Sayeh S. Wang J. Main WR. Kilby W. Maurer CR: Respiratory motion tracking for robotic radiology. In: Urschel HC (ed) Treating tumors that move with respiration. Springer, Berlin 2007. pp. 15–29.
23. Kaplan E. Meier P. NON-parametric estimation of incomplete observations. Journal of the American Statistical Association 1958;53:457–481.
24. Sener SF. Fremgen A. Menck HR. Winchester DP. Pancreatic cancer: a report of treatment and survival trends for 100,313 patients diagnosed from 1985-1995, using the National Cancer Database. J Am Coll Surg 1999;189:1–7.
25. Kalser MH. Ellenberg SS. Pancreatic cancer: Adjuvant combined radiation and chemotherapy following curative resection. Archives of Surgery 1985;120(8):899–903.
26. Klinkenbijl JH. Jeekel J. Sahmoud T. van Pel R. Couvreur ML. Veenhof CH. Arnaud JP. Gonzalez DG. de Wit LT. Hennipman A. Wils J. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. Annals of Surgery 1999;230(6):776–784.
27. Neoptolemos JP. Stocken DD. Friess H. Bassi C. Dunn JA. Hickey H. Beger H. Fernandez-Cruz L. Dervenis C. Lacaine F. Falconi M. Pederzoli P. Pap A. Spooner D. Kerr DJ. Buchler MW. European Study Group for Pancreatic Cancer. A randomized trial of chemoradiotherapy and chemotherapy after resection of

pancreatic cancer. New England Journal of Medicine 2004;350 (12):1200–1210.
28. Oettle H. Post S. Neuhaus P. Gellert K. Langrehr J. Ridwelski K. Schramm H. Fahlke J. Zuelke C. Burkart C. Gutberlet K. Kettner E. Schmalenberg H. Weigang-Koehler K. Bechstein WO. Niedergethmann M. Schmidt-Wolf I. Roll L. Doerken B. Riess H. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 2007;297(3):267–77.
29. Regine WF. Winter KA. Abrams RA. et al. Fluorouracil vs gemcitabine chemotheraphy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. JAMA 2008;299:1019–1026.
30. Riall TS. Nealon WH. Goodwin JS. Zhang D. Kuo YF. Townsend CM Jr. Freeman JL. Pancreatic cancer in the general population: Improvements in survival over the last decade. Journal of Gastrointestinal Surgery 2006;10(9):1212–1224.
31. White R. Lee C. Anscher M. Gottfried M. Wolff R. Keogan M. Pappas T. Hurwitz H. Tyler D. Preoperative chemoradiation for patients with locally advanced adenocarcinoma of the pancreas. Annals of Surgical Oncology 1999;6(1):38–45.
32. Wolff RA. Chemotherapy for pancreatic cancer: From metastatic disease to adjuvant therapy. Cancer J 2007;13:175–184.
33. Bendel J & Goldberg RM. Targeted agents in the treatment of pancreatic cancer; history and lessons learned. Current opinion in Oncology 2007;19:390–395.
34. Rocha-Lima CM. New directions in management of advanced cancers a review. Anti-cancer drugs 2008;19:435–446.
35. Minn AY. Schellenberg D. Maxim P. et al. Pancreatic tumor motion on a single planning 4D-CT does not correlate with intrafraction tumor motion during treatment. AM J Clin Oncol 2009;32:364–368.
36. Fowler JF. The linear–quadratic model and progression radiotherapy. Br J Radiol 1989;62: 679–694.
37. Whelden TE. Deehan C. Wheldon GE. et al. The linear quadratic transformation of dose-volume histogram in fractioned radiotherapy. Radiother Oncol 1998;46: 285–295.
38. Sindelar WF. Kinsella TJ. Studies of intraoperative radiotherapy in carcinoma of the pancreas. Annals of Oncology 1999;10 (Suppl 4):226–230.
39. Tepper JE. Noyes D. Krall J. Sause MD. et al. Intraoperative radiation therapy of pancreatic carcinoma: A report of RTOG-8505. Int. J. Radiation Oncology Biol Phys 1991;21:1145–1149.
40. Ko AH. Quivey JM. Venook AP. Bergsland EK. Dito E. Schillinger B. Tempero MA. A phase II study of fixed-dose rate gemcitabine plus low-dose cisplatin followed by consolidative chemoradiation for locally advanced pancreatic cancer. International Journal of Radiation Oncology, Biology, Physics 2007;68 (3):809–816.
41. Murphy JD. Adusumilli S. Griffith KA. Ray ME. Zalupski MM. Lawrence TS. Ben-Josef E. Full-dose gemcitabine and concurrent radiotherapy for unresectable pancreatic cancer. International Journal of Radiation Oncology, Biology, Physics 2007;68 (3):801–808.
42. Krishnan S. Rana V. Janjan NA. Varadhachary GR. Abbruzzese JL. Das P. Delclos ME. Gould MS. Evans DB. Wolff RA. Crane CH. Induction chemotherapy selects patients with locally advanced, unresectable pancreatic cancer for optimal benefit from consolidative chemoradiation therapy. Cancer 2007;110 (1):47–55.
43. Mahadevan A. Jain S. Goldstein M. et al. Stereotactic body radiotherapy and gemcitabine for locally advanced pancreatic cancer. International Journal of Radiation Oncology, Biology, Physics 2010 (in press). doi:10.1016/j.ijrobp.2009.08.046.
44. Polistina F. Costantin G. Casamassima F. et al. Unresectable locally advanced pancreatic cancer: A multimodal treatment using neoadjuvant chemoradiotherapy (gemcitabine plus stereotactic radiosurgery) and subsequent surgical exploration. Annals of Surgical Oncology. 2010;17(8):2092–2101.
45. Kalser MH. Barkin J. MacIntyre JM. Pancreatic cancer: Assessment of prognosis by clinical presentation. Cancer 1985;56(2):397–402.
46. Lillemoe KD. Cameron JL. Kaufman HS. Yeo CJ. Pitt HA. Sauter PK. Chemical splanchnicectomy in patients with unresectable pancreatic cancer. A prospective randomized trial. Annals of Surgery 1993;217(5):447–457.
47. Wong GY. Schroeder DR. Carns PE. Wilson JL. Martin DP. Kinney MO. Mantilla CB. Warner DO. Effect of neurolytic celiac plexus block on pain relief, quality of life, and survival in patients with unresectable pancreatic cancer: a randomized controlled trial. JAMA 2004;291(9):1092–1099.

J Gastrointest Surg (2010) 14:1560–1565
DOI 10.1007/s11605-010-1329-1

2010 SSAT PLENARY PRESENTATION

Gender-Specific Transfusion Affects Tumor-Associated Neutrophil: Macrophage Ratios in Murine Pancreatic Adenocarcinoma

Douglas D. Benson · Marguerite R. Kelher · Xianzhong Meng · David A. Fullerton · Joon H. Lee · Christopher C. Silliman · Carlton C. Barnett Jr.

Received: 5 May 2010 / Accepted: 9 August 2010 / Published online: 11 September 2010

Abstract
Introduction Perioperative blood transfusion has been linked to decreased survival for pancreas cancer. Noting clinical data associating female blood products with increased morbidity, our lab has demonstrated that transfusion of female blood augments metastatic events compared to male blood in an immunocompetent murine pancreatic cancer model. It has been suggested that tumor-associated macrophages correlate with tumor progression by promoting angiogenesis. More recently, tumor-associated neutrophils have been implicated in aggressive tumor behavior. We hypothesize that differences in gender-specific transfusion-mediated pancreatic cancer progression are due to microenvironmental changes within the tumor. To test this hypothesis, we examined tumor-associated neutrophils and macrophage ratios in male and female mice with pancreatic cancer receiving blood transfusion from male or female donors.
Methods C57/BL6 mice, age 7–9 weeks, underwent splenic inoculation with 2.5×10^5 PanO2 murine pancreatic adenocarcinoma cells. Mice were transfused on post-op day 7 with 1 ml/kg supernatant from day 42 male or female packed red cells. Necropsy was performed at 5 weeks or earlier for clinical deterioration, and tumors harvested. Frozen sections (5 μm) were stained for neutrophils and macrophages by immunofluorescence. Data were analyzed using ANOVA; $p\leq0.05$ was used to determine significance; $N\geq3$ per group.
Results Clinically, male mice had greater morbidity and mortality than female mice when receiving female blood products, with roughened hair coat, development of ascites and death due to bowel obstruction. In evaluating the tumor microenvironment from mice receiving female blood products, male mice were noted to have a greater neutrophil to macrophage ratio than female mice, 0.176±0.028 vs. 0.073±0.012, $p=0.03$. When examining neutrophil to macrophage ratio in mice receiving male blood products, no difference was noted ($p=0.48$).
Conclusions Male mice with pancreas cancer have greater morbidity than female mice when receiving female blood products. Furthermore, the difference in neutrophil to macrophage ratio suggests that gender-specific blood transfusion promotes aggressive tumor behavior in male mice via microenvironmental changes. These data warrant further study to delineate sex-related differences in pancreatic cancer progression.

D. D. Benson · C. C. Barnett Jr. (✉)
Denver Health Medical Center,
777 Bannock Street,
Denver, CO 80204-0206, USA
e-mail: Carlton.Barnett@dhha.org

D. D. Benson · X. Meng · D. A. Fullerton · J. H. Lee · C. C. Silliman · C. C. Barnett Jr.
Department of Surgery, University of Colorado,
Aurora, CO, USA

M. R. Kelher · C. C. Silliman · C. C. Barnett Jr.
Bonfils Blood Center,
Denver, CO, USA

Keywords Transfusion · Pancreas cancer · Metastasis · Erythrocytes · Immunomodulation · Neutrophil to macrophage ratio

Introduction

Perioperative blood transfusion has been linked to negative outcomes in patients undergoing pancreaticoduodenectomy for cancer.[1–4] However, a causal mechanism has not been elucidated. Allogeneic blood transfusions expose patients to

foreign cells, antigens, and cytokines and lipid mediators that may promote tumor growth or affect the host immune system. This phenomenon is termed transfusion-related immunomodulation (TRIM).[5] The affect of blood transfusion on immune function was first described in 1973 wherein it was noted that renal allografts had prolonged survival in patients who received transfusions preoperatively.[6] Gantt later proposed that transfusion may increase tumor progression.[7] Since the 1980s, many trials have examined TRIM effects on cancer progression, with similar outcomes.

When specifically examining pancreatic cancer, there is evidence that males perform more poorly than females.[3,8,9] Our laboratory has recently shown in an immunocompetent murine pancreatic cancer–transfusion model that male mice perform worse than females and have more metastatic events.[10] This was particularly noted in males who received a transfusion from a female donor. Previous work has demonstrated gender-related dimorphism in the area of transfusion-related acute lung injury (TRALI) with female blood products being deleterious.[11–16] Although the exact mechanism is unclear, differences may be due to anti-white blood cell (WBC) antibodies or anti-human leukocyte antigen (HLA) antibodies produced from alloimmunization of multiparous female donors.[13–15] Based on these data, we hypothesize that differences in gender-specific transfusion-mediated pancreatic cancer progression are due to microenvironmental changes in the inflammatory cells within the tumor.

Recent work has shown that increasing tumor-associated macrophages and tumor-associated neutrophils (TAMs and TANs, respectively) promote tumor growth and invasiveness.[17–19] Neutrophils in particular have been implicated in being effector cells in the tumor microenvironment and have been shown to be integral in the initial angiogenic switch, which preceded the metastatic phenotype.[19] Queen et al. have demonstrated that TANs correlate with tumor progression by promoting angiogenesis and cell detachment in an in vitro model of breast cancer.[20] Our laboratory has also shown that neutrophils aggregate at the leading edge of tumors, likely playing a role in tumor metastasis.[21] We propose that TAMs and TANs may be sensitive to the effects of TRIM with TANs acting as the principle effector cell.

Methods

Plasma Preparation from Stored pRBCs

With institutional review board-approved informed consent, seven healthy donors donated 450 ml of whole blood as per American Association of Blood Banks criteria.[22] Preparation of the acellular portion of packed red cells has been described previously.[23]

Murine Pancreatic Adenocarcinoma Culture

GFP-expressing PanO2 cells were maintained at 37°C in a mixture of 5% CO_2 and 95% air in RPMI 1640 medium (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin–streptomycin (Invitrogen). Subculture was performed when cells reached 70–90% confluence. Cells were harvested after washing with HEPES-buffered saline solution (Lonza, Walkersville, MD), using trypsin–EDTA (Lonza) for 5 min, and adding trypsin-neutralizing solution (Lonza). Cells were centrifuged at 1,000×*g* for 10 min, counted, and reconstituted in saline at a concentration of 5×10^6 cells per milliliter to provide a tumor inoculation of 2.5×10^5 cells per 50 μl.

Immunocompetent Murine Metastatic Model of Pancreatic Adenocarcinoma

Experiments were performed in groups of male and female C57/BL6 mice (Jackson Laboratories, Bar Harbor, ME) using the acellular component (plasma) of pRBCs from male and female donors. All studies were performed under the guidelines of the University of Colorado at Denver Institutional Animal Care and Use Committee (IACUC). After being allowed to acclimate, mice underwent general anesthesia and were inoculated by a subcapsular splenic injection of 2.5×10^5 GFP-expressing PanO2 cells. Mice were then randomized 1 week post-tumor inoculation and received a lateral tail vein (LTV) injection of saline control, blood product from male donors, or blood product from female donors. All animals were given a dose of 1 ml/kg acellular plasma diluted into 50 μl of saline. Mice were clinically followed up,[24] weighed three times weekly, and sacrificed 5 weeks after injection of tumor cells (or 4 weeks after transfusion) or earlier if there was clinical deterioration per IACUC regulations. Non-tumor-bearing mice were maintained for 4 weeks after LTV injection of plasma extract to observe for any adverse effects from injection of human plasma.

Necropsy was performed and extent of disease was quantified noting the number of gross metastatic events and clinical tumor sequelae such as ascites, bowel obstruction, jaundice, and biliary obstruction. Mice were randomized at the time of necropsy to limit interpreter bias. Organs were harvested for tissue staining, preserved in tissue freezing media (Fischer Scientific, Pittsburgh, PA), frozen, and stored at −80°C.

Tissue Immunofluorescence

Tumors from all groups of animals were examined for the presence of neutrophils and macrophages using immunofluorescence (IF). Sectioning and staining was performed in 5-μm serial sections of frozen liver

segments. Slides were fixed in a solution of 30% acetone and 70% methanol for 10 min and air dried for 2 min. After three washes with phosphate buffer solution (PBS), they were again fixed in 4% paraformaldehyde. After three washes in PBS, slides were blocked with 10% donkey serum for 30 min. They were then incubated in primary antibody, either rat anti-mouse PMN (clone 7/4; ABD Serotec, Oxford, UK) or rat anti-mouse CD68 (ABD Serotec) overnight at 4°C. They were then washed in PBS three times and incubated at room temperature in a dark environment with secondary antibodies of a donkey anti-rat CY3 IgG (imaged on the red channel), Alexa Fluor 488-labeled conjugate wheat germ agglutinin (Molecular Probes, Invitrogen), and bisBenimide H33342 trihydrochloride (Sigma-Aldrich, St. Louis, MO) for 60 min. Slides were then washed with PBS three times, rinsed with distilled water, and air dried. They were mounted with quench medium and sealed with nail polish. Slides were then examined with a Leica DMRXA digital microscope (Leica Mikroskopie und Syteme, Wetzlar, Germany), and three random pictures were taken of both areas of tumor as well as the normal surrounding liver parenchyma using the SlideBook 2.6 software (I.I.I., Denver, CO). Neutrophil and macrophage signaling was determined by two different methods. First, signals were counted by hand, with number of cells providing signal per high-power field. Antibody signal was then calculated by SlideBook using the area of the stain signal for neutrophils and macrophages compared with the total area of the picture.

Statistical Analysis

The data are expressed as the mean ± the standard error of the mean. One-way ANOVA testing was performed to determine the significance of observed differences with Fisher's exact testing for post hoc comparisons. Statistical significance was determined as $p<0.05$; $n\geq10$ per group for clinical data, $n\geq3$ for tissue IF groups with three sections per mouse.

Results

Clinically, male mice had greater morbidity and mortality when receiving female blood product, with roughened hair coat, development of ascites, and death due to bowel obstruction. Seventeen percent of male mice died (when able to examine—this was due to malignant bowel obstruction with perforation). The earliest death due to malignant cause was 9 days post-transfusion. All groups of evaluated male mice were necessarily sacrificed early, between days 21 and 25 post-transfusion due to excessive morbidity (ascites) according to IACUC regulations. Female mice groups were sacrificed at 4 weeks post-transfusion. Control male mice had a trend of more metastatic events than female mice, 3.27±0.45 vs. 1.89±0.54 ($p=0.064$). Males had significantly more metastatic events compared with females when receiving male blood product, 3.82±0.36 vs. 2.59±0.51 ($p=0.048$), and female blood product, 4.96±0.57 vs. 2.82±0.39 ($p=0.005$; Fig. 1).

In evaluating the tumor microenvironment, counts of neutrophils and macrophages were examined by tissue IF (Fig. 2). When examining neutrophil to macrophage ratios in male and female mice receiving male blood product, there was no comparative difference. However, in mice receiving female blood product, male mice were noted to have a greater neutrophil to macrophage ratio than females, 0.176±0.028 vs. 0.073±0.012 ($p=0.03$; Fig. 3), which may correlate with the poorer outcomes observed clinically. The signal area of both neutrophils and macrophages were compared with the total area of the high-power field and confirmed the above findings, as in mice receiving female blood product, male mice again had a greater neutrophil to macrophage ratio than female mice, 0.343±0.074 vs. 0.112±0.006 ($p=0.046$).

Discussion

Limitations in clinical trials for pancreatic cancer, overall poor prognosis, small sample sizes, and clinical variation inherent among patients such as stage and operative difficulty make it complicated to elicit prognostic factors that can impact survival. Therefore, preclinical models offer great opportunities to control for many of these variables and study the effects of TRIM on tumor biology in pancreatic adenocarcinoma. Herein, we have developed an

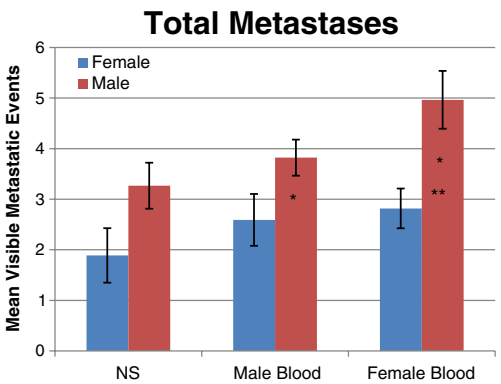

Fig. 1 Total metastatic events in male and female mice receiving male blood, female blood, or saline control. Male mice receiving male or female blood had significantly more metastatic events compared with females (*one asterisk*). Males who received female blood also had significantly more metastases than males receiving saline (*two asterisks*). Gender differences in the saline control did not reach significance

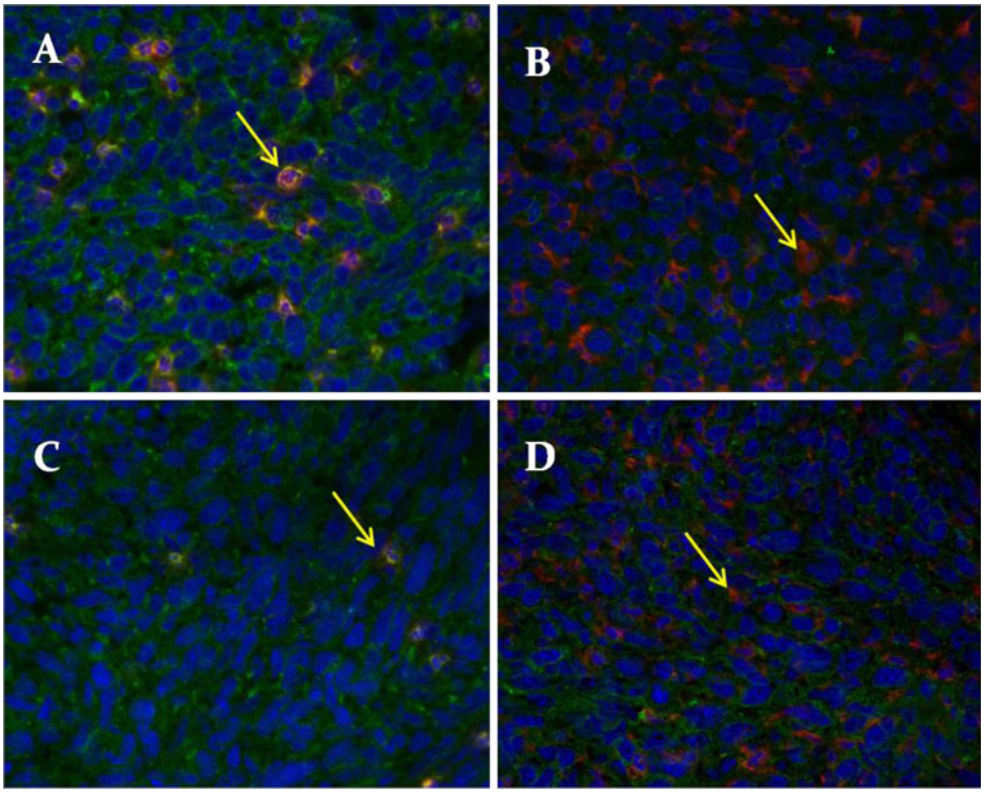

Fig. 2 Representative photomicrographs. **a** Neutrophil stain from male mouse. **b** Macrophage stain from male mouse. **c** Neutrophil stain from female mouse. **d** Macrophage stain from female mouse. *Arrows* indicate cell signal (*red*), *blue stain* is the nuclear stain (H33342), and *green stain* is wheat germ agglutinin

immunocompetent murine metastatic pancreatic cancer model[21] that allows for control of tumor burden, genetic diversity, operative trauma, and the amount of transfusion. Given the potential for gender differences in pancreatic cancer outcomes, we examined the gender-related dimorphism in blood transfusion.

The results of this pilot study indicate that male recipients of female blood products have the highest comparative tumor neutrophil to macrophage ratios and males receiving female blood had the worst clinical outcomes. This suggests that the immunomodulatory effects of blood transfusion may alter the inflammatory cell makeup of the tumor microenvironment and may be a mechanism behind the gender differences that we have observed.

Previous investigations evaluating the interaction between cancer cells and surrounding inflammatory cells[17–19] have shown that increased TAMs correlate with poorer outcomes.[18] The cytokines produced by macrophages may lead to a permissive environment via proteases and growth factors.[18] Unlike other macrophages, TAMs exhibit an alternatively activated phenotype, which has the ability to produce factors that suppress T-cell proliferation and facilitate tumor growth.[25] A recent in vitro model of murine lung adenocarcinoma showed that alternatively activated macrophages have high expression of VEGF-C and that co-incubation with cancer cells enhanced lymphangiogenesis.[25] Neutrophils have also been investigated as effector

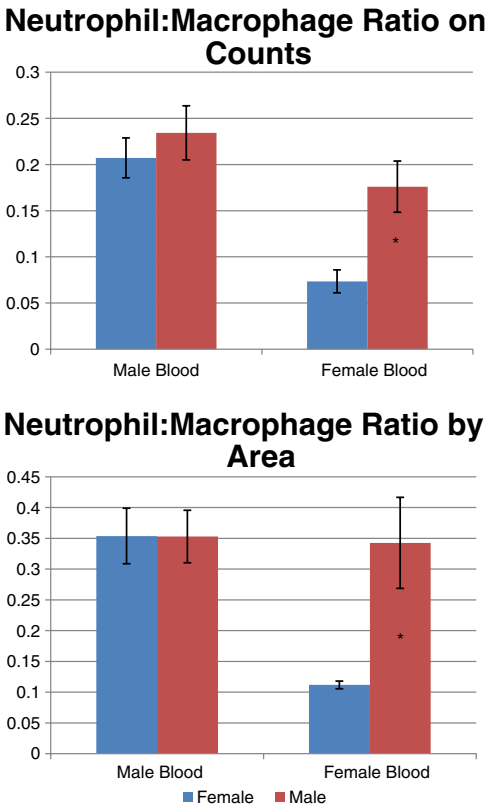

Fig. 3 Neutrophil to macrophage ratio by counts and by area. Male mice receiving female blood had a significantly higher neutrophil to macrophage ratio than females receiving female mice receiving female blood (*$p<0.05$ in both). No gender differences were noted in mice receiving male blood product

cells in tumor progression and have been shown to be critical in the angiogenic switch.[19] Neutrophils are capable modulators in metastatic capability.[21,26,27] These two inflammatory cells may act in concert to promote tumor progression, wherein the macrophages recruit the neutrophils as effectors in breaking down the extracellular matrix to cell detachment and invasion via matrix metalloproteases and VEGF. Clinically, the balance of neutrophils and macrophages play crucial roles in the control of pulmonary infections where increased neutrophils in relation to macrophages are necessary to eliminate parasite infection.[28] In examining colorectal liver metastases, high ratios of circulating neutrophils to lymphocytes are linked to poorer outcomes,[29–31] suggesting that the ratio of effector cells, not gross numbers, play an important role in the tumor metastatic phenotype. Although controversial, ratios between different inflammatory cells have been linked to clinical outcomes and suggest that microenvironmental neutrophil to macrophage ratios are an important area of investigation.

There are some limitations of this study. A xenotransfusion of human blood product may affect outcomes and may lead to inflammatory and immunological responses in the innate mouse immune system. However, in studying TRALI, it has been shown that giving fresh human blood product is safe and does not lead to a significant inflammatory injury in rats.[32] In this pilot trial examining the microenvironment of immunocompetent mice with pancreas cancer receiving either male or female blood product, there were significant gender-specific differences in clinical outcomes. In particular, male mice receiving female blood had severe clinical sequelae, including ascites, bowel obstruction, and significantly more metastatic events than their female counterparts. Finding a significant comparative difference in the neutrophil to macrophage ratio, we believe this may explain the clinical observations seen. Further examination of the neutrophil to macrophage ratio in males and female mice receiving male blood however showed similar but high ratios. The reason for these findings is unclear as these groups performed clinically better. It is possible that cross-gender transfusion of male blood into females elevated the neutrophil to macrophage ratio in females and males with pancreas cancer may have higher ratios in general. However, male mice receiving female blood performed extremely poorly with one mouse dying as soon as 9 days post-transfusion. It is our belief that, if these animals had been allowed to live longer, the neutrophil ratio would have been even higher in this group. In any event, the significant gender dimorphism observed in male mice receiving female blood and the comparative difference in neutrophil to macrophage ratio in this group warrants further study.

Conclusion

In summary, male mice receiving female blood product have the worst clinical outcomes and the highest comparative neutrophil to macrophage ratio in the tumor when compared with females, with associated higher morbidity. These data suggest that neutrophils are important effector cells in mediating tumor progression, and a threshold between increased neutrophils compared with macrophages may trigger an aggressive metastatic phenotype. These data warrant further study to delineate sex-related differences in pancreatic cancer progression.

Acknowledgments This work was supported in part by the American Cancer Society, grant number MSRG-09-034-01-CCE.

References

1. Yeh J, Gonen M, Tomlinson J, Indrees K, Brennan M, Fong Y. Effect of blood transfusion on outcome after pancreaticoduodenectomy for exocrine tumor of the pancreas. *Br J Surg*. 2007; 94:466–72.
2. Park S, Kim S, Jang J, Lee K, Park Y. Intraoperative transfusions: Is it a real prognostic factor of periampullary cancer following pancreatoduodenectomy? *World J Surg*. 2002; 26:487–92.
3. Cameron J, Crist D, Sitzmann J, Hruben R, Boitnott J, Seidler A, Coleman J. Factors influencing survival after pancreaticoduodenectomy for pancreatic cancer. *Am J Surg*. 1991; 161:120–5.
4. Millikan K, Deziel D, Silverstein J, Kanjo T, Christein J, Doolas A, Prinz R. Prognostic factors associated with resectable adenocarcinoma of the head of the pancreas. *Am Surg*. 1999; 65:618–24.
5. Vamvakas E, Blajchman M. Transfusion-related immunomodulation (TRIM): an update. *Blood Rev.* 2007; 21(6):327–48.
6. Opelz G, Senger D, Mickey M, Terasaki P. Effect of blood transfusion on subsequent kidney transplants. *Transfusion Proc*. 1973; 5:253–9.
7. Gantt C. Red blood cells for cancer patients. *Lancet*. 1981; 363.
8. Breslin T, Hess K, Harbison D, Jean M, Cleary K, Dackiw A, Wolff R, Abbruzzese J, Janjan N, Crane C, Vauthey J, Lee J, Pisters P, Evans D. Neoadjuvant chemotherapy for adenocarcinoma of the pancreas: Treatment variables and survival duration. *Ann Surg Oncol*. 2001; 8:123–32.
9. Conlon K, Klimstra D, Brennan M. Long-Term Survival After Curative Resection for Pancreatic Ductal Adenocarcinoma: Clinicopathologic Analysis of 5-Year Survivors. *Ann Surg* 1996; 223(3): 273–279.
10. Moore, P, Benson, D, Kelher, M, Moore, E, Fragoso, M, Silliman, C, Barnett, C. The plasma fraction of stored erythrocytes augments pancreatic cancer metastases in male versus female mice. *J Surg Res*. In Press.
11. Palfi M, Berg S, Emerudh J, Berlin G. A randomized Controlled Trail of transfusion-related acute lung injury: is plasma from multiparous blood donors dangerous? *Transfusion.* 2001; 41(3):317–322.
12. Triulzi D. Transfusion-related acute lung injury: current concepts for the clinician. *Anesth Analg.* 2009; 108(3): 770–776.
13. Gajic O, Yilmaz M, Iscimen R, Kor D, Winters J, Moore S, Afessa B. Transfusion from male-only versus female donors in critically ill recipients of high plasma volume components. *Crit Care Med.* 2007; 35(7):1645–8.
14. Eder A, Herron R, Strupp A, Dy B, Notari E, Chambers L, Dodd R, Benjamin R. Transfusion-related acute lung injury surveillance

(2003–2005) and the potential impact of the selective use of plasma from male donors in the American Red Cross. *Transfusion.* 2007; 47(4):599–607.
15. Imoto S, Araki N, Shimada E, Saigo K, Nishimura K, Nose Y, Bouike Y, Hashimoto M, Mito H, Okazaki H. Comparison of acute non-haemolytic transfusion reactions in female and male patients receiving female or male blood components. *Transfus Med.* 2007; 17(6):455–65.
16. Chapman C, Stainsby D, Jones H, Love E, Massey E, Win N, Navarrete C, Lucas G, Soni N, Morgan C, Choo L, Cohen H, Williamson L; Serious Hazards of Transfusion Steering Group. Ten years of hemovigilance reports of transfusion-related acute lung injury in the United Kingdom and the impact of preferential use of male donor plasma. *Transfusion.* 2009; 49(3):402–5.
17. Coussens L, Werb Z. Inflammation and cancer. *Nature*. 2002; 420:860–7.
18. Pollard J. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer*. 2004; 4:71–8.
19. Nozawa H, Chui C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci USA*. 2006; 103:12493–8.
20. Queen M, Ryan R, Holzer R, Keller-Peck C, Jorcyk C. Breast cancer cells stimulate neutrophils to produce oncostatin M: Potential implications for tumor progression. *Cancer Res*. 2005; 65:8896–904.
21. Roland C, Dineen S, Toombs J, Carbon J, Smith C, Brekken R, Barnett C. Tumor-derived intercellular adhesion molecule-1 mediates tumor-associated leukocyte infiltration in orthotopic pancreas xenografts. *Exp Biol Med*. 2010; 235: 263–70.
22. Technical Manual. American Association of Blood Banks, Bethesda. 2003.
23. Barnett C, Beck A, Holloway S, Kehler M, Schluterman M, Brekken R, Fleming J, Silliman C. Intravenous delivery of the plasma fraction of stored packed red cells promotes pancreatic cancer growth in immunocompetent mice. *Cancer*. In Press.
24. Animal Resources Body Weight Study of 1993. The Jackson Labs, 1993.
25. Zhang B, Wang J, Gao J, Guo Y, Chen X, Wang B, Gao J, Rao Z, Chen Z. Alternatively activated RAW264.7 macrophages enhance tumor lymphangiogenesis in mouse lung adenocarcinoma. *J Cell Bio*. 2009; 107:134–43.
26. Roland C, Harken A, Sarr M, Barnett C. ICAM-1 expression determines malignant potential of cancer. *Surgery.* 2007; 141:705–7.
27. Strell C, Lang K, Niggemann B Zaenker K, Entschladen F. Neutrophil granulocytes promote the migratory activity of MDA-MB-468 human breast carcinoma cells via ICAM-1. *Exp Cell Res*. 2010; 316:138–48.
28. Novais F, Santiago R, Bafica A, Khouri R, Afonso L, Borges V, Brodskyn C, Barral-Netto M, Barral A, de Oliveira C. Neutrophils and macrophages cooperate in host resistance against Leishmania braziliensis infection. *J Immunol*. 2009; 183:8088–98.
29. Kishi Y, Kopetz S, Chun Y, Palavecino M, Abdalla E, Vauthey J. Blood neutrophil-to-lymphocyte ratio predicts survival in patients with colorectal liver metastases treated with systemic chemotherapy. *Ann Surg Oncol*. 2009; 16:614–22.
30. Lui H, Lui G, Bao Q, Sun W, Bao H, Bi L, Wen W, Lui Y, Wang Z, Yin X, Bai Y, Hu X. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in rectal carcinoma. *J Gastrointest Cancer.* 2010; Feb 2 Epub.
31. Halazun K, Aldoori A, Malik H, Al-Mukhtar A, Prasad K, Toogood G, Lodge J. Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases. *Eur J Surg Oncol*. 2008; 34:55–60.
32. Silliman C, Bjornsen J, Wyman T, Kelher M, Allard J, Bieber S, Voelkel N. Plasma and lipids from stored platelets cause acute lung injury in an animal model. *Transfusion*. 2003; 43:633–40.

Discussant

Dr. Magesh Sundaram (Morgantown, WV): This is a very interesting animal model that's examining transfusion-related immunomodulation on the effects of growth of cancer. When you look at the human literature, it's hard to control for so many different variables, so your model is very nicely done. I have three questions for you.

My first question, we know that the deleterious effect of TRIM, leading to the increased recurrence rates of resected malignancies, is perhaps due to intact or active white blood cells in the transfusion, soluble white blood cell-derived mediators, or circulating HLA peptides in the plasma supernatant. Which of these factors are you checking in the plasma that you're transfusing to the mice?

My second question is, do you feel that the male mice with pancreatic cancer that you are giving the female plasma to leads to a milieu of hypogonadism and thereby promoting the progression of the pancreatic cancer. And if so, what sort sex steroid hormonal factors are you looking at in the plasma that's transfused?

In terms of the clinical implication, we know that since 2006, the American Association of Blood Banks has recommended that we give male-donated plasma to prevent the incidence of TRALI or transfusion-related acute lung injury, which was seen with female-donated plasma. So what do you feel might be the clinical implications of your work in the perioperative management of cancer patients?

Closing Discussant

Dr. Douglas Benson: To start with that last question, the clinical implication would be if we can confirm these findings and consistently show that female blood products lead to worse outcomes, especially in males, it may be indicated to have a male predominant donation strategy in surgical oncology cases. I think we are a long way away from that, but that's the clinical implication that could arise from this study.

As far as what might be happening, there could be a number of different factors for your first two questions. There are many things in female blood that aren't in male blood, as has been shown in the TRALI literature with female plasma donation; HLA antibodies and leukocyte antibodies that are more predominant in multiparous female donors. We are in the process now of looking at what specifically in the blood might be leading to the tumor progression. So it's still unclear, blood has many factors, chemokines, and lipid mediators, so there are many things to sort through. It's likely multifactorial, and we are trying to find what are the most important aspects of the blood transfusions that lead to tumor progression.

J Gastrointest Surg (2010) 14:1566–1577
DOI 10.1007/s11605-010-1338-0

2010 SSAT PLENARY PRESENTATION

Involvement of Osteopontin in the Matrix-Degrading and Proangiogenic Changes Mediated by Nicotine in Pancreatic Cancer Cells

Melissa Lazar · Jennifer Sullivan · Galina Chipitsyna · Qiaoke Gong · Chee Y. Ng · Ahmed F. Salem · Tamer Aziz · Agnes Witkiewicz · David T. Denhardt · Charles J. Yeo · Hwyda A. Arafat

Received: 30 April 2010 / Accepted: 18 August 2010 / Published online: 8 September 2010

Abstract
Background Substantial evidence indicates that exposure to cigarette smoke is associated with an elevated risk of pancreatic ductal adenocarcinoma (PDA). However, the mechanisms underlying the effects of nicotine on the development or progression of PDA remain to be investigated. Previously, we showed that nicotine promotes the expression of osteopontin c (OPNc), an isoform of OPN protein that confers on cancer cells a migratory phenotype. In this study, we explored the potential prometastatic role of nicotine in PDA through studying its effect on the expression of matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) and evaluated the role of OPN in mediating these effects.
Materials and Methods MMP-9 and VEGF mRNA and protein were analyzed in PDA cells treated with or without nicotine (3–300 nM). Transient transfection and luciferase-labeled promoter studies evaluated the effects of OPNc and OPN protein on the transcription and translation of MMP-9 and VEGF. Real-time PCR and immunohistochemistry were used to analyze the mRNA expression levels and localization of OPN, MMP-9, and VEGF proteins in matched invasive human PDA and surrounding nonmalignant tissues.
Results and Discussion Nicotine significantly enhanced the expression of MMP-9 and VEGF mRNA and protein in PDA cells. Blocking OPN with siRNA or OPN antibody prevented the nicotine-mediated increase of both MMP-9 and VEGF. Transient transfection of OPNc gene in PDA cells or their treatment with recombinant OPN protein significantly ($p<0.05$) increased MMP-9 and VEGF mRNA expression levels and induced their promoter activities. In invasive PDA lesions, MMP-9 mRNA levels were significantly ($p<0.005$) higher in smokers vs. nonsmokers. VEGF protein co-localized with MMP-9 and OPN in the malignant ducts and correlated well with their higher levels in invasive PDA lesions.
Conclusions Our data show for the first time that cigarette smoking and nicotine may contribute to PDA metastasis through inducing MMP-9 and VEGF and suggest that OPN plays a central role in mediating these effects. The presence of OPN as a downstream effector of nicotine that is capable of mediating its prometastatic effects in PDA cells is novel and could provide a unique therapeutic target to control pancreatic cancer aggressiveness, especially in the cigarette-smoking population.

Keywords Pancreatic cancer · Nicotine · Osteopontin · VEGF · MMP-9 · Angiogenesis · Metastasis

Presented as a plenary presentation at the 51st Annual Meeting of the Society for Surgery of the Alimentary Tract, May 1–5, 2010 and an oral presentation at the SSAT Residents and Fellows Research Conference, May 1, 2010, New Orleans, LA.

M. Lazar · J. Sullivan · G. Chipitsyna · Q. Gong · C. Y. Ng · A. F. Salem · T. Aziz · A. Witkiewicz · D. T. Denhardt · C. J. Yeo · H. A. Arafat (✉)
Department of Surgery, Jefferson Pancreatic, Biliary and Related Cancer Center, Thomas Jefferson University,
1015 Walnut Street,
Philadelphia, PA 19107, USA
e-mail: hwyda.arafat@jefferson.edu

Introduction

Pancreatic ductal adenocarcinoma (PDA) is an extremely aggressive cancer, and currently, there are no methods for early detection. At the time of diagnosis, more than 85% of the tumors have infiltrated into adjacent organs or have metastasized, and the overall 5-year survival rate is <5%.[1] Given the grim prognosis of this disease, it is essential to understand the mechanisms that underlie PDA aggressiveness in order to design more effective therapies.

Exposure to cigarette smoke has been shown to be strongly associated with an elevated risk of PDA. Smokers have at least twofold increase in the risk of developing PDA,[2] and it is

estimated that 25–30% of all PDA cases are related to cigarette smoking.[3] Nicotine is a major component of cigarette smoke and is an addictive agent. The US Surgeon General has characterized nicotine as a drug of abuse.[4] Pancreatic cancer has been linked to nicotine and cigarette smoking in several studies.[5, 6] However, it is not clear how nicotine contributes to the development or progression of PDA.

The majority of cancer-related deaths are caused by metastatic disease. Breakdown of the extracellular matrix (ECM) is necessary for tumor cells to invade adjacent tissue and metastasize. Tumor cells degrade components of the ECM through a family of enzymes called matrix metalloproteinases (MMPs). There are five MMP subclasses: interstitial collagenases, gelatinases, stromelysins, membrane-type MMPs, and elastases.[7] In several cancers such as ovarian, lung, prostate, breast, and pancreatic, overexpression of MMPs correlates with tumor aggressiveness and metastatic potential.[8–13] Matrix metalloproteinase-9 (MMP-9), a gelatinase, is believed to play an important role in the invasion and angiogenesis[14] of malignant tumors.

Also necessary for tumor invasion and metastasis is angiogenesis. In order for tumors to grow beyond a few millimeters in diameter, they need to build new blood vessels.[15] Vascular endothelial growth factor (VEGF) is an important angiogenic factor involved in tumor growth and survival.[16] VEGF induces the growth of new blood vessels in both normal and malignant tissues and has been associated with tumor progression and metastasis in gastric[17] and colon cancers.[18]

We have recently shown that nicotine induces the expression of osteopontin (OPN), a protein that plays important roles in tumor angiogenesis and metastasis.[19] This effect was mediated through the nicotinic acetylcholine receptors on PDA cells and through an ERK1/2-dependent pathway.[20] We identified an isoform of OPN that confers a migratory phenotype on cancer cells, OPNc, which is selectively induced by nicotine[20] and highly expressed in invasive PDA tissues from smokers. In this study, we examined the prometastatic role of nicotine in PDA through studying its effects on the expression of MMP-9 and VEGF and evaluated the role of OPN in mediating these effects. We also analyzed the expression of MMP-9 and VEGF in PDA specimens from smokers and nonsmokers.

Materials and Methods

Cell Culture The human PDA cell lines MIA PaCa-2 and AsPC-1 were purchased from the American Type Culture Collection (Manassas, VA). Cells were counted and cultured at 1×10^4 cells to near confluence in 96-well plates and maintained in DMEM supplemented with 10% fetal bovine serum in a humid atmosphere of 5% CO_2/95% air. Cells were treated with nicotine (0.3–300 nM) for 3 and 24 h and were evaluated for the expression of MMP-9 and VEGF mRNA by real-time polymerase chain reaction (PCR). Cells were also treated with OPN protein (0.15–15 nM) in the presence or absence of nicotine and were evaluated for MMP-9 and VEGF mRNA. To block the action of OPN, cells were treated with (0.4 μg/ml) rabbit polyclonal IgG OPN antibody (Santa Cruz Biotechnology, Santa Cruz, CA) with or without nicotine and were evaluated for the expression of MMP-9 and VEGF mRNA.

RNA Extraction and Real-Time Reverse Transcription PCR Total RNA was isolated from PDA cells or pancreata using Trizol reagent (Life Technologies, Gaithersburg, MD). RNAs were quantified and input amounts were optimized for each amplicon. MMP-9, VEGF, and GAPDH (internal control) primers and probes were designed with the help of Primer Express Software (Applied Biosystems, Foster City, CA). cDNA was prepared, diluted, and subjected to real-time PCR using the TaqMan technology (7500 Sequence Detector, Applied Biosystems). The relative mRNA levels are presented as unit values of $2^{\wedge}[C_{T(MMP\text{-}9/VEGF)} - C_{T(GAPDH)}]$, where C_T is the threshold cycle value defined as the fractional cycle number at which the target fluorescent signal passes a fixed threshold above baseline.

Protein Isolation and Western Blot Analysis Cell lysates were analyzed as described elsewhere.[19] Briefly, protein concentrations in the supernatant were determined using the BCA protein assay reagent (Pierce, Rockford, IL). Equal protein concentrations (40 μg) were denatured in a gel loading buffer at 85°C for 5 min, loaded onto 10% SDS-polyacrylamide slab gels, transferred to polyvinylodene difluoride membranes, and incubated at 4°C overnight with mouse monoclonal antibody diluted in phosphate-buffered saline-Tween 20 against MMP-9 (1:200). Antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). To avoid sample loading errors, β-actin expression was determined in the blots to adjust and normalize the amount of sample loaded (Sigma). The protein bands were visualized with enhanced chemiluminescence reagents (ECL Plus Western Blotting Detection System, Amersham Pharmacia Biotech), analyzed, and intensity quantified using Kodak Electrophoresis Documentation and Analysis System 290 (EDAS 290).

Enzyme-Linked Immunosorbent Assay VEGF concentration in the media was measured using a human-specific ELISA kit (Assay Design, Ann Arbor, MI). Spectrophotometric evaluation of VEGF levels was made by Synergy HT multi-detection microplate reader (BioTeck, Winooski, VT).

siRNA Sequences and Constructs Using GenBank™ sequence AK315461 for human OPN cDNA and computer analysis software developed by Applied Biosystems/Ambion, candidate sequences in the OPN cDNA sequence for RNAi with no homology with other known human genes were selected and used during transient transfection experiments. Human mismatch or scramble siRNA sequences (Applied Biosystems/Ambion, Austin, TX) possessing limited homology to human genes served as a negative control. Transfection was done with TransFast (Promega, Madison, WI) in AsPC-1 cells as directed by the manufacturer. Cells were treated with or without nicotine and examined for MMP-9 and VEGF expression by qPCR.

Confocal Microscopy For confocal analysis, 0.5×10^6 AsPC-1 cells were seeded on sterilized round glass coverslips and incubated overnight. ASPC-1 cells were rinsed three times with 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ in phosphate-buffered saline (PBS/CM), fixed with 2% paraformaldehyde in PBS/CM for 30 min, permeabilized with 0.1% Triton-X100 and 0.2% bovine albumin serum in PBS/CM (IF buffer) for 10 min, quenched with 50 mM NH_4Cl in PBS/CM for 10 min, then rinsed with IF buffer. For MMP-9/VEGF/OPN staining, cells were incubated simultaneously with mouse anti-OPN (1:200) and anti-MMP-9 antibody (1:100) or with OPN and anti-VEGF antibodies (1:200, Santa Cruz, CA) for 1 h at room temperature. After washing in IF buffer (3× 10 min), cells were incubated for 30 min with secondary antibodies: rhodamine red X goat anti-mouse IgG (for OPN) and FITC donkey anti-goat (for MMP-9) and FITC donkey anti-rabbit (for VEGF), both from Jackson ImmunoResearch. After repeating the washing steps, nuclei were stained with Hoescht 33342 for 2 min, followed by rinsing in PBS. Coverslips were mounted with ProLong Gold anit-fade (Molecular Probes, OR) and left overnight in the dark. The laser confocal microscope LSM 510 microscope (Carl Zeiss GmbH, Thornwood, NY) was used to image the cells in the respective channels at a magnification of ×60.

Transient Transfection of OPNc pDest-290 vector containing a truncated splice variant OPNc (base pairs 1-93, 175-942) was a generous gift from Dr. X Wang, Center for Cancer Research, NCI, Bethesda, MD.[21] MIA PaCa-2 cells were transfected with 0.5 μg OPNc plasmid DNA using TransFast (Promega), and lysates were harvested after 24 h for initial semiquantitative PCR testing of the expression of OPNc. For subsequent experiments to determine the levels of MMP-9 and VEGF by real-time PCR, MIA PaCa-2 cells (1×10^6) were transfected with 0.5 μg OPNc plasmid DNA using optimized nucleofection conditions (60–80% efficiency by pGEM4/enhanced green fluorescent protein visualization). We determined the levels of MMP-9 and VEGF by real-time PCR 24 h after transfection.

Semiquantitative PCR RNAs from cells that were transfected with OPNc gene construct were quantified, DNase-digested, and cDNAs were prepared using ImProm-II™ Reverse Transcription System (Promega), then subjected to semiquantitative PCR using master mix (Promega). The primers used were: OPNc human forward 5′-TCAG GAAAAGCAGAATGCTG-3′, reverse 5′-GTCAATG GAGTCCTGGCTGT-3′.

Upstream and downstream primers that could anneal with the 3′-untranslated region of human GAPDH were included in the PCR reaction as an internal standard forward 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′, reverse 5′-CATGTGGGCCATGAGGTCCACCAC-3′. The linear range of amplification for each set of primers was determined to ensure that we used a number of cycles in the linear range. PCR products were electrophoresed on 2% agarose gels and band intensities were quantified using Kodak Electrophoresis Documentation and Analysis System 290 (EDAS 290).

Promoter Studies To evaluate the effect of nicotine and OPN on MMP-9 transcription, we used the MMP-9 gene promoter in luciferase expression vector pGL_3 basic (Promega) which was kindly provided by Dr. Dina Lev, University of Texan, MD Anderson, Houston, TX.[22] To evaluate the effect of nicotine and OPN on VEGF transcription, we used the VEGF gene promoter (GenBank™ accession no. AY102626) in luciferase expression vector pGL_3 basic (Promega), kindly provided by Dr. Marta Ruiz-Ortega, Universidad Autónoma, Madrid, Spain.[23] Cells were seeded into six-well culture plates (1×10^5). At 80% confluence, they were transfected by TransFast reagent (Promega) with either the MMP-9 or VEGF luciferase-labeled promoters. Two hours later, serum-containing medium was overlaid and the cells were incubated for an additional 24 h. The cells then were incubated with serum-free medium for 18 h, after which nicotine or OPN was added for 3 h. Luciferase activities were assayed with the Dual-Luciferase Reporter Assay System (Promega) in a Veritas Microplate Luminometer (Turner Designs, Sunnyvale, CA). Transfection efficiency was normalized using the total protein concentration of the cell lysates. The results for nicotine-treated cells were expressed as a fold induction of the luciferase activity of the same construct in the control condition, taking the control (no treatment) value as 100.

Human Tissue Acquisition and Analysis Human PDA ($n=73$) and premalignant specimens (IPMN, $n=6$) were obtained from patients who underwent surgical resection at Thomas Jefferson University Hospital between 2006 and 2008. All patients signed an appropriate consent for tissue acquisition and study. The study was approved by the Institutional Review Board of Thomas Jefferson University. Patients' smoking history was examined and correlated with MMP-9 and VEGF expression levels.

Tissue samples were stored in *RNA Later* for RNA analysis or fixed in neutral formaline for histological processing. Sections at 5 μm were stained with H&E. To localize MMP-9, VEGF, and OPN, sections from the different tissues were analyzed by immunohistochemistry using MMP-9, VEGF, and OPN antibodies. A vectastain universal elite ABC kit and 3,3′-diaminobenzidine tetrahydrochloride chromogenic substrate (Vector Laboratories Inc.) was used according to the manufacturer's protocol to visualize the tissue reaction. Antibody specificity was validated with non-immune isotype serum. Negative control sections where the primary or secondary antibodies were omitted were also prepared.

Statistical Analyses All experiments were performed three to five times. Data were analyzed for statistical significance by ANOVA with post hoc Student's *t* test analysis. Data are presented as mean ± SEM. Continuous, normally distributed variables were analyzed by Student's *t* test. Spearman's rank correlation test was performed to analyze the correlation between OPN, MMP-9, and VEGF mRNA expression. Analyses were performed with the assistance of a computer program (JMP 5 Software SAS Campus Drive, Cary, NC). Differences were considered significant at $p \leq 0.05$.

Results

Nicotine Stimulates MMP-9 and VEGF mRNA Accumulation and Protein Production in Cultured PDA Cells To investigate whether nicotine can increase MMP-9 or VEGF mRNA accumulation in PDA cells, we used MIA PaCa-2 and AsPC-1 cells treated with or without nicotine (0.3–300 nM) for 3 and 24 h. Dose-dependent significant induction of MMP-9 and VEGF mRNA expression was seen with a maximum increase at 24 h in MIA PaCa-2 cells. (Fig. 1a, c). In AsPC-1 cells, doses between 0.3 and 30 nM of nicotine significantly increased MMP-9 and VEGF mRNA levels after 24 h of nicotine stimulation (Fig. 1b, d). To examine whether the increase in MMP-9 and VEGF mRNA levels in response to nicotine is associated with translation of their proteins, MMP-9 AND VEGF protein levels in the cells were determined by Western blotting and ELISA, respectively. MIA PaCa-2 (Fig. 2a) and AsPC-1 (Fig. 2b) cells showed the expression of MMP-9 protein at two molecular weight bands (~66 and 35 kDa). Addition of nicotine at 3 and 30 nM for 48 h increased the expression of both bands in MIA PaCa-2 cells (Fig. 2a) and the ~35-kDa band in AsPC-1 cells.

Using ELISA, a significant induction of VEGF protein secretion was noted: in MIA PaCa-2 cells from 38 to 280 pg/ml and 566 pg/ml after 48 h of 3 and 30 nM nicotine incubation, respectively (Fig. 2c), and in ASPC-1 cells from 119 to 1,058 pg/ml and 1464 pg/ml after 48 h of 3 and 30 nM nicotine incubation, respectively (Fig. 2d). Nicotine concentration and times were used according to our preliminary concentration studies with references to the values of VEGF release. These data indicate that MMP-9 and VEGF induction by nicotine is a general phenomenon seen in the tested PDA cells lines.

Nicotine Induces MMP-9 and VEGF Promoter Activity To investigate whether nicotine can directly increase MMP-9 or VEGF transcription, MIA PaCa-2 cells were transfected with MMP-9 or VEGF luciferase-labeled promoters and were treated with nicotine (3 and 30 nM) for 3 h. Nicotine significantly and dose-dependently activated the MMP-9 promoter (Fig. 3a) and the VEGF promoter (Fig. 3b). This suggests that MMP-9 and VEGF promoters respond directly to nicotine.

Since we showed previously that nicotine directly induces OPN transcription in PDA cells[19, 20] and since OPN was shown to increase MMP-9 and VEGF expression in other cells,[24–26] we tested the hypothesis that OPN contributes to the upregulation of MMP-9 and VEGF mRNA by nicotine.

RNAi Decreases OPN Expression and Reduces Nicotine-Mediated Upregulation of MMP-9 and VEGF We suppressed OPN expression in MIA PaCa-2 cells by selecting two 21-nt targets within the OPN cDNA for RNAi. Based on these targets, double-stranded 21-nt siRNA constructs were designed encoding sense and antisense siRNA, and the OPN levels were measured using real-time PCR. As shown in Fig. 4a, OPN mRNA expression level was significantly inhibited by ~60% with OPN siRNA construct. We added nicotine (3.30 nM) to these cells and evaluated MMP-9 and VEGF expression by real-time PCR. Addition of nicotine was unable to elicit an increase in MMP-9 (Fig. 4b) or VEGF mRNA (Fig. 4c). It also was apparent that cells lacking OPN also expressed less endogenous MMP-9 and VEGF mRNA. These data indicate that there is a relationship between the presence of intracellular OPN and the cell response to nicotine to produce MMP-9 or VEGF.

To confirm our data, we blocked OPN function by adding OPN rabbit polyclonal antibody (0.4 μg/ml) 1 h before the addition of nicotine (3 nM) for 3 h. As seen in Fig. 4d, e, blocking OPN resulted in the complete prevention of the nicotine-mediated stimulation of MMP-9 or VEGF promoter activities. Cells that expressed normal levels of OPN expressed higher levels of basal MMP-9 and VEGF promoter activities when compared with cells where OPN expression was blocked. This indicates that OPN is essential for the nicotine-mediated induction of MMP-9 and

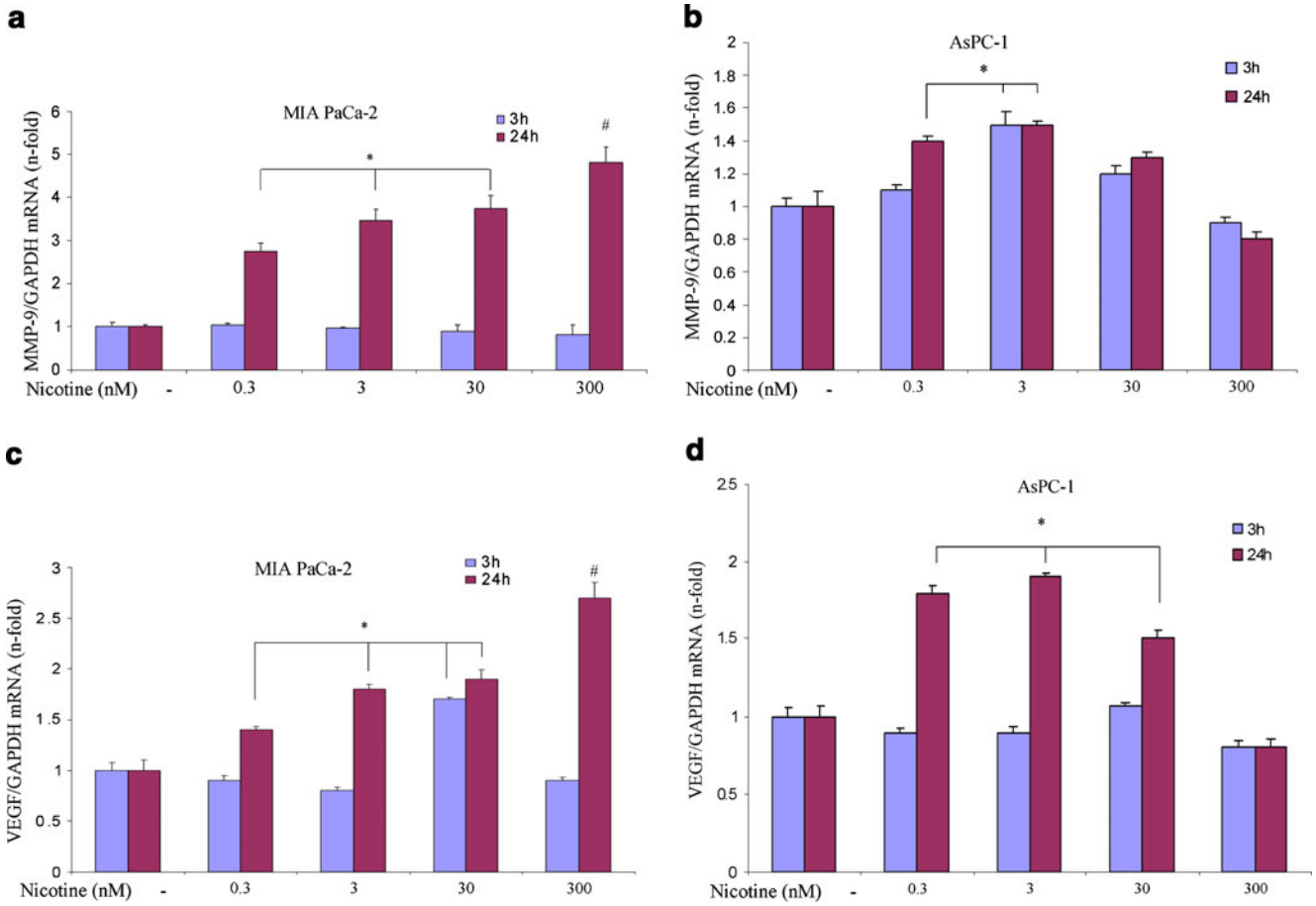

Fig. 1 Nicotine induces MMP-9 (**a**, **b**) and VEGF (**c**,**d**) mRNA accumulation in cultured PDA cells. MIA PaCa-2 (**a**) and AsPC-1 (**b**) cells were treated with nicotine (0.3–300 nM) for 3 and 24 h. Significant induction of MMP-9 mRNA expression is seen with the maximum induction after 24 h in both cell lines. MIA PaCa-2 (**c**) and AsPC-1 (**d**) cells were treated with nicotine (3–300 nM) for 3 and 24 h. Significant increase of VEGF mRNA expression is seen after 24 h in both cell lines. Values are expressed as mean ± SEM of three experiments. $^{*}p<0.05$, $^{\#}p<0.02$ vs. control untreated cells using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test

VEGF transcription. Next, we questioned whether OPN itself could induce MMP-9 or VEGF transcription in PDA cells.

OPN Increases MMP-9 and VEGF mRNA Expression in PDA Cells Recombinant OPN protein (0.15–15 nM) was added for 3 h to MIA PaCa-2 cells. OPN significantly and dose-dependently stimulated MMP-9 and VEGF mRNA expression (Fig. 5a, b).

In other experiments, OPNc plasmid was transiently transfected into MIA PaCa-2 cells. UV light illumination of ethidium bromide-stained PCR products after agarose gel electrophoresis showed a 155-bp band for OPNc and a 208-bp band of total OPN, confirming overexpression of OPNc and total OPN (Fig. 5c). In addition, ELISA analysis showed an ~14-fold increase of medium OPN (Fig. 5c). Real-time PCR analysis showed that overexpressing OPNc increased MMP-9 mRNA by ~15-fold, whereas OPN siRNA reduced MMP-9 mRNA by 30% below basal levels (Fig. 5d). OPNc also increased VEGF mRNA by twofold, whereas reduction of OPN expression was associated with an ~50% decrease of VEGF basal levels (Fig. 5e). These data suggest that high levels of intracellular OPN could on its own increase the expression levels of MMP-9 and VEGF. Next, we analyzed the localization of OPN and MMP-9 and VEGF in PDA cells in vitro.

Co-localization of OPN, MMP-9, and VEGF in PDA Cells Confocal microscopy after immunofluorescence staining of MIA PaCa-2 cells with antibodies against OPN and MMP-9 and VEGF shows co-localization of all three proteins in the cytosol of PDA cells (Fig. 6a, b). OPN (red) was expressed in the form of granules that could be localized in the cytosol and cell membrane of PDA cells. MMP-9 also has a granular appearance and was expressed similarly in the cytosol and cell membrane (Fig. 6a). VEGF was mainly expressed in the cytosol and showed a more homogenous pattern (Fig. 6b).

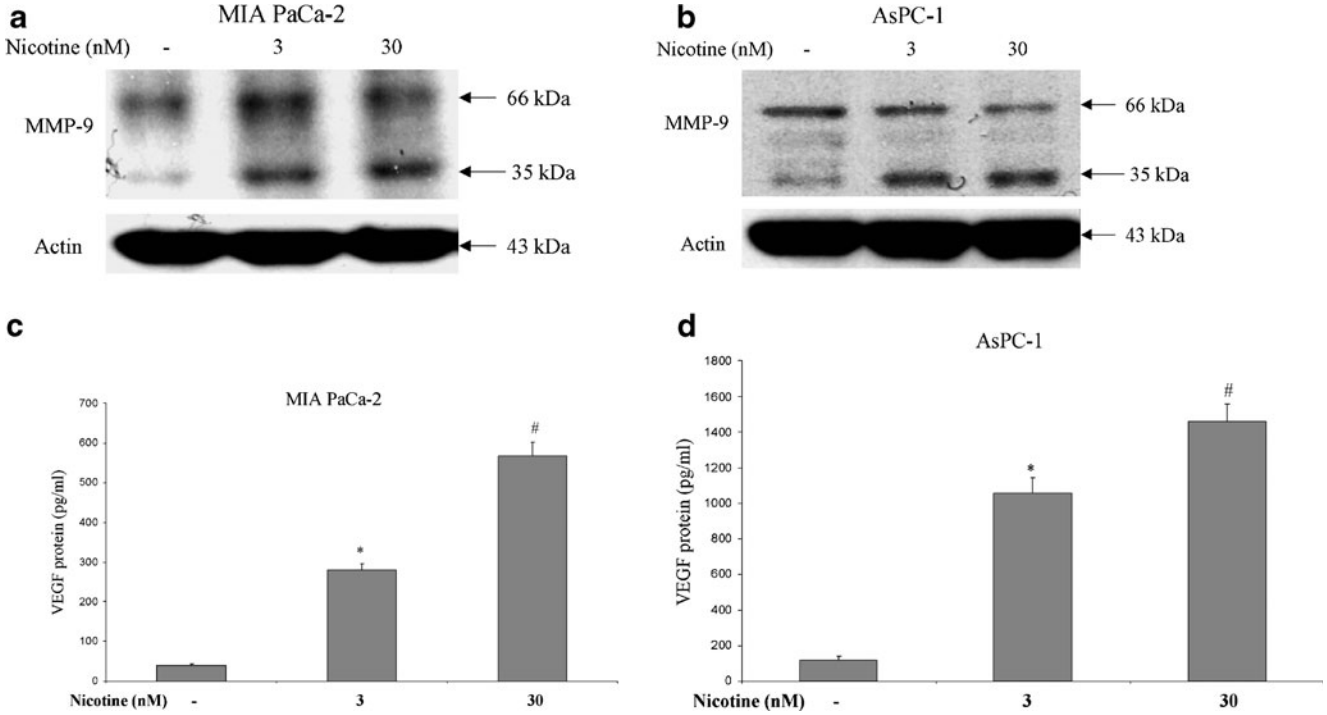

Fig. 2 Representative Western immunoblot showing the nicotine-mediated increased expression of MMP-9 protein in MIA PaCa-2 (**a**) and AsPC-1 cells (**b**) that is seen at two bands at ~66 and ~35 kDa. VEGF protein in culture media was measured using a human-specific ELISA kit. Dose-dependent increase of VEGF protein secretion is seen in MIA PaCa-2 cells (**c**) and AsPC-1 cells (**d**). Each experiment was repeated three times for reproducibility. Values are expressed as mean ± SEM of three experiments. *$p<0.05$, $^{\#}p<0.005$ vs. control levels using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test

We next examined the endogenous levels and localization of MMP-9 and VEGF and OPN in premalignant IPMN and malignant PDA lesions.

Expression of OPN in Human PDA Using immunohistochemical staining, we found that MMP-9 and VEGF were absent from the normal pancreatic ducts, whereas OPN was focally present, mostly on the apical surface of the ductal epithelium.[19] In PDA tissue from non-smokers (Fig. 7a) and smokers, OPN ductal epithelial staining was intensified and localized to the cell membrane and cytoplasm of the tumor cells. MMP-9 and VEGF co-localized with OPN in the malignant ducts. It was apparent, however, that the periductal stromal tissue was intensely stained in PDA tissue from smokers (Fig. 7b).

Analysis of quantitative PCR data of MMP-9 and VEGF mRNA corrected with GAPDH as an internal control

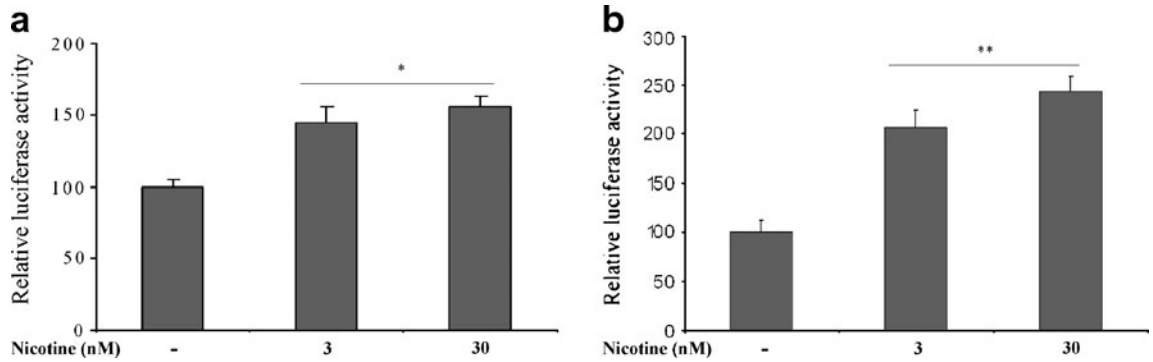

Fig. 3 Nicotine induces MMP-9 (**a**) and VEGF (**b**) promoter activity in MIA PaCa-2 cells. After 24 h of transfection with luciferase-labeled promoter, the cells were incubated with nicotine (3 and 30 nM) for 3 h. Luciferase activity in the cell lysates was measured. Relative luciferase activity was calculated after deduction of the activity levels with pGL3 vector alone. Results represent mean ± SEM of triplicate determinations. *$p<0.05$, **$p<0.02$ vs. control levels using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test

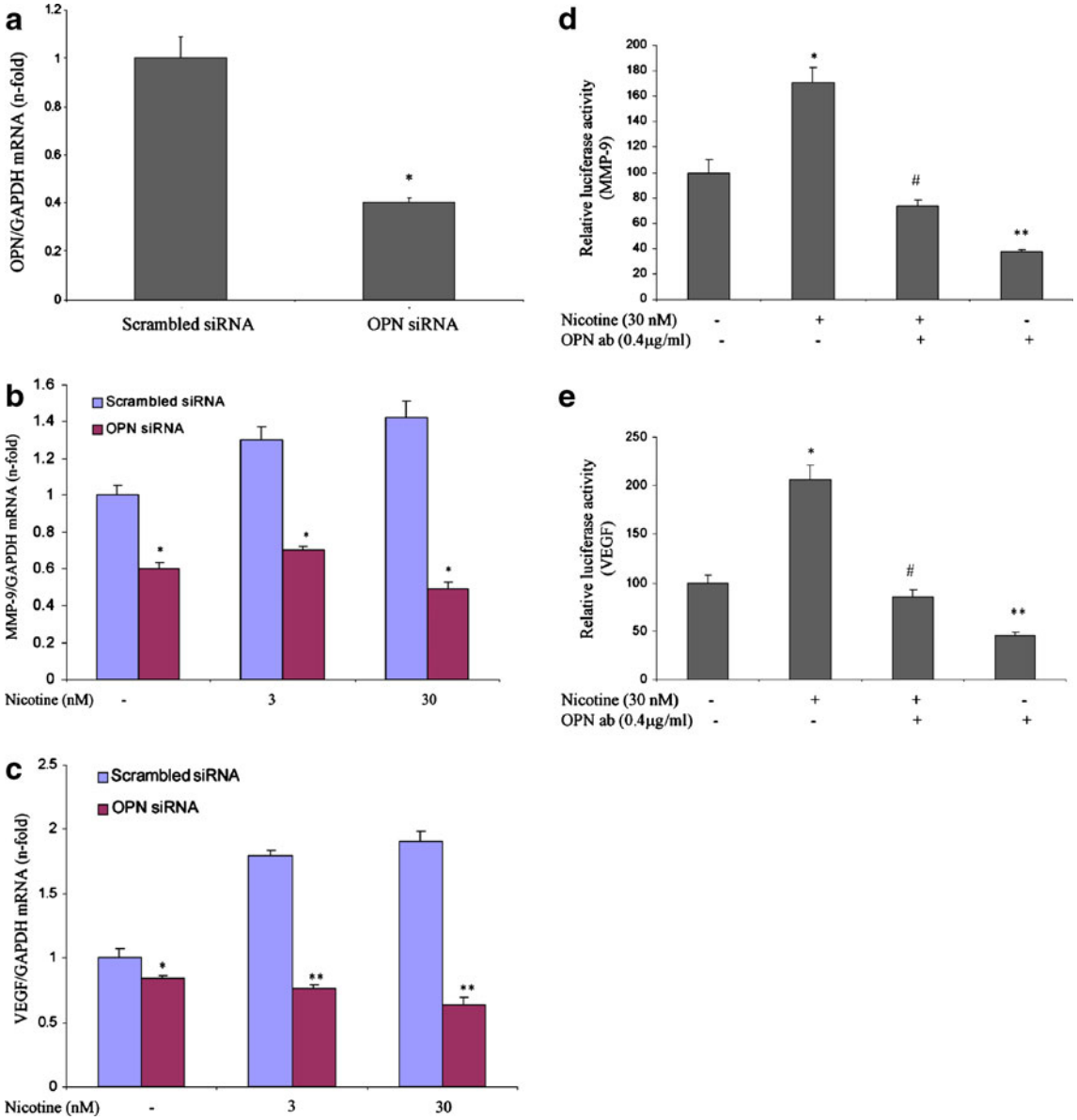

Fig. 4 **a** Real-time PCR analysis showing the specificity and level of OPN knockdown in MIA PaCa-2 cells. Cells were transfected for 24 h with either scrambled siRNA or OPN siRNA. Forty-eighthours after transfection, cells were harvested and RNA isolated. Data (OPN/GAPDH) represent mean ± SE from three independent experiments. *$p<0.005$ vs. scrambled siRNA cells using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test. MIA PaCa-2 cells transfected with OPN siRNA or scrambled siRNA for 24 h and treated with or without nicotine (3 and 30 nM) for 24 h showing the expression of MMP-9 mRNA (**b**) and VEGF (**c**). Values are expressed as mean ± SEM of three experiments. *$p<0.05$, **$p<0.005$ vs. scrambled siRNA-transfected cells levels using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test. Inhibition of MMP-9 (**d**) and VEGF (**e**) promoter activities in MIA PaCa-2 cells treated with rabbit polyclonal OPN antibody for 1 h and treated with nicotine (30 nM) for 3 h. Values are expressed as mean ± SEM of three experiments. *$p<0.05$ vs. control; $^{\#}p<0.05$, **$p<0.02$ vs. nicotine-treated cells using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test

demonstrated that invasive PDA lesions from patients who were smokers have significantly higher levels of MMP-9 (Fig. 7c) and VEGF (Fig. 7d) mRNA when compared to PDA tissue from nonsmokers ($p<0.05$) and IPMN premalignant lesions ($p<0.002$). Interestingly, 66% of the invasive lesions (48 of 73) were taken from patients who were smokers (Fig. 7c, d).

To correlate the expression levels of MMP-9 and VEGF with OPN in PDA tissue, the level of mRNA for each gene was recorded. Relative quantification values of MMP-9, VEGF, and OPN/GAPDH of >1 indicated high levels and were labeled (+++), values of 0.5–1 were labeled (++), of 0.1–0.5 were labeled (+), and of <0.1 were labeled (−). Expression of MMP-9 (Fig. 7e) and VEGF (Fig. 7f)

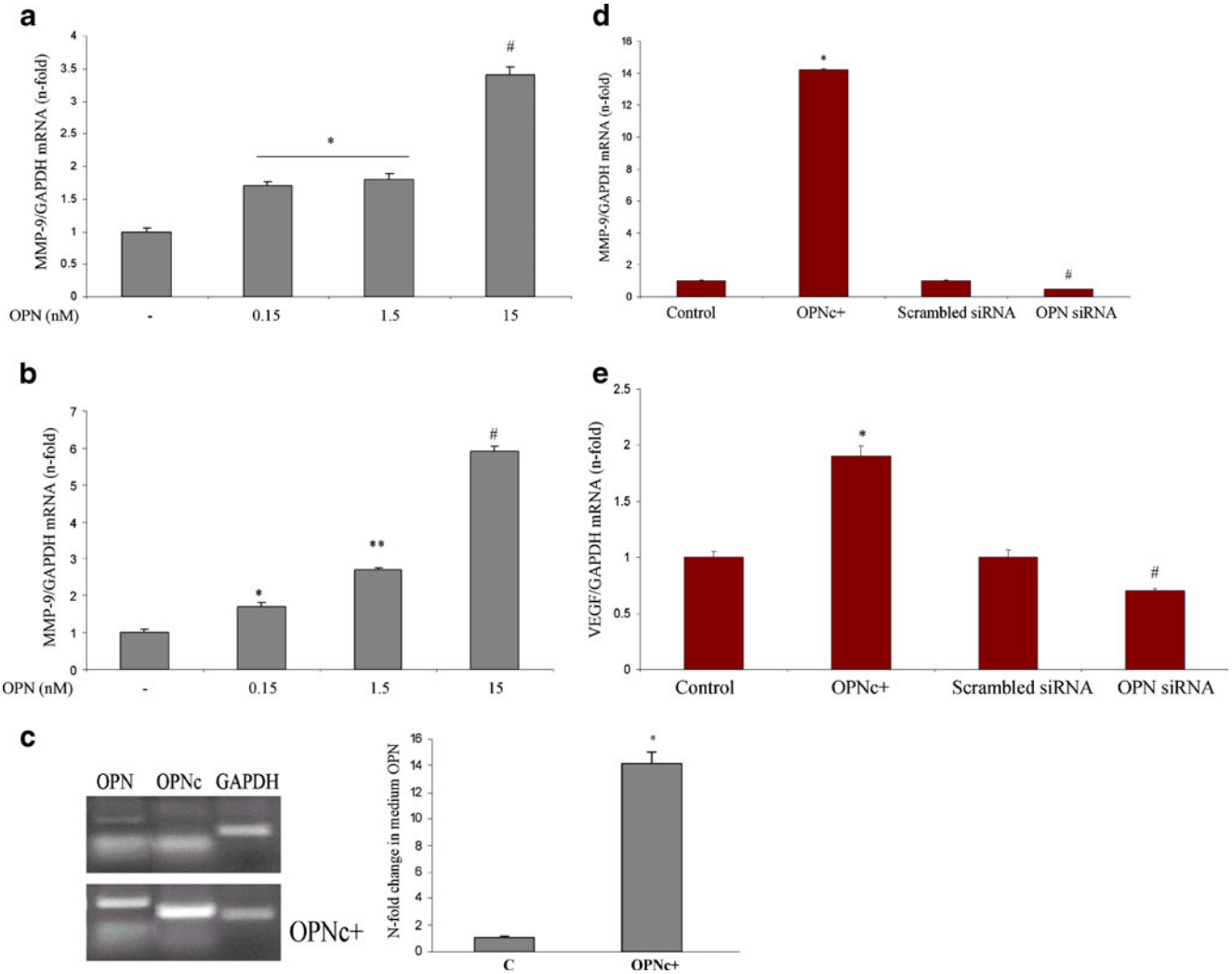

Fig. 5 Dose-dependent increase of MMP-9 (**a**) and VEGF (**b**) mRNA levels in MIA PaCa-2 cells that were incubated with OPN (0.15–15 nM) for 24 h. Results represent the mean ± SEM of triplicate determinations. *$p<0.05$, **$p<0.02$, $^{\#}p<0.002$ vs. control using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test. **c** Transient transfection of OPNc in MIA PaCa-2 cells. *Left panel* Representative agarose gel with the PCR product of PDA cells showing the expression of total OPN and OPNc (208-, 155-, and 109-bp bands correspond to the amplified OPN, OPNc, and GAPDH, respectively). *Right panel* ELISA analysis of culture media from these cells shows significant increase in OPN protein expression in the media. *$p < 0.05$ vs. control cells. Real-time PCR analyses show significant increase of MMP-9 (**d**) and VEGF (**e**) mRNA in MIA PaCa-2 cells that overexpress OPNc. In cells where OPN was knocked down by specific siRNA, significantly lower levels of both genes are seen. *$p<0.05$, $^{\#}p<0.05$ vs. control levels using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test

paralleled OPN expression levels. These data suggest that increased OPN expression in smokers is associated with the expression of MMP-9 and VEGF.

Discussion

In this study, we evaluated the potential role of nicotine as a major risk factor in PDA. We show for the first time an interesting relationship between nicotine and two metastasis-related factors: MMP-9 and VEGF. We also show that OPN, a pro-inflammatory and prometastatic protein, is involved as a mediator for this interaction.

Most deaths in PDA and other cancers are due to metastatic disease. Breakdown of the extracellular matrix and angiogenesis are required for metastasis to occur. MMP-9 and VEGF play important roles in this process. Although several studies have shown that MMPs and VEGF are overexpressed in PDA and play important roles in its progression,[27–30] and that their overexpression is regarded as a prognostic factor in PDA,[28, 30] very few have investigated their relationship to smoking and the upstream factors involved in their regulation in PDA cells. In this

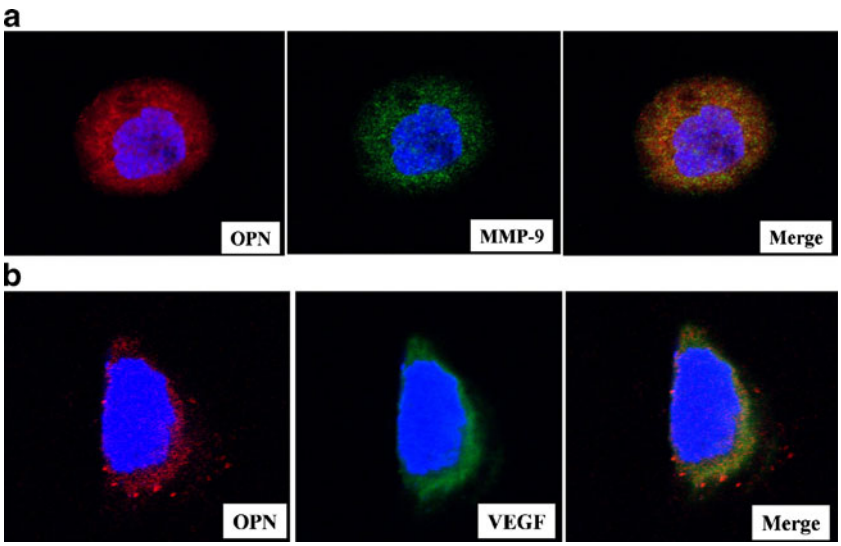

Fig. 6 Double immunofluorescence analysis of OPN and MMP-9 (**a**) and OPN and VEGF (**b**) in PDA cells. Optical sections at 2-μm intervals from the dorsal to the ventral surface of MIA PaCa-2 cells were immunostained for both OPN (*red*) and MCP-1 or VEGF (*green*) and examined by confocal microscopy. OPN and MMP-9 show granular appearance and are localized in the cytosol and cell membrane of PDA cells, whereas VEGF shows a more homogenous pattern and was present in the cytosol. The merged images show co-localization of both proteins with OPN in the cytosol of PDA cells (×600 original magnification)

study, we show for the first time that nicotine treatment time-dependently increased MMP-9 and VEGF expression in PDA cells. We also demonstrate a previously undescribed role for OPN as a mediator for these effects.

Our data show that nicotine induced MMP-9 and VEGF accumulation with significant magnitude. Dose–response studies demonstrated a significant induction of MMP-9 and VEGF mRNA and protein levels at the physiological range of blood levels of nicotine in smokers (0.3–300 nM). The maximal effective concentration (300 nM) is similar to other nicotine actions that have been previously reported.[31]

Our data further showed that the MMP-9 and VEGF promoters were significantly stimulated as early as after 3 h after exposure to nicotine. It is unknown what long-term effect this early response could have on pancreatic ductal cells that are continuously exposed to high blood nicotine levels in heavy smokers. Further studies are now required to analyze the chronic effects of nicotine on cell behavior and the expression of metastasis-related genes. In addition, the nicotine-specific *cis*-elements on both the MMP-9 and VEGF promoters and the transcription factor(s) involved in nicotine-mediated upregulation need to be evaluated. Studies in this regard are currently ongoing in our lab.

Since we showed previously that nicotine directly induces OPN transcription in PDA cells[19, 20] and since OPN was shown to increase MMP-9[24, 25] and VEGF expression in other cells,[26] we tested the hypothesis that OPN mediates the upregulation of MMP-9 and VEGF by nicotine.

We inhibited OPN synthesis by siRNA (Fig. 4a–c) or blocked its function by a polyclonal antibody against human OPN (Fig. 4d, e). In both studies, nicotine was unable to increase MMP-9 or VEGF expression or transcription in PDA cells, suggesting that OPN may play a role in mediating the effects of nicotine. Next, we treated PDA cells with recombinant human OPN protein (Fig. 5a, b) or overexpressed an OPN isoform (OPNc, Fig. 5c–e), which has been shown to promote metastasis in cancer cells.[32, 33] Exogenous addition of OPN significantly and dose-dependently increased the expression levels of both MMP-9 and VEGF (Fig. 5a, b). Elevating the intracellular levels of OPN by transfecting them with OPNc increased the basal expression levels of both MMP-9 and VEGF (Fig. 5d, e), an effect that was reversed when OPN was knocked down by siRNA (Fig. 5d, e). Previous studies have shown that nicotine induces the expression of total OPN.[19] and selectively induces the expression of OPNc in PDA cells.[20] This is the first report to demonstrate a relationship between nicotine, OPN/OPNc MMP-9 and VEGF. Confocal microscopy analysis also revealed intracytoplasmic co-localization of OPN with MMP-9 and VEGF in PDA cells (Fig. 6a, b), providing more evidence for the paracrine/autocrine relationship between the three molecules. Additional studies are now required to delineate the details of this relationship and the signaling pathways involved in mediating the increase of MMP-9 and VEGF by OPN. Furthermore, the effect of the nicotine-mediated increase of OPNc on PDA cell prometastatic and pro-angiogenic behavior and function are the subject of our currently ongoing studies in the laboratory. Such studies will have a tremendous impact on our understanding of the role of nicotine in PDA and will provide an opportunity to block its prometastatic effects in PDA and in other cancers.

Numerous studies have correlated high levels of MMP-9 expression with tumor invasion and progression in many cancers, including pancreatic cancer.[27, 28] MMP-9 promotes cell survival through inducing the expression of VEGF.[14] VEGF also promotes cell survival and angiogenesis.[29, 33] These functions are similar to those reported to be mediated by OPN.[26, 34] Our in vitro data suggest that OPN might be acting upstream of MMP-9 and VEGF to mediate these effects (Fig. 4).

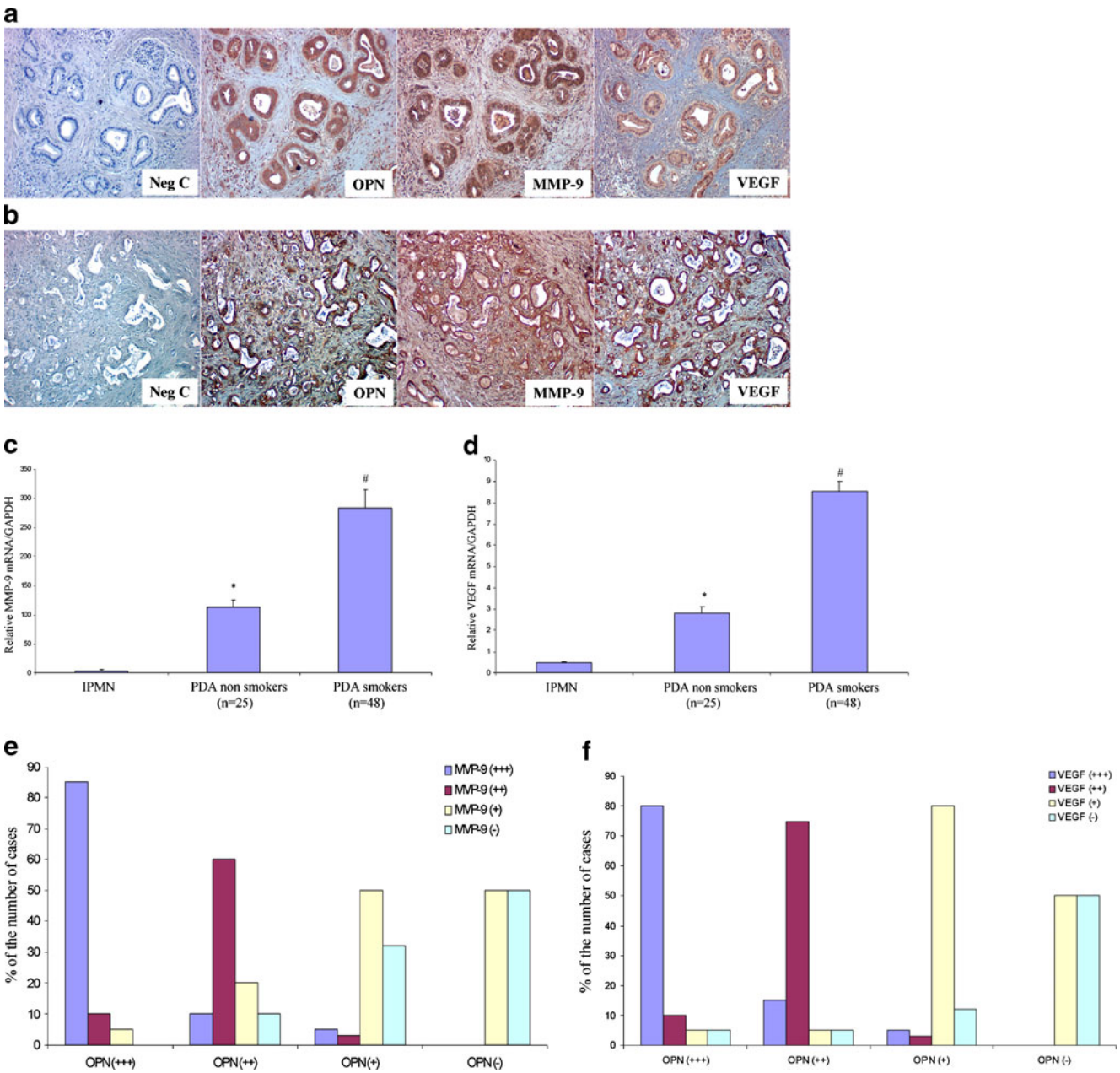

Fig. 7 **a** Representative immunohistochemical staining for OPN, MMP-9, and VEGF in malignant PDA from nonsmokers (**a**) and smokers (**b**). Serial sections of paraffin-embedded PDA sections were stained with OPN, MMP-9, and VEGF antibodies. All proteins co-localized in the malignant ductal epithelium with more stromal staining in PDA tissue from smokers (×100 original magnification). Negative control (*−ve C*) sections where the primary antibody was not added did not show non-specific reaction. Real-time PCR analysis of IPMN lesions and invasive PDA from smokers and nonsmokers show significantly higher MMP-9 (**c**) and VEGF (**d**) mRNA levels seen in invasive PDA. Analysis of patient history of the samples used for RNA analysis shows that invasive PDA patients were mostly (66%) smokers. $^{*}p<0.05$, $^{\#}p<0.005$ vs. IPMN levels using one-way repeated ANOVA with subsequent all pairwise comparison procedure by Student's *t* test. **e** Significant correlation ($p<0.05$) between tissue OPN and MMP-9. High MMP-9 (+++) was found in 85% of the invasive PDA samples that expressed high OPN (+++). **e** Significant correlation ($p<0.05$) between tissue OPN and VEGF. High VEGF (+++) was found in 80% of the invasive PDA samples that expressed high OPN (+++). Very low levels of MMP-9 and VEGF were was found in IPMN lesions that expressed very low levels of OPN

Studies in several cancers have correlated higher levels of MMP-9 and VEGF with tumor stage and high metastatic potential.[27–30] Our analyses reported here have found that MMP-9 and VEGF were found in 100% of invasive PDA lesions, of which 66% were smokers. This is the first report to examine the relationship between tumor MMP-9 and VEGF and the status of smoking in PDA patients. Our analysis also reveals that higher levels of these molecules

were seen in invasive PDA as compared to premalignant lesions (Fig. 7c, d). Immunohistochemical analysis of PDA showed that MMP-9 and VEGF co-localize with OPN in the malignant ducts and their mRNA levels significantly correlate with higher expression levels of OPN in the tissue from patients with invasive PDA (Fig. 7e, f). It remains to be determined, however, whether MMP-9 and VEGF levels correlate with pathologic stage, survival, or recurrence. We are currently performing these studies in addition to other studies to determine whether similar findings could be obtained from endoscopic ultrasound and fine needle aspiration samples.

Interestingly, our studies show that high levels of MMP-9 and VEGF and OPN exist in invasive lesions from non-smokers. This could be related to other factors that should be investigated, such as second-hand smoke (environmental tobacco smoke). It could also be related to chronic pancreatitis, which has been linked to pancreatic carcinogenesis,[35, 36] and could create a tumor microenvironment with higher levels of OPN, MMP-9, and VEGF. Additional studies addressing these possibilities are currently ongoing in our laboratory.

Our study suggests that cigarette smoking and nicotine may contribute to PDA matrix degradation, angiogenesis, and metastasis through inducing MMP-9 and VEGF and demonstrates a distinctive role of OPN in mediating these effects. Although the signaling events that involve the OPN-mediated induction of MMP-9 and VEGF in pancreatic carcinogenesis remain to be defined, the potential role of OPN as a downstream effector of nicotine, capable of mediating its prometastatic effects in PDA cells, is novel. OPN could be a unique potential target to control pancreatic cancer metastasis, especially in the cigarette-smoking population.

Acknowledgment This work was supported by NIH grant 1R21 CA133753-02.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer Statistics, 2008. CA Cancer J Clin 2008;52(2):71–96.
2. Wang XL, Wang J. Smoking-gene interaction and disease development: relevance to pancreatic cancer and atherosclerosis. World Journal of Surgery 2005;29:344–53.
3. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best Prac Res Clin Gastroenterology 2006;20:197–209.
4. US Department of Health and Human Services. The health consequences of using smokeless tobacco: a report of the advisory committee to the Surgeon General [NIH Publication no. 86-2874]. Bethesda, MD: USDHHS; April 1996.
5. Farrow DC, Davis S. Risk of pancreatic cancer in relation to medical history and use of tobacco, alcohol and coffee. Intl J Cancer 1990;45:816–20.
6. Silverman DT, Dunn JA, Hoover RN, Schiffman M, Lillemoe KD, Schoenberg JB et al. Cigarette smoking and pancreas cancer: a case control study based on direct interviews. J Natl Cancer Inst 1994;86:1510–6.
7. Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 1997;89(17):1260–70.
8. Coussens LM, Werb Z. Matrix metalloproteinases and the development of cancer. Chem Biol 1996;3:895–904.
9. Kelly T, Yan Y, Osborne RL, Athota AB, Rozypal TL, Colclasure JC, Chu WS. Proteolysis of extracellular matrix by invadopodia facilitates human breast cancer cell invasion and is mediated by matrix metalloproteinases. Clin Exp Metastasis 1998;16:501–12.
10. Nikkola J, Vihinen P, Vlaykova T, Hahka-Kemppinen M, Kahari VM, Pyrhonen S. High expression levels of collagenase-1 and stromelysin-1 correlate with shorter disease-free survival in human metastatic melanoma. Int J Cancer 2002;97:432–8.
11. Kahari VM, Saarialho-Kere U. Matrix metalloproteinases and their inhibitors in tumor growth and invasion. Ann Med 1999;31:34–45.
12. Kleiner DE, Stetler-Stevenson WG. Matrix metalloproteinases and metastasis. Cancer Chemother Pharmacol 1999;43:42–51.
13. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 1980;284:67–8.
14. Mira E, Lacalle RA, Buesa JM, de Buitrago GG, Jiménez-Baranda S, Gómez-Moutón C, Martínez-A C, Mañes S. Secreted MMP9 promotes angiogenesis more efficiently than constitutive active MMP9 bound to the tumor cell surface. J Cell Sci. 2004;117 (Pt 9):1847–57.
15. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995;1:27–31.
16. Kerbel RS. Tumor angiogenesis. The New England Journal of Medicine 2008;358(19):2039–49.
17. Takahashi Y, Cleary KR, Mai M, Kitadai Y, Bucana CD, Ellis LM. Significance of vessel count and vascular endothelial growth factor and its receptor (KDR) in intestinal-type gastric cancer. Clin Cancer Res 1996;2:1679–84.
18. Takahashi Y, Kitadai Y, Bucana CD, Cleary ER, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res1995;55:3964–68.
19. Chipitsyna G, Gong Q, Anandanadesan R, Alnajar A, Batra SK, Denhardt ST, et al. Induction of osteopontin (OPN) expression by nicotine and cigarette smoke in the pancreas and pancreatic ductal adenocarcinoma cells. Int J Cancer 2009;125:276–85.
20. Sullivan J, Blair L, Alnajar A, Aziz T, Ng CY, et al. Expression of a prometastatic splice variant of osteopontin, OPNc, in human pancreatic ductal adenocarcinoma. Surgery 2009;146:232–40.
21. Takafuji V, Forgues M, Unsworth E, Goldsmith P, Wang XW. An osteopontin fragment is essential for tumor cell invasion in hepatocellular carcinoma. Oncogene 2007;26(44):6361–71.
22. Liu J, Zhan M, Hannay JA, Das P, Bolshakov SV, Kotilingam D, Yu D, Lazar AF, Pollock RE, Lev D. Wild-type p53 inhibits nuclear factor-kappaB-induced matrix metalloproteinase-9 promoter activation: implications for soft tissue sarcoma growth and metastasis. Mol Cancer Res. 2006 Nov;4(11):803–10.
23. Sánchez-López E, López AF, Esteban V, Yagüe S, Egido J, Ruiz-Ortega M, Alvarez-Arroyo MV. Angiotensin II regulates vascular endothelial growth factor via hypoxia-inducible factor-1alpha induction and redox mechanisms in the kidney. Antioxid Redox Signal. 2005;7(9-10):1275–84.
24. Liu H, Chen A, Guo F, Yuan L. A short-hairpin RNA targeting osteopontin downregulates MMP-2 and MMP-9 expressions in prostate cancer PC-3 cells. CancerLett. 2010;295:27–37
25. Dai J, Peng L, Fan K, Wang H, Wei R, Ji G, Cai J, Lu B, Li B, Zhang D, Kang Y, Tan M, Qian W, Guo Y. Osteopontin induces angiogenesis through activation of PI3K/AKT and ERK1/2 in endothelial cells. Oncogene. 2009; 28(38):3412–22.

26. Chakraborty G, Jain S, Kundu GC. Osteopontin promotes vascular endothelial growth factor-dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms. Cancer Res. 2008 Jan 1;68(1):152–61.
27. Tian M, Cui YZ, Song GH, Zong MJ, Zhou XY, Chen Y, Han JX. Proteomic analysis identifies MMP-9, DJ-1 and A1BG as over-expressed proteins in pancreatic juice from pancreatic ductal adenocarcinoma patients. BMC Cancer. 2008;8:241
28. Mroczko B, Lukaszewicz-Zajac M, Wereszczynska-Siemiatkowska U, Groblewska M, Gryko M, Kedra B, Jurkowska G, Szmitkowski M. Clinical significance of the measurements of serum matrix metalloproteinase-9 and its inhibitor (tissue inhibitor of metalloproteinase-1) in patients with pancreatic cancer: metalloproteinase-9 as an independent prognostic factor. Pancreas. 2009;38(6):613–8.
29. Toomey DP, Manahan E, McKeown C, Rogers A, McMillan H, Geary M, Conlon KC, Murphy JF. Vascular endothelial growth factor and not cyclooxygenase 2 promotes endothelial cell viability in the pancreatic tumor microenvironment. Pancreas. 2010;39:595–603
30. Sandblom G, Granroth S, Rasmussen IC. TPS, CA 19-9, VEGF-A, and CEA as diagnostic and prognostic factors in patients with mass lesions in the pancreatic head. Ups J Med Sci. 2008;113(1):57–64.
31. Hukkanen, J., Jacob, P. 3 rd and Benowitz, N.L. Metabolism and disposition kinetics of nicotine. Pharmacol Rev 2005;57:79–115.
32. Mirza M, Shaughnessy E, Hurley JK, Vanpatten KA, Pestano GA, He B, et al. Osteopontin-c is a selective marker of breast cancer. Int J Cancer 2008;122:889–97.
33. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J Cell Mol Med. 2005;9(4):777–94.
34. Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest 2001;107:1055–61.
35. Rocca G, Gaia E, Iuliano R. Increased incidence of cancer in chronic pancreatitis. J Clin Gastroenterol. 1987;9:175–9.
36. Maisonnneuve P, Lowenfels AB. Chronic pancreatitis and pancreatic cancer. Dig Dis. 2002;20:32–7

Discussant

DR. MARY MALUCCIO (Indianapolis, IN): You make a very convincing argument for the link between nicotine, osteopontin, MMP9, and VEGF in pancreatic cancer. I think your blocking studies are quite elegant and certainly prove your point. This only adds to the long list of reasons that people should stop smoking.

Unfortunately, even with a much stronger correlation between smoking and the more prevalent lung cancer, most people do not stop smoking until the die is cast. And there are millions upon millions of people that smoke and only 40 some odd thousand cases per year of pancreatic cancer.

So surveying smokers is not a realistic option. Thus, I struggle a bit to envision how these data influence the diagnosis or treatment of pancreatic cancer.

Your data do not suggest that prevention models would work since you did not show that this pathway influences the development of cancer. A preclinical model, whereby you alter the development of cancer, like in the KRAS mutant mouse model or if you can alter the biologic behavior of an established pancreatic cancer by blocking osteopontin would make a much more convincing argument that this is a good target.

The major obstacle in pancreatic cancer continues to be that there is no truly high-risk patient population that we can survey in hopes of altering the detection and behavior of an inevitable pancreatic cancer. And therefore, our ability to intervene early is limited.

There are a couple of questions that I have for your group.

I believe that there are 10 to 20, if not more, pancreatic cancer cell lines through the ATCC, whereas you chose to study two. My question is, how did you decide to use these particular cell lines? And are there cell lines that are more or less likely to respond to nicotine-induced changes?

Secondly, your paper shows very nice images of the human tissue with avid staining of osteopontin in pancreatic cancers from smokers vs. nonsmokers. And that if osteopontin levels are high, then the remaining molecules are also high.

In your experience, if you were to take 100 pancreatic cancer samples, regardless of smoking status, how many would you expect to show significant staining for osteopontin that would suggest that targeting this protein would be of therapeutic benefit?

Closing Discussant

DR. MELISSA LAZAR: To address your first question of how we chose these two cells lines, these are two cell lines that express sort of low levels of basal OPN, which is so that is why we chose them. So when we did overexpress OPNc, that made a difference. We have also used these two cell lines previously in some of our previous other studies with nicotine.

And then, if you did take 100 samples and you stained the tissue for OPN, all of the samples would stain for OPN. When we do stain just normal pancreatic tissue, there are some low levels of OPN staining in the ducts, but there are no levels of MMP9 or VEGF.

J Gastrointest Surg (2010) 14:1578–1591
DOI 10.1007/s11605-010-1335-3

2010 SSAT PLENARY PRESENTATION

National Trends in the Management and Survival of Surgically Managed Gallbladder Adenocarcinoma Over 15 years: A Population-Based Analysis

Skye C. Mayo · Andrew D. Shore · Hari Nathan · Barish Edil · Christopher L. Wolfgang · Kenzo Hirose · Joseph Herman · Richard D. Schulick · Michael A. Choti · Timothy M. Pawlik

Received: 4 May 2010 / Accepted: 12 August 2010 / Published online: 8 September 2010

Abstract
Introduction National Comprehensive Cancer Network (NCCN) guidelines recommend hepatic resection and lymphadenectomy (LND) for gallbladder adenocarcinoma (GBA). We sought to evaluate compliance with these recommendations and to assess trends in the management and survival of patients with GBA.
Methods Using Surveillance, Epidemiology and End Results (SEER)-Medicare-linked data, we identified 2,955 patients with GBA who underwent cancer-directed surgery from 1991 to 2005. We assessed clinicopathologic data, trends in surgical management, and survival.
Results From 1991 to 2005, preoperative evaluation included CT (62%), MRI (6%), and PET (2%). Only 383 (13%) patients underwent radical resection/hepatectomy with a temporal increase over the study period (1991–1995, 12%; 1996–1999, 10%; 2000–2002, 12.0%; 2003–2005, 16%; $P<0.001$). For patients undergoing radical resection/hepatectomy, LND≥3 nodes was performed in 96 (3%) patients. Among patients who had LND, 47% had nodal metastasis. The overall 1-, 3-, and 5-year survival was 56%, 30%, and 21%. On multivariate analysis, radical resection/hepatectomy (hazard ratio (HR)=0.71) and LND≥3 nodes (HR=0.56) were independently associated with increased survival. There was no significant improvement in survival over time ($P=0.60$).
Conclusions Compliance with NCCN guidelines for GBA remains poor. Survival of patients with surgically managed GBA has not improved over time.

Presented at the Society for Surgery of the Alimentary Tract 51st Annual Meeting and 25th Annual Residents and Fellows Research Conference, New Orleans, LA, May 2nd, 2010.

S. C. Mayo · H. Nathan · B. Edil · C. L. Wolfgang · K. Hirose · R. D. Schulick · M. A. Choti · T. M. Pawlik (✉)
Department of Surgery,
The Johns Hopkins University School of Medicine,
Harvey 611, 600 N. Wolfe Street,
Baltimore, MD 21287, USA
e-mail: tpawlik1@jhmi.edu

A. D. Shore
Health Policy and Management,
The Johns Hopkins Bloomberg School of Public Health,
Baltimore, MD, USA

J. Herman
Radiation Oncology,
The Johns Hopkins Hospital,
Baltimore, MD, USA

Keywords Gallbladder cancer · Surgery · SEER · Medicare · Survival

Introduction

Gallbladder adenocarcinoma (GBA) is a relatively uncommon, but aggressive malignancy. In 2009, there were 9,760 cases of GBA with an associated 5-year survival of only 15.3%.[1] Although GBA has traditionally been associated with a poor prognosis, surgery has been advocated as a means to improve long-term survival. Based upon data from several retrospective studies that demonstrated a survival benefit,[2–8] "radical" re-operation is the current National Comprehensive Cancer Network (NCCN) guideline recommendation for patients with stages 1 to 3b GBA.[9,10] Specifically, re-operation is recommended for patients with

T1b (invasion in the muscularis layer), T2 (invades perimuscular connective tissue; no extension beyond serosa or into liver), or T3 (perforation of serosa and/or liver invasion and/or adjacent organ or structure) disease.[10] In general, "radical" re-operation includes hepatic resection, lymph node dissection, and possibly common bile duct resection with reconstructive hepaticojejunostomy. Prognosis after surgery, however, can vary dramatically with reported 5-year survival ranging from 10% to 63%,[11,12] depending on the extent of disease.

Data on compliance with the NCCN guidelines, as well as temporal trends in population-based survival of patients with GBA remain poorly defined. Most data about survival of patients with GBA comes from single institution series.[2,4,6,13–15] These data may be susceptible to publication bias, as well as not accurately reflect "true" population-based outcomes of patients treated with surgical therapy for GBA. In addition, trends in survival after surgical therapy for GBA have not been investigated in a population-based study. Similarly, while data on the utilization of surgical management of early-stage gallbladder cancer in the USA has previously been reported,[16,17] it was limited in scope. Specifically, previous reports were derived from data exclusively drawn from the Surveillance, Epidemiology and End Results (SEER) cancer registry.[14,16–18] While the SEER registry provides tumor specific data, it contains more limited data about peri-operative or surgical procedure utilization. Rather, the use of Medicare claims data linked with SEER data has been demonstrated to be more effective in accurately capturing all surgical and peri-operative procedures than use of either dataset alone.[19]

As such, the objective of the current study was to evaluate compliance with the NCCN guidelines, as well as define the specific utilization of peri-operative and operative procedures, for patients with GBA employing the SEER-Medicare-linked dataset. In addition, we sought to assess whether there have been improvements in the survival of patients with surgically managed GBA on a population basis over time.

Methods

Data Source

We performed a retrospective analysis of prospectively collected data from the linked SEER-Medicare database. These data reflect the linkage of two large population-based sources of data that provide detailed information about Medicare beneficiaries with cancer. The SEER database is maintained by the National Cancer Institute.[20] The SEER database began in 1973 and today includes data from 18 cancer registries, representing approximately 26% of the USA population. All SEER data are de-identified and publicly available. The SEER program of cancer registries collects clinical, demographic and cause of death information for persons with cancer. Available data include patient demographics, sociodemographic information, SEER stage of disease, use of cancer-directed surgery, as well as use of radiation therapy.

Data elements more specific to cancer staging and treatment (e.g., American Joint Committee on Cancer (AJCC) staging), details of surgical therapy, tumor size, lymph node involvement) are consistently available only in more recent time periods (e.g., after 1988). The SEER-Medicare data represents linkage of the SEER data to Medicare claims for covered health care services from the time of a person's Medicare eligibility until death.[21] These linked data are available from 1991 forward. Medicare's master enrollment file is used to identify persons in the SEER data who are Medicare beneficiaries. The SEER-Medicare data include over 3.3 million persons with cancer. For people who are Medicare eligible, the SEER-Medicare data include claims for covered health care services, including hospital, physician, outpatient, home health, and hospice bills. These data include the original surgical resection in addition to any peri-operative procedural interventions in both the inpatient and outpatient settings around the time of the operation. Per the data usage agreement with the National Cancer Institute, any variable totals with a value less than 11, whether directly reported or inferred, was replaced throughout the document with $n<11$ and the proportion (X%) was calculated using 11 divided by the total.

Case Definitions

Our analysis included patients in whom incident cases of gallbladder cancer were diagnosed between 1991 and 2005, corresponding to the inception of SEER-Medicare linkage to the latest update in our gallbladder cancer dataset (incidence site recode 239). Patients with gallbladder adenocarcinoma were identified by using the International Classification of Diseases for Oncology (ICD-O-3) histology codes.[22] The histology codes (Table 1) were chosen to identify only patients with gallbladder adenocarcinoma. All histology codes were reviewed by a gastrointestinal pathologist at Johns Hopkins Hospital and were selected to be the most representative of the pathology of interest (i.e., adenocarcinoma). Cases with histology codes corresponding to histologies other than adenocarcinoma (e.g., 8070=squamous cell carcinoma) were excluded from the analysis. Only patients undergoing cancer-directed surgery who were actively followed were included; all patients diagnosed at autopsy or by death certificate were excluded. Patients with AJCC stage T4 tumors and those with metastatic disease at the time of their cancer-directed operation were also excluded from the analysis.

Table 1 ICD-O-3 histology codes for identification of gallbladder adenocarcinoma

Histology code	Number of patients (%) (n=2,955)
8140	2,264 (76.6)
8260	205 (6.9)
8010	108 (3.7)
8480	86 (2.9)
8481	44 (1.5)
8210	26 (0.9)
8144	20 (0.7)
8261	20 (0.7)
8160	18 (0.6)
8263	16 (0.5)
8050	13 (0.4)
8255	<11 (<0.4%)
8020	<11 (<0.4%)
8141	<11 (<0.4%)
8000	<11 (<0.4%)
8145	<11 (<0.4%)
8211	<11 (<0.4%)
8262	<11 (<0.4%)
8470	<11 (<0.4%)
8471	<11 (<0.4%)

Per NCI data usage agreement, no cells with totals less than 11 were reported

The AJCC 7th edition[10] T-stage was derived using the extent of disease information (i.e., 20=muscularis propria=T1b).[16,17] Extent of lymph node disease could not be stratified according to the recent 7th edition that now distinguishes N1 (i.e., lymph nodes adjacent to the cystic duct, bile duct, hepatic artery and portal vein) from N2 (i.e., celiac, periduodenal, and peripancreatic lymph nodes) disease. Because such data were not available, the SEER variable "extent of nodal disease" was transformed into a categorical variable indicating N0 and N1 per the AJCC 6th edition.[23] Those patients who underwent cancer-directed surgical procedures were identified using site-specific surgery codes 10–90 or surgery of primary site codes 10–90. Patients were divided into two groups on the basis of these variables: simple resection and radical resection. Simple resection was defined within the SEER coding manual as "simple or total removal of the primary site" and herein is referred to as "simple cholecystectomy." Radical resection was defined by the SEER designation "partial or total removal of the primary site with an en bloc resection (partial or total removal) of other organs".[24] The radical resection variable and a variable from the linked Medicare database indicating a hepatectomy were subsequently combined into a composite variable to indicate that a radical resection/hepatectomy had taken place. The performance of a lymphadenectomy was determined in two different ways. Firstly, it was determined by examining the "regional nodes examined" variable with ≥1 lymph nodes examined qualifying as a lymphadenectomy. Some authors[18] have used a more strict definition of lymphadenectomy per the AJCC suggestion that lymphadenectomy be defined as ≥3 lymph nodes assessed; therefore, we also evaluated lymphadenectomy using this definition.

We were interested in evaluating utilization and incidence of select treatments, procedures, and complications within the peri-operative period (i.e., 6 months before or after the definitive cancer operation). As such, these events were identified in the Medicare portion of the database using a combination of Common Procedure Terminology codes and ICD-9-CM[22] diagnosis codes (Table 2). Several previous studies[25] of ICD-9-CM procedure and diagnosis codes for Medicare patients have demonstrated excellent agreement (>85%) between Medicare billing data and chart review for such data. Procedures reported on physician claims are considered highly valid, especially for acute complications.[26]

Statistical Analyses

Overall survival time was calculated from the date of GBA diagnosis to the date of last follow-up. The SEER database codes patients surviving less than 1 month as having zero time of survival. We redefined this zero survival time as 0.1 months. Patients surviving less than 30 days were classified as a post-operative death. Cumulative event rates were calculated using the method of Kaplan and Meier.[27] Univariate analyses were performed using the log-rank test to compare differences between categorical groups. Cox proportional hazards models[28] were developed using relevant clinicopathologic variables in order to determine the association of each with overall survival. The model was validated by checking against a forward stepwise Wald selection model as described by Hosmer and Lemeshow.[29] The overall fit of the multivariate models was assessed using the likelihood ratio test. Relative risks were expressed as hazard ratio (HR) with a 95% confidence interval (CI). The final model was evaluated for goodness-of-fit using the method proposed by May and Hosmer.[29,30] The data were separated into quartiles (1991–1995, 1996–1999, 2000–2002, and 2003–2005) based upon the year of operation. Trends in ordinal data were evaluated using the linear-by-linear association test.[31] Significance levels were set at $P \leq 0.05$. All tests were two-sided. All statistical analyses were performed using SPSS Version 18.0 (Chicago, Illinois).

Results

Of the 8,492 cases of gallbladder cancer identified from 1973 to 2005, 8,115 (95.6%) cases had a histology code

Table 2 CPT/ICD codes used in identification of Medicare claims

Procedure	CPT code	ICD-9 codes (outpatient)
Endoscopy	43234, 43235, 43239, 43241, 43242, 43245, 43250, 43251, 43256, 43258	42.24, 44.14, 45.13, 45.14, 45.16
Cholangiogram	74320	87.52, 87.53, 87.54
PTC	47500, 47505, 47510, 74363, 75980	51.98, 87.51
MRI abdomen (with and without contrast)	74181, 74182, 74183, 74185	88.97
CT abdomen (with and without contrast)	74150, 74160, 74170	87.41, 87.42, 87.72, 88.01, 88.02
PET	78811,78812, 78813, 78814, 78815, 78816	88.90, 92.04, 92.18
Portal vein embolization	37204, 75894	39.79
Diagnostic laparoscopy	49320, 49321, 49329	54.21
Laparoscopic cholecystectomy	47562, 47563	51.23, 51.24
Open cholecystectomy	47600, 47605, 47610, 47612, 47620	51.2, 51.21, 51.22
Hepatectomy		
Biopsy	47100	50.11, 50.12, 50.19
Partial	47120	50.22
Trisegmentectomy	47122	–
Right lobectomy	47125	–
Left lobectomy	47130	–
Lobectomy (either or NOS)	47125, 47130	50.3
Lymphadenectomy	38747, 38780	40.29, 40.50
Hepaticojejunostomy	47760, 47780, 47785, 47800, 47999	51.37
Excision of biliary tree	47711, 47712	51.64, 51.69
Whipple	48150, 48152, 48153, 48154	52.53, 52.7
Radiation therapy	77290, 77301, 77413, 77414, 77418, 77263, 77427, 77334	92.21–92.24, 92.26–99.29
Chemotherapy (intravenous)	96409, 96411, 96413, 96415, 96416, 96417	99.25
Re-exploration (takeback)	49000, 49002	54.11, 54.22
Percutaneous drain	47000, 49021, 49041, 49061, 75989	54.91
Accidental laceration		998.2
Post-operative hemorrhage		998.1–998.19
Post-hemorrhagic anemia		285.1
Anesthetic reaction		995.4
Wound dehiscence		998.3, 998.6, 998.83
Liver abscess		572.0
Peritonitis		567.2
Gastrointestinal hemorrhage		578.0, 578.1, 578.9
Gastrointestinal complications		997.4
Biliary fistula		576.4
Intestinal fistula		569.81
Stomach or duodenal fistula		537.4
Postoperative infection		998.5–998.59

consistent with a diagnosis of adenocarcinoma. Case selection was further restricted to patients without T4 or metastatic disease, as well as date of GBA diagnosis between 1991 and 2005 (e.g., the years available in the Medicare data set for linkage). As such, 2,955 (35.3%) cases were available for our analyses. The most prevalent histology code (n=2,264; 76.7%) was 8140 corresponding to "adenocarcinoma not otherwise specified" (Table 1).[22]

Table 3 Patient and tumor characteristics in patients with surgically managed gallbladder adenocarcinoma

Variable	1991–1995 (n=681) Number (%)	1996–1999 (n=543)	2000–2002 (n=833)	2003–2005 (n=898)	Total (n=2,955)
Percent of total	23.0	18.4	28.2	30.4	100
Mean age at diagnosis (years)±SD	76.7 (8.2)	76.2 (8.0)	77.0 (7.9)	77.0 (8.0)	76.8 (8.0)
Female	507 (74.4)	385 (70.9)	594 (71.3)	656 (73.1)	2,142 (72.5)
White	529 (77.7)	376 (69.2)	630 (75.6)	655 (72.9)	2,190 (74.1)
Married	304 (44.6)	245 (45.1)	365 (43.8)	390 (43.4)	1,304 (44.1)
Urban	622 (91.3)	487 (89.7)	782 (93.9)	833 (92.8)	2,724 (92.2)
Historic stage					
In situ	42 (6.2)	51 (9.4)	73 (8.8)	82 (9.1)	248 (8.4)
Localized	429 (63.0)	318 (58.6)	488 (58.6)	524 (58.4)	1,759 (59.5)
Regional	210 (30.8)	174 (32.0)	272 (32.7)	292 (32.5)	948 (32.1)
AJCC T-stage					
Tis	42 (6.2)	51 (9.4)	73 (8.8)	82 (9.1)	248 (8.4)
T1a	50 (7.3)	38 (7.0)	54 (6.5)	56 (6.2)	198 (6.7)
T1b	109 (16.0)	77 (14.2)	92 (11.0)	103 (11.5)	381 (12.9)
T1NOS	86 (12.6)	52 (9.6)	53 (6.4)	38 (4.3)	229 (7.7)
T2	134 (19.7)	110 (20.3)	236 (28.3)	301 (33.5)	781 (26.4)
T3	236 (38.2)	215 (39.6)	325 (39.0)	318 (35.4)	1,118 (37.8)
AJCC N-stage: N1*	78 (11.5)	59 (10.9)	115 (13.8)	145 (16.1)	397 (13.4)
Grade					
Well-differentiated	121 (17.8)	78 (14.4)	123 (14.8)	131 (14.6)	453 (15.3)
Moderately differentiated	240 (35.2)	200 (36.8)	309 (37.1)	343 (38.2)	1,092 (37.0)
Poorly differentiated	191 (28.0)	174 (32.0)	253 (30.4)	261 (29.1)	879 (29.7)
Undifferentiated/Unknown	129 (18.9)	91 (16.7)	148 (17.7)	165 (18.4)	531 (18.0)

Per NCI data usage agreement, no cells with totals less than 11 were reported

*P<0.05 significant level by test of trend

Patient and Tumor Characteristics

Table 3 shows the clinicopathologic features of the 2,955 patients. The mean age at diagnosis was 76.8 years (standard deviation 8.0 years) and the majority of patients were women (n=2,142; 72.5%). Most patients were white (n=2,190; 74.1%), lived in an urban setting (n=2,724; 74.1%), and were not married (n=1,691; 55.9%). At the time of GBA diagnosis, most patient's cancers were classified as "localized" (n=1,759; 59.5%) by the SEER historic stage[24] and over one third were AJCC stage T3[10] (n=1,118; 37.8%). Among those patients who had primary tumor grade (ICD-O-2) information available (n=2,511; 83.8%), most cancers were moderately differentiated (n=1,092; 37.0%). Tumor grade was associated with AJCC T-stage, as T3 cancers were more likely to be moderately and poorly differentiated (P<0.001). There was no association between T-stage and sex (P=0.57), marital status (P=0.42), or urban residence (P=0.23). There was no difference in the T-stage at diagnosis over the four time period examined (P=0.54).

Utilization of Peri-operative Diagnostic Testing

We examined the utilization of peri-operative diagnostic testing among patients with GBA. Overall, computed tomography (CT) was the most commonly utilized cross-sectional imaging modality (n=1828; 61.0%). Magnetic resonance imaging (MRI) (n=171; 5.7%) and positron emission tomography (PET) (n=49; 1.6%) were utilized only in a minority of patients. While cholangiography was utilized in about one third of patients (n=835; 27.8%), diagnostic laparoscopy was infrequently employed in the treatment of GBA (n=130; 4.3%). To assess for temporal trends in utilization, we examined the use of each one of these specific procedures/imaging modalities relative to the four time periods (Table 4). While the use of PET and diagnostic laparoscopy remained stable over time, the relative use of CT, MRI, and cholangiography showed a temporal trend. Specifically, the use of CT increased from 56.7% in 1991–1995 to 70.0% in 2003–2005 (P<0.001). Similarly, while MRI was utilized in less than 1.6% of cases in 1991–1995, MRI was utilized in 10.3% of GBA cases in 2003–2005 (P<0.001).

Table 4 Preoperative staging, operative, treatment details, and survival in patients with surgically managed GBA

Variable	1991–1995 (n=681) Number (%)	1996–1999 (n=543)	2000–2002 (n=833)	2003–2005 (n=898)	Total (n=2955)
Percent of total	23.0	18.4	28.2	30.4	100
Preoperative staging					
Cholangiogram*	259 (38.0)	167 (30.8)	200 (24.0)	209 (23.3)	835 (28.3)
MRI*	<11 (<1.6%)	<11 (<2.0%)	57 (6.8)	93 (10.4)	171 (5.8)
CT*	388 (57.0)	318 (58.6)	493 (59.2)	629 (70.0)	1828 (61.9)
PET	13 (1.9)	<11 (<2.0%)	<11 (<1.3%)	16 (1.8)	49 (1.7)
Diagnostic laparoscopy	39 (5.7)	16 (2.9)	34 (4.1)	41 (4.6)	130 (4.4)
Operative					
Laparoscopic cholecystectomy*	156 (22.9)	214 (39.4)	340 (40.8)	408 (45.8)	1118 (37.8)
Hepatectomy*	47 (6.9)	37 (6.8)	71 (8.5)	108 (12.0)	263 (8.9)
Partial hepatectomy*	47 (6.9)	35 (6.4)	69 (8.3)	101 (11.2)	252 (8.5)
Lymphadenectomy: ≥1 nodes examined*	162 (23.8)	138 (25.4)	252 (30.3)	292 (32.5)	844 (28.2)
Lymphadenectomy: ≥3 nodes examined*	33 (4.8)	26 (4.8)	71 (8.5)	75 (8.4)	205 (6.9)
Hepaticojejunostomy/Resection extrahepatic biliary tree*	37 (5.4)	25 (4.6)	19 (2.3)	33 (3.7)	114 (3.9)
[a]Simple resection	625 (91.8)	507 (93.4)	789 (94.7)	832 (92.7)	2753 (93.2)
[a]Radical resection	56 (8.2)	36 (6.6)	44 (5.3)	66 (7.3)	202 (6.8)
[b]Radical resection/hepatectomy*	83 (12.2)	54 (9.9)	100 (12.0)	146 (16.3)	383 (13.0)
Radical resection/hepatectomy and ≥3 nodes examined*	<11 (<1.6%)	<11 (<2.0%)	27 (3.2)	44 (4.9)	96 (3.2)
Morbidity					
Overall	249 (36.6)	191 (35.2)	244 (29.3)	285 (31.7)	969 (32.8)
Postoperative infection	30 (4.4)	23 (4.2)	27 (3.2)	37 (4.1)	117 (4.0)
Percutaneous drain	33 (4.8)	25 (4.6)	45 (5.4)	56 (6.2)	159 (5.4)
Postoperative hemorrhage	17 (2.5)	17 (3.1)	21 (2.5)	28 (3.1)	83 (2.8)
[c]Peri-operative mortality	23 (3.4)	26 (4.8)	31 (3.7)	44 (4.9)	124 (4.2)
Adjuvant Treatment					
Radiation therapy	118 (17.3)	78 (14.4)	130 (15.6)	138 (15.4)	464 (15.7)
Chemotherapy	16 (2.3)	<11 (<2.0%)	<11 (<1.3%)	20 (2.2)	53 (1.8)
Median survival (months)	16.0	15.0	16.0	15.0	16.0
5-year survival	21.4	22.8	21.3	NR	21.3

MRI magnetic resonance imaging, *CT* computed tomography, *PET* positron emission tomography

Per NCI data usage agreement, no cells with totals less than 11 were reported

*P<0.05 significant level by linear-by-linear association test of trend

[a] Simple and radical resection: data from SEER variables site-specific surgery and surgery of primary site. See "Methods" section

[b] Cases that had a radical resection per SEER data or a hepatectomy as indicated by Medicare billing data

[c] Peri-operative mortality: death within 30 days of the cancer-directed operation

The use of cholangiography decreased over time from 38.0% in 1991–1995 to 23.3% in 2003–2005 (P<0.001).

Operative Details

Of the 2,955 patients who underwent a cancer-directed operation, 93.2% (n=2,753) had a cholecystectomy alone (i.e., simple resection of the primary site) while 202 (6.8%) had a radical resection as defined by the SEER variable (Table 4). Based on the SEER data, the prevalence of radical resection did not change over time (P=0.40). However, in examining procedure-specific Medicare data, the proportion of patients who underwent any form of hepatectomy did increase over time (1991–1995: 6.9%; 1996–1999: 6.8%; 2000–2002: 8.5%; 2003–2005: 12.0%; P<0.001). When we combined the SEER radical resection variable with data from the linked Medicare database that indicated a hepatectomy had been performed into a composite variable "radical resection/hepatectomy" to improve case capture of all radical surgeries (n=383), there

was an increase in the proportion of cases from 12.2% in 1991–1995 to 16.3% in 2003–2005 (P=0.001). T-stage was associated with receipt of radical resection/hepatectomy. Specifically, the proportion of patients who underwent radical resection/hepatectomy for T1b, T2, and T3 cancers was 8.9%, 13.4%, and 18.2%, respectively (P<0.001). Receipt of radical resection/hepatectomy was not associated with patient sex, race, or geographic location (all P>0.05). However, it was associated with age (P<0.001) with older patients undergoing radical resection/hepatectomy less frequently than younger patients. Regarding the extent of the hepatectomy, a partial hepatectomy was performed in the majority of patients (n=252; 95.8%) while 6.8% (n=18) and <4.2% (n<11) patients underwent a hemihepatectomy or an extended hepatectomy/trisegmentectomy, respectively (the values do not add to 100% as some patients underwent both a combination of a partial hepatectomy and a hemihepatectomy or extended hepatectomy). The extent of hepatectomy was associated with T-stage with higher T-stage patients being more likely to undergo a partial hepatectomy (P<0.001) or a hemihepatectomy (P=0.039). Over time there was also an increase in the use of partial hepatectomy (1991–1995, 6.9% versus 2003–2005, 11.2%; P=0.001).

Overall, no lymph nodes were examined (NX) in 71.8% of patients (n=2151), while 844 (28.2%) patients had at least one lymph node evaluated (Table 4). The reporting of at least one lymph node evaluated increased over time from 23.8% (n=162) in 1991–1995 to 34.6% (n=292) in 2003–2005 (P<0.001). When stratified by surgery type, 51.2% of patients who underwent a radical resection had at least one lymph node evaluated compared with 26.8% patients who had a simple cholecystectomy (P<0.001). As expected, fewer patients (n=205; 6.9%) had ≥3 lymph nodes evaluated. In fact, of the 383 patients who underwent radical resection/hepatectomy, only 58.0% (n=222) had ≥3 lymph nodes evaluated. There was, however, an increase over time in the number of patients undergoing radical resection/hepatectomy who had a lymphadenectomy with ≥3 lymph nodes examined (1991–1995: 24.5%; 1996–1999: 25.8%; 2000–2002: 30.9%; 2003–2005: 33.6%; P< 0.001). Factors associated with ≥3 lymph nodes evaluated included younger patient age (P<0.001) and higher tumor grade (P<0.001). T-stage was also strongly associated with ≥3 lymph nodes evaluated (T1a=23.2%; T1b=25.2%; T2= 33.3%; T3=33.5%; P<0.001). Of those patients who underwent lymphadenectomy and had at least 1 lymph node examined (n=844), 397 (47.0%) had nodal metastasis (N1 disease).

At the time of surgery, resection of the extrahepatic biliary tree with concomitant hepaticojejunostomy was rare (n=114; 3.9%) and decreased over time from 5.4% in 1991–1995 to 3.7% in 2003–2005 (P=0.02).

Peri-operative Morbidity and Mortality

The proportion of overall peri-operative complications was 32.8% (Table 4). Morbidity following surgical treatment of GBA was mostly associated with post-operative infection (4.0%), need for percutaneous drain (5.4%) or post-operative hemorrhage (2.8%). There was a decrease in the overall prevalence of complications over time (P=0.01). However, there was no trend over time in postoperative complications regarding the prevalence of infections, need for percutaneous drains, or hemorrhage (all P>0.05) (Table 4). Among those patients who underwent a radical resection/hepatectomy the risk of post-operative morbidity was higher compared with patients who underwent a simple cholecystectomy (OR= 1.37; P=0.005).

Overall, 124 patients survived less than 30 days after their cancer-directed operation for a peri-operative mortality of 4.2%. The risk of peri-operative mortality tended to be higher among patients who underwent a simple cholecystectomy (4.5%) versus a radical resection/hepatectomy (1.8%; P=0.01). Only a few patients who underwent a partial hepatectomy (n=2), a hemihepatectomy (n=1) or extended hepatectomy (n=1) had a peri-operative mortality. There was no change with regard to peri-operative mortality over time (1991–1995, 3.4%; 1996–1999, 4.8%; 2000–2002, 3.7%; 2003–2005, 4.9% (P=0.36).

Adjuvant Treatment

Adjuvant therapy was utilized sparingly in patients with GBA. Specifically, radiation therapy was administered to 421 (14.2%) patients, the overwhelming majority of whom received radiation in the post-operative setting (n=412; 97.9%). Patients who underwent a radical resection/hepatectomy were more likely to receive adjuvant radiotherapy compared with patients who had a simple cholecystectomy (23.3% vs. 12.9%; P<0.001). There was no increase in the use of radiation over time (P=0.08) (Table 4). Overall, 1.8% (53) patients who had a cancer-directed operation were treated with systemic chemotherapy. There was no difference in the proportion of patients treated with systemic therapy relative to the type of surgical procedure or the time period examined (both P>0.05).

Long-term Outcome

The overall median survival was 16.0 months with a 1-, 3-, and 5-year overall survival of 55.7%, 30.3%, and 21.3%, respectively. There was no improvement in survival over time from 1991 to 2005 for patients with surgically managed GBA (P=0.60) (Table 4 and Fig. 1).

On univariate analyses, several clinicopathologic factors known to be associated with GBA were analyzed to determine their association with survival (Table 5). Factors influencing

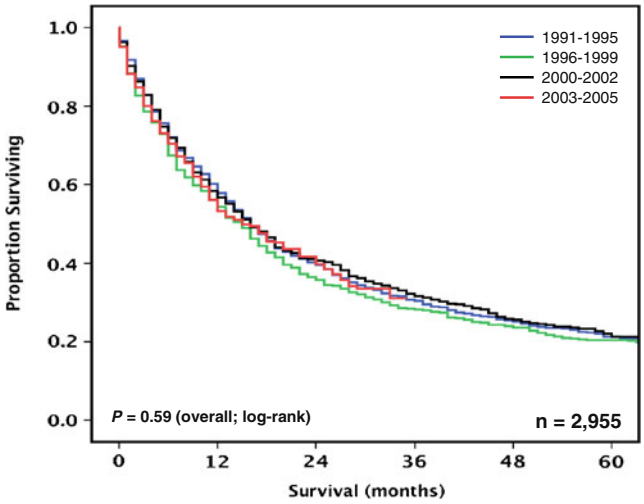

Fig. 1 Overall survival stratified of the patients by four time periods. There was difference in survival across time (P=0.59) with a median survival of 16 months and 5-year survival of 21.3%

survival included gender, race, marital status, cancer grade, T-stage, N-stage, receipt of radical surgery, as well as lymphadenectomy ≥3 lymph nodes (all P<0.05). Survival time had a direct relationship with increasing T-stage (Fig. 2a). Specifically, patients with T3 cancers had a median survival of 8 months compared with 19 months for patients with T2 cancers (P<0.001). Survival was also associated with receipt of radical resection/hepatectomy. The median survival for patients undergoing radical resection/hepatectomy was 20 months compared with 15 months for patients undergoing simple cholecystectomy (P=0.001). The impact of radical resection/hepatectomy on survival persisted regardless of T-stage (Fig. 2a). Patients with T2 cancers who had a radical resection/hepatectomy had a median survival of 53.0 months compared with 16.0 months for those who had a cholecystectomy only (P<0.001). Similarly, patients with T3 cancers who had a radical resection/hepatectomy had a median survival of 11 months compared with 8 months for those who had a simple cholecystectomy only (P<0.001).

Median survival was also influenced by N-stage (Fig. 2b). Patients with no lymph node metastasis had a median survival of 17 months compared with 11 months for patients with N1 disease (P<0.001). Performance of a lymphadenectomy with evaluation of ≥3 lymph nodes was associated with improved long-term survival compared with the evaluation of fewer nodes, especially among T2 and T3 patients (P<0.001) (Fig. 3).

Table 5 Cox regression analyses of variables associated with survival in patients with surgically managed gallbladder cancer

Prognostic factor	Univariate			Multivariate		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
Year of diagnosis 1991–1995	0.97	0.86–1.11	0.688	–	–	–
Male gender	1.19	1.08–1.30	<0.001	1.33	1.20–1.48	<0.001
White race	1.11	1.01–1.23	0.035	1.10	1.00–1.22	0.049
Unmarried	1.17	1.08–1.28	<0.001	1.26	1.15–1.39	<0.001
Rural residence	1.14	0.97–1.33	0.103	1.01	0.87–1.18	0.867
Simple cholecystectomy	1.24	1.09–1.42	0.002	1.40	1.21–1.61	<0.001
Lymphadenectomy <3 nodes	1.59	1.31–1.93	<0.001	1.78	1.45–2.17	<0.001
T-stage						
Tis		Reference				
T1a	1.20	0.93–1.56	0.169	1.24	0.94–1.63	0.134
T1b	1.40	1.12–1.75	0.003	1.47	1.15–1.88	0.002
T1NOS	2.24	1.78–2.82	<0.001	2.25	1.74–2.89	<0.001
T2	2.02	1.65–2.47	<0.001	2.08	1.65–2.63	<0.001
T3	3.77	3.01–4.59	<0.001	3.82	3.05–4.78	<0.001
Grade						
Well-differentiated		Reference				
Moderately differentiated	1.28	1.19–1.46	<0.001	1.12	0.98–1.29	0.095
Poorly differentiated	2.03	1.77–2.32	<0.001	1.64	1.42–1.88	<0.001
Undifferentiated	1.69	1.21–2.36	0.002	1.74	1.24–2.43	0.001
Unknown	0.92	0.78–1.08	0.306	1.13	0.95–1.34	0.162
Positive lymph node status (N1)	1.43	1.27–1.62	<0.001	1.17	1.03–1.33	0.015
Radiation treatment	1.10	0.99–1.22	0.086	0.85	0.76–0.95	0.005

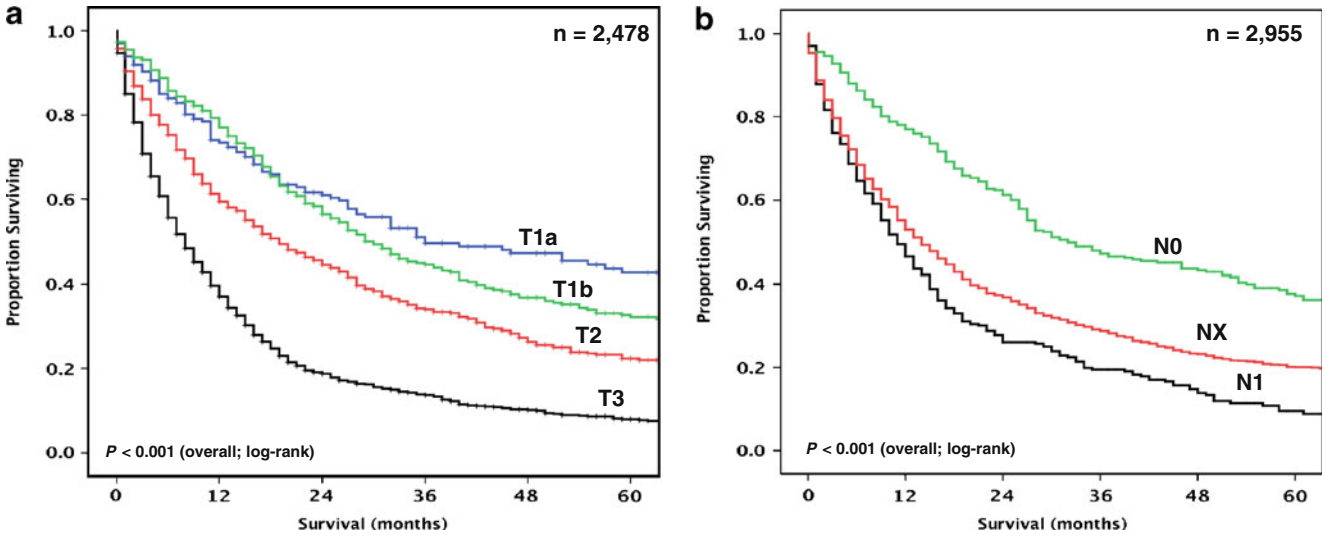

Fig. 2 Overall survival stratified by: **a** T-stage ($P<0.001$); and, by **b** N-stage ($P<0.001$)

After controlling for competing risk factors with multivariate analysis, several factors were found to be independently associated with a poor outcome (Table 5). Demographic factors associated with poor survival included male gender, white race, and unmarried persons (all $P<0.05$). Surgical factors independently associated with an increased risk of death included history of simple cholecystectomy (HR: 1.40 (95% CI: 1.21–1.61); $P<0.001$) and lymphadenectomy of fewer than three lymph nodes (HR: 1.78 (95% CI: 1.45–2.17); $P<0.001$). In addition, tumor grade and T-stage each remained independently associated with survival. Specifically, patients with T3 tumors had almost a fourfold increased risk of death compared with patients with Tis (HR=3.82, $P<0.001$).

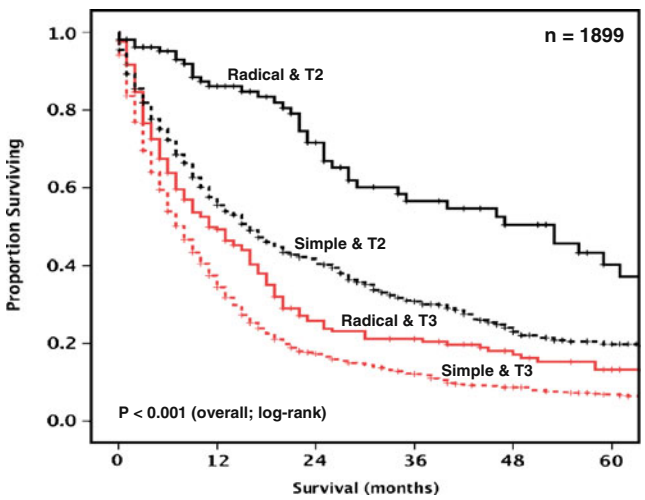

Fig. 3 Survival by T-stage and radical resection/hepatectomy for stage T2 and T3 cancers. Radical resection/hepatectomy was associated ($P<0.001$) with an increase in median survival of 37 and 3 months, for T2 and T3 cancers, respectively

Discussion

Over the past decade, there has been a trend toward investigating cancer surgery outcomes on a population-basis using data from national registries.[16–18] These data are not without their limitations[32], but the SEER dataset does provide the ability to assess the quality and outcomes of cancer surgery care from over a 25% sample of the US population. To that end, we used the SEER database to assess compliance with nationally promulgated NCCN guidelines on the surgical management of patients with GBA. In contrast to previously published studies on gallbladder cancer that used the SEER database only,[16–18] we also augmented the data by linking it with Medicare billing claims—a method that has been shown to improve the capture and accuracy of cancer surgery care analysis.[19] To date, this is the first study to combine SEER-Medicare data to assess outcomes and trends of cancer-directed surgery for GBA.

As demonstrated in the current study (Table 4), the vast majority of patients were evaluated with CT imaging, the utilization of which increased over time. Kim et al.[33] noted that the accuracy of multi-detector CT for T2 versus T3 versus T4 cancers was 79.3%, 92.7%, and 100%, respectively. Although the use of MRI and magnetic resonance cholangiography has not in general been shown to provide additional information over the use of three-dimensional helical CT alone,[34] we noted that the use of MRI to assess patients with GBA increased tenfold from 1991 to 2005. The reason for the increased use of MRI is not well defined, but may relate to the increased familiarity and utilization of MRI for a broad scope of malignancies at many institutions[35–37] PET scanning is another imaging modality that has increasingly been utilized in the staging of several gastrointestinal

malignancies. Because of the relatively high incidence of metastatic disease, some investigators have suggested that PET may be useful in the preoperative evaluation of patients with GBA[38] In one study, Corvera et al.[39] noted that the use of PET changed operative management in nearly 25% of patients with GBA. Interestingly, in the current study, we noted the overall use of PET scanning in the evaluation of patients with GBA was quite low—less than 2%.

Several population-based studies have demonstrated an association between lymphadenectomy and survival for patients with GBA.[17,18] The incidence of metastatic disease in regional lymph nodes can range from 10% to 45%.[8] In the current study, 47% of those patients who underwent lymphadenectomy had nodal metastasis. As such, repeat surgery for GBA should include a lymphadenectomy to provide important staging information, as well as possibly decrease the risk of local recurrence. Jensen et al.[17] reported that patients who underwent radical resection for gallbladder cancer had a survival advantage if a lymphadenectomy was also performed at the time of surgery. In contrast, the authors noted that patients who had no lymph nodes evaluated had a similar survival compared with patients undergoing local resection/simple cholecystectomy only. The AJCC defines an adequate lymphadenectomy as the evaluation of ≥3 lymph nodes in the surgical specimen.[10,18] Data from the current study provide evidence that the evaluation of fewer than 3 lymph nodes is independently associated with a worse survival ($P<0.001$). Compared with patients who had evaluation of fewer than 3 nodes, T2 and T3 patients who underwent a lymphadenectomy of ≥3 lymph nodes had an improvement in overall median survival of 18 and 5 months, respectively. We did note that the proportion of patients undergoing a lymphadenectomy consisting of ≥3 lymph nodes increased over the time periods examined (Table 4). The reason for the increase in lymphadenectomy of ≥3 lymph nodes, as well as the improved survival associated with a more thorough lymphadenectomy, is probably multifactorial. Some investigators[17] have hypothesized that the extent of lymphadenectomy may be a surrogate for a more complete oncologic operation by a more experienced hepatobiliary surgeon. While the removal of 1 or 2 additional lymph nodes may not have a direct therapeutic effect, the attainment of a more thorough lymphadenectomy may more likely be an overall indicator of quality of care.

While the empiric performance of a nonanatomical resection versus an anatomical resection for GBA has traditionally been controversial, recently most surgeons have advocated a more parenchymal sparing approach to resection of hepatobiliary tumors.[40] In fact, several studies[8,40,41] have demonstrated that the extent of hepatic resection did not impact survival. Rather than extent of hepatic resection, surgical margin status and the ability to obtain a microscopically negative (R0) margin of resection has been shown to be the key determinant of outcome.[8] In the current study, we assessed the utilization of various hepatectomy procedures over time. Specifically, we noted that formal hemihepatectomy or extended hepatectomy was rarely utilized in the treatment of GBA (6.8% and <4.2%, respectively). Rather, most patients with GBA who underwent a radical resection had a partial hepatectomy, with the use of partial hepatectomy increasing over time (Table 4). Most single institutions series have reported an operative mortality following hepatectomy of less than 1%,[42–48] while population-based studies[49] have noted an overall 5.6% nationwide operative mortality. In the current series, we herein report a perioperative mortality of 4.2% with no change over the time of the study period.

The 5-year population-based estimate of survival for patients with GBA was only 21.3% (Fig. 1). We did not find an improvement in survival over time. These data underscore the high case-mortality associated with the diagnosis of GBA. Several groups have advocated radical re-resection as a means to improve survival of patients with GBA, especially those with T2 or T3 disease. Investigators at the Memorial Sloan-Kettering Cancer Center reported a 5-year survival of 61% for patients with T2 GBA who were managed with re-resection compared with 19% for those patients treated with only a simple cholecystectomy.[50] Other investigators have reported a similar benefit of re-resection for patients with T3 disease.[2–4,16–18,50] Consistent with these previous reports, we similarly noted a survival benefit of radical resection among patients with T2 or T3 GBA (Fig. 4). Specifically, patients with T2 disease who underwent radical resection had a nearly threefold increase in median survival compared with patients who underwent simple cholecystectomy alone ($P<0.001$). While the benefit

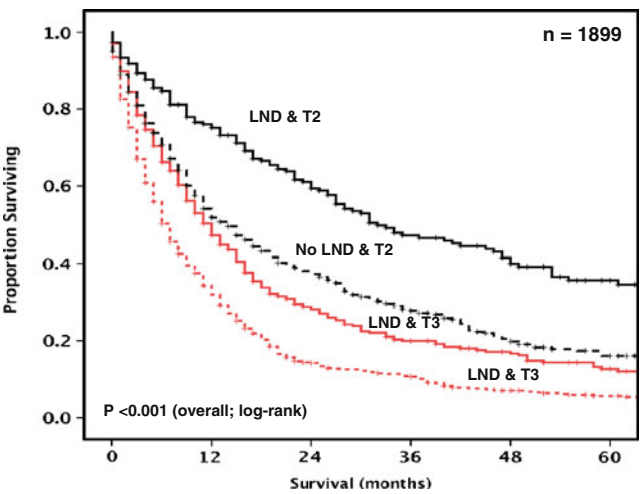

Fig. 4 Survival by lymphadenectomy for stage T2 and T3 cancers. Lymphadenectomy was associated ($P<0.001$) with an increase in the median survival of 18 and 5 months for T2 and T3 cancers, respectively

of radical resection persisted for patients with T3 disease, the gain in overall survival was considerably more modest. In aggregate, data from previously published reports and the current study strongly suggest that radical resection can potentially provide a survival benefit for patients with T2 and T3 GBA.

The NCCN currently recommends that radical repeat surgery be undertaken in patients with T1b, T2, and T3 patients with GBA. In assessing compliance with the NCCN guidelines, we noted that overall utilization of radical resection/hepatectomy and lymphadenectomy were quite poor (13.0% and 6.9%, respectively). Other authors have similarly noted a low utilization of hepatic resection[16,18,51] and lymphadenectomy[16,18,51] for GBA. However, unlike other studies, we specifically examined temporal trends in compliance with the NCCN guidelines. Overall, radical resection/hepatectomy was performed in only 8.9%, 13.4%, and 18.2% of T1b, T2, and T3 cancers, respectively. Whereas previous data[16] had not suggested a temporal change in the utilization of radical resection for GBA, we noted an increase in the proportion of patients undergoing radical resection/hepatectomy over time (1991–1995, 12.2% versus 2003–2005, 16.3%). The reason for this difference most likely relates to our use of Medicare-linked data. Unlike previous reports that examined only SEER data, the linked SEER-Medicare dataset allowed for a more complete capture of procedure-specific data related to radical resection. Moreover, when we assessed the number of patients who had both a radical resection/hepatectomy and lymphadenectomy of ≥3 lymph nodes, the compliance with the NCCN guidelines was poor, despite a temporal increase over time (1991–1995, 2.2% versus 2003–2005, 4.9%).

Inherent to analyses of many administrative databases, our study had a number of limitations. While broad in scope, the SEER-Medicare dataset lack certain detailed clinicopathologic data. Specifically, data on surgical margin status, as well as performance status, were not available for inclusion in our analyses. In addition, because we chose to use Medicare-linked data, the analyses were constrained to only those individuals 65 years or older. While this may theoretically limit the generalizability of our findings, this limitation is unlikely to have major clinical implications when examining a malignancy that has its highest incidence among older individuals.

In conclusion, the overall 5-year survival of patients with GBA was less than 25% with no significant improvement in survival over the past 15 years. Compliance with NCCN guidelines for radical resection for GBA was poor. Only a minority of patients underwent either a radical resection/hepatectomy or lymphadenectomy for GBA. Radical resection/hepatectomy and lymphadenectomy of ≥3 lymph nodes was, however, associated with a survival benefit. The benefit of radical resection appeared to be most pronounced among patients with T2 disease. Taken together, data from the current study delineate the current underutilization of radical surgery for GBA. These data should serve as the basis to inform future initiatives to enhance compliance with practice guidelines regarding GBA, as well as drive efforts to increase the number of patients offered surgical management when appropriate.

References

1. Horner MJ RL, Krapcho M, Neyman N, Aminou R, Howlader N, Altekruse SF, Feuer EJ, Huang L, Mariotto A, Miller BA, Lewis DR, Eisner MP, Stinchcomb DG, Edwards BK (eds). SEER Cancer Statistics Review, 1975–2006, National Cancer Institute. Bethesda, MD. Available at: http://seer.cancer.gov/csr/1975_2006/ . Based on November 2008 SEER data submission, posted to the SEER web site, 2009.
2. Shih SP, Schulick RD, Cameron JL, et al. Gallbladder cancer: the role of laparoscopy and radical resection. Ann Surg 2007; 245 (6):893–901.
3. Fong Y, Malhotra S. Gallbladder cancer: recent advances and current guidelines for surgical therapy. Adv Surg 2001; 35:1–20.
4. Foster JM, Hoshi H, Gibbs JF, et al. Gallbladder cancer: Defining the indications for primary radical resection and radical re-resection. Ann Surg Oncol 2007; 14(2):833–840.
5. Chan SY, Poon RT, Lo CM, et al. Management of carcinoma of the gallbladder: a single-institution experience in 16 years. J Surg Oncol 2008; 97(2):156–164.
6. Balachandran P, Agarwal S, Krishnani N, et al. Predictors of long-term survival in patients with gallbladder cancer. J Gastrointest Surg 2006; 10(6):848–854.
7. Dixon E, Vollmer CM, Jr., Sahajpal A, et al. An aggressive surgical approach leads to improved survival in patients with gallbladder cancer: a 12-year study at a North American Center. Ann Surg 2005; 241(3):385–394.
8. Pawlik TM, Gleisner AL, Vigano L, et al. Incidence of finding residual disease for incidental gallbladder carcinoma: implications for re-resection. J Gastrointest Surg 2007; 11(11):1478–86; discussion 1486–7.
9. The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines™) Hepatobiliary Cancers. (Version 1.2010). © 2010. Available at: www.NCCN.org. Accessed 8 April 2010.
10. American Joint Commission on Cancer (2010) American Cancer Society, 7th ed. New York, NY: Springer
11. Cubertafond P, Gainant A, Cucchiaro G. Surgical treatment of 724 carcinomas of the gallbladder. Results of the French Surgical Association Survey. Ann Surg 1994; 219(3):275–280.
12. Kokudo N, Makuuchi M, Natori T, et al. Strategies for surgical treatment of gallbladder carcinoma based on information available before resection. Arch Surg 2003; 138(7):741–750; discussion 750.
13. D'Angelica M, Brennan MF, Fortner JG, et al. Ninety-six five-year survivors after liver resection for metastatic colorectal cancer. J Am Coll Surg 1997; 185(6):554–559.
14. Konstantinidis IT, Deshpande V, Genevay M, et al. Trends in presentation and survival for gallbladder cancer during a period of more than 4 decades: a single-institution experience. Arch Surg 2009; 144(5):441–447; discussion 447.
15. Wise PE, Shi YY, Washington MK, et al. Radical resection improves survival for patients with pT2 gallbladder carcinoma. Am Surg 2001; 67(11):1041–1047.
16. Jensen EH, Abraham A, Habermann EB, et al. A critical analysis of the surgical management of early-stage gallbladder

cancer in the United States. J Gastrointest Surg 2009; 13(4):722–727.
17. Jensen EH, Abraham A, Jarosek S, et al. Lymph node evaluation is associated with improved survival after surgery for early stage gallbladder cancer. Surgery 2009; 146(4):706–711; discussion 711–3.
18. Coburn NG, Cleary SP, Tan JC, et al. Surgery for gallbladder cancer: a population-based analysis. J Am Coll Surg 2008; 207 (3):371–382.
19. Cooper GS, Virnig B, Klabunde CN, et al. Use of SEER-Medicare data for measuring cancer surgery. Med Care 2002; 40(8 Suppl): IV-43-8.
20. National Cancer Institute D, Surveillance Research Program, Cancer Statistics Branch. Surveillance, epidemiology, and end results (SEER) program. Available at: (http://www.seer.cancer.gov). SEER* Stat database: Incidence, SEER 17 regs public-use, Nov 2005 sub (1973–2003 varying), linked to county attributes, total US, 1969–2003 counties. Released April 2006, based on the November 2005 submission.
21. Warren JL, Klabunde CN, Schrag D, et al. Overview of the SEER-Medicare data: content, research applications, and generalizability to the United States elderly population. Med Care 2002; 40(8 Suppl):IV-3-18.
22. Fritz AG. International Classification of Diseases for Oncology: ICD-O, 3rd ed. Geneva: World Health Organization 2000.
23. American Joint Commission on Cancer, American Cancer Society. 6th ed. New York: Springer, 2002
24. SEER program coding and staging manual. Bethesda, MD: National Cancer Institute, 2007.
25. Morris AM, Baldwin LM, Matthews B, et al. Reoperation as a quality indicator in colorectal surgery: a population-based analysis. Ann Surg 2007; 245(1):73–79.
26. Potosky AL, Warren JL, Riedel ER, et al. Measuring complications of cancer treatment using the SEER-Medicare data. Med Care 2002; 40(8 Suppl):IV-62-8.
27. Kaplan EL MP. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457–481.
28. Cox D. Regression models and life tables. J R Stat Soc B 1972; 34:187–220.
29. Hosmer DW, Lemeshow S. Applied Survival Analysis: Regression modeling of time to event data. 2nd ed. New York: John Wiley and Sons, Inc., 1999.
30. May S, Hosmer DW. A simplified method of calculating an overall goodness-of-fit test for the Cox proportional hazards model. Lifetime Data Anal 1998; 4(2):109–120.
31. Agresti A. Introduction to categorical data analysis. New York: Wiley, 1996. pp. 231–236.
32. Nathan H, Pawlik TM. Limitations of claims and registry data in surgical oncology research. Ann Surg Oncol 2008; 15(2):415–423.
33. Kim SJ, Lee JM, Lee JY, et al. Accuracy of preoperative T-staging of gallbladder carcinoma using MDCT. AJR Am J Roentgenol 2008; 190(1):74–80.
34. Rao ND, Gulati MS, Paul SB, et al. Three-dimensional helical computed tomography cholangiography with minimum intensity projection in gallbladder carcinoma patients with obstructive jaundice: comparison with magnetic resonance cholangiography and percutaneous transhepatic cholangiography. J Gastroenterol Hepatol 2005; 20(2):304–308.
35. Park HS, Lee JM, Choi HK, et al. Preoperative evaluation of pancreatic cancer: comparison of gadolinium-enhanced dynamic MRI with MR cholangiopancreatography versus MDCT. J Magn Reson Imaging 2009; 30(3):586–595.
36. Manfredi R, Mehrabi S, Motton M, et al. MR imaging and MR cholangiopancreatography of multifocal intraductal papillary mucinous neoplasms of the side branches: MR pattern and its evolution. Radiol Med 2008; 113(3):414–428.
37. Guiu B, Loffroy R, Ben Salem D, et al. Combined SPIO-gadolinium magnetic resonance imaging in cirrhotic patients: negative predictive value and role in screening for hepatocellular carcinoma. Abdom Imaging 2008; 33(5):520–528.
38. Hueman MT, Vollmer CM, Jr., Pawlik TM. Evolving treatment strategies for gallbladder cancer. Ann Surg Oncol 2009; 16 (8):2101–2115.
39. Corvera CU, Blumgart LH, Akhurst T, et al. 18F-fluorodeoxyglucose positron emission tomography influences management decisions in patients with biliary cancer. J Am Coll Surg 2008; 206(1):57–65.
40. Pawlik TM, Choti MA. Biology dictates prognosis following resection of gallbladder carcinoma: sometimes less is more. Ann Surg Oncol. 2009; 16(4):787–788.
41. D'Angelica M, Dalal KM, DeMatteo RP, et al. Analysis of the extent of resection for adenocarcinoma of the gallbladder. Ann Surg Oncol 2009; 16(4):806–816.
42. Tsao JI, Loftus JP, Nagorney DM, et al. Trends in morbidity and mortality of hepatic resection for malignancy. A matched comparative analysis. Ann Surg 1994; 220(2):199–205.
43. Thompson HH, Tompkins RK, Longmire WP, Jr. Major hepatic resection. A 25-year experience. Ann Surg 1983; 197(4):375–388.
44. Bentrem DJ, Dematteo RP, Blumgart LH. Surgical therapy for metastatic disease to the liver. Annu Rev Med 2005; 56:139–156.
45. Fong Y, Fortner J, Sun RL, et al. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg 1999; 230(3):309–318; discussion 318–21.
46. Bozzetti F, Gennari L, Regalia E, et al. Morbidity and mortality after surgical resection of liver tumors. Analysis of 229 cases. Hepatogastroenterology 1992; 39(3):237–241.
47. Taylor M, Forster J, Langer B, et al. A study of prognostic factors for hepatic resection for colorectal metastases. Am J Surg 1997; 173(6):467–471.
48. Capussotti L, Polastri R. Operative risks of major hepatic resections. Hepatogastroenterology 1998; 45(19):184–190.
49. Asiyanbola B, Chang D, Gleisner AL, et al. Operative mortality after hepatic resection: are literature-based rates broadly applicable? J Gastrointest Surg 2008; 12(5):842–851.
50. Fong Y, Jarnagin W, Blumgart LH. Gallbladder cancer: comparison of patients presenting initially for definitive operation with those presenting after prior noncurative intervention. Ann Surg 2000; 232(4):557–569.
51. Wright BE, Lee CC, Iddings DM, et al. Management of T2 gallbladder cancer: are practice patterns consistent with national recommendations? Am J Surg 2007; 194(6):820–825; discussion 825–6.

Discussion

Discussant

Dr. Gerard V. Aranha (Maywood, IL): Dr. Mayo and his co authors from the Department of Surgery at Johns Hopkins University have reviewed SEER-Medicare linked data and concluded that the National Comprehensive Cancer Network guidelines for the surgical treatment of gallbladder cancer are not being followed by a majority of institutions in this country.

Unfortunately, this is not just true for gallbladder cancer but it is true for many other malignancies. Being a surgical

oncologist, I'm pretty aware of that and in my opinion, it requires urgent attention and intervention.

I have the following questions.

In your paper, you state that those patients who had extended hepatectomy had a 20% mortality compared to those who had a regular hemihepatectomy, which was 5%. Were the extended hepatectomies done at a local community hospital, low- or high-volume center, and whom in gallbladder cancer do you think should have an extended hepatectomy?

How do we change the culture so that the NCCN guidelines will be followed in this country? Do you think that, at least in gallbladder cancer, that the patients who need surgical treatment should be referred to high-volume centers where maybe the guidelines are followed? Or do you believe that this should be done through the Commission on Cancer of the American College of Surgeons? I think this is a topic that you should address in your manuscript and needs attention.

I think you did a great job with your presentation, Dr. Mayo, and I commend it to the membership at large.

Closing Discussant

Dr. Skye C. Mayo: Thank you for your excellent questions and comments, Dr. Aranha. In our manuscript, we show that there was no difference in mortality between patients undergoing an extended hepatectomy versus a partial or hemihepatectomy. The number of patients who had a hepatectomy who suffered a peri-operative mortality was low. For instance, of the people that had an extended hepatectomy, one patient died post-operatively. Amongst the patients who had a partial hepatectomy or a hemihepatectomy, there were two peri-operative deaths. The numbers are very small and statistically fragile. I would be hesitant to draw conclusions from these data.

As for patients who should undergo a more extended procedure for clearance of their cancer, I believe the recent literature supports that an extended hepatectomy should not be performed on a routine basis for patients with gallbladder cancer, but instead should be performed only for certain patients to achieve clearance of their disease. An extended hepatectomy is associated with a higher morbidity, but it has not been shown to improve long-term survival. The recent literature has shown that it's not the extent of resection that's important, but rather the status of the surgical margin that influences patient outcome. I think the surgeon's aim should be to achieve a microscopically complete resection with disease clearance and limit the resection at that.

As for the cultural change and the NCCN guidelines, I agree with you that it's a very pressing issue and one that is very difficult to address. I think it has to start with standardization of documentation at a more national level and then be disseminated to the smaller hospitals and community centers. Checklists and guidelines should be developed that allow the surgeon and the hospital to collect these data prospectively to ensure that they are following the recommended guidelines appropriately.

In regards to patient referral to high-volume centers, I really wish that we could have looked at volume within the study, but within the SEER database there's really no reliable measure of volume.

Discussant

Dr. Sharon Weber (Madison, WI): I am trying to put this in the context of what we see clinically. These are clearly unbelievably low numbers of patients that are getting referred on for definitive resection. But so often clinically what we see is the patient who had an incidental finding of gallbladder cancer after laparoscopic cholecystectomy, and we see them and obtain a re-staging MRI or CT scan and they are found to have metastatic disease, and therefore we never operate on them.

How would this dataset code that patient? Are those patients counted as having metastatic disease based on the imaging studies? Or is the pathologic staging based solely on the operation which they had, in that case, just a lap chole?

The second question I have for you is about predictors of patients that underwent radical resection. Did you do a multivariate logistical regression trying to sort this out a little more, to understand which patients actually were more likely to undergo radical resection for their gallbladder cancer?

Closing Discussant

Dr. Skye C. Mayo: Thank you for your questions, Dr. Weber. As for patients with metastatic disease being included in this database, one of our exclusion criteria was to use the SEER summary stages and the historical cancer stage to exclude patients with metastatic disease. Whether those patients are undergoing a laparoscopic cholecystectomy and later having metastatic disease added onto their record, I don't believe that is the case. The SEER database collects tumor specific information at the time of diagnosis and treatment and records only if the patient had metastatic disease at the time of their diagnosis. I'm not certain how the database handles a patient that develops metastatic disease in the time period between their cholecystectomy and their referral for re-resection.

In regards to factors associated with patients who underwent a radical resection, we found that patients who were in the younger age quartile of our cohort, and patients

who had a more recent operation were more likely to have had a radical resection.

Discussant

Dr. Henry Pitt (Indianapolis, IN): This analysis and presentation was very good. Your data suggest that an extended operation is warranted with T2 and T3 tumors. However, I didn't see you comment on an extended operation for T1b tumors. I presume the data were not robust enough to answer this question. For your information, I just reviewed a meta-analysis of the literature that suggests that extended cholecystectomy is also warranted in T1b. Can you comment further from your analysis?

Closing Discussant

Dr. Skye C. Mayo: Thank you, Dr. Pitt for your question. In the data from recently published literature, in patients with T1b cancers, approximately 10% have residual disease in their gallbladder fossa that is found at their re-resection. Due to this percentage, many surgeons advocate radical cholecystectomy for those patients with a T1b cancer discovered after their initial cholecystectomy. In our data, not surprisingly, there was a survival benefit for patients who had a T1b cancer, but we chose to focus T2 and T3 for our discussion today. It's more fully delineated in our paper.

Discussant

Dr. Fabrizio Michelassi (New York, NY): Nice presentation. My question relates to the survival curves according to extent of surgery in lymph node negative patients. I believe that you showed a graph with two distinct survival curves, one for patients who had undergone a radical resection, and the second one, for patients without a radical resection. Am I interpreting the data correctly?

Closing Discussant

Dr. Skye C. Mayo: Thank you, Dr. Michelassi. There were two different survival curves. The first one compared the survival impact of radical resection for patients with T2 versus T3 cancers. The second assessed the survival impact of lymphadenectomy on patients with T2 versus T3 cancers. It was irrespective of radical resection. We show an increase of 18 months median survival in patients with a T2 cancer who underwent a lymphadenectomy as indicated by their SEER-Medicare data.

J Gastrointest Surg (2010) 14:1592–1599
DOI 10.1007/s11605-010-1321-9

2010 SSAT PLENARY PRESENTATION

TNF-α Induces Vectorial Secretion of IL-8 in Caco-2 Cells

Dennis I. Sonnier · Stephanie R. Bailey · Rebecca M. Schuster · Alex B. Lentsch · Timothy A. Pritts

Received: 30 April 2010 / Accepted: 9 August 2010 / Published online: 9 September 2010

Abstract
Introduction Intestinal epithelial cells represent an important component of innate immunity, with sophisticated responses to inflammatory stimuli. The manner in which intestinal epithelial cell polarity affects responses to inflammatory stimuli is largely unknown. We hypothesized that polarized intestinal epithelial cells exhibit a bidirectional inflammatory response dependent upon the location of the stimulus.
Methods Caco-2 cells were grown on semi-permeable inserts in a dual-compartment culture system and treated with tumor necrosis factor-α (TNF-α; 100 ng/ml) or serum-free media in the apical or basolateral chamber. Interleukin-8 (IL-8) production in each chamber was measured by enzyme-linked immunosorbent assay. To determine receptor specificity, anti-TNF receptor antibodies were added to the apical or basolateral chamber.
Results Basolateral stimulation with TNF-α resulted in increased apical and basolateral IL-8 production. Apical TNF-α stimulation resulted in increased apical, but not basolateral IL-8 production. Receptor blockade suggested TNF receptor 1 involvement on both apical and basolateral membranes, while TNF receptor 2 was only active on the apical membrane.
Conclusion Polarized intestinal epithelial cells respond to TNF-α stimulation with focused, directional secretion of the proinflammatory cytokine IL-8. These findings are important because they suggest that intestinal epithelial cells are capable of organizing their response to inflammatory signals and producing inflammatory mediators in a bidirectional, vectorial fashion.

Keywords Vectorial · Caco-2 · TNF receptor · IL-8 · Polarized

Introduction

The intestinal mucosa plays an active role in the response to local and systemic inflammation that occurs in disease states such as trauma, sepsis, inflammatory bowel disease (IBD), lung injury, burn, and infectious diarrhea and has been dubbed a "motor" of inflammation in severe illness.[1–5] The intestinal mucosa acts as a barrier to the outside environment and must interact with this environment appropriately, either by exhibiting tolerance or forming an immune response. Mechanisms of interaction between the gut epithelium and luminal contents, including commensal and pathogenic bacteria, are complex and specific to individual types of microbes.[6–11] The mechanisms involved in controlling gut based inflammatory response are a critical, but incompletely understood, part of the innate immune system. In this setting, intestinal epithelial cells occupy a unique position in the intestinal mucosal tissue, with potential exposure to stimuli from the luminal contents (apical stimulation) as well as the lamina propria (basolateral stimulation). Although not known, it is conceivable that the responses to apical and basolateral stimulation may be distinct.

Presented at the Society for Surgery of the Alimentary Tract, New Orleans, Louisiana, May 2010

D. I. Sonnier (✉) · S. R. Bailey · R. M. Schuster · A. B. Lentsch · T. A. Pritts
Department of Surgery,
University of Cincinnati College of Medicine,
Mail Location 0558, 231 Albert Sabin Way,
Cincinnati, OH 45267, USA
e-mail: sonnieds@ucmail.uc.edu

Tumor necrosis factor-α (TNF-α) is a critical proinflammatory mediator in both acute and chronic stages of intestinal and systemic inflammatory disease states. It is a known activator of the transcription factors nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1), two key modulators of the inflammatory response.[12,13] In severe burns, gut-derived TNF-α results in local damage to intestinal mucosa, systemic vascular permeability, and lung injury.[14] Anti-TNF-α treatment is an important part of inflammatory bowel disease therapy, resulting in improved remission rates and steroid requirements.[15,16] Alteration of TNF-α immune response, however, has been associated with severe septic complications in maintenance therapy and postoperative regimens.[17,18] Gut ischemia associated with sepsis and hemorrhage alters mesenteric cytokine profiles as well as intestinal barrier function via TNF-α mechanisms.[19] In addition to IBD, there are a host of TNF-α-mediated chronic diseases such as rheumatoid arthritis, psoriasis, heart failure, bronchitis, and colon cancer.[12,20]

The intestinal epithelial layer plays a key role in gut-mediated inflammation, with capability to receive signals from multiple inputs and generate an inflammatory response. The purpose of this study is to better understand how intestinal epithelial cells actively participate in directing an inflammatory response. We hypothesized that polarized intestinal epithelial cells in culture are capable of generating a bidirectional, vectorial response to an inflammatory signal.

Methods

Materials Caco-2 cells and Eagle's minimum essential medium with Earle's balanced salt solution and 2 mM L-glutamine (EMEM) were obtained from American Type Culture Collection (Rockville, MD, USA). Sodium pyruvate, non-essential amino acids (NEAA), penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Hyclone Laboratories (Logan, UT, USA). Recombinant human TNF-α, mouse monoclonal anti-human TNF receptor 1 (TNFR1) antibody, mouse monoclonal anti-human TNF receptor 2 (TNFR2) antibody, mouse IgG_1 isotype control antibody and enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). Culture flasks and Costar Transwells were purchased from Corning, Inc (Corning, NY, USA). EVOM2 epithelial voltohmmeter was purchased from World Precision Instruments (Sarasota, FL, USA). Ninety-six-well plates were purchased from Nunc (Roskilde, Denmark).

Cell Culture Caco-2 cells were grown in flasks at 37°C in 5% CO_2 in nutrient media consisting of EMEM supplemented with 10% FBS, 1 mM sodium pyruvate, 0.1 mM NEAA, 100 U/ml penicillin, and 100 mg/ml streptomycin. Cells used for experiments were between passages 5 and 20 and were seeded at a density of 300,000 cells/well onto 24-mm-diameter Transwells permeable inserts with 0.04 μm pores. Cells were grown in supplemented EMEM for 21 days to achieve full differentiation prior to use. Transepithelial electrical resistance (TEER) values were measured in all wells and were adjusted for the area of the membrane (4.5 cm^2) and the background resistance of the media and the membrane insert. Only cells with TEER greater than 500 Ωcm^2 were considered fully differentiated and suitable for use.

Experimental Conditions Cells were placed in serum-free media for 24 h, then treated with TNF-α at a concentration of 100 ng/ml,[19] and added to the apical or basolateral compartment. After 24 h of treatment, supernatants were harvested. In additional experiments, LPS (100 ng/ml) was placed in the apical compartment for the duration of the experiment. In receptor blockade experiments, cells were treated with antibody against TNFR1 (15 μg/ml), TNFR2 (15 μg/ml), or isotype control IgG (30 μg/ml) antibody as described in the results for 24 h prior to addition of TNF-α.

Determination of Supernatant Protein Interleukin-8 (IL-8) and CD14 protein levels were determined by ELISA according to manufacturer's instructions. Due to unequal volumes in the apical and basolateral compartments, protein concentrations were multiplied by the volume of the respective compartment to normalize amount of protein secreted into each chamber.

Statistical Analysis When appropriate, results were expressed as mean ± standard error. Statistical analysis was carried out with ANOVA followed by Student–Newman–Keuls test. Statistical analysis was performed using Sigma Plot 11 software (Systat Software, Chicago, IL, USA). A p value of <0.05 was regarded as statistically significant. All experiments were performed at least three times in order to ensure reproducibility.

Results

Vectorial Secretion of IL-8 We first examined the effect of TNF-α treatment on IL-8 production in Caco-2 cells. Basolateral treatment of cells with TNF-α resulted in bidirectional IL-8 production, with significant increases in IL-8 release observed in both apical (Fig. 1a) and basolateral (Fig. 1b) compartments. Apical treatment with TNF-α resulted in release of IL-8 only into the apical compartment (Fig. 1a, b). Because intestinal epithelial cells are constantly exposed to luminal LPS, we next sought to determine if the

presence of apical LPS altered the ability of TNF-α to induce directional IL-8 secretion. LPS was added to the apical chamber and cells were treated with TNF-α in apical or basolateral compartments. The presence of apical LPS had no effect on TNF-α-induced vectorial release of IL-8 (Fig. 2a, b).

CD14 Secretion Previous studies indicate that Caco-2 cells secrete CD14, a protein associated with LPS binding.[21] In order to determine if the effects of TNF-α were a generalized, non-specific response, we examined the effect of TNF-α treatment on apical and basolateral release of CD14. Treatment with TNF-α had no effect on the release of CD14 in either apical or basolateral compartments (Fig. 3a, b).

Transepithelial Electrical Resistance TEER values were measured during each of the experimental conditions. TEER remained stable throughout the experiment, suggesting that bidirectional IL-8 production was not the result of lost barrier function (Fig. 4).

TNF Receptor Blockade In order to determine if there was differential receptor utilization for the observed vectorial secretion of IL-8 induced by TNF-α, cells were treated with neutralizing antibodies to TNFR1 or TNFR2 in either apical or basolateral compartments prior to treatment with TNF-α. Apical blockade of either TNFR1 or TNFR2 resulted in

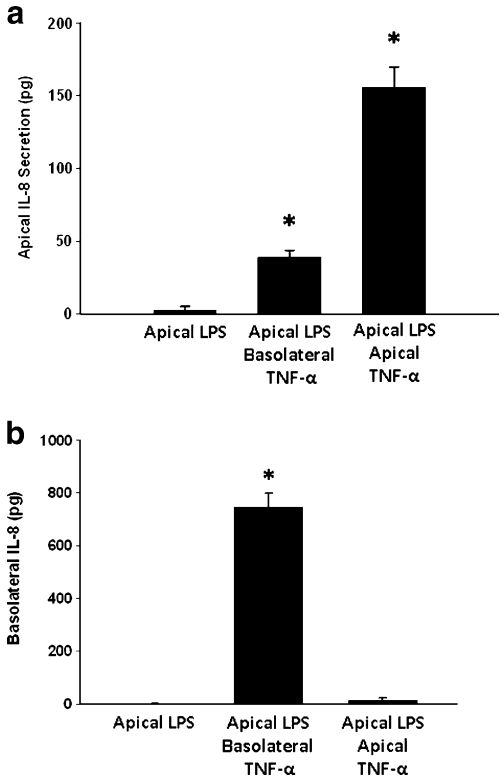

Fig. 2 Apical (**a**) and basolateral (**b**) IL-8 production in Caco-2 cells after treatment with TNF-α in the presence of apical LPS (100 ng/ml). *$p<0.05$ vs. control

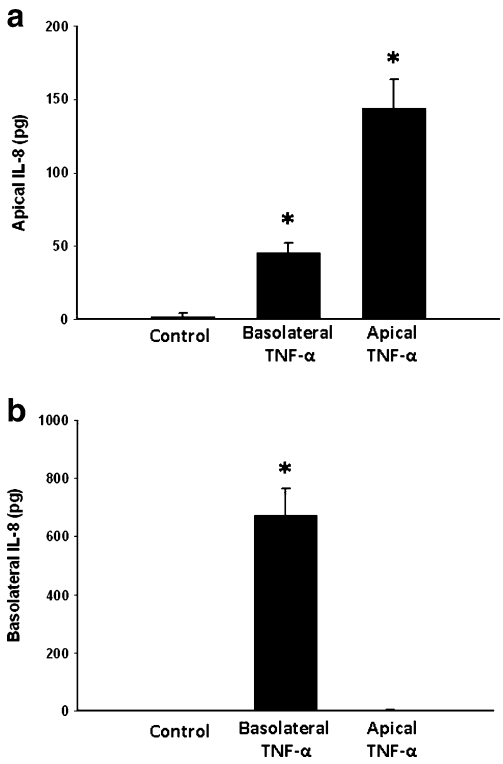

Fig. 1 Apical (**a**) and basolateral (**b**) IL-8 production in Caco-2 cells after treatment with TNF-α. *$p<0.05$ vs. control

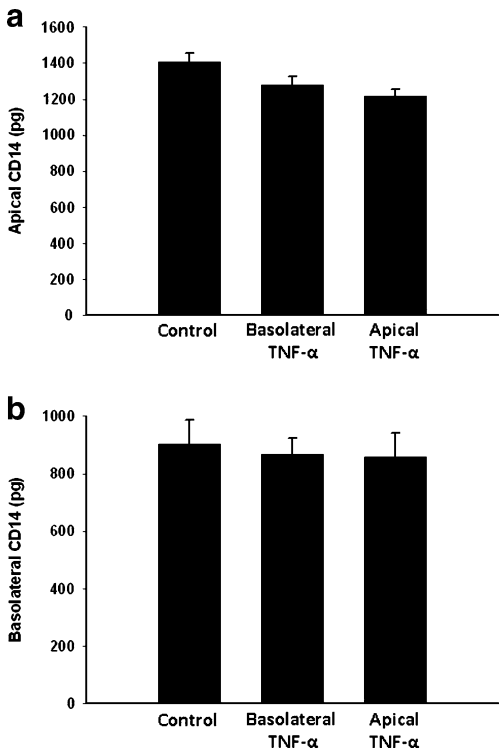

Fig. 3 Apical (**a**) and basolateral (**b**) CD14 levels in Caco-2 cells after treatment with TNF-α

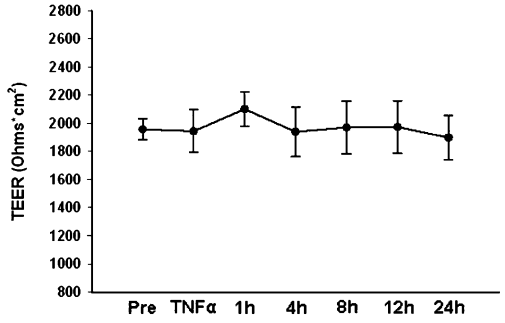

Fig. 4 Transepithelial electrical resistance of Caco-2 cells during treatment

significantly decreased secretion of IL-8 (Fig. 5a, b). Combined blockade of both TNFR1 and TNFR2 reduced IL-8 release in a similar capacity as singular receptor blockade. Blockade of basolateral TNFR1 and TNFR2, either individually or combined, had no effect on apical IL-8 release (Fig. 6a). In contrast, only blockade of basolateral TNFR1, and not TNFR2, resulted in significantly reduced basolateral IL-8 release (Fig. 6b). Basolateral blockade of both TNFR1 and TNFR2 reduced IL-8 release in a similar fashion as blockade of TNFR1 alone (Fig. 6b).

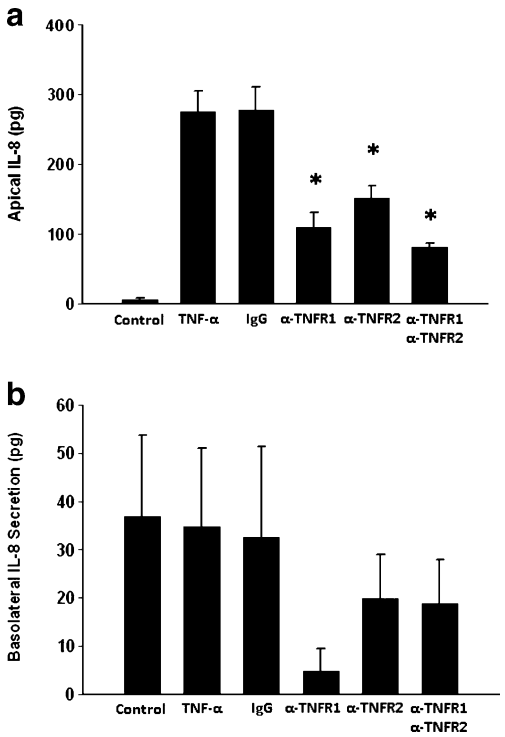

Fig. 5 IL-8 production in Caco-2 cells after treatment with TNF-α in the presence of antibodies to TNFR1 or TNFR2 in the apical chamber. *α-TNFR1* antibody to TNF-α receptor 1 (15 μg/ml), *α-TNFR2* antibody to TNF-α receptor 2 (15 μg/ml), *IgG* isotype control antibody (30 μg/ml). *$p<0.05$ vs. TNF-α alone

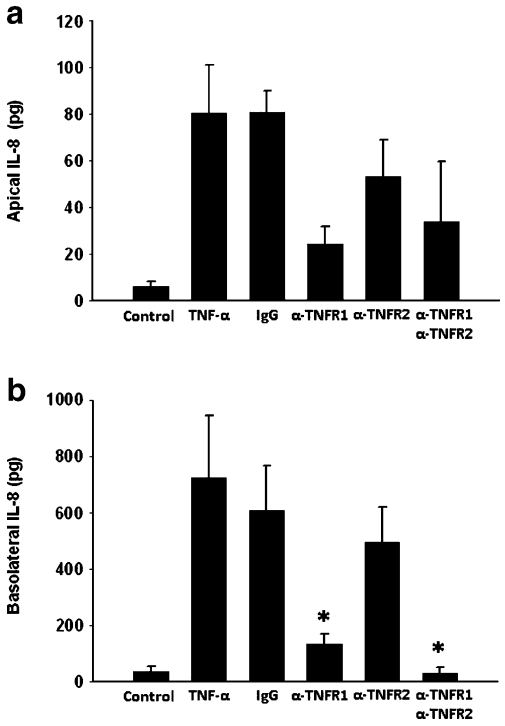

Fig. 6 IL-8 production in Caco-2 cells after treatment with TNF-α in the presence of antibodies to TNFR1 or TNFR2 in the basolateral chamber. *α-TNFR1* antibody to TNF-α receptor 1 (15 μg/ml), *α-TNFR2* antibody to TNF-α receptor 2 (15 μg/ml), *IgG* non-specific antibody (30 μg/ml). *$p<0.05$ vs. TNF-α alone

Discussion

In the present study, our data demonstrate that TNF-α-induced IL-8 production in differentiated Caco-2 intestinal epithelia cells is vectorial in nature, with the predominant response directed toward the direction of the stimulus. In addition, both apical and basolateral IL-8 secretion appears to be mediated primarily by TNFR1, while apical secretion appears to involve both TNFR1 and TNFR2. This is important because of the unique arrangement of the intestinal epithelium as part of the innate immune system. Intestinal epithelial cells are sandwiched between the antigen rich contents of the gut lumen and the largest lymphoid organ of the body, the gut-associated lymphoid tissue. Our data suggest that intestinal epithelial cells are capable of responding to proinflammatory stimuli in a nuanced fashion, with directed, vectorial secretion, rather than simply in a binary "on or off" manner.

Bidirectional, vectorial secretion, similar to that seen in the current study, has been observed in other epithelial cells. In a combined in vitro and ex vivo model of ophthalmologic inflammation, IL-6 and IL-8 were secreted in a vectorial manner after treatment with IL-1β. Retinal pigment epithelial (RPE) cells were used, including both ARPE-19 cell cultures and donor RPE cells cultures grown

in a dual chamber system. Similar to intestinal epithelial cells, the RPE forms an important barrier layer between body compartments and is also associated with both acute and chronic inflammatory disorders.[22] In another study using a model of differential cytokine expression in testicular inflammation and spermatogenesis, primary culture Sertoli cell cultures were shown to exhibit bidirectional, vectorial secretion of IL-1β and IL-6 following treatment with microbial antigens.[23] Other examples of vectorial secretion show interesting interactions between an inflammatory or other signal, with a specific directional cytokine or other cellular response.[24–29]

In this study, we used differentiated, polarized Caco-2 cells as a model of human intestinal epithelium in a dual-chambered culture system. Caco-2 cells were chosen because, when allowed to fully differentiate, they express characteristics similar to mature enterocytes. In dual-chambered systems, Caco-2 cells spontaneously organize into a polarized monolayer with expression of apical tight junctions as evidenced by formation of domes on microscopy.[30] Caco-2 cells also have the ability to transport ions in a vectorial manner, one of the crucial functions of in vivo enterocytes. This cell line also develops an apical brush border with associated brush border enzymes such as lactase, sucrase, dipeptidylpeptidase, aminopeptidase, and alkaline phosphatase.[30–32] Thus, this cell line provides an ex vivo reductionist model with several relevant in vivo characteristics.

The gut mucosa is somewhat unique in that it receives constant exposure to a high level of endotoxin in the intestinal lumen. In experiments designed to determine if the presence of apical LPS altered TNF-α-induced IL-8 production, we found no evidence to suggest that apical LPS regulates this response. These data suggest that Caco-2 cells are appropriately resistant to LPS stimulation, a classic characteristic of intestinal epithelial cells.[33] Additionally, it showed that presence of an apical stimulus did not change the vectorial secretion signaled by treatment with TNF-α.

TEER measurements are traditionally measured as a correlate to level of differentiation, tight junction integrity,[34] and monolayer permeability.[35] Our experimental conditions did not significantly alter TEER levels from baseline, suggesting that leakage or diffusion of TNF-α or IL-8 is not an alternate explanation for our results. The effects of TNF-α on mediator release was not a global, generalized effect as there was no effect of TNF-α on CD14 release.

TNF-α exerts its effects via two specific cell membrane bound receptors, TNFR1 or TNFR2. Our data show differential TNF-α receptor expression on Caco-2 cells. Apical TNF-α signaling appears to take place via both TNFR1 and TNFR2 receptors, while basolateral TNF-α signaling appears to take place primarily via TNFR1. TNFR1 is commonly found in most tissues, where TNFR2 is typically found in immune cells and is more strictly regulated.[36–38] Upon ligand binding, TNFR1 interacts with many complex intracellular signaling factors, including TNF receptor-associated death domain protein and TNF receptor-associated factors (TRAFs), TRAF1 and TRAF2, resulting in potent induction of NF-κB and AP-1 gene expression. TNFR1 also signals apoptosis via its Fas-associated death domain protein.[39–41] TNFR2 appears to modulate inflammation via TRAF2 and contains no death domain. TNFR2 also influences TNFR1-related mechanisms, where it can temper or intensify responses. Also, TNFR2 appears to be more prominent in chronic disease states.[42] It is clear that differential signaling through one or both of these receptors influences the character of inflammatory response to TNF-α.

The proinflammatory chemokine, IL-8, is crucial to the intestinal response to injury and systemic inflammation. IL-8 is regulated by the transcription factor NF-κB[43] and is produced by many cell types including macrophages, endothelial cells, fibroblasts, and various epithelial cell types.[44–46] In the intestinal mucosa, IL-8 has been shown to be a potent stimulator of neutrophil recruitment to the lamina propria.[47] Though additional signals may be required,[48] IL-8 has repeatedly been demonstrated to participate in migration of neutrophils across the intestinal epithelium in response to acute inflammation.[49–51] Both inflammatory disorders of the intestine and hypoperfusion due to ischemia or shock may result in damage to the intestinal mucosa. Intestinal restitution, the in vivo response to a mucosal injury, consists of three stages: de-differentiation, migration, and re-differentiation of intestinal epithelial cells. Together, this process leads to healing of mucosal lesions.[52] IL-8 has been shown to play a significant role in all three stages of this process via the CXCR1 receptor.[53,54] Human intestinal microvascular cells treated with IL-8 show increased chemotaxis, proliferation, and tube formation via the CXCR2 receptor.[55]

Vectorial secretion of IL-8 may be an important factor in one or more of the above functions. Apical IL-8 released after an apical stimulus may improve the chemotactic gradient and facilitate neutrophil transepithelial migration. Alternatively, a basolateral stimulus resulting in bidirectional IL-8 secretion could result in neutrophil recruitment to the lamina propria and improved epithelial restitution luminally.

Conclusion

In conclusion, our data demonstrated that differentiated, polarized Caco-2 cells respond to TNF-α by vectorially secreting IL-8. This response appeared to be mediated by differential TNF-α receptor expression: TNFR1 basolater-

ally and TNFR1 and TNFR2 apically. Our findings provide important clues to the mechanism by which the intestinal epithelium regulates local inflammation.

References

1. Gosain, A. and R.L. Gamelli, *Role of the gastrointestinal tract in burn sepsis.* J Burn Care Rehabil, 2005. 26(1): p. 85–91.
2. Diebel, L.N. and D.M. Liberati, *Intestinal epithelial cells mediate lung injury after ethanol exposure and hypoxic insult.* J Trauma, 2009. 67(2): p. 296–302.
3. Clark, J.A. and C.M. Coopersmith, *Intestinal crosstalk: a new paradigm for understanding the gut as the "motor" of critical illness.* Shock, 2007. 28(4): p. 384–93.
4. Lenz, A., G.A. Franklin, and W.G. Cheadle, *Systemic inflammation after trauma.* Injury, 2007. 38(12): p. 1336–45.
5. Rogler, G. and T. Andus, *Cytokines in inflammatory bowel disease.* World J Surg, 1998. 22(4): p. 382–9.
6. Alverdy, J.C. and E.B. Chang, *The re-emerging role of the intestinal microflora in critical illness and inflammation: why the gut hypothesis of sepsis syndrome will not go away.* J Leukoc Biol, 2008. 83(3): p. 461–6.
7. McCormick, B.A., et al., *Apical secretion of a pathogen-elicited epithelial chemoattractant activity in response to surface colonization of intestinal epithelia by Salmonella typhimurium.* J Immunol, 1998. 160(1): p. 455–66.
8. Neal, M.D., et al., *Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier.* J Immunol, 2006. 176(5): p. 3070–9.
9. Reilly, N., et al., *Probiotics potentiate IL-6 production in IL-1beta-treated Caco-2 cells through a heat shock-dependent mechanism.* Am J Physiol Regul Integr Comp Physiol, 2007. 293(3): p. R1169–79.
10. Wehkamp, J., et al., *NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by Escherichia coli Nissle 1917: a novel effect of a probiotic bacterium.* Infect Immun, 2004. 72(10): p. 5750-8.
11. Wu, L., et al., *Recognition of host immune activation by Pseudomonas aeruginosa.* Science, 2005. 309(5735): p. 774–7.
12. Bradley, J.R., *TNF-mediated inflammatory disease.* J Pathol, 2008. 214(2): p. 149–60.
13. Wajant, H., K. Pfizenmaier, and P. Scheurich, *Tumor necrosis factor signaling.* Cell Death Differ, 2003. 10(1): p. 45–65.
14. Spies, M., et al., *Role of TNF-alpha in gut mucosal changes after severe burn.* Am J Physiol Gastrointest Liver Physiol, 2002. 283 (3): p. G703–8.
15. Colombel, J.F., et al., *Infliximab, azathioprine, or combination therapy for Crohn's disease.* N Engl J Med., 2010. 362(15): p. 1383–95.
16. Owczarek, D., et al., *Biological therapy of inflammatory bowel disease.* Pol Arch Med Wewn, 2009. 119(1–2): p. 84–8.
17. Appau, K.A., et al., *Use of infliximab within 3 months of ileocolonic resection is associated with adverse postoperative outcomes in Crohn's patients.* J Gastrointest Surg, 2008. 12(10): p. 1738–44.
18. Marehbian, J., et al., *Adverse events associated with common therapy regimens for moderate-to-severe Crohn's disease.* Am J Gastroenterol, 2009. 104(10): p. 2524–33.
19. Tamion, F., et al., *Gut ischemia and mesenteric synthesis of inflammatory cytokines after hemorrhagic or endotoxic shock.* Am J Physiol, 1997. 273(2 Pt 1): p. G314–21.
20. Onizawa, M., et al., *Signaling pathway via TNF-alpha/NF-kappaB in intestinal epithelial cells may be directly involved in colitis-associated carcinogenesis.* Am J Physiol Gastrointest Liver Physiol, 2009. 296(4): p. G850–9.
21. Bocker, U., et al., *Responsiveness of intestinal epithelial cell lines to lipopolysaccharide is correlated with Toll-like receptor 4 but not Toll-like receptor 2 or CD14 expression.* Int J Colorectal Dis, 2003. 18(1): p. 25–32.
22. Holtkamp, G.M., et al., *Polarized secretion of IL-6 and IL-8 by human retinal pigment epithelial cells.* Clin Exp Immunol, 1998. 112(1): p. 34–43.
23. Cudicini, C., et al., *Vectorial production of interleukin 1 and interleukin 6 by rat Sertoli cells cultured in a dual culture compartment system.* Endocrinology, 1997. 138(7): p. 2863–70.
24. Kruger, S., et al., *Interleukin-8 secretion of cortical tubular epithelial cells is directed to the basolateral environment and is not enhanced by apical exposure to Escherichia coli.* Infect Immun, 2000. 68(1): p. 328–34.
25. Panigrahi, P., et al., *Escherichia coli transcytosis in a Caco-2 cell model: implications in neonatal necrotizing enterocolitis.* Pediatr Res, 1996. 40(3): p. 415–21.
26. Nasreen, N., et al., *Polar production of interleukin-8 by mesothelial cells promotes the transmesothelial migration of neutrophils: role of intercellular adhesion molecule-1.* J Infect Dis, 2001. 183 (11): p. 1638–45.
27. Sitaraman, S.V., et al., *Neutrophil–epithelial crosstalk at the intestinal lumenal surface mediated by reciprocal secretion of adenosine and IL-6.* J Clin Invest, 2001. 107(7): p. 861–9.
28. Vreugdenhil, A.C., et al., *Lipopolysaccharide-binding protein is vectorially secreted and transported by cultured intestinal epithelial cells and is present in the intestinal mucus of mice.* J Immunol, 2000. 165(8): p. 4561–6.
29. Zeillemaker, A.M., et al., *Polarized secretion of interleukin-8 by human mesothelial cells: a role in neutrophil migration.* Immunology, 1995. 84(2): p. 227–32.
30. Chantret, I., et al., *Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cells: a survey of twenty cell lines.* Cancer Res, 1988. 48(7): p. 1936–42.
31. Grasset, E., J. Bernabeu, and M. Pinto, *Epithelial properties of human colonic carcinoma cell line Caco-2: effect of secretagogues.* Am J Physiol, 1985. 248(5 Pt 1): p. C410–8.
32. Grasset, E., et al., *Epithelial properties of human colonic carcinoma cell line Caco-2: electrical parameters.* Am J Physiol, 1984. 247(3 Pt 1): p. C260–7.
33. Abreu, M.T., M. Fukata, and M. Arditi, *TLR signaling in the gut in health and disease.* J Immunol, 2005. 174(8): p. 4453–60.
34. Duizer, E., et al., *Absorption enhancement, structural changes in tight junctions and cytotoxicity caused by palmitoyl carnitine in Caco-2 and IEC-18 cells.* J Pharmacol Exp Ther, 1998. 287(1): p. 395–402.
35. Li, N., et al., *Glutamine supports recovery from loss of transepithelial resistance and increase of permeability induced by media change in Caco-2 cells.* J Nutr Biochem, 2003. 14(7): p. 401–8.
36. Baud, V. and M. Karin, *Signal transduction by tumor necrosis factor and its relatives.* Trends Cell Biol, 2001. 11(9): p. 372–7.
37. Ferrero, E., et al., *Roles of tumor necrosis factor p55 and p75 receptors in TNF-alpha-induced vascular permeability.* Am J Physiol Cell Physiol, 2001. 281(4): p. C1173–9.
38. Orlinick, J.R. and M.V. Chao, *TNF-related ligands and their receptors.* Cell Signal, 1998. 10(8): p. 543–51.
39. Edelblum, K.L., et al., *TNFR1 promotes tumor necrosis factor-mediated mouse colon epithelial cell survival through RAF activation of NF-kappaB.* J Biol Chem, 2008. 283(43): p. 29485–94.
40. Heyninck, K. and R. Beyaert, *Crosstalk between NF-kappaB-activating and apoptosis-inducing proteins of the TNF-receptor complex.* Mol Cell Biol Res Commun, 2001. 4(5): p. 259–65.

41. Neurath, M.F., C. Becker, and K. Barbulescu, *Role of NF-kappaB in immune and inflammatory responses in the gut.* Gut, 1998. 43 (6): p. 856–60.
42. Holtmann, M.H. and M.F. Neurath, *Differential TNF-signaling in chronic inflammatory disorders.* Curr Mol Med, 2004. 4(4): p. 439–44.
43. Vallee, S., et al., *Cytokine-induced upregulation of NF-kappaB, IL-8, and ICAM-1 is dependent on colonic cell polarity: implication for PKCdelta.* Exp Cell Res, 2004. 297(1): p. 165–85.
44. Gross, V., et al., *Regulation of interleukin-8 production in a human colon epithelial cell line (HT-29).* Gastroenterology, 1995. 108(3): p. 653–61.
45. Schroder, J.M. and E. Christophers, *Secretion of novel and homologous neutrophil-activating peptides by LPS-stimulated human endothelial cells.* J Immunol, 1989. 142(1): p. 244–51.
46. Strieter, R.M., et al., *Monokine-induced neutrophil chemotactic factor gene expression in human fibroblasts.* J Biol Chem, 1989. 264(18): p. 10621–6.
47. Kucharzik, T., et al., *Acute induction of human IL-8 production by intestinal epithelium triggers neutrophil infiltration without mucosal injury.* Gut, 2005. 54(11): p. 1565–72.
48. McCormick, B.A., *Bacterial-induced hepoxilin A3 secretion as a pro-inflammatory mediator.* FEBS J, 2007. 274(14): p. 3513–8.
49. Blake, K.M., et al., *Neutrophils migrate across intestinal epithelium using beta2 integrin (CD11b/CD18)-independent mechanisms.* Clin Exp Immunol, 2004. 136(2): p. 262–8.
50. Kim, J.M., et al., *Nuclear factor-kappa B activation pathway in intestinal epithelial cells is a major regulator of chemokine gene expression and neutrophil migration induced by Bacteroides fragilis enterotoxin.* Clin Exp Immunol, 2002. 130(1): p. 59–66.
51. Kucharzik, T. and I.R. Williams, *Neutrophil migration across the intestinal epithelial barrier—summary of in vitro data and description of a new transgenic mouse model with doxycycline-inducible interleukin-8 expression in intestinal epithelial cells.* Pathobiology, 2002. 70(3): p. 143–9.
52. Mammen, J.M. and J.B. Matthews, *Mucosal repair in the gastrointestinal tract.* Crit Care Med, 2003. 31(8 Suppl): p. S532–7.
53. Maheshwari, A., et al., *Effects of interleukin-8 on the developing human intestine.* Cytokine, 2002. 20(6): p. 256–67.
54. Sturm, A., et al., *CXCL8 modulates human intestinal epithelial cells through a CXCR1 dependent pathway.* Cytokine, 2005. 29(1): p. 42–8.
55. Heidemann, J., et al., *Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2.* J Biol Chem, 2003. 278(10): p. 8508–15.

Discussant

Dr. Edward E. Whang (Boston, MA): Good job with the studies and very clear presentation. TNF-stimulated IL-8 secretion in this cell line is a well-known phenomenon. Your identification of directional asymmetry to this process is intriguing. I will ask you to address issues related to validity and biological significance of these findings.

First, you have suggested but not actually shown differential distribution of TNF receptor subtypes. Have you done these studies?

Second, Caco-2 cells are commonly used to model small intestinal epithelium, but they are colon cancer cells, after all. Are you aware whether normal small bowel enterocytes express TNF receptors and whether they respond to TNF by secreting IL-8? How would you validate that the directionality of TNF-stimulated IL-8 secretion you have reported today exists in normal intestine?

Finally, let us assume you were to figure out the detailed mechanisms responsible for this directional phenomenon. What would you do with that information? What would be the potential biological or clinical significance of this information?

Closing Discussant

Dr. Dennis Sonnier: Thank you very much for your comments and insightful questions. I think that looking at this in an ex vivo setting or in other cell lines would be helpful.

In addition, the IBD literature contains data regarding expression of various inflammatory markers in the stool, and they use this to track disease. This suggests that findings similar to ours make occur in the in vivo setting. IBD patients also show considerable responses to anti-TNF receptor therapy. The presence and function of these receptors on the apical membrane in tissue specimens and animal models is something else we plan on looking at as well.

What do we plan to do with this? Transferring this into a mouse model after we work out some of the cellular mechanisms will be going to be very important for studying various causes of intestinal inflammation as well as possible use in assessment of clinical severity of intestinal inflammation after trauma.

Regarding your questions about receptor expression, I agree that we have not demonstrated receptor expression during these studies. We have performed some initial confocal microscopy studies to help determine receptor expression.

Discussant

Dr. Michael Sarr (Rochester, MN): There are several other enterocyte-like cell models, such as RIE and IEC-6 cells. And they are more from younger animals. Have you thought about looking at those cell lines as well?

Closing Discussant

Dr. Dennis Sonnier: Thank you for your comments and questions. We have not yet looked at those specific cell lines, but these and other cell lines should be considered. We used Caco-2 cells in the current experiments because of their known ability to polarize when differentiated on Transwells.

Discussant

Dr. Michael Sarr (Rochester, MN): Why would a cell secrete something into the lumen when it is polarized. We have been struggling with that, looking at the effects of some hormones that are secreted into the lumen that have an effect. Why does this cell line secrete IL-8 into the lumen?

Closing Discussant

Dr. Dennis Sonnier: Dr. Sarr, that is a great question. By way of pure speculation, I think luminal secretion of cytokines may be a way of autocrine or paracrine control of inflammation. Upstream, intestinal epithelial cells can control a response downstream by the mediators that they secrete into the lumen in a way that cannot be achieved by secreting basolaterally into the bloodstream.

Additional effects of IL-8 specifically related to cell restitution or angiogenesis could also be important. In other words, mucosal healing, I think, could also be affected by mediators from cells upstream.

Discussant

Dr. Carlos Chan (Montreal, Canada): I have one quick question about the slide that you showed regarding LPS treatment on the apical side. As far as I understand, the Caco-2 cells do not express Toll-like receptor 4, which is the receptor for the LPS, although you show that CD14 is expressed. So it may not respond. So have you thought of using other cell lines that you have that expresses Toll-like receptor 4?

Closing Discussant

Dr. Dennis Sonnier: Thank you for your comments and questions. Some investigators have demonstrated TLR4 expression in Caco 2 cells, at least under some conditions, but they generally are hyporesponsive to LPS due to lack of MD2 and other proteins related to LPS signaling.

Discussant

Dr. Carlos Chan (Montreal, Canada): Actually, there are some papers that show there is no receptor and some papers show there is a receptor. Have you shown on your study that these cells that you have actually have Toll-like receptor 4?

Closing Discussant

Dr. Dennis Sonnier: I have not demonstrated that myself, but unpublished data from previous residents in our lab indicate that Caco-2 cells do respond to LPS stimulation if MD2 is added to the treatments.

I would like to thank the Society for the privilege of presenting our data.

J Gastrointest Surg (2010) 14:1600–1607
DOI 10.1007/s11605-010-1316-6

2010 SSAT PLENARY PRESENTATION

Comparison of Outlier Identification Methods in Hospital Surgical Quality Improvement Programs

Karl Y. Bilimoria · Mark E. Cohen · Ryan P. Merkow · Xue Wang · David J. Bentrem · Angela M. Ingraham · Karen Richards · Bruce L. Hall · Clifford Y. Ko

Received: 27 April 2010 / Accepted: 9 August 2010 / Published online: 8 September 2010

Abstract
Background Surgeons and hospitals are being increasingly assessed by third parties regarding surgical quality and outcomes, and much of this information is reported publicly. Our objective was to compare various methods used to classify hospitals as outliers in established surgical quality assessment programs by applying each approach to a single data set.
Methods Using American College of Surgeons National Surgical Quality Improvement Program data (7/2008–6/2009), hospital risk-adjusted 30-day morbidity and mortality were assessed for general surgery at 231 hospitals (cases=217,630) and for colorectal surgery at 109 hospitals (cases=17,251). The number of outliers (poor performers) identified using different methods and criteria were compared.
Results The overall morbidity was 10.3% for general surgery and 25.3% for colorectal surgery. The mortality was 1.6% for general surgery and 4.0% for colorectal surgery. Programs used different methods (logistic regression, hierarchical modeling, partitioning) and criteria ($P<0.01$, $P<0.05$, $P<0.10$) to identify outliers. Depending on outlier identification methods and criteria employed, when each approach was applied to this single dataset, the number of outliers ranged from 7 to 57 hospitals for general surgery morbidity, 1 to 57 hospitals for general surgery mortality, 4 to 27 hospitals for colorectal morbidity, and 0 to 27 hospitals for colorectal mortality.
Conclusions There was considerable variation in the number of outliers identified using different detection approaches. Quality programs seem to be utilizing outlier identification methods contrary to what might be expected, thus they should justify their methodology based on the intent of the program (i.e., quality improvement vs. reimbursement). Surgeons and hospitals should be aware of variability in methods used to assess their performance as these outlier designations will likely have referral and reimbursement consequences.

K. Y. Bilimoria (✉) · M. E. Cohen · R. P. Merkow · X. Wang · A. M. Ingraham · K. Richards
Division of Research and Optimal Patient Care,
American College of Surgeons,
633 N. St. Clair Street, 22nd Floor,
Chicago, IL 60611, USA
e-mail: kbilimoria@facs.org

K. Y. Bilimoria · D. J. Bentrem
Department of Surgery, Feinberg School of Medicine,
Northwestern University,
Chicago, IL, USA

K. Y. Bilimoria
Division of Surgical Oncology, M.D. Anderson Cancer Center,
Houston, TX, USA

B. L. Hall
Department of Surgery,
John Cochran Veterans Affairs Medical Center,
Saint Louis, MO, USA

B. L. Hall
Department of Surgery, School of Medicine,
Washington University in St. Louis and Barnes Jewish Hospital,
Saint Louis, MO, USA

B. L. Hall
Center for Health Policy and the Olin Business School at
Washington University in St Louis,
St Louis, MO, USA

C. Y. Ko
Department of Surgery, University of California, Los Angeles (UCLA) and VA Greater Los Angeles Healthcare System,
Los Angeles, CA, USA

Keywords Surgery · Hospital · Quality · Morbidity · Mortality · Morbidity · Complications · Deaths · National surgery quality improvement program · Outlier

Introduction

In response to calls to measure the quality of medical care provided in the USA,[1–3] hospital quality assessment initiatives have been developed by government oversight agencies, payers, professional societies, and commercial entities.[4,5] This information is increasingly being reported publicly,[4,5] and patients and referring providers are selecting hospitals based on performance rankings from these hospital quality assessment reports.[6] In addition, an increasing number of surgical quality measurement programs focus on providing hospitals with self-assessment data to help direct internal quality improvement initiatives in order to improve care.

Most quality assessment initiatives seek to identify hospitals with outlying performance or outcomes in order to direct referrals and to guide targeted quality improvement efforts. Thus, being identified as an outlier can be a critical designation that can have considerable consequences. The objectives of this study were to examine how different hospital surgical quality assessment programs identify outliers and how the number of outliers varies with the different methods and criteria employed.

Methods

Data Acquisition and Patient Selection

Hospitals participating in the American College of Surgeons National Surgical Quality Improvement Program (ACS NSQIP) were examined.[7] The structure of ACS NSQIP, including sampling strategy, data abstraction procedures, variables collected, and outcomes have been extensively described previously.[7–12] Briefly, the program prospectively collects detailed data regarding patient demographics, preoperative comorbidities and other risk factors, laboratory values prior to the index surgical procedure, and certain operative variables.[13] Patients are followed for postoperative outcomes for 30 days after the index operation. Morbidity and mortality are identified using comprehensive strategies.[14] The surgical clinical reviewers (SCR) examine inpatient records, review outpatient physician office charts, and contact patients directly to accurately assess outcomes.

ACS NSQIP samples cases from general surgery, vascular surgery, and certain subspecialties. Data are abstracted at each site by SCRs who complete intensive training programs and continuing education courses to standardize data collection.[6] Data definitions are rigorous and standardized across all participating institutions. Data consistency and reliability are assessed periodically at each hospital through an on-site inter-rater reliability audit program.[15] ACS NSQIP then provides participating hospitals with risk-adjusted morbidity and mortality data in comparison to the other participating hospitals in order to identify areas for improvement.[15]

All patients were identified from the ACS NSQIP database from July 1, 2008 through June 30, 2009. We examined two groups: (1) all patients who underwent a general surgery operation and (2) all patients who underwent a colorectal operation requiring resection of a part of the colon or rectum. Assessment of colorectal operations allows comparisons of a common group of procedures with relatively high morbidity and mortality rates and minimizes the issues related to case mix when examining all general surgery operations. Only hospitals that reported at least 100 cases for each category were included to minimize issues related to hospitals with small sample sizes as is done in some quality improvement programs.[16,17]

Outcomes

Outcomes of interest were overall morbidity (occurrence of any of 19 morbidity events collected by ACS NSQIP) and mortality within 30 days of the index operation. Complications generally applicable to all operations are assessed by ACS NSQIP and include superficial surgical site infection (SSI), deep SSI, organ space SSI, wound disruption/dehiscence, pneumonia, unplanned intubation, pulmonary embolism, ventilator dependence more than 48 h, progressive renal insufficiency, acute renal failure, urinary tract infection, stroke or cerebrovascular accident, coma lasting more than 24 h, cardiac arrest, myocardial infarction, bleeding requiring transfusion, deep venous thrombosis, sepsis, and septic shock.

Statistical Methods

Programs used a variety of methods (logistic regression, hierarchical modeling, partitioning) and criteria ($P<0.01$, $P<0.05$, $P<0.10$) to identify outliers. Separately for general surgery and colorectal surgery, the standard ACS NSQIP modeling approach was employed.[15] Forward stepwise logistic regression models were constructed for overall morbidity and mortality. Demographics, comorbidities, surgical subtype and/or indication, and preoperative laboratory variables were used in the modeling (Table 1). Missing data (almost entirely limited to laboratory values) were imputed using the method of Buck.[18] Regression equations yield expected event probabilities for individual

Table 1 Factors included in risk models

Patient demographics	Age (<65, 65 to 74, 75 to 84, 85+ years)
	Gender
	Race (white, black, Asian, Hispanic, other)
Lifestyle factors	Smoking status (within 1 year of surgery)
	Alcohol consumption (>2 drinks/day for 2 weeks before admission)
Overall health assessments	ASA class (I/II—normal healthy/mild systemic disease, III—severe systemic disease, IV/V—severe systemic disease that is a constant threat to life/moribund),
	Preoperative functional status (independent, partially dependent, totally dependent)
Comorbidities	Dyspnea (none, moderate exertion, at rest),
	Body mass index (BMI: normal [<18.5 to ≤25], underweight [≤18.5], overweight [<25 to ≤30], three levels of obese [<30 to ≤35, <35 to ≤40, <40]).
	Ventilator dependence
	Sepsis (systemic inflammatory response syndrome, sepsis, septic shock)
	History of chronic obstructive pulmonary disease (COPD)
	Hypertension requiring medication
	Current pneumonia
	Ascites
	Coronary heart disease (includes angina, myocardial infarction within 30 days prior to surgery, percutaneous cardiac intervention, or cardiac artery bypass surgery)
	Peripheral vascular disease (includes revascularization for peripheral vascular disease, claudication, rest pain, amputation, or gangrene)
	Neurologic event/disease (includes stroke with or without residual deficit, transient ischemic attack, hemiplegia, paraplegia, quadriplegia, or impaired sensation)
	Diabetes (oral medication or insulin dependent)
	Disseminated cancer
	Steroid use
	Weight loss (>10% in last 6 months)
	Bleeding disorders
	Current chemotherapy or radiotherapy
Surgical factors	Operation (based on CPT groupings)
	Wound class (clean, clean/contaminated vs. contaminated, dirty/infected)
	Indication for surgery (based on ICD-9 codes)
	Emergent procedure
Laboratories	Sodium, albumin, blood urea nitrogen, creatinine, hematocrit, platelet count, white blood count (WBC), partial thromboplastin time (PTT), and prothrombin (PT)

patients, and the sum of these probabilities for patients at each hospital is the expected (E) in the hospital observed-to-expected (O/E) ratio. *P* values for these ratios were then computed using an exact procedure.[19] An O/E ratio of 1.0 indicates that the number of observed events is equal to the number of expected events. Ratios of less than 1.0 indicate outcomes which are better than expected based on the regression model, while O/E ratios greater than 1.0 indicate outcomes which are worse than expected. If the O/E ratio confidence interval does not include 1.0, then these differences are deemed statistically significant.

To determine whether the hospital was an outlier, we reproduced the various strategies currently used by different hospital surgical quality improvement programs as well as some alternatives that have been suggested in the literature. For standard logistic regression models, standard confidence intervals of 99%, 95%, and 90% were examined.[15] In addition, the Bonferroni correction ($P<0.05$ divided by number of hospitals) was used as a particularly rigorous criterion for outlier detection.[20] A false-discovery rate criterion was also examined (controls for the expected proportion of type I errors).[20,21] Finally, we also assessed the effect of multi-level random effects modeling applying a P value <0.05. For each outcome, variables selected for inclusion in the logistic model ($P<0.05$ and quadrature estimation) were then included in a random intercepts, fixed slopes hierarchical model using the adaptive quadrature likelihood approximation method in SAS PROC GLIM-

MIX.[22–24] Patient level-predicted probabilities were estimated using only the fixed portion of the model (SAS GLIMMIX option NOBLUP), so that O/E ratios would appropriately identify outliers.[25] All data manipulation and analyses were done with SAS version 9.1.3 (Cary, NC, USA).

Results

For general surgery, 217,630 patients underwent operations at 231 hospitals in the data. For colorectal surgery, 17,251 patients underwent operations at 109 hospitals. The smaller number of hospitals examined for colorectal surgery was due to the requirement to report at least 100 cases during the year. The median number of cases per hospital was 951 (range: 110 to 2,785) for general surgery and 149 (range: 101 to 528) for colorectal surgery. The overall morbidity was 10.3% for general surgery and 25.3% for colorectal surgery. The mortality was 1.6% for general surgery and 4.0% for colorectal surgery.

Ten established surgical quality improvement programs were examined to determine how they designated outliers. Seven programs used standard logistic regression models with various P values or confidence intervals including $P<0.01$ or a 99% confidence interval (ACS and VA NSQIP for morbidity[16]), $P<0.05$ or a 95% confidence interval (ACS and VA NSQIP for morbidity, New York State Cardiac Surgery Report Card[26], California Office of Statewide Health Planning and Development Quality Ratings[27], Surgical Care and Outcomes Assessment Program [SCOAP][17], and the American Society of Clinical Oncology's Quality Oncology Practice Initiative[28]), and $P<0.10$ or a 90% confidence interval (ACS and VA NSQIP mortality, HealthGrades[29]). One program clearly noted that they use hierarchical models to identify outliers and employ a criterion of $P<0.05$ (Society for Thoracic Surgery[30]). Some programs identified outliers as those hospitals in the bottom quintile (Medicare[31]) or quartile (Leapfrog[21] and SCOAP). We reproduced the outlier methodology of each of these programs using a single dataset.

For general surgery morbidity, we identified 17 outliers with $P<0.01$, 33 outliers with $P<0.05$, 36 outliers with $P<0.10$, 46 outliers with quintiles, 57 outliers with quartiles, and 35 outliers by hierarchical modeling (Table 2). For general surgery mortality, we identified one outlier with $P<0.01$, five outliers with $P<0.05$, ten outliers with $P<0.10$, 46 outliers with quartiles, 57 outliers by quintiles, and six outliers with hierarchical modeling.

For colorectal surgery morbidity, we identified six outliers with $P<0.01$, eight outliers with $P<0.05$, ten outliers with $P<0.10$, 21 outliers with quintiles, 27 outliers with quartiles, and eight outliers with hierarchical modeling (Table 2). For colorectal surgery mortality, we identified

Table 2 Comparison of outliers identified using different detection methods and criteria for general surgery and colorectal surgery

		Number (%) of high-outlier hospitals (poor performers)			
		General surgery		Colorectal surgery	
		Morbidity (22,485 patients)	Mortality (3,395 patients)	Morbidity (4,357 patients)	Mortality (690 patients)
Methods currently utilized by hospital surgical quality assessment programs					
Logistic regression					
P value ≤0.01	(ACS and VA NSQIP morbidity)	17 (7%)	1 (<1%)	6 (6%)	1 (1%)
P value ≤0.05	(ACS and VA NSQIP morbidity, HealthGrades, New York Cardiac Surgery, California OSHPD, SCOAP, QOPI)	33 (14%)	5 (2%)	8 (7%)	1 (1%)
P value ≤0.10	(ACS and VA NSQIP mortality)	36 (16%)	10 (5%)	10 (9%)	1 (2%)
Hierarchical modeling					
P value ≤0.05	(Society for Thoracic Surgery)	35 (15%)	6 (3%)	8 (7%)	1 (1%)
Partitioning					
Quintiles	(Medicare)	46 (20%)	46 (20%)	21 (19%)	21 (19%)
Quartiles	(Leapfrog, SCOAP)	57 (25%)	57 (25%)	27 (25%)	27 (25%)
Alternative methods proposed in the literature					
$P \leq 0.05$ with Bonferroni correction		7 (3%)	0	4 (4%)	0
5% false discovery rate		20 (9%)	1 (<1%)	7 (6%)	0

Hospitals are considered for outlier determination if the number of patients reported is ≥100 per hospital

one outlier with $P<0.01$, one outlier with $P<0.05$, one outlier with $P<0.10$, 21 outliers with quintiles, 27 outliers with quartiles, and one outlier with hierarchical modeling.

Though we did not find they were currently being used, other methods have been suggested in the literature as possibilities for hospital quality outlier identification, thus these were examined as well. For general surgery morbidity, the Bonferroni correction yielded seven outliers and the false discovery rate criterion yielded 20 outliers (Table 2). For general surgery mortality, the Bonferroni correction yielded zero outliers and the false discovery rate criterion yielded one outlier. For colorectal surgery morbidity, the Bonferroni correction yielded four outliers and the false discovery rate criterion yielded seven outliers. For colorectal surgery mortality, the Bonferroni correction yielded zero outliers and the false discovery rate criterion yielded zero outliers.

Discussion

With increasing emphasis on quality assessment, numerous programs have been developed to measure hospital surgical quality and employ different statistical methodologies to detect outliers. Many of the programs offer public comparisons and have an impact on where patients receive their care.[6] Alternatively, many quality assessment programs provide hospitals with personalized quality improvement data in order to facilitate targeted quality improvement initiatives. In either case, there can be profound implications when hospitals are designated as outliers. We found that the different methods used by these programs to identify outlying hospitals resulted in substantial variation in the number of outliers detected, when sequentially applied to a single (ACS NSQIP) dataset.

In examining the established hospital surgical quality assessment programs, the methods and criteria used to identify outliers varied. Most commonly, programs used standard logistic regression models with the typical p value of 0.05 to identify hospitals with outlying performance, and an example of how this is reported back to hospitals by ACS NSQIP is shown in Fig. 1. A distinction should be made between outlier detection methods and criteria. Specifically, methodology refers to the statistical approach used to analyze the data, while the criteria refers to the significance threshold the different methods employ. Three *methods* were used: logistic regression, hierarchical modeling, and simple partitioning (i.e., quartiles and quintiles). Within the regression and hierarchical modeling, various *criteria* can be employed (i.e., $P<0.01$, $P<0.05$, or $P<0.10$).

We previously demonstrated that the number of outliers identified by logistic and hierarchical methods does not differ appreciably, and we found this to be generally true in this study as well.[25] There are multiple nuances of hierarchical models that have also been shown to affect the number of outliers, almost always reducing the number of outliers in comparison to non-multilevel approaches.[32,33] The various criteria employed with logistic regression models resulted in a range of outliers for general surgery morbidity (7 to 36), general surgery mortality (0 to 10), colorectal morbidity (4 to 10), and colorectal mortality (0 to 1). The overall number of outliers differed between the general surgery and colorectal groups primarily due to the smaller sample sizes per hospital (median number of cases per hospital: 951 vs. 149), where smaller samples lead to wider confidence intervals and fewer definitive outliers, as well as different event rates and differences in intra-institutional correlation. However, simply partitioning the hospitals into roughly equal size groups and denoting the lowest performing

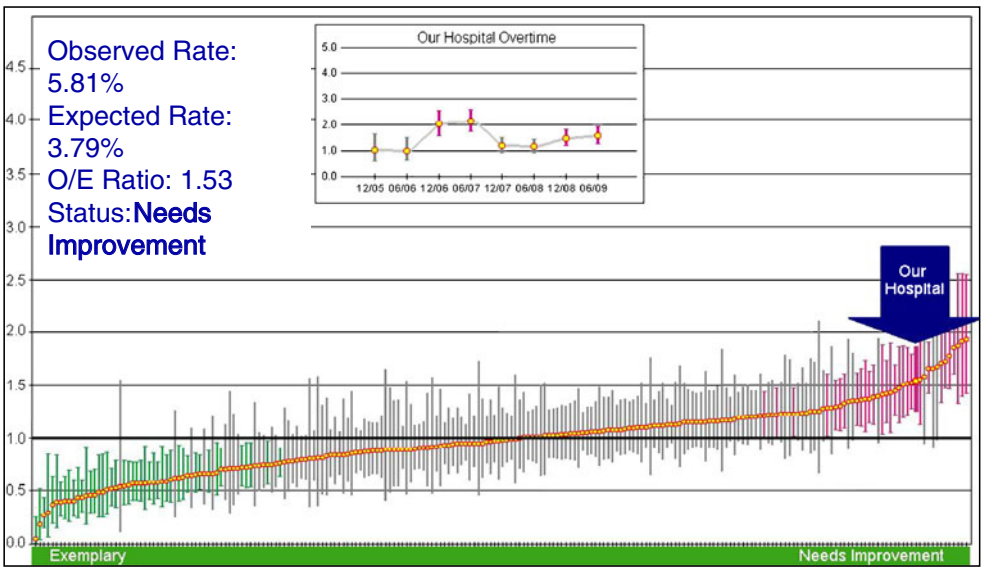

Fig. 1 Sample graph demonstrating how ACS NSQIP presents hospitals as outliers to participating institutions

group as outliers led to a considerably more outliers than when statistical methods were employed. The statistical details of these approaches are highly technical, thus it is simply important to note that different approaches exist and result in a highly variable number of outliers.

Typically, the purpose of identifying hospital quality outliers is either to help patients assess hospitals or to help hospitals identify areas for quality improvement. Although we could not review every piece of documentation for each program, we found it rare that programs explicitly justified their outlier identification methodology. In principle, if the program could have detrimental effects on a hospital designated as an outlier (i.e., if the purpose of the program is to direct referrals, help patients in selecting a hospital, or influence reimbursement), then more strict criteria to identify outliers may be merited. However, if the intent of the program is to help hospitals identify quality improvement targets, then identifying more hospitals as outliers may not be particularly disadvantageous. For example, if a hospital in the 24th percentile is identified as an outlier, having that hospital make an attempt to improve care would likely be helpful for the overall care of patients. In our assessment of the ACS NSQIP hospitals, few if any hospitals were outstanding for every outcome assessed. Thus, most hospitals have room for improvement. Broader designation as outliers may help trigger quality improvement initiatives. However, it seems as though the intent does not often match the outlier detection method as we might expect. Programs like ACS NSQIP are using strict criteria for quality improvement purposes; whereas, programs like LeapFrog are using quartiles for public reporting. This is the opposite of what is expected.

This study should be interpreted in light of certain limitations. First, this is not an all-inclusive list of hospital surgical quality assessment programs, but rather those that are generally well-established and had their methodology easily available on the internet. In addition, some programs employ different criteria and methods depending on the measure being examined. Furthermore, we used data from more than 200 ACS NSQIP hospitals, but there may be a hospital selection bias in this group as all of these institutions elect to participate in the program. Thus, assessing these hospitals may result in identification of fewer outliers. Finally, detection of outliers is very sensitive to the sample size of the hospital. In this study, we only examined hospitals that reported a minimum of 100 eligible cases. Including hospitals with smaller case numbers would likely alter the number of outliers, but these estimates are unreliable. Alternate methods such as reliability adjustment have been described to resolve this issue with varying sample sizes[34], but the issues surrounding how to detect outliers persists.

Conclusion

There is considerable variation in the number of outliers identified by various hospital surgical quality assessment programs when applying their approaches to a single data set, resulting from differences in the outlier detection methodologies and criteria employed. The different approaches to identifying outliers may have advantages in specific situations depending on the intent of the program. Quality assessment programs should clearly define the methods used to identify outliers and the reasoning for any particular approach. Surgeons and hospitals should be aware of variability in methods used to assess their performance as these outlier designations will likely have referral and reimbursement consequences.

References

1. Institute of Medicine (US). Committee on Quality of Health Care in America. Crossing the quality chasm a new health system for the 21st century. Washington, DC: National Academy, 2001.
2. Institute of Medicine (US). Committee on Quality of Health Care in America, Kohn LT, Corrigan J, Donaldson MS. To err is human: building a safer health system. Washington, DC: National Academy, 2000.
3. Institute of Medicine (US). Committee on Redesigning Health Insurance Performance Measures Payment and Performance Improvement Programs. Performance measurement: accelerating improvement. Washington, DC: National Academies, 2006.
4. Fung CH, Lim YW, Mattke S, et al. Systematic review: the evidence that publishing patient care performance data improves quality of care. Ann Intern Med 2008; 148(2):111–23.
5. Russell EM, Bruce J, Krukowski ZH. Systematic review of the quality of surgical mortality monitoring. Br J Surg 2003; 90(5):527–32.
6. Faber M, Bosch M, Wollersheim H, et al. Public reporting in health care: how do consumers use quality-of-care information? A systematic review. Med Care 2009; 47(1):1–8.
7. American College of Surgeons. National Surgical Quality Improvement Program. Available at www.facs.org/acsnsqip.html. Accessed April 15, 2009.
8. Khuri SF, Daley J, Henderson W, et al. The Department of Veterans Affairs' NSQIP: the first national, validated, outcome-based, risk-adjusted, and peer-controlled program for the measurement and enhancement of the quality of surgical care. National VA Surgical Quality Improvement Program. Ann Surg 1998; 228(4):491–507.
9. Daley J, Khuri SF, Henderson W, et al. Risk adjustment of the postoperative morbidity rate for the comparative assessment of the quality of surgical care: results of the National Veterans Affairs Surgical Risk Study. J Am Coll Surg 1997; 185(4):328–40.
10. Khuri SF, Henderson WG, Daley J, et al. The patient safety in surgery study: background, study design, and patient populations. J Am Coll Surg 2007; 204(6):1089–102.
11. Davis CL, Pierce JR, Henderson W, et al. Assessment of the reliability of data collected for the Department of Veterans Affairs national surgical quality improvement program. J Am Coll Surg 2007; 204(4):550–60.
12. Khuri SF, Henderson WG, Daley J, et al. Successful implementation of the Department of Veterans Affairs' National Surgical Quality Improvement Program in the private sector: the Patient Safety in Surgery study. Ann Surg 2008; 248(2):329–36.

13. American College of Surgeons. National Surgical Quality Improvement Program: Resources, Program Information. Available at https://acsnsqip.org/main/resources_downloads.asp. Accessed April 15, 2009.
14. Bilimoria K, Cohen M, Ingraham A, et al. Effect of post-discharge morbidity and mortality on comparisons of hospital surgical quality. Ann Surg 2010 (in press).
15. American College of Surgeons. National Surgical Quality Improvement Program: Semiannual Report. Available at https://acsnsqip.org/main/resources_semi_annual_report.pdf. Accessed April 15, 2009.
16. American College of Surgeons. National Surgical Quality Improvement Program. Available at www.facs.org/acsnsqip.html. Accessed April 9, 2010.
17. Surgical Care and Outcomes Assessment Program (SCOAP). Available at http://www.scoap.org/downloads/Q1-2009-Hospital-Report-XX.pdf. Last accessed April 9, 2010.
18. Hamilton BH, Ko CY, Richards K, Hall BL. Missing data in the American College of Surgeons National Surgical Quality Improvement Program are not missing at random: implications and potential impact on quality assessments. J Am Coll Surg; 210 (2):125–139 e2.
19. Sun J, Ono Y, Takebuchi Y. A simple method for calculating the exact confidence interval of the standardized mortality ratio with an [sic] SAS function. Journal of Occupational Health 1996; 38:196–197.
20. Jones HE, Ohlssen DI, Spiegelhalter DJ. Use of the false discovery rate when comparing multiple health care providers. J Clin Epidemiol 2008; 61(3):232–240.
21. Austin PC, Naylor CD, Tu JV. A comparison of a Bayesian vs. a frequentist method for profiling hospital performance. J Eval Clin Pract 2001; 7(1):35–45.
22. Dai J, Li Z, Rocke D. Hierarchical logistic regression modeling with SAS GLIMMIX. Available at: www.lexjansen.com/wuss/2006/Analytics/ANL-Dai.pdf, 2006.
23. Houchens R, Chu B, Steiner C. Hierachical modeling using HCUP data. US Agency for Healthcare and Research Quality 2007.
24. SAS. The GLIMMIX Procedure, June 2006. Available at: http:/support.sas.com/rnd/app/papers/glimmix.pdf, 2006.
25. Cohen ME, Dimick JB, Bilimoria KY, et al. Risk adjustment in the American College of Surgeons national surgical quality improvement program: a comparison of logistic versus hierarchical modeling. J Am Coll Surg 2009; 209(6):687–93.
26. New York State Department of Health. Adult Cardiac Surgery in New York State. Available at http://www.nyhealth.gov/statistics/diseases/cardiovascular/. Last accessed April 9, 2010.
27. California Office of Statewide Health Planning and Development (OSHPD). Available at http://www.oshpd.ca.gov/HID/DataFlow/HospQuality.html. Last accessed April 9, 2010.
28. The Quality Oncology Practice Initiative (QOPI). Available at http://qopi.asco.org/index. Last accessed April 9, 2010.
29. HealthGrades. Available at http://www.healthgrades.com. Last accessed April 9, 2010.
30. Society of Thoracic Surgeons. http://www.sts.org/sections/stsnationaldatabase/ Accessed April 9. 2010.
31. US Department of Health and Human Resources. Hospital Compare. http://www.hospitalcompare.hhs.gov. Accessed April 15, 2009.
32. Fedeli U, Brocco S, Alba N, et al. The choice between different statistical approaches to risk-adjustment influenced the identification of outliers. J Clin Epidemiol 2007; 60(8):858–62.
33. Glance LG, Dick A, Osler TM, et al. Impact of changing the statistical methodology on hospital and surgeon ranking: the case of the New York State cardiac surgery report card. Med Care 2006; 44(4):311–9.
34. Ghaferi AA, Birkmeyer JD, Dimick JB. Variation in hospital mortality associated with inpatient surgery. N Engl J Med 2009; 361(14):1368–75.

Discussant

Dr. Thomas J. Watson (Rochester, NY): The issue of quality assessment, and how the data might be utilized by patients, payers, and regulatory agencies for directing care, as well as by hospitals for targeting their improvement initiatives, is certainly gaining a lot of attention among surgeons. Yet as the authors so nicely demonstrate, the manner in which quality outliers are identified varies widely based on lack of uniformity, methodology, and cut-off criteria. We are all quite indebted to the authors for bringing these inconsistencies into the light.

The manuscript is likely to fuel a significant debate regarding which methods and boundaries are appropriate for different purposes. The results of such a debate could have significant impact on institutions that fall just above or just below established thresholds.

I have two questions for the authors.

Number one, is a certain methodology more suitable than others based upon the width or standard deviation of the outcomes' distribution? As an example, ranking hospitals in quintiles may not make sense when the outcomes are clustered closely together. Perhaps setting a minimum threshold would be more appropriate in such a circumstance.

Number two, if you were appointed health care czar today, which methodology and cut-offs would you choose?

Closing Discussant

Dr. Karl Y. Bilimoria: I think that the method selected obviously depends on the measure. And certainly, if it's something like beta blocker post MI, where everybody is 95% plus, the range is going to be narrow. So setting up different criteria for that, a sort of a basement threshold, would be better.

The vast majority of measures that we see that are like this-where there is wide variation. I think it depends entirely upon the intent, whether it's for a quality improvement initiative or whether it's to be publicly disseminated with referral and reimbursement consequences.

Similarly, it would depend what I was using the measure for. But for NSQIP, I favor using quintiles or quartiles.

Discussant

Dr. Keith D. Lillemoe (Indianapolis, IN): I'm not going to make you the health care czar, but I'm going to make you the chair of a department of surgery. I get these kind of numbers and they are not made up. What would you recommend for either myself, your chair, Dr. Soper and any other surgical chair do with this data and to try to institute quality improvement, because this isn't so much about persecuting the bad people, it's trying to lift up the quality.

Regardless of the metric that you look at, we are all going to have some underperformers or outliers. What's the step in instituting quality improvement?

Closing Discussant

Dr. Karl Y. Bilimoria: I think the first step will be bringing it to light and providing people their data and making sure it is high quality data. I think that we have a lack of that right now. Although you may get some reports, I think, providing detailed, high quality data back to the individual performers is something that's been lacking in general.

Also, it's not about the absolute number or where you rank. It's about just showing, what half of the group you are in. And if you are in the lower half, that's something to act on.

Finally, actually demonstrating performance improvement or at least some activity toward improving performance is needed. In some of these measures, the numbers are very small, so demonstrating absolute improvement in outcomes would be difficult. But at least on process measures, those are more absolute, and maybe we can improve there in these circumstances.

Discussant

Dr. Sharon Weber (Madison, WI): I find this whole concept a little bit disturbing in light of the era of public reporting of the outcomes. And I would be even more disturbed if the hospitals identified as low outliers changed when different methodologies were applied. Did you evaluate the specific hospitals that were identified at each end of the scale and whether they changed position when different methodologies were applied, especially for the low outliers?

Closing Discussant

Dr. Karl Y. Bilimoria: For the most part, the really low-performing hospitals are the same across most of the models. When you get to the better performing of the low-performers, there is some variation in the nature of the hospitals. So some do flip in and out of being an outlier.

J Gastrointest Surg (2010) 14:1608–1612
DOI 10.1007/s11605-010-1238-3

CASE REPORT

An Unusual Complication Encountered Incidentally at Laparoscopic Cholecystectomy: A Case Series

Mohammed Mohsin Uzzaman · Manojkumar S. Nair · Fiona Myint

Received: 13 December 2009 / Accepted: 25 May 2010 / Published online: 21 July 2010

Abstract
Introduction This is a case series of erosion of the common bile duct by an in situ stent found incidentally during laparoscopic cholecystectomy (LC). To the best of our knowledge, this is one of the first reported incidences of this nature.
Method Four individual case reports.
Results Thirty nine patients with an in situ CBD stent underwent LC for symptomatic gallstones in our institution over a 4-year time period (2005 to 2009). Four patients were found to have the stent eroding through the wall of the CBD. In these four patients, endoscopic retrograde cholangiopancreatography (ERCP) had previously been performed — extracting stone (s) — followed by sphincterotomy and insertion of a 7 Fr pigtail stent (measuring 4 cm). The operation was converted to open in two patients, and the procedure was abandoned in one of these cases. In the other two patients, the anatomy of Calots triangle was delineated well, and the operator was able to complete LC. The duration between initial pigtail stent insertion and LC ranged from 32 to 400 days. None of our patients required a definitive surgical repair of the CBD or T-tube placement. The stent was removed during surgery in one case, removed endoscopically at a later date in two patients, and passed spontaneously in one patient. All four patients made a good postoperative recovery.
Conclusion CBD erosion is a complication of plastic biliary stent insertion. CBD stent erosion will make surgery more hazardous especially if it remains in situ for a significant period of time. CBD erosion can generally be managed conservatively without the need for surgical repair. Awareness of this complication should prompt earlier surgery or earlier removal of plastic pigtail stents.

Keywords CBD · Stent · Erosion · Laparoscopic cholecystectomy · Case series

Introduction

Laparoscopic cholecystectomy (LC) has become a standard operation for gallstones. In the last 15 years, it has become established practice to perform endoscopic retrograde cholangiopancreatography (ERCP) in patients presenting with the complications of gallstones in whom stones in the common bile duct (CBD) are suspected. More recently, some clinicians routinely insert a CBD stent at the time of ERCP. These are usually plastic pigtail stents. Such patients may then be presented for LC with CBD stents in situ for an undefined time.

M. M. Uzzaman (✉) · M. S. Nair · F. Myint
Department of General Surgery,
North Middlesex University Hospital,
62 Kensington Avenue,
London E12 6NP, UK
e-mail: mohsinuzzaman@yahoo.co.uk

Materials and Methods

There were around 350 ERCPs performed per annum and around 190 biliary stents placed per annum at our institute. All endoscopies were performed by several experienced consultant physicians (gastroenterologists and gastrointestinal surgeons). 39 patients with an in situ CBD stent underwent LC for symptomatic gallstones at our institution over a 4-year time period (June 2005 to June 2009). Four patients were found to have the stent eroding through the wall of the CBD. This represents around 0.53% of the population who were stented. To the best of our knowledge,

this is one of the rare reports of CBD erosion by an in situ stent found incidentally at LC.

Results

Case 1

A 50-year-old female underwent laparoscopic cholecystectomy (LC) for symptomatic gallstones. She had an in situ pigtail CBD stent at the time of LC. The duration between initial stent insertion and LC was 400 days. The reason for the delay was continual nonattendance at clinic appointments and deferring her operation date. She was known to have sickle cell disease and was initially presented with acute cholecystitis with deranged liver function test (LFT: ALP 220, AST 84, Bili 55) but normal amylase. Ultrasound scanning (USS) revealed inflamed thick walled gallbladder and a markedly dilated CBD (12 mm). ERCP was performed — extracting one stone — followed by sphincterotomy and insertion of a 7 Fr pigtail stent (measuring 4 cm). The position of the stent was confirmed to be in the correct position radiologically. At laparoscopy, the anatomy of Calots triangle was indefinable and hence the procedure was converted to open. In this patient, the surgeon could see the stent eroding out of the CBD and disappearing behind the first part of the duodenum. On reviewing the previous imaging, it was clear that the stent had been correctly positioned initially. The operation was abandoned as the anatomy of the biliary tree was dangerously distorted. This patient made a good postoperative recovery and was transferred to a tertiary hepato-biliary unit where definitive treatment, including removal of the CBD stent, was performed.

Case 2

A 40-year-old female underwent laparoscopic cholecystectomy (LC) for symptomatic gallstones. She had an in situ pigtail CBD stent at the time of LC. She initially presented to the general surgical clinic with a 1-year history of biliary colic. As a result of a raised alkaline phosphatase (ALP, 162) and amylase (249), a magnetic resonance cholangiopancreatogram (MRCP) was performed which showed a dilated CBD (7 mm) and multiple stones in the CBD (largest measuring 4 mm). ERCP was performed — extracting three stones — followed by sphincterotomy and insertion of a 7 Fr pigtail stent (measuring 4 cm). The position of the stent was confirmed to be in the correct position radiologically. The duration between initial stent insertion and LC was 32 days. At laparoscopy, the proximal limb of stent was found eroding out of the CBD. As the anatomy of the Calots triangle was delineated clearly, the operator was able to complete LC. The stent was left alone with a temporary drain beside it. The time from umbilical port insertion to removal of all ports was 2 h. This patient made a good postoperative recovery with no postoperative pyrexia or bile leak. She was discharged on the third postoperative day and was followed up with an ERCP 6 weeks later when the eroded stent was successfully removed. It was noted on ERCP that there was free flow of contrast into the duodenum after the procedure and normal biliary anatomy with no evidence of retained stones. Further follow-up visits in outpatient clinics were unremarkable.

Case 3

A 57-year-old female underwent laparoscopic cholecystectomy (LC) for symptomatic gallstones. She had an in situ pigtail CBD stent at the time of LC. The duration between initial stent insertion and LC was 34 days. She initially presented with recurrent epigastric pain and weight loss. Esophagogastroduodenoscopy (OGD) revealed gastritis. She did not respond to proton-pump inhibitors and the pain persisted. She had a raised Ca19-9 level (55 ng/ml) and erythrocyte sedimentation rate (ESR, 46 mm) but normal LFT and amylase. Computed tomography (CT) abdomen showed dilated CBD measuring 9 mm with mild intrahepatic duct dilatation. MRCP revealed two stones in the distal CBD. During ERCP, stones were extracted followed by sphincterotomy and insertion of a 7 Fr pigtail stent (measuring 4 cm). At laparoscopy, the anatomy of Calots triangle was definable. The surgeon could see the distal segment of the stent eroding partially out of the CBD (see Fig. 1). On reviewing the previous imaging, it was clear that the stent had been correctly positioned initially. The operation was completed laparoscopically. The time from umbilical port insertion to removal of all ports was 1 h 45 min. There were no postoperative problems such as

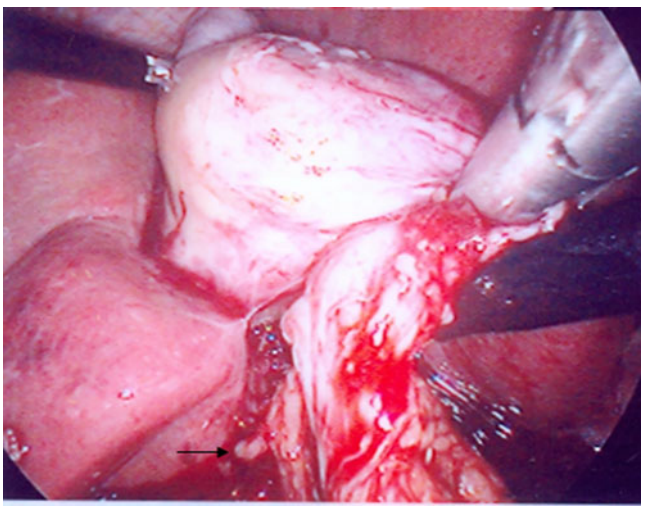

Fig. 1 CBD stent erosion for case 3 (*arrowed*).

pyrexia or bile leak. She was discharged on the second postoperative day. She had a follow-up ERCP 4 weeks later when the eroded stent was successfully removed. It was noted on ERCP that there was a free flow of contrast into the duodenum after the procedure and normal bilary anatomy with no evidence of retained stones. Further follow-up visits in outpatient clinics were unremarkable.

Case 4

A 64-year-old female underwent laparoscopic cholecystectomy (LC) for symptomatic gallstones. She had an in situ pigtail CBD stent at the time of LC. The duration between initial stent insertion and LC was 124 days. The reason for the delay was constant deferral of operation and removal of stent. She initially presented with acute cholecystitis. She had deranged LFTs (AST 130, Bili 45, ALP 187) but normal amylase. Subsequent USS abdomen revealed multiple stones in the gallbladder and dilated CBD (8 mm). During ERCP, stones were extracted followed by sphincterotomy and insertion of a 7 Fr pigtail stent (measuring 4 cm). At laparoscopy, the anatomy of Calots triangle was indefinable and so was converted to open. The patient was noted to have an edematous, thick-walled gall bladder with adherent omentum. Dissection of Calots triangle was made difficult by extensive fibrosis. There was also a cholecysto-duodenal fistula which was repaired. In this patient, the surgeon could see the stent eroding out of the CBD (see Fig. 2). The protruding CBD stent was reduced back into the duct but was not removed intraoperatively. The patient did not require an intraoperative T-tube or surgical repair of the CBD. The time from umbilical port insertion to removal of all ports was 2 h 35 min. There was no postoperative bile leak although the patient developed superficial wound infection which was treated successfully with antibiotics. She was discharged on the tenth postoperative day. She then had a follow-up ERCP 6 weeks later when the eroded stent was not present within the biliary tract. The patient presumably passed the stent spontaneously through the enteral route. Further follow-up visit in outpatient clinic was unremarkable.

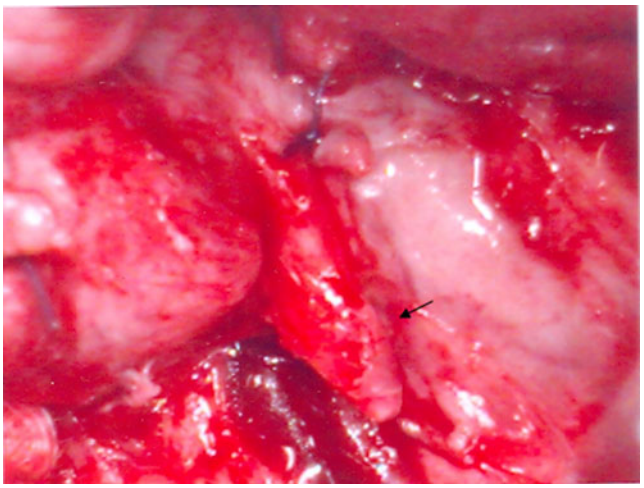

Fig. 2 CBD stent erosion for case 4 (*arrowed*).

Discussion

ERCP was first described in 1968. Since then, it has increasingly become an established tool in the diagnosis and management of pancreatic and biliary disease. It is performed in patients presenting with the complications of gallstones in whom stones in the CBD are present. More recently, some clinicians routinely insert a CBD stent at the time of ERCP after duct clearance. This is done to keep the CBD patent and to prevent further occlusion by gallstone until patients have their gall bladder removed. Biliary stents are commonly used in obstructive malignant conditions and less commonly in benign conditions to bypass bile flow. In selected conditions, they are used preoperatively and also form the mainstay in treating bile leak after LC.

CBD stents are of two types: (1) plastic and (2) metallic. For benign stenosis and CBD stones, plastic pigtail stents remain the standard,[1, 2] while in a malignant disease, metal straight stent (stainless steel or nickel–titanium alloy) implantation can be performed for palliative purposes.[3] Straight metallic stents are more likely to migrate distally in around 7% cases,[2] causing cases of gastrointestinal perforation,[4, 5] intestinal obstruction,[6, 7] and intraabdominal fistulae.[8, 9] There have been reports of perforation of hollow viscous including duodenum,[10, 11] jejunum,[12] and sigmoid colon.[12] Other reported complications of CBD stents include hemobilia,[13] cholangitis,[14] and stent fracture.[15] We report an addition to the list of complications — stent erosion of CBD.

To the best of our knowledge, there are only four published data addressing the issue of stent erosion of the biliary tree. Dundee el al.[16] described erosion of the proximal arm of a 6-Fr JJ Zimmon stent through the common hepatic duct and into the gallbladder. This was detected incidentally 6 weeks later during LC. The stent was removed laparoscopically and the cystic duct and fibrous tract from around the stent were both ligated. Jendresen et al. described one case of erosion of a stent, inserted 3 months earlier, through the CBD wall. They were able to demonstrate on MRI that the distal end of the stent had impacted in the pancreas following proximal migration.[17] Liebich-Bartholain et al.[18] reported proximal migration and erosion of a biliary stent, inserted 3 months earlier, through the liver capsule and pleural space resulting in biliary pneumonitis. A case of portobiliary fistula secondary to biliary ductal erosion by a stent of 10 days duration was reported by Chaitowitz et al.[19]

On reviewing the previous imaging, it was clear that the four patients in our series had their stents correctly positioned initially. These pigtail stents are foreign bodies and are made of plastic such as teflon, polyethylene, or polyurethane.[14] Unlike straight stents, pigtail stents are more likely to travel proximally into narrower biliary lumina with rates of up to 5% being reported in the literature.[20] This can compromise bile drainage with resultant jaundice and cholangitis. Proximal migration also increases the risk of developing biliary tract erosion. This is because such migration creates an incorrect alignment in a curved duct with the distal ends of the stent impinging against the wall on the bend of the duct. All our patients had endoscopic sphincterotomy. This is regarded as a risk factor for distal but not proximal migration.[18] The risk of proximal stent migration has been reported to increase with malignant strictures, as well as with the use of larger diameter (>10 F) and shorter stents (<7 cm).[21] The stent used in our cases were small in diameter (7 F) and short 4-cm French pigtail type. Therefore, the length of the stent used in our patients may have contributed to the degree of migration up the biliary tree and therefore biliary erosion.

We found that two of the four LC in our series had to be converted to an open procedure. In both cases, the anatomy around the Calots triangle was distorted due to dense adhesions. Interestingly, in both cases, the stents were left in situ for a significant period of time (>120 days). There are currently no specific guidelines on the duration of time that bile duct stents should remain in situ . Williams et al.[22] published guidelines on the management of CBD stones but only recommended the "short-term" use of stents prior to surgery. These guidelines did not specify the maximum duration of stent placement or whether these stents should be removed prior to surgery. Despite this, most clinicians electively remove stents prior to surgery. However, long-term use of CBD stents are sometimes employed in patients awaiting transplantation, where the goal is to avoid gallbladder surgery pending availability of a new liver.[23–25]

We feel that the short term placement of an in situ stent may help with dissection during LC as it allows early identification of the CBD, especially in the presence of scar tissues. However, the effect of long-term stent placement on LC is unclear. There are various previous reports of inflammation of the liver parenchyma in the presence of biliary disease and stents.[26–28]; However, there are no reports of the effects of biliary stents on the extrahepatic tree. As a result, Karston et al.[29] studied the effects of biliary stents on the extrahepatic bile ducts in mongrel dogs. They showed that 4 weeks of stenting of a normal or obstructed CBD resulted in fibrosed bile ducts, showing severe chronic inflammation with papillary hyperplasia of the epithelium. Two months after stent removal, Karsten et al.[29] showed reduced inflammation was present in the extrahepatic ducts. Another study by the same authors looked at histopathological specimens in patients with biliary obstruction secondary to tumor.[30] They showed that bile duct obstruction was associated with a mild inflammation, a moderate degree of fibrosis, and local epithelial disintegration. The presence of a biliary stent, however, induced severe inflammatory changes with considerable fibrosis and ulcerative lesions, resulting in markedly thickened ducts with lumina approximating the diameter of the stent.[30] Our study provides preliminary data suggesting that the long-term use of stents may cause subtle fibroproliferative reaction and erosion within the CBD lumen. This may be enough to distort the extrahepatic architecture, complicate elective LC, and result in a greater chance of converting to an open procedure.

Most patients at our institute who had ERCP followed by insertion of prophylactic biliary endoprosthesis were followed up routinely around 6 weeks later with removal of stents at this time. Cases 1 and 4 were unusual as they kept deferring their appointments. Our study reinforces the need to keep track of patients that have stents placed and then skip appointments to have them removed. Extra efforts must be made to inform these patients of the danger they are placing themselves in by such lack of action to having a stent removed in a timely fashion.

We considered removing the stent at the time of performing open cholecystectomy in case 4, where suture closure of a small hole can be done if necessary with temporary subhepatic drainage. However, we felt that removing the stent would be particularly difficult considering the dense adhesions around the biliary tree. Ultimately, we felt that the presence of an in situ stent, together with the intense fibrosis that it stimulates, would be helpful in reducing the risks of postoperative bile leaks. This is why we elected to wait for a period of time (4–6 weeks) before endoscopic removal of the CBD stent. Removal of the stents and repair of the bile duct will be more difficult to perform laparoscopically; hence, we chose to delay removal of stents in cases 2 and 3. In all three cases, there was no evidence of leakage through the eroded segment of the bile duct at the time of cholecystectomy as the defects were small, so we felt that performing any intraoperative manipulation and repair of the bile duct will pose an unnecessary risk. The fact that none of our patients in this series suffered from postoperative bile leak vindicates this management approach.

It is plausible that there could have been biliary tract leakage when these stents were removed endoscopically at the delayed interval period due to perforation of the biliary tree caused by the ductal erosion. However, there were no further complications encountered after removal of the stents, suggesting that the eroded segment was too small to allow any free spillage of bile. In addition, we believe the

inflammatory process that developed within the biliary tree as a result of an in situ stent had reduced the incidence of bile leak during the time of endoscopic removal of the bilary endoprostheisis. As a result, we feel that, even if a stent has migrated, observation and endoscopic removal at a later date is a justifiable means of managing patients who encounter problems with CBD erosion.

Conclusion

We report four cases of CBD erosion by in situ plastic stent found incidentally during laparoscopic cholecystectomy. To the best of our knowledge, there are only four previous reports of similar cases in the literature. If stents are placed in the CBD for a long period of time, this can make LC more difficult to perform. Most cases of CBD erosion found during LC can be managed conservatively without the need for a T-tube or surgical repair. In some cases, biliary stents can be safely removed endoscopically at a later date. Awareness of CBD erosion should prompt earlier surgery and/or removal of biliary stents.

References

1. De Palma GD, Cantanzano C. Senting or surgery for treatment of irretrievable common bile duct calculi in elderly patients? Am J Surg 1999;178:390–3.
2. Diller R, Senninger N, Kautz G, Tübergen D. Stent migration necessitating surgical intervention. Surg Endosc 2003 Nov;17 (11):1803–7.
3. Davids PHP, Groen AK, Rauws EAJ, Tytgat GNJ, Huibregtse K. Randomised trial of self expanding metal stents versus polyethylene stents for malignant biliary obstruction. Lancet 1992;340:1488–92.
4. Levey JM. Intestinal perforation in a parastomal hernia by a migrated plastic biliary stent. Surg Endosc 2002;16:1636–7.
5. Størkson RH, Edwin B, Reiertsen O, Faerden AE, Sortland O, Rosseland AR.Gut perforation caused by biliary endoprosthesis. Endoscopy 2000 Jan;32(1):87–9.
6. Ikeda T, Nagata S, Ohgaki K. Intestinal obstruction because of a migrated metallic biliary stent. Gastrointest Endosc 2004 Dec;60 (6):988–9.
7. Mistry BM, Memon MA, Silverman R, Burton FR, Vanna CR, Solomon H, Garvin PJ. Small bowel perforation from a migrated biliary stent. Surg Endosc 2001;15:1043.
8. Fiori E, Mazzoni G, Galati G, Lutzu SE, De Cesare A, Bononi M, Bolognese A, Tocchi A. Unusual breakage of a plastic biliary endoprosthesis causing an enterocutaneous fistula. Surg Endosc 2002;16:870.
9. Pathak AK, de Souza LJ. Duodenocolic fistula: an unusual sequela of stent migration. Endoscopy 2001;33:731.
10. Bharathi RS, Rao PP, Ghosh K. Intra-peritoneal duodenal perforation caused by delayed migration of endobiliary stent: a case report. Int J Surg 2008;6(6):478–80.
11. Miller G, Yim D, Macari M, Harris M, Shamamian P. Retroperitoneal perforation of the duodenum from biliary stent erosion. Curr Surg 2005 Sep–Oct;62(5):512–5.
12. Ruinemans GM, van Zanten RA, Rakic S, van den Hout JH, Veneman TF. Abdominal pain following placement of an endoprosthesis in the biliary duct. Ned Tijdschr Geneeskd 2006 Jul 1;150(26):1466–9.
13. Frost A, Taylor MA, Parks RW. An unusual cause of haemobilia — beware the forgotten stent! Int J Clin Pract 2007 May;61(5):877–8.
14. Donelli G, Guaglianone E, Di Rosa R, Fiocca F, Basoli A. Plastic biliary stent occlusion: factors involved and possible preventive approaches. Clin Med Res 2007 Mar;5(1):53–60.
15. Yoshida H, Mamada Y, Taniai N, Mizuguchi Y, Shimizu T, et al. Fracture of an expandable metallic stent placed for biliary obstruction due to common bile duct carcinoma. J Nippon Med Sch 2006 Jun;73(3):164–8.
16. Dundee PE, Franzi SJ, Thomas PR. Unique complication of a biliary stent: erosion through the common hepatic duct wall. ANZ J Surg 2007 Mar;77(3):194.
17. Jendresen MB, Svendsen LB. Endoscopy 2001;33:195.
18. Liebich-Bartholain L, Kleinau U, Elsbernd H, Büchsel R. Biliary pneumonitis after proximal stent migration. Gastrointest Endosc 2001 Sep;54(3):382–4.
19. Chaitowitz IM, Heng R, Bell KW. Management of iatrogenic porto-biliary fistula following biliary stent. Australas Radiol 2007 Dec;51 Suppl:B316–8.
20. Johanson JF, Schmalz MJ, Geenen JE. Incidence and risk factors for biliary and pancreatic stent migration. Gastrointest Endosc 1992;38:341–6.
21. Arhan M, Odemiş B, Parlak E, Ertuğrul I, Başar O. Migration of biliary plastic stents: experience of a tertiary center. Surg Endosc 2009 Apr;23(4):769–75.
22. Williams EJ, Green J, Beckingham I, Parks R, Martin D, Lombard M. Guidelines on the management of common bile duct stones (CBDS). British Society of Gastroenterology. Gut 2008 Jul;57 (7):1004–21.
23. Schlenker C, Trotter JF, Shah RJ, Everson G, Chen YK, Antillon D, Antillon MR. Endoscopic gallbladder stent placement for treatment of symptomatic cholelithiasis in patients with end-stage liver disease. Am J Gastroenterol 2006 Feb;101(2):278–83.
24. Conway JD, Russo MW, Shrestha R. Endoscopic stent insertion into the gallbladder for symptomatic gallbladder disease in patients with end-stage liver disease. Gastrointest Endosc 2005 Jan;61(1):32–6.
25. Shrestha R, Trouillot TE, Everson GT. Endoscopic stenting of the gallbladder for symptomatic gallbladder disease in patients with end-stage liver disease awaiting orthotopic liver transplantation. Liver Transpl Surg 1999 Jul;5(4):275–81.
26. Triger DR, MacIver AG, Gamlen TR, Wilken BJ. Liver abnormalities and gallstones: a prospective combined clinical, histological and surgical study. Br J Surg 1976 Apr;63(4):272–7.
27. Flinn WR, Olson DF, Oyasu R, Beal JM. Biliary bacteria and hepatic histopathologic changes in gallstone disease. Ann Surg 1977 May;185(5):593–7.
28. Burdiles P, Csendes A, Díaz JC, Smok G, Bastias J, Palominos G, Braghetto I, Maluenda F. Histological analysis of liver parenchyma and choledochal wall, and external diameter and intraluminal pressure of the common bile duct in controls and patients with common bile duct stones with and without acute suppurative cholangitis. Hepatogastroenterology 1989 Jun;36 (3):143–6.
29. Karsten TM, Davids PH, van Gulik TM, Bosma A, Tytgat GN, Klopper PJ, van der Hyde MN. Effects of biliary endoprostheses on the extrahepatic bile ducts in relation to subsequent operation of the biliary tract. J Am Coll Surg 1994 Apr;178(4):343–52.
30. Karsten TM, Coene PP, van Gulik TM, Bosma A, van Marle J, James J, Lygidakis NJ, Klopper PJ, van der Heyde K. Morphologic changes of extrahepatic bile ducts during obstruction and subsequent decompression by endoprosthesis. Surgery 1992 May;111(5):562–8.

J Gastrointest Surg (2010) 14:1613–1618
DOI 10.1007/s11605-010-1244-5

HOW I DO IT

Technique of Minimally Invasive Ivor Lewis Esophagogastrectomy with Intrathoracic Stapled Side-to-Side Anastomosis

Kfir Ben-David · George A. Sarosi · Juan C. Cendan · Steven N. Hochwald

Received: 1 March 2010 / Accepted: 25 May 2010 / Published online: 8 June 2010

Abstract
Objective An intrathoracic linear stapled side-to-side anastomosis for gastroesophageal junction malignancy is feasible, results in low leak rates and less stenosis.
Design Retrospective case series.
Setting University tertiary care center.
Patients Between March 2008 and January 2009, six patients with gastroesophageal junction malignancy undergoing minimally invasive esophagectomy with an intrathoracic linear stapled side-to-side anastomosis were identified and their clinicopathological data analyzed.
Main Outcome Measures Technique of a 6-cm side-to-side stapled intrathoracic esophagogastric anastomosis.
Results Six patients underwent a minimally invasive esophagectomy with a side-to-side stapled intrathoracic esophagogastric anastomosis. Median age was 61.5 years. All patients had gastroesophageal junction adenocarcinoma and completed neoadjuvant chemoradiation therapy. The median operative time was 360 min. No patient received a blood transfusion. The 30-day mortality was 0. The median length of hospital stay was 8 days. The median number of nodes harvested was 18. At a median follow-up of 9 months, all patients were alive. There have been no anastomotic strictures to date.
Conclusion A 6-cm side-to-side stapled intrathoracic esophagogastric anastomosis is feasible and is associated with a low anastomotic leak rate.

Keywords Minimally invasive esophagectomy · Intrathoracic anastomosis

Introduction

Minimally invasive esophagectomy was first described by Depaula and colleagues in 1995.[1] Since then, a number of authors have contributed to the description of the technique and outcomes. Luketich et al. first described the combined thoracoscopic esophageal mobilization followed by fashioning of the gastric conduit laparoscopically and construction of a cervical esophagogastric anastomosis.[2] In his large series, he was able to illustrate that a minimally invasive esophagectomy results in a low incidence of mortality, respiratory complications, blood loss, and length of hospital stay, while still maintaining a similar oncological principle to the open technique.[3] Subsequently, the same authors published their approach for minimally invasive Ivor Lewis esophageal resection and intrathoracic anastomosis and concluded that this type of procedure is feasible. Similarly, the rate of perioperative complications, including anastomotic leak, pneumonia, and mortality were low and comparable with their established technique of minimally invasive esophagectomy[4] and cervical anastomosis. While the ideal type of minimally invasive esophageal resection and intrathoracic anastomosis is not known, most have reported the use of end to end anastomotic (EEA) stapler to facilitate the procedure. Nguyen and colleagues published their series of Ivor Lewis esophageal resection with a thoracoscopic

K. Ben-David (✉) · G. A. Sarosi · J. C. Cendan · S. N. Hochwald
Department of Surgery,
University of Florida College of Medicine,
1600 SW Archer Road, P.O. Box 100109, Gainesville,
FL 32610, USA
e-mail: kfir.bendavid@surgery.ufl.edu

anastomosis using a circular 25-mm stapled esophagogastrostomy reconstruction. Their anastomotic leak and stricture rates were 9.6% and 26%, respectively.[5] During open esophagectomy, many experienced esophageal surgeons have moved away from EEA stapled anastomosis in the right chest due to the small anastomotic lumen and need to use the end of the esophagus with possible higher leak rates and, instead, favor a stapled or sutured side-to-side anastomosis. However, a minimally invasive thoracoscopic linear side-to-side stapled anastomosis is technically more difficult than an EEA stapled anastomosis. We present our technique and outcomes in patients undergoing an intrathoracic linear stapled side-to-side anastomosis for gastroesophageal (GE) junction malignancy.

Methods

Our protocol for patient evaluation includes an endoscopic ultrasound followed by a positron emission tomography (PET) and computerized tomography (CT) scans to determine if they are appropriate surgical candidates. Patients receive preoperative neoadjuvant chemoradiation therapy as determined by our multidisciplinary team for any GE junction malignancy staged as T2–T4 or regional node positive. Following neoadjuvant therapy, patients are reassessed with an additional PET/CT scan to evaluate response and to ensure that there is no evidence of metastatic disease. Our standard approach to minimally invasive esophagectomy includes thoracoscopic mobilization of the esophagus and peri-esophageal lymph nodes, laparoscopic gastric conduit formation and lymph node dissection and a cervical anastomosis. All patients in this series were not appropriate candidates for our routine minimally invasive esophagectomy due to previous extensive left carotid surgery, the size of the tumor at the GE junction with extension on to the cardia of the stomach, previous esophageal or gastric surgery such as Nissen fundoplication or previous vagotomy, and inability for the gastric conduit to reach up to the neck. Hence, it was decided preoperatively that a thoracoscopic esophagogastrostomy would be performed using an intrathoracic 6-cm linear stapled anastomosis.

Operative Description

Abdominal Dissection

The patient is placed in the supine position and intubated with a double lumen endotracheal tube. The abdominal cavity is accessed via a 5-mm left subcostal incision under direct visualization with the aid of a 5-mm visiport. Three additional trocars are placed under direct visualization. A 5-mm 30° angle scope is utilized throughout the duration of the case. A 5-mm trocar is placed in the supraumbilical region just left of the midline for the laparoscope. A 12-mm trocar is placed in the right mid abdominal region and an additional 12-mm trocar is placed in the right subcostal region along the mid clavicular line for surgeon's right and left hand respectively. The Nathanson liver retractor is utilized through a 5-mm subxiphoid incision in order to elevate the left lobe of the liver and expose the GE junction. The surgeon stands on the patient's right side with the assistant on the opposite side operating the camera and retraction instruments via the left subcostal 5-mm trocar.

Initially, the abdomen is explored for any metastatic disease by carefully evaluating the peritoneum and liver surface. Any questionable lesions are biopsied and evaluated by our pathologist at the time of the operation. The patient is placed in a reverse Trendlenburg position and the gastrohepatic ligament is divided in order to expose the GE junction. A retrogastric window is created from the right crus to the angle of His and a Penrose is placed around this area which will later allow retraction of the GE junction during this dissection. The right and left crus of the diaphragm are widely and freely dissected from the phrenoesophageal ligament. Care is taken not to enter the thoracic cavity during this portion of the procedure. Following the utilization of neoadjuvant chemoradiation therapy, there may be inflammatory changes at the GE junction with thickening of the phrenoesophageal ligament. Care must be taken to identify the diaphragmatic crura and dissect them away from the GE junction. On occasion, it is helpful to mobilize the greater curvature of the stomach prior to attempting to define the retrogastric plane.

Next, the greater curvature of the stomach is mobilized from the origin of the right gastroepiploic artery and vein to the angle of His while preserving the right gastroepiploic arcade. All adhesions between the stomach and pancreas are divided. A limited mobilization of the first and second portions of the duodenum is performed. The left gastric artery and vein and associated lymph nodes are then elevated and both are mobilized to the origins of these vessels. Nodes along the superior border of the pancreas are reflected towards the specimen. The left gastric pedicle is then divided using a vascular load on a 60-mm stapler. On occasion, the planes near the left gastric pedicle may be edematous or difficult to visualize due to the use of neoadjuvant chemoradiotherapy or with extensive nodal disease. In this situation, meticulous dissection to separate the left gastric vein from the artery should be done with early division of the left gastric vein. Subsequently, the left gastric artery is further mobilized and lymphatic tissue along the superior border of the pancreas mobilized en bloc

with the artery. The nodal tissue is then separated from the artery utilizing the Enseal and the artery stapled and divided. The right gastric artery is also divided using a vascular stapler. A point approximately 3 cm proximal to the pylorus is chosen along the lesser curvature as the start of the formation of the gastric conduit. This is constructed with multiple firings of a laparoscopic 60-mm stapler towards the angle of His ensuring appropriate margins from the tumor. Care is taken to keep the gastric conduit approximately 6–7 cm in diameter. Three to four Lembert sutures are placed along the gastric staple line junctions to reinforce it and to provide a handle for further manipulation in the right chest. The gastric conduit is completely transected and then re-sutured to the proximal stomach along the lesser curvature side. Following completion of the gastric conduit, the distal aspect of the esophagus in the posterior mediastinum is circumferentially dissected.

A 16 French T-tube is used for our feeding jejunostomy which is placed approximately 30 cm distal to the ligament of Treitz. None of the patients had a pyloroplasty performed, while two patients underwent an intraoperative Botox injection to the pylorus. All trocars and retractors are removed, and the skin incisions are reapproximated.

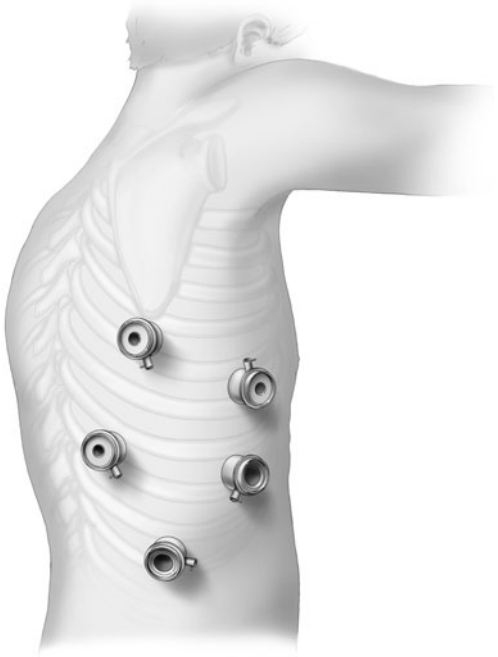

Fig. 1 Thoracoscopic trocar placement.

Thoracic Dissection

The patient is placed in the left lateral decubitus position and the right lung is collapsed. The right chest cavity is entered through a 5-mm incision in the subscapular region. This trocar is for the 5-mm 30° angled scope. Four additional trocars are placed under direct visualization. A 12-mm trocar is placed in the seventh intercostal space anterior to the mid axillary line and is used to retract the lung anteriorly. A 5-mm port is placed in the fifth intercostal along the same axes as the previously placed port and is used by the assistant for retraction. A 5-mm trocar is placed in the seventh intercostal space posterior to the mid axillary line and is used as the surgeon's left while standing posterior to the patient. Lastly, a 12-mm port is placed in the ninth intercostal space along the mid axillary line which is used as the surgeon's right hand when using the stapler and suturing (Fig. 1).

The posterior mediastinum is opened at the level of the inferior pulmonary ligament and the esophagus is circumferentially dissected with the aid of a Penrose drain and the Realize™ dissector to a point above the level of the azygos vein (Figs. 2 and 3). The azygos vein is circumferentially dissected and divided using a vascular stapler through the 12-mm trocar placed in the 10th intercostal space (Fig. 4). Once the esophagus is completely mobilized from the hiatus to 3 cm above the azygos vein, it is divided using a 60-mm stapler. The gastric conduit is delivered from the abdominal cavity up to the transected esophagus. The previously placed sutures on the gastric tube are utilized to help deliver the stomach into the right chest. The transected proximal esophagus and gastric conduit are aligned with 2–0 non-absorbable braided suture. An esophagotomy is created at the distal end of the transected esophagus. To facilitate this, a large bore nasogastric tube or bougie dilator is carefully placed down the esophagus and an esophagotomy performed using the tube as a guide. Similarly, a gastrotomy is performed 8 cm proximal to the end of the gastric conduit. With the aid of the previously placed traction sutures, a side-to-side intrathoracic 6-cm linear stapled esophagogastrostomy is performed (Fig. 5) by placing the envil portion of the stapler through the esophagotomy and the cartridge through the gastrotomy.

Once the isoperistaltic anastomosis is completed, the common opening of the gastrotomy and esophagotomy are sutured with an absorbable braided inner layer followed by a non-absorbable outer layer suture using the Endo Stitch™ device. This enables us to suture with fewer difficulties and at various angles within the thoracic cavity. The anastomosis is then inspected with endoscopy to ensure patency and that no leak is present during insufflation of intraluminal air while submerging the anastomosis under fluid. The gastric tube is sutured to the diaphragm with two sutures and to the pleura if necessary. Following this, the specimen is removed through a 3-cm non-rib spreading incision along the sixth intercostal space. A 24 French thoracostomy tube is placed along the posterior mediastinum. The nasogastric tube was left in place for four days. All six patients underwent a gastrograffin swallow on postoperative day 5

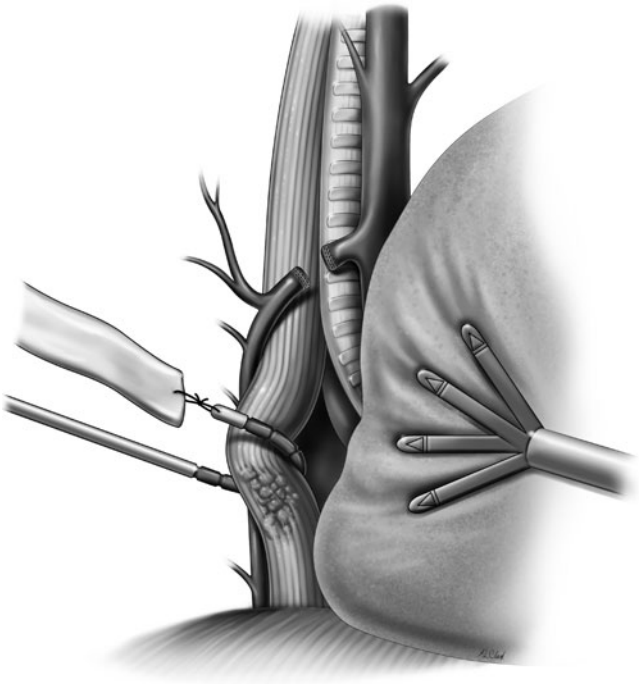

Fig. 2 Circumferential dissection of the esophagus with the aid of a Penrose drain and the Realize™ dissector.

with no evidence of leak and adequate emptying of the stomach confirmed. All were discharged on postoperative day eight tolerating a diet.

Results

Six male patients underwent the procedure with a median age of 61.5 years (range=43–66). The median American Society of Anesthesiologists (ASA) grade was 3 and body mass index was 23 (range=22–28). All six patients had gastroesophageal junction adenocarcinoma and completed neoadjuvant chemoradiation therapy for ≥T2 or node positive disease as noted by endoscopic ultrasound or imaging. Two patients had a previous Nissen Fundoplication

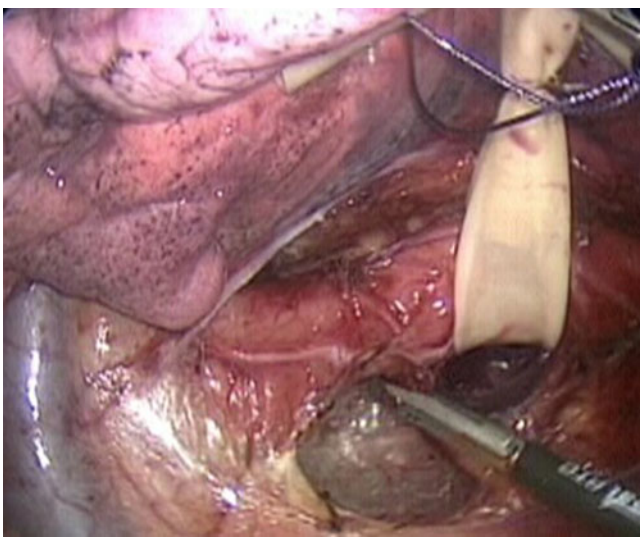

Fig. 3 Mobilization of the intrathoracic esophagus.

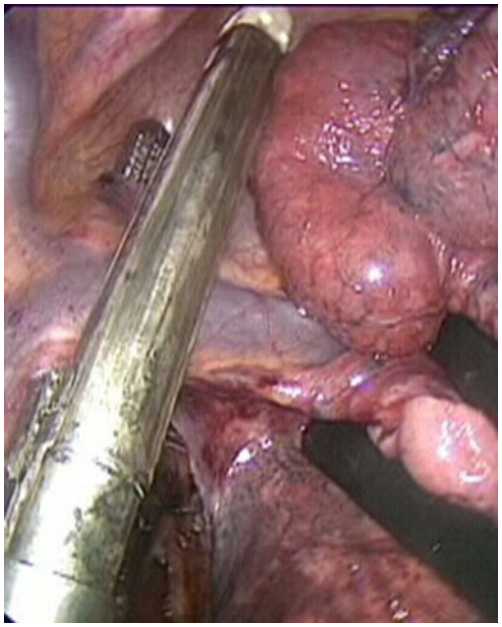

Fig. 4 Division of the azygos vein.

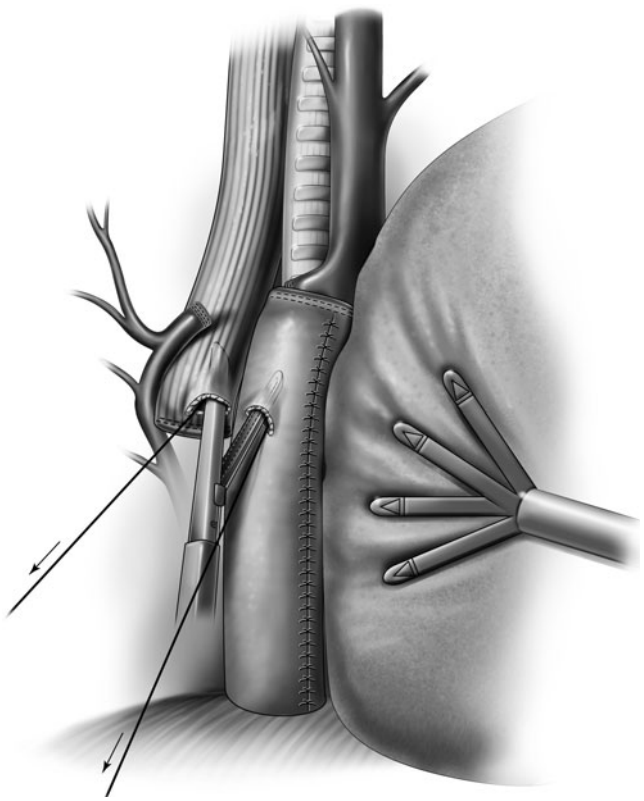

Fig. 5 Side-to-side intrathoracic stapled esophagogastric anastomosis.

Table 1 Characteristics of the Six Patients

Age	Median, 61.5 years (range, 43–66)
Gender	6 males
American Society of Anesthesiologists grade	3
Body mass index	23 kg/m^2 (range, 22–28)
Neoadjuvant chemoradiation	6
Operative time	360 min (range, 300–480)
Blood transfusion	0
Pyloroplasty	0
Intraoperative Botox Injection to pylorus	2
30-day mortality	0
Active smoker	4 yes, 2 no
Neoadjuvant therapy	4 docetaxel, cisplatin, fluorouracil
	2 cisplatin, fluorouracil
	RT dose, 4,500–5,400
Time from neoadjuvant therapy to surgery	Median 7 weeks (range, 5–8)
Length of hospital stay	Median 8 days (range, 6–8)

and the remaining four patients had a large gastroesophageal junction tumor extending 3 to 5 cm onto the proximal stomach. The median duration of operation was 360 min (range=300–480). No patients received a blood cell transfusion. The 30-day mortality was 0 (Table 1). Transient postoperative dysphagia developed in one patient, and there were no documented incidences of aspiration or delayed gastric empting. The median length of hospital stay was 8 days (range=7–8). The median number of nodes harvested was 18 (range, 6–30). The wide range in nodal yield appeared to be related to the use of neoadjuvant chemoradiotherapy. The sites of the tumors resected and pathologic stages are given in Table 2. At a median follow-up of 9 months, all six patients were alive. There have been no postoperative strictures to date. One patient had complaints of dysphagia in the early postoperative period that has subsequently resolved.

Discussion

Various laparoscopic, thoracoscopic, and robotic techniques for minimally invasive esophagectomy have been described with varying opinions regarding the location and technique of esophagogastric anastomosis.[5–15] Avoiding anastomotic complications is essential for minimizing the morbidity and maximizing the operative outcome. Anastomotic leaks have been reported to occur up to 15% of the time and are the most frequent cause of immediate postoperative mortality. In addition, anastomotic leaks can result in long-term stricture formation in up 50% of resected patients[16,17] impacting long-term functional results and quality of life.[18,19] Behzadi et al. concluded that a linear stapled anastomosis is associated with lower leak rates and need for long-term dilatation as compared to a hand sewn anastomosis regardless of the anastomotic location.[16] Similarly, the Mayo Clinic and University of Pittsburgh have both shown that a side-to-side isoperistaltic anastomosis resulted in lower rates of stenosis.[16,20,21] Hence, a successful anastomosis is essential for decreasing long-term morbidity associated with patients undergoing an esophagectomy. It is for these reasons, that we perform an intrathoracic 6-cm side-to-side linear stapled anastomosis for patients that are not appropriate candidates for our traditional thoracoscopic esophageal mobilization, laparoscopic gastric conduit formation, and cervical esophagogastrostomy. Therefore, this procedure is best suited for patients with gastric cardia, GE junction or distal esophageal tumors or for patients where there is not enough stomach available for a cervical anastomosis.

Although our results are very compelling, yet limited by our sample size, the difficulty of performing this procedure is challenging and requires expertise in both minimally invasive surgery and esophageal surgery. This technique

Table 2 Tumor Characterization and Survival

Patient	Preoperative clinical stage	Postoperative pathologic stage	Postoperative pathologic diagnosis	Number of lymph nodes harvested
1	T3N0	T3N1	Poorly differentiated adenocarcinoma	17
2	T3N1	T2N1	Moderately differentiated adenocarcinoma	6
3	T2N1	T2N1	Moderately differentiated adenocarcinoma	19
4	T2N0	T0N0	No residual tumor	10
5	T2N0	T2N1	Moderately differentiated adenocarcinoma	21
6	T3N1	T2N0	Poorly differentiated adenocarcinoma	30

requires being able to suture in the thoracic cavity that is not as easily adaptable as the abdomen. Potential advantages of a long (6 cm) side-to-side stapled anastomosis results includes lower leak rates and long-term stenosis. The disadvantages of an intrathoracic esophagogastric anastomosis are well known including increased morbidity to the patient if a leak were to occur. The advantages of an intrathoracic anastomosis include avoidance of cervical dissection of the esophagus with increased risk for recurrent laryngeal nerve injury and postoperative strictures.

In summary, we describe a new and technically feasible thoracoscopic technique for construction of an esophagogastrostomy using a 6-cm side-to-side linear stapled anastomosis. This method is applicable to patients who require an intrathoracic anastomosis. More importantly, the large size of the anastomosis should result in decreased anastomotic leaks and strictures. Although performing this complex procedure requires a unique learning curve, this procedure can be mastered when performed by surgeons interested in performing intrathoracic side-to-side stapled anastomosis rather than a circular stapled anastomosis. As with any new technique, having the prerequisite technical skills will ensure safe and similar results as those described in this article.

References

1. DePaula AL, Hashiba K, Ferreira EA, de Paula RA, Grecco E. Laparoscopic transhiatal esophagectomy with esophagogastroplasty. Surg Laparosc Endosc 1995;5:1-5.
2. Luketich JD, Nguyen NT, Weigel T, Ferson P, Keenan R, Schauer P. Minimally invasive approach to esophagectomy. JSLS 1998;2:243-247.
3. Luketich JD, Alvelo-Rivera M, Buenaventura PO, Christie NA, McCaughan JS, Litle VR, Schauer PR, Close JM, Fernando HC (2003) Minimally invasive esophagectomy: outcomes in 222 patients. Ann Surg 238:486-494.
4. Bizekis C, Kent MS, Luketich JD, Buenaventura PO, Landreneau RJ, Schuchert MJ, Alvelo-Rivera M. Initial experience with minimally invasive Ivor Lewis esophagectomy. Ann Thorac Surg 2006;82:402-406.
5. Nguyen NT, Hinojosa MW, Smith BR, Chang KJ, Gray J, Hoyt D. Minimally Invasive Esophagectomy, Lessons Learned From 104 Operations. Ann Surg 2008;248(6):1081-1091
6. Benzoni E, Bresadola V, Terrosu G, Uzzau A, Cedolini C, Intini S, Noce L, Bresadola F. Minimally invasive esophagectomy: a comparative study of transhiatal laparoscopic approach versus laparoscopic right transthoracic esophagectomy. Surg Laparosc Endosc Percutan Tech 2008;18:178-187.
7. Bernabe KQ, Bolton JS, Richardson WS. Laparoscopic hand-assisted versus open transhiatal esophagectomy: a case-control study. Surg Endosc 2005;19:334-337.
8. Bintintan VV, Mehrabi A, Fonouni H, Esmaeilzadeh M, Muller-Stich BP, Funariu G, Ciuce C, Gutt CN. Feasibility of a high intrathoracic esophagogastric anastomosis without thoracic access after laparoscopic-assisted transhiatal esophagectomy: a pilot experimental study. Surg Innov 2009;16:228-236.
9. Braghetto I, Csendes A, Cardemil G, Burdiles P, Korn O, Valladares H. Open transthoracic or transhiatal esophagectomy versus minimally invasive esophagectomy in terms of morbidity, mortality and survival. Surg Endosc 2006;20:1681-1686.
10. Dapri G, Himpens J, Cadiere GB. Robot-assisted thoracoscopic esophagectomy with the patient in the prone position. J Laparoendosc Adv Surg Tech A 2006;16:278-285.
11. Fabian T, McKelvey AA, Kent MS, Federico JA. Prone thoracoscopic esophageal mobilization for minimally invasive esophagectomy. Surg Endosc 2007;21:1667-1670.
12. Gerhart CD. Hand-assisted laparoscopic transhiatal esophagectomy using the dexterity pneumo sleeve. JSLS 1998;2:295-298.
13. Verhage RJ, Hazebroek EJ, Boone J, Van Hillegersberg R. Minimally invasive surgery compared to open procedures in esophagectomy for cancer: a systematic review of the literature. Minerva Chir 2009;64:135-146.
14. Horgan S, Berger RA, Elli EF, Espat NJ. Robotic-assisted minimally invasive transhiatal esophagectomy. Am Surg 2003;69:624-626.
15. Zhou J, Chen H, Lu JJ, Xiang J, Zhang Y, Hu H, Zhou X, Luo X, Yang F, Tam J. Application of a modified McKeown procedure (thoracoscopic esophageal mobilization three-incision esophagectomy) in esophageal cancer surgery: initial experience with 30 cases. Dis Esophagus 2009;22:687-693.
16. Behzadi A, Nichols FC, Cassivi SD, Deschamps C, Allen MS, Pairolero PC. Esophagogastrectomy: the influence of stapled versus hand-sewn anastomosis on outcome. J Gastrointest Surg 2005;9:1031-1040
17. Cassivi SD. Leaks, strictures, and necrosis: a review of anastomotic complications following esophagectomy. Semin Thorac Cardiovasc Surg 2004;16:124-132.
18. McLarty AJ, Deschamps C, Trastek VF, Allen MS, Pairolero PC, Harmsen WS. Esophageal resection for cancer of the esophagus: long-term function and quality of life. Ann Thorac Surg 1997;63:1568-1572.
19. Headrick JR, Nichols FC, 3rd, Miller DL, Allen MS, Trastek VF, Deschamps C, Schleck CD, Thompson AM, Pairolero PC. High-grade esophageal dysplasia: long-term survival and quality of life after esophagectomy. Ann Thorac Surg 2002;73:1697-1702.
20. Sanders G, Borie F, Husson E, Blanc PM, Di Mauro G, Claus C, Millat B. Minimally invasive transhiatal esophagectomy: lessons learned. Surg Endosc 2007;21:1190-1193.
21. Santos RS, Raftopoulos Y, Singh D, DeHoyos A, Fernando HC, Keenan RJ, Luketich JD, Landreneau RJ. Utility of total mechanical stapled cervical esophagogastric anastomosis after esophagectomy: a comparison to conventional anastomotic techniques. Surgery 2004;136:917-925.

J Gastrointest Surg (2010) 14:1619–1628
DOI 10.1007/s11605-010-1189-8

REVIEW ARTICLE

The Incidence and Risk Factors of Post-Laparotomy Adhesive Small Bowel Obstruction

Galinos Barmparas · Bernardino C. Branco · Beat Schnüriger · Lydia Lam · Kenji Inaba · Demetrios Demetriades

Received: 1 February 2010 / Accepted: 23 February 2010 / Published online: 30 March 2010

Abstract
Introduction The purpose of this review was to assess the incidence and risk factors for adhesive small bowel obstruction (SBO) following laparotomy.
Methods The PubMed database was systematically reviewed to identify studies in the English literature delineating the incidence of adhesive SBO and reporting risk factors for the development of this morbidity.
Results A total of 446,331 abdominal operations were eligible for inclusion in this analysis. The overall incidence of SBO was 4.6%. The risk of SBO was highly influenced by the type of procedure, with ileal pouch–anal anastomosis being associated with the highest incidence of SBO (1,018 out of 5,268 cases or 19.3%), followed by open colectomy (11,491 out of 121,085 cases or 9.5%). Gynecological procedures were associated with an overall incidence of 11.1% (4,297 out of 38,751 cases) and ranged from 23.9% in open adnexal surgery, to 0.1% after cesarean section. The technique of the procedure (open vs. laparoscopic) also played a major role in the development of adhesive SBO. The incidence was 7.1% in open cholecystectomies vs. 0.2% in laparoscopic; 15.6% in open total abdominal hysterectomies vs. 0.0% in laparoscopic; and 23.9% in open adnexal operations vs. 0.0% in laparoscopic. There was no difference in SBO following laparoscopic or open appendectomies (1.4% vs. 1.3%). Separate closure of the peritoneum, spillage and retention of gallstones during cholecystectomy, and the use of starched gloves all increase the risk for adhesion formation. There is not enough evidence regarding the role of age, gender, and presence of cancer in adhesion formation.
Conclusion Adhesion-related morbidity comprises a significant burden on healthcare resources and prevention is of major importance, especially in high-risk patients. Preventive techniques and special barriers should be considered in high-risk cases.

Keywords Adhesive small bowel obstruction · Early small bowel obstruction · Late small bowel obstruction · Postoperative small bowel obstruction

G. Barmparas · B. C. Branco · B. Schnüriger · L. Lam · K. Inaba · D. Demetriades (✉)
Division of Trauma, Emergency Surgery and Surgical Critical Care, Los Angeles County Medical Center–University of Southern California,
1200 North State Street, Inpatient Tower (C)–Room C5L100, Los Angeles, CA 90033, USA
e-mail: demetria@usc.edu

Introduction

In 2006, almost 1.4 million patients underwent a surgical procedure involving the digestive system in the USA, including colectomy, appendectomy, cholecystectomy, and lysis of peritoneal adhesions.[1] Additionally, almost 1.3 million women underwent cesarean section during the same year. The development of intra-abdominal adhesions following such procedures is considered an inevitable consequence. In a postmortem study conducted in the early 1970s, Weibel and Majno found that of all subjects who had previously undergone a minor, a major, or multiple operations, 51%, 72%, and 93%, respectively, had intra-abdominal adhesions.[2] Menzies and Ellis found that, of a series of 210 patients who had previously had one or more

abdominal procedures, 93% had intra-abdominal adhesions at re-laparotomy.[3]

Prevention of the development of intra-abdominal adhesions has been the focus of several investigators, and various products have been tested. While the efficacy of such products remains under evaluation, the true incidence of adhesive-related morbidity and especially that of adhesive small bowel obstruction (SBO) has not been fully assessed. Additionally, since it is known that most adhesions remain silent, the factors that may make patients more prone to developing adhesion-related morbidity are not fully understood.

In the present review, we aim to comprehensively assess the incidence of adhesive SBO and report the available evidence identifying risk factors predisposing to this condition.

Methods

The National Library of Medicine MEDLINE database was utilized to identify all articles related to the incidence and risk factors associated with adhesive SBO. English language citations published from January 1980 to May 2009 were included. The "related articles" option in the PubMed Entrez interface was utilized. The bibliography in the identified articles was also reviewed. Case reports, letters to editors, and review articles were excluded.

To identify risk factors related to the development of adhesive morbidity, we utilized the search terms "abdominal adhesions AND risk factors" and "adhesive small bowel obstruction AND risk factors". In addition, specific risk factors that possibly predispose to adhesion development were queried to identify their association with intestinal obstruction. These risk factors included age, gender, immunosuppression, gallstone spillage, use of starch-containing surgical gloves, and closure of the peritoneum following laparotomy.

Incidence

Determining the true incidence of adhesion-related morbidity following a laparotomy is difficult. While it is known that the vast majority of patients undergoing an abdominal procedure will develop intra-abdominal adhesions, the short- and long-term risks for developing adhesion-related morbidity cannot be predicted. The complex natural history of the disease, in addition to the significant heterogeneity between the studied populations and the failure of a reliable follow-up, enhance the difficulty of assessing the true risk associated with the presence of intra-abdominal adhesions.

The incidence of adhesion-related readmissions and adhesive SBO is available in the literature from several sources. One major source is the trilogy of the Surgical and Clinical Adhesions Research (SCAR) studies, which utilized the Scottish National Health Service medical record linkage database.[4–9] These large-scale studies had the endpoint "adhesion-related readmissions". Retrospective reports with a non-standardized follow-up, having as endpoints "early or late SBO requiring surgical intervention" is another source. A third source is the reported incidence of adhesive SBO in control patients of randomized controlled trials assessing the efficacy of adhesion preventive methods. Finally, patients enrolled in randomized controlled trials which aim to assess the cost-efficacy and safety of laparoscopic vs. open abdominal surgical procedures, comprise another source of data.

The Surgical and Clinical Adhesions Research Studies

One of the most comprehensive efforts to evaluate the burden of adhesion-related morbidity was undertaken by the SCAR group. The investigators of this group utilized the Scottish National Health Service Medical Record Linkage Database, which holds data on individual linked patients' records on every inpatient and day-case hospital admission from 1981 onwards in Scotland, excluding psychiatric or maternity admissions.

The first SCAR study[4] focused on assessing the frequency of complications from adhesions in the general population. Patients undergoing initial abdominal or pelvic surgery in 1986 were analyzed, excluding those who had undergone abdominal or pelvic surgery in the previous 5 years. All patients were followed up for readmissions for defined outcomes over a period of 10 years. Despite all efforts to eliminate overestimation of the burden of adhesion-related readmissions, it was found that over 5.7% of all readmissions were directly related to adhesions, with 66.7% of these patients requiring adhesiolysis (Table 1). Overall, 7.3% of patients who had undergone a mid- and hindgut procedure were readmitted for reasons directly related to adhesions. This proportion was lower among patients who had undergone for gut or other abdominal procedures (4.6%) or female reproductive tract procedures (4.4%). One in three patients was readmitted at least twice over the 10-year study period, and at least one in 18 outcome readmissions (for operative and non-operative) were directly related to adhesions.

Parker et al.[5] used subsequently the same data and methodology to look specifically at patients who had undergone lower abdominal surgery (mid- and hindgut). It was found that patients who had undergone an initial operation involving the rectum had the highest rate of readmissions directly related to adhesions (8.8%), followed by those who had undergone an operation involving the small bowel (7.6%) and the colon (7.1%). Similarly, Lower et al.[6] examined specifically patients who had undergone an

Table 1 Summary of the Results from the First Study from the Surgical and Clinical Adhesions Research (SCAR) Group[4]

	Readmissions directly related to adhesions	Readmissions possibly related to adhesions	Readmissions directly or possibly related to adhesions
Operation/adhesions	808 (2.7%)	3,186 (10.7%)	3,994 (13.4%)
Non-operative management	401 (1.4%)	5,054 (17.0%)	5,455 (18.4%)
Total	1,209 (4.1%)	8,240 (27.7%)	9,449 (31.7%)

Incidence of adhesion-related readmissions after open abdominal or pelvic surgery (N=29,790)

The study population comprises of patients who had undergone open abdominal or pelvic surgery in the year 1986 and no other abdominal or pelvic surgery in the previous 5 years. Patients were followed up for 10 years

open gynecological operation and found that patients who had undergone an ovarian operation had the highest risk for readmission directly related to adhesions, at 7.1% (Table 2).

An additional significant finding from these studies was that the greatest percentage of readmissions (22.1%) occurred in the first year after the index operation and continued to rise steadily over the 10-year follow-up for all outcome measures.[4–6]

The SCAR-2 study[7] aimed to examine the real-time burden of adhesion-related readmissions following colorectal surgery and to assess the impact of previous surgery on adhesion-related outcomes. The findings of this study demonstrated that the rates of adhesion-related readmissions (directly and possibly related) were 12.4%, 19.5%, 25.7%, and 29.7% at 1, 2, 3, and 4 years after the index surgery, respectively (Table 3). Lower et al.[8] used subsequently the same methodology to examine these outcomes in female patients undergoing open or laparoscopic gynecological procedures. The results from this examination demonstrated that with the exception of laparoscopic sterilization, which is considered a low-risk gynecological procedure for adhesion development, open and laparoscopic gynecological procedures are associated with comparable risks for adhesion-related readmissions (Table 4).

The SCAR-3 study[9] focused on aspects such as the nature of the surgery, age, comorbid conditions, and history of previous surgery. The findings of this study will be discussed in the following sections (Table 5).

Despite the serious limitations of registry-based studies, the SCAR studies comprise the first and most comprehensive to date efforts to quantify the problem of adhesion-related readmissions. A major benefit of utilizing this database was the demography of Scotland, which, geographically, is self-contained and has a stable population of about 5.1 million, with less than 1% annual migration.[10]

Risk Factors

Identification of patients who are at high risk of developing adhesions is important in prevention strategies. True risk factors that predispose patients to develop adhesion-related morbidity, however, and especially adhesive SBO, have not been clearly identified. Several have been proposed, but level I evidence is lacking in most instances.

Type of Surgery

The type of surgery may be the most important factor that determines the incidence of adhesion-related morbidity, especially adhesive SBO. As mentioned, the SCAR studies have demonstrated a higher incidence of adhesion-related admissions for patients undergoing a mid- and hindgut surgery. Additionally, the SCAR-3 study demonstrated that patients undergoing an index surgery involving the ileum had the highest risk (7.7%), followed by those having abdominal wall and colorectal surgery (Table 5).

In our collective review of the literature, we analyzed 62 published studies in the English literature with 448,718 patients who underwent an abdominal operation. Overall,

Table 2 Incidence of Adhesion-Related Readmissions After Open Surgery to the Reproductive System[6]

	Readmissions directly related to adhesions	Readmissions possibly related to adhesions	Readmissions directly or possibly related to adhesions
Operation/adhesions	245 (2.9%)	1,278 (15.1%)	1,523 (18.0%)
Non-operative management	–	1,201 (14.1%)	1,201 (14.1%)
Total	245 (2.9%)	2,479 (29.2%)	2,724 (31.1%)

Results from the Scottish National Health Service Medical Record Linkage Database (N=8,489)

Study population: Women with open surgery to the reproductive system

Table 3 Summary of the Results from the Second Study of the Surgical and Clinical Adhesions Research (SCAR) Group[7]

Years following operation	Readmissions directly related to adhesions (%)	Readmissions possibly related to adhesions (%)	Total readmissions (%)
1	2.1	6.1	8.2
2	3.2	9.4	12.6
3	4.1	11.3	15.4
4	4.5	12.5	17.0

The results represent the directly and possibly adhesion-related readmissions for the subgroup of patients who had not undergone abdominopelvic procedure in the previous 5 years (N=2,067)

Study population: Patients with open colorectal surgery in 1996–1997 and no abdominal surgery in the previous 5 years

20,635 patients (4.6%) required adhesion-related readmission, mostly due to adhesive SBO (Table 6). The incidence varied widely according to procedure. The highest incidence was reported in patients with open adnexal surgery (23.9%), followed by patients with ileal pouch–anal anastomosis (19.3%), open total abdominal hysterectomy (15.6%), and open colectomy (9.5%).

The method of operation (open vs. laparoscopic) also plays an important role in the development of adhesive SBO. Collective review of the literature shows an incidence of 7.1% in open cholecystectomy vs. 0.2% in laparoscopic cholecystectomy, 15.6% in open total abdominal hysterectomy (TAH) vs. 0.0% in laparoscopic TAH, and 23.9% in open vs. 0.0% in laparoscopic adnexal operations. In the case of appendectomies, it seems that there is no difference between the open and laparoscopic techniques (1.4% vs. 1.3%; Table 6).

Due to the high incidence of SBO associated with colectomies, it would be expected that the beneficial effect of laparoscopy would be apparent. Despite the fact, however, that laparoscopy has been shown to be associated with a decreased adhesion formation,[11] this has not been shown to be associated with a lower incidence of SBO in colorectal surgery. A recent Cochrane meta-analysis assessing the long-term results of colorectal cancer resection failed to show a benefit with regards to reoperation for adhesions in patients undergoing a laparoscopic procedure when compared with those undergoing an open procedure.[12] In addition, the conventional vs. Laparoscopic-Assisted Surgery In Colorectal Cancer trial, which is attempting to evaluate the incidence of adhesion-related complications, particularly SBO, after laparoscopic and open colorectal surgery has failed to date to show any significant difference between the two approaches.[13]

In our collective review of the literature, however, we found that the incidence of SBO is twofold higher in open when compared with laparoscopic procedures (Table 6). It should be noted, however, that the studies reporting the incidence of SBO after the various types of surgery are highly heterogeneous. The follow-up is insufficient in most instances, while comorbid conditions are rarely accounted for. Selection bias puts into question the reported incidence.

Table 4 Adhesion-Related Readmissions within 4 Years After Open or Laparoscopic Gynecological Surgery[8]

Method of operation	Readmissions directly related to adhesions (%)	Readmissions possibly related to adhesions (%)	Total readmissions (%)
Laparoscopic (N=15,197)	1.5	16.1	17.6
Open (N=8,849)	2.0	14.5	16.5

Results from the Scottish National Health Service Medical Record Linkage Database (N=24,046)

Table 5 Summary of the Results from the Third Study of the Surgical and Clinical Adhesions Research (SCAR) Group[9]

Site of surgery	Admissions directly related to adhesions within 5years of operation (%)
Duodenum (N=685)	1.8
Ileum (N=912)	7.7
Colon (N=3176)	5.0
Rectum (N=1,690)	5.2
Abdominal wall (N=2,180)	5.4
Appendix (N=4,113)	0.9

Readmissions directly related to lower abdominal surgery (excluding gynecological procedures) according to site and type of operation (N=12,756)

Study population: Patients with open lower abdominal surgery (excluding gynecological operations) during the period 1996–1997

Gender

Only a few studies examining the role of gender in the development of adhesion-related complications were identified, and the reported results were significantly conflicting. Riber et al.[14] examined the role of gender in patients undergoing open appendectomy and found that female patients had an almost fourfold higher overall risk for SBO requiring surgical intervention. Contrarily, Andresson[15] found that female patients were at a slightly lower risk for developing this complication [adjusted hazard ratio 0.8 (0.8–0.9)] in a similar population. The SCAR-3 study[9] did not report the results of the effect of gender on readmissions directly related to adhesions due to the significant skewness of the data towards women. Therefore, conclusions with regards to the role of gender cannot be withdrawn.

Table 6 Overall Incidence of Adhesion-Related Readmissions According to the Type of Surgery

Surgery	Total number of patients	Adhesion-related readmissions	References
Open appendectomy	266,695	3,663 (1.4%)	5,9,14,15,22,23,45–65
Laparoscopic appendectomy	4,445	57 (1.3%)	16,22,45,46,48–60,62,63,66
Open cholecystectomy	141	10 (7.1%)	67,68
Laparoscopic cholecystectomy	7,103	11 (0.2%)	66–68
Open colectomy	121,085	11,491 (9.5%)	4,5,7,9,23,69–73
Laparoscopic colectomy	930	40 (4.3%)	66,72,74
Ileal pouch–anal anastomosis	5,268	1,018 (19.3%)	75–89
Laparotomy for trauma	1,913	48 (2.5%)	23–25,90,91
Gynecological procedures	38,751	4,297 (11.1%)	
Open TAH	20,377	3,182 (15.6%)	6,8,92
Laparoscopic TAH	303	0 (0.0%)	6,92
Open adnexal surgery	4,621	1,105 (23.9%)	6,8,92
Laparoscopic adnexal surgery	470	0 (0.0%)	6,92
Cesarean section	12,980	10 (0.1%)	6,8,92
Overall incidence	446,331	20,635 (4.6%)	

Age

The role of age as a risk factor predisposing to adhesion-related morbidity has been examined in very few studies. The SCAR-3 study[9] found that patients <60 years old undergoing a colorectal surgery had a higher overall risk for readmission directly related to adhesions compared with their ≥60-year-old counterparts, even after censoring the data for mortality. This difference applied both, to patients who had or had not undergone an abdominopelvic procedure in the previous 5 years. Additionally, it was found that patients ≥16 years old undergoing an appendectomy were at higher risk for readmission directly related to adhesions over the following 5 years, when compared with young patients <16 years old. Contrarily, Andersson found that of all patients undergoing appendectomy, those in the age group 20–39 years had the lowest risk for SBO requiring surgery, while patients >70 years old had a twofold higher risk, compared with patients <20 years old.[15]

Age <40 years was identified as an independent risk factor for recurrence of adhesive SBO in a multicenter prospective study conducted in France with a median follow-up of 41 months (range, 1–75 months).[16]

Immunosuppression and Comorbidities

Whether the difference in the risk for adhesion-related morbidity between the various age groups is attributed to immunosuppression associated with age, cannot be easily determined. Several studies suggest that immunosuppressed patients undergoing transplantation may have a decreased risk for adhesion formation due to the suppression of the inflammatory response. In a retrospective review of 4,001 patients undergoing orthotopic liver transplantation, only 19 (0.5%) had postoperative SBO directly related to peritoneal adhesions.[17] Similarly, pancreas transplant recipients[18] demonstrate comparative low incidence of adhesive SBO.

Wasserberg et al.[19] in an experimental study in which groups of rats underwent small bowel transplantation and were subsequently randomized for tacrolimus immunosuppression versus no immunosuppression, it was found that postsurgical adhesion formation was significantly reduced in the immunosuppressed group of rats.[19]

Very few studies have evaluated this factor in the general surgery population and most of them have only looked at cancer patients. The SCAR-3 study demonstrated that patients with colorectal cancer had a significantly lower risk for adhesion-related readmissions.[9] The authors, however, attributed this difference to the type of surgery performed in this group, which was mostly right hemicolectomy and which was associated with a lower overall incidence of adhesion-related readmissions. Most of the other studies have demonstrated that patients with cancer are at higher risk for adhesive SBO. Park et al.[20] in a randomized controlled trial evaluating the efficacy of Seprafilm® reported an incidence of 7% for early in-hospital SBO and 4.6% for readmissions for SBO in the control group of cancer patients undergoing radical resection of their sigmoid or rectal cancer. However, there was no comparative group with no cancer patients in this study. Shin et al.[21] found that poor general condition, defined as American Society of Anesthesiologists (ASA) grade ≥3 and local remnant tumor were factors independently associated with early adhesive SBO in patients undergoing pelvic

surgery for colorectal cancer. Recently, Leung et al. reported that for patients undergoing an appendectomy, the risk for SBO is more than sevenfold higher in those with pathology of cancer or chronic appendicitis.[22] It is of note though, that other parameters, such as radiotherapy or chemotherapy, have not been accounted for.

The role of other comorbid conditions has been evaluated by the SCAR-3 study.[9] Patients with diverticulitis (without peritonitis) or Crohn's disease, conditions associated with inflammatory reaction in the abdomen, were not at higher risk for readmissions directly related to adhesions. The presence of peritonitis in patients who underwent appendectomy had a slightly higher risk to be readmitted when compared with patients who did not have peritonitis. This difference was most prominent in patients who had undergone previous surgery.

Abdominal Trauma

Table 7 summarizes the incidence of SBO associated with negative or non-therapeutic laparotomy for trauma. Penetrating injuries and injuries to the small bowel seem to increase the risk of early SBO requiring surgery.[23]. Tortella et al.[24] in a prospective study of 298 patients undergoing celiotomy for penetrating trauma found that the incidence of SBO in these patients was high, reaching 7.3%. In the same study, gunshot wounds and injury of the small or large bowel were found to increase this risk. In a prospective observational study of trauma patients undergoing laparotomy, Weigelt et al. found that only five of the 248 patients developed SBO during their follow-up.[25] All five patients had intra-abdominal injuries and underwent extensive exploration of the abdominal cavity, with access to the retroperitoneum.

Closure of the Peritoneum After Midline Laparotomy

The association between suturing of the peritoneum on abdominal closure and adhesion formation is highly debated due to the lack of clinical evidence. Several studies in the general surgery literature have suggested that non-closure of the peritoneum after midline laparotomy is associated with reduced operative time and decreased rate of wound-related postoperative complications.[26–28] Evaluation of adhesion formation in these patients, however, was not feasible, and SBO was not reported as an outcome.

In obstetrics, however, several studies have evaluated this association. Komoto et al.[29] randomized 124 women undergoing cesarean section into two groups, closure vs. non-closure of the peritoneum. These patients were evaluated at a second cesarean section for adhesion formation. The study reported that patients who had their peritoneum sutured had a higher incidence of extensive adhesions and required more frequently adhesiolysis. This study, however, did not utilize a scoring system for the adhesions, and the exclusion criteria were not adequate. Recently, a meta-analysis from Cheong et al.[30] which utilized strict quality criteria for inclusion of the studies, concluded that according to current data in the literature, there is some evidence to suggest that non-closure of the peritoneum after cesarean section is associated with more adhesion formation compared with closure.

Malvasi et al.[31] in a prospective, randomized study of women undergoing cesarean sections found that at repeat operation, women with peritoneal closure had a significantly higher incidence of adhesions compared with those with non-closure (57.0% vs. 20.6%, $p<0.05$). Although no scoring system was utilized, these investigators found on microscopy increased mesothelial hyperplasia, fibrosis, and neoangiogenesis in the group with peritoneal closure, and they concluded that this practice may predispose to inflammatory reaction and adhesion formation.

Despite the conflicting results of the available literature, it seems that non-closure of the peritoneum might be beneficial in reducing the incidence of postoperative intra-abdominal adhesions.

Use of Starch-Free Gloves

Since the introduction of starch gloves in the late 1940s, the association between starch granules and adhesion formation has been studied extensively. Starch is an absorbable material and does not remain in the peritoneal cavity indefinitely. The time for this absorption to occur, however, has not been clarified. Sheikh et al.[32] showed that most of the starch powder granules had been disappeared by the fourth week in rats undergoing a laparotomy. Cade and Ellis, however, found that, in rats undergoing laparotomy,

Table 7 The Incidence of Small Bowel Obstruction (SBO) After Negative or Non-Therapeutic Laparotomy for Trauma

Study	Number of patients	SBO	Mean follow-up
Tortella et al.[24]	154	5 (2.3%)	6 months
Weigelt et al.[25]	186	5 (2.7%)	57 months
Renz et al.[90]	254	6 (2.4%)	36 months
Morrison et al.[91]	80	0 (0.0%)	36 months
Total	674	16 (2.4%)	

starch granules could be detected even after 15 months, but only with PAS staining of the peritoneal tissue.[33] Examining the association of starch-powdered gloves with the development of adhesions in the clinical setting is hardly feasible. Cooke et al.[34] excised peritoneal nodules and band adhesions for pathological examination from patients undergoing re-laparotomy for several reasons. It was found that, in the vast majority of patients who had undergone the first laparotomy within the previous 2 years, starch granulomas could be detected and they were responsible for the development of intestinal obstruction. In most patients who had undergone the first laparotomy more than 2 years before the second laparotomy, starch granules could not be detected, but the associated band adhesions persisted. Luijendijk et al.[35], in a similar study, found that, when granulomas were present, the median interval between present and most recent laparotomy was significantly shorter than when no granulomas were found. Additionally, in patients with adhesions who had had the previous operation less than 6 months previously, granulomas were present in 71%. In contrast, only 13% of the patients operated upon longer than 6 months previously had granulomas.

Gallstone Spillage During Cholecystectomy

Iatrogenic perforation of the gallbladder during laparoscopic cholecystectomy is common. In a review of the literature, Woodfield et al.[36] estimated that, in a total of 7.3% of patients undergoing laparoscopic cholecystectomy, gallstones will be spilt in the peritoneal cavity and approximately 33% of these patients will be discharged having retained gallstones.

The presence of gallstones in the peritoneal cavity has been associated with serious complications, including several types of intra-abdominal abscesses, postoperative fever, and development of enterocutaneous fistulae.[37] Despite the availability of animal data suggesting an association between retained gallstones and adhesion formation, such clinical consequences are rarely reported.[38,39] Examining this phenomenon in patients can be hardly achieved. Gallstone ileus due to stone erosion into the small bowel is a known entity, but the development of adhesions due to the presence of gallstones is far from understood. Adhesion formation after gallstone spillage may be highly related to the inflammatory response that the gallstones provoke as foreign bodies. In one of the largest series examining the complications associated with gallstone spillage during laparoscopic cholecystectomy, only one out of 547 patients developed ileus.[40] It is unclear however, if this ileus was due to adhesion formation. In a prospective study over a 7-year period of 106 patients who had gallstone spillage, none developed complications directly related to adhesions.[41] Similarly, Manukian et al.[42] reported on 21 such patients who were followed up for a period of 121 months. None of these patients had any complication related to adhesion formation. Hui et al.[43] also found that retained gallstones did not have any significant effect on patients after a median follow-up of 44 months, while Assaff et al.[44] found that spillage of gallstones did not affect the overall in-hospital course of patients.

In summary, with the exception of small number of case reports, the overall association of gallstone spillage with formation of intra-abdominal adhesions in humans has not been clearly determined. Due to the available animal data and the rare, but serious other complications associated with retained gallstones, every effort should be made to remove any spilt stones in the peritoneal cavity during laparoscopic cholecystectomy.

Conclusion

Adhesion-related morbidity comprises a significant burden on healthcare resources, and prevention is of major importance, especially in high-risk patients. The most important risk factor is the type of surgery, with open surgical interventions in the lower abdomen carrying the highest risk. Laparoscopic procedures appear to be associated with lower incidence of adhesive SBO when compared with open procedures. This, however, does not apply to appendectomy. Closure of the peritoneum, use of gloves containing starch granules, and gallstone spillage during cholecystectomy all increase the risk for adhesion formation. Further understanding of the risk factors for developing adhesion-related morbidity is important for the development of preventive strategies.

References

1. Deference CJ, Lucas CA, Buie VC, Golosinskiy A. 2006 National Hospital Discharge Survey. National Health Statistics Reports; 2008. Report No. 5
2. Weibel MA, Majno G. Peritoneal adhesions and their relation to abdominal surgery. A postmortem study. Am J Surg [Internet]. 1973;126(3):345–353.
3. Menzies D, Ellis H. Intestinal obstruction from adhesions—how big is the problem? Ann R Coll Surg Engl [Internet] 1990;72 (1):60–63.
4. Ellis H, Moran BJ, Thompson JN, Parker MC, Wilson MS, Menzies D, McGuire A, Lower AM, Hawthorn RJ, O'Brien F, Buchan S, Crowe AM. Adhesion-related hospital readmissions after abdominal and pelvic surgery: A retrospective cohort study. Lancet [Internet]. 1999;353(9163):1476–1480.
5. Parker MC, Ellis H, Moran BJ, Thompson JN, Wilson MS, Menzies D, McGuire A, Lower AM, Hawthorn RJ, O'Briena F, Buchan S, Crowe AM. Postoperative adhesions: Ten-year follow-up of 12,584 patients undergoing lower abdominal surgery. Dis

Colon Rectum [Internet]. 2001;44(6):822–829; discussion 829–830.
6. Lower AM, Hawthorn RJ, Ellis H, O'Brien F, Buchan S, Crowe AM. The impact of adhesions on hospital readmissions over ten years after 8849 open gynaecological operations: An assessment from the surgical and clinical adhesions research study. BJOG [Internet]. 2000;107(7):855–862.
7. Parker MC, Wilson MS, Menzies D, Sunderland G, Thompson JN, Clark DN, Knight AD, Crowe AM, Surgical and Clinical Adhesions Research (SCAR) Group. Colorectal surgery: The risk and burden of adhesion-related complications. Colorectal Dis [Internet]. 2004;6(6):506–511.
8. Lower AM, Hawthorn RJ, Clark D, Boyd JH, Finlayson AR, Knight AD, Crowe AM, Surgical and Clinical Research (SCAR) Group. Adhesion-related readmissions following gynaecological laparoscopy or laparotomy in scotland: An epidemiological study of 24 046 patients. Hum Reprod [Internet]. 2004;19(8):1877–1885.
9. Parker MC, Wilson MS, Menzies D, Sunderland G, Clark DN, Knight AD, Crowe AM, Surgical and Clinical Adhesions Research (SCAR) Group. The SCAR-3 study: 5-year adhesion-related readmission risk following lower abdominal surgical procedures. Colorectal Dis [Internet]. 2005;7(6):551–558.
10. London: Central Statistical Office. Social Trends. 1995. Report No. 25
11. Dowson HM, Bong JJ, Lovell DP, Worthington TR, Karanjia ND, Rockall TA. Reduced adhesion formation following laparoscopic versus open colorectal surgery. Br J Surg [Internet]. 2008;95 (7):909–914.
12. Kuhry E, Schwenk WF, Gaupset R, Romild U, Bonjer HJ. Long-term results of laparoscopic colorectal cancer resection. Cochrane Database Syst Rev [Internet]. 2008 16;(2)(2):CD003432.
13. Taylor GW, Jayne DG, Brown SR, Thorpe HC, Brown JM, Dewberry SC, Quirke P, Guillou PJ. Adhesive intestinal obstruction and incisional herniation following laparoscopic-assisted and open colorectal cancer surgery: A supplementary analysis of the MRC CLASICC trial. Brit J Surg/Wiley, New York, 2007, p 42. AGSBI Abstracts
14. Riber C, Soe K, Jorgensen T, Tonnesen H. Intestinal obstruction after appendectomy. Scand J Gastroenterol [Internet]. 1997;32 (11):1125–1128.
15. Andersson RE. Small bowel obstruction after appendectomy. Br J Surg [Internet]. 2001;88(10):1387–1391.
16. Duron JJ, Silva NJ, du Montcel ST, Berger A, Muscari F, Hennet H, Veyrieres M, Hay JM. Adhesive postoperative small bowel obstruction: Incidence and risk factors of recurrence after surgical treatment: A multicenter prospective study. Ann Surg [Internet]. 2006;244(5):750–757.
17. Blachar A, Federle MP. Bowel obstruction following liver transplantation: Clinical and ct findings in 48 cases with emphasis on internal hernia. Radiology [Internet]. 2001;218(2):384–388.
18. Sugitani A, Gritsch HA, Shapiro R, Bonham CA, Egidi MF, Corry RJ. Surgical complications in 123 consecutive pancreas transplant recipients: Comparison of bladder and enteric drainage. Transplant Proc [Internet]. 1998;30(2):293–294.
19. Wasserberg N, Nunoo-Mensah JW, Ruiz P, Tzakis AG. The effect of immunosuppression on peritoneal adhesions formation after small bowel transplantation in rats. J Surg Res [Internet]. 2007;141(2):294–298.
20. Park CM, Lee WY, Cho YB, Yun HR, Lee WS, Yun SH, Chun HK. Sodium hyaluronate-based bioresorbable membrane (seprafilm) reduced early postoperative intestinal obstruction after lower abdominal surgery for colorectal cancer: The preliminary report. Int J Colorectal Dis [Internet]. 2009;24(3):305–310.
21. Shin JY, Hong KH. Risk factors for early postoperative small-bowel obstruction after colectomy in colorectal cancer. World J Surg [Internet]. 2008;32(10):2287–2292.
22. Leung TT, Dixon E, Gill M, Mador BD, Moulton KM, Kaplan GG, MacLean AR. Bowel obstruction following appendectomy: What is the true incidence? Ann Surg [Internet]. 2009;250(1):51–53.
23. Stewart RM, Page CP, Brender J, Schwesinger W, Eisenhut D. The incidence and risk of early postoperative small bowel obstruction. A cohort study. Am J Surg [Internet]. 1987;154(6):643–647.
24. Tortella BJ, Lavery RF, Chandrakantan A, Medina D. Incidence and risk factors for early small bowel obstruction after celiotomy for penetrating abdominal trauma. Am Surg [Internet]. 1995;61 (11):956–958.
25. Weigelt JA, Kingman RG. Complications of negative laparotomy for trauma. Am J Surg [Internet]. 1988;156(6):544–547.
26. Ellis H, Heddle R. Does the peritoneum need to be closed at laparotomy? Br J Surg [Internet]. 1977;64(10):733–736.
27. Gilbert JM, Ellis H, Foweraker S. Peritoneal closure after lateral paramedian incision. Br J Surg [Internet]. 1987;74(2):113–115.
28. Hugh TB, Nankivell C, Meagher AP, Li B. Is closure of the peritoneal layer necessary in the repair of midline surgical abdominal wounds? World J Surg [Internet]. 1990;14(2):231–233; discussion 233–234.
29. Komoto Y, Shimoya K, Shimizu T, Kimura T, Hayashi S, Temma-Asano K, Kanagawa T, Fukuda H, Murata Y. Prospective study of non-closure or closure of the peritoneum at cesarean delivery in 124 women: Impact of prior peritoneal closure at primary cesarean on the interval time between first cesarean section and the next pregnancy and significant adhesion at second cesarean. J Obstet Gynaecol Res [Internet]. 2006;32(4):396–402.
30. Cheong YC, Premkumar G, Metwally M, Peacock JL, Li TC. To close or not to close? A systematic review and a meta-analysis of peritoneal non-closure and adhesion formation after caesarean section. Eur J Obstet Gynecol Reprod Biol [Internet]. 2009;147 (1):3–8
31. Malvasi A, Tinelli A, Farine D, Rahimi S, Cavallotti C, Vergara D, Martignago R, Stark M. Effects of visceral peritoneal closure on scar formation at cesarean delivery. Int J Gynaecol Obstet [Internet]. 2009;105(2):131–135.
32. Sheikh KM, Duggal K, Relfson M, Gignac S, Rowden G. An experimental histopathologic study of surgical glove powders. Arch Surg [Internet]. 1984;119(2):215–219.
33. Cade D, Ellis H. The peritoneal reaction to starch and its modification by prednisone. Eur Surg Res [Internet]. 1976;8 (5):471–479.
34. Cooke SA, Hamilton DG. The significance of starch powder contamination in the aetiology of peritoneal adhesions. Br J Surg [Internet]. 1977;64(6):410–412.
35. Luijendijk RW, Wauters CC, Voormolen MH, Hop WC, Jeekel J. Intra-abdominal adhesions and foreign-body granulomas following earlier laparotomy. Ned Tijdschr Geneeskd [Internet]. 1994;138(14):717–721.
36. Woodfield JC, Rodgers M, Windsor JA. Peritoneal gallstones following laparoscopic cholecystectomy: Incidence, complications, and management. Surg Endosc [Internet]. 2004;18 (8):1200–1207.
37. Zehetner J, Shamiyeh A, Wayand W. Lost gallstones in laparoscopic cholecystectomy: All possible complications. Am J Surg [Internet]. 2007;193(1):73–78.
38. Sax HC, Adams JT. The fate of the spilled gallstone. Arch Surg [Internet]. 1993;128(4):469.
39. Huynh T, Mercer D. Early postoperative small bowel obstruction caused by spilled gallstones during laparoscopic cholecystectomy. Surgery [Internet]. 1996;119(3):352–353.
40. Schafer M, Suter C, Klaiber C, Wehrli H, Frei E, Krahenbuhl L. Spilled gallstones after laparoscopic cholecystectomy. A relevant problem? A retrospective analysis of 10,174 laparoscopic cholecystectomies. Surg Endosc [Internet]. 1998;12(4):305–309.

41. Memon MA, Deeik RK, Maffi TR, Fitzgibbons RJ Jr. The outcome of unretrieved gallstones in the peritoneal cavity during laparoscopic cholecystectomy. A prospective analysis. Surg Endosc [Internet]. 1999;13(9):848–857.
42. Manukyan MN, Demirkalem P, Gulluoglu BM, Tuney D, Yegen C, Yalin R, Aktan AO. Retained abdominal gallstones during laparoscopic cholecystectomy. Am J Surg [Internet]. 2005;189 (4):450–452.
43. Hui TT, Giurgiu DI, Margulies DR, Takagi S, Iida A, Phillips EH. Iatrogenic gallbladder perforation during laparoscopic cholecystectomy: Etiology and sequelae. Am Surg [Internet]. 1999;65 (10):944–948.
44. Assaff Y, Matter I, Sabo E, Mogilner JG, Nash E, Abrahamson J, Eldar S. Laparoscopic cholecystectomy for acute cholecystitis and the consequences of gallbladder perforation, bile spillage, and "loss" of stones. Eur J Surg [Internet]. 1998;164(6):425–431.
45. Lujan Mompean JA, Robles Campos R, Parrilla Paricio P, Soria Aledo V, Garcia Ayllon J. Laparoscopic versus open appendicectomy: A prospective assessment. Br J Surg [Internet]. 1994;81(1):133–135.
46. Ortega AE, Hunter JG, Peters JH, Swanstrom LL, Schirmer B. A prospective, randomized comparison of laparoscopic appendectomy with open appendectomy. laparoscopic appendectomy study group. Am J Surg [Internet]. 1995;169(2):208–212; discussion 212–213.
47. Walker SJ, West CR, Colmer MR. Acute appendicitis: Does removal of a normal appendix matter, what is the value of diagnostic accuracy and is surgical delay important? Ann R Coll Surg Engl [Internet]. 1995;77(5):358–363.
48. Cox MR, McCall JL, Toouli J, Padbury RT, Wilson TG, Wattchow DA, Langcake M. Prospective randomized comparison of open versus laparoscopic appendectomy in men. World J Surg [Internet]. 1996;20(3):263–266.
49. Hansen JB, Smithers BM, Schache D, Wall DR, Miller BJ, Menzies BL. Laparoscopic versus open appendectomy: Prospective randomized trial. World J Surg [Internet]. 1996;20(1):17–20; discussion 21.
50. Mutter D, Vix M, Bui A, Evrard S, Tassetti V, Breton JF, Marescaux J. Laparoscopy not recommended for routine appendectomy in men: Results of a prospective randomized study. Surgery [Internet]. 1996;120(1):71–74.
51. Williams MD, Collins JN, Wright TF, Fenoglio ME. Laparoscopic versus open appendectomy. South Med J [Internet]. 1996;89 (7):668–674.
52. Heikkinen TJ, Haukipuro K, Hulkko A. Cost-effective appendectomy. open or laparoscopic? A prospective randomized study. Surg Endosc [Internet]. 1998;12(10):1204–1208.
53. Kazemier G, de Zeeuw GR, Lange JF, Hop WC, Bonjer HJ. Laparoscopic vs open appendectomy. A randomized clinical trial. Surg Endosc [Internet]. 1997;11(4):336–340.
54. Laine S, Rantala A, Gullichsen R, Ovaska J. Laparoscopic appendectomy-is it worthwhile? A prospective, randomized study in young women. Surg Endosc [Internet]. 1997;11(2):95–97.
55. Macarulla E, Vallet J, Abad JM, Hussein H, Fernandez E, Nieto B. Laparoscopic versus open appendectomy: A prospective randomized trial. Surg Laparosc Endosc [Internet]. 1997;7 (4):335–339.
56. Hellberg A, Rudberg C, Kullman E, Enochsson L, Fenyo G, Graffner H, Hallerback B, Johansson B, Anderberg B, Wenner J, Ringqvist I, Sorensen S. Prospective randomized multicentre study of laparoscopic versus open appendicectomy. Br J Surg [Internet]. 1999;86(1):48–53.
57. Ozmen MM, Zulfikaroglu B, Tanik A, Kale IT. Laparoscopic versus open appendectomy: Prospective randomized trial. Surg Laparosc Endosc Percutan Tech [Internet]. 1999;9(3):187–189.
58. Huang MT, Wei PL, Wu CC, Lai IR, Chen RJ, Lee WJ. Needlescopic, laparoscopic, and open appendectomy: A comparative study. Surg Laparosc Endosc Percutan Tech [Internet]. 2001;11(5):306–312.
59. Pedersen AG, Petersen OB, Wara P, Ronning H, Qvist N, Laurberg S. Randomized clinical trial of laparoscopic versus open appendicectomy. Br J Surg [Internet]. 2001;88(2):200–205.
60. Milewczyk M, Michalik M, Ciesielski M. A prospective, randomized, unicenter study comparing laparoscopic and open treatments of acute appendicitis. Surg Endosc [Internet]. 2003;17 (7):1023–1028.
61. Tingstedt B, Johansson J, Nehez L, Andersson R. Late abdominal complaints after appendectomy—readmissions during long-term follow-up. Dig Surg [Internet]. 2004;21(1):23–27.
62. Katkhouda N, Mason RJ, Towfigh S, Gevorgyan A, Essani R. Laparoscopic versus open appendectomy: A prospective randomized double-blind study. Ann Surg [Internet]. 2005;242(3):439–448; discussion 448–450.
63. Moberg AC, Berndsen F, Palmquist I, Petersson U, Resch T, Montgomery A. Randomized clinical trial of laparoscopic versus open appendicectomy for confirmed appendicitis. Br J Surg [Internet]. 2005;92(3):298–304.
64. Khairy GA, Afzal MF, Murshid KR, Guraya S, Ghallab A. Post appendectomy small bowel obstruction. Saudi Med J [Internet]. 2005;26(7):1058–1060.
65. Cueto J, D'Allemagne B, Vazquez-Frias JA, Gomez S, Delgado F, Trullenque L, Fajardo R, Valencia S, Poggi L, Balli J, Diaz J, Gonzalez R, Mansur JH, Franklin ME. Morbidity of laparoscopic surgery for complicated appendicitis: An international study. Surg Endosc [Internet]. 2006;20(5):717–720.
66. Duron JJ, Hay JM, Msika S, Gaschard D, Domergue J, Gainant A, Fingerhut A. Prevalence and mechanisms of small intestinal obstruction following laparoscopic abdominal surgery: A retrospective multicenter study. French Association for Surgical Research. Arch Surg [Internet]. 2000;135(2):208–212.
67. Kiviluoto T, Siren J, Luukkonen P, Kivilaakso E. Randomised trial of laparoscopic versus open cholecystectomy for acute and gangrenous cholecystitis. Lancet [Internet]. 1998;351(9099):321–325.
68. Lujan JA, Parrilla P, Robles R, Marin P, Torralba JA, Garcia-Ayllon J. Laparoscopic cholecystectomy vs open cholecystectomy in the treatment of acute cholecystitis: A prospective study. Arch Surg [Internet]. 1998;133(2):173–175.
69. Parikh JA, Ko CY, Maggard MA, Zingmond DS. What is the rate of small bowel obstruction after colectomy? Am Surg [Internet]. 2008;74(10):1001–1005.
70. Mohri Y, Uchida K, Araki T, Inoue Y, Tonouchi H, Miki C, Kusunoki M. Hyaluronic acid-carboxycellulose membrane (sepra-film) reduces early postoperative small bowel obstruction in gastrointestinal surgery. Am Surg [Internet]. 2005;71(10):861–863.
71. Beck DE, Opelka FG, Bailey HR, Rauh SM, Pashos CL. Incidence of small-bowel obstruction and adhesiolysis after open colorectal and general surgery. Dis Colon Rectum [Internet]. 1999;42(2):241–248.
72. Duepree HJ, Senagore AJ, Delaney CP, Fazio VW. Does means of access affect the incidence of small bowel obstruction and ventral hernia after bowel resection? laparoscopy versus laparotomy. J Am Coll Surg [Internet]. 2003;197(2):177–181.
73. Nieuwenhuijzen M, Reijnen MM, Kuijpers JH, van Goor H. Small bowel obstruction after total or subtotal colectomy: A 10-year retrospective review. Br J Surg [Internet]. 1998;85 (9):1242–1245.
74. Sonoda T, Pandey S, Trencheva K, Lee S, Milsom J. Longterm complications of hand-assisted versus laparoscopic colectomy. J Am Coll Surg [Internet]. 2009;208(1):62–66.
75. Poppen B, Svenberg T, Bark T, Sjogren B, Rubio C, Drakenberg B, Slezak P. Colectomy–proctomucosectomy with S-pouch:

Operative procedures, complications, and functional outcome in 69 consecutive patients. Dis Colon Rectum [Internet]. 1992;35 (1):40–47.
76. McMullen K, Hicks TC, Ray JE, Gathright JB, Timmcke AE. Complications associated with ileal pouch–anal anastomosis. World J Surg [Internet]. 1991;15(6):763–766; discussion 766–767.
77. Skarsgard ED, Atkinson KG, Bell GA, Pezim ME, Seal AM, Sharp FR. Function and quality of life results after ileal pouch surgery for chronic ulcerative colitis and familial polyposis. Am J Surg [Internet]. 1989;157(5):467–471.
78. Becker JM, Stucchi AF. Proctocolectomy with ileoanal anastomosis. J Gastrointest Surg [Internet]. 2004;8(4):376–386.
79. Oresland T, Fasth S, Nordgren S, Hulten L. The clinical and functional outcome after restorative proctocolectomy. A prospective study in 100 patients. Int J Colorectal Dis [Internet]. 1989;4 (1):50–56.
80. Young CJ, Solomon MJ, Eyers AA, West RH, Martin HC, Glenn DC, Morgan BP, Roberts R. Evolution of the pelvic pouch procedure at one institution: The first 100 cases. Aust N Z J Surg [Internet]. 1999;69(6):438–442.
81. Vasilevsky CA, Rothenberger DA, Goldberg SM. The S ileal pouch-anal anastomosis. World J Surg [Internet]. 1987;11(6):742–750.
82. Nicholls RJ, Holt SD, Lubowski DZ. Restorative proctocolectomy with ileal reservoir. Comparison of two-stage vs. three-stage procedures and analysis of factors that might affect outcome. Dis Colon Rectum [Internet]. 1989;32(4):323–326.
83. Fonkalsrud EW, Stelzner M, McDonald N. Experience with the endorectal ileal pullthrough with lateral reservoir for ulcerative colitis and polyposis. Arch Surg [Internet]. 1988;123(9):1053–1058.
84. Marcello PW, Roberts PL, Schoetz DJ Jr, Coller JA, Murray JJ, Veidenheimer MC. Obstruction after ileal pouch–anal anastomosis: A preventable complication? Dis Colon Rectum [Internet]. 1993;36(12):1105–1111.
85. Francois Y, Dozois RR, Kelly KA, Beart RW Jr, Wolff BG, Pemberton JH, Ilstrup DM. Small intestinal obstruction complicating ileal pouch–anal anastomosis. Ann Surg [Internet]. 1989;209(1):46–50.
86. Galandiuk S, Pemberton JH, Tsao J, Ilstrup DM, Wolff BG. Delayed ileal pouch-anal anastomosis. Complications and functional results. Dis Colon Rectum [Internet]. 1991;34(9):755-758.
87. Fazio VW, Ziv Y, Church JM, Oakley JR, Lavery IC, Milsom JW, Schroeder TK. Ileal pouch-anal anastomoses complications and function in 1005 patients. Ann Surg [Internet]. 1995;222(2):120–127.
88. MacLean AR, Cohen Z, MacRae HM, O'Connor BI, Mukraj D, Kennedy ED, Parkes R, McLeod RS. Risk of small bowel obstruction after the ileal pouch-anal anastomosis. Ann Surg [Internet]. 2002;235(2):200–206.
89. Nyam DC, Brillant PT, Dozois RR, Kelly KA, Pemberton JH, Wolff BG. Ileal pouch-anal canal anastomosis for familial adenomatous polyposis: Early and late results. Ann Surg [Internet]. 1997;226(4):514–519; discussion 519–521.
90. Renz BM, Feliciano DV. Unnecessary laparotomies for trauma: A prospective study of morbidity. J Trauma [Internet]. 1995;38 (3):350–356.
91. Morrison JE, Wisner DH, Bodai BI. Complications after negative laparotomy for trauma: Long-term follow-up in a health maintenance organization. J Trauma [Internet]. 1996;41(3):509–513.
92. Al-Sunaidi M, Tulandi T. Adhesion-related bowel obstruction after hysterectomy for benign conditions. Obstet Gynecol [Internet]. 2006;108(5):1162–1166.

J Gastrointest Surg (2010) 14:1629–1631
DOI 10.1007/s11605-010-1224-9

GI IMAGE

Multiple Bile Duct Hamartomas Mimicking Diffuse Hepatic Metastasis: GI Image

Ashish Singhal · Rajesh Kanagala · Harlan I. Wright · Vivek Kohli

Received: 8 April 2010 / Accepted: 28 April 2010 / Published online: 18 May 2010

Abstract Bile duct hamartomas (von Meyenburg complex) are the rare benign neoplasm of the liver due to dysembryogenesis; constituted historically, cystic dilatations of the bile duct encompassed by fibrous stroma. Usually, they are asymptomatic and are not detected on routine radiological examinations including ultrasound or CT scan. Magnetic resonance cholangiography has been suggested as the best investigation for their imaging diagnosis. Their presence can cause diagnostics confusion and complicate the patient's management. We report a 45-year-old female with symptomatic cholelithiasis, whose liver on laparoscopy mimicked multiple hepatic metastases.

Keywords Bile duct hamartomas · von Meyenburg complex · Ductal plate abnormalities · Liver

Clinica Data

A 45-year-old female suffering with gall-stone-induced acute cholecystitis was referred to us following an abandonment of laparoscopic cholecystectomy elsewhere. Procedure was abandoned due to confusing intraoperative findings of numerous irregular whitish lesions of various sizes scattered on the hepatic surface imitating metastatic deposits; however, examination of the peritoneal cavity failed to reveal any abnormality or peritoneal carcinomatosis. Review of preoperative clinical and laboratory tests showed no abnormality except an increase in total leukocyte count. Preoperative abdominal ultrasonography revealed multiple gallbladder calculi with slight thickening of the gallbladder wall, characteristic of acute cholecystitis, but did not display any alterations in the hepatic parenchyma. Contrast-enhanced CT scan (abdomen) showed innumerable subcentimeter low-density lesions scattered diffusely throughout the liver representing tiny cysts. Consequently, patient was counseled and the laparoscopic cholecystectomy with wedge biopsy of liver was performed (Fig. 1). Liver biopsy was suggestive of multiple bile duct hamartomas with pus cells in few bile ducts (cholangitis) and bridging fibrosis (trichrome positive; Fig. 2a and b). Postoperative course was uneventful and currently, she is doing well 12 months post-surgery.

A. Singhal · R. Kanagala · H. I. Wright · V. Kohli (✉)
Nazih Zuhdi Transplant Institute,
INTEGRIS Baptist Medical Center,
3300 NW Expressway,
Oklahoma City, OK 73112, USA
e-mail: vivek.kohli@gmail.com

Discussion

Multiple bile duct hamartomas (MBDHs), also known as von Meyenburg complexes are infrequently observed benign hepatic tumor-like lesions, consisting of dilated bile ducts structures with a surrounding fibrous stroma.[1] They were first described by von Meyenburg in 1918. These are considered to be part of the spectrum of ductal plate abnormalities and are caused by arrest or perturbation of the ductal plate remodeling during the embryogenesis of intrahepatic bile ducts. Embryologically, intrahepatic bile ducts develop from the hilum of the liver to the distal parts and undergo remodeling. It seems that arrest occurs in the later phases, as their morphology represent the partially fibrosing remnant of the smaller, more peripheral, and interlobular bile ducts.[2] They may occur in otherwise normal liver or in association with other ductal plate abnormalities such as Caroli's disease, congenital hepatic fibrosis, and autosomal dominant polycystic kidney disease.[2] Trichrome positivity in the present case may represent the association between MBDHs and hepatic fibrosis.

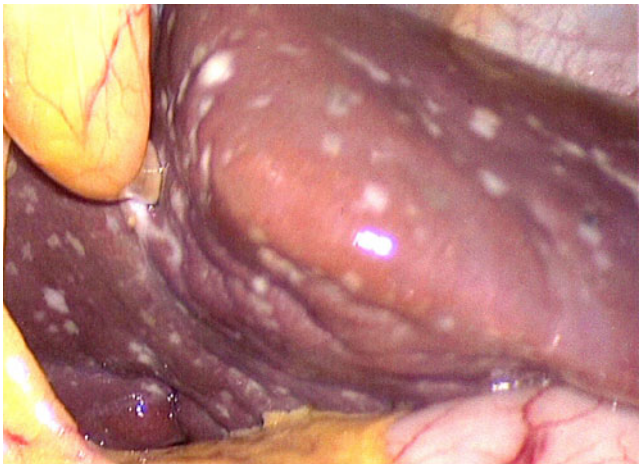

Fig. 1 Macroscopic appearance of multifocal bile duct hamartomas.

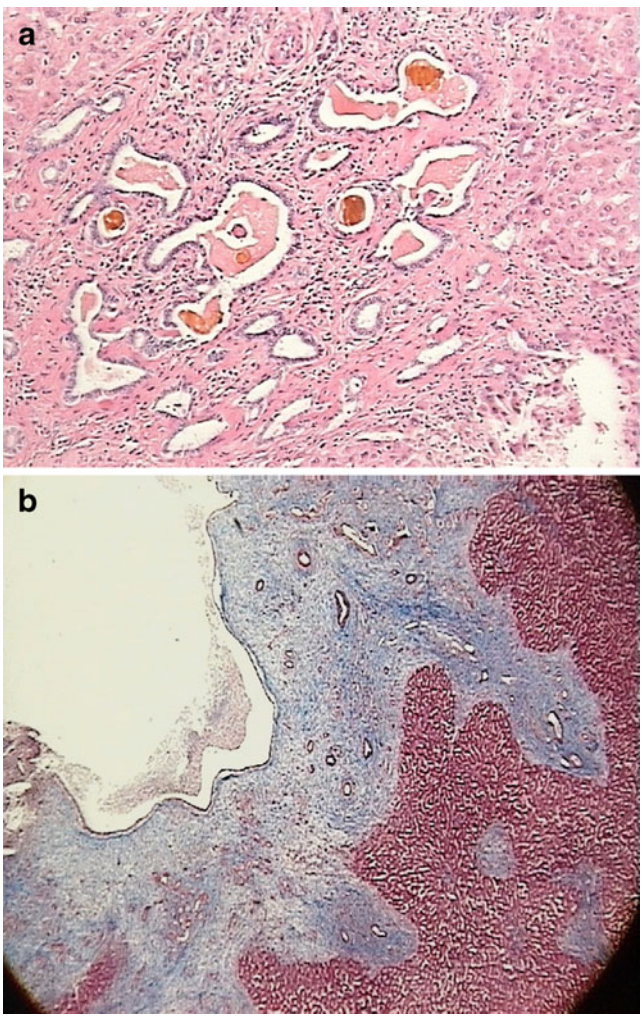

Fig. 2 **a** Microscopic appearance (hematoxylin–eosin staining) of bile duct hamartomas. **b** Bile duct hamartomas with bridging fibrosis (trichrome positivity).

Usually, they are detected as an incidental finding on laparotomy or autopsy, or diagnosed during the management of patients with other ductal plate malformations.[1–4] The incidence based on a study of consecutive autopsies is approximately 5.6% in adults and 0.9% in children.[1] Among adults with APKD, biliary hamartomas were found in 97% and hepatic cysts in 88%. Because APKD can account for only 11% of the patients with biliary hamartomas, authors suggested that MBDHs, in the absence of APKD, are a manifestation of other disease, which could be genetic or secondary to inflammation or ischemia.[1] However, these genetic or non-genetic factors are not known.

MBDHs are small (0.1–1.0 cm), grayish-white or green, multiple nodular lesions below the Glisson's capsule, usually scattered in both lobes.[1–3] Microscopically, the lesions are discrete, round to irregular in shape, and typically paraportal in location. They comprise of cluster of dilated bile ducts of various sizes, peri-ductal glands, and encompassed by fibrous stroma.[4,5]

Being small in diameter (0.5–1.0 cm), they are often not detected on routine radiological examinations including ultrasound, or CT scan.[6] If detected, on ultrasonography, appear as multiple small areas of high and low echogenicity, which assume the appearance of target lesions, with a hyperechogenic center and a hypoechogenic periphery, and a posterior echo resulting from the presence of cholesterol crystals in the interior of the dilated bile duct.[6] On CT, appear as small intrahepatic cystoid lesions and are frequently located adjacent to the portal veins.[7] A magnetic resonance cholangiography is the best imaging investigation for diagnosis of biliary hamartomas.[8,9] It can also distinguish the different forms of dilatation of the bile duct, such as sacular dilatation of the biliary system (Caroli disease), periductal cystic dilatation (multiple abscess or polycystic disease) and can even detect the presence of intrahepatic cholangiocarcinoma or diffuse metastases.[8,9]

Generally, they are asymptomatic with normal liver function tests and are not considered of any pathological value. However, a case report has described a patient who suffered with fever, jaundice, and right upper quadrant pain due to biliary hamartomas with microabscess formation associated with biliary stones and biliary tract infection.[10] Multifocal occurrence may occur and mimic multiple hepatic metastases as observed in our patient necessitating histopathological examination for diagnosis.[11,12] In addition, recent reports suggested their potential of malignant transformation to intrahepatic cholangiocarcinoma.[13–16] Fine needle aspiration is not diagnostic owing to sampling errors and performance difficulties due to very small size of the lesions. Liver biopsy is the gold standard for diagnosis of this rare hepatobiliary condition and underlines the importance of exact diagnostic measures in cases of unexpected intraoperative findings.

Conclusion

von Meyenburg complexes are rare benign liver lesions, usually subcentimeter in size that can escape preoperative routine radiological diagnostics. Their association with other congenital anomalies, resemblance with liver metastases causing diagnostic dilemma, and potential of malignant transformation underlines the importance of histopathological diagnosis by liver biopsy or intraoperative frozen sections.

IRB Approval Done.

Conflict of Interest None.

References

1. Redston MS, Wanless IR. The hepatic von Meyenburg complex: prevalence and association with hepatic and renal cysts among 2843 autopsies. Mod Pathol 1996;9:233–237. PMID: 8685220
2. Desmet VJ. Pathogenesis of ductal plate abnormalities. May Clin Proc 1998;73(1):80–89. PMID: 9443684
3. Siddiqui MA, McKenna BJ. Hepatic mesenchymal hamartoma: a short review. Arch Pathol Lab Med 2006;130:1567–1569. PMID: 17090204
4. Wei SC, Huang GT, Chen CH, et al. Bile duct hamartomas. A report of two cases. J Clin Gastroenterol 1997;25:608–611. PMID: 9451673
5. Zen Y, Terahata S, Miyayama S, et al. Multicystic biliary hamartoma: a hitherto undescribed lesion. Hum Pathol 2006;37:339–344. PMID: 16613329
6. Bravo SM, Laing FC. Multiple bile duct hamartomas: von Meyenburg complexes detected on sonography and CT scanning. J Ultrasound Med 1994;13:649–651. PMID: 7933036
7. Luo TY, Itai Y, Eguchi N, et al. von Meyenburg complexes of the liver: imaging findings. J Comput Assist Tomogr 1998;22:372–378. PMID: 9606376
8. Mortele B, Mortele K, Seynaeve P, et al. Hepatic bile duct hamartomas (von Meyenburg Complexes): MR and MR cholangiography findings. J Comput Assist Tomogr 2002;26(3):438–443. PMID: 12016376
9. Nagano Y, Matsuo K, Gorai K, et al. Bile duct hamartomas (von Mayenburg complexes) mimicking liver metastases from bile duct cancer: MRC findings. World J Gastroenterol 2006;12:1321–1323. PMID: 16534895
10. Chen YC, Chien RN, Chen MF, et al. Biliary hamartomas associated with biliary stones presenting as multiple microabscesses. Chang Gung Med 2000;23:56–565. PMID: 11092146
11. Fritz S, Hackert T, Blaker H, et al. Multiple von Meyenburg complexes mimicking diffuse liver metastases from esophageal squamous cell carcinoma. World J Gastroenterol 2006;12(26):4250–4252. PMID: 16830386
12. Eisenberg D, Hurwitz L, Yu AC. CT and sonography of multiple bile-duct hamartomas simulating malignant liver disease (case report). AJR Am J Roentgenol 1986;147:279–280. PMID: 3524159
13. Orii T, Ohkohchi N, Sasaki K, Satomi S, Watanabe M, Moriya T. Cholangiocarcinoma arising from preexisting biliary hamartoma of liver—report of a case. Hepatogastroenterology 2003;50:333–336. PMID: 12749215
14. Rocken C, Pross M, Brucks U, Ridwelski K, Roessner A. Cholangiocarcinoma occurring in a liver with multiple bile duct hamartomas (von Meyenburg complexes). Arch Pathol Lab Med 2000;124:1704–1706. PMID: 11079031
15. Jain D, Sarode VR, Abdul-Karim FW, Homer R, Robert ME. Evidence for the neoplastic transformation of Von-Meyenburg complexes. Am J Surg Pathol 2000;24:1131–1139. PMID: 10935654
16. Xu AM, Xian ZH, Zhang SH, Chen XF. Intrahepatic cholangiocarcinoma arising in multiple bile duct hamartomas: report of two cases and review of the literature. Eur J Gastroenterol Hepatol 2009;21:580–584. PMID: 19282767

J Gastrointest Surg (2010) 14:1632–1633
DOI 10.1007/s11605-010-1286-8

LETTER TO THE EDITOR

Damage Control Principles for Pancreatic Surgery

Chad G. Ball · Camilo Correa-Gallego · Thomas J. Howard · Nicholas J. Zyromski · Keith D. Lillemoe

Received: 31 March 2010 / Accepted: 30 June 2010 / Published online: 17 August 2010

To the Editor:

We read with great interest the article entitled "Not Just for Trauma Patients: Damage Control Laparotomy in Pancreatic Surgery" by authors Morgan, Mansker, and Adams.[1] This manuscript discusses the authors' experience with damage control laparotomy (DCL) by describing eight patients who received various elements of a damage control procedure after either massive hemorrhage (6) or intraperitoneal/systemic sepsis (2) during elective pancreatic operations. While Dr. Stone and colleagues[2] are appropriately credited with the first published report describing the early termination of laparotomies for multiply injured patients with major coagulopathies (non-mechanical bleeding), the conceptual underpinning for DCL was introduced by Lucas and Ledgerwood[3] before a meeting of the American Association for the Surgery of Trauma in 1975. This report detailed their experience using hemostatic techniques in liver injury with an emphasis that the most crucial determinant of survival was control of bleeding, regardless of technique. In three patients (1%), perihepatic packing followed by ongoing resuscitation and reoperation 3 to 5 days later proved successful. The validity of this strategy was confirmed in 1981 by Feliciano and Mattox[4] detailing their experience in ten patients (2.2%) where "following all attempts at surgical control, the technique of intra-abdominal packing to the liver was then applied as a last desperate maneuver to control exsanguinating hepatic hemorrhage." An unexpectedly robust 90% survival rate was reported in this highly selected, critically ill patient population. Of note is that these groups of authors collectively represent some of the most influential minds in the history of trauma and critical care.

These seminal concepts have evolved to our current level of understanding where damage control is now viewed as a philosophy, not a destination. As a result, damage control resuscitation (DCR) is now the preferred terminology and incorporates multiple facets including: (1) early initiation of blood product transfusions (massive transfusion protocols), (2) reduced administration of crystalloid fluid resuscitation, (3) permissive hypotension in selected (e.g., penetrating torso) patients, (4) immediate hemorrhage control (angiographic or operative), and (5) a structured, team-based system that functions independently of a patient's hospital location (Emergency Department, Angiography Suite, Operating Room, Intensive Care Unit). Although the foundation of this extended philosophy remains the prompt control of bleeding with restoration of blood volume and correction of hypothermia and acidosis, the role of coagulopathy has become a dominant concept in modern DCR. While the conventional view of DCL focused on reversing acidosis and hypothermia to prevent the development of a coagulopathy, recent evidence[5, 6] suggests that up to one third of all severely injured patients actually arrive to the ED with a coagulopathy, even those who do not suffer from a prolonged prehospital "lag time".

Stimulated by this paper, we reviewed our own experience using DCR over a 5-year period in 178 patients undergoing operative debridement for necrotizing pancreatitis. Of these, 12 (7%) patients required an open abdomen/laparotomy (6=hemorrhage; 6=repeat debridements). The need for DCR was limited to cases of massive hemorrhage with concurrent physiologic exhaustion (6/178, 3%), as evidenced by a mean base deficit of −18 in this patient cohort. Among the four survivors, only half underwent

C. G. Ball (✉) · C. Correa-Gallego · T. J. Howard · N. J. Zyromski · K. D. Lillemoe
Department of Surgery, Indiana University School of Medicine, 545 Barnhill Drive, Emerson Hall #203, Indianapolis, IN 46202, USA
e-mail: ball.chad@gmail.com

primary abdominal fascial closure (mean=6.9 days) during their initial hospitalization. While the risk of uncontrolled exsanguinating hemorrhage during elective pancreatic surgery is always present, based on both Morgan's series (1% in all pancreas cases),[1]as well as our own data (3% in necrotizing pancreatitis operations), the need to initiate DCR is relatively uncommon. This point is particularly important because, while we fundamentally support the life-saving power of DCR when applied for specific well-defined criteria, over-utilization can have deleterious effects on both patients and hospital systems. In high-volume trauma centers, indications for initiating DCR are an initial body core temperature less than 35°C, arterial pH less than 7.2, and/or an initial base deficit greater than −15.[7, 8]Adherence to these principles results in a civilian DCR rate of less than 3% in all severely injured patients (7% in military).[9]Furthermore, in patients who stabilize and begin correcting these physiologic variables once bleeding has been controlled, DCR should be reversed and operations/repairs completed with primary fascial abdominal closure at the end of the operation.

Based on the above-stated criteria, only three patients (#1 [temp], #5 [pH, temp], #6 [pH]) in this current series appeared to be approaching physiologic exhaustion and therefore qualify for DCR. Do the authors have corresponding base deficit values (Table 3) for these patients to justify the initiation of DCR? Progression of a patient's base deficit can be particularly helpful not only in assessing for physiologic exhaustion but also in predicting subsequent mortality.[10]Were there any patients in the authors' experience where DCR was initiated, then reversed due to stabilization, adequate resuscitation, and correction of physiologic parameters? Seventy-five percent (6/8) of their patients had primary fascial closure during their initial hospitalization, a higher percentage than we would have anticipated given the level of physiologic insult inherent with the use of DCL. Can the authors tell us how many days from initial operation to subsequent abdominal wall closure did each of these patients require? This time interval can often be used as a surrogate indicator for the "sickness" of a patient, providing insight into their physiologic status at the time of injury or clinical decompensation. Lastly, in the two patients (#7, #8) who required reoperations following their prior elective pancreatic surgeries where sepsis initiated the DCR, were not these simply truncated operations following source control rather than true DCR—please comment? We subscribe to the DCR philosophy and commend the authors for highlighting its potential utility in the context of elective pancreatic surgery. As we move forward, it will be important to apply these principles based on defined criteria and not simply on the intuitive judgment of experienced pancreatic surgeons. We very much appreciate the opportunity to comment on the manuscript.

References

1. Morgan K, Mansker D, Adams DB. Not just for trauma patients: damage control laparotomy in pancreatic surgery. J Gastrointest Surg 2010:Published online, March 12.
2. Stone HH, Strom PR, Mullins RJ. Management of the major coagulopathy with onset during laparotomy. Ann Surg 1983; 197:532-535.
3. Lucas CE, Ledgerwood AM. Prospective evaluation of hemostatic techniques for liver injuries. J Trauma 1976; 16:442-451.
4. Feliciano DV, Mattox KL, Jordan GL Jr. Intra-abdominal packing for control of hepatic hemorrhage: a reappraisal. J Trauma 1981; 21:285-290.
5. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. J Trauma 2003; 54:1127-1130.
6. Holcomb JB, Jenkins D, Rhee P, Johannigman J, Mahoney P, Metha S, Cox ED, Gehrke MJ, Beilman GJ, Schreiber M, Flaherty SF, Grathwohl KW, Spinella PC, Perkins JG, Beekley AC, McMullin NR, Park MS, Gonzalez EA, Wade CE, Dubnick MA, Schwab CW, Moore FA, Champion HR, Hoyt DB, Hess JR. Damage control resuscitation: directly addressing the early coagulopathy of trauma. J Trauma 2007; 62:207-310.
7. Lier H, Krep H, Schroeder S, Stuber F. The influence of acidosis, hypocalcemia, anemia and hypothermia on functional hemostasis in trauma. J Trauma 2008; 65:951-960.
8. Cushman JG, Feliciano DV, Renz BM, Ingram WL, Ansley JD, Clark WS, Rozycki GS. Iliac vessel injury: operative physiology related to outcome. J Trauma 1997; 42:1033-1040.
9. Como JJ, Dutton RP, Scalea TM, Edelman BB, Hess JR. Blood transfusion rates in the care of acute trauma. Transfusion 2004; 44:809-813.
10. Tremblay LN, Feliciano DV, Rozycki GS. Assessment of initial base deficit as a predictor of outcome: mechanism of injury does make a difference. Am Surg 2002; 68:689-694.

J Gastrointest Surg (2010) 14:1634
DOI 10.1007/s11605-010-1287-7

LETTER TO THE EDITOR

Not Just for Trauma Patients: Damage Control Laparotomy in Pancreatic Surgery (Response to Letter to the Editor)

Katherine Morgan · David B. Adams

Received: 26 May 2010 / Accepted: 30 June 2010 / Published online: 30 July 2010

We appreciate your excellent commentary on damage control surgery and particularly the appropriate acknowledgement of the icons of modern trauma surgery.

The magnitude of your experience with acute pancreatitis at Indiana is as always impressive. This large volume makes your descriptive data an important contribution to the accurate understanding of this challenging disease. Certainly, much can be learned from your conduct in the management of these ill patients. It is notable that we excluded patients undergoing laparostomy or debridement for severe acute pancreatitis with necrosis from our report on elective pancreas surgery. The inflammatory process is a likely contributor to the physiologic derangements surrounding perioperative events. Presumably, patients with necrotizing pancreatitis are a sicker subgroup of pancreas surgery patients.

The trauma literature is robust with helpful guidelines for the institution of damage control surgery, given the large numbers of evaluable patients. The criteria for application of damage control in the previously healthy trauma warrior, however, are potentially quite different from the criteria for its optimal use in a patient with catastrophe while undergoing elective pancreatic surgery, with the attendant metabolic disadvantage of chronic illness (cancer or pancreatitis) and the resultant limited physiologic reserve. Therefore, the appropriate parameters for applying damage control in the pancreas surgery patient are currently uncertain, and the judgment of the experienced pancreatic surgeon remains important.

We reported pH as a marker of acidosis. The median base deficit in our series was 11.1 (range 0.4 to 16.6). The interval to abdominal closure in those patients who achieved it was a median of 3 days (range 1 to 3 days). Patient #7 was relegated to damage control due to intraoperative coagulopathy from sepsis. Patient #8 underwent damage control as a result of intraoperative acidosis and coagulopathy also due to sepsis.

We greatly appreciate your thoughtful comments and common interest in taking optimal care of the pancreas surgery patient. Given the relatively rare need for damage control in elective pancreas surgery, perhaps more experience or more likely a collaborative effort could help to more precisely define the role of damage control.

K. Morgan (✉) · D. B. Adams
Medical University of South Carolina,
Charleston, SC, USA
e-mail: morganka@musc.edu