J Gastrointest Surg (2009) 13:1747–1753
DOI 10.1007/s11605-009-0996-2

PRESIDENTIAL ADDRESS

"Above Average" SSAT Presidential Address 2009

David McFadden

Received: 10 August 2009 / Accepted: 10 August 2009 / Published online: 2 September 2009

First and foremost, I offer a sincere thank you to the Society for Surgery of the Alimentary Tract for the great privilege and honor of serving as your president this year. It is the singular honor of my career. Twenty-five years ago, I made my first national scientific presentation at this meeting. Dr. Michael Zinner has already alluded to my beta-blockade for that presentation. I assure you that my heart rate is considerably faster today. At one time, I thought I could spend the duration of my presidential address just thanking all of the people who have meant so much to me and my career. It would be easier, and more appropriate, to talk about their accomplishments than mine. Plus, it would be easier than coming up with an original presidential address. Still, with just 30 min or so, I'd inevitably leave somebody out and offend them; there have been that many.

I must thank and publicly acknowledge the six surgical chairmen for whom I have had the privilege and advantage of working. Dr. George Zuidema was the Chairman at Johns Hopkins who took a risk on a southern boy and admitted me to his housestaff. Dr. Bernie Jaffe started my surgical research career at SUNY Downstate and introduced me to the SSAT. Dr. John Cameron finished my training at Johns Hopkins, is always "the boss" and a profound supporter of his trainees. My first academic faculty position was under Dr. Josef Fischer at the University of Cincinnati. Joe presaged Nike with his "just do it" work ethic. Dr. Michael Zinner was my chair during my initial UCLA days but has been my mentor, role model, leader, and friend for a quarter of a century. Finally, Dr. E. Carmack Holmes, who succeeded Mike as Chair at UCLA, taught me the philosophy of servant leadership. I must also mention and thank a few of the many who have shaped my career and philosophy: Mike Sarr, Tom Gadacz, John Tarpley, Russ Postier, Larry Pennington, Ken Cherry, Charles Yeo, Keith Lillemoe, Henry Pitt, Richard Bell, Chip Souba, Mike Nussbaum, Stan Ashley, and Bob D'Allesandri. Charles DeGaulle famously said that the "graveyards are full of indispensable men"; Bob Jones and Jon Blackstone are indispensable for the SSAT, and especially for the office of the president. Thank you. And of course, I am so very grateful to the officers, board members, and members and guests of this great organization.

I must ardently thank my family. Chip Souba notably said, "Your family never reads your CV." That is a good thing, as they would never believe it after what they see of me at home. I have three children, each born in a different state. Bill is my Baltimore firstborn, a pilot and an aeronautical engineering student. Hunter is my Cincinnati-born NYU student, and the prototypical middle child. Nora, my Southern California-born valley girl, is sitting here with my wife Nancy. I have no pictures of my mother or wife, the two most important women in my life, as I was strictly forbidden of showing any. So, I will show a slide of my dogs. Apparently, every time one of our children goes off to college, we get a big slobbering Newfoundland dog to replace them. I am so grateful to my mother, who broke a lot of rules for women in the 1960s and especially women in the south. She made a lot of personal and professional sacrifices for her children. Finally, there is Nancy, who has raised the kids, kept the house, cooked gourmet meals, carpooled, and kept me sane and grounded these past 26 years. If you only want to remember one thing I say

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today, remember this advice attributed to the Dalai Lama: Every day of your life tell someone (and today I say them publicly to my family):

Thank you.
Forgive me.
I love you.

Now, I have never been a raging fan of presidential addresses. I have slept through a few and skipped a few for a nap or for 30 min on a hotel treadmill. I know to give one is a great privilege, but we have to remind ourselves that The Sermon on the Mount lasted approximately 7 min, the Gettysburg address, around 6 min, and Martin Luther King's "I have a dream" lasted approximately 9 min. The SSAT Presidential Address, weighing in at a potential 45 min, seems bloated and inappropriate in comparison. John F. Kennedy said that public speaking is the art of diluting a 2-min idea with a 2-h vocabulary. I will try not to do that today.

The title of this talk is "above average." A search of the Internet can find a lot about this simple phrase:

> A particularly fine head on a man usually means that he is stupid; particularly deep philosophers are usually shallow thinkers; in literature, talents not much above the average are usually regarded by their contemporaries as geniuses.
>
> Robert Musil

Let me tell those here today, a group of overachievers, where the title, the muse, for this talk arose. As a young faculty member at the University of Cincinnati, I had the opportunity of helping my friend and senior partner, Dr. Richard Bell, move from his office. Dick was an officer at the time of the Association for Academic Surgery, so some of the items we transported were the membership files. During this endeavor, I happened to come across the letters of recommendation about Charles Yeo and Keith Lillemoe written by John Cameron. Charles and Keith are recognized now as bona fide leaders of this organization and of American surgery, and John's letters were honest, prophetic, and extraordinarily flattering about these two recent graduates of his training program. It is said that, "pride goeth before a fall," but the actual quote from Proverbs 16 is "Pride goeth before destruction, and a haughty spirit before a fall." Proudly I dug out my own file, only to read my letter where I was described as an "above average" resident with a "future in academic surgery." Fallen of spirit, I spent considerable time evolving through Kubler-Ross's stages until acceptance was achieved. But, I also spent considerable time thinking about the concept of average, and above average, and that will be the subject of my brief discussion today.

Average is Not Normal, but Average is Relative

I will discuss the "Lake Wobegone Effect" later today, but for the statistically minded here, let us first discuss a few basic issues. First, people believe that most distributions are normal, so that a Lake Wobegone, whose offspring are all above average, cannot exist. Undeniably they cannot, but all *could nearly be* above (or below) average. Take baseball, like the airline industry, a frequent analogy for surgery. In 2006, 588 players received at least one at-bat in the National League, collecting 23,501 hits in 88,844 at-bats, for a collective batting average of 0.265. But, only 182 players had individual batting averages over 0.265. In other words, 69% of the 2006 NL major league baseball players were "below average"!

A related problem is our reliance on the Likert Scale, whose five-item variant is the most widely used psychometric scale in survey research. It is quick, and dirty, but it is flawed, and inevitably steers us to the "4, or above average" side of the street. A rating of 3 is just so bland and indecisive. Likert evaluations are subject to misrepresentation by several reasons. There is the central tendency bias, an avoidance of using extreme responses, the acquiescence bias, or effortless agreement with statements as presented, and the dreaded social desirability bias, which favors the depiction of oneself or an organization favorably. Hence, an "average" Likert Score of 3.0 commonly exceeds 4.0. Also, for many of us just being "above average" is unacceptable, and average, used alone, seems pejorative. No one really wants to be average at anything. Therefore, the average has been promoted to above average, and we all start to believe it when applied to ourselves. For example, Americans typically perform poorly on international tests while seldom scoring poorly on measures of self-confidence. Svenson found that 80% of subjects rated themselves in the top 30% of all drivers.

So here is the issue: starting from an early age, we have elevated average into above average, and hence above average is…well, average. This calls the question—who believes us anymore? Who will believe us? Lowering expectations for our children, our students, our residents, and ourselves to improve self-esteem or to relieve responsible parties may have short-term advantages, but is unkind and unfair as an enduring solution. As surgeons, we are frequently immersed in real life or death situations where we succeed or fail based only our actual skills. We have a moral and public health obligation to create world-class standards and follow them.

The classic end-around starts early, as in our "No Child Left Behind" initiative. If your son's class is surpassing the national average and he is not, you focus on your child. If the entire school is below average, you tackle the school board. Unfortunately, most states have established their

own diluted standards and tests, pacifying the public with an artificial confidence in their schools. In Mississippi, 89% of fourth graders meet state reading requirements whereas only 18% pass the National Assessment of Educational Progress (NAEP) test. In Oklahoma there is a 50-point gap while Wisconsin has a 53-point gap.

The results may be perhaps seen in the genuinely noble intent of the HOPE Scholarship Program. Unfortunately, it often leads to false hope. In Georgia, for example, high school grades climbed after HOPE scholarships were awarded to students with a 3.0 grade point average (GPA). However, HOPE also requires students to maintain that 3.0 GPA in college. Over 50% of awardees lost eligibility before earning just 30 credits. In my former state of West Virginia, the overcrowded freshman dormitory rooms quickly became spacious single-dweller units after first semester grades were distributed. Students report on surveys taken during the SAT or ACT exams that GPA's have risen faster than their test scores, suggesting that rising grades are not just because our students are getting smarter. Even straight A students frequently arrive on campus supremely unaware of how unprepared they are to achieve at the collegiate level.

It must be tough to be a Dean of Admissions. One recent year, UCLA had 47,317 applications, of which 21,000 had GPA's greater or equal to a perfect 4.0. Reportedly, some high schools graduate with 30–40 valedictorians, so as to not cause undue stress. Recognized grade inflation leads to a general reliance on standardized test scores for college admissions. Standardized tests (think ABSITE) cannot by themselves predict with perfect accuracy who will or will not succeed, but must be a considerable part of the mixture. It is no longer an excuse to say "I am not a good test taker." That option, a pseudodisability, has become extinct.

This trend of universal "above average-ness" continues up the educational food chain: 82% of the Harvard graduating class of 2000 received some sort of honors, 43% of all grades awarded at Brown are A's, 46% of all grades at Northwestern, and *Newsweek* reports that the "average" grade at Duke University now approaches an A minus. We used to call a gift from a professor a "gentleman's C" at the University of Virginia. I suspect it is now a "gentleman's A−." The College Board Review succinctly concluded that, "college grades are no longer accurate indicators of what students know."

Stuart Rojstaczer, a Duke University professor and visiting scholar at Stanford University published "Where All Grades are Above Average." In this compelling article, he comments that university officials give fallacious reasons for rising grade point averages, including that the teaching is more effective, and of course, today's students are just plain smarter and better than those of previous decades. Sadly, parents and students believe and accept this false flattery as the truth. He comments that:

> The last time I gave a C was over two years ago. Once commonly accepted, a "C" is now the equivalent of the mark of Cain. The previous signs of academic disaster, D and F, went by the wayside in the Vietnam era, when flunking out meant becoming eligible for the draft …. GPA's are rising approximately 0.15 points per decade. A's are common as dirt in universities nowadays …. If I sprinkle my classroom with the C's some students deserve, it will suffer from declining enrollments. Low enrollments are taken as a sign of poor-quality instruction. I have no interest in being known as a failure. Parents and students want high grades … they are consumers of an educational product for which they pay dearly. I am expected to cater to their desires not just to be educated well but to receive a positive reward for their enrollment. So I don't give C's anymore, and neither do most of my colleagues. I can easily imagine a time when I'll say the same thing about B's.

Rojstaczer summarily opines that with a dearth of fair grading, our universities' success in providing this country with a truly educated public is reduced. The implications of such failure for a free society are tremendous.

> Of all upon the earth that breathes and creeps nothing is more miserable than man.
>
> Homer

So, if everyone is above average, how can we truly evaluate? Am I just being a middle-aged curmudgeon, as suggested by Homer? Actually, grade inflation is akin to rudeness, reckless driving, poor surgical training, or even long-winded presidential addresses: we all know it happens, and we are uniformly certain that *someone else* does it. We are all sure that we trained under an exacting bell curve; subsequently, we become convinced that these standards have now disappeared into a fog of relativism and "A for effort." Consider our locker room reminiscences about pyramidal surgical training programs and every other night call.

In our medical schools that still issue grades, the average GPA of 3.65 represents 2/3 grades awarded being A's. None of us wants the kid who got a "C" in surgery on our housestaff, but none of us can now tell who the "C" student is, he received an A−! Do not worry, though, grades are also considered stressful; only 25 allopathic medical schools still use them. A recent study from my alma mater, the University of Virginia, concluded that changing medical school grading to pass/fail improves student psychological

well-being. There were no statistically significant variations between the graded and ungraded students as to course performance, national examination scores, class attendance, or quality of residency placement. However, a graded curriculum was said to be more of an incentive to compete with classmates and to achieve honors. "With medicine being practiced in more of a team approach, eliminating grades may facilitate team learning and an increase in support among students, since they would not be competing against each other." It is "T" ball practiced at a ridiculously advanced level. Surgical residencies today can no longer assume that a medical degree guarantees a solid level of education. And unlike in the past, when in error, we can no longer presume or assign extra hours of work, more call, or a year or so of seasoning in a laboratory to make a weak trainee stronger.

So if grades are meaningless and stressful, by all means, let's eliminate them. But, this again leaves us with the question, how do we evaluate quality? How are we to be judge and be judged? Our unwillingness to defy grade inflation only allows the private sector to resolve it for academia. Now that grades are no longer a reliable tool, most employers are creating their own implements, with a dramatic increase in pre-employment examinations that evaluate everything from aptitude to personality. There is also more value placed on personal recommendations. The question for us continues—if we are all above average, how do we judge quality?

The Average Surgeon

A website, medschoolhell.com, exposes the perception of the average general surgeon for future generations. The major caveat is that we have poor lifestyles. The third ranked reason by practicing general surgeons for dissatisfaction with their careers was "lifestyle issues," behind reimbursement and medical liability issues, respectively. Our unpredictable hours, exceeding 60 h per week, are unattractive. Students are warned about the burdens of "rounding on your patients both pre- and post-op, as well as a rigorous schedule in the operating room." Our spouses are exposed as the major decision makers at home and are unlikely to give credit to us for contributing to household duties and childcare. Their final caveat is that the surgical life requires many sacrifices, often involving personal and family lives. On the up side, they report that the salary of a general surgeon is "above average" for medical specialties. Published physician satisfaction scales show we are below average for medical specialties (Table 1).

The old joke that if one asked any surgeon who the best three surgeons in the world are, he (or she) would have trouble naming the other two, is perhaps based in fact. We recognize our own inadequacies with great difficulty, and in a gradeless world where all are above average, that puts us and our patients at risk. Landmark findings by Dunning and Kreuger, two psychology professors at Cornell, are worth discussion. In their article, a 2000 "igNobel Prize" winner, entitled "Unskilled and Unaware of It: How Difficulties in Recognizing One's Own Incompetence Lead to Inflated Self-Assessments" explains on an experimental psychological level our above-average culture. The authors discovered that subjects who perform poorly on sundry tasks are usually unaware of their incompetence. They posit that the internal skills needed to evaluate how well the subjects are doing are often the same as those needed to do the job in the first place. People who tell one bad joke after another are not only incapable of making us laugh, but incapable as well of recognizing that we are not laughing. It takes some nominal skill and judgment to acknowledge that we have little skill or judgment and hence that we may be average or even below average. In Dunning and Kreuger's study, subjects underwent written humor, grammar, and logic tests, rated their own abilities relative to their peers, and predicted their test scores. Unsurprisingly, everybody rated their abilities as *above average*. Subjects who performed best slightly underestimated their ability; subjects who performed worst grossly overestimated theirs.

One of the baffling results is how the unskilled fail, through life, to learn that they are incompetent. If incompetent individuals are unable to spot their own poor performances, it is not inevitable that negative feedback would have been received at some juncture? Unfortunately, people seldom receive negative feedback about their skills and abilities from others. Even young children are familiar with the views that "if you do not have something nice to say, don't say anything at all."

If people receive negative feedback, they must understand it accurately to accept it. One difficulty with failure is that it is prone to more "attributional ambiguity" than success. For success to occur, many factors must go right, including skill, effort, and perhaps luck. The lack of any one of these components is sufficient to induce failure. Hence, even if people receive feedback that identifies a lack of skill as the culprit, they may attribute failure to some other factor. Again, here's the analogy—residents complain most about a lack of timely feedback—negative and positive.

The Lake Wobegone Effect

Weinstein coined this "everybody is above average" phenomenon the Lake Woebegone effect over 20 years ago. It persists and is pervasive. A *Business Week* survey of over 2,000 executives and middle managers revealed that

Table 1 The Average Surgeon

	Average surgeon	Average American worker	Difference
Weekly work hours	>60	37.5	23.5 h, or a factor of 1.66
Annual compensation	$330,215	$44,413	$285,802, or factor of 7.44
Lifestyle satisfaction	0.85	n/a	1.0 average for all MDs
Average practice insurance	$23,600	0	Priceless

90% of managers believe that they are among the top 10% of performers in their workplace. A *Journal of Financial Economics* article entitled "CEO Pay and the Lake Wobegon Effect" revealed how some companies exploit this and pay less-skilled CEOs highly to keep up the firm's stock prices. If a firm hires a CEO and pays a low salary, investors might infer that the CEO is average and downgrade the firm's stock. If the firm pays its CEO like a superstar, investors might believe it, and share prices soar. Federal regulation was not the solution. In 1993, a million-dollar rule held that companies must confirm that any CEO salary over one million dollars was linked to firm accomplishments in order to be tax deductible. Organizations responded by limiting salaries and increasing bonuses. We have some real above-average health care CEO's (Table 2).

This is not the case for surgeons, though. Of course it is. A classic study has shown that most physicians (61%) believe that they are uninfluenced by gifts from pharmaceutical representatives; however, they believe the same is true for only 16% of their colleagues. The social science literature demonstrates that, although bias is identifiable, it tends to be preferentially attributed to others. Little to no data exists for our patient care and operating skills. However, a study from the University of Virginia demonstrated that academic surgical educators' self-perceptions differed significantly from resident assessments. Furthermore, attendings who chose not to evaluate themselves scored lower than their peers. Attendings whose scores were significantly below their peers were most likely to overrate their ability. Finally, none of the surgeons who attained scores significantly below their peers were able to identify the weaknesses indicated by the residents. It is Dunning and Kreuger déjà vu.

Table 2 Average Health Care CEO

Average salary 2005	$24.3 million
Preceding rolling 5-year average salary	$129 million
Average hospital CEO salary	$644,843
Average hospital CEO bonus	$411,466
Average hospital CEO total compensation	$1,252,755

Interestingly, patients do not see our felicities as a weakness, if properly disclosed. Beach et al. reported that physician self-disclosure is associated with higher patient satisfaction ratings for surgical visits and surprisingly lower patient satisfaction ratings for primary care visits. In a study of 1,265 patients and 65 surgeons, disclosure occurred in only 20% of visits. But, more surgical patients reported feelings of warmth/friendliness, reassurance/comfort and being "very satisfied" with these visits (all P values <0.05).

So, where am I, famously above average, going with this? Earlier, I mentioned how the private sector resolves its issues for academia. Look at the myriad of health care evaluation websites. We are certainly not all above average. Perception is reality, especially on the Internet. HealthGrades.com, the self-proclaimed leading independent health care ratings organization, implores patients to rate their doctors so that "It is time to stop choosing on prestige." So far, over 750,000 physicians, 5,000 hospitals, and 16,000 nursing homes are evaluated. Another, Dr.Score.com, claims 89,604 physician evaluations, with the average rating a solid Likert six out of ten.

The importance cannot be understated because health care is dangerous, and its delivery is a privilege. Activities with less than one death per 100,000 encounters include the nuclear power industry, and scheduled airlines. Between one in 1,000 and one in 100,000 deaths include driving and the chemical manufacturing industry. Activities where death is expected in more than one per 1,000 encounters include bungee jumping, mountain climbing, and health care. The surgeon Atul Gawande stated (parentheses mine) that "The real problem isn't how to stop bad doctors from harming, even killing their patients. It's how to prevent good (*above average*) doctors from doing so."

Among the most difficult lessons for many people to learn, and perhaps the easiest to forget, is that we do not always know what is going on, that our opinions may not always be facts, that our intuitions, although useful, are not proof. It is not possible to learn if we do not realize that we have something to learn. These "cold light of day appraisals" are inherent in the universal training standards of the American Council of Graduate Medical Education

and the American Board of Medical Specialties. Both entities include language pertaining to practice-based learning and improvement and lifelong learning and self-assessment through continuing education and periodic self-assessment. The language of maintenance of surgical certification includes the dictum "evaluation of performance in practice through tools such as outcome measures and quality improvement programs, and the evaluation of behaviors such as communication and professionalism." Can we truly all be above average?

Malcolm Gladwell, in his newest book *Outliers*, gives some indirect insight into surgical excellence. In one section, he posits how greater than 10,000 h of practice appears to be a magic number for superior performance, or greatness. He provides evidence with descriptions of The Beatles, Bill Gates, Mozart, and others. We can extrapolate that the average surgical resident works 19,200 h in a 5-year residency ($48 \times 80 \times 5$). Surveys have demonstrated that approximately 20.6% of training is spent in the operating room, or roughly 4,000 h in total. Hence, the average resident, above average or not, has only achieved 40% of the number of hours of surgical practice to achieve greatness, as per Gladwell. If the average surgeon works 60 h a week, and operates 25% of this time, he or she will require over eight more years before "success." Factor in a 5–10% "unlearning" index per annum, and you can see that while not exactly a Zeno's paradox, surgical success or greatness is a lifetime aspiration.

Here is some educational advice to avoid the "above average" state of mind:

1. Be aware that some of the things you say or do may in fact cause your trainees to become unrealistically optimistic about their skills
2. Do not make higher-order skills look or sound easy. Never show off.
3. Discuss the potential consequences of errors that trainees make. Do not play down or joke about mistakes or poor performance.
4. Avoid comments that imply that a trainee's ability is above average or that he has mastered complex skills.
5. When a trainee has demonstrated satisfactory performance, acknowledge that they have met the standard and move on. Treat with caution the adult learning principle of overlearning.
6. Do not make higher-order skills look or sound easy.
7. When a trainee has demonstrated satisfactory performance, simply acknowledge that they have met the standard and move on.
8. Reward cautious behavior.
9. Train your trainees to recognize their own failures and give themselves constructive feedback. Ask them always to rate their behavior against the standard.
10. Avoid making derogatory remarks about others. The more critical drivers become of others, the more they tend to elevate themselves.
11. At the end, emphasize that most learning occurs after training. Trainees should not think that they have mastered skills during training.

The above caveats derive from automobile driving academies in the United Kingdom, but can and should be applied to surgical education in the US. It was discovered that some driver training programs actually increased drivers' chances of crashing because they promoted an exaggerated sense of control ("above average").

The Legitimately Above-Average Surgeon: A Lifelong Process

> The average estimates themselves by what they do, the above average by what they are.
>
> Johann Friedrich von Schille

The legitimately above-average surgeon requires a lifelong process of learning and the enduring practice of candid evaluation and assessment. Because of the exaggerated grading standards from kindergarten through college, the selection of surgical trainees, faculty recruits, or partners should not be based on grades; rather personal statements, honest letters and conversations with their evaluators, interviews, and standardized tests should be more valued. Our students and residents should receive honest, timely, and "relative" feedback, as we ourselves.

In conclusion, I stand here grateful to have been dubbed merely above average by a great surgeon and leader. If he had said otherwise, I might have believed him, and not been able to as humbly pursue this wonderful craft. Furthermore, I have learned that it is okay to be average, and even better to be "above average" especially if you are a surgeon, pilot, or a major league baseball player.

So, that's the news from Lake Michigan where all the men are handsome, all the women strong, and all the surgeons above average. Ladies and gentlemen, it has been a great privilege serving as your president. Thank you for your time and attention.

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J Gastrointest Surg (2009) 13:1754–1761
DOI 10.1007/s11605-009-0957-9

2009 SSAT PLENARY PRESENTATION

Anti-ERBB2 sh-RNA Suppress Both Cell Growth and Tumor Growth in ERBB2-Overexpressing Upper Gastrointestinal Adenocarcinomas

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Received: 28 May 2009 / Accepted: 12 June 2009 / Published online: 15 July 2009

Abstract
Introduction ERBB2 is overexpressed in 15–25% of upper gastrointestinal adenocarcinomas. We use a stable lentiviral shRNA model to demonstrate that ERBB2 suppression in upper gastrointestinal adenocarcinomas with documented ERBB2 amplification effectively decreases ERBB2 protein levels and decreases cell viability. Further, we evaluate tumor growth of cells treated with the ERBB2 shRNA.
Methods Three upper gastrointestinal adenocarcinoma cells lines with varying ERBB2 levels were treated with one of three separate lentiviral green fluorescent protein (GFP)-labeled ERBB2 shRNA vectors or a nonsilencing control shRNA vector for 6 h. Protein levels on day 6 and cell viability was evaluated on days 3–10. A xenograft in vivo experiment was performed using OE19 cells pretransduced with ERBB2 shRNA to evaluate tumor growth.
Results ERBB2 protein levels decreased by 80%. ERBB2 knockdown significantly decreased cell viability in cell lines with high ERBB2 levels. In vivo tumor growth was suppressed in ERBB2-shRNA-treated groups.
Conclusion ERBB2 suppression based on a stable lentiviral shRNA transfection system effectively decreases cell viability in cell lines with amplification of ERBB2 as compared to cell lines without overexpression. ERBB2 knockdown significantly decreases tumor growth in vivo. ERBB2-directed therapy may be of benefit in the subset of patients with gastrointestinal adenocarcinomas exhibiting overamplification of ERBB2.

Keywords ERBB2 · Esophageal adenocarcinoma · Gastric adenocarcinoma · Lentivirus shRNA

Introduction

The incidence of esophageal adenocarcinoma and esophagogastric junction tumors has increased sixfold since 1970.[1–4] As shown in a Surveillance, Epidemiology, and End Results (SEER) database review by Devesa et al., the annual rate of esophageal adenocarcinoma alone increased by more than 350% from 1974 to 1994.[3] This rate of increase has made esophageal adenocarcinoma the fastest expanding type of cancer in Western countries when compared to all other cancers known to be increasing in incidence (e.g., two- to threefold increase for melanoma and prostate cancer). Unfortunately, the 5-year survival of esophageal adenocarcinoma is dismal even in the subset of patients who undergo appropriate surgical intervention. The mainstay of treatment is surgery if the primary tumor is resectable. Chemotherapy, usually consisting of 5-fluorouracil, cisplatin, and epirubicin, does not change the overall poor prognosis. Improvements in the treatment of unresectable and late-stage disease remain insignificant. Therefore, the development of other treatment strategies is critical.

Several studies have evaluated genes that are commonly deleted or amplified in upper gastrointestinal (GI) adeno-

Digestive Disease Week Plenary Session June 2, 2009 Chicago, IL

This project was partly supported by NIH/NIDDK R01 DK063615 and NIH/NCI R01 CA094084 (Yamamoto).

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carcinomas. Of all genes evaluated, reported proto-oncogenes in upper gastrointestinal adenocarcinomas include c-myc, ERBB2, CCNE1,mdm2, GATA-4, Cathepsin B, CMet, epidermal growth factor receptor (EGFR), and Ras.[5,6] Of these proto-oncogenes, ERBB2 is amplified in a much higher proportion of samples. In the case of esophageal adenocarcinoma, ERBB2 has been shown to be the most commonly amplified proto-oncogene and is overexpressed in approximately15–40% of tumors evaluated.[5–11] Similar amplification of ERBB2 has also been noted in gastroesophageal junction and gastric cardia adenocarcinomas.[4,12,13]

As a member of the epidermal growth factor receptor family, ERBB2 (*c-erb*-B2, HER-2/*neu*) is a transmembrane tyrosine kinase receptor. Located on chromosome 17q12, the ERBB2 gene plays a key role in growth factor signal transduction and is also involved in the regulation of cell growth, survival, and differentiation.[14,15] In its normal state, ERBB2 has no known ligand[16] and must pair with other EGFR receptors for activation. As a tyrosine kinase receptor, it is then autophosphorylated and initiates a phosphorylation cascade that results in activation of multiple intracellular pathways, including MAP kinase and phosphoinositol 3 kinase. However, when ERBB2 is overexpressed as the result of a gene amplification event, it is hypothesized that the receptor homodimerizes. In this state, it remains constitutively active and promotes downstream signaling.

To better understand the effect of ERBB2 knockdown in esophageal and gastric adenocarcinomas, we previously used a transient siRNA model to suppress ERBB2 levels in esophageal and gastric adenocarcinoma cell lines with known ERBB2 amplification. Our study reported that ERBB2 inhibition significantly decreases cell viability via an apoptotic pathway.[17] Of note, there were no significant changes in cell cycle seen in our study. However, siRNA is a transient model in which the toxicity of the transfection reagent used as well as the toxicity of the siRNA itself prevents higher transfection efficiencies. Furthermore, siRNA is readily degraded by RNAses. Therefore, in vitro work performed with siRNA technology cannot proceed to an in vivo model; nor can it be translated into the clinical trial setting. Given these inherent limitations of siRNA, we proceeded to evaluate ERBB2 knockdown on upper gastrointestinal adenocarcinomas using a stable transfection model, via lentiviral ERBB2 shRNA vectors. The purpose of this study was to generate a stable transfection model in order to analyze the effect of ERBB2 knockdown on (1) cell viability in upper gastrointestinal adenocarcinoma cell lines with different levels of ERBB2 expression, and (2) tumor growth in an in vivo model.

We believe that in cell lines with ERBB2 amplification there will be a more significant increase in cell death, correlating with our previous siRNA work. Further, we hypothesize that tumor growth of cells treated with the stable ERBB2 shRNA will be significantly inhibited. We show that ERBB2 knockdown via lentiviral shRNA vectors (Fig. 1 and Table 1) in upper gastrointestinal adenocarcinoma cell lines significantly decreases cell viability in the cell lines known to have ERBB2 amplification. Further, tumor growth is inhibited in cell lines treated with shRNA. Our current data correlate with our previous siRNA study and further strengthen the potential benefit of ERBB2-directed therapies in the subset of patient with ERBB2-amplified upper GI adenocarcinoma tumors.

Materials and Methods

Cell Lines

In the present set of experiments, three upper GI adenocarcinoma cell lines were chosen due to their known varying amount of ERBB2 and compared. A gastric adenocarcinoma cell line, MKN45, was obtained from the Japanese Cancer Research Bank. MKN45 has approximately tenfold amplification of ERBB2.[18] An esophageal adenocarcinoma cell line, OE19, was obtained from the European Collection of Cell Cultures. This cell line has 100-fold amplification of ERBB2.[18] Finally, a cell line with a baseline level of ERBB2, Seg-1 (a gift from Dr. David Beer, University of Michigan, Ann Arbor), was used as a control. All cell lines were cultured in Dulbecco's modified essential medium (DMEM), 5% fetal bovine serum (FBS), 1% penicillin–streptomycin, and 1% amphotericin B. HEK293T cells (ATCC) were used for lentiviral

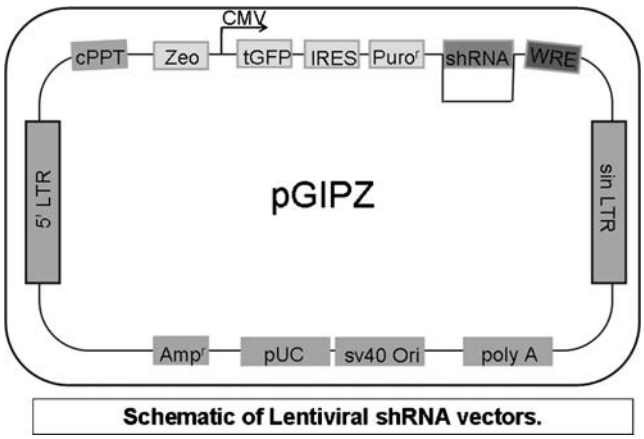

Figure 1 Schematic of Lentiviral shRNA vectors. All of the lentiviral ERBB2 shRNA vectors contain a GFP-label and a puromycin selection marker. This allows for the preferential selection of cells infected with the appropriate lentiviral shRNA vector. Abbreviations are shown in Table 1.

Table 1 Abbreviations of Structures Included in the Lentiviral Vectors

Abbreviations of structures included in the lentiviral pGIPz vectors	
CMV	RNA polymerase II promoter
Ψ (Psi)	Region of viral RNA responsible for directing packaging
PPT	Purine-rich sequence cleaved during reverse transcription to produce RNA primer for viral DNA synthesis
wpre	Woodchuck hepatitis virus post-transcriptional regulatory element
5′ LTR	Long terminal repeat (LTR)
SIN	Deletion of the transcriptional enhancers and promoter in the U3 region of the 3′ LTR
RRE	Binding site for the Rev protein
TurboGFP	Green fluorescent protein utilized to track shRNAmir expression
Puro	Puromycin-*N*-acetyl transferase, mammalian drug selectable marker
polyA	Polyadenylation site
pUC Ori	Permits high-copy replication and maintenance in *Escherichia coli*
Amp	Allows selection of the plasmid in *E. coli*

As shown, each lentiviral vector is under a CMV promoter and includes a poly-A tail

viral tittering and maintenance. This cell line was maintained in DMEM, 5% FBS, and 1% penicillin–streptomycin. All cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Lentiviral shRNA Generation

Three green fluorescent protein (GFP)-labeled ERBB2 shRNA sequences were purchased from OpenBiosytems (Huntsville, AL, USA), as well as a nonsilencing control shRNA sequence (to determine off-target effects). The referenced names as for the ERBB2 shRNA are V2LHS_17669, V2LHS_17671, and V2LHS_17672. Detailed sequence information for each shRNA used is shown in Table 2. The University of Minnesota RNAi Core Facility (Minneapolis, MN, USA) then produced Pgipz™ lentiviral shRNA vectors according to the manufacturer's instructions (OpenBiosystems, Huntsville, AL, USA). The TransLentiviral™ GIPZ Packaging System is based on lentiviral vectors developed by Kappes and Wu.[19] In order to select out transfected cells, these lentiviral vectors not only include a GFP label, but they also have a puromycin selection marker as shown in Fig. 1 and Table 1. Treatment with puromycin after transduction eliminates all non-infected cells.

Treatment of Cells with Lentiviral shRNA Vectors

Prior to starting treatments, viral titers of all vectors were routinely found to be 10^6–10^7 transforming units (TU) per milliliter. Optimization experiments were carried out with varying amounts of lentivirus (1–100 multiplicity of infection, MOI) to determine that the optimal MOI for transduction without toxicity for each lentiviral vector was a MOI of 10. Finally, a puromycin kill curve was performed with each cell line to determine the optimal amount of puromycin needed for selection without toxicity to transduced cells. In all cell lines, the optimal concentration of puromycin was 7.5 μg/mL.

For generation of a stable transduced cell line, cells were plated in a six-well plate at a density of 2×10^5 cells/well and incubated overnight at 37°C and 5% CO_2. A transduction media was made, consisting of serum-free antibiotic-free DMEM media and 8 μg/mL of Polybrene. Each lentiviral vector was diluted with transduction media to a MOI 10. Cells were washed with phosphate-buffered saline (PBS) and

Table 2 Details of ERBB2 shRNA Sequences

	Sense sequence	Loop sequence	Antisense sequence
V2LHS_17669	AGCGCAGATGCGGATCCTGAAA	TAGTGAAGCCACAGATGTA	TTTCAGGATCCGCATCTGCGCC
V2LHS_17671	CGCCCTGGCCGTGCTAGACAAT	TAGTGAAGCCACAGATGTA	ATTGTCTAGCACGGCCAGGGCA
V2LHS_17672	CGCTGAACTGGTGTATGCAGAT	TAGTGAAGCCACAGATGTA	ATCTGCATACACCAGTTCAGCA

Each shRNA sequence is preceded and is followed by a 30-mir sequence which increases subsequent Dicer recognition and specificity. The loop sequences for all shRNAs are identical. ERBB2 shRNA sequences vary slightly in the sense and antisense sequences

then 1 mL of diluted virus was added. After 6 h, the lentiviral shRNA media was removed and replaced with DMEM containing serum and antibiotic. Cells treated with serum-free media served as the control and are referred to as nontreated cells. On post-transduction day 1, media was removed and replaced with 2 mL of DMEM containing serum, antibiotic, and 7.5 μg/mL puromycin. After 3–5 days, all non-infected cells were killed, and remaining cells had GFP expression, indicating 100% transduction efficiency. Once cells reached 80% confluence, they were then trypsinized with 0.25% trypsin and placed in a 100-mm culture dish for propagation of cell lines.

For cell viability assays, 1×10^3 cells per well were seeded in 96-well plates and then incubated overnight at 37°C. Fifty microliters of the appropriate diluted virus at MOI of 10 in transduction media (described above) was then placed on the appropriate wells for 6 h. After 6 h, the viral-containing media was removed and replaced with serum-containing DMEM. Cells treated with serum-free media served as the control and are referred to as nontreated cells. Cells were then incubated for 3–10 days at 37°C and 5% CO_2 until collection for cell viability.

Determination of ERBB2 by Western Blot Analysis

Cells were treated with shRNA lentivirus as described above. Once placed in 100-mm culture plate, cells were grown to 70% confluence and then collected by trypsinization (0.25% trypsin). Cells were washed with PBS thoroughly. Next, cells were lysed with lysis buffer (25 mM Tris–HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), complete Protease Inhibitor (Roche, Indianapolis, IN, USA) for 2 h at 4°C and cleared by centrifugation for 10 min at 10,000×*g* twice. Supernatants were collected and then stored at −80°C. Total protein concentration was determined using the Bio-Rad DC protein assay (Bio-Rad Hercules, CA, USA). ERBB2 protein expression was analyzed by Western blotting by loading 75–100 μg samples of total protein from the supernatants onto a 10%SDS-polyacrylamide gel electrophoresis gel. The separated proteins were transferred onto polyvinyllidene diflouride membranes. Nonspecific binding to the membranes was blocked by 2-h incubation in a buffer containing Tris-buffered saline Tween-20 (TBS-T; 10 mM Tris–HCl, pH 7.4, 150 NaCl, 0.05% Tween-20) and 5% nonfat dry milk. The ERBB2 protein was subsequently detected by overnight incubation at 4°C with a monoclonal anti-ERBB2 antibody (sc-7301; 1:250 dilution; from Santa Cruz, Santa Cruz, CA, USA) and then 1-h incubation with a peroxidase-conjugated sheep anti-mouse IgG antibody (1:4,000 dilution), both in TBS-T and 5% bovine serum albumin. Bound secondary antibody was visualized by enhanced chemiluminescense (Thermo Fisher Scientific, Rockford, IL, USA) and autoradiography. Actin expression was used as an internal control (1:10,000 dilution).

Determination of Cell Viability

Cell viability was determined using MTS assay (CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay from Promega, Madison, WI, USA). Briefly, cells were seeded into a 96-well plate at a density of 1×103 cells/well and allowed to adhere overnight. After treatment with lentiviral shRNA, cells were incubated for 3–10 days. Next, 20 μL of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] and phenazine methosulfate (MTS-PMS) solution was added to each well. Plates were incubated at 37°C for 2 h, after which the absorbance at 490 nm was measured.

In Vivo Antitumor Effect in Esophageal Adenocarcinoma Xenograft Model

To study the effect of ERBB2 shRNA on tumor growth of esophageal adenocarcinoma in vivo, the cell line with 100-fold ERBB2 amplification, OE19, was used. OE19 cells were pretransduced with lentiviral shRNA vectors as described above. After puromycin treatment, all remaining cells expressed GFP, indicating 100% transduction efficiency. Once cells were infected with the appropriate lentiviral ERBB2 shRNA vector, cells were maintained and grown as described above.

Female *nu/nu* nude mice (Frederick Cancer Research, Frederick, MD, USA; 6–8 weeks of age) were used to establish Esophageal Adenocarcinoma xenografts. The mice were randomized into four groups of five mice each prior to inoculation. The pretransduced lentiviral shRNA OE19 cells were harvested, resuspended in cold PBS, and kept on ice until injected. Per injection site, 4×10^6 cells were inoculated into both flanks of each animal. Therefore, each treatment group had a total of ten tumor sites. The condition of the mice was monitored daily, and the tumor diameter was measured twice a week with calipers. The tumor volume was calculated using the formula: tumor volume=$(\text{width}^2\times\text{length})/2$. In accordance with institutional approved animal experimental protocol, mice were euthanized when tumors ulcerated or at time of overgrowth of the tumors. All of the animals received humane care based on the guidelines set by the American Veterinary Association. All of the experimental protocols involving live animals were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Subcutaneous tumors rapidly excised at the end of the treatment and evaluation period. Tumors were then snap-frozen with dry ice and stored at −80°C for protein extraction and analysis of ERBB2 by Western blot, as described above.

Statistical Analysis

Statistical analysis of lentiviral shRNA treatment in vitro and in vivo was performed with a two-tailed *t* test. Data are expressed as a mean ±SD of at least three sets of results. Results were considered statistically significant when $P \leq 0.05$.

Results

Transduction efficiency was measured after puromycin selection by the amount of GFP expression and was consistently 100% for each cell line.

ERBB2 shRNA Treatment of Gastrointestinal Adenocarcinoma Decreases Protein Expression

Transduction with lentiviral ERBB2 shRNA decreased protein levels significantly at 72 h. ERBB2 protein levels decreased by 80% with siRNA treatment in OE19, with similar results seen in both MKN4 and Seg-1 (Fig. 2). Results were reproduced with all three ERBB2 shRNA.

ERBB2 shRNA Treatment of Gastrointestinal Adenocarcinoma Decreases Cell Viability

The effect of ERBB2 shRNA on the viability of the upper GI adenocarcinoma cancer cells was examined after 6 h incubation with shRNA and incubation with serum containing media for 3–10 days post-infection. The treatment with ERBB2 shRNA significantly reduced cell viability of the two cell lines with known ERBB2 amplification as shown by cell death of 55% by day 7 in MKN45 cells and up to 80% in OE19 cells on day 7 (Table 3; Fig. 3). The cell line with a normal ERBB2 levels, Seg-1, exhibited no significant reduction in cell viability when compared to nontreated cells and to cells treated with control siRNA (Table 3; Fig. 3).

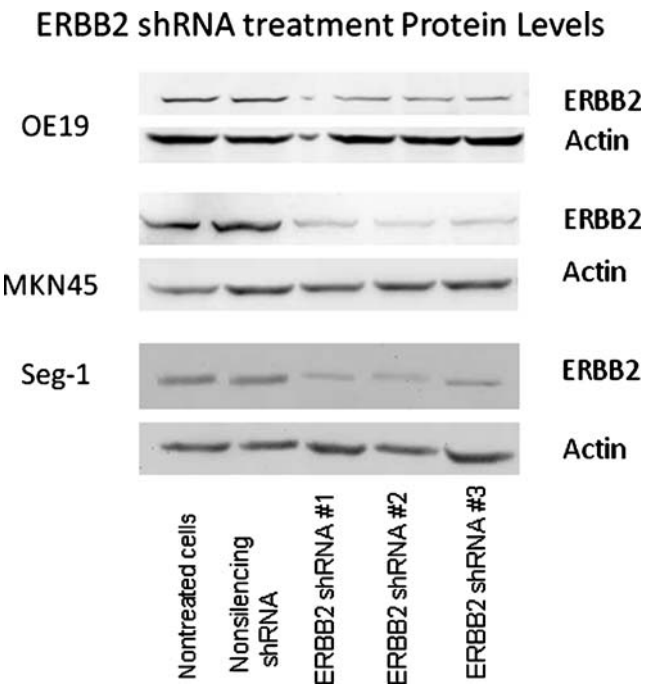

Figure 2 ERBB2 protein levels after treatment with lentiviral ERBB2 shRNA. All cell lines showed protein suppression with shRNA treatment. Membranes were stripped and reprobed for actin to show equal protein loading.

ERBB2 shRNA Treatment of Gastrointestinal Adenocarcinoma In Vitro Decreases Tumor Growth In Vivo

The cell line with the highest amount of ERBB2 amplification, OE19, was chosen for the in vivo model. Tumors were palpable in the control group (mice injected with nonsilencing control shRNA) by day 4. All mice developed tumors. However, given that one animal in the control group developed tumor only in one injection site, the total number of tumors evaluated in the control group was nine. On day 14 after injection, the nonsilencing control shRNA group (relative tumor volume 33.85±14.26; $n=9$) had significantly larger tumors in comparison to the ERBB2-shRNA-treated groups (Table 4; Fig. 4). All ERBB2 shRNA tumors had a significantly slower rate of tumor growth as compared to the control group. By day 25, tumors in the control (nonsilencing shRNA) group were 2.5 times larger than all ERBB2-shRNA-treated tumors. Of the tumors evaluated, ERBB2 protein levels were suppressed in five of the six ERBB2 shRNA tumors as compared to the nonsilencing control shRNA tumors (Fig. 4). This indicates that ERBB2 protein continued to be suppressed in the majority of ERBB2 shRNA tumors. Thus, the in vivo data indicate that ERBB2 suppression does inhibit tumor growth of upper GI adenocarcinomas.

Discussion

In the present study, ERBB2 shRNA treatment significantly decreased protein levels of ERBB2 in all cell lines, indicating that the lentiviral shRNA model is an effective tool to evaluate ERBB2 suppression. Further, ERBB2 shRNA treatment significantly decreased cell viability in our two upper GI adenocarcinoma cell lines with known ERBB2 overexpression (OE19 and MKN45), though it did not affect that of a cell line with a normal level of ERBB2 (Seg-1). These results correlate with results in our earlier work with ERBB2 siRNA in which we found that ERBB2 siRNA significantly decreases cell viability by up to 60% in 3 days.[17] Given that a decrease in cell viability can be the result of an increase in apoptosis, an increase in cell cycle arrest, or a combination of both mechanisms, we also reported that decreased cell viability in this model occurred via an

Table 3 Cell Viability Assay of Upper GI Adenocarcinoma After ERBB2 shRNA Treatment Day 7

Cell line	Nontreated cells	Nonsilencing control shRNA	ERBB2 shRNA #1	ERBB2 shRNA #2	ERBB2 shRNA #3
Seg-1	100 (±11.13)%	94.93 (±9.42)%	103.08 (±2.21)%	100.79 (±5.88)%	93.26 (±5.51)%
OE-19	100 (±8.00)%	91.50 (±5.57)%	29.09 (±9.33)%*	31.10 (±12.57)%*	21.80 (±1.14)%*
MKN45	100 (±8.61)%	88.17 (±9.07)%	52.83 (±7.11)%*	45.19 (±2.95)%*	52.40 (±8.40)%*

Effect of ERBB2 shRNA treatment on upper GI adenocarcinoma cell viability. After 6 h transduction with ERBB2 siRNA, cell viability was significantly decreased in ERBB2 amplified cells lines, OE19 (B.) and MKN45 (C.). In comparison, there were no significant changes in treatment groups in the cell line with a baseline ERBB2, Seg-1 (A.). $n=4$

*$p<0.001$ when compared to nonsilencing control shRNA

apoptotic pathway. Of note, cell cycle did not significantly change in cell lines treated with ERBB2 siRNA.[17] When evaluated in vivo, we find that treating the cell line with the highest level of ERBB2 amplification with shRNA, tumor growth is significantly slower and less than in tumors of cells treated with a nonsilencing control shRNA. These results also correlate with a similar model by Yang et al. in which breast cancer cells were treated with ERBB2 siRNA and found to have decreased cell viability, increased apoptosis, and decreased tumor growth.[20]

Advanced upper gastrointestinal adenocarcinomas are highly aggressive cancers that portray poor long-term prognoses.[3, 21, 22] Curative treatment consists of surgical resection for early-stage disease. Unfortunately, only 20–30% of patients are eligible for surgery secondary to metastases at time of diagnosis, invasion of surrounding structures, or due to multiple comorbidities of the patients.[23,24] Under current standards of clinical care, there are few treatment options for the majority of patients who cannot undergo surgical resection of their tumors. Neoadjuvant and adjuvant chemotherapy provides patients with little improvement in overall survival rates. Therefore, new therapeutic approaches must be investigated to offer a more effective treatment for unresectable disease. Given that upper gastrointestinal adenocarcinoma is easily accessible via endoscopy, direct injection of ERBB2-directed therapies could provide a beneficial treatment strategy without the systemic toxicities.

The best documented studies involving ERBB2-directed therapy are using the breast cancer model.[25,26] As seen in these breast cancer models, ERBB2 amplification is

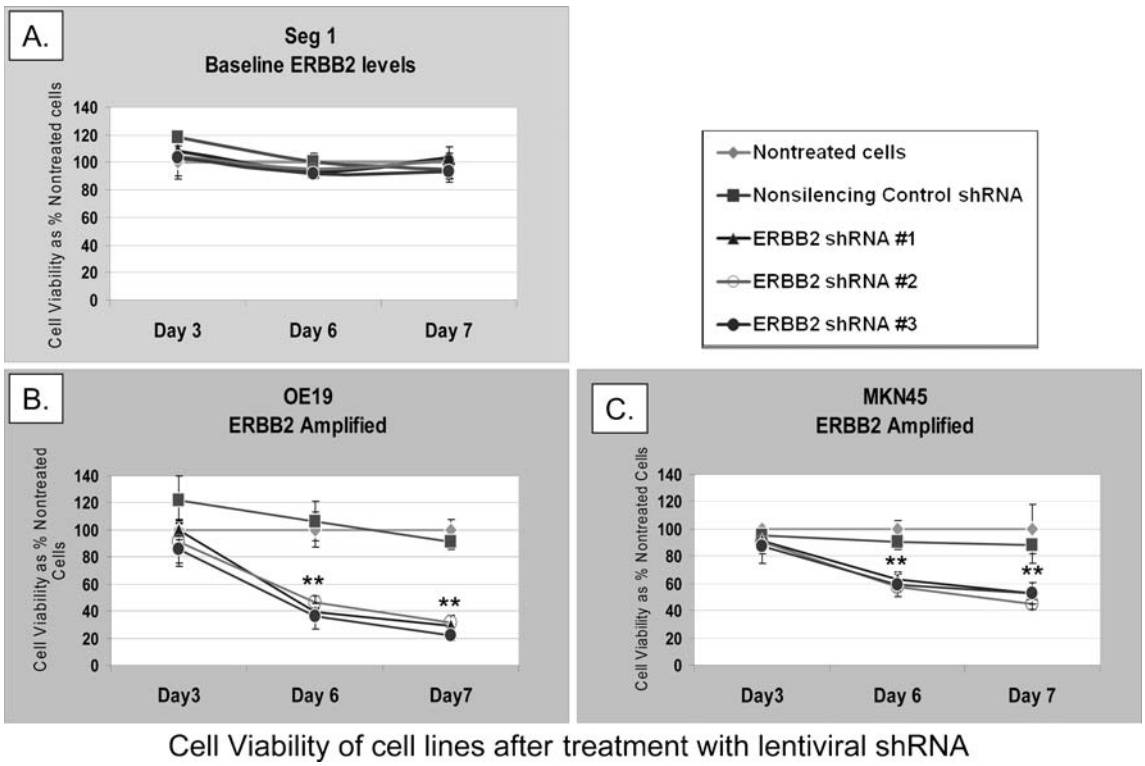

Figure 3 Cell viability assay of upper GI adenocarcinoma after ERBB2 shRNA. Effect of ERBB2 shRNA treatment on upper GI adenocarcinoma cell viability. After 6 h transduction with ERBB2 shRNA, cell viability was significantly reduced in OE19 and MKN45, with no significant change in Seg-1. $n=4$. **$p<0.001$ when compared to nonsilencing control shRNA.

Table 4 Tumor Growth In Vivo of OE19 Pretransduced Cells

Day	Nonsilencing control shRNA (mm^3)	ERBB2 shRNA #1 (mm^3)	ERBB2 shRNA #2 (mm^3)	ERBB2 shRNA #3 (mm^3)
0	0	0	0	0
4	6.22 (±7.91)	0.25 (±0.42)	6.80 (±6.93)	2.75 (±4.40)
7	20 (±11.22)	8.82 (±5.67)*	10.41 (±6.54)*	6.36 (±7.08)*
11	31.66 (±15.650	13.01 (±7.82)*	12.38 (±5.79)*	9.58 (±8.86)*
14	33.85 (±14.26)	15.83 (±6.40)**	16.03 (±9.40)**	21.08 (±17.20)**
18	80.54 (±19.06)	26.44 (±10.40)**	20.19 (±8.33)**	32.75 (±19.73)**
21	155.26 (±42.75)	49.63 (±27.29)**	44.81 (±20.94)**	53.77 (±26.81)**
25	241.83 (±32.00)	98.48 (±62.12)**	72.79 (±51.69)**	89.66 (±59.17)**

Tumor growth was significantly slower and less in the ERBB2-shRNA-treated cells as compared to cells treated with nonsilencing control shRNA. Tumors were measured every 3–4 days with calipers. Tumor volume was calculated as [tumor volume=(width^2 ×length)/2]

*p<0.01 when compared to nonsilencing control shRNA; **p<0.001 when compared to nonsilencing control shRNA

documented in 25–40% of tumors evaluated.[25,26] ERBB2 overepression in breast cancer tumors has been found to be an independent prognostic predictor of overall survival and time to relapse, perhaps due to the increased association with estrogen receptor negativity and early metastasis of these tumors. In this subset of patients, ERBB2-targeted therapies have significantly improved overall survival and disease-free rates of survival.[25,26]

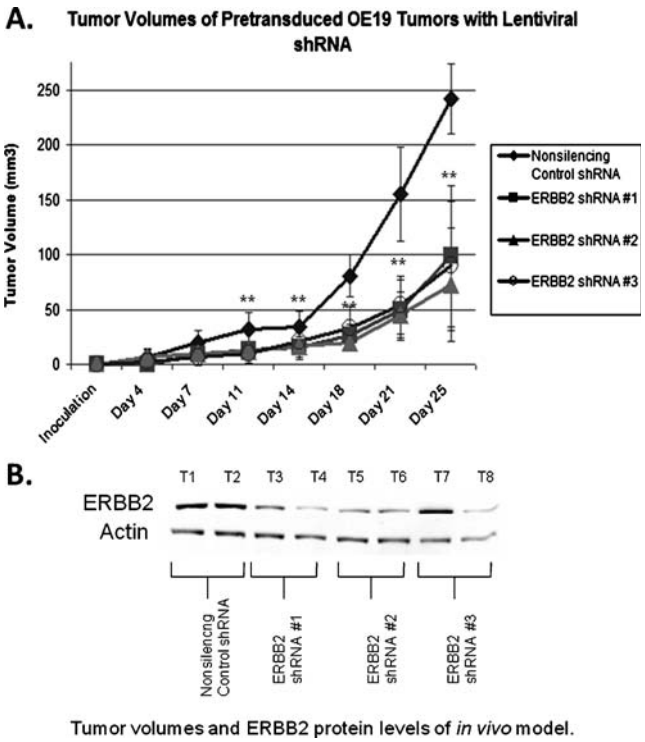

Figure 4 Tumor growth in vivo of OE19 pretransduced cells. **a** Tumor growth was significantly slower and less in the ERBB2-shRNA-treated cells as compared to cells treated with nonsilencing control shRNA. Tumors were measured every 3–4 days with calipers. Tumor volume was calculated as [tumor volume=(width^2×length)/2]. *p<0.01 when compared to nonsilencing control shRNA. **p<0.001 when compared to nonsilencing control shRNA. **b** ERBB2 protein levels were decreased in five of the six ERBB2-shRNA-treated tumors as compared to the nonsilencing control tumors.

The ERBB2 gene is amplified in several cancers other than breast and upper GI adenocarcinomas, including cancers of the prostate,[27] pancreas,[28] lung,[29,30] and ovary.[14] Trastuzumab (Herceptin), a humanized monoclonal antibody which binds to the extracellular domain of the HER2 protein, has been used clinically to treat patients with ERBB2-amplified metastatic breast cancers.[26,31] In previous studies by our lab, this drug also inhibited growth of OE19, an esophageal adenocarcinoma with 100-fold amplification of ERBB2. However, in cell lines with normal ERBB2 levels, cell growth was not inhibited.[5] No clinical trials have yet tested trastuzumab in the subset of patients with known ERBB2-amplified upper GI adenocarcinomas. Unfortunately, there are many obstacles to overcome with the use of herceptin, including the significant side effects (in particular, cardiotoxicity) secondary to systemic administration as well as the cost of treatment (ranging from $20,000 to $80,000/year). Although herceptin therapy may be an effective strategy in a high-risk population, development of treatment with local administration could potentially bypass these obstacles. Therefore, the future direction of our lab is to generate a cost-effective stable transfection model with the potential of local administration.

Lentiviral shRNA vectors provides several advantages over siRNAs. Unlike siRNA, lentiviral vectors are more stable and are not degraded by RNAses. Unlike siRNAs in which expression of suppression is transient, lentiviruses integrate into the host genome as part of their life cycle, and thus their genomic backbone provides a means for life-long expression of ERBB2 shRNA. Further, lentiviruses infect all cells, including actively dividing cells as well as resting and differentiated cells. Given that the lentivirus used is nonreplicative, ERBB2 shRNA is continually expressed, but infectious virus is not generated.

However, there are limitations to this model. For example, endogenous expression of shRNA can cause side effects such as the activation of innate immunity via induction of an interferon response.[32] As with siRNA, lentiviral shRNA vectors also have the potential of off-target gene silencing. However, by evaluating several ERBB2 shRNA in comparison to a nonsilencing control shRNA, the potential of off-target effects is essentially eliminated in this study. Finally, shRNAs have the risk of competition with cellular miRNAs given that they use come of the miRNA machinery for their generation and export.

A stable cost-effective transfection model for ERBB2 suppression of upper GI adenocarcinomas could be an optimal treatment adjunct. Our lab is currently generating replication-incompetent adenoviral ERBB2 shRNA vectors to repeat all experiments. There are several benefits in an adenoviral system, including providing us with a much higher level of initial transient expression. Further, as with the lentiviral model, replication-incompetent adenoviruses are limited in their ability to spread beyond the local injection site, thus generating little systemic effects.

In summary, we have shown that ERBB2 suppression significantly decreases cell viability via an apoptotic pathway[17] and inhibits tumor growth in upper gastrointestinal adenocarcinomas. ERBB2-directed therapy may be of benefit in the subset of patients with gastrointestinal adenocarcinomas exhibiting overamplification of ERBB2. Further studies are clearly warranted to generate improved treatment strategies for patients with unresectable disease.

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J Gastrointest Surg (2009) 13:1762–1772
DOI 10.1007/s11605-009-0912-9

2009 SSAT PLENARY PRESENTATION

Duodenal-Jejunal Exclusion Improves Glucose Tolerance in the Diabetic, Goto-Kakizaki Rat by a GLP-1 Receptor-Mediated Mechanism

Tammy L. Kindel · Stephanie M. Yoder · Randy J. Seeley · David A. D'Alessio · Patrick Tso

Received: 13 March 2009 / Accepted: 15 April 2009 / Published online: 12 May 2009

Abstract
Background Gastric bypass results in the rapid resolution of type 2 diabetes. No causal evidence exists to link specific gut hormone changes with improvements in glucose homeostasis post-operatively. We hypothesized that surgical augmentation of the glucoregulatory factor GLP-1 would improve glucose tolerance in diabetic GK rats. We compared two procedures that increase distal small bowel stimulation, ileal interposition (IT), and duodenal-jejunal exclusion (DJE).
Methods DJE, IT, DJE Sham, or IT Sham were performed in GK rats. Glucose tolerance was tested at 4 and 6 weeks, the latter with and without Exendin-[9-39], a GLP-1 receptor antagonist. Small bowel segments were harvested for GLP-1 protein content 2 weeks after DJE or Sham surgery.
Results Despite similar weight profiles, a significant improvement in the OGTT was noted at 4 weeks after DJE and IT. Plasma GLP-1 levels were significantly elevated after DJE and IT. Intestinal GLP-1 was increased in the mid-jejunum and ileum after DJE. Exendin-[9-39] abolished the improvement in glucose tolerance after DJE.
Conclusions DJE increased GLP-1 secretion and improved glucose tolerance, an effect that was reversed by GLP-1 receptor antagonism. This study provides direct evidence that improvement of glucose tolerance following a gastric bypass-like surgery is mediated by enhanced GLP-1 action.

Keywords Gastric bypass · Glucagon-like peptide-1 · Ileal interposition · Incretin

Grant Support: NIH DK082205 (TK); NIH DK056863, DK059630, and DK076928 (PT), NIH DK57900 (DD).

Manuscript was presented at Digestive Disease Week 2009, SSAT plenary session Chicago, IL; June 1, 2009

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Abbreviations

RYGB	Roux-en-Y gastric bypass
GK	Goto-Kakizaki
DJE	Duodenal-jejunal exclusion
IT	Ileal interposition
OGTT	Oral glucose tolerance test
GLP-1	Glucagon-like peptide-1
LOT	Ligament of Treitz
AUC	Area under the curve

Introduction

Roux-en-Y gastric bypass (RYGB), the most commonly performed bariatric surgery in the United States, results in the rapid improvement of type 2 diabetes for morbidly obese patients.[1] The reported rate of resolution of diabetes after RYGB is approximately 80%.[2–7] Mechanisms beyond weight loss and calorie restriction are quite probable given the rapid and sustained improvement in type 2 diabetes

found in post-RYGB patients. Common explanations for this response are based on changes in gastrointestinal hormone release that occur due to alterations in gastrointestinal anatomy.[8–14] However, there is as yet no direct evidence from animal or human studies that changes in gastrointestinal hormone secretion cause the improvement of glucose tolerance seen after gastric bypass surgery.

The distal jejunum and ileum contain the majority of enteroendocrine L cells, which secrete the incretin hormone, glucagon-like peptide-1 (GLP-1). The incretin hormones GLP-1 and gastric inhibitory polypeptide are responsible for up to 70% of post-prandial insulin secretion.[15,16] GLP-1 is a 30 amino acid peptide secreted by intestinal L cells in response to enteral carbohydrates and fats.[16] GLP-1 also decreases glucagon secretion, suppresses endogenous glucose production, and enhances peripheral glucose uptake.[17–19] In addition, GLP-1 functions as an "ileal brake" by slowing gastric emptying, inhibiting food intake, and prolonging intestinal transit.[20–22] The administration of GLP1R agonists or DPPIV inhibitors, which retard the degradation of endogenous GLP-1, improve HgbA1c levels, and fasting and postprandial glucose concentrations of type 2 diabetic patients.[23–25] Post-prandial plasma GLP-1 levels are almost universally increased after RYGB, as early as 2 days after surgery, and this is likely due to increased delivery of nutrients to distal small bowel L cells.[11,26–28]

Duodenal-jejunal exclusion (DJE) is an experimental, metabolic surgery similar to RYGB, including duodenal and proximal jejunal exclusion to nutrients, a jejunal Roux-en-Y reconstruction, and early nutrient delivery to the distal small bowel. Several authors have shown dramatic, early improvements in glucose homeostasis in rodents following DJE surgery.[9,12,29] Ileal interposition (IT) is another experimental, metabolic, gastrointestinal surgery originally described in rats by Koopmans.[30] In an IT surgery, a distal segment of ileum is moved more proximally in the small bowel resulting in increased secretion of the ileal gut hormones, including GLP-1 and peptide YY.[31–33] Previously, a study comparing DJE and IT surgeries in lean, diabetic Goto-Kakizaki (GK) rats found that both surgeries resulted in the same improvement in glucose homeostasis, leading the authors to postulate that the distal small bowel was the responsible factor.[34] However, rats in this study had a significant weight loss after DJE and IT surgeries compared to sham controls, rendering definitive differentiation between the effect of weight loss and the surgical procedure itself difficult.

We hypothesized that DJE and IT surgeries would improve glucose tolerance in GK rats through early stimulation of the distal small bowel by nutrients resulting in increased secretion of GLP-1. We therefore directly compared the effects of DJE and IT on glucose tolerance and GLP-1 secretion in GK rats without a difference in post-surgical weight profiles. To further test if GLP-1 was the responsible hormone released from the distal small bowel, we acutely administered the GLP1R antagonist, Exendin-[9-39] (Ex-9), during an oral glucose tolerance test (OGTT) performed 6 weeks after surgery in DJE and DJE Sham rats.

Methodology

Animals and Experimental Design At the time of study initiation, 12- to 14-week old, male, GK rats (Taconic, Germantown, NY), or age-matched Wistar rats (Charles River Laboratories, Wilmington, MA) were housed individually. GK rats are an inbred, lean model of type 2 diabetes derived from Wistar rats. Rats were allowed to acclimate to their environment for 1 week prior to the beginning of the study. All animal procedures and protocols were approved by the University of Cincinnati's Internal Animal Care and Use Committee.

The first experiment involved rats in five different study groups (n=9 per group). These groups included: (1) GK DJE, (2) GK DJE Sham, (3) GK IT, (4) GK IT Sham, and (5) Wistar IT Sham. A Wistar IT Sham group allowed for a comparison to non-diabetic animals. Food intake and body weight were followed for 30 days post-operatively. An OGTT was performed pre-operatively and at 2 and 4 weeks post-operatively. An insulin tolerance test (ITT) was performed at 3 weeks post-operatively. At 5 weeks post-operatively, a mixed meal test was performed following the insertion of a jugular cannula for the measurement of systemic incretin hormones.

The second experiment included GK rats in two different study groups, DJE (n=7) and DJE Sham (n=6 Sham). At 2 weeks after surgery, intestinal segments from the duodenum, mid-jejunum, and ileum were harvested for GLP-1 protein content. We chose this time point because we had seen from Experiment #1 an improvement in glucose tolerance in DJE rats compared to Sham rats during an OGTT as early as 2 weeks after surgery.

The third experiment again had two different groups of GK rats, DJE (n=8) and DJE Sham (n=6). Animals were followed for 6 weeks after surgery. After 6 weeks, the animals were acutely challenged with Exendin-9 during the administration of an OGTT to test the involvement of GLP1R signaling in the improvement in glucose homeostasis after duodenal-jejunal exclusion.

Surgical Procedures

(1) Duodenal-jejunal exclusion. Animals were fasted for 18 h pre-operatively. Under isoflurane anesthesia, the peritoneum was entered through a midline incision. Similar to the duodenal exclusion described by Rubino

et al.[29] the most proximal portion of the duodenum and the jejunum 10 cm distal to the Ligament of Treitz (LOT) were divided (Fig. 1). The proximal segment of duodenum was anastamosed to the distal segment of divided jejunum in end-to-end fashion. The distal stump of duodenum was sewn closed. A partial enterotomy was made 15 cm distal to the duodeno-jejunostomy and a jeju-jejunostomy was made with the proximal segment of divided jejunum in end-to-side fashion. The abdomen was irrigated and closed in two layers. Rats had free access to water for the first 24 h post-operatively. Twenty-four hours after surgery, the rats were started on an ad libitum liquid diet (Regular Ensure, Abbott Laboratories, Columbus, OH). After 24 h of a liquid diet (post-operative day 2), the rats were transitioned back to their pre-operative standard chow diet (Harlan Teklad diet 7012).

(2) Ileal interposition. An ileal interposition was performed similar to the procedure previously described by Strader et al.[31] Rats were also fasted for 18 h pre-operatively. The abdomen was entered under isoflurane anesthesia. The cecum was identified and the ileum was divided at 5 and 15 cm proximal to the cecum (Fig. 1). After division of the jejunum 10 cm distal to the LOT, the isolated segment of ileum was interposed into the divided segment of proximal jejunum. The divided segment of proximal and distal ileum were then re-anastamosed in end-to-end fashion. The abdomen was irrigated and closed in two layers. The post-operative care was the same as that described for DJE above.

(3) Sham surgeries. All Sham rats received the same pre- and post-operative care as the DJE and IT rats. For the DJE Sham surgery, a full enterotomy with division of the mesentery and re-anastamosis in end-to-end fashion was made at the proximal duodenum, 10 cm distal to the LOT and 25 cm distal to the LOT. The IT Sham surgery included an enterotomy, mesenteric division, and re-anastamosis at 10 cm distal to the LOT, 5 cm proximal to the cecum, and 15 cm proximal to the cecum.

(4) Jugular cannulation and gastric tube insertion. Animals were fasted overnight. Under isoflurane anesthesia, the right internal jugular vein was identified and isolated. A catheter (0.014 ID/0.033 OD, Braintree Scientific, Braintree, MA) was inserted in the jugular vein and advanced to the level of the right atrium. The distal catheter was tunneled subcutaneously and exteriorized at the posterior aspect of the neck. Under the same anesthetic period, the abdomen was entered through the previous midline incision. The stomach was mobilized and a small enterotomy was made along the anterior aspect of the greater curvature. A catheter (0.04 ID/0.085 OD, VWR International, West Chester, PA) was inserted into the stomach and secured with a purse-string stitch. The gastric catheter was exteriorized through the right flank, and the abdomen was closed in two layers. Animals were kept in restraint cages post-operatively. The mixed meal study for experiment #1 was started after 2 h of anesthetic recovery.

Insulin Tolerance Test An ITT was performed at 3 weeks post-operatively in experiment #1. Insulin, 0.5 U/kg, was administered subcutaneously followed by blood sample collection from the tail vein at 15, 30, 45, and 60 min post-injection. Blood samples were immediately assayed in duplicate for glucose concentration using a handheld glucometer. Due to unacceptable hypoglycemia in Wistar rats, a 0.5 U/kg dose of insulin could not be used and subsequently the Wistar IT group was not used for comparison of insulin sensitivity.

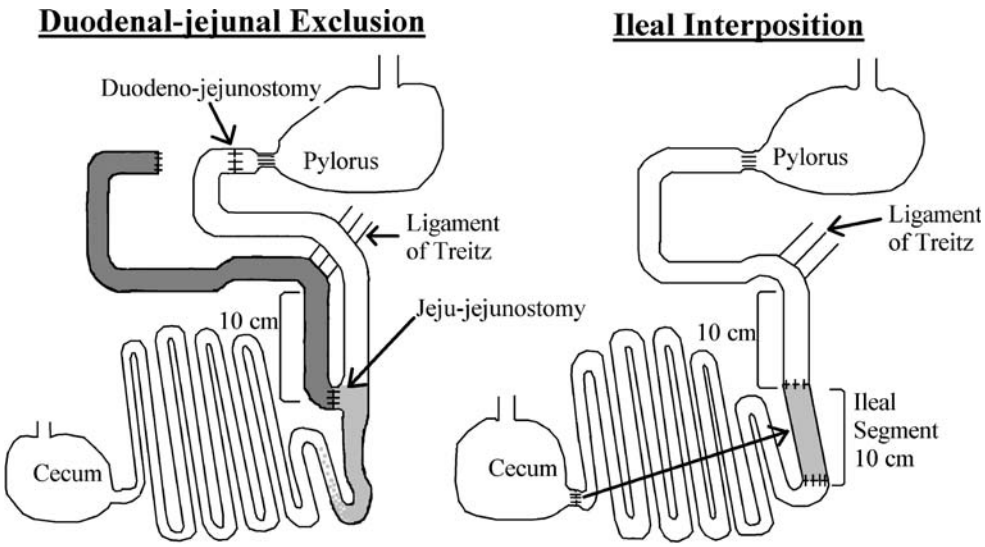

Figure 1 Duodenal-jejunal exclusion (DJE) and ileal interposition (IT) are two experimental, metabolic surgeries used for the investigation and treatment of type 2 diabetes mellitus. As diagramed on the *left*, DJE bypasses the entire duodenum and 10 cm of proximal jejunum (*dark grey color*). IT (*right panel*) leaves anatomically normal nutrient flow to the proximal small bowel. Both surgeries offer early nutrient delivery to the distal small bowel (*light grey color*).

Oral Glucose Tolerance Test For experiment #1, a 2 g/kg D-glucose OGTT was performed pre-operatively and at 2 and 4 weeks post-operatively. Blood samples were collected from the tail vein at 0, 10, 30, 60, and 120 min after the glucose gavage and immediately assayed in duplicate for glucose concentration using a handheld glucometer. Blood samples from the 4 week OGTT were also collected in EDTA coated collecting tubes. Samples were spun at 4,000×*g* for 10 min at 4°C, and the plasma was stored at −20°C until assayed for insulin concentration using a commercially available ELISA kit (Millipore, St Charles, MO). For experiment #3, an OGTT was performed on two separate days at 6 weeks post-operatively with the co-administration of either subcutaneous saline or the GLP-1R antagonist Ex-9 as described below. To better characterize the glucose response, blood samples were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min after the glucose challenge.

Mixed Meal Test We have previously shown that GK rats have a more robust secretion of GLP-1 to a mixed meal bolus over a solitary nutrient, such as glucose.[35] We therefore used a mixed meal test in experiment #1 to maximize GLP-1 secretion and plasma measurement. A mixed meal of Regular Ensure (7.68 ml/kg) was given intragastric to all rats in Experiment #1 at 5 weeks post-operatively. Blood samples were collected from the jugular catheter at 0 and 30 min after the mixed meal bolus. Blood samples were collected into EDTA-coated collecting tubes with the addition of a 1% DPPIV inhibitor (Millipore, St Charles, MO) and spun at 4000×*g* for 10 min at 4°C. Plasma was stored at −20°C until assayed for GLP-1 concentration. GLP-1 samples were assayed using an active GLP-1 ELISA kit (Millipore, St Charles, MO).

Small Bowel GLP-1 Protein Content For experiment #2, 2 cm intestinal segments were isolated from three separate sections of small intestine under anesthesia. These sections included (1) the second segment of the duodenum, (2) 25 cm distal to the LOT (Sham animals) or just distal to the jeju-jejunostomy (DJE animals), and (3) the distal ileum. Tissues were weighed and frozen at −20°C. Frozen segments were homogenized in 2 M glacial acetic acid (5 ml/g tissue weight). Samples were incubated at 95°C for 10 min followed by a 10-min incubation on ice. After centrifugation at 4,000×*g* for 10 min at 4°C, the supernatant was removed, frozen, and lyophilized. Once lyophilized, the segments were resuspended in dH_2O, diluted, and assayed the same day for total protein concentration and GLP-1 concentration using an active GLP-1 ELISA kit (Millipore, St Charles, MO).

GLP1R blockade with Ex-9 In experiment #3, GK rats at 6 weeks after surgery were given either a subcutaneous dose of 200 μl of saline or 25 nM of Ex-9 (Bachem, Torrence, CA). This was followed 10 min later by a 2 g/kg D-glucose OGTT as described above.

Statistical Analysis Area under the curve (AUC) was calculated using the trapezoidal rule. Comparisons between surgical groups were made using a one-way analysis of variance (ANOVA) or a two-way ANOVA for the Exendin-9 study to account for separate treatments and surgeries. Comparisons between surgical groups over time were performed using a two-way repeated-measures ANOVA. A student's *t*-test was used to compare GLP-1 content of the intestinal segments. All values are presented as the mean±standard error. Values were determined as statistically significant if $p<0.05$.

Results

DJE and IT do not Affect Body Weight or Food Intake in GK Rats

In experiment #1, Wistar IT rats weighed significantly more than all of the GK surgical groups for every time point of the study (Fig. 2a). There was no difference in body weight between any of the GK surgical groups for each day measured post-operatively. As shown in Fig. 2b, Wistar IT rats also ate significantly more food per day compared to all GK rat groups (excluding post-operative days 0–2 when rats were fasted or on a liquid diet). GK DJE rats ate the same amount of daily chow as GK DJE Sham rats except for post-operative day 28 (GK DJE 26.9 g±2.20 vs. GK DJE Sham 22.1±2.48, $p<0.05$). Similarly, GK IT rats ate the same amount of daily chow as GK IT Sham rats except for post-operative day 12 (GK IT 22.5±0.98 versus GK IT Sham 26.8 g±2.25, $p<0.05$). In experiment #2 and 3, there was no difference in post-operative body weights for any day measured between GK DJE and GK DJE Sham rats (data not shown).

DJE and IT Significantly Improves Glucose Tolerance by 4 weeks after Surgery in GK Rats Without Changing Plasma Insulin Concentrations

An OGTT was performed at 0, 2, and 4 weeks post-operatively in experiment #1. There was no difference in pre-operative glucose tolerance AUC among the 4 GK groups (Fig. 3), and pre-operative Wistar IT Sham rats had a significantly lower glucose concentration throughout the OGTT compared to the GK groups (data not shown). As shown in Fig. 3, by 4 weeks after surgery, both GK DJE and GK IT rats had a significantly lower late-phase glucose AUC (60–120 min) compared to GK DJE Sham and GK IT Sham

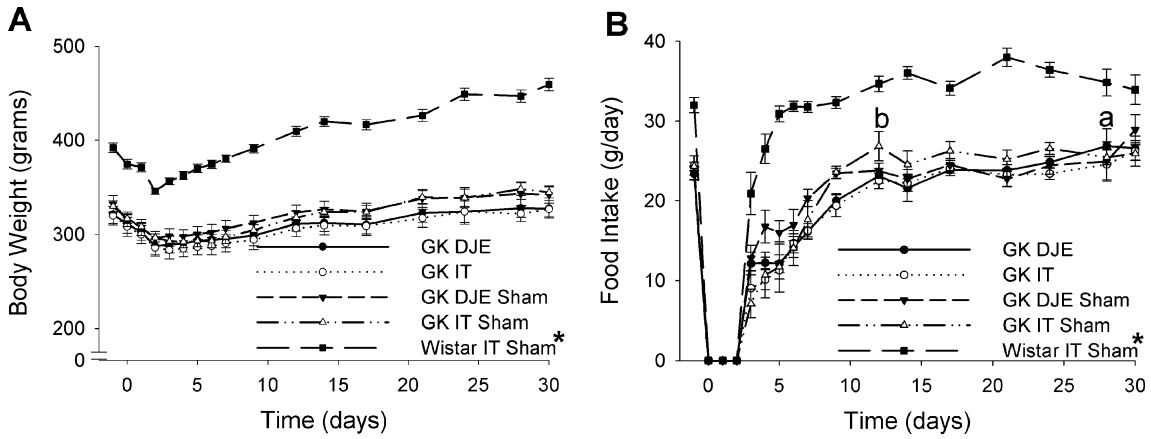

Figure 2 Body weight and food intake after gastrointestinal surgery in GK and Wistar rats. Body weights (**a**) and food intake (**b**) were assessed daily pre-operatively and for 30 days post-operatively. *Statistically different for all days when comparing Wistar IT sham to all GK surgical groups when $p<0.05$. Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$. Data are presented as mean±SE.

rats (GK DJE 13,267 (mg/dl)min±457 vs. GK DJE Sham 15,696 (mg/dl)min±663, $p<0.05$; GK IT 13,327 (mg/dl)min±936 vs. GK IT Sham 15,769 (mg/dl)min±360, $p<0.05$).

At 2 weeks after surgery, both DJE and IT rats had a lower glucose concentration at 120 min compared to their respective sham groups during an OGTT (GK DJE 189.3 mg/dl±8.5 vs. GK DJE Sham 237.1 mg/dl±15.3, $p<0.05$; GK IT 197.4 mg/dl±13.3 vs. GK IT Sham 238.0 (mg/dl)min±17.6, p=NS; data not shown). As reflected in Fig. 4a, the glucose concentration over time during an OGTT at 4 weeks was significantly lower in GK DJE compared to GK DJE Sham rats at 60 min (244 mg/dl±7.7 vs. 282 mg/dl±15.7, respectively, $p<0.05$) and 120 min (198 mg/dl±10.6 vs. 241 mg/dl±15.0, respectively, $p<0.05$). Similar to GK DJE rats, GK IT rats had a significantly lower glucose concentration compared to GK IT sham rats at 120 min (GK IT 192 mg/dl±17.4 vs. GK IT Sham 242 mg/dl±8.2, $p<0.05$). Surprisingly, there was no difference in insulin secretion profiles (Fig. 4b) during the 4 week OGTT between the GK experimental and their respective GK sham group at any time point. GK rats lacked a rapid increase and peak in insulin secretion seen at 30 min in Wistar IT Sham rats (30 min insulin concentration, 2.1 ng/ml±0.16, $p<0.05$ compared to all GK groups).

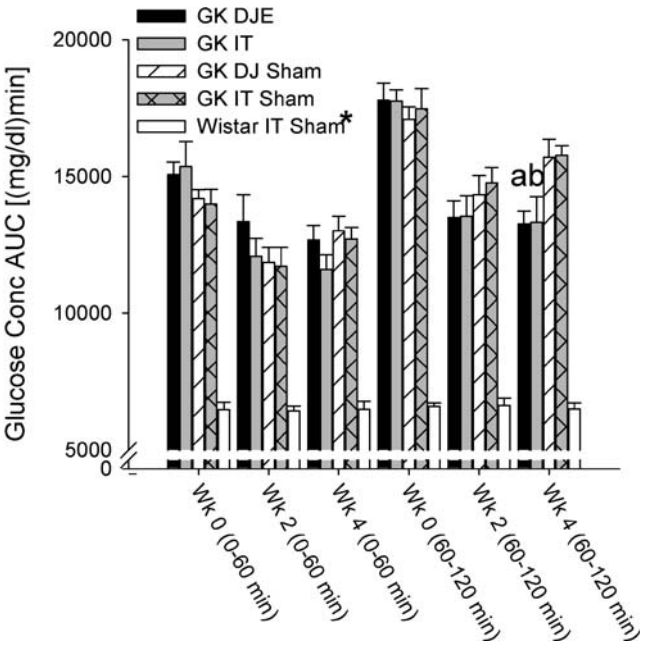

Figure 3 Oral glucose tolerance test AUC was determined by measuring glucose concentrations before and after (10, 30, 60, and 120 min) the administration of an oral glucose load (2 g/kg D-glucose). AUC was determined using the trapezoidal rule. Glucose tolerance tests were performed pre-operatively and at 2 and 4 weeks post-operatively. *Statistically different AUC when comparing Wistar IT sham to all GK surgical groups when $p<0.05$ (intra-week comparisons only). Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$ (intra-week comparisons only). Data are presented as mean±SE.

DJE and IT do not Affect Insulin Sensitivity in GK Rats

Plasma glucose concentrations were determined after the administration of 0.5 U/kg of insulin subcutaneously to all GK surgical groups (Fig. 5) at 3 weeks post-operatively. There was no statistical difference in glucose concentrations at any time point between any of the GK surgical groups, suggesting that neither DJE nor IT surgery acutely affects insulin sensitivity in GK rats after surgery.

DJE and IT Increase Post-prandial Plasma GLP-1 Concentrations

Plasma GLP-1 levels were measured from the jugular vein after administration of a mixed meal tolerance test at

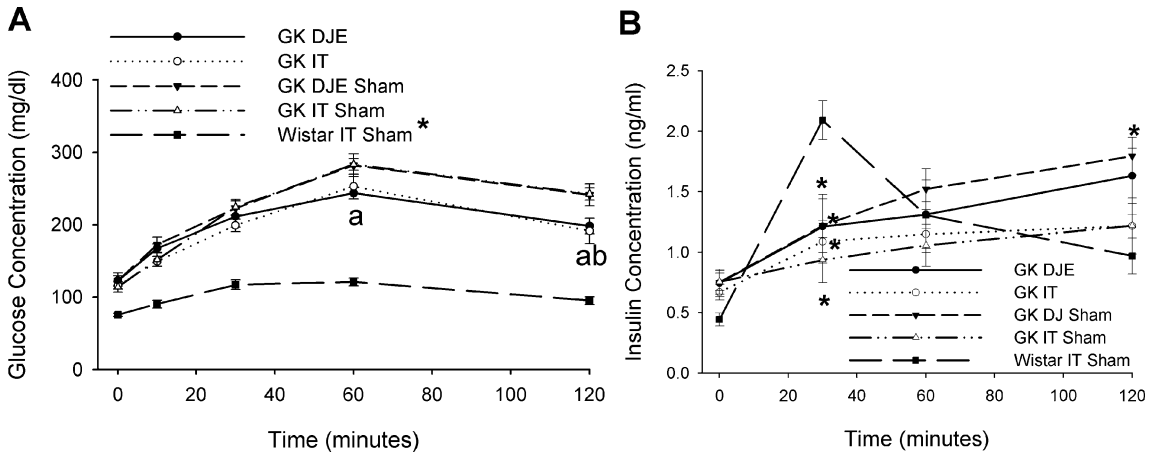

Figure 4 Plasma glucose (**a**) and insulin (**b**) concentrations were measured before and after (10, 30, 60, and 120 min) the administration of an oral glucose tolerance test (2 g/kg D-glucose) at 4 weeks post-operatively. *Statistically different for all time points (**a**) or designated time points and groups (**b**) when comparing Wistar IT sham to all GK surgical groups when $p<0.05$. Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$. Data are presented as mean±SE.

5 weeks after surgery (Fig. 6). There was a statistically significant increase in fasting GLP-1 levels of GK DJE rats, 3.5 pM±0.20, compared to Wistar IT Sham rats, 2.3 pM±0.19, ($p<0.05$). Both GK DJE and IT surgical groups had significantly higher plasma GLP-1 concentrations at 30 min post-prandial compared to their respective GK sham groups (GK DJE 4.5 pM±0.36 versus GK DJE Sham 2.7 pM±0.22, $p<0.05$; GK IT 4.4 pM±0.52 versus GK IT Sham 3.1 pM±0.25, $p<0.05$). Both GK DJE and IT groups also had a significantly higher GLP-1 concentration at 30 min compared to the Wistar IT Sham group, 2.5 pM±0.11.

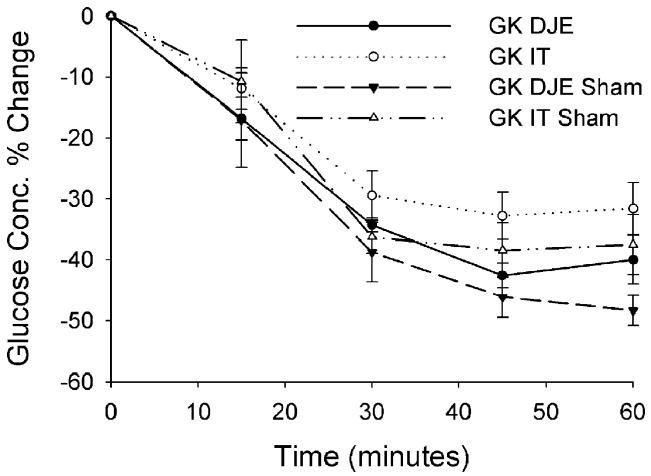

Figure 5 An insulin tolerance test was performed at 3 weeks post-operatively. Plasma glucose concentrations were measured before and after (15, 30, 45, and 60 min) the administration of insulin (Humalin 0.5 U/kg for GK rats). Values are presented for each surgical group as a percent glucose concentration change compared to each groups respective fasting values. There were no differences in glucose concentrations between any of the GK surgical groups at any time point after insulin administration with significance determined as $p<0.05$.

DJE Increases Distal Small Bowel GLP-1 Protein Content

By 2 weeks after surgery, there was a significant increase in the GLP-1 content of the distal small intestine (Fig. 7). As expected, DJE did not significantly alter the duodenal GLP-1 concentration compared to sham animals (0.33×10^{-6}%±0.067 vs. 0.263×10^{-6}%±0.039, respectively, $p=0.43$). DJE compared to Sham surgery significantly increased both mid-jejunal GLP-1 content (2.34×10^{-6}%±0.29 vs. 1.44×10^{-6}%±0.22, respectively, $p=0.03$) and ileal GLP-1 content compared to sham rats (5.19×10^{-6}%±0.42 vs. 2.88×10^{-6}%±0.24 respectively, $p<0.001$).

Ex-9 Administration Ablates the Significant Improvement in Glucose Tolerance at 6 weeks after DJE in GK Rats

Similar to experiment #1 as seen 4 weeks after surgery, there was a statistically significant late-phase improvement in glucose concentrations in DJE rats compared to DJE Sham rats at both 60, 90, and 120 min after an oral glucose load performed at 6 weeks after surgery (Fig. 8a). DJE rats at 60 min had an average glucose concentration of 285.0 mg/dl±5.9 compared to 316.9 mg/dl±4.1 for Sham rats, $p=0.007$. At 120 min, the average glucose concentration for DJE rats was 211.1 mg/dl±10.3 compared to 255.7 mg/dl±13.5 for Sham rats, $p<0.001$. As shown in Fig. 8c, there was a significant improvement in glucose concentration AUC for DJE rats (28,786 (mg/dl)min±571) compared to Sham rats (32,113 (mg/dl)min±593, $p=0.035$). The administration of Exendin (9-39) to DJE and DJE Sham rats resulted in similar glucose concentration curves, with the loss of the statistically significant late-phase improvement for the DJE group over time (Fig. 8b). As shown in Fig. 8c, there was no difference ($p=0.439$) in

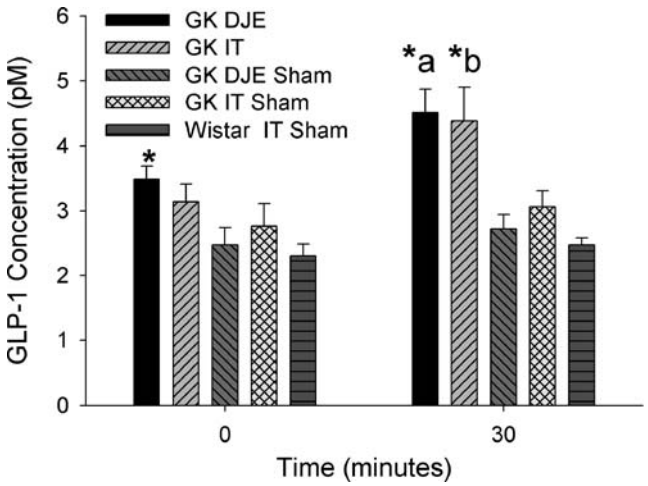

Figure 6 GLP-1 concentrations were measured from jugular plasma samples before and 30 min after a mixed meal bolus of Ensure (7.68 ml/kg) via an intragastric catheter. *Statistically different when compared to Wistar IT Sham rats when $p<0.05$. Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$. Data are presented as mean±SE.

OGTT AUC observed between the two groups after the administration of Ex-9.

Discussion

In this study, we found that independent of weight loss, both DJE and IT in GK rats result in a statistically significant improvement in glucose tolerance by 4 weeks

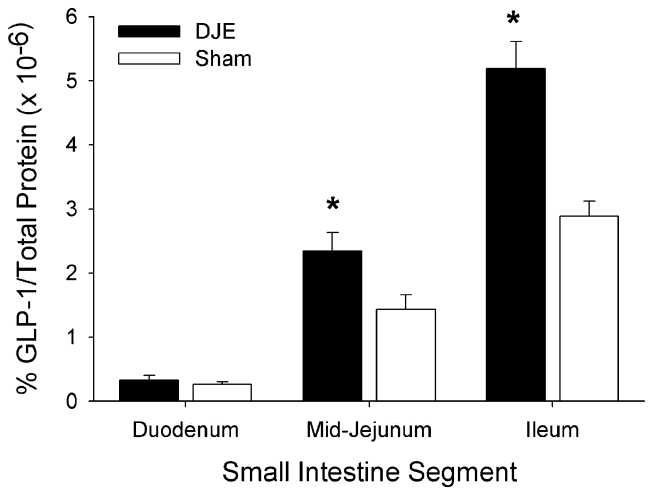

Figure 7 Percentage intestinal GLP-1 protein content was determined at 2 weeks after DJE ($n=7$) or DJE Sham ($n=6$) surgery in GK rats. Intestinal segments were taken from the second segment of the duodenum, mid-jejunum (distal to the jeju-jejunostomy in DJE rats or 25 cm distal to the ligament of Treitz in Sham rats), and distal ileum. *Statistically different for the tested segment of small bowel between DJE and Sham rats when $p<0.05$. Data are presented as mean±SE.

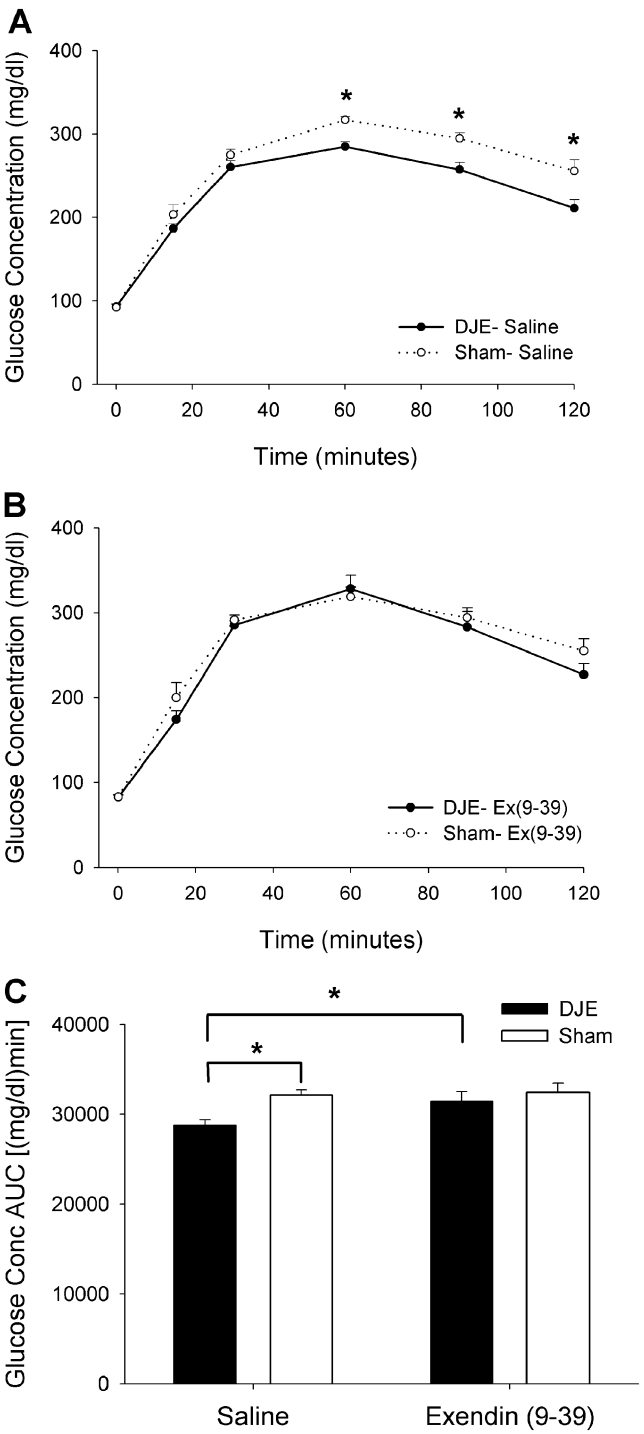

Figure 8 An OGTT was performed in male, GK rats 6 weeks after DJE ($n=8$) or DJE Sham ($n=6$) surgery. Plasma glucose concentrations were measured at 0, 15, 30, 60, 90, and 120 min after a 2 g/kg D-glucose oral gavage with the co-administration of 200 µl of saline (**a**) or 200 µl of 25 nM of the GLP1R antagonist Ex-9 (**b**) subcutaneously. (**c**) depicts glucose concentration AUC for the 6-week OGTT. *Statistically different for the designated time points (**a** and **b**) or between groups (**c**) when $p<0.05$. Data are presented as mean±SE.

after surgery. Both metabolic surgeries did not acutely change plasma insulin concentrations or insulin sensitivity. Supporting a mechanism mediated by enhanced nutrient delivery to the distal small bowel, the common feature of DJE and IT, we found a similar magnitude of elevation of post-prandial plasma GLP-1. Furthermore, intestinal GLP-1 protein levels were significantly increased by 2 weeks after surgery in not only the ileum (the major focus of L cells in the non-operated gut) but also the mid-jejunum at the new post-surgical site of primary nutrient absorption. The administration of the GLP1R antagonist, Ex-9, ablated the significant improvement in glucose tolerance seen after DJE surgery at 6 weeks. Thus, the improvement in glucose tolerance noted after DJE in this model is mediated by GLP1R signaling.

RYGB results in the early and sustained improvement in glucose homeostasis for the majority of morbidly obese, type 2 diabetic patients. Multiple mechanisms stemming from the rearrangement of small bowel anatomy may be involved beyond weight loss and calorie restriction. For this reason, we compared two different experimental, metabolic surgeries, DJE and IT, to determine if one surgery offered an advantage over the other regarding glucose tolerance in a lean, rodent model of diabetes. Both surgeries increase distal small bowel exposure to nutrients but only DJE, like RYGB, bypasses the duodenum and proximal jejunum. It has been proposed that exclusion of the duodenum from nutrient stimulation is a predominant mechanism responsible for the improvement in glucose homeostasis after RYGB.[8,9] Results of this study indicate that increased GLP-1 secretion and GLP1R stimulation, and not duodenal exclusion, is the predominate mechanism involved in the early improvement in glucose tolerance after DJE surgery in GK rats.

The GLP1R is a specific G-protein coupled receptor located on the lung, brain, kidney, pancreatic islets and gastrointestinal tract.[36–38] We were unable to specifically identify which action of GLP1R signaling was responsible for the improvement in glucose tolerance. Although we did not detect an absolute increase in plasma insulin levels following DJE, this does not exclude the possibility that the surgery enhances insulin secretion via increased GLP1R stimulation. Because both DJE and IT result in reduced glucose concentrations without a change in insulin sensitivity, it is possible that a relatively greater secretion of insulin for the given glucose concentration accounts for some of the effect of surgery. This relative increase in insulin secretion could be the dominate GLP1R mechanism in this model, as some clinical studies have found an increase in post-prandial insulin secretion after RYGB.[39,40]

Ayala et al. have shown that GLP1R −/− mice have an impaired suppression of hepatic glucose production independent of insulin secretion.[19] Activation of GLP1R signaling suppresses glucagon secretion and could possibly mediate the suppression of hepatic glucose production. Le Roux et al. administered octreotide as a non-specific blocker of GLP-1 and PYY to post-RYGB and gastric banding patients and found an increase in meal size and decrease in satiety unique to the RYGB group; however, the effect on glucose tolerance, insulin, and glucagon secretion was not assessed.[28] There is a lack of consensus regarding the changes in glucagon secretion after RYGB, including a decrease, no change, or increase in glucagon secretion.[41–44] Because we did not measure plasma, or more specifically, portal vein glucagon concentrations, we cannot exclude the possibility that the effects of DJE surgery are mediated by the suppression of glucagon secretion via a GLP1R mechanism.

The administration of Ex-9 in vivo completely abolishes the stimulatory effect of endogenous GLP-1 on insulin secretion, with no effect on co-stimulators of insulin such as gastric inhibitory polypeptide and vasoactive intestinal polypeptide.[45,46] While Ex-9 is specific for the GLP1R, there are cross-reactive hormones of the GLP1R besides GLP-1, including the intestinal proglucagon alternative splice product oxyntomodulin. Oxyntomodulin as yet does not have an identified separate receptor and has been found to mediate glucoregulatory actions including stimulation of insulin secretion through a functional GLP1R.[47,48] We did not measure oxyntomodulin concentrations in this study and are unaware of any published reports regarding the effect of RYGB on oxyntomodulin secretion. However, oxyntomodulin acts only partially via the GLP1R. Because we found a full reversal of the improvement in glucose tolerance after DJE with use of the GLP1R antagonist, we expect that the hormone involved is mediated only by GLP1R signaling, making GLP-1 the likely candidate. The increase in GLP1R signaling could be from a physiologically relevant increase in GLP-1 or due to increased sensitivity and enhanced incretin effect of GLP-1 on the GLP1R, regardless of the quantitative changes in GLP-1 secretion.

We found no change in insulin sensitivity assessed by a subcutaneous ITT. While the euglycemic-hyperinsulinemic clamp offers greater precision compared to an ITT in assessing peripheral insulin sensitivity, we were not surprised to find that insulin sensitivity was not acutely affected by DJE or IT. Some studies have suggested unique changes in insulin sensitivity after RYGB.[49] However, when RYGB patients are compared to patients with similar degrees of weight loss (gastric banding patients), the improvement in insulin sensitivity correlates to the magnitude of post-surgical weight loss,[50,51] and thus would not be expected in our surgical model.

Our study does not find the robust improvement in glucose tolerance as previously reported by some investigators after DJE in GK rats.[12,29,45] Differences in surgical technique and post-operative care are possible reasons for

this difference. Also, there are differences in phenotypic severity between different colonies of GK rats.[52] The GK rat is a lean, inbred model of type 2 diabetes derived from Wistar rats. These rats have reduced β-cell mass, decreased pancreatic insulin reserves, and a defective secretion of insulin to a glucose stimulus.[53,54] With age, GK rat islets have a decreased number of β-cells, reduced islet insulin content, and exhibit abnormal islet morphology.[55,56] It is possible that in a rat strain dominated by pancreatic insulin insufficiency, there is a point of "no return" in reversing pancreatic failure and a sub-maximal amount of recovery that can be obtained with DJE surgery. Recent data has shown that the rate of resolution of diabetes after RYGB is highest for patients who have had a short duration of disease (less than 4 or 5 years) or mild disease (diet-controlled).[4,57] The lack of a consentient improvement in glucose tolerance after DJE surgery points to the need for further research to determine what factors (duration of diabetes, type of diabetes, insulin requirements, beta cell reserve, etc.) enable or prevent a maximum surgical response.

While this study did not produce dramatic improvements in glucose tolerance by 4 to 6 weeks after DJE or IT, our results parallel the findings of recently published results with IT surgery. IT performed in streptozocin-induced diabetic rats had a similarly significant although small improvement in glucose homeostasis by 4 weeks after surgery without a change in insulin secretion.[32] By 11 weeks, IT surgery in these rats resulted in a more dramatic improvement in glucose concentrations after a glucose tolerance test. We suspect that with a longer observation period, improvements in glucose tolerance would have been more pronounced for both surgeries due to β-cell recovery as seen by other investigators after IT or with exogenous GLP-1 treatment.[58,59]

Conclusion

To our knowledge, this study offers the first direct evidence documenting a causal relationship between a change in GLP-1 signaling induced by bypass surgery and the subsequent improvement in post-operative glucose tolerance. It is possible that in other animal models, specifically in a diet-induced obesity model, DJE may cause other positive hormonal changes beyond GLP1R signaling that affect glucose tolerance. Clinically, it is yet unknown if the combination of effects that bypass surgery can achieve induced by weight loss, calorie restriction, and augmented hormone signaling are superior to pharmacologic intervention in a population of type 2 diabetic patients with a BMI< 35 (especially when considering cost effectiveness, morbidity, and mortality). However, evidence, as shown in this study, that RYGB-like surgeries, independent of weight loss and calorie restriction, can benefit type 2 diabetes mellitus in animal models by enhancing incretin signaling, supports the further careful and cautious investigation of RYGB for the use as a treatment for type 2 diabetic patients without morbid obesity.

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J Gastrointest Surg (2009) 13:1773–1780
DOI 10.1007/s11605-009-0958-8

2009 SSAT QUICK SHOT PRESENTATION

An Analysis of Trends and Growth Factor Receptor Expression of GI Carcinoid Tumors

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Received: 29 May 2009 / Accepted: 12 June 2009 / Published online: 7 July 2009

Abstract
Introduction The purpose of our study was twofold: (1) to determine the incidence, patient and tumor characteristics, and outcome of patients with gastrointestinal carcinoid tumors using the Surveillance, Epidemiology and End Results (SEER) database, and (2) to delineate the expression pattern of growth factor receptors (GFRs) in carcinoid tumors.
Materials and methods The SEER database search provided information on patients diagnosed with carcinoid tumors from 1990 to 2002. Carcinoid tumor sections (n=46) were stained for the GFRs: epidermal growth factor receptor, insulin-like growth factor receptor (IGFR), vascular endothelial growth factor receptor (VEGFR), and HER-2/neu.
Results Over the 12-year analysis period, 18,180 patients were identified with carcinoid tumors of the foregut, midgut, and hindgut; the incidence of carcinoid tumors increased ~2-fold during this time period. Of the patients with carcinoid tumors, there was a trend of increased expression of VEGFR and IGFR, particularly in the foregut and midgut carcinoids. Analysis of the SEER database confirms that the incidence of carcinoid tumors is increasing with an approximate doubling in the number of carcinoid cases from 1990 to 2002. Furthermore, an increase in VEGFR and IGFR expression suggests that GFR inhibitors may be effective adjuvant therapy for carcinoid cancer.

Keywords VEGFR · IGFR · Carcinoid tumors

Introduction

Carcinoid tumors are uncommon, slow-growing neuroendocrine neoplasms arising from the enterochromaffin cells of the gut.[1] Although uncommon, carcinoids are increasing in incidence at a rate greater than other cancers.[2] Due to the indolent nature of carcinoid cancers, these tumors are usually not detected until after the development of metastases or intestinal fibrosis.[3] Patients with metastatic disease may present with carcinoid syndrome, a set of symptoms including flushing, diarrhea, bronchospasm, and hypotension, or with manifestations of peritumoral and distant fibrosis.[4] Common amine and peptide products secreted from carcinoids include serotonin, chromogranin A, and neurotensin.[5]

Currently, surgical resection is the only treatment with the possibility of achieving a cure and remains the mainstay of treatment for all patients with primary carcinoid tumors.[6] Although there are ongoing research and clinical trials aimed at increasing survival of patients with metastatic disease, there has been no significant increase in survival in

Kanika A. Bowen presented at the Society for Surgery of the Alimentary Tract on June 2, 2009, Chicago, IL.

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the past decade due, in part, to the lack of response to standard chemotherapeutic treatments.[7] Somatostatin analogues have commonly been used for symptomatic relief of carcinoid syndrome after the development of hepatic metastasis.[8] We have previously shown that administration of the somatostatin analogue octreotide significantly decreases hepatic metastasis using an in vivo mouse model, suggesting an antiproliferative effect of octreotide.[9] Current combination chemotherapeutic regimens with streptozocin, 5-fluorouracil, and doxorubicin are commonly used in the treatment algorithms of highly proliferating carcinoid tumors.[10]

The lack of in vitro and in vivo model systems for carcinoid tumors has limited our understanding of the progression of this disease. We are fortunate to have established the novel carcinoid cell line BON, derived from a pancreatic carcinoid metastasis.[11] We have utilized the BON cell line to delineate signaling pathways regulating carcinoid cell growth and secretion.[9,11] BON cells express growth factor receptors (GFRs), including epidermal growth factor receptor (EGFR) and HER-2/neu, that may contribute to the development and sequelae of carcinoid tumors.[12,13] Recently, using the BON cell line, we developed a novel in vivo model of carcinoid syndrome which recapitulates many of the clinical sequelae noted in humans and determined that treatment with the vascular endothelial growth factor receptor (VEGF) inhibitor bevacizumab significantly inhibited tumor growth.[14]

Alterations in GFR expression have been linked to an increased risk of neoplastic transformation.[15] Overexpression of HER-2/neu occurs in several cancers such as ovarian, stomach, breast, and aggressive forms of uterine cancer.[16,17] With ligand binding, EGFR stimulates intrinsic intracellular protein-tyrosine kinase activity which results in autophosphorylation of tyrosine residues. Downstream signaling proteins then initiate several signal transduction cascades, including the MAPK, phosphoinositide 3-kinase (PI3K), and JNK pathways, which are involved in important functions such as DNA synthesis and cell proliferation.[18,19] Insulin-like growth factor receptor (IGFR) is another receptor-tyrosine kinase that plays a critical role in cell survival and proliferation.[20] IGFR binding to its ligand activates the same pathways as EGFR to promote cell proliferation and suppress apoptosis.[21,22]

In the current study, we analyzed carcinoid tumor incidence using The Surveillance, Epidemiology and End Results (SEER) registry database of the National Cancer Institute and compared this to our institutional incidence. Furthermore, we analyzed the expression of various GFRs known to be involved in cancer development, including VEGFR, EGFR, IGFR, and HER-2/neu in a set of carcinoid tumors from our institutional tumor bank as well as from commercial tissue arrays.

Materials and Methods

Materials

Rabbit monoclonal anti-chromogranin, anti-synaptophysin, anti-VEGFR, anti-IGFR, anti-EGFR, anti-platelet-derived growth factor receptor (PDGFR), and anti-HER-2/neu antibodies were purchased from Cell Signaling (Danvers, MA, USA). Carcinoid tissue arrays were purchased from Biomax (Rockville, MD, USA). Immunostaining was performed using a DAKO EnVision Kit (Carpinteria, CA, USA).

Study Design

The histopathology and clinical course of patients undergoing carcinoid resection from 1986 to 2006 at The University of Texas Medical Branch (UTMB) were retrospectively analyzed. UTMB Institutional Review Board approval was obtained for the collection of patient data, tissue acquisition, and subsequent use. A comprehensive search of the medical records was first performed using ICD-9 Common Procedure Terminology codes for "carcinoid," "malignant carcinoid," "carcinoid syndrome," and "neuroendocrine tumor." Histopathology reports were then obtained for all patients during the specified time period. Patients with a pathologically confirmed diagnosis of carcinoid (typical or atypical) were then entered into the UTMB Carcinoid Database. Demographic data (e.g., age, gender, race), tumor–node–metastases (TNM) stage, lymph node status, presence of distant metastasis, and presence or absence of synchronous lesions was collected for all patients. For tissue analysis, paraffin-embedded blocks of resected carcinoid tissue were obtained from 20 UTMB patients with carcinoid tumors. Blocks were sectioned for immunohistochemistry.

SEER Database

The National Cancer Institute's SEER-9 program was used to collect national data on carcinoid incidence, patient age and gender distribution, tumor histology, TNM stage, lymph node status, and tumor size for the years 1990–2002. SEER data are compiled from population-based cancer data from nine cancer registries in geographically distinct areas of the USA (five states: Connecticut, Hawaii, Iowa, New Mexico, and Utah; four cities: Atlanta, Detroit, San Francisco, and Seattle).[23] Population data for additional statistical analysis was obtained from the 2000 US Census Bureau (available at: www.census.gov).

Immunohistochemistry

Paraffin-embedded carcinoid blocks were sectioned (5 μm) and deparaffinized in xylene and rehydrated in a descending

Table 1 Comparison of University of Texas Medical Branch Patient Demographics with Surveillance, Epidemiology and End Results Database

	SEER 1990–2002 (*N*=18,180)	UTMB 1986–2006 (*N*=44)	*p* Value
Age; median	61.9±14.4	59.3±11.7	0.09
Gender			0.54
Male	47.7%	52.3%	
Female	52.3%	47.7%	
Race			0.14
White	74.3%	75.0%	
Black	12.6%	13.6%	
Hispanic	5.9%	11.4%	
Other	7.6%	0.0%	
Site of carcinoid			<0.0001
Small intestine	20.8%	36.4%	
Colorectal/anal	28.0%	9.1%	
Appendix	3.3%	2.3%	
Stomach	6.3%	6.8%	
Pancreas	4.4%	6.8%	
Liver/gall bladder/ bile ducts/ampulla	1.7%	0.0%	
Other digestive	0.9%	0.0%	
Lung/respiratory	34.0%	31.8%	
Esophagus	0.2%	0.0%	
Retroperitoneal	0.4%	0.0%	
Unknown	0.0%	6.8%	
Nodal Status			<0.0001
Negative	49.4%	20.5%	
Positive	15.8%	65.9%	
Unknown	34.8%	13.6%	
Stage			<0.0001
Localized	48.0%	20.5%	
Regional	21.0%	20.5%	
Distant	23.4%	54.5%	
Unknown	7.6%	4.5%	
Survival (Overall)			0.52
1 year	77.4%	80.9%	
3 year	65.3%	77.4%	
5 years	57.9%	61.2%	
Median	93 months	138 months	

ethanol series. Immunostaining was performed using a DAKO EnVision Kit (Dako Corp) as we have described previously.[24] Briefly, sections were incubated overnight at 4°C with monoclonal antibodies diluted 1:100 in 0.05 M Tris–HCL with 1% bovine serum albumin against anti-chromogranin A, anti-synaptophysin, anti-EGFR, anti-VEGFR, anti-IGFR, and anti-HER-2/neu antibodies (Cell Signaling). After three washes with Tris-buffered saline Tween-20 (TBST), sections were incubated for 30 min with secondary antibody labeled with peroxidase, then washed three times with TBST. Lastly, peroxidase substrate diaminobenzidinetetrahydrochloride was added for staining. All sections were counterstained with hematoxylin and observed by light microscopy. All specimens were reviewed by a pathologist in a blinded fashion.

Statistical Analysis

Differences in GFR expression were assessed using Pearson chi-square test. Comparison of UTMB and SEER data was tested using the Pearson chi-square test for GFRs, gender, race, and tumor stage. The median test was used for age, presence of positive lymph nodes, and synchronous lesions. Association between carcinoid location and data set (UTMB or SEER), controlling for gender or race, was assessed using the Cochran–Mantel–Haenszel test. Associations between carcinoid tumors and GFRs were assessed using the Pearson chi-square test for gender, tumor stage, presence of positive lymph nodes, presence of synchronous lesions, and using the median test for age and tumor size. All statistical computations were carried out using SAS statistical software (release 9.1; SAS Institute).

Results

UTMB Patient Demographics

Between January 1986 and December 2006, 44 patients had resections performed for gastrointestinal carcinoid tumors at UTMB. There was a total of 21 women (47.7%) and 23 men (52.3%), with a mean age of 59 years (range 34–77 years). The average age was 59.3 years with a standard deviation of 11.7 years. Caucasians represented 75% of all

Table 2 Comparison of Gender and Frequency of Carcinoid Tumor Location for University of Texas Medical Branch and Tissue Array Patients

	N (%)			*N*
	Foregut	Midgut	Hindgut	Total
Female				
UTMB	5 (42%)	5 (42%)	2 (16%)	12
Array	7 (78%)	1 (11%)	1 (11%)	9
Male				
UTMB	3 (38%)	4 (50%)	1 (12%)	8
Array	16 (94%)	1 (6%)	0 (0%)	17

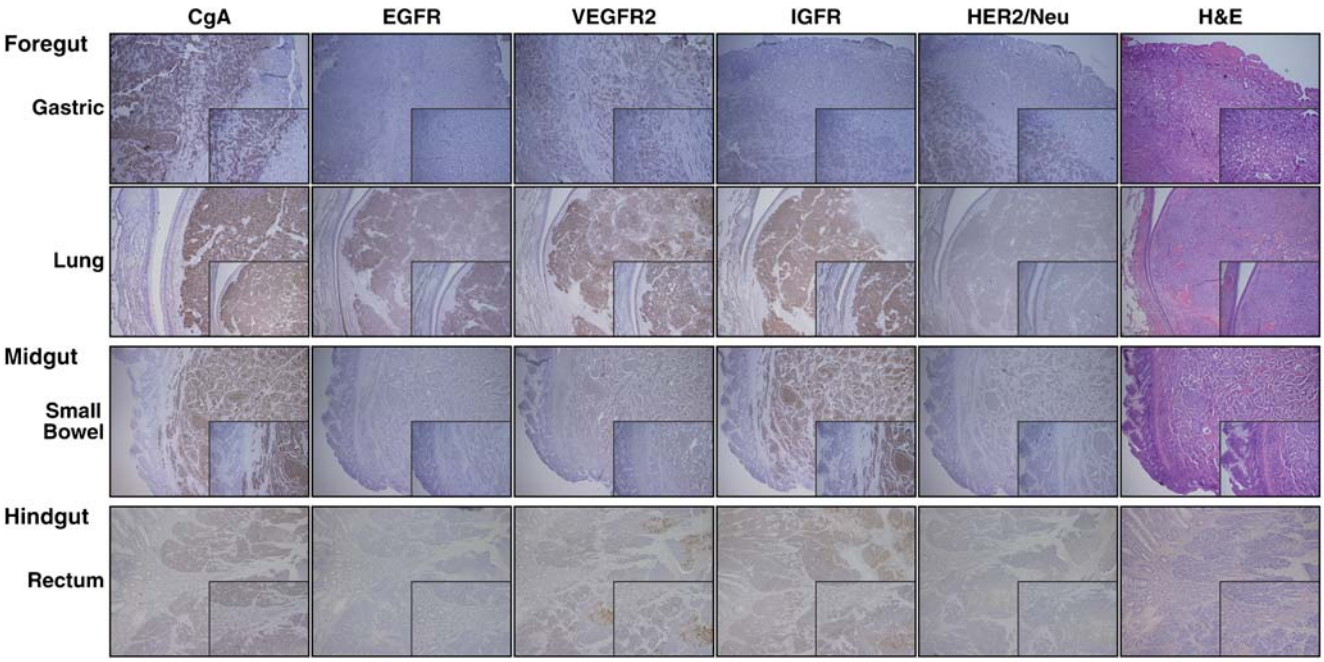

Figure 1 Expression of growth factor receptors in carcinoid cancer tissues. Immunohistochemical analysis of chromogranin A (*CgA*), epidermal growth factor receptor (*EGFR*), vascular endothelial growth factor receptor (*VEGFR*), insulin-like growth factor receptor (*IGFR*), and HER-2/neu in representative carcinoid cancer tissues derived from the foregut, midgut, and hindgut (×4 magnification with ×10 inlay).

patients, followed by African Americans (13.6%) and Hispanics (11.4%). The most common site of occurrence was the foregut (45.4%), followed by midgut (39.2%) and hindgut (9.1%). When carcinoid tumors were further divided by organ system, the small intestine was the most common site (36.4%), followed by the respiratory tract (31.8%), colorectal/anal (9.1%), stomach (6.8%), pancreas (6.8%), and appendix (2.3%). Of patients with disseminated disease at the time of presentation, 6.8% had an unknown primary tumor. Lymph node metastases were present in 29 patients (65.9%), nine patients (20.5%) had node-negative disease, and six patients (13.6%) had unknown nodal status. The majority of patients (54.5%) presented to UTMB with disseminated distant disease, and 25.5% of patients had localized or regional disease. Tumor stage was unknown in 4.5% of patients.

Comparison of UTMB Patients with the SEER Database

Demographics for patients with carcinoid tumors diagnosed at UTMB and those recorded in the SEER database are presented in Table 1. A search of the SEER database identified 18,180 patients with carcinoid tumors during the study period. The incidence of carcinoid tumors in the foregut, midgut, and hindgut increased ~2-fold during this time period. The two populations had a similar ratio of male and female patients. Median age was slightly higher in SEER patients (62 versus 59; $p=0.09$). Racial distribution was also noted to be similar between the two populations. UTMB had a slightly higher percentage of African-American and Hispanic patients compared to the SEER database. The majority of SEER patients presented with localized disease (48%); 23% of patients presented with distant disease. In contrast, ~54.5% of UTMB patients presented with distant disease.

Comparison of GFR Expression in Carcinoid Tumors

Specific immunohistochemistry staining for carcinoid tissues using chromogranin A and synaptophysin verified that the tissue sections were carcinoid tumors. Associations between outcome measures, tumor location (foregut, midgut,

Table 3 University of Texas Medical Branch vs. Tissue Array Growth Factor Receptor Staining

	Number (%)				
	CgA	EGFR	VEGFR	IGFR	HER-2/neu
UTMB	20 (100%)	3 (15%)	12 (60%)	18 (90%)	3 (15%)
Array	21 (84%)	3 (13%)	10 (42%)	3 (12%)	7 (29%)

Table 4 Comparison of Gender and Frequency of Carcinoid Tumor Growth Factor Receptor Staining

Gender	Number (%)				
	CgA	EGFR	VEGFR	IGFR	HER-2/neu
Female	20 (95%)	5 (25%)	12 (60%)	11 (52%)	6 (30%)
Male	21 (88%)	1 (4%)	10 (42%)	10 (42%)	7 (17%)

or hindgut), patient age, and sex were assessed using the multiple logistic regression model. Hindgut carcinoids were excluded from the multiple logistic regression model due to very small sample size (n=4).

Of 46 samples stained for immunohistochemistry, all stained positive for synaptophysin. Association between carcinoid location and gender are summarized for UTMB or commercial arrays in Table 2. A significant association was not observed between gender and carcinoid location adjusting for UTMB versus tissue array (p=0.64). This indicates that the ratio of men to women from each carcinoid location was similar. However, a significant association was observed between UTMB or tissue array and location adjusting for gender (p=0.005). This suggests that the number of samples from each carcinoid location were significantly different between UTMB and array tissues when adjusted for gender. The majority of tissues from tissue arrays were from the foregut, whereas tissues from UTMB patients were similarly distributed between foregut and midgut tumors.

Mean patient age for each carcinoid location with number of samples and standard deviation was analyzed. There were no significant differences in patient age compared with carcinoid location (p=0.38); however, there was a statistically significant age difference between foregut and midgut carcinoid tumors among men at UTMB. The mean ages were 48 and 68 with a standard deviation of 12 and 9 years for foregut and midgut tumors, respectively (p<0.05).

Representative tissue staining patterns from selected UTMB patients are shown in Fig. 1. Similar sections of carcinoid tumors with areas of normal tissue were chosen to illustrate changes in the expression of various proteins. These samples were representative of all specimens analyzed. Expression of CgA was observed in the selected foregut, midgut, and hindgut tissues. Strong EGFR expression was observed in lung carcinoids, whereas weak expression was observed in small-bowel sections. Lung, gastric, and small-bowel carcinoids all stained strongly positive for VEGFR expression. Weak VEGFR expression was also noted in hindgut carcinoids. Strong IGFR expression was found in lung, gastric, and small-bowel carcinoids, whereas weak expression was observed in hindgut carcinoids. HER-2/neu was expressed in all of the (Fig. 1) tissues except for hindgut carcinoids.

Comparison of receptor staining between UTMB patient samples and commercial tissue arrays is summarized in Table 3. Some carcinoid sections were unable to be stained due to tissue loss which is reflected in the number of stained tissues. Ninety percent of UTMB samples (18 of 20) stained positive for IGFR. On the other hand, 88% of array samples (22 of 25) were negative for IGFR expression. Twenty-nine percent of array samples (seven of 24) were HER-2/neu positive, while only 15% of UTMB samples (three of 20) stained positive for HER-2/neu . When all carcinoid tissues were categorized based on tissue location and receptor staining, VEGFR expression was fairly evenly distributed among foregut and midgut carcinoids, and all hindgut carcinoids exhibited positive expression (Table 4). IGFR expression was greater in midgut carcinoids compared to foregut carcinoids. Gender did not significantly influence the outcome of GFR expression (Table 5). Although not statistically significant (p=0.10), men were ~8 times less likely to have positive staining for EGFR. Patients less than 50 years of age were ~9 times (p= 0.07) more likely to have positive staining for VEGFR than patients between 50 and 59 (Table 6). The odds ratio of exhibiting positive VEGFR staining in patients 60 years or older was almost identical to those between 50 and 59. Patients under age 50 years of age were ~24 times more likely (p=0.048) to have positive staining for IGFR than patients between the ages of 50 and 59. The odds ratio of

Table 5 Comparison of Carcinoid Tumor Location and Frequency for Growth Factor Receptor Staining

	Number (%)				
	CgA	EGFR	VEGFR	IGFR	HER-2/neu
Foregut	26 (87%)	4 (13%)	14 (47%)	9 (30%)	8 (27%)
Midgut	11 (100%)	2 (20%)	4 (40%)	9 (82%)	2 (20%)
Hindgut	4 (100%)	0 (0%)	4 (100%)	3 (75%)	0 (0%)

Table 6 Comparison of Age and Frequency for Growth Factor Receptor Staining

Age Group	Number (%)				
	CgA	EGFR	VEGFR	IGFR	HER-2/neu
24–49	10 (100%)	0 (0%)	8 (89%)	8 (80%)	3 (33%)
50–59	15 (88%)	5 (29%)	7 (41%)	6 (35%)	3 (17%)
60–79	16 (89%)	1 (6%)	7 (39%)	7 (39%)	4 (22%)

patients 60 years or older demonstrating IGFR expression was almost identical to those between the ages of 50 and 59.

Discussion

In 1907, Siegfried Obendorfer, a German pathologist, coined the term "karzinoide" to describe a benign tumor resembling a carcinoma microscopically.[25] In 1948, serotonin was identified from the Kulchitsky cell and later hypothesized to be the hormone responsible for carcinoid syndrome.[26] Since this time, studies have attempted to determine targets for the treatment of carcinoid tumors. Recently, there has been growing interest in the use of GFR inhibitors in the treatment of carcinoid tumors. Endostatin, sunitinib, sorafenib, and bevacizumab are drugs that are under investigation and have shown promise.[27–29] Although carcinoid tumors are mostly slow growing and indolent in nature, current chemotherapeutic options have not altered survival. Somatostatin analogues attenuate the symptoms of carcinoid syndrome, but their effects on tumor growth are controversial. Currently, platinum-based chemotherapy is not useful in these patients, and traditional single agents are only minimally effective in a small number of patients.[30,31]

Increased expression of GFRs has previously been demonstrated in neuroendocrine tumors and has been implicated as a possible mechanism for tumor development and progression. Carcinoid tumors are highly vascular in nature with many of these tumors exhibiting a marked desmoplastic reaction. Expression of VEGF has been demonstrated in both gastrointestinal and pulmonary carcinoids.[32,33] Recently, overexpression of VEGF was found to promote the growth of human neuroendocrine tumors through the up-regulation of angiogenesis; bevacizumab significantly reduced tumor angiogenesis and impaired tumor growth in vivo.[34] Similarly, our group found that treatment with bevacizumab significantly inhibited tumor growth using a novel in vivo metastasis model.[14] Increased VEGF expression appears to correlate with metastases and decreased progression-free survival.[35] In our current study, we demonstrated that carcinoid tumors from patients under 50 years of age were more likely to express VEGFR. These findings are intriguing and suggest that targeting VEGFR may represent a treatment option in a subset of patients with carcinoid tumors.

IGFR is implicated as an important component of growth factor signaling in neuroendocrine tumors.[36] We found increased IGFR expression in UTMB patient tissues when compared to the commercial array tissues, which may be attributed to the increased number of midgut carcinoids in the UTMB patient population. Patients under 50 years of age were also more likely to have increased expression of IGFR. Exogenous IGF has been shown to activate mTOR and increase cellular proliferation in carcinoid cells.[37] Due to the importance of IGFR signaling in carcinomas, it has become another possible target for kinase inhibitors. Our findings suggest that IGFR is important in carcinoid tumors, specifically in younger patients. In the future, this finding may lead to the investigation of patient-specific treatment therapies based on patient age. Although EGFR expression has been identified in carcinoid tumors of the gastrointestinal tract, we did not observe a significant trend in EGFR expression in our current study. Previously, EGFR and p-EGFR were found to be more highly expressed in small bowel carcinoid tumors compared with islet-cell tumors, and p-EGFR expression was associated with decreased survival among patients with pancreatic endocrine tumors.[38] Even though previous studies have shown increased expression of PDGFR in carcinoid tumors, we did not detect PDGFR expression in carcinoid tumors from our patient population. In a phase-II study of imatinib, which is specific for the tyrosine kinase domain in PDGFR, there was no significant regression of carcinoid disease, but a significant number of patients with progressive disease did achieve disease stabilization.[39] This suggests that although imatinib may not be a future first-line agent in the treatment of carcinoid tumors it may play a role in palliative treatment.

Slow-growing carcinoids are often indolent in nature, and like other cancers with a similar course, they are often discovered in advanced stages of the disease. Currently, complete operative resection is the only option for cure. Recent clinical trials evaluating the effectiveness of GFR inhibition show promise in disease stabilization, but more research in combination drug therapy is forthcoming. Our study highlights the importance of GFR signaling in

carcinoid tumors, specifically VEGFR and IGFR, and provides evidence to support current investigations into the inhibition of these GFRs as a novel treatment strategy for carcinoids. Evaluation of the SEER database demonstrated a twofold increase in the number of carcinoids over the last decade. During that time, the most common site of occurrence was in the foregut followed by hindgut and midgut locations. When divided by gastrointestinal organ system, the colorectal location intestine was the most common site of occurrence. Although there has not been a significant change in survival, new information about the occurrence of GFRs in carcinoid tumors will provide novel treatment options in the future.

Acknowledgments This work was supported by grants RO1 CA104748, RO1 DK48498, PO1DK35608, R01 CA125454 (to BPZ) and T32DK07639 from the National Institutes of Health. KB is a recipient of a Jeane B. Kempner Scholar Award. The authors thank Karen Martin for manuscript preparation and Tatsuo Uchida for statistical analysis.

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J Gastrointest Surg (2009) 13:1781–1790
DOI 10.1007/s11605-009-0948-x

2009 SSAT POSTER PRESENTATION

Effective Treatment of Advanced Colorectal Cancer by Rapamycin and 5-FU/Oxaliplatin Monitored by TIMP-1

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Received: 25 May 2009 / Accepted: 3 June 2009 / Published online: 30 June 2009

Abstract
Aim The mTOR-inhibitor rapamycin has shown antitumor activity in various tumors. Bedside observations have suggested that rapamycin may be effective as a treatment for colorectal carcinomatosis.
Methods We established an orthotopic syngenic model by transplanting CT26 peritoneal tumors in Balb/C mice and an orthotopic xenograft model by transplanting SW620 peritoneal tumors in nu/nu mice. Expression levels of tissue inhibitor of matrix-metalloproteinases 1 (TIMP-1) in the tumor and serum was determined by enzyme-linked immunosorbent assay.
Results Rapamycin significantly suppressed growth of syngenic and xenografted peritoneal tumors. The effect was similar with intraperitoneal or oral rapamycin administration. Tumor suppression was further enhanced when rapamycin was combined with 5-fluorouracil and/or oxaliplatin. The combination treatment showed no acute toxicity. TIMP-1 serum levels correlated well (CC=0.75; $P<0.01$) with rapamycin treatment.
Conclusions Rapamycin suppressed advanced stage colorectal cancer, even with oral administration. Combining rapamycin with current chemotherapy regimens significantly increased antitumor efficacy without apparent toxicity. The treatment efficacy correlated with serum TIMP-1 levels, suggesting its potential as a surrogate marker in future clinical trials.

Keywords mTOR inhibitor · Rapamycin · 5-FU · Oxaliplatin · TIMP-1 · Colorectal cancer

Introduction

About 25% of patients with symptoms from colorectal cancer show peritoneal seeding of tumor cells (peritoneal carcinomatosis).[1] The prognosis for patients with peritoneal carcinomatosis is somber. The estimated 5-year survival is less than 10%,[2] despite improved tumor responses to combinations of basic chemotherapy (5-fluorouracil [5-FU] + leucovorin) with either Irinotecan or oxaliplatin (FOLFOX) and more recently with bevacizumab. To significantly improve survival for these patients, novel treatment strategies are needed.

Rapamycin is a bacterial macrolide that forms a complex with the FK506-binding protein (FKBP-12) and inactivates the mammalian target of rapamycin (mTOR). mTOR is activated through the phosphoinositide 3 kinase (PI3K), protein kinase B/rat sarcoma (Akt/Ras) pathway and leads to protein synthesis and cell proliferation. Accordingly, aberrant activation of mTOR has been observed in more than 30% of epithelial cancers (reviewed in[3,4]). Nearly 40% of carcinomas of the colorectum exhibit either partial or complete positive staining for a downstream factor of mTOR, phospho-S6K. This suggests that they are rapamycin-sensitive lesions.[5] However, the antitumor activity of rapamycin is two-pronged; it has direct antiproliferative effects and also inhibits tumor-angiogenesis.[6,7]

Paper presented at Digestive Disease Week, May 2009, Chicago, USA

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Seeliger et al. reported that rapamycin increased antitumor activity by counteracting 5-FU-induced expression of angiogenetic 2-deoxy-ribose (dRib) in a mouse colorectal tumor model.[8] Other groups have reported various synergistic effects of rapamycin with experimental agents, including radiation therapy and the DNA-damaging agents, cisplatin, oxaliplatin, and doxorubicin.[9–14]

Several synthetic rapamycin homologues have been tested in clinical trials for brain tumors, renal cell carcinoma, gynecologic cancer, lung cancer, and sarcomas (reviewed in Figlin[15]).[16–19] However, early clinical trials have been hampered by difficulties in demonstrating biologic activity of the tested compound in advanced disease.

In order to set the stage for rapamycin therapy in advanced colorectal cancer, it would be desirable to identify a suitable surrogate marker to monitor antitumor activity. The tissue inhibitor of matrix metalloproteinases 1 (TIMP-1) has been shown to correlate with disease stage and tumorigenicity of colorectal cancer[20,21](and reviewed in[22]). TIMP-1 shares homology with its three other family members, and all display a robust sensitivity to changes in pH, temperature, and denaturing conditions due to their disulphide bonds.[23] The N-terminal domain displays inhibitory activity against matrix metalloproteinases (MMPs) and contains the consensus sequence VIRAK.[24] TIMP-1 is expressed and secreted by many cells of most tissues. The TIMP-1 protein includes a signal peptide that directs its secretion into the extracellular space. One of the main functions of TIMP-1 is the inhibition of MMPs. This activity is involved in the tissue remodeling observed in inflammation, wound healing, and cancer invasion.[25,26] TIMP-1 binding to the cell surface activates the PI3K signaling pathway, which leads to cell proliferation.[27,28] Conversely, inhibition of the PI3K pathway through PTEN (a tumor suppressor that prevents phosphorylation of Akt) resulted in down-regulation of epidermal growth factor-induced TIMP-1 expression.[29] Because it has also been reported that rapamycin caused down-regulation of TIMP-1 expression in experimental transplant rejection, we were interested in whether serum TIMP-1 expression levels might serve as a surrogate marker for the efficacy of rapamycin-based anticancer regimens.[30,31]

Material and Methods

Cell Lines and Chemicals

SW620 human colonic carcinoma cells and CT26 mouse colon carcinoma cells (derived from a murine Balb/c colon carcinoma) were obtained from the American Type Culture Collection (Rockville, MD, USA). We obtained 3-(4,5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), tetrazolium salt, and all other chemicals and reagents from Sigma Chemical Corp. (Buchs, Switzerland). Immunohistochemistry was performed with rabbit anti-TIMP-1 (H-150; Santa Cruz Biotechnology; LabForce AG, Nunningen, Switzerland). The substrate solution for enzyme-linked immunosorbent assay (ELISA; ImmunoPure TMB Substrate Kit) were from Pierce (Perbio Science, Lausanne, Switzerland). The DuoSet kit (ELISA Development System) was used with mouse (DY980) and human anti-TIMP-1 (DY970) antibodies from R&D Systems Inc. (Minneapolis, USA). Rapamycin (Rapamune ®) was from Wyeth Pharmaceuticals (Zug, Switzerland), 5-FU was from Roche Pharma (Reinach, Switzerland), and oxaliplatin was from Sanofi-Synthélabo (Meyrin, Switzerland).

Cell Cytotoxicity Assay

Cell cytotoxicity was determined as described previously[32] by colorimetric assays (MTT dye reduction assay) performed with the Ultra-microplate reader EL808 (Bio-Tek instruments Inc, Winooski, USA). Cells were plated in triplicate at a density of 1×10^4 in 96-well plates in 0.2 ml normal growth medium and incubated at 37°C, with 5% CO_2. All assays were repeated at least three times.

Orthotopic, Syngenic, and Xenograft Animal Models

Four- to 6-week-old female, 20–25 g Balb/c OlaHsd mice and athymic Nude-Foxn1^{nu} mice (Harlan Netherlands B.V., Horst, Netherlands) were housed in individual ventilated cages under sterile conditions according to the Swiss guidelines for the care and use of laboratory animals. Sterile food and water were provided ad libitum. Animal procedures were approved by the regional authorities according to Swiss animal-care regulations. To create an orthotopic model of peritoneal carcinomatosis, CT26 or SW620 cells were injected at a density of 5×10^5 cells/50 µl into the peritoneal cavity of BALB/c (syngenic model) or nude mice (xenograft model), respectively. Preliminary analysis of stably transplanted CT26 cells by bioluminescence imaging showed that 100% of mice developed tumors, and the localization of tumors was reminiscent of peritoneal carcinomatosis in humans. Five days after tumor-cell inoculation, once peritoneal carcinomatosis was established, treatment was initiated. Mice were followed daily and killed by CO_2 euthanasia when ascites or tumor development interfered with the well-being of the animals or at the end of experiments. Tumors from the peritoneal cavity were carefully excised under a surgical microscope (magnification ×4). The total excised tumor was weighed, and the ascites volume was measured. Specimens from the tumor, colon, liver, and lungs were embedded in paraffin.

Antitumor Agents

Rapamycin was diluted in water and administered orally or intraperitoneally (i.p.) at 0.15 or 1.5 mg/kg every 2 days, respectively. In mice, a dosage of 1.5 mg kg^{-1} day^{-1} has been shown to produce steady-state rapamycin serum levels in a range similar to that used on a long-term basis in organ transplantation to prevent allograft rejection.[6] 5-FU was diluted in 0.9% saline and administered i.p. at 100 mg/kg on days 7 and 14. Thereafter, 5-FU was given at 50 mg/kg every 7 days to the end of the experiment, as described previously.[6] It was reported that 100 mg/kg 5-FU was the maximum tolerated dose in mice.[33] Oxaliplatin (Eloxatin ®) was prepared according to the description of the manufacturer and administered i.p. at 5 mg/kg/d for 5 days. For combination treatment experiments with rapamycin + 5-FU/Oxal, half of the above dose of Oxaliplatin was used. Cyclosporine A was given orally by gavage at a dose of 15 mg/kg every 2 days as described previously.

Determination of TIMP-1

Cell lysates, tissue lysates, and tail vein serum samples spun at 1,500×*g* were analyzed with enzyme-linked immunosorbent assay for TIMP-1 quantitation according to manufacturer specifications (DuoSet, ELISA Development System, mouse TIMP-1 or human TIMP-1; R&D Systems Inc., USA). Wells were incubated for 20 min in substrate solution (ImmunoPure TMB Substrate Kit, Pierce, USA). The optical density of each well was determined immediately with a microplate reader (EL808, Microplate Reader, Bio-Tek Instruments Inc., USA) at a wavelength of 450 nm (wavelength correction set to 562 nm). A standard curve was created by reducing the data to a four-parameter logistic (4-PL) curve fit with KC4-Software (Kineticalc for Windows; version # 3.03, Rev. # 4, Bio-Tek Instruments Inc., USA). Comparison of the optical density of each well to the standard curve provided a relative measurement of protein concentration. Measurements were performed in triplicate.

Immunohistochemistry

Tumor tissue samples were fixed in 4% formalin, processed, and embedded in paraffin. Paraffin embedded tissue samples were sliced into 5 μm sections and processed according to the manufacturer's protocol (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA). For antigen retrieval, samples were digested with Proteinase K at a concentration of 5 μg/ml at 37°C for 20 min. Rabbit polyclonal TIMP-1 antibodies (H-150) were added at a final concentration of 1 μg/ml, and sections were incubated overnight at 4°C. Sections were developed with 3,3′-diaminobenzidine (SigmaFast 3,3′-diaminobenzidine tablet sets, Sigma-Aldrich, USA) substrate for a maximum of 10 min.

Statistics

Statistical software NCSS (Kaysville, UT, USA) was used to analyze the data. One-way analysis of variance (ANOVA) and *t* tests were used where appropriate. The significance level was set at 0.05. Whiskers (10th and 90th), box margins (25th and 75th), and the midline of box plots (50th) depict the percentiles of the respective variable. Linear regression modeling was used for the estimation of correlations. The regression coefficient and R^2 were calculated. The Spearman rank correlation coefficient was used to estimate confidence levels and probabilities.

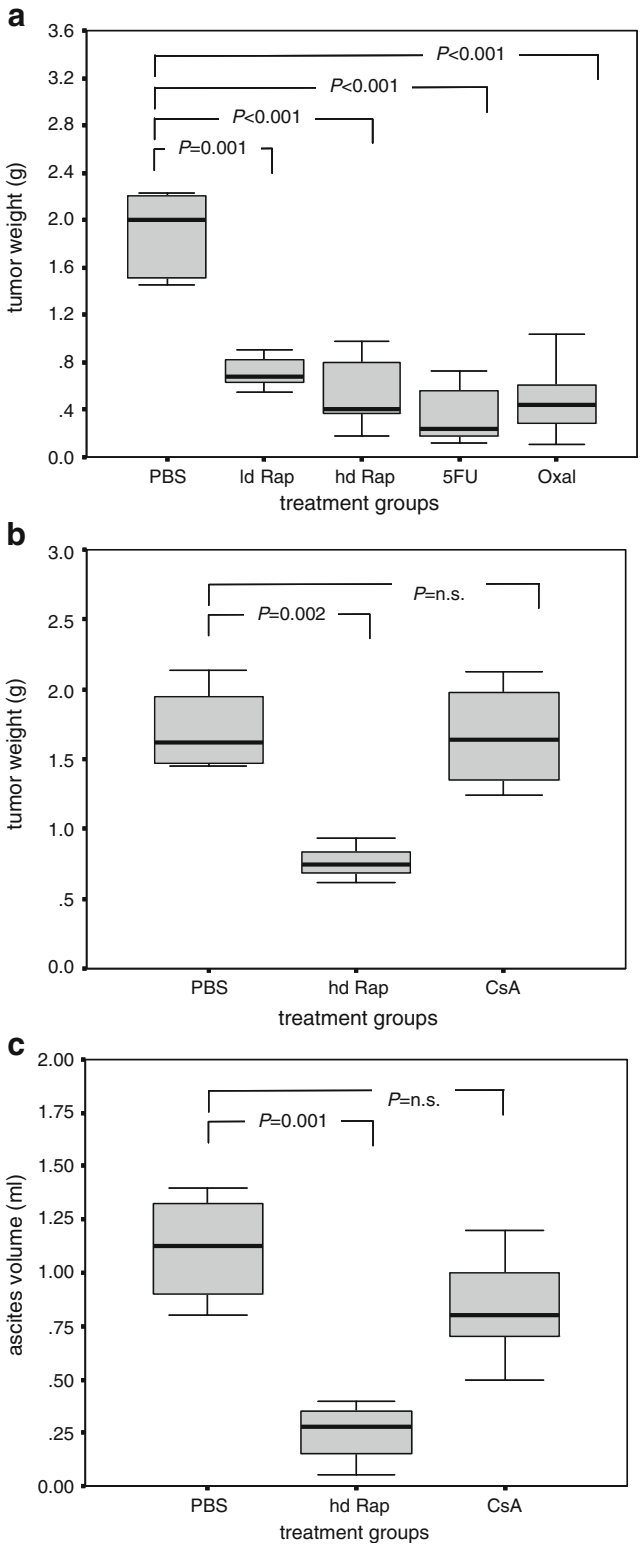
Results

Rapamycin Inhibited Growth of Peritoneal Carcinomatosis

A dose escalation of rapamycin on CT26/SW620 cells in vitro showed direct cytotoxicity (determined by MTT assays) only at very high concentrations (>100 ng/ml; data not shown). In vivo, rapamycin administered orally (gavage) or intraperitoneally (i.p.) caused significant suppression of peritoneal tumor growth in Balb/c mice (syngenic model with CT26 cells; Fig. 1a, b). The antitumor efficacy of rapamycin at "pharmacological doses"[6] (hd Rap) and at one tenth of that concentration (ld Rap) was similar to the tumor suppressive activity of 5-FU or oxaliplatin (Fig. 1a). In contrast, treatment with the immunosuppressive agent cyclosporine A, another immunosuppressive drug used to inhibit transplant rejection, did not decrease tumor growth (Fig. 1b). Mice in the rapamycin-treated group showed very low amounts of ascites, but mice in the cyclosporine A and control groups showed marked formation of malignant ascites (Fig. 1c).

Combination of Rapamycin with 5-FU- and/or Oxaliplatin-Enhanced Growth Inhibition of Syngenic Tumors with no Apparent Toxicity

Rapamycin combined with either 5-FU or oxaliplatin showed superior tumor suppression compared to rapamycin as a single agent therapy (Fig. 2a, b). No acute or chronic toxicity was noted, but two mice of the oxaliplatin alone group showed subcutaneous necrosis at the site of injection, likely due to an inaccurate injection. Body weight was maintained in balance over most of the observation period. However, in control treated animals, weight increased in proportion to apparent ascites and tumor load. This finally



led to early killing of the animals because interference with well-being was noted (Fig. 2c). H&E staining of paraffin-embedded liver samples did not reveal ultrastructural changes that indicated acute or chronic hepatic toxicity (analysis by an independent pathologist; Fig. 2d).

◀ **Figure 1** **a** Intraperitoneal administration of rapamycin, 5-FU, or oxaliplatin resulted in tumor suppression. Box plots represent tumor weights at the end of the experiment. Mouse colon carcinoma cells (CT26; 5×10^5 cells) were injected into the peritoneal cavity of syngenic Balb/c mice. Twenty-two days after intraperitoneal injection of CT26 cells and 17 days after treatment initiation, mice were killed. Treatment groups were: *PBS*; *ld Rap* low-dose rapamycin, 0.15 mg/kg every 2 days; *hd Rap* high-dose rapamycin, 1.5 mg/kg every 2 days; *5-FU* 100 mg/kg every 7 days for the first 2 weeks, then 50 mg/kg every 7 days until the end of the experiment; and oxaliplatin 5 mg kg^{-1} day^{-1} for 5 days). The group size was $n=8$. Significant tumor growth suppression compared to controls was noted in the various treatment groups (*t* test). **b** Tumor growth was significantly inhibited by orally administered rapamycin but not by the immunosuppressive agent cyclosporine A. Box-plots represent the weight of peritoneal tumors. The tumor model is the same as in **a**. Treatment groups were: *PBS*; *hd Rap* high dose rapamycin, 1.5 mg/kg every 2 days; cyclosporine A, 15 mg/kg every 2 days. The group size was $n=8$. **c** Significantly smaller tumor size and less ascites formation were noted in hd Rap compared to controls but not in the CsA group (*t* test).

Rapamycin Treatment Alone and in Combination Inhibited Tumor Growth of Human Xenograft Peritoneal Carcinomatosis

Both rapamycin and "FOLFOX" (5-FU+oxaliplatin) treatments caused significant suppression of the peritoneal tumor growth induced by human colorectal SW620 cancer in nude mice. The antitumor effect was enhanced by a combination of rapamycin with 5-FU+oxaliplatin (Fig. 3). At low doses of rapamycin (one tenth of the "standard" dose), tumor suppression was enhanced compared to 5-FU+oxaliplatin treatment alone. The effect was further enhanced when rapamycin was given at a high dose. Again, no acute or chronic toxicity was observed as confirmed by histological analysis of liver tissue and body weight curves.

TIMP-1 Was Down-Regulated by Rapamycin Treatment in Cancer Cells and Tumors

CT26 or SW620 cell lysates showed a dose-dependent decrease in TIMP-1 protein levels after addition of rapamycin to the culture media (Fig. 4a; CT26). Hence, low to medium-high doses of rapamycin down-regulated TIMP-1 expression without apparent toxic effects, as shown by immunohistochemistry (Fig 4b) and western blots (not shown). Compared to phosphate-buffered saline (PBS)-treated mice, the combination of 5-FU and oxaliplatin also lowered intratumoral TIMP-1 expression but significantly less than rapamycin treatment.

Serum Levels of TIMP-1 Correlated with Antitumor Activity of Rapamycin

The expression levels of TIMP-1 in serum samples from mice with syngenic CT26 tumors correlated well with tumor weight (CC=0.76; Fig. 4c). The correlation was

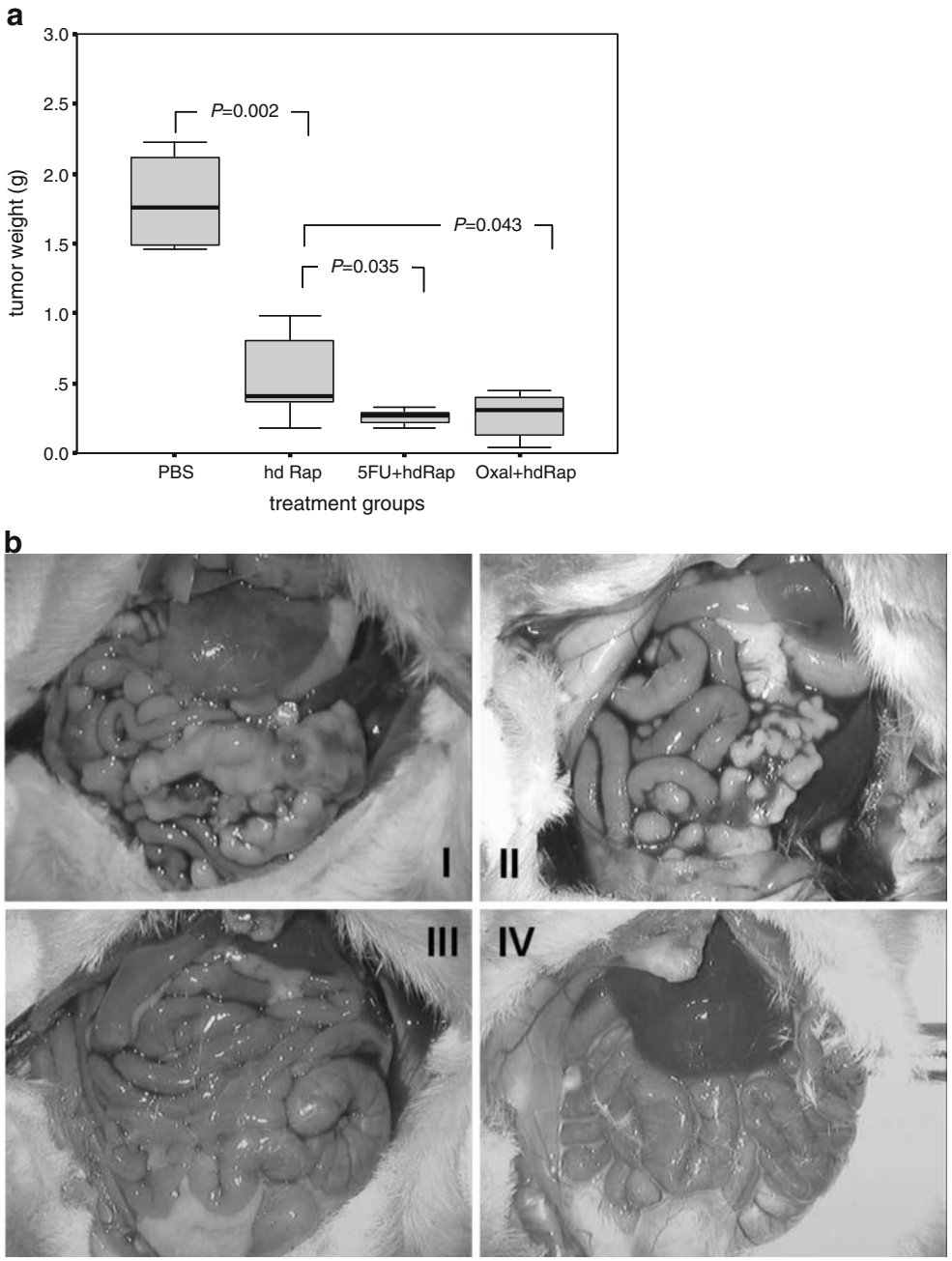


Figure 2 **a**, **b** Rapamycin combined with 5-FU or oxaliplatin inhibited tumor growth more efficiently than rapamycin alone. Boxplots represent weight of peritoneal tumors (**a**). Animals (n=8/group) with established syngenic, orthotopic tumors (5 days after injection of 5×10^5 CT26 cells) were treated with rapamycin (*hd Rap*, 1.5 mg/kg every 2 days) in combination with either 5-FU (100 mg/kg every 7 days for the first 2 weeks, then 50 mg/kg every 7 days) or oxaliplatin (5 mg kg^{-1} day^{-1} for 5 days). All compounds were administered i.p. Mice were killed 22 days after tumor inoculation. Rapamycin combined with 5-FU or oxaliplatin inhibited tumor growth more effectively than rapamycin treatment alone (t test). **b** Picture of mice described in **a**: *I* PBS; *II* hd Rap; *III* 5-FU + hd Rap; *IV* Oxal + hd Rap. Tumor load was significantly reduced by rapamycin alone and in combination treatments of rapamycin with 5-FU or oxaliplatin. **c** Treatment with rapamycin alone or in combination did not influence body weight (n=8/group). Graphs depict body weight of mice from **a** measured every second day after tumor cell inoculation. Mice were treated with PBS, rapamycin, or a combination of rapamycin and 5-FU or oxaliplatin as stated above (**a**). The body weight of control mice (PBS), with tumor loads of up to 2.5 g and ascites formations, was significantly different from that of the most effective treatment group, with no macroscopic tumor and no ascites (hd Rap + 5-FU; P=0.04). For the other groups, body weight tended to be lower than that of control animals. No significant intergroup differences were noted between treatments (one-way ANOVA). **d** Treatment with rapamycin alone or in combination did not alter the ultrastructure of the liver. H&E staining of liver samples (n=8/group) displayed no change in the ultrastructure of the liver parenchyma after 17 days of treatment with PBS, oxaliplatin, or a combination of rapamycin with oxaliplatin and 5-FU.

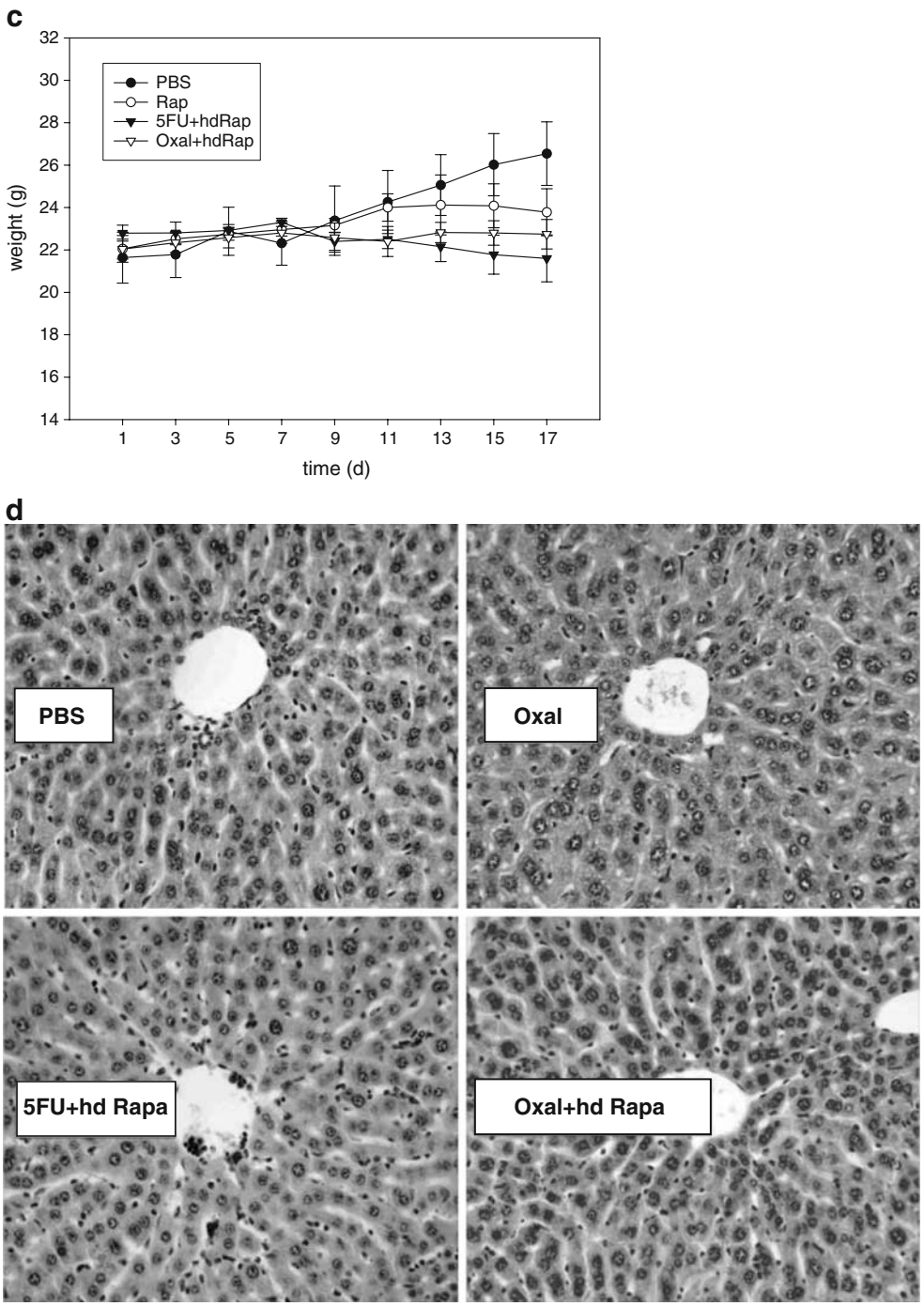


Figure 2 (continued).

more pronounced in mice treated with the combination of rapamycin and 5-FU/Oxal compared to mice treated without rapamycin, where no significant correlation to tumor weight was observed. This suggested that both TIMP-1 secretion from the tumoral tissue and the secretion from healthy tissues were affected by direct TIMP-1 suppression and indirect suppression by mTOR inhibition (the biological activity of rapamycin), respectively. Peritumoral tissue is likely to contribute to TIMP-1 serum levels. However, the fact that human TIMP-1 serum levels correlated well (correlation coefficient=0.75) with tumor weight in mice carrying a human SW620 xenograft tumor suggested that peritumoral TIMP-1 expression due to inflammation or a reaction to increased tissue levels of MMPs was not the dominant source of the TIMP-1 correlation.

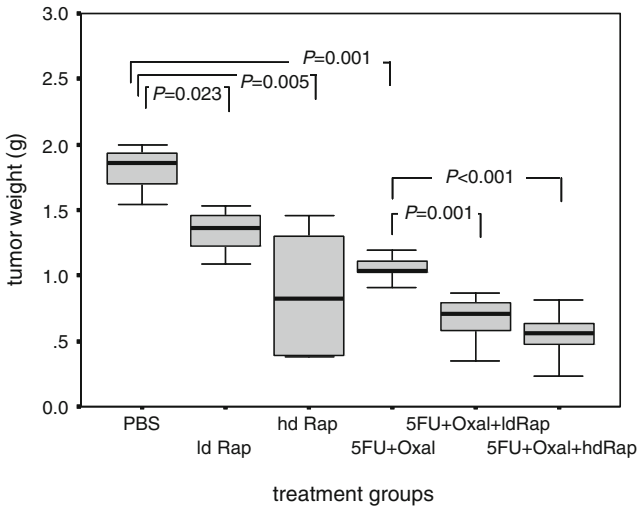


Figure 3 Growth of peritoneal xenograft tumors was significantly suppressed by rapamycin alone or in combination with 5-FU +oxaliplatin. Box-blots represent tumor weight in different treatment groups. SW620 cells (5×10^5) were injected intraperitoneally in athymic nude mice. Treatment was initiated after 7 days for a total of 35 days, when mice were killed. Treatment groups ($n=8$) were: Controls (*PBS*); low-dose rapamycin (*ld Rap*, 0.15 mg/kg every 2 days) per gavage; high-dose rapamycin (*hd Rap*, 1.5 mg/kg every 2 days) per gavage; 5-FU+oxaliplatin (*5-FU*, 100 mg kg^{-1} $week^{-1}$, i.p. for the first 2 weeks, thereafter 50 mg/kg every week + Oxal: 5 mg kg^{-1} day^{-1}, i.p. for 5 days); and a combination of rapamycin with 5-FU+Oxal (at above doses). Significant suppression of tumor growth compared to controls was noted following oral administration of rapamycin (ld Rap and hd Rap). Likewise, a combination treatment of 5-FU + oxaliplatin significantly inhibited tumor growth. The antitumor effect was synergistically enhanced by combination of rapamycin with 5-FU+oxaliplatin compared to 5-FU+Oxal alone (*P* values between groups: *t* test; intergroup differences: one-way ANOVA).

Discussion

Our results corroborate preclinical and early clinical findings from various tumors that the mTOR-inhibitor rapamycin has antitumor efficacy. Our motivation to undertake this study was the observation that a 62-year-old patient, immunosuppressed due to liver transplantation for 2 years, was treated for adenocarcinoma of the colon with peritoneal carcinomatosis by local resection and adjuvant chemotherapy (FOLFOX). At that time, immunosuppression was changed to rapamycin. Two years later, he experienced an incisional hernia that required repair. At that time, peritoneal carcinomatosis had resolved but for one small lesion. Now, 6 years after the initial diagnosis, the patient is alive with no manifestations of peritoneal carcinomatosis (personal observation).

In our syngenic and xenograft orthotopic mouse models for peritoneal carcinomatosis, rapamycin treatment significantly inhibited tumor growth. The pronounced antitumor effect was consistently observed in our experiments. This study extends other work that showed rapamycin activity against colorectal liver metastases.[6,8,34] We demonstrated that this anticancer effect occurs even in more advanced, late-stage disease and with either intraperitoneal application or oral administration of rapamycin. This is relevant because rapamycin (Rapamune ®) is a highly lipophilic solution given orally to transplanted patients. In previous studies on colorectal cancer, solvents (dimethylsulfoxide or ethanol) that are cytotoxic were used for intraperitoneal rapamycin injections.[6] Here, we emulsified rapamycin in distilled water and then applied this emulsion intraperitoneally and orally, in order to minimize direct cytotoxic effects. The route of application did not influence the antitumor activity of rapamycin.

In order to mimic treatment regimens in potential future clinical trials (e.g., FOLFOX plus rapamycin), we combined rapamycin treatment with 5-FU and/or oxaliplatin. The combination therapy showed improved efficacy for colorectal carcinoma without increasing toxicity. In addition, when rapamycin was combined with half the recommended dosing of 5-FU/Oxaliplatin, tumor suppression was equally effective. Thus, systemic toxicity and side effects can be reduced with this chemotherapy in the future.[35] A recent study showed that a combination of rapamycin with irinotecan had pronounced antitumor effects on colorectal xenografts, dependent on the hypoxic state of cells.[34] In histologic evaluation of tumors, we did not find necrotic patterns typical of oxygen dependency.

Early studies in transplant rejection showed that rapamycin down-regulated TIMP-1, a factor overexpressed in patients with advanced colorectal cancer and associated with advanced tumor stages, poor outcome, and chemoresistance.[21,22,36] In this study, treatment with rapamycin caused nearly complete disappearance of TIMP-1 in tumor tissue samples. Because TIMP-1 has broad tumor-promoting activity (pro-angiogenesis, anti-apoptosis; promotion of cell growth and proliferation), one could speculate that its down-regulation by rapamycin could be another mechanism through which rapamycin mediates its antitumor effects.[37,38] We found that TIMP-1 expression in the tissue and the serum correlated well with the antitumor effect of rapamycin. A high rapamycin dose yielded lower TIMP-1 than a low rapamycin dose for similar tumor sizes. This suggested that TIMP-1 serum levels mirrored both antitumor activity and biological activity. In addition, we found that human TIMP-1 serum levels in mice carrying human SW620 tumors correlated well with tumor weight and that TIMP-1 levels were not influenced by treatment with the immunosuppressive agent cyclosporine A. This provided corroborating evidence that serum levels of TIMP-1 reflected primarily tumor load and proliferation rather than a reaction of the peritumoral tissue.[39]

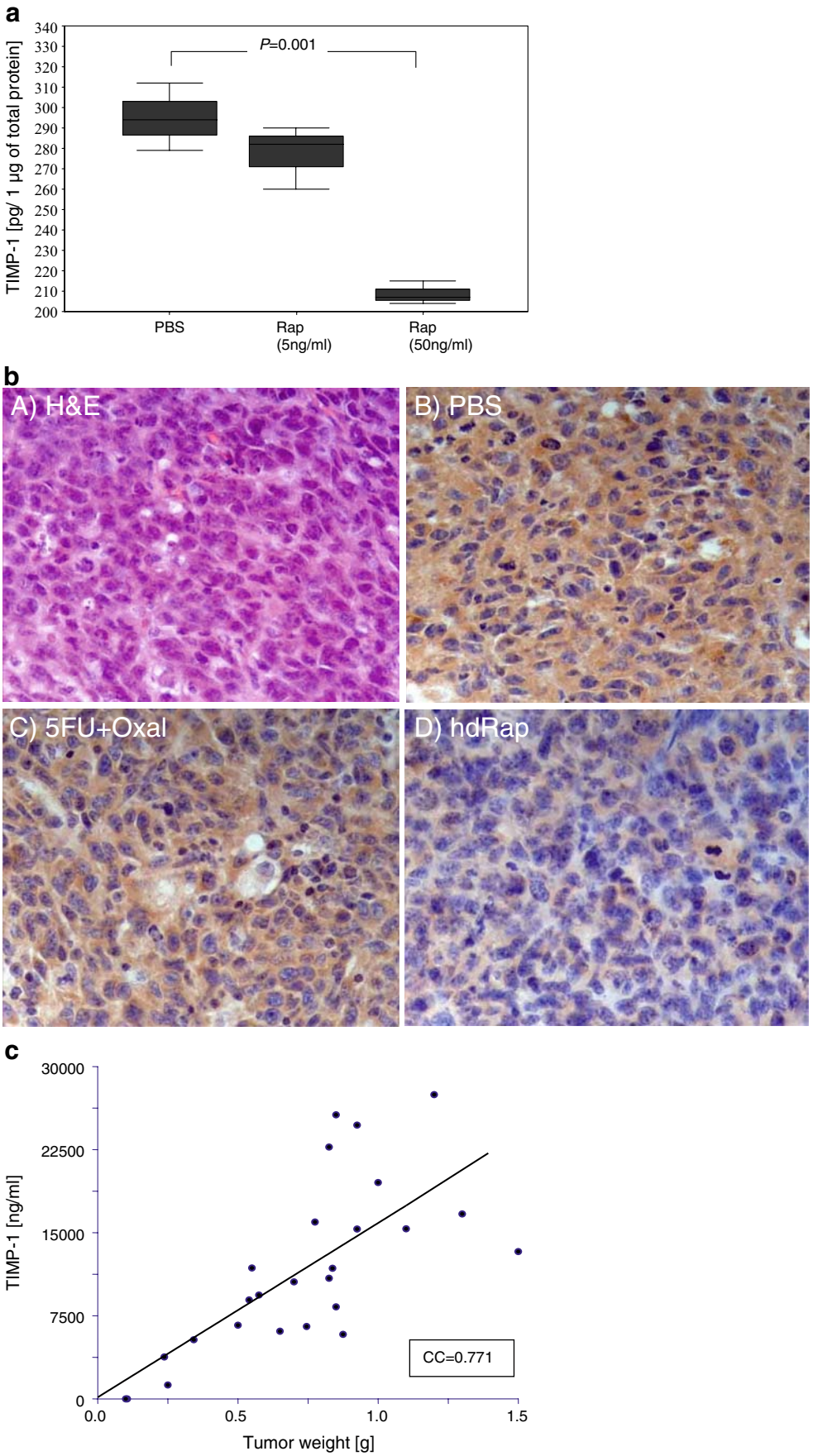


Figure 4 **a** Rapamycin down-regulated TIMP-1 expression. Forty-eight hours after cell-cultures were treated with PBS, 5 ng/ml, or 50 ng/ml rapamycin, the whole cell lysates of CT26 cells were analyzed for TIMP-1 expression by ELISA (normalized to 1 µg of total protein). Compared to PBS-treated cells, a significant down-regulation of TIMP-1 protein levels was observed in the cells treated with high-dose rapamycin ($P<0.001$; *t* test). **b** Intratumoral TIMP-1 was strongly down-regulated after rapamycin treatment. Hematoxylin–eosin staining of peritoneal CT26 syngenic tumor from experiment in **a** (*A*). Sections were subjected to immunohistochemistry for TIMP-1 (*brown staining*) and counterstained for nuclei (*blue*; *B–D*). Cytoplasmic and extracellular expression of TIMP-1 was strongly reduced after rapamycin treatment (*D*) compared to controls (*B*). 5-FU+Oxal therapy (*C*) led to a weak down-regulation of TIMP-1 in the tumors. **c** TIMP-1 serum levels correlated with tumor weight. Whole blood taken from the tail vein of CT26 tumor-bearing Balb/c mice at the time of killing was spun down and the serum analyzed for TIMP-1 expression by ELISA. Linear regression analysis showed that tumor weight was correlated with TIMP-1 expression in mice treated with PBS, FU/Oxal alone, and in combination with low- or high-dose rapamycin. Data are representative of one experimental run. A very good positive correlation between tumor weights and TIMP-1 serum levels was calculated (correlation coefficient=0.77 (Spearman rank); $P<0.005$).

Conclusion

The combination of rapamycin with 5-FU and oxaliplatin had strong synergistic effects against late-stage colorectal cancer even at reduced dosing. Hence, our findings suggest that rapamycin combined with current state-of-the-art chemotherapy should enter phase I/II clinical trials as a treatment for late-stage colorectal carcinoma. TIMP-1 serum levels can easily be monitored as a surrogate marker to measure antitumor activity in patients with peritoneal carcinomatosis.

Acknowledgment We would like to thank Cynthia Fuhrer (University of Bern) for her help with immunohistochemistry.

Grant support This work was supported by the 3R Research Foundation Switzerland (no 94/04); Swiss National Foundation (SNF 3100A0-104023); Oncosuisse (OCS 01431-08-2003) (SAV).

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J Gastrointest Surg (2009) 13:1791–1797
DOI 10.1007/s11605-009-0916-5

2008 SSAT POSTER PRESENTATION

Very High Serum CA 19-9 Levels: A Contraindication to Pancreaticoduodenectomy?

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Received: 25 February 2009 / Accepted: 15 April 2009 / Published online: 21 May 2009

Abstract
Aim To assess the outcome of patients with resectable pancreatic adenocarcinoma (PA) associated with high serum CA 19-9 levels.
Methods From 2000 to 2007, 344 patients underwent pancreatoduodenectomy for PA. Fifty-three patients (*elevated* group) had preoperatively elevated serum CA 19-9 levels (>400 IU/ml) after resolution of obstructive jaundice. Of these, 27 patients had high levels (400–899 IU/ml (HL)) and 26 patients had very high levels ≥900 IU/ml (VHL). Fifty patients with normal preoperative serum CA 19-9 levels (<37 IU/ml) comprised the *control* group.
Results Median survival of the *control* group ($n=50$) versus *elevated* group ($n=53$) was 22 versus 15 months ($p=0.02$) and overall 3-year survival was 32% versus 14% ($p=0.03$). There was no statistical difference in the median and 3-year overall survival between patients with HL and VHL. Patients in the *elevated* group who normalized their CA 19-9 levels after surgery ($n=11$) had a survival equivalent to patients in the *control* group.
Conclusions Patients who normalized their CA19-9 levels postoperatively had equivalent survival to patients with normal preoperative CA 19-9 levels. Preoperative serum CA 19-9 level by itself should not preclude surgery in patients who have undergone careful preoperative staging.

Keywords CA 19-9 · Pancreatic cancer · Pancreatic adenocarcinoma · Pancreaticoduodenectomy

Introduction

Pancreatic adenocarcinoma (PA) remains one of the deadliest cancers with incidence nearly equivalent to mortality. A minority of individuals (<15%) with pancreatic adenocarcinoma are candidates for "curative" surgical resection. Despite surgical resection in this minority, long-term survival remains low (<15%).[1] This observation may be explained by occult metastatic disease which leads to early recurrence despite margin negative (R0) resection. As diagnostic imaging improves detection of occult metastasis, patients with advanced disease who would not benefit from surgery will be identified.[2] Currently, however, most resected patients despite favorable preoperative staging have early recurrence reinforcing the knowledge that PA is present systemically at the time of diagnosis. Accurate preoperative biomarkers are needed to determine which patients with PA will benefit from surgical resection. Positron emission tomography (PET) and laparoscopy have been employed to identify occult metastases but with limited success in subgroups of patients.[3, 4]

Serum carbohydrate tumor-associated antigen (CA19-9) is a biomarker used for the diagnosis, prognosis, and monitoring of pancreatic cancer patients.[5–7] Several reports have shown

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that a serum CA19-9 level above 200 or 300 IU/ml correlates with poor outcome and conclude that such patients will likely not benefit from surgery.[8–17] A minority of patients with resectable PA have high levels (>400 IU/ml) of serum CA19-9. Even when patients with high serum CA 19-9 are determined to be radiographically resectable, they will often have metastasis discovered at the time of laparoscopy or laparotomy.[8] The purpose of this study was to determine the outcome of patients with resectable head PA associated with highly elevated preoperative serum CA19-9 who underwent pancreatoduodenectomy (PD). First, we aimed to determine whether preoperative serum CA19-9 levels can predict patient's survival. Second, we sought to determine if postoperative serum CA19-9 normalization was a significant positive predictor of overall survival.

Patients and Methods

Patient Selection From January 1, 2000 to December 31, 2007, 344 consecutive patients with resectable head PA underwent PD at Indiana University Hospital (Indianapolis, IN, USA). All patient data were entered prospectively into a clinical database approved by the Indiana University Institutional Review Board. Pancreatic cancer was staged by history and physical examination, serum laboratory studies, chest radiography, endoscopic ultrasound (EUS), dual phase computed tomography (CT scan), and magnetic resonance imaging (MRI). All serum CA19-9 levels were determined using radioimmunoassay. The normal range for CA19-9 is 0–37 IU/ml.

Inclusion Criteria To be included in this study, patients needed to have (a) a preoperative serum CA19-9 value available, (b) a serum bilirubin of ≤2 mg/dl at the time of serum CA 19-9 determination, and (c) no neoadjuvant treatment.

Exclusion Criteria Patients with serum bilirubin of >2 mg/dl at the time of serum CA19-9 measurement were excluded even if biliary stenting was already performed. Patients with CA 19-9 levels >37 IU/ml and <400 IU/ml were not included in this study.

Study Groups Patients with serum CA 19-9 levels >400 IU/ml were designated the *elevated* group. Patients were also sub-categorized as having a high level (*HL*) if the serum CA19-9 level was 400 IU/ml to 899 IU/ml or a very high level (*VHL*) if the serum CA19-9 level was >900 IU/ml. During the same period, patients with normal preoperative serum CA 19-9 levels (≤37 IU/ml) comprised our *control* group. The 400 IU/ml cut-off level was chosen because based upon recent literature this level was at or above majority the level considered to dictate a poor prognostic outcome (Table 1). By using this 400 IU/ml cut-off, we sought to compare the survival of patients anticipated to have the worst prognosis with the survival of patients anticipated to have the best prognosis according to preoperative serum CA19-9 level.

Surgery All patients underwent PD with curative intent. Laparoscopy was not performed routinely; however, one patient of *control* group and three patients of *elevated* group had laparoscopy prior to PD.

End Points Studied The variables evaluated included age, sex, weight loss, pre- and postoperative serum CA 19-9 level (from 1 to 3 months after surgery and before any adjuvant treatment), maximal tumor size (cm) defined as maximum diameter at pathologic analysis, histologic differentiation (well, moderate, or poor), margin of resection (positive or negative), node stage (positive nodes; number of examined nodes), metastasis stage, and perineural, vascular, and lymphatic invasion. Margins assessed included the pancreatic neck, bile duct, uncinate/retroperitoneal, and duodenal.

Statistical Analysis Data analyses were carried out with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) and Excel 2004 (Microsoft, Seattle, WA, USA). Survival time was measured from the time of PD until death

Table 1 Case Series of Resected Pancreatic Cancer Patients with Preoperative Serum CA 19-9 Elevation

	Year	*N*	Cut-off (IU/ml)	Ca19-9>1,000 (*n*)
Berger et al.[11]	2008	385	180[a]	None
Zhang et al.[12]	2008	104	353	None
Smith et al.[13]	2008	52	150	None
Halloran et al.[14]	2008	94	150	None
Ong et al.[15]	2008	53	473	NA
Karachristos et al.[5]	2005	63	100	None
Berger et al.[16]	2004	129	200	NA
Nakao et al.[17]	1998	148	2,000	yes (>15)
Montgomery et al.[10]	1997	40	180[a]	None

[a] Post-resection CA 19-9 cut-off; *NA* information was not available

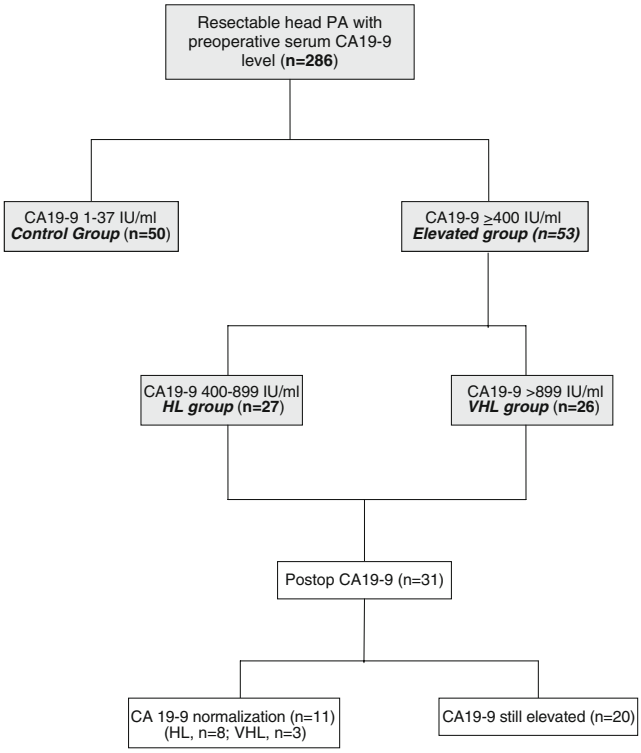


Figure 1 Selection of patients.

or last follow-up (censor date was November 1, 2008). Statistical associations between categorical factors were assessed using the Fisher exact test. The association of categorical factors with survival was assessed using the Kaplan–Meier method and was tested using the log-rank test. Statistical significance was set at p value <0.05.

Results

Of the 344 consecutive patients with resectable PA who underwent PD, 286 patients matched the inclusion criteria for this study. Fifty patients met criteria for the *control* group (CA 19-9≤37 IU/ml). Fifty-three patients met criteria for the *elevated* group (CA19-9≥400 IU/ml) after matching the exclusion criteria. Within the *elevated* group, *HL* (CA19-9 400–899 IU/ml) and *VHL* (CA 19-9≥900 IU/ml) subgroups comprised 27 patients and 26 patients, respectively (Fig. 1).

Clinical Characteristics Clinical and pathological characteristics of the *elevated* and *control* groups were comparable (Table 2). No patients were lost to follow-up and median follow-up was 47 months (95% CI [45.4–56.3]). Overall perioperative morbidity and mortality for all 103 patients

Table 2 Comparison of Clinical and Pathologic Parameters in *Control* (CA 19-9≤37 IU/ml) and *Elevated* (CA 19-9≥400 IU/ml) Serum CA 19-9 Groups

	Control group (n=50)	Elevated group (n=53)	p value
Age	64 (45–82)	66 (44–80)	Ns
Male n (%)	19 (38)	21(40)	Ns
Weight loss n (%)	28 (56)	32 (60)	Ns
Preoperative biliary stenting n (%)	14 (28)	23 (43)	Ns
Preoperative CA 19-9 (IU/ml)	33 (1–36)	1,756 (400–13,100)	0.02
Median follow-up (months)	42 ([37.6–53.5])	51 ([47.9–63])	Ns
Operative duration (min)	344 (182–561)	324 (190–697)	Ns
Blood loss (ml)	724 (150–5,000)	1,082 (300–4,000)	Ns
Vascular resection n (%)	12 (24)	14 (26)	Ns
Tumor size (cm)	3 (range 0.6–4.5)	3 (range 0.9–5.9)	Ns
Tumor differentiation n (%)			
Poor	19 (38)	29 (55)	Ns
Moderate	24 (48)	21 (40)	Ns
Well	7 (14)	3 (5)	Ns
Positive margin n (%)	9 (18)	13 (24)	Ns
Examined lymph nodes	12 (5–29)	12 (4–28)	Ns
N1 status n (%)	29 (58)	39 (73)	Ns
Perineural invasion n (%)	20 (40)	29 (55)	Ns
Perivascular invasion n (%)	19 (38)	27 (51)	Ns
Morbidity n (%)	18 (36)	15 (28)	Ns
Mortality n (%)	0	0	Ns
LOS (days)	13 (range 6–68)	11 (range 6–27)	Ns
Adjuvant treatment n (%)	28 (56)	34 (64)	Ns

Results are shown as median (range) except follow-up which is expressed as median with 95% confidence interval ([CI]). Other parameters are expressed as n (%) where n=number of patients and %=percentage of patients

LOS length of hospital stay

Table 3 Patients with Normalized (CA 19-9≤37 IU/ml) Postoperative Serum CA 19-9 Level

Patients	Preoperative CA19-9 serum level (IU/ml)	Postoperative CA19-9 serum level (IU/ml)	Survival (months)
1	408	27	11[b]
2	467	30	17[b]
3	543	15	23[b]
4	560	33	29[b]
5	564	17	20[b]
6	569	25	26[b]
7	571	20	31[b]
8	847	34	6[b]
9	*992*	*31*	*62*[b]
10	*4,649*	*18*	*82*[a]
11	*13,100*	*16*	*12*[b]

Patients in *italics* are patients with preoperative VHL (>900 IU/ml)

[a] Alive without recurrence

[b] Death

was 32% and 0%, respectively. Postoperative serum CA 19-9 level was available in 31 patients of the *elevated* group (58%). Postoperative serum CA 19-9 level normalization occurred in 11 patients (eight HL, three VHL; 21%) (Table 3). Conversely, 20 patients (38%) had postoperative CA19-9 serum level decreasing without reaching the normal range or increasing compared to their preoperative value.

Survival Analysis The median overall survival of the *control* group (n=50) versus the *elevated* group (n=53) was 22 vs. 15 months, respectively (p=0.02). Overall 3-year overall survival was 32% in the *control* group vs. 14% in the *elevated* group, respectively (p=0.03) (Fig. 2). Within the *elevated* group, there was no statistical difference in median survival (15 and 12 months) and 3-year overall survival (13% and 15%) between patients with HL or VHL (Fig. 3). The median overall survival of patients who normalized serum CA 19-9 level post-resection (n=11) or not (n=20) was 23 and 16 months, respectively (p=0.02) (Fig. 4). There was no statistical difference in median (23 and 22 months) and 3-year overall survival (32% and 27%) between patients who normalized their serum CA 19-9 level and patients in the *control* group (Fig. 4).

Discussion

The radiographic ability to identify metastatic or locally unresectable PA continues to improve. Nonetheless, even with careful preoperative staging using state-of-the-art technology, the prevalence of undetectable metastatic or locally advanced disease remains approximately 15–20%.[18]The median survival for patients with unresectable disease is 6–12 months and only systemic therapies have demonstrated a potential survival benefit.[19] Sparing patients with unresectable disease who will not obtain a survival benefit from surgery remains a major challenge in the current care of patients with pancreatic cancer.

The serum CA 19-9 tumor antigen is currently the most clinically useful serologic marker for pancreatic cancer. The majority of PA will secrete CA 19-9 and have measurable serum levels.[20] Thus, many investigators have turned to serum CA 19-9 as a possible prognostic marker for tumor

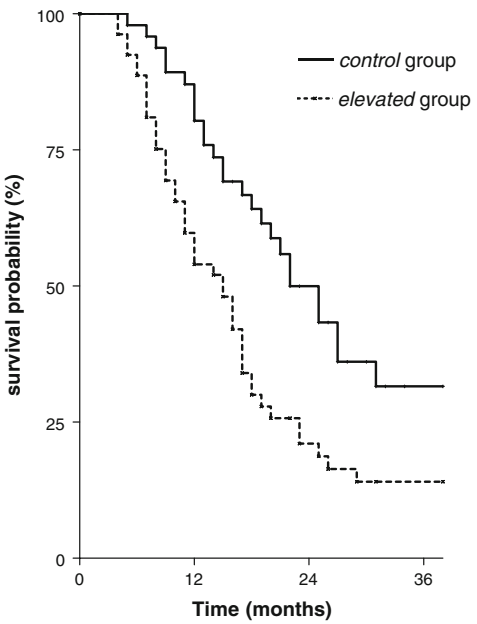
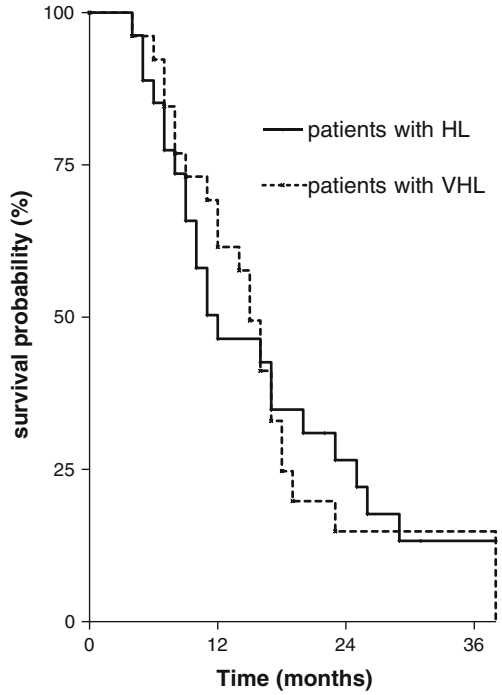


Figure 2 Survival of patients having pancreatoduodenectomy and with preoperative normal (CA19-9≤37 IU/ml) (n=50) or elevated (CA 19-9≥400 IU/ml) (n=53) CA19-9 serum level.



(log-rank test p=0.95)

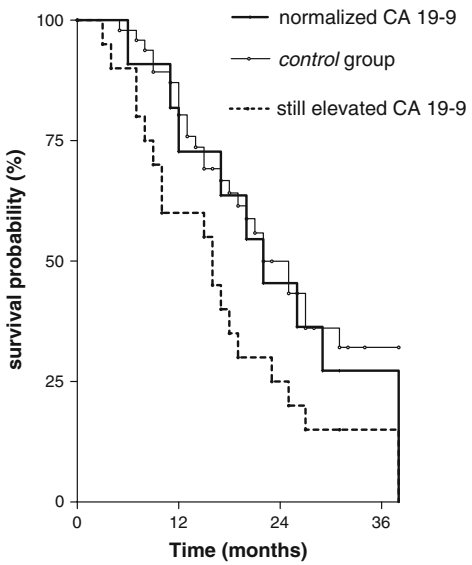
Patients alive at risk

Time (months)	0	12	24	36
HL group	27	13	7	3
VHL group	26	18	4	4

Figure 3 Survival of patients having pancreatoduodenectomy and with preoperative high level (*HL*, CA 19-9 400–899 IU/ml) (n=27) or very high level (*VHL*, CA19-9>899 IU/ml) (n=26) serum CA 19-9.

resectability, recurrence, and patient survival. Importantly, CA 19-9 levels detected by conventional antibody tests may be affected by Lewis blood group phenotypes.[20] In fact, pancreatic cancer patients with a Lewis negative (a-, b-) phenotype will have an undetectable CA 19-9 level. Moreover, 7% to 10% of patients may have undetectable CA 19-9 levels even in the face of metastatic or recurrent disease.[21] Berger et al. showed that patients with undetectable CA 19-9 levels actually had improved survival.[16] The findings of our study are not statistically different by including or excluding patients with undetectable levels which reinforces our findings that normalization of preoperatively elevated serum CA 19-9 is a good prognostic sign and confers a survival advantage. Corroborating our study in part is Ferrone et al.[9] who found that a postoperative decrease in serum CA 19-9 level and an absolute postoperative serum CA 19-9 value less than 200 IU/ml were both significant predictors of survival in patients with PA.

Another use of preoperative serum CA 19-9 level may be in its ability to detect patients at greater risk for having undetected occult metastatic or locally advanced disease.[12] Moreover, some investigators have found that elevated preoperative serum CA 19-9 levels are significantly associated with tumor unresectability, although the cut-off levels reported range from 100 to 350 IU/ml.[8–17] Based on these data, some speculate that preoperative staging of a potentially resectable PA should routinely include serum CA 19-9 levels after biliary decompression. In the case where careful preoperative staging indicates a radiographically resectable PA without evidence of distant metastasis but the serum CA 19-9 levels are highly elevated, the staging might be better clarified by the use of laparoscopy. Indeed, the benefit of laparoscopy in the radiographically resectable patient with normal CA 19-9 is still under debate. However, laparoscopy has a higher yield in detecting unknown carcinomatosis or liver metastasis in 5% to 10% of patients if performed in patients with high serum CA 19-9 levels.[4, 5, 8] Alternatively, a neoadjuvant approach would allow time and follow-up restaging which may spare resection in patients where progressive disease is imminent. The time selection of the neoadjuvant approach, however, is also not uniformly reliable in weeding out micrometastatic and early recurrence patients.[22]



(log rank test normalized vs. control p=0.78
log rank test normalized vs. still elevated **p=0.02**)

Patients alive at risk

Time (months)	0	12	24	36
normalized group	11	9	6	3
still elevated group	20	14	6	3
control group	50	39	16	5

Figure 4 Survival of patients in the *elevated* group who normalized (CA19-9≤37 IU/ml) (n=11) serum CA 19-9 level post-resection are compared to patients who failed to normalize (n=20) post-resection and patients of the *control* group (i.e., normal preoperative CA 19-9 serum level).

Our study confirmed that elevated serum CA 19-9 level correlates with a poor survival as corroborated by several studies.[8–17, 23] Our study also found that patients with VHL (over 800 IU/ml) had equivalent survival as patients with HL. Thus, although a serum CA 19-9 level of 150 or 300 IU/ml preoperatively may discriminate between patients with good and poor outcome,[5, 12–14, 16] levels over 400 IU/ml, however high, have no additional effect on survival. Median survival of patients with ≥400 IU/ml serum CA19-9 levels is higher than patients with metastatic or unresectable disease.[18] Thus, patients with resectable PA must be given the benefit of the doubt and be offered resection despite high serum levels of CA 19-9 levels. Consideration may be given in these patients to enrollment in an aggressive adjuvant regimen to improve survival.[24, 25]

Postoperative changes of serum CA 19-9 levels have been examined previously. Indeed, Montgomery et al.[10] demonstrated that normalization of serum CA 19-9 was a good prognostic factor. On the other hand, the cut-off in this study was 180 IU/ml and very few patients with high serum CA 19-9 levels (>400 IU/ml) were enrolled. Nakao et al.[17] published over 10 years ago a report of 15 resected patients with serum CA 19-9 levels >2,000 IU/ml. These patients had a median survival of 6 months and were all dead after 19 months of follow-up. Recently, Hernandez et al.[26] reported the importance of velocity of normalization in predicting a favorable prognosis. However, Hernandez's series did not include patients with very high serum CA 19-9 levels. In our series, normalization of serum CA 19-9 levels was not rare and occurred in 21% of patients. Moreover, the novel aspect of this study is that even patients with preoperative VHL (992, 4,649, and 13,100 IU/ml) may normalize their serum CA 19-9 level and have equivalent overall survival compared to patients with normal preoperative serum CA 19-9 levels. We would speculate that absence of normalization is a marker of persistent tumor burden after PD. Unfortunately, no preoperative criteria permitted us to predict which patients would normalize serum CA 19-9 levels and likely to benefit from surgery. Thus, we propose that patients with highly elevated serum CA 19-9 level (after biliary decompression) have optimal and timely (<1 month preoperatively) preoperative imaging. If distant metastasis are not detected, strong consideration should be given to laparoscopy to detect occult metastases. If optimal preoperative imaging, explorative laparoscopy, and laparotomy are negative, however, then patients should undergo resection. Postoperative serum CA19-9 measurement appears to be helpful in determining patient's prognosis and may be useful for planning care and family support.[10, 11] We further speculate that prospective evaluation of a neo-adjuvant therapy in this subpopulation of patients may have merit.

Conclusions

Serum CA 19-9 level helps discriminate pancreatic cancer patients with good and poor prognosis. Nonetheless, patients with very high serum CA 19-9 levels may still potentially benefit from surgery. Thus, careful preoperative staging and possibly laparoscopic staging are recommended to spare patients with unresectable or metastatic disease from surgery. If staging is negative, however, such patients should be explored and not denied surgical resection on the basis of high serum CA 19-9 levels.

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J Gastrointest Surg (2009) 13:1798–1805
DOI 10.1007/s11605-009-0936-1

2009 SSAT POSTER PRESENTATION

National Complication Rates after Pancreatectomy: Beyond Mere Mortality

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Received: 27 February 2009 / Accepted: 20 May 2009 / Published online: 9 June 2009

Abstract
Introduction National studies on in-hospital pancreatic outcomes have focused on mortality. Non-fatal morbidity affects a greater proportion of patients.
Methods The Nationwide Inpatient Sample 1998–2006 was queried for discharges after pancreatectomy. Rates of major complications (myocardial infarction, aspiration pneumonia, pulmonary compromise, perforation, infection, deep vein thrombosis/pulmonary embolism, hemorrhage, or reopening of laparotomy) were assessed. Predictors of complication(s) were evaluated using logistic regression. Their independent effect on in-hospital mortality, length of stay, and discharge disposition was assessed.
Results Of 102,417 patient discharges, 22.7% experienced a complication. Complication rates did not decline significantly over time, while mortality rates did. Independent predictors of complications included age ≥75 [referent, 19–39; adjusted odds ratio (OR) 1.34, 95% confidence interval (CI) 1.2–1.5, $p<0.0001$], total pancreatectomy (vs proximal, OR 1.29, 95%CI 1.1–1.5, $p=0.0025$), and low hospital resection volume (vs high, OR 1.61, 95%CI 1.4–1.8, $p<0.0001$). Complications were a significant independent predictor of death (OR 7.76, 95%CI 6.7–8.8, $p<0.0001$), prolonged hospital stay (OR 6.94, 95%CI 6.2–7.7, $p<0.0001$), and discharge to another facility (OR 0.28, 95%CI 0.26–0.3, $p<0.0001$).
Conclusions Despite improvements in mortality, complication rates remain substantial and largely unchanged. They predict in-hospital mortality, prolonged hospital stay, and delayed return to home. The impact on healthcare costs and quality of life deserves further study.

Keywords Pancreatectomy · Morbidity · Nationwide inpatient sample

To be presented in part at the Society for Surgery of the Alimentary Tract, Digestive Diseases Week, Chicago, May 30–June 4, 2009.

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Introduction

Pancreatic resection is a technically complex operation with significant attendant morbidity and mortality. While much literature has focused on recent improvements in perioperative mortality,[1–3] complication rates remain high in reported series.[4,5] The technical nature of the operation with requisite multi-organ resection, usually performed on an older population with significant comorbid illness, contributes to the potential for complications.[6] The aim of this study was to assess the rates of major perioperative complications and their associated risk factors.

Using a nationally representative administrative database, we assessed rates of major in-hospital complications following pancreatectomy as well as their associated risk

factors. We also assessed the effect of major postoperative complications on the likelihood of in-hospital mortality, prolonged length of stay, and discharge to another facility rather than home.

Materials and Methods

Patient Sources and Cohort Assembly

The Nationwide Inpatient Sample (NIS) was queried between 1998 and 2006 for patient discharges for pancreatic resection (NCHS[7]; ICD-9-CM procedure codes 52.6, 52.7, 52.51, 52.52, 52.53, and 52.59). As part of the government-sponsored Healthcare Cost and Utilization Project, the NIS is a national, all-payer discharge database containing information for approximately seven million hospital discharges annually. This represents a stratified sample of 20% of nonfederal US community hospitals from participating states, including academic and specialty hospitals. The NIS weighting strategy facilitates population-based estimates to be drawn at the national level. All statistical analyses were performed based on these survey weights; results are presented as either weighted (national) or unweighted (actual) frequencies.

Patient Discharge and Hospital Characteristics

Demographic information, including age at admission, sex, and race was collected. Race information was excluded from all explanatory analyses because of the high rate of missing values. Records for patients aged <18 or >95 years old were also excluded. Patient discharges were assigned a Charlson comorbidity score,[8] based on concurrent ICD-9-CM diagnoses, following the method described by Deyo et al.[9] Because of the relative rarity of records with scores greater than 5, Charlson score was collapsed into four groups as follows: group 1, Charlson score of 0 or 1; group 2, score of 2 or 3; group 3, score of 4 through 7; and group 4, score of ≥8. Indication for operation was defined as benign disease (including pancreatitis and cystic disease, ICD-9 577.0-9; and benign neoplasms of islet cells, the duodenum and ampulla, ICD-9 codes 211.7, 211.2, 211.5, respectively; and duodenal diverticular disease, ICD-9 code 562.0), malignant neoplasm (including malignancies of the pancreas, extrahepatic bile ducts, duodenum, ICD-9 codes 157.0-9, 156.1, 152.0, respectively), or other indication (including trauma and those without an indication reported).

Hospital surgical volume for pancreatectomy was assessed over the time period of the study. It was divided into equal thirds and defined as low (average of eight or less resections per year), medium (average of nine to 32 resections per year), or high (average of >32 resections per year).

Outcome Measures

The identified cohort of patient discharges was analyzed for codes for major postoperative complications. These diagnoses and codes were chosen based on their validation as true complications rather than comorbidities in methods developed by Lawthers et al.[10] These were defined as secondary diagnoses of (1) postoperative infection (except wound and pneumonia), (2) acute myocardial infarction, (3) aspiration pneumonia, (4) deep venous thrombosis and pulmonary embolism, (5) postoperative pulmonary compromise, (6) postoperative gastrointestinal hemorrhage (7) reopening of laparotomy, and (8) procedure-related lacerations or perforations. Complete listing of ICD-9-CM codes used is found in the Appendix. Complications specific to pancreatectomy, such as pancreatic leak or fistula, were not examined since the current ICD-9-CM codes do not capture these accurately.

The secondary outcome of in-hospital mortality was defined as death due to any cause prior to discharge regardless of the time from operation. Prolonged length of stay was defined as a hospital stay that was more than one standard deviation above the mean length of stay for the cohort. Discharge disposition was dichotomized into either discharge to home or discharge to another facility, including skilled nursing facility or nursing home; patients who died in-hospital, who left against medical advice, or whose disposition was unknown were excluded from these analyses. Adjustments were not made for the specific hospital or region when analyzing this endpoint.

Statistical Analysis

Predictors of occurrence of any identified complication were evaluated using logistic regression. Covariates controlled for in this model included: sex, age, indication for operation, Charlson score, hospital teaching status, hospital annual resection volume, and type of resection. Predictors of in-hospital death, prolonged length of stay, and discharge disposition were evaluated in an analogous fashion, but with presence of a complication also used as an additional covariate in these models. Trend analyses were conducted to evaluate yearly overall rates of complications and in-hospital death, as well as the relationship between hospital volume and these outcomes. All statistical analyses were performed with advanced survey procedures using SAS (v9.1, Cary, NC, USA).

Results

Cohort Characteristics

There were 102,417 patient discharge records identified between 1998 and 2006. Of these, mean age at admission was 60.1 years, 51,175 (50.0%) were male, and 58,276 (76.5%) of those with race recorded were white (of note is that race was not available for 25.6% of the cohort). Most patients were in the Charlson group 1, with a score of 0 or 1 (33.2%, n=33,971), with group 2 (score of 2 or 3) comprising 28.8% (n=29,524), group 3 (score of 4–7) having 5.6% (n=5739), and the highest score group (score ≥8) containing 32.4% (n=33,183). The majority of operations were performed for malignant disease (52.0%, n=53,223), with the most frequent procedure being proximal pancreatectomy (54.9%, n=56,207). Most procedures were performed at teaching hospitals (74.4%, n=76,160).

Overall, 23,238 (22.7%) experienced a major postoperative complication as defined above. The overall in-hospital mortality rate was 6.3% (n=6415). Mean length of stay was 16.5 days (standard deviation, 16.1). After excluding records for which the discharge disposition was not known, not an in-hospital death, and not recorded as discharge again medical advice, the majority was discharged to home (87.2%, n=83,571). A comparison of the demographics for the group with a complication and the group without a complication is provided in Table 1. The most frequent complication was postoperative pulmonary compromise (51.7%, n=12,013). On unadjusted analysis, those in the complication group were 8.92 times more likely to die in-hospital than those without a complication [95% confidence interval (CI) 7.69–10.34, p<0.0001].

Trend Analyses

There was no significant change in the rate of major complication over the time period studied (p=0.069). The rate was 23.3% in 1998 and 22.5% in 2006, with a peak in 2002 of 24.2%. However, there was a significant linear decline in in-hospital mortality over this same period (p<0.0001). In 1998, the rate was 8.5%, but declined to 4.8% by 2006, its nadir (Fig. 1).

An inverse correlation was also seen for complication rates and annual hospital resection volume. High-volume hospitals had the lowest overall complication rate (17.8%) compared with medium-volume (23.1%) and low-volume hospitals (27.2%). This was significant on trend test (p<0.0001). Similarly for in-hospital death, a significant linear downtrend was seen (p<0.0001). For high-volume hospitals, the in-hospital mortality rate was 3.3% compared with medium volume, 6.4%, and low volume, 9.1%.

Table 1 Patient Demographics for Patient Discharges with a Complication and Without a Complication

Factor	Complication group	No complication group	p value
Mean age in years (SD)	61.1 (0.33)	59.8 (0.22)	<0.0001
	Weighted N (weighted %)	Weighted N (weighted %)	
Male sex	13,414 (57.7)	37,761 (47.7)	<0.0001
Race	(% missing=23.8)	(% missing=35.4)	<0.0001
White	12,857 (72.6)	45,419 (77.7)	
Black	2,112 (11.9)	5,523 (9.4)	
Other	2,742 (15.5)	7,519 (12.9)	
Died in-hospital	4,395 (18.9)	2,019 (2.6)	<0.0001
Indication for operation			<0.0001
Malignant neoplasm	11,473 (49.4)	41,750 (52.7)	
Benign disease	6,768 (29.1)	26,078 (32.9)	
Other indication	4,998 (21.5)	11,351 (14.3)	
Hospital resection volume			<0.0001
Low (≤8)	9,351 (40.2)	25,055 (31.6)	
Medium (9–32)	7736 (33.3)	25,743 (32.5)	
High (>32)	6,151 (26.5)	28,381 (35.8)	
Hospital teaching status			<0.0001
Teaching	16,084 (69.2)	50,077 (75.9)	
Non-teaching	7,150 (30.8)	19,080 (24.1)	
Charlson score			<0.0001
0–1	7,657 (32.9)	26,314 (33.2)	
2–3	6,570 (28.3)	22,954 (29.0)	
4–7	1,779 (7.7)	3,961 (5.0)	
≥8	7,233 (31.1)	25,950 (32.8)	
Pancreatectomy type			<0.0001
Total	1,729 (7.4)	4,237 (5.4)	
Proximal	12,857 (55.3)	43,351 (54.8)	
Distal/middle	8,653 (37.2)	31,591 (39.9)	
Pulmonary compromise	12,013 (51.7)	N/A	N/A
Infection (excludes wound and pneumonia)	1,610 (6.9)	N/A	N/A
Myocardial infarction	793.2 (3.4)	N/A	N/A
Aspiration pneumonia	506.4 (2.2)	N/A	N/A
Deep venous thrombosis and/or pulmonary embolism	1,670 (7.2)	N/A	N/A
Gastrointestinal hemorrhage	4,129 (17.8)	N/A	N/A
Reopening of laparotomy	1,334 (5.7)	N/A	N/A
Procedure-related perforation or laceration	1,183 (5.1)	N/A	N/A

Primary Endpoint, Major Postoperative Complications

After adjusting for other factors, significant predictors of having a complication included age ≥75 years [referent,

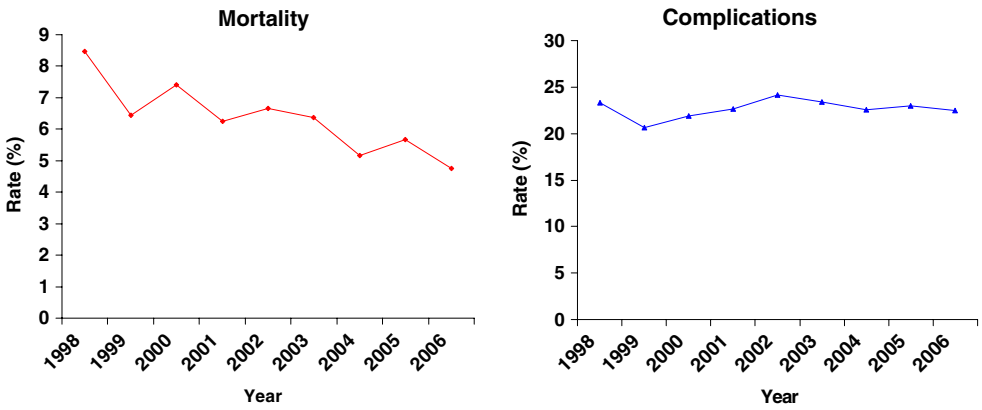

Figure 1 Trends in in-hospital mortality (**a**) and complication rates (**b**), 1998 to 2006.

19–39; adjusted odds ratio (OR) 1.34, 95%CI 1.17–1.52, $p<0.0001$], total pancreatectomy compared with proximal pancreatectomy (OR 1.29, 95%CI 1.09–1.53, $p=0.0025$), indication for procedure other than benign or malignant disease (referent, malignant; OR 1.75, 95%CI 1.56–1.97, $p<0.0001$), hospitals with low annual resection volume (OR 1.61, 95%CI 1.41–1.84, $p<0.0001$) or medium volume (OR 1.35, 95%CI 1.19–1.54, $p<0.0001$) compared with high volume, and Charlson score of 4–7 (OR 1.32, 95%CI 1.13–1.55, $p=0.0006$) as compared with a score of 0 or 1 (Table 2). Significant protective factors included female sex (OR 0.67, 95%CI 0.63–0.72, $p<0.0001$), age groups 40–54 (OR 0.82, 95%CI 0.72–0.93, $p=0.0014$) and 55–64 (OR 0.84, 95%CI 0.74–0.96, $p=0.0083$) versus age 19–39, distal/middle pancreatectomy compared with proximal pancreatectomy (OR 0.74, 95% CI 0.68–0.81, $p<0.0001$), and procedure at a teaching hospital (OR 0.89, 95%CI 0.80–0.98, $p=0.0229$).

Secondary Endpoint, In-Hospital Mortality

On multivariable analysis, the presence of complication was the strongest predictor of in-hospital death, increasing the odds nearly eightfold (OR 7.76, 95%CI 6.69–8.78, $p<0.0001$). Other significant independent predictors included older age (vs <55) with a magnitude of effect ranging from 1.45 for those 55–64 (95%CI 1.08–1.94, $p=0.0131$) to 3.29 for those ≥75 (95%CI 2.49–4.34, $p<0.0001$), performance of a total pancreatectomy (referent, proximal pancreatectomy; OR 2.90, 95%CI 2.22–3.79, $p<0.0001$), and both low and medium annual hospital resection volume (referent, high >32; OR 2.33, 95%CI 1.88–2.90, $p<0.0001$ and OR 1.75, 95%CI 1.43–2.15, $p<0.0001$, respectively). Significant protective factors included female sex (OR 0.79, 95% CI 0.70–0.89, $p=0.0002$), benign disease (referent, malignant; OR 0.55, 95%CI 0.42–0.72, $p<0.0001$), distal/middle pancreatectomy compared with proximal pancreatectomy (OR 0.80, 95%CI 0.68–0.94, $p=0.0070$) and Charlson score of 2 or 3 (OR 0.57, 95%CI 0.44–0.73, $p<0.0001$) or ≥8 (OR 0.54, 95%CI 0.42–0.70, $p<0.0001$), both compared with score of 0 or 1. The complete regression is presented in Table 3.

Secondary Endpoint, Prolonged Length of Stay

On multivariable analysis, the presence of a complication was the strongest predictor of prolonged length of stay, increasing the odds nearly sevenfold (OR 6.94, 95%CI 6.24–7.73, $p<0.0001$). Operations performed at teaching hospitals (OR 1.41, 95%CI 1.23–1.61, $p<0.0001$) and

Table 2 Multivariable Analysis of Predictors of Having a Complication

Factor	Adjusted odds ratio (95%CI)	*p* value
Female sex	0.67 (0.63, 0.72)	<0.0001
Age group (ref=19–39 years)		
40–54	0.82 (0.72, 0.93)	0.0014
55–64	0.84 (0.74, 0.96)	0.0083
65–74	1.03 (0.91, 1.17)	0.6191
≥75	1.34 (1.17, 1.52)	<0.0001
Indication for operation (ref = malignant)		
Benign disease	1.10 (0.96, 1.26)	0.1701
Other indication	1.75 (1.56, 1.97)	<0.0001
Hospital resection volume (ref = high >32)		
Low (≤8)	1.61 (1.41, 1.84)	<0.0001
Medium (9–32)	1.35 (1.19, 1.54)	<0.0001
Teaching hospital	0.89 (0.80, 0.98)	0.0229
Charlson score (ref = group 1, score 0 or 1)		
2–3	0.93 (0.82, 1.05)	0.2387
4–7	1.32 (1.13, 1.55)	0.0006
≥8	0.90 (0.78, 1.03)	0.1106
Pancreatectomy type (ref = proximal)		
Total	1.29 (1.09, 1.53)	0.0025
Distal/middle	0.74 (0.68, 0.81)	<0.0001

Ref referent

Table 3 Logistic Regression Model of the Independent Effect of Having a Complication on the Odds of In-Hospital Mortality

Factor	Adjusted odds ratio (95%CI)	*p* value
Complication present	7.76 (6.69, 8.78)	<0.0001
Female sex	0.79 (0.70, 0.89)	0.0002
Age group (ref=19–39 years)		
40–54	1.25 (0.95, 1.65)	0.1158
55–64	1.45 (1.08, 1.94)	0.0131
65–74	2.06 (1.55, 2.74)	<0.0001
≥75	3.29 (2.49, 4.34)	<0.0001
Indication for operation (ref = malignant)		
Benign disease	0.55 (0.42, 0.72)	<0.0001
Other indication	1.46 (1.18, 1.80)	0.0004
Hospital resection volume (ref = high >32)		
Low (≤8)	2.33 (1.88, 2.90)	<0.0001
Medium (9–32)	1.75 (1.43, 2.15)	<0.0001
Teaching hospital	0.96 (0.81, 1.12)	0.5855
Charlson score (ref = group 1, score 0 or 1)		
2–3	0.57 (0.44, 0.73)	<0.0001
4–7	0.77 (0.57, 1.04)	0.0880
≥8	0.54 (0.42, 0.70)	<0.0001
Pancreatectomy type (ref = proximal)		
Total	2.90 (2.22, 3.79)	<0.0001
Distal/middle	0.80 (0.68, 0.94)	0.0070

Ref referent

Table 4 Multivariable Analysis of the Independent Effect of Complications on the Odds of Having a Prolonged Length of Stay

Factor	Adjusted odds ratio (95% CI)	*p* value
Complication present	6.94 (6.24, 7.73)	<0.0001
Female sex	0.81 (0.73, 0.89)	<0.0001
Age group (ref=19–39 years)		
40–54	0.99 (0.81, 1.22)	0.9548
55–64	0.99 (0.80, 1. 22)	0.9177
65–74	0.97 (0.80, 1.19)	0.7844
≥75	1.16 (0.94, 1.43)	0.1652
Indication for operation (ref = malignant)		
Benign disease	1.28 (1.06, 1.54)	0.0096
Other indication	1.21 (1.01, 1.45)	0.0342
Hospital resection volume (ref = high >32)		
Low (≤8)	2.10 (1.78, 2.48)	<0.0001
Medium (9–32)	1.68 (1.44, 1.96)	<0.0001
Teaching hospital	1.41 (1.23, 1.61)	<0.0001
Charlson score (ref = group 1, score 0 or 1)		
2–3	0.88 (0.74, 1.05)	0.1512
4–7	0.80 (0.62, 1.04)	0.0880
≥8	0.78 (0.64, 0.95)	0.0906
Pancreatectomy type (ref = proximal)		
Total	1.06 (0.85, 1.32)	0.6186
Distal/middle	0.59 (0.52, 0.67)	<0.0001

Ref referent

those with low (OR 2.10, 95%CI 1.78–2.48, $p<0.0001$) or medium (OR 1.68, 95%CI 1.44–1.96, $p<0.0001$) annual resection volumes, compared with high volume, were also more likely to be associated with prolonged lengths of stay. Female sex (OR 0.81, 95%CI 0.73–0.89, $p<0.0001$) and distal/middle pancreatectomy (referent, proximal; OR 0.52–0.67, $p<0.0001$) were significantly protective against prolonged hospital stays (Table 4).

Secondary Endpoint, Discharge to Home

After implementing the exclusion criteria described in "Materials and methods," 95,899 patient discharges were analyzed. On multivariable modeling (Table 5), the presence of a complication reduced the odds of discharge to home by 72% (OR 0.28, 95%CI 0.26, 0.31, $p<0.0001$). Those aged 65–74 and those ≥75 were also less likely to be discharged home compared to patients aged 19–39 years (OR 0.42, 95%CI 0.34–0.52, $p<0.0001$ and OR 0.15, 95%CI 0.12–0.18, $p<0.0001$, respectively). Compared with hospitals with high annual resection volume, both low- and medium-volume hospitals decreased the odds of discharge to home (OR 0.51, 95%CI 0.42–0.62, $p<0.0001$ and OR 0.81, 95%CI 0.66–0.99, $p=0.0401$, respectively). Distal/middle pancreatectomy, compared with proximal pancreatectomy, increased the odds of discharge to home (OR 1.41, 95%CI 0.52–0.67, $p<0.0001$), as did age 40–54 (referent 19–39; OR 1.48, 95%CI 1.18–1.84, $p=0.0006$).

Discussion

In this study, we found that major postoperative complications occur with far greater frequency than perioperative death, affecting approximately one quarter of all patients. There was a significant inverse correlation between annual hospital resection volume and rates of complication and in-hospital death. For medium-volume hospitals, the complication rate increases by 23% over high-volume hospitals; for low-volume hospitals, the rate of complication increases another 23% over the medium-volume hospital rate. Postoperative complications are also correlated with a nearly eightfold increase in the risk of in-hospital death,

Table 5 Multivariable Analysis of the Independent Effect of Complications on the Odds of Discharge to Home

Factor	Adjusted odds ratio (95%CI)	*p* value
Complication present	0.28 (0.26, 0.31)	<0.0001
Female sex	0.87 (0.79, 0.96)	0.0043
Age group (ref=19–39 years)		
40–54	1.48 (1.18, 1.84)	0.0006
55–64	0.93 (0.75, 1.15)	0.5126
65–74	0.42 (0.34, 0.52)	<0.0001
≥75	0.15 (0.12, 0.18)	<0.0001
Indication for operation (ref = malignant)		
Benign disease	0.95 (0.78, 1.16)	0.6248
Other indication	0.66 (0.57, 0.77)	<0.0001
Hospital resection volume (ref = high >32)		
Low (≤8)	0.51 (0.42, 0.62)	<0.0001
Medium (9–32)	0.81 (0.66, 0.99)	0.0401
Teaching hospital	0.93 (0.82, 1.06)	0.2866
Charlson score (ref = group 1, score 0 or 1)		
2–3	0.99 (0.83, 1.19)	0.9438
4–7	0.87 (0.69, 1.08)	0.2077
≥8	1.08 (0.89, 1.30)	0.4321
Pancreatectomy type (ref = proximal)		
Total	0.92 (0.73, 1.16)	0.4781
Distal/middle	1.41 (0.52, 0.67)	<0.0001

Ref referent

as well as prolonged hospital stays, and reduced likelihood of discharge to home. Over the time period of the study, a significant decline in in-hospital mortality was seen, while the rate of major complication has not similarly improved. As perioperative death rates improve, complication rates deserve increasing attention.

Several authors have noted a decrease in the perioperative mortality of pancreatectomy in recent years.[3,11–13] While more patients are surviving operation, this shifts the attention from mere survival to expected recovery from operation and on the morbidity associated with pancreatectomy. Previous studies that have examined morbidity have focused primarily on complications specific to pancreatic surgery, most notably pancreatic fistula.[11,14–17] Their work has shed much needed light on the risks and benefits or lack thereof of specific practices such as octreotide administration, drain and stent placement, and feeding tube use. While every effort must be made to prevent complications such as pancreatic fistula, biliary leak with possible subsequent intra-abdominal abscess, other more general postoperative complications involving the cardiovascular and pulmonary systems are critically important and may, in fact, be a more ready target for systematic quality improvement.

Patients undergoing complex surgical procedures including pancreatectomy are at risk for a host of general postoperative complications, including myocardial infarction, pneumonia, and pulmonary embolism. These complications have been shown to increase risk of death, even in previously healthy patients.[18] Complications have also been correlated with longer mean lengths of stay and an increased likelihood of readmission.[19] Additionally, patients who experience one complication have been shown to be at increased risk for subsequent complications.[20,21] These negative outcomes associated with postoperative complications demonstrate the importance of studying their risk factors in an effort to gain insight into preventative strategies and early intervention.

Our work represents an updated national perspective on this important issue of major postoperative complications after pancreatectomy. Unlike previous studies that report on either single-institution experiences,[14,19] or less recent time periods,[11] this analysis includes patient discharges from across the USA at both teaching and non-teaching hospitals. It also focuses on the impact of general, multi-system complications rather than pancreatectomy-specific technical complications. As the US population ages and an increasing number of operations are performed on older patients with more comorbid illness,[22,23] this type of complication may have increasing relevance. Unlike pancreatectomy-specific complications that may be best addressed by surgical technique,[6] these more general complications could be targeted using principles of medical management and perioperative prevention techniques. Care should be taken to reduce both types of complications, since some general complications may arguably be related to the occurrence of a pancreatectomy-specific complication.

Since this study was conducted in an administrative claims database, there is the potential that the ICD-9 diagnosis codes could represent comorbidities rather than complications. In an effort to minimize this risk, we used only codes that had been previously validated.[10] This necessarily limited our analysis to standard postoperative

Table 6 Overall In-Hospital Mortality and Complication Rates for Three Complex Procedures, 1998–2006

Procedure	In-hospital mortality (%)	Postoperative complication (%)
Pancreatectomy	6.3	22.7
Esophagectomy	3.4	16.6
Coronary artery bypass graft	7.3	31.2

complications rather than those specific to pancreatectomy such as pancreatic fistula and intra-abdominal abscess. There have been several reports, mostly from single institutions, on pancreatectomy-specific complications; the rates of pancreatic leak, for example, have ranged widely, from 5% to 20%.[11,12,24] However, the effect of this, along with the fact that complications in general may be underreported,[25,26] is that our results thus represent a systematic underestimate of true complication rates. In light of this, our finding of a 22.7% complication rate for pancreatectomy should be viewed as a conservative figure.

In order to provide some context for this work, we also looked at two other complex procedures: coronary artery bypass graft (CABG) and esophagectomy. For comparison, the CABG cohort had an overall in-hospital mortality of 7.3% and an overall complication rate of 31.2%. The esophagectomy cohort had an overall mortality rate of 3.4% and an overall complication rate of 16.6% (see Table 6). On trend analysis of the time period studied, both operations succeeded in displaying a significant downward trend in mortality (both $p<0.0001$). In contrast to pancreatectomy, both esophagectomy and CABG had significant linear trends in complication rates ($p<0.0001$), but in opposite directions; esophagectomy complications have significantly decreased (31.5% to 29.8%), while CABG complication rates have increased (15.1% to 20.2%). This underscores the importance of considering these endpoints together, and in context with other findings, in order to generate hypotheses for systematic improvements in patient care. The relationship between perioperative mortality rates and procedural complication rates is complex.

What remains clear is that postoperative complications represent a substantial consideration, particularly as perioperative mortality for pancreatectomy declines. The findings of this study may be useful for preoperative patient counseling, in particular as a way of helping to set appropriate expectations for the postoperative course. The identified cascade of risks for poorer outcomes that accompany a complication also helps to underscore the importance of prevention of complications when possible.

These findings warrant further study, including the use of institutional databases to look at the contribution of specific practices for perioperative medical optimization, such as beta-blockers, deep venous thrombosis prophylaxis, and early extubation guidelines. Also, the effect of major postoperative complications on patient quality of life should be examined. The prevalence of complications and their association with prolonged hospital stays and discharge to other facilities suggests that cost analyses could highlight the importance of prevention strategies.

Conclusion

While mortality rates for pancreatic resection have improved, pancreatectomy remains a morbid operation. Having a complication significantly increases the risk of in-hospital death, prolonged hospital stay, and discharge to another facility rather than to home. The importance of this lies not in dissuading people from undergoing appropriate procedures but in making explicit the risks of pancreatic surgery. If patients and providers share a data-driven, appropriate expectation for the convalescence period, patient satisfaction and quality of life stand to gain immensely.

Acknowledgments The contributions of Dr Fred Anderson in database provision and statistical analysis are greatly appreciated. Dr Simons is funded by the Pancreatic Cancer Alliance. Dr Tseng is funded by a Howard Hughes Medical Institute Early Career Award and the American Surgical Association Foundation.

Appendix

Codes Used to Identify Postoperative Complications

Diagnosis	ICD-9-CM codes
Postoperative infection	008.45, 320.00-.99, 510.0, 510.9, 513.1, 519.2, 590.10-590.11, 590.80, 683
Myocardial infarction	410.00-410.91
Aspiration pneumonia	507.0
Deep venous thrombosis/ pulmonary embolism	415.1, 451.11, 451.19, 451.2, 451.81, 453.8
Pulmonary compromise	514, 518.4, 518.5, 518.81, 518.82
Gastrointestinal hemorrhage	530.82, 531.00-.21, 531.40-.41, 531.60-.61, 532.00-.21, 532.40-.41, 532.60-.61, 533.00-.21, 533.40-41, 533.60-.61, 534.00-.21, 534.40-.41, 534.60-.61, 535.01, 535.11, 535.21, 535.31, 535.41, 535.51, 535.61, 578.9
Reopening of laparotomy	01.23, 03.02, 06.02, 34.03, 35.95, 39.49, 54.12, 54.61
Procedure-related perforation or laceration	530.4, 569.83, 575.4, 29.51, 31.61, 33.41, 33.43, 42.82, 44.61, 46.71, 46.75, 48.71, 50.61, 51.91, 55.81, 56.82, 57.81, 58.41, 69.41

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J Gastrointest Surg (2009) 13:1806–1812
DOI 10.1007/s11605-009-0965-9

SSAT POSTER PRESENTATION

A Cost and Benefit Study of Esophagectomy for Patients with Esophageal Cancer

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Received: 11 May 2009 / Accepted: 22 June 2009 / Published online: 28 July 2009

Abstract
Introduction The incidence of esophageal cancer is increasing all over the world but the cost-and-benefit of esophagectomy for esophageal cancer patients was rarely studied. The aim of this study is to compare the cost-and-benefit of esophagectomy in different stages of esophageal cancer.
Materials and Methods Clinical and utilization data, including medical expenses and reason for treatment, of esophageal cancer patients were collected, summed and followed up for 5 years. The patients were divided into two groups according to their treatments, with or without esophagectomy. The monthly medical expense and relative expense performance index (REPI) were then calculated. Factors influenced total and monthly medical expense and survival time were further analyzed.
Results A total of 310 esophageal cancer patients, 281 male and mean age of 64.3, were included in this study. One hundred forty-nine patients had undergone esophagectomy. The 5-year survival rate, total and monthly medical expense for two groups was 36.0% and 10.2% ($p<0.001$), USD \$22,532.8 vs. 12,256.4 ($p<0.001$) and USD \$2,101.65 vs. 2,033.94 ($p=0.831$), respectively. The REPIs in four different stages were 7.573, 2.422, 2.446 and 0.705. Both esophagectomy and tumor stage were the sole factors that could influence total and monthly medical expense respectively. Both esophagectomy and tumor stage could influence a patient's survival time.
Conclusions Esophagectomy has better performance than non-esophagectomy for patients with stages I to III esophageal cancer. Therefore, adding economical considerations, esophagectomy is recommended for patients, at least earlier than stage III.

Keywords Cost-benefit study · Esophageal cancer · Esophagectomy

Introduction

The incidence of esophageal cancer is increasing all over the world, not only in the West but also in the East. The difference between these cancer cases is in histology. In Western countries, the incidence of adenocarcinoma of esophagus is increased quickly, while the incidence of squamous cell carcinoma remains constant. But in oriental countries, squamous cell carcinoma still accounts for more than 80% of all esophageal cancer cases, and the incidence is also increased quickly.[1–3] The main treatment methods now for esophageal cancer are surgical resection, chemotherapy, and radiotherapy. However, even though the treatment of esophageal cancer has advanced greatly in recent decades, results of esophageal cancer treatments are still poor, and 5-year survival rate is less than 20%.[4–6]

There were several previous studies which studied cost and outcomes among different cancer treatments. However,

Poster presented at the 49th annual meeting of The Society for Surgery of the Alimentary Tract, San Diego, CA, May 17–21, 2008

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most of them were more interesting in discussing results of different treatment methods, follow-up protocols, or both.[7–15] Till now, there are only a few literatures which had discussed about the potentially large medical expense in treating esophageal cancer patients.[16–17] For the era of limited medical budget, appropriate selection of treatment strategies should include the consideration of treatment expense. The aim of this study is to assess cost and benefit of esophagectomy in patients with different stages of esophageal cancer.

Materials and Methods

From Jan. 2000 to June 2003, 327 esophageal cancer patients who were diagnosed of having esophageal cancer and underwent treatments in Taipei Veterans General Hospital (TVGH), a 3,000-acute-bed medical center, were included in this study. Esophageal cancer patients who did not receive treatments in TVGH were excluded from this study. Clinical data of these patients including patients' characteristics, treatment methods, and results of follow-up were collected from their chart records. Stage of esophageal cancer was classified according the American Joint Committee on Cancer criteria.[18] All patients were routinely followed up every 3–6 months. The first date of visit was defined as the date of hospital visit when a patient was impressed as esophageal cancer (ICD-9: 150.0–150.9). The survival time was calculated in month from the first date of visit for treating esophageal cancer to the date when a patient expired or end of the study. Patients were divided into two groups according to the patients who received surgical resection of esophagus and reconstruction or not.

Medical Expense

Because Taiwan has implemented its National Health Insurance (NHI) since 1995, almost all hospitals in Taiwan, including TVGH, were under contracts with the NHI. Therefore, a patient's total medical expense could be abstracted and summed from TVGH's claim files which were submitted to Bureau of National Health Insurance every month. Total medical expense included doctor's fee, ward fee, examination or lab-test fee, operation and anesthesia fee, treatment or procedure fee, medication and service fee, blood and other blood products fee, special material fee, and nutrition fee. Total medical expense of a patient was summed up from the first date of visit for treating esophageal cancer to the last date of receiving treatment for esophageal cancer for five consecutive years unless a patient died. Patients whose total medical expenses were extra-high or belonged to the top 5% of all patients were excluded from this study to prevent outlier effects. The monthly medical expense was calculated by dividing total medical expense with survival time in months. The relative expense performance index (REPI) was calculated by the ratio of survival benefit divided by the following formula:

$$\left(\text{Survival time in month}_{\text{with esophagectomy}} \div \text{survival time in month}_{\text{without esophagectomy}}\right) /$$

$$\left(\text{Monthly expense}_{\text{with esophagectomy}} \div \text{monthly expense}_{\text{without esophagectomy}}\right)$$

REPI is a referential index used by the Advisory Committee of Taiwan's Bureau of National Health Insurance (BNHI) in assessing necessity of alternative treatments for a disease. The unit of medical expense in this study was expressed in USD and 1 USD was averaged approximately 31 New Taiwan Dollar (NTD) during study period.

Statistical Analysis

The relationships between nominal variables were analyzed by χ^2 test. The relationships between continuous variables were analyzed by Student's t test. Linear multiple regression models were used to determine independent variables (factors) of total medical expense, monthly medical expense, and other dependent variables. The Kaplan–Meier product-limit estimator was used to estimate survival for subgroups of patients with esophagectomy or without esophagectomy and compared with the log-rank test. Cox's proportional hazard regression analysis was used to determine the association between survival and potential factors. A p value less than 0.05 was considered statistically significant in this study. Statistical analysis was performed by applying SPSS version 15.0 (SPSS, Chicago, IL, USA).

Results

A total of 310 patients were enrolled in this study. The mean age was 64.3 years old, ranging from 36 to 92, and 281 (90.6%) patients were male. There were 149 patients (48.1%) who underwent esophagectomy, including six patients who received esophagectomy after chemoradiotherapy. The flowchart of patients' treatment was shown as Fig. 1. Patients'

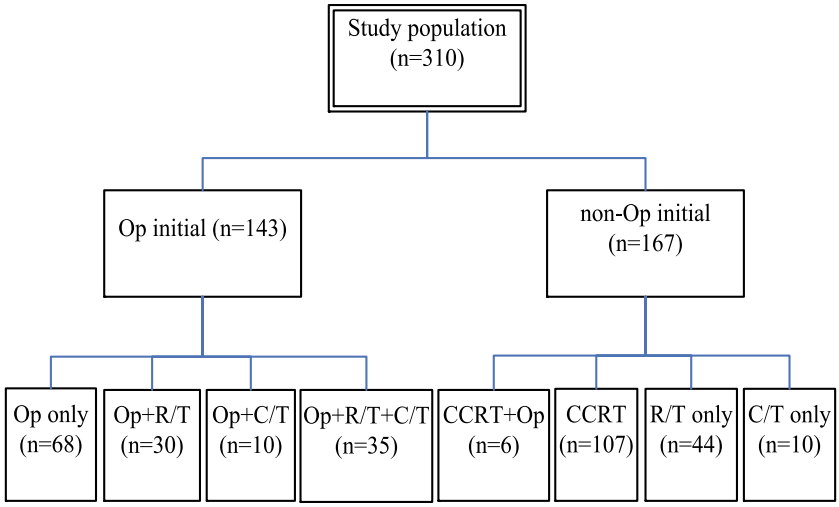

Figure 1 The flowchart for patients' treatment. *Op* esophagectomy with reconstruction, *R/T* radiotherapy, *C/T* chemotherapy, *CCRT* concurrent chemoradiotherapy

characteristics were described in Table 1. Patients in esophagectomy group were younger than those without esophagectomy. In esophagectomy group, more patients had a middle to lower-third tumor and in early stages.

The overall survival was shown in Fig. 2. The 5-year survival rates in patient with esophagectomy and without esophagectomy were 36.0% and 10.2%; the difference was significant ($p<0.001$). There were 26 patients with stage I esophageal cancer, 93 patients with stage II, and 106 patients with stage III, and the other 85 patients were with stage IV cancers. The survival rates between esophagectomy and no esophagectomy were also compared. Patient who underwent esophagectomy had a better survival rate for patients with stages I, II, and III esophageal cancer and had no significant difference for patients with stage IV cancers.

The total medical expense within 5 years after first date of visit for patients with esophagectomy and without esophagectomy were USD $22532.8±8876.00 and $12256.4±7364.58, respectively; the difference was significant ($p<0.001$). The monthly expenses within 5 years were $2101.65±3485.58 and $2033.94±1763.19, respectively; there was no significant difference between two groups ($p=0.831$).

In patients with different stage of esophageal cancer, the total medical expense and monthly medical expense between esophagectomy and no esophagectomy groups were shown in Table 2. In patient who underwent esophagectomy, the total medical expense was not significantly different among different stages ($p=0.960$). Also, in patients without esophagectomy, the total medical expense was not significantly different among stages ($p=0.772$).

Table 1 The Characteristics of Study Subjects

Variable	Item	Esophagectomy ($n=149$)	No esophagectomy ($n=161$)	p value
Age[a] (years)		61.6±11.5	66.7±12.9	<0.001
Gender[b]	(M/F)	132/17	149/12	0.248
Location[b]	Cervical	4	11	<0.001
	Upper thoracic	21	35	
	Middle thoracic	81	71	
	Lower thoracic	41	22	
	Long segment	2	22	
Cell type[b]	Squamous cell carcinoma	136	135	0.059
	Others	13	26	
Stage[b]	I	21	5	<0.001
	II	59	34	
	III	41	65	
	IV	28	57	

[a] t test

[b] χ^2 test

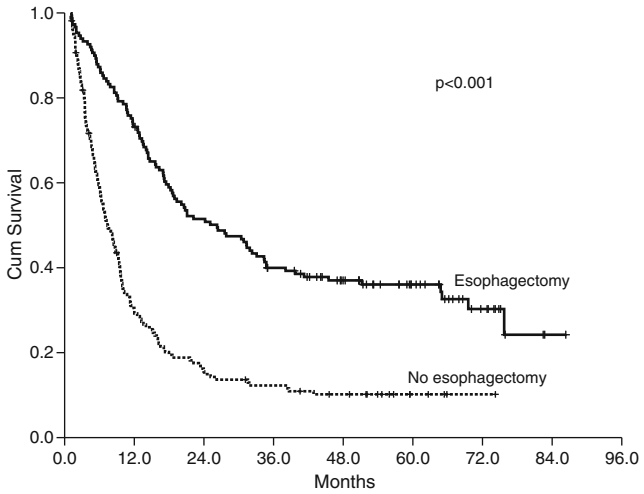

Figure 2 The overall survival curve in this study population, solid line indicated patients with esophagectomy and the dotted line indicated patients without esophagectomy

But, in monthly medical expense, patients with early stages in esophageal cancer spent significantly less medical expense than those in late stages in esophagectomy treatment ($p<0.001$). Post hoc test showed patients with stage IV cancer had a higher monthly medical expense than those with other stages. In patients without esophagectomy, the treatment expense for late-stage cancer was also higher than early stages ($p=0.008$). In each stage, patient who underwent esophagectomy had a higher total medical expense than patient without esophagectomy. But, the monthly medical expense was not significantly different between patient who underwent esophagectomy and no esophagectomy.

The REPI in each stage was also shown in Table 2. The REPI was 7.573 in patient with stage I esophageal cancer. This means that esophagectomy was more cost-beneficial than no esophagectomy in patient with stage I esophageal cancer. In patient with stages II and III of esophageal cancer, the REPIs were also more than 1. But, in patient with stage IV of esophageal cancer, esophagectomy was less cost-beneficial than no esophagectomy because the REPI was smaller than 1.0.

The linear regression model for the total medical expense was shown in Table 3. Esophagectomy, age, sex, tumor stage, and survival time were independent variables (factors) included in the model. Esophagectomy was the only variable which could influence total medical expense ($p<0.001$). Tumor stage and patient survival time did not influence total medical expense.

The linear regression model for the monthly medical expense was shown in Table 4. Esophagectomy, age, sex, and tumor stage were independent variables (factors) included in the model. Tumor stage was the only variable that could influence the monthly medical expense, especially in stages III and IV. Comparing with patients with

Table 2 Survival Time, Total Medical Expense, Monthly Medical Expense, and REPI in Different Stages of Esophageal Cancer

Stage	Variable	Esophagectomy ($n=149$)	No esophagectomy ($n=161$)	p value
I	n	21	5	
	Survival time[a]	75.7	11.3	0.003
	Total expense[b]	23075.7	10026.3	0.013
	Monthly expense[b]	762.03	861.46	0.784
	REPI[c]	7.573		
II	n	59	34	
	Survival time	39.7	15.1	0.003
	Total expense	22063.9	12319.0	<0.001
	Monthly expense	1450.85	1336.77	0.827
	REPI	2.422		
III	n	41	65	
	Survival time	14.4	6.0	0.002
	Total expense	22877.9	11821.8	<0.001
	Monthly expense	2042.74	2081.96	0.922
	REPI	2.446		
IV	n	28	57	
	Survival time	8.5	6.6	0.112
	Total expense	22608.6	12910.2	<0.001
	Monthly expense	4563.98	2497.94	0.071
	REPI	0.705		

[a] Survival time was presented as median survival time in months

[b] Total (medical) expense and monthly (medical) expense were calculated for survival time in months or up to 5 years at most since first date of hospital visit

[c] REPI was calculated by the ratio of survival benefit divided by the ratio of expense per month

Table 3 Regression Analysis of Independent Factors Influence Total Medical Expense

Variable	Item	B	95% C.I.	*p* value
Constant		13946.1	6667.1, 21225.1	<0.001
Age		−35.52	−111.56, 40.52	0.359
Gender	Female	Reference		0.995
	Male	−10.20	−3184.60, 3164.20	
Stage	I	Reference		
	II	−38.04	−3654.76, 3578.69	0.984
	III	271.33	−3485.05, 4027.70	0.887
	IV	912.00	−3030.26, 4854.25	0.649
Esophagectomy	No	Reference		<0.001
	Yes	9946.34	7863.94, 12028.73	
Survival time		20.23	−34.90, 75.36	0.471

stage I esophageal cancer, patients in late cancer stage had more monthly medical expense; the differences were \$766.67 in stage II (p=0.192), \$1529.15 in stage III (p=0.010), and \$2652.80 in stage IV (p<0.001). Patients who underwent esophagectomy cannot influence the monthly medical expense (p=0.106).

The result of Cox hazard regression analysis was shown in Table 5; patient who underwent esophagectomy reduced the risk (hazard ratio (HR)=0.348; confidence interval (CI), 0.260–0.466), higher monthly medical expense increased the risk (HR=1.004, CI, 1.004–1.005), and tumor stage influenced the survival, respectively.

Discussion

The incidence of esophageal cancer has increased fast in recent decades. For example, the new case number was double from 1995 to 2005 and thus has become the ninth leading cause of death by cancer in Taiwan since 2005.[19] Although the progression in treatment methods and molecular biology applications for cancers was quick, the improvement of survival rate was limited. And, the treatment results for esophageal cancer were still poor in the last decades.[20] Since 1970, the 5-year survival rate was only raised from 4% to 14%.[4] Rapid increase in growth of esophageal cancer also raised social concern about medical expenditure used in treating esophageal cancer patients. Even in a country with universal health insurance like Taiwan, economic burden of esophageal cancer patients is still significant in comparison to other diseases. Especially in today's managed care environments, although cancers were classified as major catastrophic diseases and esophageal cancer patients are waived from deductibles and copayments under the NHI, cost consciousness and cost containment strategies of all hospitals still result in additionally high charges related to treating esophageal cancer. Besides, recent changes in payment systems, from fee-for-service to global budget and pay-by-performance, signal that cost and benefit issues have become more and more important for clinical strategies.[21] However, in previous literatures of esophageal cancer, cost and benefit

Table 4 Regression Analysis of Independent Factors Influence Monthly Medical Expense

Variable	Item	B	95% C.I.	*p* value
Constant		736.98	−1492.61, 2966.57	0.516
Age		−9.21	−33.53, 15.12	0.457
Gender	Female	Reference		0.686
	Male	208.11	−805.53, 1221.75	
	I	Reference		
	II	766.67	−385.83, 1919.16	0.192
Stage	III	1529.15	371.09, 2687.22	0.010
	IV	2652.80	1459.24, 3846.36	<0.001
Esophagectomy	No	Reference		0.106
	Yes	524.52	−111.49, 1160.54	

Table 5 Cox Regression Analysis of Independent Factors Influence Survival Time

Variable	Item	Hazard ratio	95% C.I.	*p* value
Age		1.007	0.996, 1.017	0.211
Gender	Female	Reference		0.344
	Male	1.259	0.781, 2.031	
Stage	I	Reference		
	II	1.151	0.613, 2.164	0.662
	III	2.244	1.205, 4.179	0.011
	IV	3.419	1.273, 4.598	0.007
Esophagectomy	No	Reference		<0.001
	Yes	0.348	0.260, 0.466	
Monthly medical expense[a]		1.004	1.004, 1.005	<0.001

[a] Hazard per 10 USD

studies were rare. Most of the cost and benefit studies in esophageal cancer focused on metallic stent placement and the screening expenses of gastroesophageal reflux or Barrett's esophagus.[22–24]

Besides, how long medical expenses should be included was another issue for previous studies. The calculation period of expense in this study was different from previous studies. Some studies focused only on consequences of different treatments; their calculation period was short, such as the expense between chemotherapy and brachytherapy in nasopharyngeal cancer and conservative surgery compared with radical surgery in breast cancer.[9–10] Some studies focused on post-operative follow-up program in colorectal cancer, their studied period was 5 years but did not include the initial treatment expenses.[11–12] The period of this study was 5 years, but initial treatment expenses, following treatment expenses, and the follow-up examination expenses were all included in calculation of total medical expense. This method is similar to the report by Wilson et al.[13] because the detection and treatment for the recurrent disease is as important as initial treatment which influences overall survival.

In the report by Farndon et al., total expense of esophagectomy was higher than other treatments but monthly medical expense was not when survival time was considered. So, surgical resection was as cost-beneficial as other treatment methods in treating esophageal cancer.[17] This study had similar result as Farndon's study; even the expense within 5-year follow-up time was calculated. Furthermore, this study demonstrated that esophagectomy was more cost-beneficial in patients in stages I to III of esophageal cancer if survival was considered; but esophagectomy was less cost-beneficial in stage IV of esophageal cancer.

In lung cancer study by Fleming et al., stage of a cancer was the factor independently related to the total treatment expense within 1 year after diagnosis of lung cancer.[25] In this study, using the regression model, total medical expense was only related to esophagectomy and not influenced by age, sex, tumor stage, and even patient survival time. But, if the survival time was considered, the monthly medical expense was only influenced by late cancerous stage, especially in stages III and IV. Fleming's report did not consider survival time of each patient. It implied that using esophagectomy to treat patients may consume more medical resources, but it is still cost-beneficial if individual patient survival time and the whole treatment expense(till death or 5 years after diagnosis) for esophageal cancer were calculated. The phenomenon is significant in patient with stages I to III esophageal cancer.

There were many factors related to the survival in esophageal cancer. Surgical resection and stage were usually discussed in this kind of literatures. Most of them concluded that surgical resection had benefit for early stage cancer and controversy in relatively late stage. This was also shown in the study; cancer stage and esophagectomy were the two independent variables (factors) related to survival. In addition, we also found that elevation of monthly medical expense also accompanied increased risk of death. It may indicate the presence of comorbidity or development of complications. Both of them usually decreased the survival time and increased medical expenses.[25–26]

Tumor stage in diagnosis usually influences initial choice of treatment. At present, surgical resection remains the main treatment for early stage of esophageal cancer, and chemo-radiotherapy is the main treatment option for late-stage esophageal cancer.[27–28] The boundary of early and late stage is blurred due to inaccurate staging even with modern technology in examination tools, such as computerized tomography, magnetic resonance, positron emission tomography, and endoscopic ultrasound.[29] Certainly, economic issue is becoming one major factor to consider when an esophageal cancer patient needs to be treated. Based on this study, considering both survival and medical expense, surgical resection is recommended for patients with esophageal cancer, not only in stage I but even in stages II and III.

Conclusions

Esophagectomy was more cost-beneficial than non-esophagectomy treatment for patients with stages I to III esophageal cancer. Therefore, adding the economic consideration to survival results, esophagectomy is recommended for patients at least earlier than stage III.

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J Gastrointest Surg (2009) 13:1813–1820
DOI 10.1007/s11605-009-0960-1

SSAT QUICK SHOT PRESENTATION

Outcome after Resection of Hepatic and Pulmonary Metastases of Colorectal Cancer

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Received: 22 January 2009 / Accepted: 22 June 2009 / Published online: 11 July 2009

Abstract
Introduction Multimodal therapies (especially surgery of metastases and "aggressive" chemotherapy) in patients with metastases of colorectal cancers (CRC) are increasingly performed and may provide long-term survival in selected patients with more than one location of metastases. In the current literature, there are only few studies with relatively low patient numbers reporting on the outcome after resection of both hepatic and pulmonary metastases of CRC. We therefore evaluated survival of patients who underwent sequential resection of hepatic and pulmonary metastases under potentially curative intention.
Material and Methods From 1987 until 2006, 44 patients (32% female; median age, 58 years) with hepatic and pulmonary CRC metastases underwent resections at both metastatic sites. The primary CRCs were in 50% rectal and in 50% colonic carcinomas (61% node positive, all with free resection margins). Metastases occurred synchronously (regarding primary CRC) in 32% of the patients. In 86%, liver resection was performed prior to pulmonary resection. The first resection of metastases was performed a median of 16 months after resection of the primary CRC; the median interval between the first and the second resection of metastases was 7 months. Forty-seven percent of the patients also underwent at least a third metastasectomy. During resection of the first and second site of metastases, free margins were achieved in 98% and 95%, respectively. Survival analysis was performed using Kaplan–Meier and Cox regression methods.
Results The 5-year survival rates (SV) were 64% after initial surgery of CRC, 42% after the first resection of metastases, and 27% after the last metastasectomy. Patients with synchronous metastases had a 5-year SV after first metastasectomy of 43% and in patients with metachronous metastases of 41% (n.s.). The location of the primary tumor (20% 5-year SV in rectal vs. 57% in colonic cancer; $p<0.02$) and the lung as primary site of metastatic disease (5-year SV 0% vs. 60% in patients with primarily hepatic metastases only; $p<0.001$) significantly influenced survival in univariate analysis. Patients with rectal cancer had a significantly higher frequency of the lung as first metastatic site (46%) compared to patients with colonic cancer (14%; $p<0.03$). Multivariate survival analysis revealed the lung as first metastatic site and as the sole significant independent factor for the outcome ($p<0.001$; relative risk vs. liver first metastases 4.7).

Dedicated to Eva Fischer (died 2008).

Presented (Quick shot) at the 49th Annual Meeting of the Society for Surgery of the Alimentary Tract, May 23, 2008 San Diego, CA, USA.

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Conclusion In selected patients with metastasized CRC resection of both hepatic and pulmonary metastases may improve survival rates or even provide long-term survival. Patients with lung as the first site of metastatic disease (either lung only or in combination with hepatic metastases) have a significantly worse outcome than patients with metastases primarily confined to the liver.

Keywords Colorectal cancer · Liver metastases · Pulmonary metastases · Surgery · Survival

Introduction

The liver and the lung are the most frequent sites of metastases of colorectal cancer (CRC). During the last two decades, liver surgery for CRC metastases evolved in many centers with several of them reporting more than 400 resections.[1–6] Perioperative mortality has been reported to be clearly below 5%.[6–9] Most important, however, is the fact that curative resection of isolated liver metastases may achieve long-term survival in many of those patients, with current 5-year survival rates of up to 58%. As for isolated liver metastases, resection may also be beneficial in selected patients with pulmonary metastases. Since the early 1990s, several larger series have reported 5-year survival rates of higher than 50% after resection of isolated lung metastasis.[10] In patients eventually presenting with both hepatic and pulmonary metastases, the role of surgery is less well defined as in patients with a single metastatic site. Regarding the literature, there are only five articles reporting the outcome of more than 30 patients,[11–14] including only one with more than 100 patients.[15]

The aim of this study was to analyze the experience with 44 patients who underwent both hepatic and pulmonary resection for CRC metastases at our institution during a 20-year period.

Material and Methods

Patients undergoing resection of both hepatic and pulmonary metastases at our institution between 1987 and 2006 were identified in the hospital information system using the International Classification of Disease and procedure codes. The detailed information required for our analyses was then mainly gained by retrospective chart reviews. In addition, further data were retrieved from our prospective hepatic surgery database (data included since 1996). Data principally assessed were patient demographics, time point of treatment, type of surgical treatment, tumor characteristics, neo-/adjuvant therapy, and survival. Survival information was obtained from the tumor registry at the Comprehensive Cancer Center at the University Hospital Freiburg. Our analysis included 44 patients whose primary colorectal tumor had been resected at our as well as other institutions (who were then referred to our center for the treatment of metastatic disease). In all patients, complete information regarding the primary CRC and further surgical treatment were available. Synchronous metastatic disease in this series was defined as the presence of metastases at the time of resection of the primary CRC. The patient demographics and tumor characteristics at the time of first CRC resection are shown in Table 1.

Patient selection for resectional treatment was done individually. There were not always predefined criteria or algorithms applied in our institutions. Until 2004, no strict internal protocol of staging procedures and or neoadjuvant/adjuvant therapy was followed. Patients were, in general, treated according to the current guidelines of the German Cancer Society. Since 2004, patients with CRC (including metastatic disease) are treated according to internal guidelines of our Comprehensive Cancer Center after case presentation and discussion in the interdisciplinary tumor board. In general, the indication for metastasectomy was

Table 1 Patient Demographics and Tumor Characteristics in 44 Patients at the Time of First Colorectal Cancer (CRC) Resection

	Number of patients	Percent
Gender		
Male	30	68
Female	14	32
Location of primary CRC		
Colon	22	50
Rectum	22	50
Nodal status of primary CRC		
Node-positive	28	63
Node-negative	16	37
Grading primary CRC		
G1	–	–
G2	34	77
G3	5	11
G4	–	–
Unknown	5	11
Free margins CRC resection	44	100
T stage		
T 1	–	–
T 2	6	14
T 3	34	77
T 4	4	9
Median age; years (range)	58 (38–71)	

given when preoperative staging indicated that margin-negative resections were achievable in patients otherwise fit to undergo (major) surgery. In the presence of simultaneous hepatic and pulmonary metastases, hepatic resection was done prior to pulmonary resection at our institution.

The extent of liver resection was defined as wedge or segmental resection, hemihepatectomy, or extended hemihepatectomy. Hemihepatectomy was referred to as resection of Couinaud's segments 5–8 (right) or 2–4 (left). Extended hemihepatectomy was referred to as resection of segments 4–8 (extended right hemihepatectomy) or 2–5 plus 8 (extended left hemihepatectomy).[16] The extent of pulmonary resection was defined as wedge resection (including segmentectomy), lobectomy or pneumonectomy. Actuarial survival was calculated for the whole patient group after resection of the primary CRC, after the first metastasectomy and after the last resection of a metastatic lesion. Various parameters like the site of primary colorectal disease, primary nodal disease, synchronous vs. metachronous metastatic disease, site of first metastases, and others were assessed regarding their influence on survival after first metastasectomy.

Actuarial survival was estimated by the Kaplan–Meier method using SPSS® for Windows™ (version 15.0, Chicago, IL, USA). In subgroup analyses differences between groups were assessed by a log-rank test. Multivariate survival analysis was performed using the Cox proportional hazard model.

Results

In 31 of the 44 patients (70%), the liver was the first site of metastatic CRC (without lung metastases); six patients (14%) presented with lung first metastases and seven patients (16%) had both lung and liver disease at first diagnosis of metastatic disease. The site of first metastases correlated significantly with the location of primary CRC: Of the 22 patients with rectal cancer, ten (46%) presented with lung first metastases (lung or lung plus liver), whereas only three of the 22 patients (14%) with colonic cancer had lung first metastases ($p<0.03$).

In the 44 patients of our study, a total of 155 resectional procedures (including resection of the primary CRC; mean, 3.5 per patient) ranging from two (initially simultaneous colonic resection plus metastasectomy) to six per patient were performed (Table 2). In five cases, a resection of liver metastases was performed simultaneously with the resection of the colonic primary tumor. In all other 39 patients, the first metastasectomies were performed as either staged procedures (synchronous metastases) after resection of the primary or after later detection of the metastases (metachronous metastases). In 86%, liver resection was the first metastasectomy. Four of the 111 resectional procedures after the initial operation were colorectal re-resections (without metastasectomy) for recurrent locoregional disease. During five metastasectomies (three times at first metastasectomy and twice at second metastasectomy), resection was simultaneously performed at both sites (liver and lung). The other 107 metastasectomies were undertaken at "only" one site for either hepatic or pulmonary metastases. After their initial metastasectomy in the liver and lung, 21 of the 44 patients (48%) underwent at least one further metastasectomy. Median follow-up after resection of the primary CRC was 4.9 years (range, 1.2–14.8) and 3.3 (0.5–14.2) years after first metastasectomy.

Table 2 One Hundred Seventeen Resections Performed During 112 Metastasectomies (Five Patients Simultaneously had Resection of Hepatic and Pulmonary Metastases During One Operation)

	Number of patients
Hepatic resections	60
Wedge resection	18
Resection of one segment	13
Resection 2–3 segments	4
Hemihepatectomy	16
Extended hemihepatectomy	9
Pulmonary resections	57
Wedge resection	48
Lobectomy	6
Resection of 2 lobes (bilobectomy)	2
Pneumonectomy	1

Surgical Treatment

The procedures performed during the 112 metastasectomies are given in Table 2. For liver metastases, "larger" resections (multiple segments, hemihepatectomy, and extended hemihepatectomy) were performed in about half of the patients, whereas the vast majority of pulmonary metastasectomies consisted of wedge resections. Three patients had simultaneous (one stage) bilateral procedures of the liver, and seven patients underwent simultaneous bilateral pulmonary resections.

During the initial removal of metastases, free resection margins were achieved in 43 of 44 patients (98%) at the first site and in 42 of 44 (95%) patients at the second site of metastases. The median number of resected metastases during first metastasectomy was one (range, 1–5). Twenty-six patients had one metastasis, ten patients had two, five patients had three, one patient had four, and one five metastases removed initially (number unknown in one patient). Two patients underwent local ablation (one cryotherapy and one thermoablation) of a liver metastasis in addition to resection.

Additional Treatment

A total of 35 of the 44 patients (80%) received chemotherapy between the time of initial diagnosis of CRC and last surgery. Five patients received 5-FU-based chemotherapy as a part of neoadjuvant or adjuvant chemoradiation of rectal cancers. Twenty patients received 5-FU-based adjuvant chemotherapy after resection of their primary CRC, but the exact type and number of cycles was not available in all of those. Before the first metastasectomy, four further patients received oxaliplatin-based (n=3) or irinotecan-based (n=1) chemotherapy under initially palliative or neoadjuvant intention. The remaining six patients received an irinotecan-based (n=3) or oxaliplatin-based (n=3) chemotherapy after their first metastasectomy. After their last metastasectomy, most patients were followed and treated by external oncologists. We, therefore, could not obtain reliable data on chemotherapeutic regimens administered after last metastasectomy (most of those eventually under palliative intention) by our retrospective analysis.

Survival

During the median follow-up of almost 5 years after initial surgery for CRC, 25 of the 44 patients died. Up to now, three patients are alive for more than 10 years after their first metastasectomy (10, 11, and 14 years, respectively). Cumulative five-year survival rates were 64% after initial surgery for CRC (Fig. 1), 42% after first metastasectomy (Fig. 2), and 27% after the last metastasectomy. The detailed univariate subgroup survival analyses after first metastasectomy are given in Table 3. Patients with colonic cancer had a significant better survival after their first metastasectomy than patients with rectal cancer (p<0.02; Fig. 3). In patients with lung as the first metastatic site

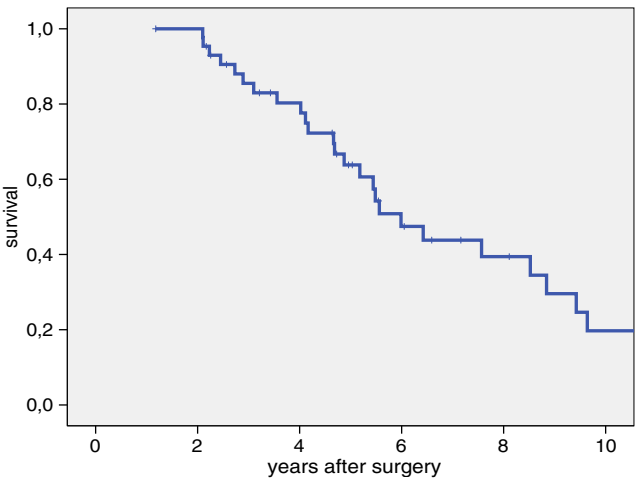

Figure 1 Actuarial survival after resection of the colorectal primary (n=44).

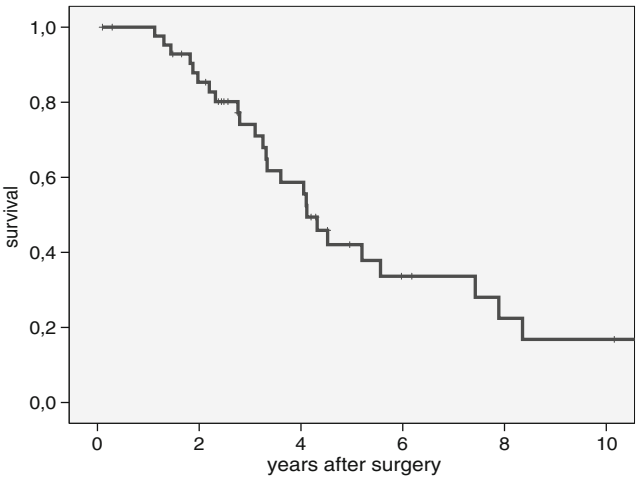

Figure 2 Actuarial survival after resection of the first metastasis (n=44).

(n=13, including seven patients with synchronous lung and liver metastases), actuarial 5-year survival was 0%, whereas 5-year survival was 60% in the 31 patients with liver metastases only as the first metastatic site (p<0.001; Table 3 and Fig. 4). In the subgroup of patients with lung first metastases, survival was not influenced by the presence of additional liver metastases. In both the group of patients with lung first (n=6) and the group of patients with synchronous lung and liver first metastases (n=7), the last patient at risk died in the fifth year after first metastasectomy (Table 3). The nodal status of the primary CRC did not significantly influence survival after first metastasectomy. Further factors like gender, age, time interval (synchronous vs. metachronous), number of metastases initially resected, and chemotherapy administered also did not correlate with survival (Table 3). Survival in the subgroup of patients with only one metastasectomy per site (i.e., one liver and one lung resection; n=23) and in the subgroup of patients with more than two metastasectomies (n=21) was comparable (5-year survival 40% and 42%, respectively; p=0.34).

The lung as the site of first metastasis (lung or lung and liver) was the sole and very strong prognostic factor influencing survival in multivariate analysis (p<0.001; relative risk compared to liver first metastases, 4.7; 95% confidence interval, 1.9–11.8).

Discussion

Published series reporting the outcome after resection of both hepatic and pulmonary colorectal metastases including relevant numbers of patients are rare.[11–15] In addition, the results of most studies (including our series) are somewhat limited by the retrospective nature and a long inclusion period (with evolving diagnostic and treatment modalities).

Table 3 Univariate Actuarial Survival Analysis in 44 Patients After First Metastasectomy of Hepatic or Pulmonary Colorectal Metastases

Parameter	Number of patients	3 years (%)	5 years (%)	10 years (%)	*p* value
Gender					
Male	30	84	49	16	0.37
Female	14	56	32	24	
Age (first operation)					
<60 years	26	69	40	17	0.85
≥60 years	18	83	46	17	
Site of first metastases					
Liver only	31	82	60	24	0.001
Lung only	6	80	0	0	
Liver and lung	7	43	0	0	
Site of first metastases					
Liver only	31	82	60	24	0.001
Lung/liver and lung	13	54	0	0	
Site of primary tumor					
Rectum	22	61	20	0	0.01
Colon	22	85	57	30	
Time of first metastasis					
Synchronous	14	76	43	–	0.93
Metachronous	30	73	41	18	
Nodal status primary tumor					
Positive	28	67	37	7	0.14
Negative	16	87	52	35	
Number of metastases[a,b]					
1	26	74	37	20	0.52
>1	17	72	54	14	
Chemotherapy[b]					
Yes	34	77	42	13	0.74
No	9	63	47	47	
All patients	44	74	42	17	

The numbers of patients at risk was 24 (3 years), ten (5 years), and three (10 years)

[a] At time of first metastasectomy

[b] Unknown in one patient

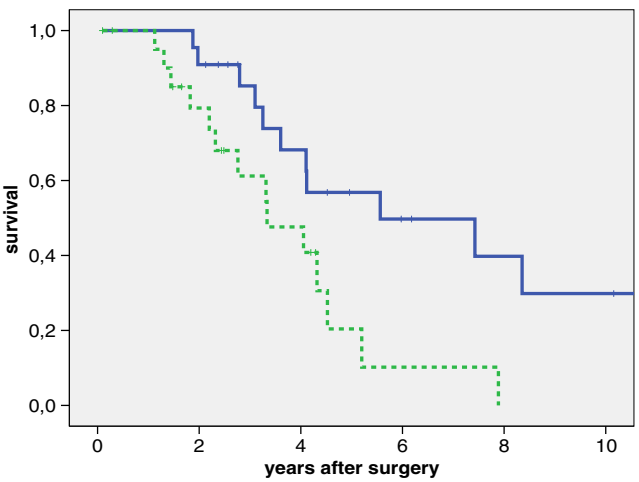

Figure 3 Subgroup survival analysis after resection of the first metastasis by location of the primary CRC. *Continuous line* colon cancer, *dotted line* rectal cancer (p=0.013; log-rank test).

The study by Miller et al.[15] from the Memorial Sloan Kettering Cancer Center reporting the results of 131 patients is the only one including more than 60 patients. Our study is the first from our country assessing the results of more than 30 patients after resection of hepatic and pulmonary metastases. The fact that "only" 44 patients were resected at both sites during a period of almost 20 years demonstrates the relative rare indication for these procedures, at least during the past.

Five-year survival after the first metastasectomy was 42% in our patients. Ten-year survival was "only" 17%. However, the fact that we already observed three patients surviving more than 10 years after metastasectomy underlines the possibility of definitive cure by these procedures in selected patients.

Published survival rates of larger studies (Table 4) show large variations of 5-year survival after metastasectomy ranging between 11% and 74%. However, these results are difficult to compare due to different patient selection and

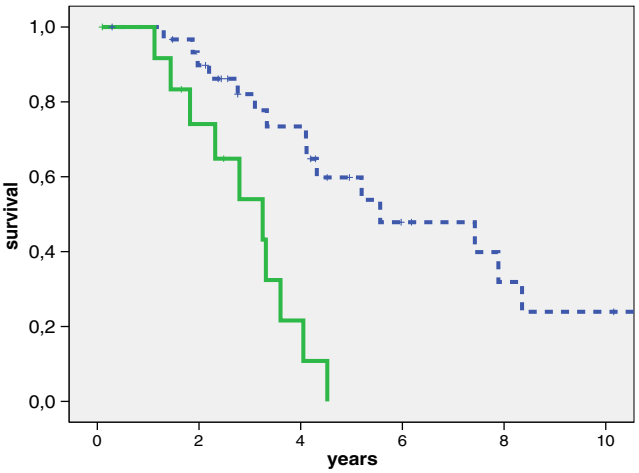

Figure 4 Subgroup survival analysis after resection of the first metastasis by site of the first metatases. *Continuous line* lung first metastases (n=13, including seven patients with additional hepatic metastases), *dotted line* liver first metastases (n=31; p<0.001; log-rank test).

different time points of the calculation of survival (some studies calculated after first metastasectomy, others after first pulmonary resection, see footnotes of Table 4). The reported survival rates after resection of hepatic and pulmonary metastases are in the range of current outcomes after resection of hepatic metastases alone.[2,3,5,17] Current 5-year survival after resection of CRC liver metastases since 1998 was 46% in our own institution (n=214; data not shown).

In contrast to the outcomes after resection of primary CRC, of isolated liver metastases,[2,3,5,17] or of isolated pulmonary metastases,[18–21] only few data are known to predict prognosis after resection of both hepatic and pulmonary metastases. The main reason for this is clearly the low number of evaluated patients. In the (only) larger series from the Memorial Sloan Kettering Cancer Center, Miller et al.[15] could demonstrate that a longer disease-free interval after first metastasectomy, the presence of only one liver metastasis, and a younger age were associated with a better outcome. In the second largest series reporting 58 patients, by contrast, Headrick et al.[11] from the Mayo Clinic identified the carcinoembryonic antigen level before metastasectomy as the sole prognostic factor. In our evaluations, we found that patients with lung first metastases (including patients with concomitant liver metastases) had a significantly worse outcome than patients presenting with liver metastases only as the first metastatic site. This clear prognostic difference might partially be explained by a higher rate of lung first metastases in patients with rectal cancer. A possible explanation for this prognostic finding, which has not been described as in the other series, is the route of venous tumor cell dissemination from the primary CRC (possible systemic venous drainage in lower rectal cancers vs. portal venous drainage in colonic cancers). It is of note that the groups of patients with rectal or colonic cancer were comparable regarding other potential risk factors like the time interval of the occurrence of metastasis (synchronous vs. metachronous) or the frequency of primary nodal disease.

Metastatic involvement of more than one organ is often believed to represent disseminated disease, which contributes to the reluctance in proposing metastasectomy. Since data are still scarce, it is difficult to define whether metastasectomy should be offered in the presence of multiple sites of metastatic disease. In the presence of isolated hepatic metastases, in contrast, prognostic factors and scores have been derived from large series.[2,3,17] There are arguments, however, that may favor a more aggressive approach with current treatment modalities in patients with more than one metastatic site: Very low mortality rates after hepatic and pulmonary resections in experienced centers have made those resections safe during the last two decades.[6–9,18–21] It has also been shown that selected patients with hepatic and extrahepatic disease[22] or even with peritoneal carcinosis[23] may benefit from surgical resection. More importantly modern chemotherapeutical regimens (including oxaliplatin, irinotecan, and targeted

Table 4 Outcome Results of Selected Published Series After Resection of Hepatic and Pulmonary Colorectal Metastases

Author	Year	Number of patient	5-year survival after primary CRC	5-year survival after 1st metastasectomy
Miller et al.[15]	2007	131	65%	49%
Shah et al.[14]	2006	39	84%	74%
Headrick et al.[11]	2001	58	–	30%[a]
Kobayashi et al.[12]	1999	47	–	22%/50%[b]
Regnard et al.[13]	1998	43	64%	11%[c]
Own results	2009	44		42%

[a] After first lung resection

[b] Twenty-two percent in synchronous hepatic and pulmonary metastases, 50% in sequential (hepatic followed by pulmonary) metastases

[c] After first lung resection (=second metastasectomy)

therapy with antibodies such as bevacizumab/cetuximab) have been shown to better control or even downstage metastatic disease to render a subset of patients resectable.[24–26] In addition to possible effective downstaging or downsizing, the early response evaluation after intensive chemotherapy given for metastatic disease may further delineate the potential biological behavior of the disease and may help select patients for metastasectomy. In this context, it might also be helpful to study the role of newer imaging modalities like positron emission tomography (PET) in disease and response assessment in patients with multiple site CRC metastases.[27]

As already outlined above, the indication for metastasectomy was potentially given in our experience when preoperative staging indicated that margin-negative resections were achievable in patients otherwise fit to undergo surgery. Although prognosis is worse in subgroups (e.g., patients with lung first or synchronous hepatopulmonary metastases, with multiple metastases or a short disease-free interval after first metastasectomy), surgery should still be offered to those patients with good biology (e.g., response to systemic chemotherapy, younger age, and low perioperative risk) when an R0 resection can be obtained.

Conclusion

We conclude that resection of both hepatic and pulmonary metastases may prolong survival in selected patients with CRC. However, patients with lung as the first site of metastatic disease (more frequent in rectal cancer) clearly have a poorer outcome than patients with metastases primarily confined to the liver. Further studies should be performed to define the exact role of combined metastasectomy in the context of modern chemotherapeutic (e.g., targeted therapy) and staging (e.g., PET) modalities.

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J Gastrointest Surg (2009) 13:1821–1830
DOI 10.1007/s11605-009-0973-9

ORIGINAL ARTICLE

Association of Allogeneic Blood Transfusions and Long-Term Survival of Patients with Gastric Cancer after Curative Gastrectomy

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Received: 18 May 2009 / Accepted: 15 July 2009 / Published online: 5 August 2009

Abstract
Introduction The relationship between perioperative allogeneic blood transfusions and poor prognosis in patients with gastric cancer remains controversial. The aim of this study is to examine the effect of perioperative blood transfusions on long-term survival of patients undergoing curative gastric resection for gastric cancer.
Methods Eight hundred fifty-six consecutive patients with gastric cancer who underwent curative gastrectomy (R0) from January 1, 1991 through December 31, 2002 were enrolled in this retrospective study.
Results A multivariate overall survival analysis using Cox proportional hazard regression model revealed macroscopically infiltrative tumor, tumor infiltration of serosa, lymph node metastasis, blood transfusions (hazard ratio, 2.69), pulmonary disease, and liver dysfunction as prognostic factors for long-term survival. Blood transfusion was an independent prognostic factor at all stages of disease. Disease-specific and overall survival showed significant differences between the transfused and nontransfused groups (log-rank, $P<0.0001$). Based on multivariate logistic regression analysis, the need for blood transfusion was significantly associated with advanced age (≥65 years), long duration of operation (≥300 min), massive blood loss (≥1,000 ml), and anemia (Hb<10 g/dl).
Conclusions Allogeneic blood transfusion is an independent prognostic factor for long-term survival in gastric cancer patients.

Keywords Blood transfusion · Gastric cancer

Introduction

It is generally supported that allogeneic blood transfusions have various adverse outcomes after cancer surgery. In particular, blood transfusions have been associated with decreased survival of patients with hepatocellular carcinoma, lung cancer, breast cancer, head and neck cancer, colorectal cancer, and prostate cancer.[1–6] The most frequently suggested explanation for this association centers on non-specific immunosuppression arising from increased activities of regulatory T lymphocytes, decreased natural killer cell activity, stimulated anti-idiotype antibody production, and impaired lymphocyte blastogenesis.[7]

Gastric cancer remains the second leading cause of death worldwide, and it is the most common malignancy in Japan, Asia, South America, and Eastern Europe.[8] In Japan and Asia, most surgeons consider D2 gastrectomy to be the standard and optimal surgical procedure for patients with advanced gastric cancer.[9] Blood transfusions are often needed when performing gastrectomy with radical lymph nodes dissection for gastric cancer; however, the relationship between perioperative blood transfusions and poor prognosis in patients with gastric cancer remains controversial. Although many studies do not support this relationship,[10–14] some studies have affirmed that it exists.[15–19]

The aim of this study was to examine the effect of perioperative allogeneic blood transfusions on long-term

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survival in patients undergoing curative gastric resection for gastric cancer.

Materials and Methods

Patients

From January 1, 1991, through December 31, 2002, a total of 1,122 patients underwent surgery for gastric cancer at Wakayama Medical University Hospital. Of these patients, 856 underwent curative gastrectomy (International Union Against Cancer [UICC] R0 resection), which is defined as an absence of microscopic residual tumor.[20] Patients with cancer in another organ or patients who underwent gastrectomy with pancreaticoduodenectomy, gastrectomy with additional hepatic resection, or gastrectomy with thoracotomy were excluded. None of the patients received preoperative neoadjuvant chemotherapy. The 856 patients were followed for at least 5 years or until death. The lost cases were treated as censored data for the analysis of survival rates. The median follow-up interval for patients from the date of surgery was 78 months. Follow-up data were obtained from the hospital database, which includes the patients' background, surgical data, tumor characteristics, and survival time. Perioperative periods were defined as 1 week before and after the operation. Tumor invasion (T) and lymph node status (N) were classified by International Union Against Cancer (UICC) criteria.[20]

Concomitant Disease

Patients with clinically diagnosed hypertension and patients with cardiovascular disease, such as angina pectoris or previous myocardial infarction, were defined as having cardiovascular disease. Patients with abnormal pulmonary function on spirograms (vital capacity ration <0.7 or forced expiratory volume in one second/forced vital capacity <0.6) were defined as having pulmonary disease as a comorbidity.[21] Patients with an estimated creatinine clearance lower than 60 ml/min or a rising serum creatinine (>2 mg/dl) were defined as having renal dysfunction.[22] Patients with liver cirrhosis (per the Child–Pugh classification), patients receiving treatment for liver disease, and patients with a serum aspartate aminotransferase (AST) greater than twice the normal upper limit of serum AST were defined as having liver dysfunction.[23] Diabetes mellitus was noted if the patient had a fasting blood glucose concentration >126 mg/dl or was receiving antidiabetic therapy. Otherwise, the results of a 75-g oral glucose tolerance test were used to diagnose diabetes mellitus.[24] Anemia was defined as preoperative total hemoglobin <10 g/dl.[25]

Surgical Treatment

Standard radical gastrectomy (distal gastrectomy, total gastrectomy, or proximal gastrectomy) was performed in all 856 patients. The extent of lymph node dissection was adjusted for the location of primary tumor according to the Japanese Research Society for Gastric Cancer rules.[26] Laparoscopy-assisted gastrectomy was used to treat early gastric cancers.

Blood Transfusions

The general indication for blood transfusions was intraoperative blood loss of >1,000 ml or a hemoglobin concentration of <8 g/dl, although transfusions were done depending on the discretion of the anesthetist and the surgical team responsible for the care of the patient in the perioperative period. In the period of this research, packed red blood cells were separated from whole blood and stored in citrate–phosphate–dextrose–adenine anticoagulant solution without leukodepletion.

Statistical Analysis

StatView 5.0 software (Abacus Concepts, Inc., Berkeley, CA, USA) was used for all statistical analyses. Quantitative results are expressed as the mean±standard deviation (SD). Statistical comparisons between the transfused and non-transfused groups were performed with χ^2 statistics. Survival curves were computed using the Kaplan–Meier method and compared by means of the log-rank test; $P<0.05$ was considered significant. Univariate and multivariate Cox proportional hazards model was used to evaluate factors that independently affected postoperative survival. Prognostic factors with a univariate $P<0.1$ were included in the multivariate analysis. Prognostic factors with a multivariate $P<0.05$ were defined as independent prognostic factors. Univariate and multivariate logistic regression analyses were performed to identify risk factors influencing blood transfusion requirements on perioperative periods. Risk factors with a univariate $P<0.1$ were included in the multivariate analysis. Risk factors with a multivariate $P<0.05$ were defined as independent risk factors.

Results

Patient Characteristics

Patient characteristics are detailed in Table 1. Among the 856 patients, 154 (18.0%) underwent perioperative allogeneic blood transfusions; the remaining 702 received no transfusions. In transfused patients, 50 patients received 400 ml of

Table 1 Clinicopathological Characteristics of the Patients

	All patients (n=856)	Transfused (n=154)	Non-transfused (n=702)	P value
Age, years (mean±SD)	64±12	67±10	63±12	0.0004
Sex (male/female)	610/246	111/43	499/203	NS
BMI (mean±SD)	22±3	22±4	22±3	NS
Approach (Open/Lap)	794/62	149/5	645/57	0.038
Type of gastrectomy (DG/TG/PG)	498/322/36	47/104/3	451/218/33	<0.0001
Splenectomy (yes/no)	245/611	82/72	163/539	<0.0001
Pancreaticosplenectomy (yes/no)	38/818	18/136	20/682	<0.0001
Lymph node dissection (D1/D2/D3)	264/472/120	38/76/40	226/396/80	<0.0001
Duration of operation, min (mean±SD)	290±86	349±100	277±77	<0.0001
Blood loss, ml (mean±SD)	589±646	1,190±1,214	458±301	<0.0001
Amounts of transfusions[a], ml (mean±SD)		960±762		
Amounts of transfusions[a], ml (0–400/401–800/>800)		50/51/53		
Tumor size, mm (mean±SD)	39±31	57±38	35±29	<0.0001
Macroscopic type (localized/infiltrative)	586/270	77/77	509/193	<0.0001
Histological type (differentiated/undifferentiated)	484/372	80/74	404/298	NS
Tumor infiltration[b] (T1/T2/T3/T4)	474/220/149/13	39/52/54/9	435/168/95/4	<0.0001
Lymph node status[b] (N0/N1/N2/N3)	562/205/60/29	69/52/20/13	493/153/40/16	<0.0001
Stage[b] (IA/IB/II/IIIA/IIIB/IV)	423/149/126/85/36/37	32/29/33/31/12/17	391/120/93/541/24/20	<0.0001
Adjuvant chemotherapy (yes/no)	206/650	55/99	151/551	0.0004

SD standard deviation, *NS* not significant, *BMI* body mass index, *Open* open gastrectomy, *Lap* laparoscopy-assisted gastrectomy, *DG* distal gastrectomy, *TG* total gastrectomy, *PG* proximal gastrectomy

[a] Autologous transfusions were not included

[b] UICC TNM classification

blood or less, whereas 53 patients received more than 800 ml. In both transfused and nontransfused groups, distributions were similar with regard to sex, body mass index (BMI), and histological differentiation (P>0.05). The transfused patients tended to be older (P=0.0004), and among the transfused patients, there was a significantly higher proportion for whom open gastrectomy, total gastrectomy, additional organ resection (splenectomy or pancreaticosplenectomy), and extended para-aortic D3 lymphadenectomy were needed (P<0.05). Duration of operation was longer and intraoperative blood loss was greater in the transfused patients (P<0.0001). In addition, transfused patients tended to have larger tumors and macroscopically infiltrative tumors (P<0.0001). Tumors in the transfused group were more advanced with regard to depth of invasion and nodal stage (P<0.0001). The patients in the transfused group underwent adjuvant chemotherapy more frequently than did the nontransfused patients (P=0.0004).

Univariate and Multivariate Cox Analysis of Prognostic Factors

Univariate and multivariate overall survival analysis was calculated by the Cox proportional hazard regression model. In univariate analysis, tumor size (≥40 mm; P<0.0001), differentiated type of tumor in histology (P=0.010), macroscopically infiltrative tumor (P<0.0001), tumor infiltration of serosa (P<0.0001), lymph node metastasis (P<0.0001), tumor invasion of lymphatic vessel (P<0.0001), tumor invasion of vein (P<0.0001), duration of operation (<300 min; P=0.004), massive blood loss (P<0.0001), blood transfusions (P<0.0001), postoperative complications (P=0.018), pulmonary disease (P=0.0004), and liver dysfunction (P=0.003) predicted decreased overall survival in all gastric cancer patients who underwent gastrectomy (Table 2). The multivariate analysis revealed macroscopically infiltrative tumor (P=0.040, hazards ratios [HR] = 1.39), tumor infiltration of serosa (P<0.0001, HR=2.43), lymph node metastasis (P=0.0010, HR=1.82), blood transfusions (P<0.0001, HR=2.69), pulmonary disease (P=0.014, HR=1.88), and liver dysfunction (P<0.0001, HR=2.67) as independent prognostic factors in gastric cancer patients (Table 3). We also studied prognostic factors according to stage. In the stage I subgroup, blood transfusions, pulmonary disease, and liver dysfunction were prognostic factors; the HR were 3.65, 3.43, and 3.17, respectively. In the stage II subgroup, only blood transfusions (HR=3.25) predicted independent prognostic factors. In stages III and IV, only

Table 2 Univariate Cox Proportional Hazard Model Analysis for Prognostic Factors

Risk factors	Categories	*P* value	Hazards ratio (95% CI)
Tumor size (mm)	≥40 vs. <40	<0.0001	3.13 (2.38–4.10)
Histological type	Differentiated vs. undifferentiated	0.010	0.71 (0.54–0.92)
Macroscopic type	Infiltrative vs. localized	<0.0001	2.90 (2.23–3.79)
Serosal invasion	Yes vs. no	<0.0001	5.06 (3.86–6.62)
Lymph node metastasis	Yes vs. no	<0.0001	3.93 (2.99–5.16)
Tumor invasion			
Lymphatic vessel	Yes vs. no	<0.0001	3.67 (2.68–5.04)
Vein	Yes vs. no	<0.0001	3.11 (2.37–4.08)
Duration of operation (min)	<300 vs. ≥300	0.004	0.68 (0.52–0.88)
Blood loss (ml)	≥1,000 vs. <1,000	<0.0001	2.72 (1.98–3.73)
Blood transfusions[a]	Yes vs. no	<0.0001	4.12 (3.13–5.43)
Postoperative complications[b]	Yes vs. no	0.018	1.76 (1.16–2.82)
BMI	≥25 vs. <25	0.665	1.08 (0.75–1.56)
Concomitant disease			
Cardiovascular	Yes vs. no	0.106	1.35 (0.94–1.95)
Renal	Yes vs. no	0.262	1.59 (0.71–3.58)
Pulmonary	Yes vs. no	0.0004	2.44 (1.49–4.01)
Liver	Yes vs. no	0.003	1.90 (1.24–2.90)
Diabetes	Yes vs. no	0.816	1.06 (0.65–1.72)
Anemia	Yes vs. no	0.955	0.97 (0.36–2.61)

CI confidence interval, *BMI* body mass index

[a] Autologous transfusions were not included

[b] Anastomotic leakage, pancreatic fistula, and intra-abdominal abscess were defined as postoperative complications

blood transfusions (HR=1.75) could be identified in the univariate analysis regarding the prognostic factors (Table 4).

Survival Rates

In overall and disease-specific survival, there were significant differences between the transfused and nontransfused groups (both $P<0.0001$). When patients were stratified by stage, there still were significant differences between the two groups ($P<0.01$ for all comparisons of overall and disease-specific survival (Figs. 1 and 2).

Furthermore, we studied survival rates according to the amount of blood transfusions. The overall survival rate was significantly higher in the nontransfused than in the transfused group, regardless of the amount of transfused blood ($P<0.0001$; Fig. 3). In addition, a dose–response relationship between the amount of transfused blood and the survival rate was not recognized ($P>0.05$; Fig. 3).

Table 3 Multivariate Cox Proportional Hazard Model Analysis for Prognostic Factors

Risk factors	Categories	*P* value	Hazards ratio (95% CI)
Tumor size (mm)	≥40 vs. <40	0.262	1.21 (0.87–1.68)
Histological type	Differentiated vs. undifferentiated	0.503	1.10 (0.83–1.47)
Macroscopic type	Infiltrative vs. localized	0.040	1.39 (1.02–1.90)
Serosal invasion	Yes vs. no	<0.0001	2.43 (1.73–3.42)
Lymph node metastasis	Yes vs. no	0.001	1.82 (1.27–2.59)
Tumor invasion			
Lymphatic vessel	Yes vs. no	0.385	1.21 (0.79–1.87)
Vein	Yes vs. no	0.184	1.25 (0.90–1.74)
Duration of operation (min)	<300 vs. ≥300	0.084	1.31 (0.97–1.76)
Blood loss (ml)	≥1,000 vs. <1,000	0.309	0.81 (0.55–1.21)
Blood transfusions[a]	Yes vs. no	<0.0001	2.69 (1.92–3.77)
Postoperative complications[b]	Yes vs. no	0.178	1.40 (0.86–2.29)
Concomitant disease			
Pulmonary	Yes vs. no	0.014	1.88 (1.14–3.09)
Liver	Yes vs. no	<0.0001	2.67 (1.71–4.15)

CI confidence interval

[a] Autologous transfusions were not included

[b] Anastomotic leakage, pancreatic fistula, and intra-abdominal abscess were defined as postoperative complications

Table 4 Univariate and Multivariate Cox Proportional Hazard Model Analysis for Prognostic Factors (Subgroup Analysis)

Risk factors	Categories	Univariate analysis		Multivariate analysis	
		P value	Hazards ratio (95% CI)	*P* value	Hazards ratio (95% CI)
A. Stage I[a] (*n*=572)					
Tumor size (mm)	≥40 vs. <40	0.021	1.79 (1.09–2.95)	0.318	1.33 (0.76–2.33)
Macroscopic type	Infiltrative vs. localized	0.003	2.20 (1.32–3.67)	0.076	1.71 (0.95–3.08)
Tumor invasion					
Lymphatic vessel	Yes vs. no	0.032	1.65 (1.04–2.60)	0.669	1.14 (0.63–2.05)
Vein	Yes vs. no	0.049	1.66 (1.00–2.74)	0.583	0.83 (0.43–1.62)
Blood loss (ml)	≥1,000 vs. <1,000	0.017	2.26 (1.16–4.39)	0.388	0.69 (0.30–1.59)
Blood transfusions[b]	Yes vs. no	<0.0001	3.99 (2.39–6.67)	0.0001	3.65 (1.89–7.05)
Concomitant disease					
Cardiovascular	Yes vs. no	0.011	2.02 (1.18–3.46)	0.411	1.29 (0.70–2.39)
Pulmonary	Yes vs. no	<0.0001	4.81 (2.39–9.67)	0.001	3.43 (1.63–7.23)
Liver	Yes vs. no	<0.0001	3.47 (2.00–6.03)	0.0003	3.17 (1.70–5.91)
B. Stage II[a] (*n*=126)					
Tumor size (mm)	≥40 vs. <40	0.908	1.03 (0.58–1.83)		
Macroscopic type	Infiltrative vs. localized	0.417	0.79 (0.45–1.39)		
Tumor invasion					
Lymphatic vessel	Yes vs. no	0.784	1.18 (0.37–3.79)		
Vein	Yes vs. no	0.114	1.65 (0.89–3.05)		
Blood loss (ml)	≥1,000 vs. <1,000	0.348	1.47 (0.66–3.26)		
Blood transfusions[b]	Yes vs. no	<0.0001	3.16 (1.80–5.56)	<0.0001	3.25 (1.85–5.73)
Concomitant disease					
Cardiovascular	Yes vs. no	0.058	1.96 (0.98–3.92)	0.228	1.70 (0.72–4.02)
Pulmonary	Yes vs. no	0.570	1.40 (0.44–4.51)		
Liver	Yes vs. no	0.067	2.61 (0.94–7.27)	0.368	1.79 (0.50–6.39)
C. Stage III/IV[a] (*n*=158)					
Tumor size (mm)	≥40 vs. <40	0.554	1.19 (0.67–2.10)		
Macroscopic type	Infiltrative vs. localized	0.625	1.12 (0.70–1.80)		
Tumor invasion					
Lymphatic vessel	Yes vs. no	0.808	1.15 (0.36–3.64)		
Vein	Yes vs. no	0.442	1.21 (0.74–1.99)		
Blood loss (ml)	≥1,000 vs. 1,000	0.185	1.33 (0.87–2.04)		
Blood transfusions[b]	Yes vs. no	0.007	1.75 (1.16–2.64)	0.007	1.75 (1.16–2.64)
Concomitant disease					
Cardiovascular	Yes vs. no	0.643	0.83 (0.39–1.80)		
Pulmonary	Yes vs. no	0.893	1.06 (0.43–2.62)		
Liver	Yes vs. no	0.520	1.39 (0.51–3.83)		

CI confidence interval

[a] TNM classification

[b] Autologous transfusions were not included

Risk Factors Influencing Blood Transfusion Requirement

Univariate and multivariate analyses were performed to identify risk factors influencing perioperative blood transfusion requirement. Table 5 shows the results of 16 parameters univariately and multivariately examined as potential risk factors for the 154 patients with blood transfusions versus the 702 patients without blood transfusions. The logistic regression analysis identified that blood transfusion requirements were significantly associated with high age (≥65 years), long duration of operation (≥300 min), massive blood loss (≥1,000 ml), and anemia

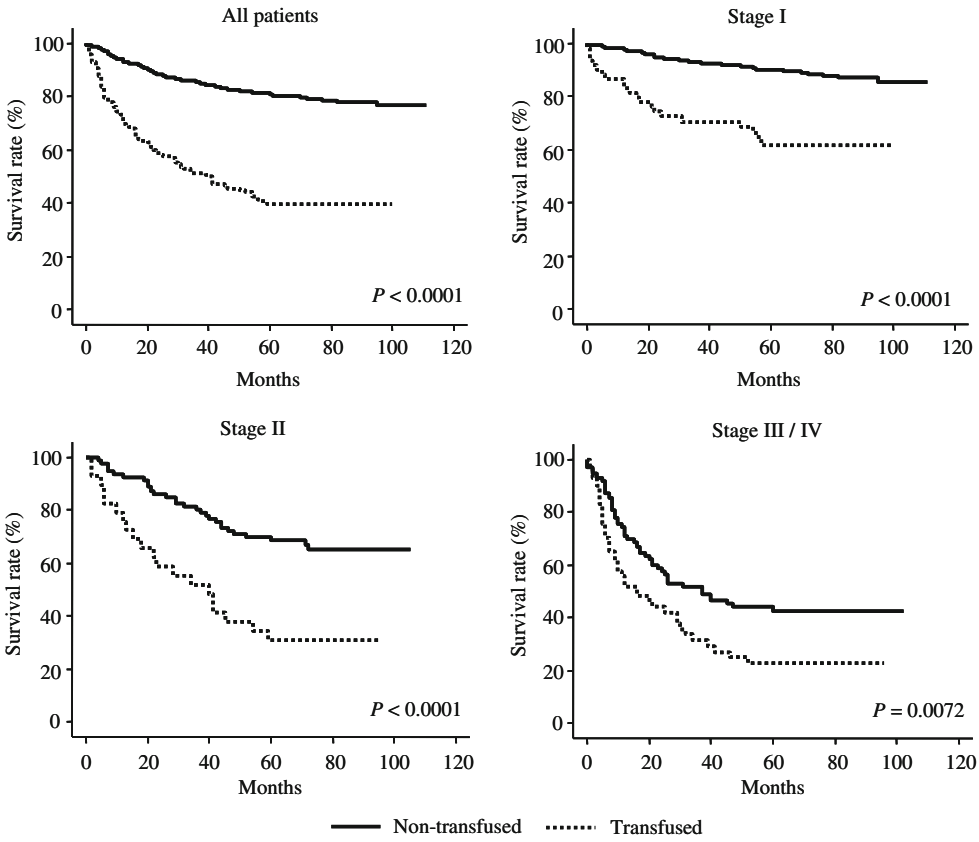

Figure 1 Overall survival rates.

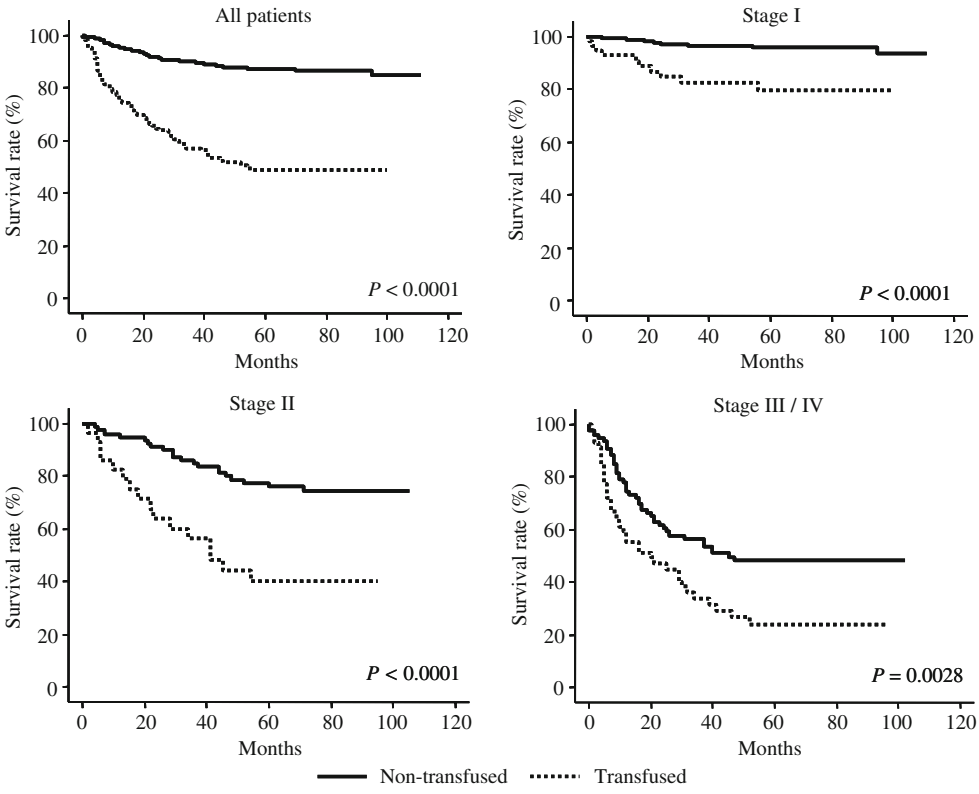

Figure 2 Disease-specific survival rates.

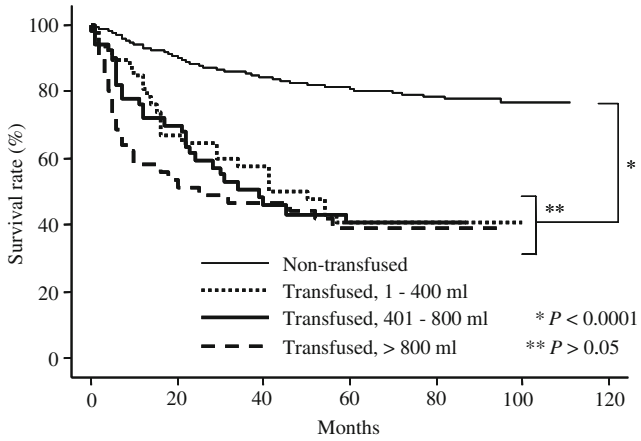

Figure 3 Overall survival rates according to the amount of transfusions.

(Hb<10 g/dl); the odds ratios were 3.15, 2.46, 11.62, and 8.08, respectively.

Discussion

The relationship between perioperative blood transfusions and survival in gastric cancer remains controversial. A previous study with 1,015 patients by Kampschöer et al.[10] showed no difference between 5-year survival rates in transfused and nontransfused patients grouped by stage. A study on 568 patients by Moriguchi et al.[11] also showed no relationship between perioperative blood transfusions and survival time of patients who underwent curative resection for gastric cancer. They described that effects of blood transfusions are closely associated with other prognostic covariates and there is no prognostic significance of blood transfusions on survival time, a finding repeated in several other studies.[12–14] On the other hand, some studies have shown an adverse relationship. Kaneda et al.[17] first proposed that blood transfusions could have a negative influence on surviving gastric cancer. Their study showed that, in subgroups of patients stratified for stage, there was a significant difference in the range of survival time for stage I patients but not for patients at other stages. However, their analysis was performed on a relatively small group, and only univariate analyses were used for comparison. A large retrospective study by Dhar et al. have shown that the 5-year disease-free survival was significantly worse in the transfused group and blood transfusion became an independent prognosticator in the multivariate analysis.[15] According to a recent study by Hyung et al. that was based on 1,710 patients, survival in transfused patients was clearly poorer than that in nontransfused patients with stage III and IV gastric cancer.[16] However, significant differences in survival rates were not found in stage I and II patients. The authors described how immunosuppression of transfusions may cause progression of metastatic foci and failure to remove circulating cancer cells at an advanced cancer stage.[16]

We performed multivariate analysis with the use of the Cox regression model, adjusting all the covariates simultaneously. Allogeneic blood transfusion was seen to have prognostic significance when all the 13 covariates were included in the Cox regression analysis of the 856 patients. When patients were stratified by stage, transfusion was independently predictive of shorter survival in patients at all disease stages. In most stage I and II patients, the spread of cancer is limited enough that cancer cells can be completely excised by the surgical procedure. However, it has been reported that some patients have minimal residual disease, even with early stages of gastric cancer.[27] Minimal residual disease is one of the major causes for tumor relapse after curative resection of the primary tumor in gastric cancer.[28] Heiss et al.[29] showed that the poorer prognosis linked to transfusion is mediated through an impact on minimal residual disease in gastric cancer patients after curative resection, and they described how transfusion-related immunosuppression affects minimal residual disease after curative tumor resection. These studies may support our findings that blood transfusions are independent prognostic factors for long-term survival even for early stage patients after curative gastrectomy.

In Japan, the current blood transfusions have routine leukodepletion, although blood transfusions at our facility did not routinely undergo leukodepletion during the time period of this study. Therefore, our results of the relationship between allogeneic blood transfusions and poor prognosis in gastric cancer patients might be reversed in the future.

As compared with a previous study at Yonsei University College of Medicine by Hyung et al.,[16] our results showed significantly lower survival rates in transfused patients. Several speculations can be formed based on this difference. First, the transfused patients managed by Yonsei University College of Medicine were significantly younger than those in our institution (55 years vs. 67 years). Second, our data included five transfused patients who died within 1 month after the operation, whereas operative mortality cases were treated as censored data in the other study. Third, Hyung et al. excluded from their study patients who had undergone only D1 lymph node dissection due to concomitant disease. Therefore, we deduce that there was a low number of patients with serious concomitant disease in the study from Korea.

There have been very few reports on the relationship between the amount of transfused blood and survival rates. In gastrectomy, only the abovementioned study described a significant difference in the survival rates according to the

amount of transfused blood.[16] In hepatic resection for colorectal metastases, patients with one- or two-unit transfusions had no significant difference in long-term survival than nontransfused patients.[30] On the other hand, our results demonstrated that allogeneic blood transfusions had an important effect on prognosis, even if the amount of transfused blood was small. Allogeneic blood transfusions generally cause down-regulation of cellular immunity, with decreased cutaneous delayed type hypersensitivity, T-cell proliferation, and natural killer cell function, and it seems to drive the immunosystem toward a T helper type 2 (Th 2) response and away from a Th 1 response.[31] It was recently reported that $CD4^+CD25^+$ regulatory T cells are implicated in immunosuppression of transfusions.[32,33] Furthermore, it is reported that this blood transfusion-related immunosuppression occurs regardless of the amount of transfused blood.[31–34] That is consistent with our findings. On the other hand, our findings showed that the short-term survival of patients with massive blood transfusion >800 ml was poorer than that of patients with blood transfusion <800 ml. The 1-year survival

Table 5 Univariate and Multivariate Analysis of Risk Factors Influencing Blood Transfusion Requirements

Risk factors	Categories	Univariate analysis		Multivariate analysis	
		P value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)
Sex	Male	0.805	1.05 (0.71–1.55)		
	Female				
Age	≥65	<0.0001	2.20 (1.51–3.18)	<0.0001	3.15 (1.97–5.02)
	<65				
BMI	≥25	0.026	1.66 (1.06–2.58)	0.265	0.71 (0.39–1.30)
	<25				
Type of gastrectomy	TG/PG	<0.0001	4.09 (2.81–5.96)	0.060	1.80 (0.98–3.33)
	DG				
Splenectomy	Yes	<0.0001	3.77 (2.62–5.41)	0.693	1.14 (0.60–2.17)
	No				
Pancreaticosplenectomy	Yes	<0.0001	4.51 (2.33–8.76)	0.665	0.82 (0.34–2.01)
	No				
Lymph node dissection	D3	<0.0001	2.73 (1.78–4.19)	0.139	1.55 (0.87–2.76)
	D1/D2				
Approach	Open	0.042	2.63 (1.04–6.68)	0.353	1.63 (0.58–4.60)
	Lap				
Duration of operation (min)	≥300	<0.0001	4.75 (3.24–6.97)	0.0002	2.46 (1.52–3.99)
	<300				
Blood loss (ml)	≥1,000	<0.0001	16.24 (10.24–25.76)	<0.0001	11.62 (6.69–20.20)
	<1,000				
Cardiovascular disease	Yes	0.137	1.45 (0.89–2.35)		
	No				
Pulmonary disease	Yes	0.446	1.37 (0.61–3.08)		
	No				
Renal dysfunction	Yes	0.219	0.28 (0.04–2.13)		
	No				
Liver dysfunction	Yes	0.109	1.66 (0.89–3.06)		
	No				
Diabetes	Yes	0.918	1.04 (0.54–1.99)		
	No				
Anemia	Yes	<0.0001	7.33 (2.94–18.26	0.0002	8.08 (2.74–23.79)
	No				

CI confidence interval, *BMI* body mass index, *DG* distal gastrectomy, *TG* total gastrectomy, *PG* proximal gastrectomy, *Open* open gastrectomy, *Lap* laparoscopy-assisted gastrectomy

rates were 93.2% for the nontransfused group, 80.6% for the group transfused with 1 to 400 ml, 72.0% for the transfused group with 401 to 800 ml, and 58.0% for the transfused group with more than 800 ml. We consider that massive blood transfusion may cause immunosuppression immediately after transfusion. Furthermore, serious complications associated with massive transfusion itself might be related to poor prognosis. Indeed, in our data, the rates of perioperative infectious complications were significantly higher in the transfused patients (4.3% vs. 13.0%).[35] Consequently, if at all possible, we should avoid giving allogeneic blood transfusions when performing gastrectomy in gastric cancer patients.

However, Hb of <10 g/dl and an expectation of intraoperative blood loss exceeding 1,000 ml indicate a necessary transfusion in gastric cancer patients.[36] In our multivariate logistic regression analyses, high age (≥65 years), long duration of operation (≥300 min), massive blood loss (≥1,000 ml), and anemia (Hb<10 g/dl) were the significant risk factors influencing blood transfusion requirements. The odds ratio of massive blood loss was 11.6, and it was the highest value in these risk factors. Therefore, we must prevent unnecessary transfusions by meticulously limiting intraoperative bleeding through careful anatomical dissection and controlling bleeding with electrocoagulation, ultrasonic, laser devices, and collagen-sealing devices. According to a meta-analysis of laparoscopic and open gastrectomy for gastric cancer, laparoscopy-assisted gastrectomy was associated with a significantly reduced rate of intraoperative blood loss.[37] Indeed, our data showed that the mean intraoperative blood loss was larger in the open gastrectomy group (620 + 658 ml) than in the laparoscopy group (197 + 245 ml). Considering avoidance of transfusion, laparoscopic approaches for early gastric cancer can be considered a valid option. In our data, the mean intraoperative bleeding was larger in stage III and IV groups than in stage I and II groups, and significantly more patients in stage III and IV groups required blood transfusion (data not shown). Therefore, neoadjuvant chemotherapy might play a pivotal role to improve the anatomical dissection of invasive malignancies when performing gastrectomy with radical lymph nodes dissection for advanced gastric cancer.

These abovementioned in potential risks of allogeneic blood transfusions have heightened interest in the use of autologous blood transfusion. However, the effects of autologous blood transfusion on immune function were yet unclear.[34,38] In addition, the use of the supply of red blood cell substitutes, such as perfluorocarbon emulsions or liposome-encapsulated hemoglobin, has been reported to reduce the need for blood transfusions in patients undergoing major surgery.[39] In fact, these red blood cell substitutes do not pose an infectious risk and have favorable O_2 transport properties.[40] The use of these materials may reduce the incidence of intraoperative allogeneic blood transfusions in gastric cancer patients undergoing gastrectomy.

In conclusion, allogeneic blood transfusion was an independent prognostic factor for long-term survival in gastric cancer patients. As far as possible, we should avoid transfusing when performing gastrectomy for gastric cancer. Moreover, massive intraoperative bleeding was the most significant risk factor for blood transfusion requirements. Therefore, we should make an increased effort to reduce blood loss during the operation.

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J Gastrointest Surg (2009) 13:1831–1838
DOI 10.1007/s11605-009-0964-x

ORIGINAL ARTICLE

Association of Pregnane X Receptor with Multidrug Resistance-Related Protein 3 and its Role in Human Colon Cancer Chemoresistance

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Received: 7 May 2009 / Accepted: 22 June 2009 / Published online: 11 July 2009

Abstract
Background Pregnane xenobiotic receptor (PXR), a ligand-activated transcription factor, regulates the drug metabolism and transport. Its activation can reduce the efficacy of antineoplastic agents. The aim of this study was to investigate the role of PXR and the relationship between PXR and multidrug resistance-related protein 3 (MRP3) in human colon cancer chemoresistance.
Results The results showed that both the mitochondrial RNA (mRNA) and protein levels of PXR and MRP3 were much higher in colon cancer tissues than that in nonneoplastic tissues by reverse transcriptase polymerase chain reaction and Western blot analysis. MRP3 mRNA was significantly correlated with PXR mRNA in cancerous (P=0.001) and nonneoplastic (P<0.001) colon tissues with Pearson correlation test. The expressions of PXR, SP1, and MRP3 were markedly enhanced after rifampicin treatment. On the other hand, the protein level of MRP3 decreased after stable RNA interference of PXR. It also observed that PXR, activated by rifampicin or knocked down via short hairpin RNAs, could enhance or reduce cells resistance to the chemotherapeutic agents through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.
Conclusions The results suggested that PXR, associated with MRP3, may play an important role in human colon cancer resistance to chemotherapeutics and SP1 may be involved in the induction of MRP3 by PXR activation.

Keywords PXR · MRP3 · Colon cancer · Drug resistance · Nuclear receptor

Introduction

Colon cancer is one of the most frequent malignant tumors in the world. The morbidity and mortality of colon cancer have been increasing year by year in China because of the great changes of Chinese dietary pattern. Chemotherapy is one of the most common treatments for colon cancer, but its efficacy is limited by the resistance of cancer cells to drugs. The inductions of drug-metabolizing enzymes and ATP-binding cassette (ABC) transporters are reported to be involved in the cancer cell multidrug resistance, but the mechanism has not been clearly clarified.[1,2]

Pregnane xenobiotic receptor (PXR; also called SXR, PAR, or NR112) is an orphan nuclear receptor that regulates a large number of genes including phase I drug-metabolizing enzymes (cytochrome P450 3A4, CYP3A4), phase II drug-metabolizing enzymes (UDP-glucuronosyltransferases, glutathione S-transferases), and drug transporters such as P-glycoprotein (MDR1), multidrug resistance-related proteins 2, 3 (MRP2, MRP3), etc. Most of them are involved in cancer multidrug resistance.[3–6] It seems that PXR can alter the metabolism and transport of chemotherapeutic drugs in tumor cells and individuals via regulating the transcription of its target genes. Therefore, it can play a

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pivotal role in the resistance to chemotherapy with inter-individual variability. Recently, it has been reported that PXR was expressed in some cancerous tissues such as breast, endometrial, prostate, and ovarian cancer, and its relationship with MDR1 and CYP3A4 in drug resistance was also studied by the other groups.[7–11] However, the expression of PXR and its possible role in colon cancer remain unknown.

The overexpression of ABC transporters is one of the primary causes of multidrug resistance in cancer cell lines and tumors.[12,13] MRP3, also known as ABCC3, is one of several ABC paralogs located in the basolateral membrane of polarized cells, and it also constitutively expressed in the gut.[14–16] The role of MRP3 in drug resistance is well known,[17,18] but the study on its relationship with the colon cancer chemotherapy is rare. Furthermore, although the induction of MRP3 by PXR ligands has been reported,[19,20] it is still unclear that whether the induction of MRP3 by PXR is involved in colon cancer resistance to chemotherapy.

This study is the first one that examined the mitochondrial RNA (mRNA) and protein expressions of PXR and MRP3 in cancerous and matched nonneoplastic colon tissues. Correlation of PXR mRNA and MRP3 mRNA was also investigated. We further studied the role of PXR in the resistance to chemotherapy in human colon cancer cell lines. The results suggested that PXR may play an important role in colon cancer resistance to chemotherapy and the induction of MRP3 by PXR activation might be involved in the mechanisms. PXR may be an essential target in colon cancer individual chemotherapy to overcome drug resistance.

Methods

Materials and Patients

The human colon cancer cell lines LS174T, LOVO, HCT116, and HT29 were purchased from American Type Culture Collection (Manassas, VA, USA). Cell culture reagents and transfection reagent were purchased from Invitrogen (Carlsbad, CA, USA). Chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA), except when the source was specified. Clinical-grade oxaliplatin and 5-fluorouracil were obtained from our pharmacy. PXR short hairpin RNA (shRNA) plasmids were constructed by Genechem (Shanghai, China) and confirmed by sequencing. The validated target sequences of PXR shRNA were obtained from Cold Spring Harbor Laboratory as follows: 1#, 5′-CTTCTCCCATTTCAAGAAT-3′; 2#, 5′-GCATCC ATTTGAACACATT-3′. A corresponding random siRNA sequence(5′-TTCTCCGAACGTGTCACGT-3′) was used as a negative control of PXR shRNA. From November 2007 to March 2008, colon cancer tissues and matched adjacent nonneoplastic tissues were obtained from 17 patients who had undergone surgical resection at the local Department of General Surgery. The tissue samples from each patient were determined by histopathologic diagnosis and frozen in liquid nitrogen until RNA and protein extractions. No patient had received chemotherapy before surgery. The research was approved by the local ethics board and informed consent was obtained from each patient preoperatively.

Cell Culture and Stable Transfection

Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator with an atmosphere of 5% carbon dioxide at 37°C. LS174T cells were transfected with shRNA constructs at a 80% to 90% confluence using Lipofectamine 2000 transfection reagent (Invitrogen) according to manufacturer's instructions. For stable transfection, cells were passaged at 1:10 into fresh growth medium 24 h after transfection. G418 was added at final concentration of 600 μg/ml to eliminate non-transfected cells in the following day and 200 μg/ml to maintain transfected cells. Surviving single colonies with fluorescence were cloned, expanded, and tested for the expression of PXR and a further study.

Semiquantitative RT–PCR

The expressions of PXR, SP1, and MRP3 were evaluated by semiquantitative reverse transcription–polymerase chain reaction (RT-PCR). In brief, total RNA was extracted from the tissues or cultured cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. RNA quantity and quality of the different samples were determined by the 260:280 nm absorbance ratio using a Bio-Rad SmartSpec 3000 Spectrophotometer. Two micrograms total RNA from each sample was used to generate cDNA by reverse transcription with the M-MLV reverse transcriptase (Promega, Madison, WI, USA). PCR was performed with 2× Taq PCR master mix (TIANGEN, Beijing, China) using the selective primers for PXR, 5′-AGAGCGCATGAAGAAGGAG ATG-3′ (forward)/5′-GAAATGGGAGAAGGTAGTGTCA AAGG-3′ (reverse); SP1, 5′-GCCGCTCCCAACTTACA GAA-3′ (forward)/5′-CCCATCAACGGTCTGGAACT-3′ (reverse); MRP3, 5′-AAAAGCAGACGGCACGACA-3′ (forward)/5′-GCAGGCACTGATGAGGAAGC-3′ (reverse); GAPDH, 5′-GAGTCAACGGATTTGGTCGTATTG-3′ (forward)/5′-CCTGGAAGATGGTGATGGGATT-3′ (reverse). The PCR mixtures were initially denatured at 94°C for 4 min, followed by denaturation for 30 s at 94°C, primer

annealing for 30 s at 63°C, and extension for 1 min at 72°C for 26 (MRP3, GAPDH, SP1) or 28 cycles(PXR) in cell samples and 30 cycles in tissue samples with the iCycler thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). A final extension for 7 min at 72°C ensured complete extension of the PCR products. PCR products were separated on 2% agarose gel, and band intensity was quantified using QuantityOne 4.2.0 software (Bio-Rad) after background subtraction from each band. The GAPDH was used for normalization.

Western Blot Analysis

Total membrane proteins were isolated from 200-mg tissues or cultured cells grown in 550-ml flasks. Samples were homogenized sufficiently at 4°C in lysis buffer (pH 7.5, 250 mM sucrose, 100 mM Tris-base, 120 mM KCl, 1 mM EDTA, and 1 mM EGTA) containing protease inhibitors (0.2 mM phenylmethylsulphonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin, and 1 μg/ml aprotinin) and centrifuged (1,000×*g* at 4°C, 10 min) to separate the membranes from the cell nuclei. Membrane fractions were collected by ultracentrifugation (100,000×*g*, at 4°C, 60 min) and resuspended in buffer (pH 7.5, 300 mM sucrose, 10 mM HEPES, one table/10 ml complete protease inhibitor). Nuclear proteins were prepared using the NE-PER kit (Pierce, Rockford, IL, USA) according to the manufacturer's protocol. Protein concentration was determined according to Pierce bicinchoninic acid protein assay kit instructions, and protein samples were stored at −80°C. For Western blot analysis, 50 μg nuclear extract, or 100 μg membrane protein was separated on 12% or 7.5% sodium dodecyl sulfate–polyacrylamide gels. After transfer to polyvinylidene fluoride microporous membranes (Bio-Rad), the membranes were blocked with 5% nonfat dry milk and then incubated sequentially with the primary antibody (PXR, 1:400; SP1, 1:200; MRP3, 1:50), followed by incubation with the corresponding horseradish peroxidase-conjugated antibody (1:2,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The immune complexes were detected with the enhanced chemiluminescence reagent (Pierce). The PXR (H-11), SP1 (H-225), and MRP3 (C-18) antibodies were purchased from Santa Cruz Biotechnology.

Chemotherapeutic Sensitivity Assay

LS174T cells were trypsinized, resuspended, and counted, and 5,000 cells were seeded into 96-well plates in triplicate. After attachment, cells were treated with 0.1% dimethyl sulfoxide or 10 μM rifampicin for 48 h and then treated with oxaliplatin or 5-fluorouracil for a further 48 h. Cell viability was measured by 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Plates were incubated with MTT at 37°C for 4 h, and the absorbance at 490 nm was measured in a plate reader (Bio-Rad Model 550 Microplate Reader, USA).

Statistical Analysis

Statistical analysis was performed using the SPSS 13.0 software package (SPSS, Chicago, IL, USA). Values were expressed as mean±SD of at least three experiments. The difference between two groups was analyzed by paired-samples *t* test or independent-samples *t* test (two-tailed) as where indicated. The correlation between PXR and MRP3 mRNA was assessed with Pearson correlation test. Value of $P<0.05$ was considered statistically significant.

Results

Increased Expressions of PXR and MRP3 in Colon Cancer Tissues as Compared with Nonneoplastic Colon Tissues

To compare the expressions of PXR and MRP3 between human colon cancer tissues and matched adjacent nonneoplastic tissues, the mRNA levels of PXR and MRP3 were examined in surgically removed colon samples from 17 patients using RT-PCR. As shown in Fig. 1a, PXR and MRP3 were expressed in both cancerous and nonneoplastic colon tissues. Furthermore, the mRNA levels of PXR and MRP3 increased visibly in colon cancer tissues on the gels. The mRNA levels of PXR (0.6794±0.2623 vs 0.3894±0.2712, $P<0.001$) and MRP3 (0.6682±0.2032 vs 0.4412±0.1932, $P<0.001$) were significantly higher in colon cancer tissues than that in the matched nonneoplastic tissues (Fig. 1b) with paired-samples *t* test. Consistent with the mRNA levels, the protein expressions of PXR and MRP3 also increased in colon cancer tissues compared with that in nonneoplastic colon tissues (Fig. 1c).

Significant Correlation Between PXR mRNA and MRP3 mRNA Transcripts in Clinical Specimens

Correlation between PXR and MRP3 mRNA levels in tissue samples was tested using Pearson correlation analysis (Fig. 2). The MRP3 mRNA was positively correlated with the PXR mRNA both in colon cancer tissues ($r=0.735$, $P=0.001$; Fig. 2a) and in matched nonneoplastic colon tissues ($r=0.759$, $P<0.001$; Fig. 2b). To reduce the experimental errors resulting from the exposure differences of individual gels, we further tested the correlation between the relative mRNA expression of PXR and MRP3, which represented the ratio of mRNA expression between cancerous and nonneoplastic colon tissues. A statistically positive correla-

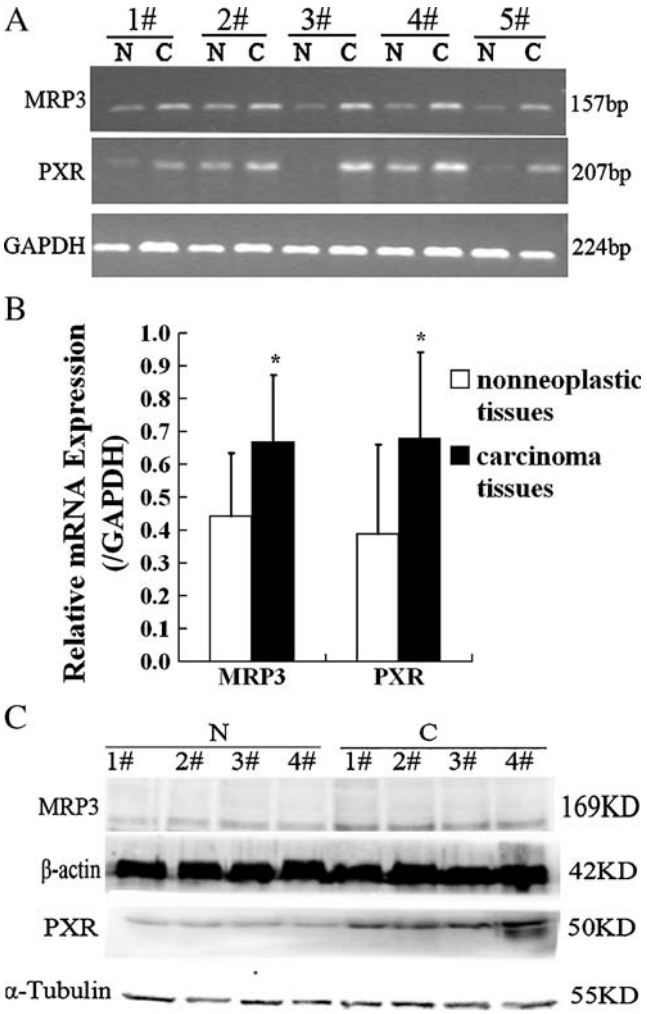

Figure 1 Expression of PXR and MRP3 in colon cancer tissues and matched nonneoplastic colon tissues. **a** The mRNA expression of PXR and MRP3 in cancerous and matched nonneoplastic colon tissues. GAPDH was used as an internal control. **b** Semiquantitative analysis of PXR and MRP3 mRNA levels in 17 tissue samples. Band intensity of PCR products was quantified and normalized to GAPDH. Data were assayed by paired-samples *t* test. **P*<0.01 when compared with nonneoplastic colon tissues. *Columns*, mean (*n*=17); *bars*, SD. **c** Protein expression of PXR and MRP3 in cancerous and nonneoplastic colon tissues. β-actin or α-tubulin was used to confirm the equal loading of membrane or nuclear protein, respectively. *C* cancerous colon tissues, *N* nonneoplastic colon tissues.

tion was also shown between them (r=0.629, P<0.007; Fig. 2c).

PXR Activation by Rifampicin Increased the Expression of PXR, SP1, and MRP3

In order to investigate the potential role of PXR in human colon cancer chemoresistance via regulating the expression of MRP3, the expression of PXR in human colon cancer cell lines was determined. As shown in Fig. 3a, LS174T cells had the highest level of PXR mRNA, but it was hardly detected in LOVO, HT29, and HCT116 cells by RT-PCR. Western blot analysis with a specific PXR antibody also showed that PXR protein level in LS174T cells was the highest among all the colon cancer cell lines. As shown in Fig. 3b, after the treatment of rifampicin, the MRP3 mRNA began to increase with the PXR mRNA at 8 h, and both decreased remarkably at 72 h. The expressions of PXR, SP1, and MRP3 in LS174T cells were also examined after the treatment of rifampicin. Both the mRNA and protein levels of PXR, SP1, and MRP3 were remarkably increased after rifampicin treatment (Fig. 3c). The results indicated that SP1 might be involved in the induction of MRP3 by PXR ligand.

PXR Activation by Rifampicin Increased Resistance of LS174T Cells to Chemotherapeutics

To study whether above-described increased MRP3 and PXR expressions were involved in drug resistance, the effects of rifampicin pretreatment on the resistance of LS174T cells to oxaliplatin and 5-fluorouracil which are two well-known chemotherapeutics drugs for colorectal cancer were further examined. It was found that the survival of LS174T cells with rifampicin pretreatment was increased compared with the vehicle-treated group after exposure of oxaliplatin or 5-fluorouracil with different doses (Fig. 4a, b). For oxaliplatin, IC_{50} without or with rifampicin pretreatment were 8.80±0.26 and 19.37±4.22 μg/ml (P=0.012; Fig. 4c, left), respectively, and for 5-fluorouracil, the corresponding IC_{50} were 32.27±2.92 and 62.3±9.08 μg/ml (P=0.005; Fig. 4c, right).

PXR Knocking Down Via shRNA Increased Colon Cancer Cell Sensitivity to Chemotherapeutics

To further confirm the role of PXR in the resistance of LS174T cells to chemotherapeutics, the expression of PXR was knocked down in LS174T cells with the shRNA constructs. In the wild-type and PXRi control LS174T cells, PXR was detected as a band of 207 bp using RT-PCR, whereas for the knockdown clones, PXR was hardly detected (Fig. 5a). The knockdown of PXR expression in LS174T cells was further confirmed by Western blot analysis. As shown in Fig. 5b, in PXRi 1# and PXRi 2# clones after stable selection with G418, the PXR protein expression was markedly knocked down compared with those in the wild-type and PXRi control cells. Corresponding to the expression of PXR, the protein level of MRP3 also was decreased in PXRi 1# and PXRi 2# clones. Finally, the wild-type, PXRi control, PXRi 1# cells, and PXRi 2# cells were subjected to chemotherapeutics. We found that PXRi 1# cells and PXRi 2# cells were more sensitive to oxaliplatin and 5-fluorouracil than the wild-type and PXRi control cells (Fig. 5c). The results further suggested the role of PXR in the responses of colon cancer cells to chemotherapy.

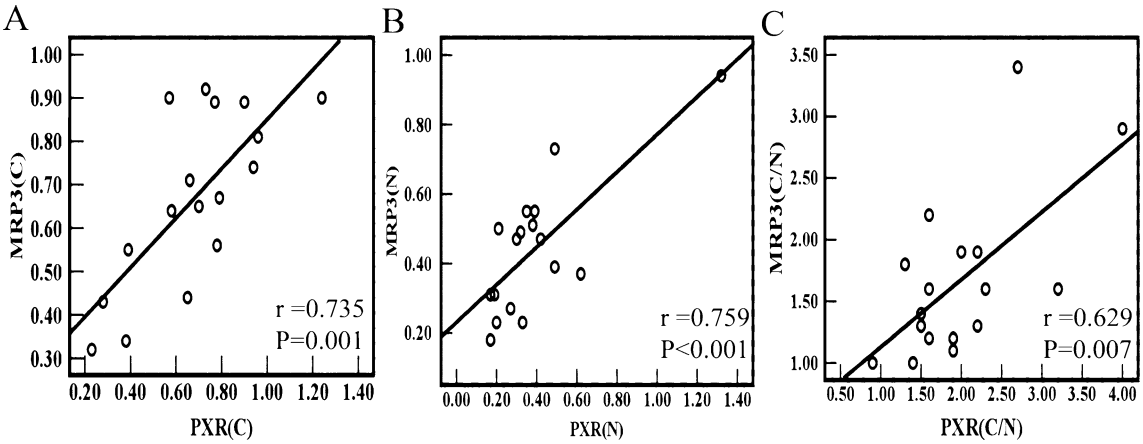

Figure 2 Correlation between PXR and MRP3 mRNA in colon tissue samples. Significant correlation between PXR and MRP3 mRNA in cancerous colon tissues (**a**) and in matched nonneoplastic colon tissues (**b**). **c** Significant correlation between the relative PXR and MRP3 mRNA in tissue samples. A ratio of mRNA between cancerous and nonneoplastic colon tissues was taken as the relative PXR and MRP3 mRNA. Data were analyzed by Pearson correlation test. *C* cancerous colon tissues, *N* nonneoplastic colon tissues, *r* Pearson correlation coefficients, $n=17$.

Discussion

In the present study, overexpression of PXR and MRP3 were detected in human colon cancer tissues compared with those in the matched adjacent nonneoplastic colon tissues. The positive correlation between PXR and MRP3 mRNA was also shown in these tissues. Furthermore, with the treatment of rifampicin which is a well-known agonist of PXR, a remarkably increased mRNA and protein levels of PXR, SP1, and MRP3 in LS174T cells and increased survival of LS174T cells towards oxaliplatin and 5-fluorouracil were found. Finally, knocking down PXR via shRNAs decreased the expression of MRP3 and sensitized cells to the chemotherapeutic agents. Our findings sug-

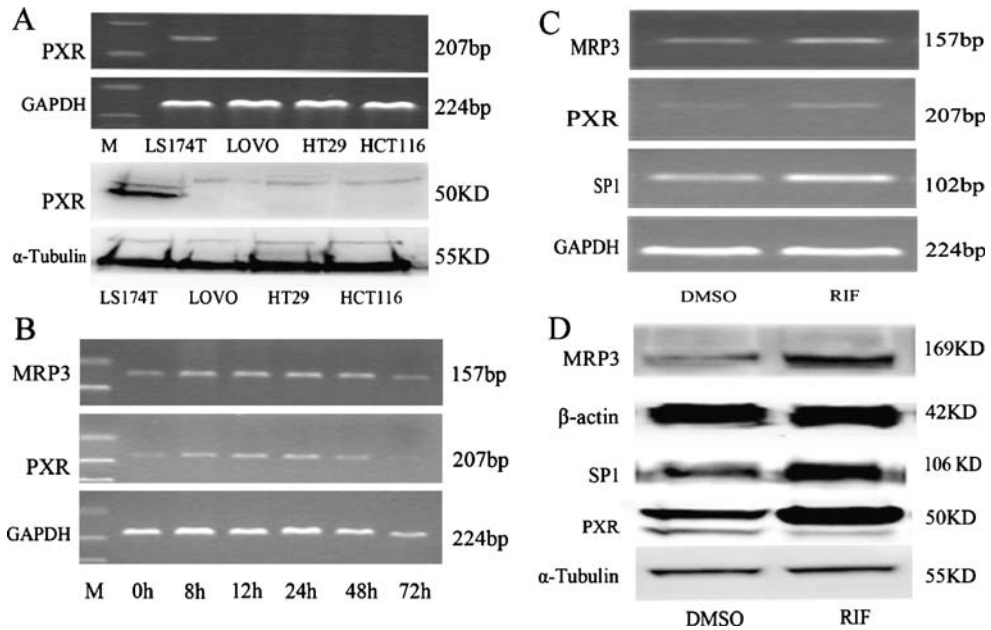

Figure 3 Expression of PXR, MRP3, or SP1 in human colon cancer cell lines with or without rifampicin. **a** Expression of PXR in LS174T, LOVO, HT29, and HCT116. *Top*, RT-PCR detection; *bottom*, Western blot analysis. **b** The mRNA levels of MRP3 and PXR in LS174T cells after rifampicin treatment for 0 h (DMSO), 8, 12, 24, 48, or 72 h. **c** RT-PCR detection of MRP3, PXR, and SP1 expression in LS174T cells treated for 24 h. **d** Western blot analysis of MRP3, PXR, and SP1 in LS174T cells treated for 48 h. The cells were cultured in DMEM containing 10% FBS, treated with 0.1% DMSO or 10 μM rifampicin, and harvested for detection at the indicated time. GAPDH or β-actin, α-tubulin was used as a control for RT-PCR or Western blot, respectively. Each experiment was performed three times at least and the best result was shown here.

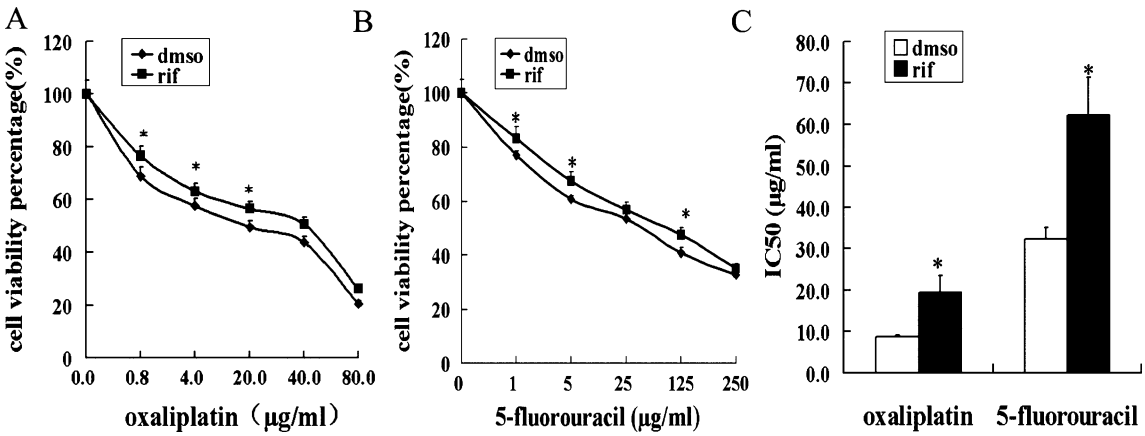

Figure 4 Increased chemoresistance in LS174T cells after PXR activation by rifampicin. The cells were cultured in DMEM containing 10% FBS, pretreated with 0.1% DMSO or 10 μM rifampicin for 48 h, and followed by 48-h treatment of oxaliplatin (**a**) or 5-fluorouracil (**b**). *Points*, mean viability as a percentage of control (cells without chemotherapeutics treatment, 100%); *bars*, SD. **c** IC_{50} of oxaliplatin or 5-fluorouracil in LS174T cells pretreated by rifampicin. *Columns*, mean; *bars*, SD. **P*<0.05 when compared with 0.1% DMSO-treated group using independent-samples *t* test. Each experiment was performed three times in triplicate.

gested an important role of PXR in human colon cancer resistance to chemotherapy, and the induction of MRP3 by PXR activation via SP1 might be involved in the process.

PXR, a ligand-activated transcription factor, regulates the drug metabolism and transport. Its activation is responsible for the important inductive drug interactions.[5,6] Conversely, blocking the activation of PXR by ketoconazole can inhibit the drug metabolism.[21,22] As the ligands of PXR are promiscuous, most of clinical drugs including some chemotherapeutic agents can serve as its ligands.[23,24] Therefore, especially for cancer patients who are in clinical settings with several kinds of drugs, PXR can be activated by antineoplastic agents themselves or by other co-administered drugs.[25,26] PXR activation can change the metabolism and transport of antineoplastic agents and contribute to the chemoresistance. In this study, both the mRNA and protein levels of PXR were significantly higher in colon cancer tissues than those in nonneoplastic colon tissues. The overexpression of PXR in cancer tissues might alter the local concentrations of antineoplastic agents in colon tumor cells and then reduced the clinical efficacy of these agents. On the other hand, PXR expression in the nucleus is considered to be activated, so our results also implied that activated PXR expression was much higher in cancerous tissues than that in nonneoplastic colon tissues. However, further studies should be preformed to determine the potential mechanism of PXR activation in the normal and cancerous colon tissues. The study in vitro further suggested that PXR, activated by rifampicin or knocked down by shRNAs, can enhance or reduce cell resistance to oxaliplatin and 5-fluorouracil. This was consistent with the previous studies which linked PXR to drug resistance in tumors.[9–11] Finally, both the differential upregulation of PXR in certain human cancers and the fact that the receptor

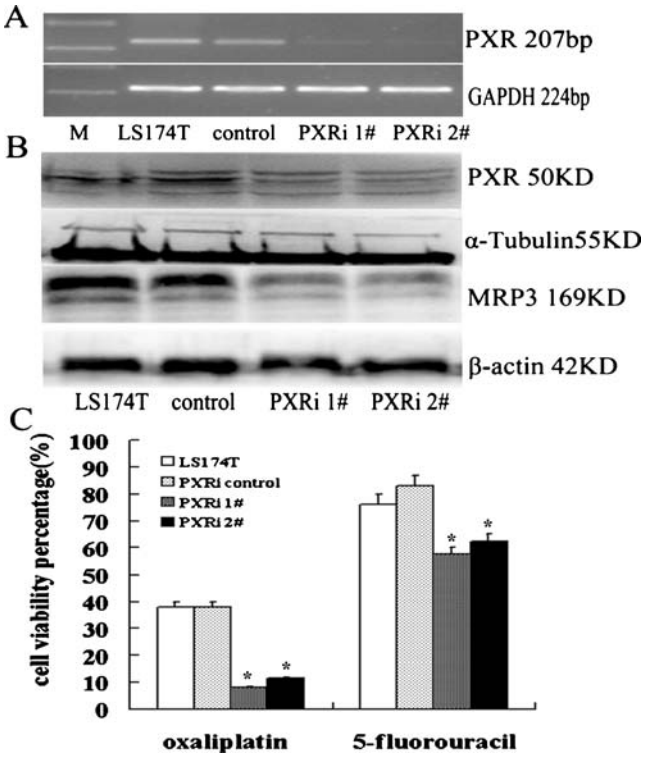

Figure 5 Reduced chemoresistance in LS174T cells with PXR knocked down. **a** The mRNA level of PXR and **b** Western blot analysis of PXR and MRP3 in wild-type LS174T cells or stable transfected cells. GAPDH or β-actin, α-tubulin was used as a control for RT-PCR or Western blot, respectively. LS174T cells were seeded in 24-well plate, transfected with PXR shRNA plasmid 1#, 2# or negative control plasmid, and selected by G418 for stable transfected cells (PXRi 1#, PXRi 2#, and PXRi control). **c** Cell viability of wild-type LS174T cells or stable transfected cells treated with oxaliplatin (20 μg/ml) or 5-fluorouracil (5 μg/ml). **P*<0.05 when compared with PXRi control cells. *Columns*, average from three separate experiments in triplicate; *bars*, SD.

is easily activated by its ligands suggested that the selective PXR modulators might be useful in cancer treatment.

MRP3 serves as an organic anion transporter and transports substances including bile acids, glucuronide conjugates, as well as some anticancer drugs and their metabolic conjugates.[17,18] MRP3 was correlated with resistance to platinum drugs in cancer cell lines.[27–29] Two MRP3 polymorphisms were identified to influence the response to platinum-based therapy in lung cancer patients.[30] Furthermore, Campa et al.[31] have reported the association between MRP3 polymorphism and colon cancer risk and provided information potentially relevant for pharmacogenetics in colon cancer chemotherapy. All these studies implied that MRP3 might play an important role in platinum-based colon cancer chemotherapy. In this study, the expression of MRP3 was significantly higher in colon cancer tissues than in matched nonneoplastic colon tissues, but the mRNA levels of CYP3A4 and MDR1 showed no significant differences between them (data not shown), which suggested that MRP3 might play a more important role in intrinsic multidrug resistance in colon cancer than CYP3A4 and MDR1. However, the evidence of MRP3 linkage to colon cancer chemotherapy will require further investigations.

Little is known about the mechanism behind the regulation of MRP3 expression. Recent studies have also indicated an involvement of nuclear receptors in MRP3 regulation, including PPARα, VDR, and RARα.[32–34] The involvement of PXR has not been determined. In this study, it was found that MRP3 mRNA significantly correlated with PXR mRNA both in colon cancer tissues and in nonneoplastic colon tissues. The activation of PXR by its ligands, rifampicin or paclitaxel (data not shown), could also induce the expression of MRP3 in LS174T cells. Furthermore, the expression of MRP3 was decreased after stable RNA interference of PXR. The overexpression of PXR in cell lines via stable plasmid transfection would enhance the observation of the effect of PXR on MRP3 in the further study. However, these results indicated that PXR could participate in the regulation of MRP3. The proximal promoter region of the rat Mrp3 gene was found to contain putative binding sites for LRH-1 and SP-1 that were essential for Mrp3 transcriptional activity.[35,36] In the study, the mRNA and nuclear protein levels of SP1 were also increased after PXR activation (Fig. 3c) in LS174T cells, but LRH-1 was not detected in this cell line (data not shown). So it suggested that SP1, not LRH-1, may be involved in the induction of MRP3 by PXR activation in LS174T cells. We hypothesized that PXR might enhance SP1 activity in GC-rich regions of the MRP3 promoter, However, the details should be further clarified. Finally, it should be noted that the induction of MRP3 by PXR is not the only possible role of PXR in cancer multidrug resistance which is multiple and complex. For example, the role of PXR in anti-apoptosis and cell proliferation in tumor cells has been reported recently.[37,38] Therefore, further studies on the PXR-mediated pathways in colon cancer chemotherapy will be useful to clarify these issues.

Conclusions

In summary, the results suggested that PXR, associated with MRP3, may play an important role in human colon cancer resistance to chemotherapy. SP1 may be involved in the induction of MRP3 by PXR activation. Because of the important roles of PXR in drug resistance, PXR activation should be considered when treating cancer patients according to the expression status of PXR in cancerous tissues or developing a novel chemotherapeutic drug to prevent the PXR-mediated drug resistance.

Acknowledgments This work was supported by the National Natural Science Foundation of China (grant no. 30570842). We thank Dr. Yingxue Hao for his generous help in collecting up patients and Mr. Wen Qin for his excellent writing assistance.

Competing interests The authors declare that they have no competing interests.

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J Gastrointest Surg (2009) 13:1839–1844
DOI 10.1007/s11605-009-0970-z

ORIGINAL ARTICLE

Perioperative Selective Decontamination of the Digestive Tract (SDD) in Elective Colorectal Surgery

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Received: 12 April 2009 / Accepted: 15 July 2009 / Published online: 28 July 2009

Abstract
Background Selective decontamination of the digestive tract (SDD) decreases morbidity and mortality in critically ill patients and morbidity in patients undergoing esophageal resection. This study analyzes the effect of perioperative SDD in patients undergoing elective colorectal surgery on postoperative infections and anastomotic leakage.
Methods This is a retrospective analysis of prospectively collected data in a 3-year cohort of 162 patients undergoing elective resection of colon and or rectum. Of these patients, 76 (47%) received SDD (polymyxine B sulfate, tobramycin, and amphotericin) perioperatively. The control group consisted of 86 patients who were not treated with SDD. Postoperative complications, hospital stay, and mortality were analyzed.
Results In the SDD group, there were six patients (7.9%) with infectious complications compared with 17 patients (19.8%) in the control group ($p=0.031$). The incidence of the combined endpoint infectious complications and anastomotic leakage was 8 (11%) in the SDD group vs. 22 (26%) in the control group ($p=0.014$). Multivariate analysis showed that no-SDD, aged above 60 years and diabetes were independent predictors of postoperative complications.
Conclusion Perioperative SDD in elective colorectal surgery seems to reduce postoperative surgical complications including infectious complications and anastomotic leakage. Prospective, randomized, placebo-controlled studies are needed to confirm this conclusion.

Keywords Selective decontamination · Colorectal surgery · Postoperative complications · Infections · Anastomotic leakage

Introduction

Despite the use of perioperative antibiotic prophylaxis, improvements in surgical techniques, and perioperative care, the complication rate after abdominal surgery remains high (30–51%) with specific incidences up to 28% after colon surgery.[1–3] The most common infectious complications are urinary tract, wound, and pulmonary infections. These nosocomial infections usually occur after 48 h of admission and are mostly caused by aerobe Gram-negative microorganisms.[4,5] These Gram negatives generally originate from the patient's digestive tract. Colonization of the digestive tract with potentially pathogenic microorganisms (PPMs) can harm the patient in two different ways. Apart from PPMs causing postoperative infections due to translocation to organ sites, permeation of endotoxins from the gut into the systemic circulation can cause sepsis if the gut barrier function fails, for example during surgery.[6] While the intact anaerobe intestinal flora protects against second-

Results were presented at a scientific meeting: Frontiers in Critical Care, NVIC, November 2008, Beurs van Berlage, Amsterdam, The Netherlands.

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ary colonization with PPMs, reduction of normal flora as a result of systemic antibiotics increases the risk of infection.

It is generally accepted that antimicrobial prophylaxis is indicated for contaminated surgery where gross contamination is inevitable and the risk of wound infection is high as in colorectal surgery. Although different regimens have been evaluated, a single dose administered immediately before operation, preferably 1 h to 30 min before incision, has shown to be as effective as long-term prophylaxis after surgery.[7]The prophylaxis must cover both aerobic and anaerobic bacteria with minimal toxicity and costs. However, some antibiotics disturb the normal balance and lead to bacterial overgrowth.[8] Secondary colonization is additionally enhanced by disturbed bowel movements and decreased immune incompetence due to the underlying disease. Factors that lower colonization resistance are host-associated (for instance old age, severe physical trauma, major surgery, malignancy, kidney and liver disease, diabetes, diminished peristalsis of the digestive tract as in ileus) or can be iatrogenic (antacida, anesthetica, antibiotics, endotracheal tube, catheters, and drains).

Selective decontamination of the digestive tract (SDD) was introduced into intensive care medicine as an infection prophylaxis regimen to reduce or even eradicate aerobic PPMs from the oropharynx to the rectum while leaving the normal anaerobic flora largely undisturbed.[9,10] The use of SDD for the critically ill patients on an intensive care unit (ICU) has been assessed in 56 randomized controlled trials (RCT) and eight meta-analyses including only RCTs.[11] Lower airway and bloodstream infections were significantly reduced by 65% and 37%, respectively, in two recent meta-analysis.[12,13] Mortality was also significantly reduced by 22% and 20%, respectively, in these two systematic reviews. In 1990, Stoutenbeek and van Saene[14] advocated the use of SDD as a perioperative infection prophylaxis in elective surgery including liver transplantation,[15] esophagectomy,[16] and gastrectomy.[17]

Since 1984, our hospital has used SDD in all critically ill patients. Encouraged by the good results in this population,[9,18,19] perioperative SDD was introduced in 1999 in elective esophageal and colorectal surgery on the surgical ward. The hypothesis was that SDD, initiated before surgery on the ward, reduces postoperative infectious complications and anastomotic leakage.

Material and Methods

This study enrolled 162 consecutive patients who were admitted to the surgical ward for elective colorectal surgery, including closure of a temporary colostomy, between January 1, 1999 and December 31, 2001. Patients who needed acute surgical intervention because of infection, perforation, ileus, and/or bleeding were excluded. Figure 1 shows the number of patients admitted for colorectal surgery and number of excluded patients. Data were collected from a database of all patients admitted to the department of surgery of the OLVG. This database contained data concerning therapy, all postoperative complications, and duration of hospital stay.

At the time of this study, SDD was given randomly, according to the surgeon's opinion, especially to patients undergoing colorectal surgery. SDD was thus not standardized.

If a diverting ileostomy was created, it was separately noted. Operating time, open vs. laparoscopic surgery, type of anastomosis, and type of surgeon (resident or attending surgeon) were registered as well as the pathological reports of the specimen. The SDD regimen consisted of polymyxine B sulfate 100 mg, tobramycine 80 mg, and amphotericine B 500 mg (PTA). It was taken orally four times daily or through a nasogastric tube if the patient had one after surgery. In that case, half of the SDD was administered in the mouth and half via the nasogastric tube. The optimal SDD regime started 2 days before surgery and was continued postoperatively until normal bowel passage was achieved (normal intake and/or passage of stool) or at least until the third day after surgery. The data concerning the use of SDD were obtained from the pharmacy information system and checked in the patient files. In addition, all patients received parenteral antibiotics perioperatively for 24 h (cefuroxim 1,500 mg and metronidazol 500 mg at 8-h interval) starting 30 min before surgery. In this study, we used an osmotic laxans (macrogol and electrolytes) named Klean-Prep® (Norgine B.V., Amsterdam, The Netherlands) as

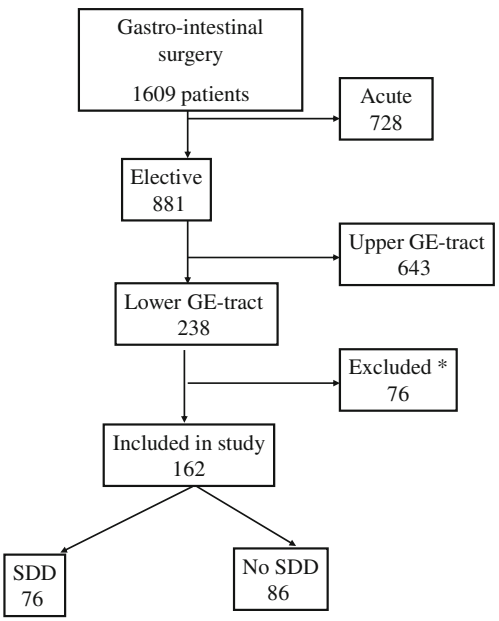

Figure 1 Study flow chart. *Asterisk*, 76 patients were excluded because of operation for rectal prolapse, rectovaginal fistulas, or underwent an abdominal perineal resection without anastomosis.

mechanical bowel preparation. Two to 4 liters of Klean-Prep® were administered in 24 h and/or a fluid diet was given starting 1 day before surgery. In rectal surgery, also an enema was applied. Complication data (during hospital stay and at least 30 days post-surgery) were obtained from a prospectively collected database as mentioned above. Infectious complications (wound infections, urinary tract infections, pneumonia, and intra-abdominal abscesses) and the incidence of anastomotic leakage were analyzed. Anastomotic leakage was determined by clinical examination (fever, abdominal pain, passage problems) and blood results (elevated white blood count and/or raised C-reactive protein). The diagnosis was confirmed by CT scan and X-ray with rectal contrast or relaparotomy.

Infectious complications and anastomotic leakage together were called "the combined endpoint." Abdominal wound dehiscence was a clinical diagnose, confirmed by an attending surgeon. Cardiac failure, deep venous thrombosis, pulmonary embolism, and cerebrovascular accidents were all diagnosed and confirmed by the consulting specialists. Mortality was defined as in-hospital death or death within 30 days post-surgery.

Statistical Analysis

Continuous variables were expressed as means±SD or median (interquartile range) and were compared with the use of an unpaired Student's *t* test or Mann–Whitney *U* test. Categorical data were compared using Fisher's exact test or Pearson chi-square test where appropriate. All reported *p* values were two-sided; differences were considered significant when p was <0.05. Analysis was on intention to treat; all patients receiving at least one dose of SDD preoperatively were analyzed. Univariate analysis was performed to identify other prognostic parameters for the endpoint infectious complications (including anastomotic leakage). We used multivariate logistic regression analysis to identify which of these were independent risk factors.

Results

Of the 162 patients undergoing elective colorectal surgery, 76 (47%) received SDD. Eighty-six patients (53%) did not receive SDD and were used as the control group. The administration of SDD was optimal (given at least until the third day after surgery) in 47 (62%) patients.

The patient characteristics are shown in Table 1. In the control group, more patients had a history of abdominal surgery and the incidence of diabetes tended to be higher. Surgical characteristics are shown in Table 2. In the SDD group, more patients underwent anterior and low anterior resections and the percentage of closure of temporary colostomy was higher. In both groups, open abdominal surgery was performed more often than laparoscopic surgery and a stapled technique was used more frequently than a sutured anastomosis. There was no difference in type (side-to-side, side-to-end, or end-to-end) of anastomosis or operation by a resident or supervising surgeon. The operating time was not different between groups. The pathology reports showed no difference in malignancy between groups [46 patients in the SDD group (61%) vs. 40 patients in the control group (47%) had a malignant disease, $p=0.074$]. In most patients, a mechanical bowel preparation (Klean-Prep ®) was used. In only a few cases was an enema given.

Table 3 shows the complications during follow-up. In the control group, there were 32 patients (38%) with 49 complications and in the SDD group 19 patients (25%) with 29 complications. There was a significant reduction in the rate of infectious complications (urinary tract infection, pneumonia, wound infection, and intra-abdominal abscesses) in the SDD group. The incidence of the combined endpoint infectious complications and anastomotic leakage was lower in the SDD group (8 vs. 22 in the control group). Three (4%) patients died in the control group vs. none in the SDD group ($p=0.248$). These three patients died of sepsis and multi-organ failure due to anastomotic leakage. There was no significant difference in hospital stay (median 10 days in both groups).

Table 1 Patients' Characteristics

	SDD ($n=76$)	Control ($n=86$)	p value
Male, *n* (%)	33 (43)	41 (48)	0.59
Age	64±15	63±15	0.70
Comorbidity			
Cardiovascular (%)	28 (37)	34 (40)	0.73
Pulmonary (%)	12 (16)	10 (12)	0.44
Previous abdominal surgery (one or more) (%)	24 (32)	45 (52)	0.008
Diabetes (%)	5 (7)	14 (16)	0.06
Immune deficiency (HIV, use of corticosteroids) (%)	4 (5)	1 (1)	0.19
Inflammatory bowel disease (%)	7 (9)	8 (9)	0.98

Values are presented as mean±SD or number (%) as appropriate

Table 2 Surgical Characteristics

	SDD (n=76)	Control (n=86)	p value
Type of surgery			
Hemicolectomy (right sided)[a] (%)	16 (21)	26 (30)	0.18
Hemicolectomy (left-sided)[a] (%)	7 (9)	8 (9)	0.98
Transversectomy (%)	1 (1)	3 (3.5)	0.62
(Subtotal) colectomy (%)	3 (4)	0	0.10
Sigmoid resection (%)	15 (20)	23 (27)	0.29
(Low) anterior resection (%)	30 (40)	13 (15)	0.00
Closure of temporary colostomy (%)	5 (7)	14 (16)	0.06
Operating time (min)	195±63	181±49	0.13
Laparoscopic surgery (%)	2 (3)	9 (11)	0.05
Diverting ileostomy (%)	7 (9)	2 (2)	0.08
Stapled (%)[b]	48 (64)	65 (78)	0.05
Resident (%)	41 (54)	50 (58)	0.59

Values are presented as mean±SD or number (%) as appropriate

[a] One patient in each group had a right- and left-sided hemicolectomy in one session

[b] Three patients in the control group and one in the SDD group had both stapled and partial sutured anastomosis and were counted as stapled

Univariate as well as multivariate analysis showed that not receiving SDD, diabetes, and age above 60 years were significant and independent predictors for the combined endpoint infectious complications and anastomotic leakage (Table 4).

Discussion

This non-randomized controlled study on the use of perioperative SDD in elective colorectal surgery in addition to standard parenteral prophylaxis shows a significant decrease of infectious complications and of the combined endpoint with anastomotic leakage when SDD is applied. The size of the present study is too small to draw conclusions about a possible effect of SDD on mortality. Since this is the first study on the use of perioperative PTA-SDD in elective colorectal surgery, initiated 1 to 2 days before surgery on the ward, comparison with the literature is not possible. However, our results are in accordance with the application of the same SDD regimen in patients undergoing esophageal resection.[16,20] The hallmark of this PTA-SDD regimen is that the antibiotics remain in the digestive tract and are not adsorbed. Taylor and Lindsay[21] evaluated oral ciprofloxacin (which has systemic effects as

Table 3 Endpoints

	SDD (n=76)	Control (n=86)	p value
Hospital stay in days, median (IQR)	10 (8–13)	10 (7–13)	0.980
Patients with infectious complications[a], n (%)	6 (8)	17 (20)	0.031
Urinary tract infection	2 (3)	9 (11)	0.048
Pneumonia	3 (4)	5 (6)	0.724
Wound infection	0	4 (5)	0.123
Intra-abdominal abscess	2 (3)	1 (1)	0.601
Patients with anastomotic leakage, n (%)	2 (3)	6 (7)	0.284
Patients with infectious complications and anastomotic leakage (combined endpoint), n (%)	8 (11)	22 (26)	0.014
Patients with non-infectious complications, n (%)[a]	15 (20)	19 (22)	0.713
Cardiopulmonary[b]	9(12)	10(12)	0.966
Neurologic (CVA, delier)	2 (3)	3 (4)	1.00
Abdominal (non-infectious)[c]	7 (9)	13 (15)	0.254
Patients with a relaparotomy, n (%)	6 (8)	7 (8)	0.937
No. of patients with complications, n (%)	19 (25)	32 (38)	0.085
Mortality, n (%)	0	3 (4)	0.248

IQR interquartile range (25%–75%)

[a] Some patients had more than one complication

[b] AF, infarct, pulmonary embolism, respiratory insufficiency, decompensatio cordis

[c] Urinary retention, ileus, wound dehiscence, hematoma

Table 4 Multivariate Analysis of the Combined Endpoint: Infectious Complications and Anastomotic Leakage

	Univariate			Multivariate		
Variable	OR	95% CI	*p* value	OR	95% CI	*p* value
SDD	0.3	0.14–0.82	0.017	0.4	0.14–0.84	0.021
Previous abdominal surgery	1.2	0.55–2.72	0.617			
(Low) anterior resection	1.2	0.52–2.96	0.635			
Diabetes	4.0	1.45–11.07	0.008	2.9	0.97–8.51	0.057
Age >60 years	3.2	1.24–8.40	0.016	3.4	1.26–9.17	0.016
Converting ileostomy	0.5	0.06–4.37	0.552			
Closure of temporary colostomy	0.5	0.11–2.21	0.349			

well) for 24 h preoperatively as a prophylaxis in colorectal surgery and found a reduction of incidence of infectious complications from 32.7% to 14.5%.On the contrary, Espin-Basany et al.[22] found no advantage in postoperative complications in 300 patients who were divided into three groups. One group received three doses of oral antibiotics (1 g of neomycin and 1 g of metronidazole three times a day) preoperatively, one group one dose of neomycin and metronidazole, and the third group no oral antibiotic. They all received perioperative parenteral antibiotics (cefotaxitim 1 g intravenous before skin incision and two postoperative doses). In the present series of patients, selective decontamination of the digestive tract was achieved by administration of the oral antibiotics four times a day, at least one and preferably 2 days prior to surgery. This regimen differs from those described in previous studies in several ways. First, the orally administered antibiotics remain in the digestive tract and have no systemic effects. Furthermore, the antibiotics are optimally given for at least 2 days prior to surgery to reach the entire digestive tract before surgery commences and are continued postoperatively until normal bowel movements are attained. Although the present results are convincing, the study has several limitations.

Most importantly, the intervention was not randomized and placebo controlled. Due to the non-randomized design of this study, we could not correct for all potential interacting factors (known and unknown).

Furthermore, for an optimal effect of SDD, bowel movements are necessary for the antibiotics to reach the entire gut. It therefore takes at least 2 days before selective decontamination is achieved, or longer if bowel movements are delayed. In this study, the SDD was started 2 days before surgery in only 67% of the patients. As a result, one third of the patients were likely not sufficiently decontaminated before surgery. When the peristalsis is diminished, for instance during obstruction or paralytic ileus, the topical antibiotics are only transported by diffusion and decontamination of the digestive tract is insufficient. Also, SDD needs to be continued until oral intake is resumed and normal bowel passage is achieved because then, secondary colonization of PPMs is prevented by natural mechanisms of defense. Due to the retrospective analysis of clinical data, it was not always clear whether SDD was continued until normal bowel passage was achieved. Therefore, if SDD would have been given at a strict regimen before surgery and until normal bowel function had been achieved postoperatively, it would probably have resulted in a further decrease of infectious complications.

It should be noted that there is a difference in administration of the SDD on the surgical ward or on the ICU.[23] On the ICU, the SDD regime includes five components. First, for gastro-enteral decontamination, polymyxine, tobramycin, and amphotericin (PTA) are administered in the stomach by the gastric tube. Second, for oral decontamination, a sticky paste containing PTA is applied in the mouth. Oral decontamination is crucial for intubated patients who lack oral feeding. Third, for treatment of (possible) infections on ICU admission, the patients receive intravenous cefotaxim 1 g four times daily until site cultures are negative. Fourth, the topical antibiotic treatment is combined with an optimal hygienic strategy preventing exogenous infections. Fifth, surveillance cultures are taken three times a week to determine colonization on admission, to monitor whether decontamination is adequate, and to detect possible acquisition of exogenous resistant microorganisms. On the ward, however, the patient population is not intubated and is not critically ill; therefore, a different regimen of administration may be applicable. Our patients did not receive additional oropharyngeal decontamination and surveillance cultures were not taken.

In literature, high American Society of Anesthesiologists score, (low) rectal surgery, an operative time of 3 h or longer, and a high body mass index are factors associated with complications after colorectal surgery.[24–26] In our series of patients, the surgeons tended to apply SDD in patients with a high risk for infectious complications [in this case (low) anterior resections]. However, this selection bias did not result in a higher complication rate in the SDD group. Multivariate analysis did not show low anterior operation as an independent predictor, although this subgroup might be too small and its interaction with the use of SDD too strong to draw a firm conclusion. Also, previous abdominal surgery and a diverting ileostomy above a low anastomosis had no effect on the outcome. Altogether, the SDD group had less infectious

complications even though there were more low-anterior and anterior resections in the SDD group.

In our study, diabetes and higher age were additional independent predictors of the combined endpoint. Although more patients had diabetes mellitus in the control group, the use of SDD was associated with less infectious complications independent of diabetes and higher age. There could be a possible advantage in the use of perioperative SDD for this specific group of patients who (a priori) have a higher chance of postoperative complications.

Conclusion

The present non-randomized controlled study of a 3-year cohort of 162 patients is the first study that analyzes the use of perioperative SDD in elective colorectal surgery. The study shows that perioperative SDD initiated 1 to 2 days before surgery using oral antibiotics that remain in the digestive tract reduces postoperative infectious complications combined with anastomotic leakage. To confirm this conclusion, we initiated a placebo-controlled randomized trial.

Acknowledgments The authors thank Dr. van Saene for his comments and helpful advice in the revision of the manuscript. We also thank Dr. M. Simons for supplying the database of prospectively collected data on postoperative complications of all patients admitted to our hospital.

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J Gastrointest Surg (2009) 13:1845–1851
DOI 10.1007/s11605-009-0974-8

ORIGINAL ARTICLE

The Role of "Fatty Pancreas" and of BMI in the Occurrence of Pancreatic Fistula After Pancreaticoduodenectomy

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Received: 29 May 2009 / Accepted: 15 July 2009 / Published online: 29 July 2009

Abstract
Introduction Pancreatic fistula (PF) after pancreaticoduodenectomy (PD) is still a serious complication. We hypothesized that the amount of fatty tissue in the pancreatic parenchyma could be associated with the occurrence of PF after PD with pancreatogastrostomy.
Material and methods From January 2004 to December 2006, 111 consecutive patients underwent PD with pancreatogastrostomy. The microscopic amount of fatty tissue in the pancreas was evaluated.
Results The morbidity and mortality rates were 35.1% and 1.8%, respectively. PF occurred in 10.8% (n=12). PF was of grade A in nine, grade B in two, and grade C in one patient. Univariate analysis showed that a body mass index (BMI)>25 (P=0.035), a soft pancreatic parenchyma (P=<0.003), a pancreatic duct size <3 mm (P=0.015), and a fatty infiltration of the pancreas of more than 10% (P=0.0003) were associated with the occurrence of PF. The advanced age (P=0.049) and the BMI (P<0.0001) were significantly associated with the presence of >10% of pancreatic fat.
Conclusions A pancreatic fatty infiltration of the pancreas over 10% constitutes a risk factor for PF after PD. Age and BMI are useful preoperative predictors of the percentage of pancreatic fat.

Keywords Pancreatic fistula · Pancreaticoduodenectomy · Fatty pancreas

Introduction

Pancreaticoduodenectomy (PD) is the standard treatment for pancreatic head and periampullary malignant tumours. During the last decade, while the operative mortality rate after PD significantly decreased, the incidence of postoperative morbidity still remains high, ranging from 25% to 50%.[1–5] Pancreatic fistula (PF) is the major source of complications, and leakage rate varies from 2.3% to 25%.[6–10] Several risk factors for PF after PD have been reported. They include age, sex, jaundice, operative time, intraoperative blood loss, type of pancreatico-digestive anastomosis, texture of the pancreas, pancreatic duct size, hospital volume, and surgeon's experience.[11–17] The identification of patients at high risk for PF during the operation may induce the choice of an alternative strategy, such as the use of a pancreatic stent or an invaginating anastomosis, in order to prevent or reduce the severity of such a complication.[18–19] Recently, Mathur et al.[20] have shown that fatty infiltration of the pancreatic parenchyma constitutes a risk factor for PF after PD. However, a certain degree of fatty infiltration can be detected in almost all pancreatic parenchyma. Therefore, the percentage of fatty infiltration of the pancreas constituting a risk for PF after PD still needs to be defined, and the correlation between the level of pancreatic fatty infiltra-

Grant support: None

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tion, the body mass index (BMI), and the pancreatic texture (assessed intraoperatively by the surgeon) has not yet been evaluated.[20] The aim of the present study was to analyze the impact of the amount of fatty infiltration of the pancreatic parenchyma on postoperative outcome after PD with pancreatogastrostomy and to investigate its correlation with other clinical and pathological relevant features such as the texture of the pancreas and the BMI.

Patients and Methods

Study Population

From January 2004 to December 2006, 111 consecutive patients underwent PD with pancreatogastrostomy in our institution. The clinicopathological data of these patients were prospectively collected and retrospectively reviewed. All the operations were performed by four senior surgeons who each had already performed previously at least 50 pancreaticoduodenectomies. A patient was considered malnourished when the weight loss was more than 10% of the usual weight during the last 3 months before the operation.

Surgical Procedure

All PD were performed according to a standardized technique as previously reported.[6] Particularly, all patients underwent a double-layer invaginated pancreatogastrostomy.[13,21] A single abdominal drain was systematically inserted through the hiatus of Winslow near the pancreatogastrostomy. Amylase concentration in the drainage fluid was measured daily during 7 days. The abdominal drain was removed at day 7 after an upper gastrointestinal tract opacification with a water-soluble contrast in order to visualize a potential anastomotic leak. In case of PF, the drain was maintained in place until the complete healing of the PF. The postoperative care was supervised by the senior surgeons following a standardized protocol.[6]

Diagnosis of PF

The diagnosis of PF was established according to the definition of the International Study Group on Pancreatic Fistula Definition (ISGPF).[22] Therefore, PF was defined as any measurable drainage output from an intraoperatively placed drain (or a postoperatively placed percutaneous drain on, or after, postoperative day 3), with amylase content greater than three times the upper limit of normal serum amylase level according to the ISGPF.[22]

Evaluation of the Pancreatic Parenchyma

The pancreatic parenchyma consistency, soft or hard, was evaluated intraoperatively by the surgeon by manual palpation of the pancreatic remnant.

Postoperative Mortality and Morbidity

Postoperative mortality included intraoperative death, death within 30 days of surgery, and in-hospital death. All postoperative complications were registered and classified into four grades according to Dindo et al.[23] Delayed gastric emptying was diagnosed according to the ISGPS.[7] Only grades B and C were considered as in our practice during the study period; nasogastric tube was kept systematically until postoperative day 7.

Pathological Analysis

The amount of microscopic fatty infiltration of the pancreas was evaluated by two pathologists, who were not aware of patient's clinical data, in a simple and reproducible way. Slides of 4 μm thick were prepared from formalin-fixed and paraffin-embedded sections of pancreatic neck specimen and stained with hematoxylin–eosin. For each patient, three representative slides of non-tumoral pancreatic tissue located at least at 1 cm from the tumour were analyzed by two different pathologists. The amount of fatty infiltration of the pancreas was expressed as a ratio of fat cells both intralobular and interlobular/overall tissue surface (Figs. 1 and 2). The presence or absence of pancreatic fibrosis was evaluated in all specimens.

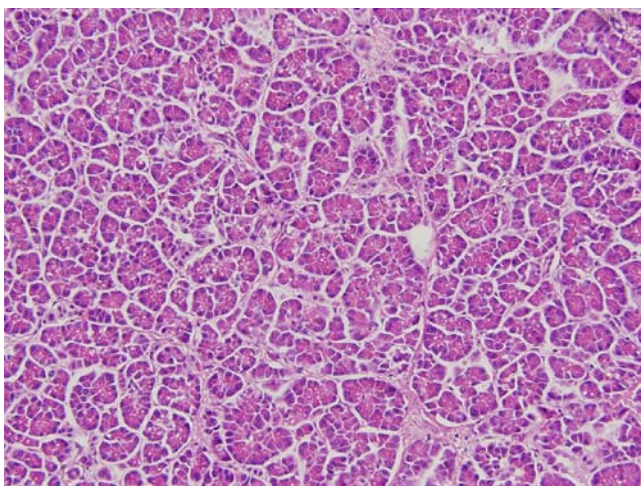

Figure 1 Microscopic photo of a typical appearance of histology specimen of the neck of the pancreas in a patient with fat infiltration of the pancreas <10% (magnification ×20, H&E stain).

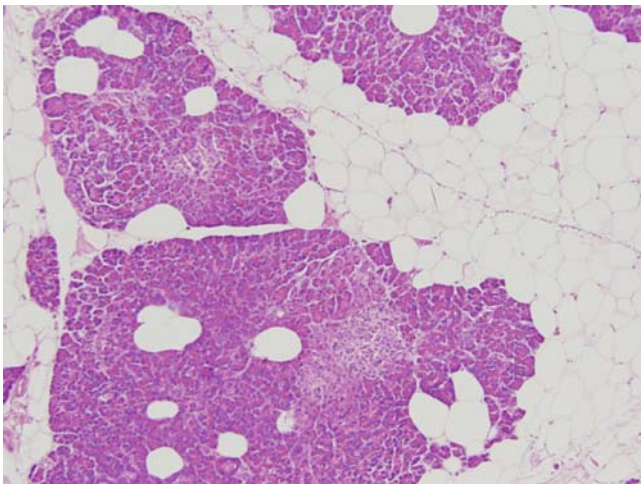

Figure 2 Microscopic photo of a typical appearance of histology specimen of the neck of the pancreas in a patient with fat infiltration of the pancreas >50% (magnification ×10, H&E stain).

Statistical Analysis

Data were expressed as mean±SD. The chi-square test, unpaired *t* test, and Mann–Whitney *U* test were appropriately used. A difference was considered significant when $P<0.05$. All statistical analysis including the receiver operating curve (ROC) were calculated by the SPSS statistical package.

Results

Patient Characteristics and Surgical Indications

There were 65 men and 46 women. The mean patient's age at the time of PD was 65±11 years (range 19–84 years). ASA score was 1 in 28 patients, 2 in 61, and 3 in 22 patients. Twelve patients were malnourished while 52 were overweight (BMI>25). A preoperative biliary drainage was used in 13 patients. The pancreatic parenchyma was soft in 62 patients, and 47 patients had a pancreatic duct size of less then 3 mm. Twenty patients underwent a PD associated with a PV resection; no interposition graft was used. The indications for resection were listed in Table 1.

Postoperative Mortality

Two patients died postoperatively (1.8%). One patient died on postoperative day 5 following a cardiac failure due to thrombosis of a mechanical aortic valve. The second patient died on postoperative day 20 following a multi-organ failure after the rupture of a mycotic aneurysm of the hepatic artery resulting in massive haemorrhage.

Postoperative Morbidity

A total of 55 non-lethal complications developed in 40 patients (36%) and were summarized in Table 2. Among them, 27 patients developed a single complication, 11 had two complications, and two patients had three complications. A total of 12 patients had PF (10.8%), of which nine with grade A, two with grade B, and one with grade C. Postoperative hospital stay was significantly longer in patients with postoperative morbidity compared to those without postoperative morbidity (30±5 vs. 14±3, $P\leq 0.001$). The occurrence of PF was not associated with the need for re-laparotomy, abdominal collection, delayed gastric emptying, or hemorrhage (Table 3).

Univariate Analysis for Risk Factors for Pancreatic Fistula

The univariate analysis showed that a BMI over 25 ($P=0.035$), a soft pancreatic parenchyma ($P<0.003$), a pancreatic duct size less than 3 mm ($P=0.015$), and a fatty infiltration of the pancreas of more than 10% ($P<0.0003$) were significantly associated with a higher risk of occurrence of PF (Table 4). PF (grade A) were similarly distributed in non-overweight and overweight patients ($P=0.218$); however, symptomatic PF (grades B and C) occurred only in overweight patients. Even for non-overweight patients, the presence of more than 10% of pancreatic fat was significantly associated with the occurrence of PF ($P=0.025$). The sensitivity, specificity, and positive and negative predictive value of fatty infiltration of the pancreas for PF were 100%, 53.5%, 20.6%, and 100%, respectively. The area under the ROC curve was 0.69. The ideal cutoff point for the percentage of pancreatic fat was 14 (Fig. 3). For this ideal cutoff value, the sensitivity, specificity, and positive and negative predictive value for the occurrence of PF were 91.7%, 55.5%, 19.6%, and 98.2%, respectively ($P=0.03$). Multivariate analysis did not show any independent factor.

Table 1 Indications for Pancreaticoduodenectomy

Indication	No. of patients
Adenocarcinoma of the head of the pancreas	61
Duodenal adenocarcinoma	4
Bile duct carcinoma	10
Adenocarcinoma of the ampulla of Vater	1
Endocrine carcinoma	4
Intraductal papillary mucinous tumour	4
Adenoma of the ampulla of Vater	8
Chronic pancreatitis	5
Others	14
Total	111

Table 2 Postoperative Morbidity According to Dindo et al.[23]

Type of complication	Grade I (n=9)	Grade II (n=27)	Grade III (n=15)	Grade IV (n=4)
Pancreatic fistula	9	1	1	1[a]
Delayed gastric emptying	0	6	0	0
Biliary leakage	0	0	2	0
Chylous ascites	0	1	0	0
Abdominal collection	0	0	3	0
Haemorrhage from pancreatic cut edge	0	0	2[a]	2[a]
Left liver lobe necrosis	0	2	0	0
Cholangitis	0	3	0	0
Partial thrombosis of the SMV	0	1	0	0
Small bowel occlusion	0	0	1[a]	0
Persistent diarrhea	0	1	0	0
Pneumothorax	0	0	1	0
Wound infection	0	0	2	0
Wound haematoma	0	0	1	0
Persistent hyperthermia	0	2	0	0
Deep vein thrombosis	0	1	0	0
Postoperative diabetes	0	5	0	0
Lung infection	0	1	0	0
Pleural effusion	0	1	1	0
Tachyarrhythmia	0	1	0	0
Intracardiac thrombosis	0	0	1	0
Heart failure	0	0	0	1
Confusion	0	1	0	0

SMV superior mesentric vein

[a] A re-laparotomy was performed for a total of six re-laparotomy in six patients

Table 3 Outcome of Pancreatic Fistula According to the Definition ISGPF[22]

	Bassi A	Bassi B	Bassi C	No pancreatic fistula
N	9	2	1	99
Hospital Stay	21±7	26±2	30	16±6
Mortality	0	0	0	2
Re-laparotomy	0	0	1	5
Abdominal collection	0	0	0	3
Delayed gastric emptying	0	0	0	6
Haemorrhage	0	0	1	3

Table 4 Univariate Analysis for Risk Factors for PF

	Pancreatic fistula (n=12)	No pancreatic fistula (n=99)	P value
ASA			0.093
1	4	24	
2	5	56	
3	3	19	
Preoperative biliary drainage			0.687
Yes	1	12	
No	11	87	
Malnutrition			0.355
Yes	0	12	
No	12	87	
BMI>25			0.035
Yes	9	43	
No	3	56	
Pancreatic parenchyma			<0.003
Hard	1	48	
Soft	11	51	
Pancreatic duct size (mm)			0.015
≥3	3	61	
<3	9	38	
External drainage of the Wirsung			0.348
Yes	1	3	
No	11	96	
Pylorus preservation			0.463
Yes	3	16	
No	9	83	
Portal vein resection			0.119
Yes	0	20	
No	12	79	
Perioperative transfusion			0.758
Yes	4	42	
No	8	57	
Operation time (mean)	585±246	703±202	0.133
Fibrosis			
Yes	4	55	0.220
No	8	44	
Pancreatic Fat			0.0003
>10%	12	46	
≤10%	0	53	

Univariate Analysis for Predictors of Fatty Pancreas

Univariate analysis showed that advanced age (P=0.049) and high BMI (P≤0.0001) were significantly associated with a fatty pancreas (>10% of pancreatic fat; Table 5). The amount of pancreatic fat infiltration was divided according

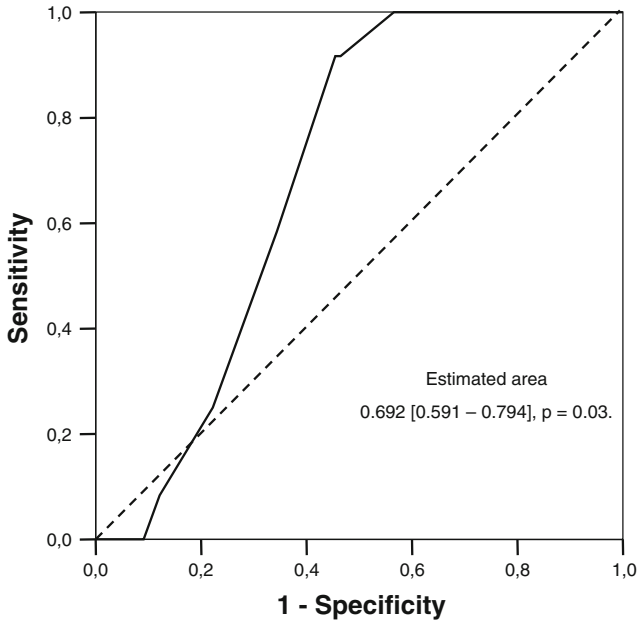

Figure 3 The ROC curve analysis of diagnostic sensitivity and specificity of fat infiltration of the pancreas for PF.

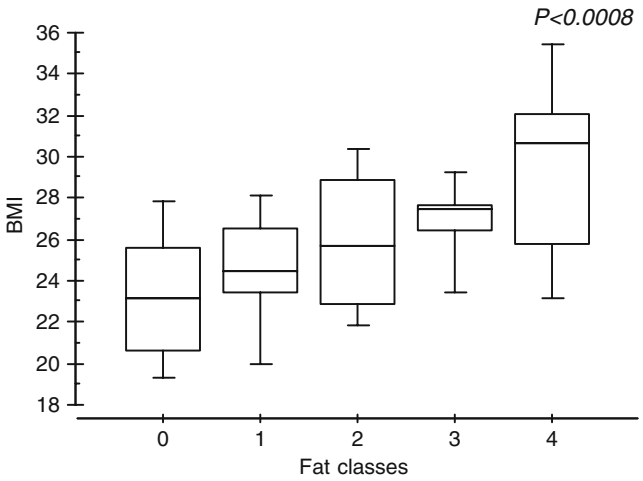

Figure 4 Correlation of pancreatic fat classified in class (0=0–9%, 1=10–19%, 2=20–29%, 3=30–39%, 4≥40%) and of the body mass index (*BMI*). The mean BMI was 23.5 in group 0, 24.6 in group 1, 25.8 in group 2, 26.9 in group 3, 29.5 in group 4.

to the five following groups: group 0=0–9%, 1=10–19%, 2=20–29%, 3=30–39%, 4≥40%. Figure 4 showed a significant correlation between the fatty infiltration of the pancreas and the BMI ($P<0.0008$).

Table 5 Univariate Analysis for Risk Factors for Fat Infiltration of the Pancreas

	Fat>10% (n=58)	Fat≤10% (n=53)	P value
Age	67.4±9.9	62.5±13.1	0.049
Sex			0.988
Male	34	31	
Female	24	22	
ASA			0.093
1	11	17	
2	34	27	
3	13	9	
Preoperative diabetes			0.187
Yes	4	1	
No	54	52	
BMI	26.2±3.8	23.5±3.5	<0.0001
Pancreatic parenchyma			0.167
Hard	22	27	
Soft	36	26	
Fibrosis			0.948
Yes	31	28	
No	27	25	

Factors Associated with the Pancreatic Parenchyma Texture

The consistency of the pancreatic parenchyma was significantly associated with the presence of fibrosis (77.5% in hard pancreas vs. 32.3% in soft pancreas, $P\leq0.0001$, respectively), while the pancreatic fat percentage was not ($P=0.837$).

The percentage of fatty infiltration of the pancreas was not associated with the presence or absence of pancreatic fibrosis ($P=0.651$); the mean percentage of pancreatic fat was 15.5±14.9 in patients with pancreatic fibrosis and 14.5±10.2 in patients without pancreatic fibrosis. Moreover, the percentage of fatty infiltration of the pancreas was not associated with the presence of an adenocarcinoma of the pancreas ($P=0.138$); the mean percentage of pancreatic fat was 13.5±12.8 in patients with pancreatic adenocarcinoma and 16.7±12.8 in patients without pancreatic adenocarcinoma.

Discussion

The present study showed that the fatty infiltration of the pancreas constituted a risk factor for PF after PD and that more than 10% of fatty infiltration of the pancreatic parenchyma represented a limit above which the risk of PF became significant. Age and BMI can be useful preoperative predictors to identify patients with a fatty pancreas.

Mathur et al.[20] showed that patients developing a postoperative PF after PD had significantly more intralobular, interlobular, and total pancreatic fat. In their study,

they selected 40 patients with PF and established a pancreatic fat score from 1 to 4 based on the addition of intra- and interlobular fat. They found that patients with PF were more likely to have a high pancreatic fat score (>3) than patients without PF (50% vs. 13%, $P<0.001$). The present study is based on a homogeneous series of patients who underwent PD with pancreatogastrostomy in a single institution. The pancreatic fat infiltration was measured in a simple and reproducible way. Interestingly, pancreatic fat infiltration was confirmed to be a risk factor for the occurrence of PF, particularly, a pancreatic fat infiltration over 10% which is the limit above which the risk of PF becomes significant. On the other hand, none of the patients with a pancreatic fat percentage less than 10% developed a PF. Moreover, a modest fatty infiltration of the pancreas is sufficient to increase significantly the risk of PF as shown by the ROC curve, which identified the ideal cutoff point for pancreatic fat infiltration at 14%.

Previous publications have demonstrated that obese patients undergoing surgery are at high risk for postoperative complications.[16,24,25] Sledzianoski et al.[25] showed that overweight patients (BMI>25 kg/m^2) had an increased PF rate after distal pancreatectomy. Our results confirmed that overweight was a risk factor for PF even after PD with pancreatogastrostomy. Moreover, all overweight patients developing PF in this study were symptomatic (grade B or C). Further study should clarify if the severity of the PF is associated with the patient's BMI.

Saisho et al.[26] showed that in adults, pancreatic fat content increased with aging and obesity, but it was not influenced by the presence or absence of type 2 diabetes. Age and BMI were associated with a pancreatic fat content of more than 10%. For the BMI, this correlation was almost linear (Fig. 3); therefore, older and overweight patients should be considered as a high-risk population for fatty pancreas.

In the present study, the multivariate analysis for risk factors for PF did not show any independent factor; therefore, it can be argued that an increased rate of PF in overweight patients may be more related to the technical performance of pancreatic anastomosis in this group of patients than to the fatty infiltration of the pancreas. However, a subgroup analysis showed that a pancreatic fat infiltration of more than 10% still constituted a risk factor for PF even in non-overweight patients.

As previously reported, pancreatic duct size and pancreatic parenchyma texture (soft vs. hard) were significantly related to the occurrence of PF.[13,15] Mathur et al.[20] suggested that fat infiltration of the pancreas would increase the softness of the gland. However, our data did not confirm this hypothesis. In the present series, we did not find any correlation between the pancreatic parenchyma texture and the amount of fatty infiltration of the pancreas. On the contrary, the presence of pancreatic fibrosis was the main feature determining the consistency of the pancreas. In the present study, the softness or the hardness of the pancreatic remnant was related to pancreatic fibrosis; therefore, the fatty infiltration of the pancreas cannot be assessed by intraoperative palpation of the pancreas. Whether the frozen sections which are routinely performed to analyze the pancreatic resection margin are useful to evaluate the fatty infiltration of the pancreas is a major concern during PD. While the frozen hematoxylin/eosin-stained sections have a low accuracy, they may identify patients with high or low fatty infiltration.[27] According to our data, fatty infiltration of the pancreas may be suspected preoperatively in elderly and overweight patients; moreover, CT scan seems useful in detecting the presence of fatty infiltration of the pancreas.[28,29]

Interestingly, the percentage of fatty infiltration of the pancreas was not associated with the pancreatic fibrosis or the presence of a pancreatic adenocarcinoma. The biological mechanisms at the origin of the fatty infiltration of the pancreas seem different from those inducing pancreatic fibrosis. According to our data, the pancreatic fat is associated with the weight and the age of the patients, whereas pancreatic fibrosis is induced by pro-fibrogenic mediators including ethanol and pancreatic cancer.[30]

In conclusion, pancreatic fatty infiltration of the pancreas over 10% constitutes a risk factor for PF after PD with pancreatogastrostomy. Age and BMI are useful preoperative predictors of the percentage of pancreatic fat. On the contrary, an intraoperative assessment of the pancreatic texture by palpation gives no information about the fatty contents of the pancreatic parenchyma, but is correlated with the presence or absence of pancreatic fibrosis.

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J Gastrointest Surg (2009) 13:1852–1858
DOI 10.1007/s11605-009-0978-4

ORIGINAL ARTICLE

S100A4 mRNA is a Diagnostic and Prognostic Marker in Pancreatic Carcinoma

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Received: 2 June 2009 / Accepted: 21 July 2009 / Published online: 4 August 2009

Abstract
Objective The aim of this study is to evaluate the clinical significance of *S100A4* mRNA expression in pancreatic cancer.
Materials and Methods We obtained invasive ductal carcinoma (IDC) cells from ten lesions, intraductal papillary mucinous neoplasm (IPMN) cells from 20 lesions, and normal ductal cells from 20 normal pancreatic tissues by laser microdissection of frozen tissues. *S100A4* expression was examined in the microdissected cells and in formalin-fixed paraffin-embedded (FFPE) samples of 87 pancreatic cancers by quantitative reverse transcription-polymerase chain reaction.
Results IDC cells expressed higher levels of *S100A4* than IPMN cells ($P=0.002$) and normal ductal cells ($P<0.001$), although the difference between IPMN cells and normal ductal cells was not statistically significant ($P=0.070$). Analysis of FFPE samples revealed that high *S100A4* expression was significantly associated with a shorter overall survival ($P=0.023$). In immunohistochemical analysis, the extent of *S100A4* mRNA expression was significantly correlated with the expression of S100A4 protein ($P=0.028$).
Conclusion S100A4 could be a marker for malignancy in pancreatic tumors and for poor prognosis in patients with pancreatic cancer.

Supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and grants from the Takeda Science Foundation, Pancreas Research Foundation of Japan, and Nakajima Foundation.

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Keywords S100A4 · Pancreatic carcinoma · Prognostic marker · Diagnostic marker · Formalin-fixed paraffin-embedded (FFPE)

Introduction

Pancreatic cancer is one of the most lethal tumors and is the fourth leading cause of tumor-related deaths in the industrialized world.[1,2] Only 10–20% of patients with pancreatic cancer have a chance of curative resection because most patients are at advanced stages of the disease at the time of diagnosis.[3,4] Therefore, early diagnosis of pancreatic cancer is critical to improve survival. On the other hand, many asymptomatic pre-invasive pancreatic neoplasms with cystic lesions have been found as a result of recent advances of diagnostic tools and screening strategy. This poses a dilemma for clinicians because it is often difficult to distinguish between pancreatic cancers and nonhazardous tumors. Intraductal papillary mucinous neoplasm (IPMN), which is recog-

nized as a precursor of pancreatic ductal adenocarcinoma, is representative of such neoplasms. Prognosis is favorable for patients with IPMN without invasion but poor for those with invasion, which accounts for a rate of death of about 30% of patients with IPMNs.[5] To determine the nature of pancreatic lesions preoperatively, novel modalities are needed. A promising approach is to measure molecular markers that could classify patients into different risk categories and aid clinicians in choosing suitable treatments for individual patients. To date, p53, transforming growth factor-β, basic fibroblast growth factor,[6] Bcl-2,[7–9] matrix-metalloproteinases,[10] β-catenin/E-cadherin,[11] vascular endothelial derived growth factor,[12,13] platelet-derived endothelial growth factor,[14,15] and human equilibrative nucleoside 1[16] have been suggested as biomarkers to predict the prognosis of pancreatic cancer patients. However, there are conflicting findings with regard to their validity as prognostic markers,[6] and none of the markers described above are used in clinical practice.

S100A4 is a member of the S100 family of calcium-binding proteins, which is characterized by two distinct EF-hand structural motifs.[17,18] *S100A4* is known to be overexpressed in many solid tumors, including breast carcinoma,[19] gastric carcinoma,[20] and colorectal adenocarcinoma,[21] while S100A4 has historically been referred to as fibroblast-specific protein 1 (FSP1), as a marker of fibroblasts.[22] There are also alternative names for S100A4 including mts1, pEL-98, 18A2, p9Ka, CAPL, and calvasculin. *S100A4* promotes cell motility and invasion in cancer[21,23–25] and induces remodeling of the extracellular matrix,[26–29] suggesting that *S100A4* is a mediator of tumor metastasis.[30] *S100A4* has also been reported to be a prognostic marker in a number of human cancers, including esophageal-squamous cancers,[31] non-small-cell lung cancers,[32] gastric cancers,[20] and bladder cancers.[33] In pancreatic cancers, it was reported that *S100A4* overexpression is associated with poor differentiation[34] and poor prognosis.[35,36] Recently, Mahon et al.[37] showed that S100A4 contributed to chemoresistance and the inhibition of apoptosis in pancreatic cancer.

The aim of this study was to evaluate the clinical significance of *S100A4* mRNA expression in pancreatic cancers as a diagnostic and prognostic marker. Using quantitative reverse transcription-polymerase chain reaction (qRT-PCR), we evaluated *S100A4* mRNA expression in invasive ductal carcinoma (IDC) cells, nonmalignant IPMN cells, and normal ductal cells of pancreatic tissues obtained by laser microdissection. Moreover, we investigated the association between *S100A4* expression and the prognosis of patients with pancreatic cancers using formalin-fixed paraffin-embedded (FFPE) samples.

Materials and Methods

Patients and Pancreatic Tissues Tissue samples were obtained from primary pancreatic tumors at the time of surgery at Kyushu University Hospital (Fukuoka, Japan) between 1992 and 2007. Normal pancreatic tissues were taken from peripheral tissues away from the tumor or from nonneoplastic pancreas resected due to bile duct disease. The tissue samples were removed as quickly as possible after resection, and a part of each sample was embedded in ornithine carbamyl transferase compound (Sakura, Tokyo, Japan), snap-frozen for analysis by microdissection, and stored at −80°C. The remainder was fixed in formalin and embedded in paraffin for pathological diagnosis. Tissues adjacent to the specimens were evaluated histologically according to the criteria of the World Health Organization.[38] Two pathologists were in agreement with regard to the pathological features of all cases, and the diagnoses were confirmed. In IPMNs, main-duct IPMNs or branch-duct IPMNs which were larger than 3 cm in diameter were removed on suspicion of being high-risk lesions. We only used IPMNs diagnosed with nonmalignant cystic tumors, which were confirmed to be intraductal papillary mucinous adenocarcinoma or intraductal papillary mucinous borderline tumor, not intraductal papillary mucinous carcinoma (IPMC), by pathological examination. Overall survival analysis was conducted for 87 patients who underwent pancreatic resection for pancreas cancer (85 ductal adenocarcinomas and two adenosquamous cell carcinomas). The patients comprised 53 men and 34 women with a median age of 65 years (range, 36–86 years). Survival was measured from the time of pancreatic resection, with death as the endpoint. Prognosis was examined in October 2008. The median observation time for overall survival was 16.3 months, ranging from 1 to 108 months. Sixty-four patients died during the follow-up, and the other patients were alive and censored. This study was approved by the Ethics Committee of Kyushu University and conducted according to the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government and the Helsinki Declaration.

RNA Isolation from Microdissected Samples and FFPE Samples Frozen tissue samples were cut into 8-μm-thick sections. One section was stained with hematoxylin and eosin (H&E) for histological examination, and the diagnosis of target cells was confirmed by the expert pathologist. Target cells (IDC cells from ten lesions; IPMN cells from 20 lesions, excluding IPMCs; and normal ductal epithelial cells from 20 tissues with the histological appearance of normal pancreas) were isolated selectively with a laser-microdissection and pressure catapulting system (P.A.L.M.

Microlaser Technologies, Bernried, Germany) in accordance with the manufacturer's protocol.[39] We microdissected 500–1,000 target cells to perform reliable and reproducible measurements of mRNA levels. We obtained 20 μl of RNA per lesion with the concentration of 10–50 ng/μl. The 28S/18S rRNA ratios ranged from 0.5 to 2.5. Total RNA was extracted from microdissected cells by a microdissection technique using a High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany) and treated with DNase I (Roche Diagnostics) according to the manufacturer's instructions. The total RNA derived from FFPE samples was isolated using the RNeasy FFPE kit (Qiagen, Tokyo, Japan), as previously described.[40] We used FFPE samples from 87 IDC patients with available prognostic data. After a review of representative H&E-stained slides, four to seven sections of 5-μm thickness were obtained from FFPE blocks of pancreatic cancers for macrodissection. Adjacent normal tissues, including normal acinar tissues and adipose tissues, were removed macroscopically using a scalpel. Only the cancerous parts of the sections were used for the isolation of mRNA. The extracted RNA was quantified by reading the absorbance of 260 nm and 280nm (A260/280) with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA, USA).

Quantitative Reverse Transcription-Polymerase Chain Reaction Quantitative RT-PCR was performed with a Chromo4 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) for 40 cycles of 15 s at 94°C and 30 s at 55°C with a QuantiTect SYBR Green Reverse Transcription-PCR kit (Qiagen) according to the manufacturer's instructions.

We designed specific primers for *S100A4* (forward, 5′-atcgccatgatgtgtaacga-3′; reverse, 5′-cccaaccacatcagaggagt-3′) and *β-actin* (forward, 5′-aaatctggcaccacaccttc-3′; reverse, 5′-ggggtgttgaaggtctcaaa-3′) using primer 3 and performed BLAST searches to confirm primer specificity. The PCR product sizes of these primers are small (*S100A4*, 85 base pairs (bp); *β-actin*, 139 bp, respectively), which allowed accurate and sensitive qRT-PCR despite the fragmented RNA extracted from FFPE tissue specimens.[41,42] The *S100A4* and *β-actin* expression levels were calculated for all cases using a standard curve constructed with total RNA from SUIT-2, a pancreatic cancer cell line. One microliter of RNA was used in qRT-PCR despite the concentration of RNA. *S100A4* mRNA expression levels were normalized using *β-actin* as an internal control and expressed as the ratio of expression of *S100A4* mRNA to that of *β-actin* mRNA. All samples were run in triplicate. The accuracy and integrity of the PCR products were confirmed with an Agilent 2100 Bioanalyzer (Agilent Technologies Inc.).

Immunohistochemical Procedures and Evaluation Sections (4 μm thick) were cut from paraffin-embedded tissues, deparaffinized in xylene, and rehydrated through a graded ethanol series. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in methanol for 30 min. Antigen retrieval was achieved by autoclaving the sections in citrate buffer at pH 6.0. A Histofine SAB-PO(R) kit (Nichirei, Tokyo, Japan) was used for immunohistochemical labeling. Each section was exposed to 10% non-immunized goat serum for 10 min to block nonspecific antibody binding, followed by incubation with a rabbit polyclonal anti-S100A4 antibody (NeoMarkers, Fremont, CA, USA; 1:100 dilution) at 4°C overnight. The sections were then sequentially incubated with a biotinylated anti-rabbit immunoglobulin solution for 20 min followed by peroxidase-labeled streptavidin for 20 min. The reaction products were visualized using 3,3′-diaminobenzidine as a chromogen, followed by nuclear counterstaining with hematoxylin. Cells were considered positively immunostained when nuclei and cytoplasm were stained. The distribution of stained S100A4 was evaluated as the percentage of stained cells, which was scored as 0, <5%; 1, 5–25%; 2, 26–50%; and 3, >51%, and as staining

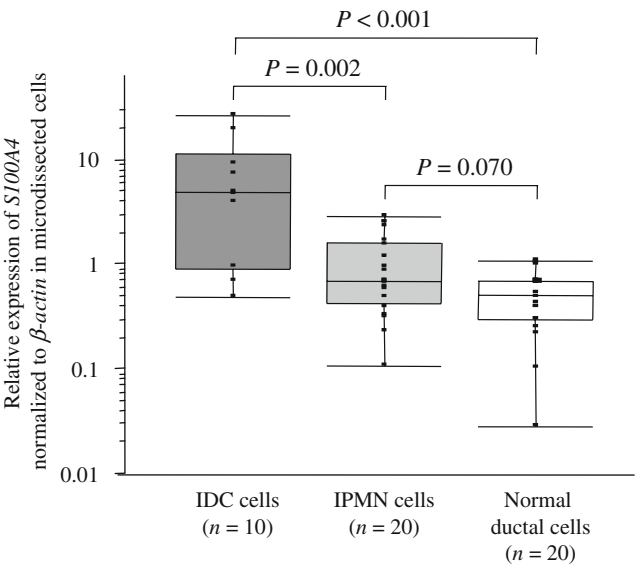

Figure 1 qRT-PCR analysis of *S100A4* mRNA expression in IDC, nonmalignant IPMNs, and normal ductal epithelial cells. IDC cells expressed higher levels of *S100A4* compared with IPMNs (P=0.002) and normal ductal cells (P<0.001). IPMNs tended to express higher levels of *S100A4* compared with normal ductal cells, although the difference did not reach statistical significance (P=0.070). The expression of *S100A4* was normalized to that of *β-actin.* The scale is logarithmic.

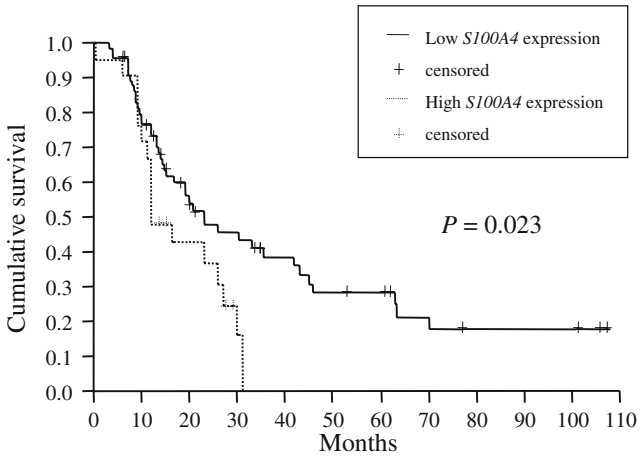

Figure 2 Overall survival after resection of pancreatic cancers with high *S100A4* expression versus low *S100A4* expression. High *S100A4* expression was significantly associated with shorter survival (P= 0.023). The S100A4 expression levels were normalized by *β-actin*.

intensity, which was scored as 0, no staining; 1, weak; 2, moderate; and 3, strong. When the multiplication product of the two scores was greater than 2, S100A4 was considered positively stained. In the immunohistochemical staining, we performed additional staining without primary antibodies in parallel to confirm that no staining was seen. All slides were evaluated independently by two investigators (NI and KN) without any knowledge of the background of each case.

Statistical Analysis Data were analyzed using the Kruskal–Wallis test if comparisons involved three groups and the Mann–Whitney U test if comparisons involved two groups. *S100A4* expression was split into high- and low-level groups using recursive descent partition analysis, as described by Hoffmann et al.[43] Survival curves were constructed with the Kaplan–Meier product-limit method and compared by log-rank test. The statistical significance was defined as a P value <0.05. All statistical analyses were performed with JMP 7.01 software (SAS Institute, Cary, NC, USA).

Results

Quantitative Analysis of S100A4 mRNA Expression in IDC, Nonmalignant IPMN, and Normal Ductal Epithelial Cells We measured the *S100A4* mRNA expression levels in IDC cells, nonmalignant IPMN cells, and normal ductal epithelial cells by qRT-PCR after laser-microdissection from frozen sections to determine whether *S100A4* is differentially expressed between pancreatic cancer cells and cells from nonmalignant tumors or normal ductal cells. *S100A4* mRNA expression was significantly higher in IDC cells than in IPMN (P=0.002) and normal ductal cells (P< 0.001), as shown in Fig. 1. IPMNs tended to express higher levels of *S100A4* compared with normal ductal cells,

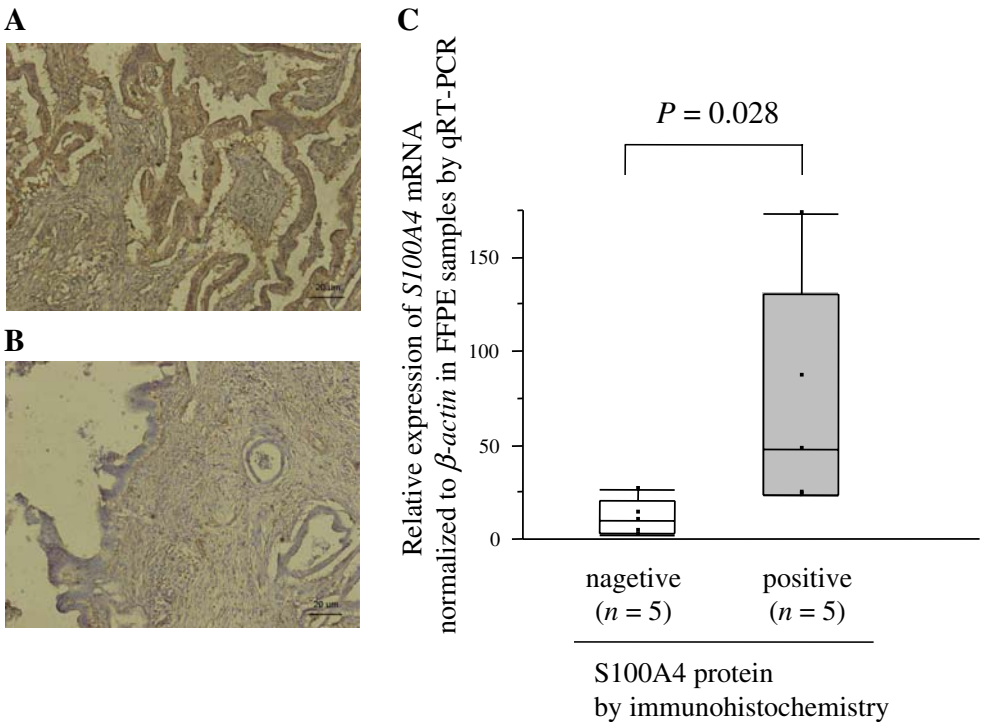

Figure 3 Positive (**a**) and negative (**b**) expression of S100A4 in pancreatic cancers (original magnification: ×100). **c** The extent of *S100A4* mRNA expression was correlated with the expression of S100A4 protein.

although the difference did not reach statistical significance (P=0.070).

S100A4 mRNA Expression Was Correlated with Prognosis of Patients with Pancreatic Cancers To investigate the correlation between *S100A4* expression and prognosis in patients with pancreatic ductal carcinomas, we isolated total RNA from FFPE samples from 87 patients with pancreatic cancers and measured the levels of *S100A4* expression. After normalizing *S100A4* mRNA expression to *β-actin* expression, we obtained two groups with high versus low *S100A4* expression (cutoff value, 20.5). The high- and low-expression *S100A4* groups comprised 21 and 66 cases, respectively. High *S100A4* expression was significantly associated with a shorter overall survival (P=0.023, Fig. 2). The median survival time of the patients with high and low S100A4 expression was 12 and 23 months, respectively.

S100A4 mRNA Expression Was Correlated with the Expression of S100A4 Protein S100A4 was immunoreactive in cytoplasm and nuclei of cancer cells (Fig. 3a, b). Cancer cells were highly stained with S100A4 compared with that in fibroblast in the stroma, even though S100A4 has been called "fibroblast-specific protein 1". The level of *S100A4* mRNA expression was significantly correlated with the expression of S100A4 protein, as shown in Fig. 3 (P=0.028).

Discussion

We measured the *S100A4* mRNA expression levels in IDC cells, nonmalignant IPMN cells, and normal ductal cells with qRT-PCR and found that IDC cells expressed the highest levels of *S100A4* among the cell types analyzed in the present study. To our knowledge, this is the first study to evaluate the correlation of *S100A4* expression in pancreatic cancers and IPMN. We have previously reported that IDC cells expressed higher levels of *S100A2*, another S100 family member, than premalignant cells and that IPMN cells with high-grade atypia expressed higher levels of *S100A2* than IPMN with low-grade atypia and normal ductal cells.[40] In the present study, a trend for a stepwise increase in *S100A4* mRNA expression from normal ductal cells to IDC cells was shown, suggesting that *S100A4* may also be involved in pancreatic carcinogenesis, similar to *S100A2*.

We quantitatively measured *S100A4* mRNA expression by qRT-PCR using FFPE samples of surgically resected pancreatic cancers. We found that high *S100A4* expression was significantly associated with a shorter overall survival, suggesting that *S100A4* mRNA could be a prognostic marker in pancreatic cancers. This finding supports a report of an immunohistochemical analysis of 62 surgical cases with pancreatic cancers, in which overexpression of S100A4 was significantly correlated with tumor size, tumor–node–metastases stage, and poor prognosis.[35] These consistent results also indicate that quantitative analysis of *S100A4* mRNA by qRT-PCR could be a reliable modality to contribute to the prediction of the prognosis of patients with pancreatic cancer. In fact, *S100A4* mRNA expression was correlated with the expression of S100A4 protein. The measurement of *S100A4* mRNA expression by qRT-PCR offers a high level of objectivity and quantitative performance compared with immunohistochemical examination. Additionally, the evaluation of *S100A4* mRNA expression of the tumor could be also performed from tiny tissue samples, resulting in a clinically informative technique.

Cytological specimens obtained by endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) and by endoscopic retrograde cholangiopancreatography (ERCP) have played an important role in the diagnosis of pancreatic cancer. However, cytological interpretation of clinical specimens obtained by these techniques is often difficult because samples are scant and bloody.[44–47] Therefore, molecular markers are needed to aid the diagnosis in indeterminate cytological samples.[48] The present study revealed that *S100A4* mRNA expression level was significantly higher in cancer cells than in nonmalignant IPMN cells or normal ductal cells. The merit of the analysis used in the present study is that we can sensitively and accurately measure the mRNA expression levels using gene-specific primers that generate short PCR products, even for tiny tissue samples or fragmented RNA obtained by EUS-FNA or ERCP. The measurement of *S100A4* mRNA for clinical samples could give clinicians important information, including tumor nature and the patient's prognosis, because *S100A4* expression was correlated with prognosis, although further studies are required to confirm this clinical application.

In summary, *S100A4* mRNA was expressed at higher levels in pancreatic cancer cells than in cells derived from nonmalignant tumors or nonneoplastic epithelium. The level of *S100A4* expression was significantly correlated with the prognosis of patients with pancreatic cancer. Thus, *S100A4* could be a marker of malignancy in pancreatic tumors and for poor prognosis in patients with pancreatic cancer.

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J Gastrointest Surg (2009) 13:1859–1863
DOI 10.1007/s11605-009-0985-5

ORIGINAL ARTICLE

Careless Use of Turban Pins: A Possible Problem for Turbaned Patients

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Received: 12 July 2009 / Accepted: 24 July 2009 / Published online: 5 August 2009

Abstract
Introduction Foreign body ingestion is rare in adults. In recent years, however, ingestion of the pins that are used for securing turbans has frequently been observed among young Islamic women. This article reviews the patients who were admitted to our emergency unit for turban pin ingestion.
Methods Between 2005 and 2009, 42 patients were admitted to our emergency unit with problems involving turban pins. The patients' characteristics were analyzed, including age; marital status; career; type, number, and location of pins; and history of gastrointestinal surgery.
Results The patients ranged in age between 11 and 48 years. Of the patients, 22 were single, and 20 were married; 19 were students, and 23 were housewives. The patients visited the emergency unit within 1 to 12 h after they had ingested the pins. Eight of the patients had ingested two pins each, while the others had ingested one pin each. The pins ingested most frequently were those with ball heads. Spontaneous excretion took 3 to 16 days. Of the patients who did not pass the pins spontaneously with feces, the pins were extracted at endoscopy in three and at laparotomy in one. The patients were followed up for 4 to 49 months. No pathological problems were noted during follow-up.
Conclusions Turban pin ingestion is common in Islamic populations, and the treatment requires a systemic approach and careful follow-up. Pin ingestion can be prevented by increasing public awareness and avoiding holding pins in the mouth when fixing a turban or wearing a type of turban that does not require pins.

Keywords Turban pin · Foreign body · Straight pin · Headscarf pin · Islamic women

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Introduction

In Islamic countries, girls start to wear turbans with the onset of puberty. Turban pins are used to attach the layers of the turban to each other in order to maintain a steady position around the head. Generally, six to eight pins are used to hold the turban in place (Fig. 1). According to our observations, the wearers hold the pins between their lips for easy use, pinheads outward, picking one at a time to attach the turban. During this process, any careless behavior, such as talking, laughing, or opening the mouth in a moment of distraction to breathe, can cause one or more of the pins to enter the gastrointestinal system or respiratory tract. We performed PubMed and library

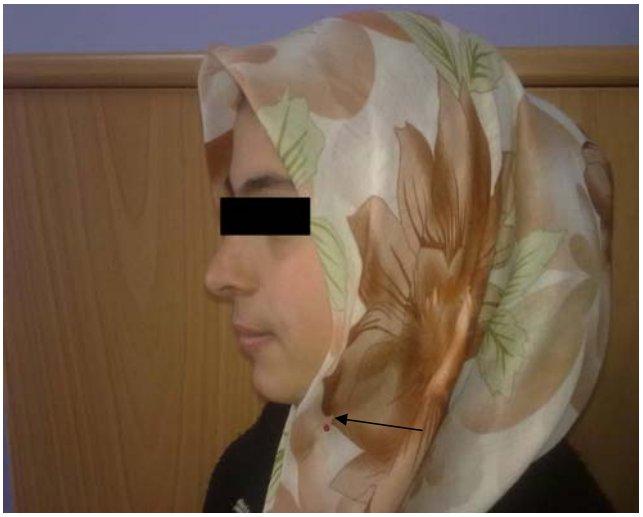

Figure 1 The most common way a turban is worn.

searches on the keywords "turban pin," "turban pin aspiration," and "turban pin ingestion." To our knowledge, only seven studies on turban pin aspiration have been published,[1–7] while we found only one study about turban pin ingestion.[8] This article reviews 42 patients who were admitted to our emergency unit for turban pin ingestion.

Materials and Methods

From 2005 to 2009, 42 patients were managed for turban pin ingestion in the Department of Surgery and the Emergency Unit of Diyarbakir Education and Research Hospital. Their medical records were evaluated retrospectively to obtain follow-up and clinical data, including age; marital status; career; time of admission after ingestion; type, number, and location of pins; and surgical history, management, outcome, and follow-up. The clinic variables of the patients ingesting turban pins are summarized in Table 1.

Results

Forty-two patients with turbans who had ingested pins were examined retrospectively. The patients ranged in age from 11 to 48 years (average 22.3). Of the patients, 22 were single, and 20 were married; 19 were students, and 23 were housewives. The interval between when the patients swallowed the pins and when they visited the emergency unit ranged from 1 to 12 h. Eight of the patients swallowed two pins each, while the others swallowed one each (Fig. 4a, b). Reviewing their surgical histories, one had a previous appendectomy, and another had a pyloromyotomy for pyloric stenosis. Three types of turban pin were ingested. Pins with ball heads were ingested most frequently (Fig. 1). In one patient, abdominal computed tomography (CT) showed that the pin had migrated through the stomach wall into the third liver segment, causing irritation of the diaphragm (Fig. 2c). The pin was removed at laparotomy. The pin ingested by the patient with a pyloromyotomy was removed endoscopically after it had remained in the stomach for 7 days. In another patient, the pin remained close to the ileocecal valve for 9 days and was removed via colonoscopy.

The most interesting patient in this group was an 11-year-old patient who had carelessly swallowed two pins and suffered from a stomach ache for days. X-rays showed two pins: one was located in the stomach and the other in the left upper quadrant of the abdomen. Abdominal CT and gastroduodenographs showed one pin embedded in the antrum perpendicularly (Fig. 3a, b). This pin was removed at endoscopy with the help of a tripot (Fig. 3c). The other pin traveled to the rectum and sigmoid within 8 days and was passed spontaneously (Fig. 2a, b).

The patients were followed up for between 4 and 49 months. During follow-up, no complications developed in any patient, including those who had required a procedure to remove the pins and those in whom the pins had passed spontaneously.

Discussion

Most ingested foreign bodies pass through the gastrointestinal tract uneventfully within one week.[9,10] We found that ingested pins passed spontaneously with defecation in 3 to 16 days. The reported incidence of foreign bodies penetrating the gastrointestinal wall is less than 1%, and most such objects are pointed or sharp, including tooth picks, sewing needles, dental plates, and fish and chicken bones.[11,12] In

Table 1 The Clinic Characteristics of the Patients Who Ingested Turban Pins

Patient characteristics	Results
Total patient number	42
Age (year)	11 to 48
Marital status	
Single	22
Married	20
Career	
Student	19
Housewife	23
Admission to emergency unit after ingestion (h)	1 to 12
Pins passed spontaneously with defecation (day)	3 to 16
Follow-up (month)	4 to 49

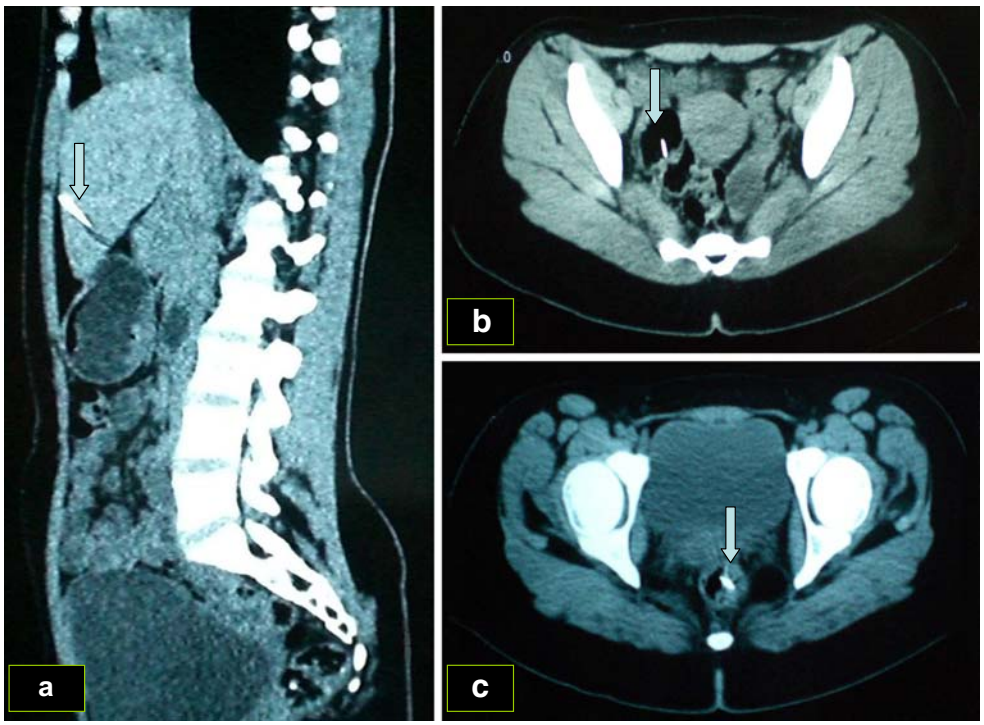

Figure 2 A pin that migrated to liver segment 3 is seen in sagittal sections on contrast abdominal CT (**a**). CT images of a patient who swallowed two pins at the same time, showing one pin in the sigmoid colon (**b**) and the other in the rectum (**c**).

one of our patients, a pin passed through the gastrointestinal wall and penetrated the third liver segment; in another patient, the shaft of the pin passed through the antrum, while the head remained inside the stomach.

The most foreign bodies were found to be ingested primarily by infants, while ingestion of turban pins was mostly seen in adolescent Islamic girls who covered their heads.[8] Kaptanoglu et al.[2,3] examined turban pin aspiration

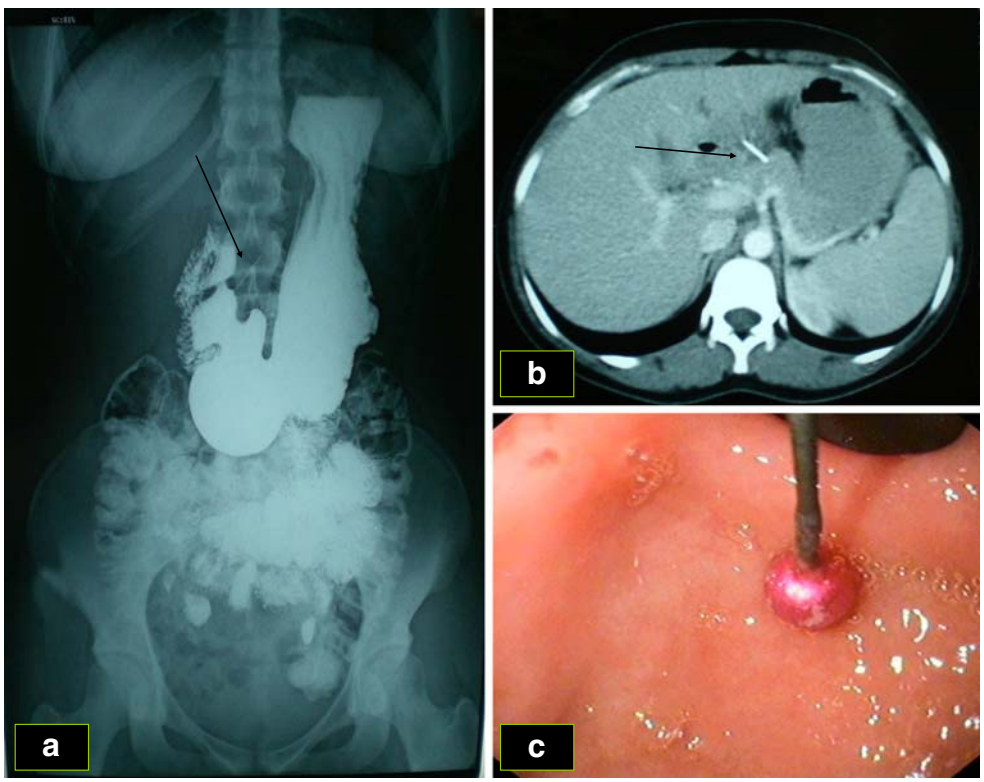

Figure 3 Image of the pin localized in the antrum seen on oral contrast esophagogastroduodenography (**a**). Contrast CT image of the same case (**b**). Endoscopic image of a pin stuck in the anterior wall of the antrum (**c**).

into the respiratory tract in two different studies and found that the average patient age was 14~15 years. In other studies of turban pin aspiration, the average patient age was 16 years in Gencer et al.[6] and 19 years in Al-Ali et al.[7] By contrast, our patients averaged 22 years old. Age may be an important factor differentiating pin ingestion and aspiration, but this should be confirmed by further studies.

The promptness with which the patients visited the hospital after pin aspiration also varied and was 2.7 days in Gencer et al.,[6] 6 h in Al-Ali et al.,[7] and 6 h in Kaptanoglu et al.[2] In our series, the average interval before the patient visited the emergency unit was 4.7 h. In our series, the patients visited the hospital equally quickly, regardless of whether symptoms developed.

Traditionally, Muslim women start wearing a scarf at the onset of puberty; therefore, all our patients were either older children or young adults.[6] In our series, more than half of the patients were age ≤18. Accidental ingestion of foreign bodies such as turban pins occurs frequently in Islamic girls. As stated above, holding six to eight turban pins between the lips is a big risk factor in this patient group. Happily, the majority of pins are passed spontaneously, and generally, conservative management is recommended for foreign bodies in the stomach and duodenum. In some cases, however, operative intervention should be considered to prevent undesirable complications, such as gastric or intestinal perforation. In our series, intervention was required in four cases, while the other cases were treated conservatively.

There is a strong, traditional belief in Turkish communities that eating lots of mashed potatoes will push a foreign body through the gastrointestinal system without complications. Most of the patients visiting our emergency service observed this tradition, although we do not specifically recommend it.

Many articles have been written on the migration of ingested foreign matter, especially pins that migrate to peripheral tissues. However, we did not see any hard-to-explain cases of pin migration. In one case, a pin became stuck in the stomach wall but did not pass through it completely because the pin head remained in the stomach (Fig. 4).

Gastric perforation secondary to foreign body ingestion is uncommon. It usually presents with peritonitis, although in some cases the perforation may seal spontaneously and the patient remains asymptomatic; in other cases, an intra-abdominal abscess may develop.[13–15] In our two patients with partial or complete stomach wall perforation, there were no notable symptoms of irritation or upset.

Shabb et al.[5] reported the use of fiberoptic bronchoscopy to treat five Middle Eastern women who aspirated straight pins used to hold veils. As veils are not used in Turkey, such cases are almost never encountered here. In a study of 332 cases of foreign body aspiration, Kaptanoğlu et al.[2] found that 121 were turban pins. Hasdiraz et al.[1] evaluated 105 patients visiting hospital after pin aspiration and explained how pins in different locations were extracted using different instruments.

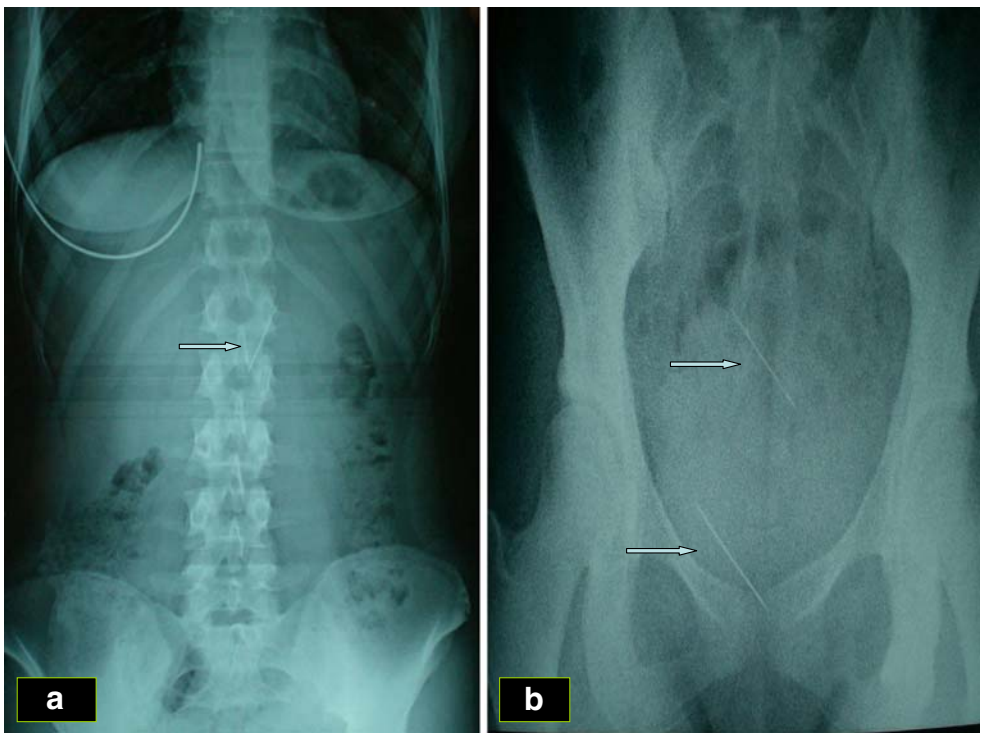

Figure 4 Upright plain abdominal X-ray images of patients who swallowed one (**a**) or two pins (**b**).

Conclusion

Many members of the Islamic community wear turbans because of their religious beliefs. Considering the number of Islamic women worldwide, turban pin ingestion is a serious problem. Pin ingestion is also a potential problem among women who wear head scarves for various reasons. Pin ingestion can be prevented by increasing public awareness and avoiding holding pins in the mouth when fixing a turban or wearing a type of turban that does not require pins.

Conflict of interest The authors declare that they have no competing interests.

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J Gastrointest Surg (2009) 13:1864–1868
DOI 10.1007/s11605-009-0926-3

CASE REPORT

Pancreatic Serous Cystadenocarcinoma: A Case Report and Review of the Literature

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Received: 16 April 2009 / Accepted: 28 April 2009 / Published online: 21 May 2009

Abstract
Background Serous cystic neoplasms of the pancreas are benign lesions with little chance for malignant degeneration. We report a case of malignant serous cystadenocarcinoma of the pancreas and review the literature.
Methods Structured review of the literature was performed using PubMed and MEDLINE searches, and cases of serous cystadenocarcinoma of the pancreas were compiled.
Results A 70-year-old man diagnosed with a serous cystadenoma was managed expectantly until he became symptomatic, and studies revealed an increase in the size of the lesion as well as duodenal invasion. The patient underwent a pancreaticoduodenectomy, and histopathological examination revealed a locally invasive cystadenocarcinoma without metastatic disease. Seven years later, the patient remains disease-free. Review of the literature identified 25 cases of serous cystadenocarcinoma published to date. The mean age at diagnosis is 68±2 years (range, 52 to 81), and women are affected more commonly (2:1).
Conclusions We conclude that there is a small but finite risk of malignancy for serous cystic neoplasms of the pancreas. The clinician should bear this in mind when faced with decisions regarding patient management. Prognosis is excellent with multiple reports of long-term survival even in the face of metastatic disease.

Keywords Pancreas · Oncology · Serous cystic lesion · Serous cystadenoma · Serous cystadenocarcinoma

Introduction

Malignant cystic neoplasms are rare entities that account for only 1% of all pancreatic tumors.[1] Serous and mucinous cystic neoplasms are tumors of the exocrine pancreas with different biological behaviors. Mucinous cystic tumors are typically slow-growing but carry a significant potential for malignancy, and thus, resection is often indicated.[2,3] In contrast, serous cystadenomas are considered benign tumors with almost no malignant potential. They are often observed with serial imaging or managed expectantly.[4] In the absence of symptoms, surgery is not usually recommended.

The first case of a pancreatic serous cystadenocarcinoma was reported by George et al. in 1989. The authors described the malignant characteristics of a serous cystic tumor of the pancreas with invasion into the spleen, stomach, and liver. The patient expired intra-operatively due to hemorrhage.[5] Subsequently, additional reports have documented similar findings of serous cystic neoplasms with malignant behavior. The histological characteristics of serous cystadenocarcinoma are indistinguishable from its benign counterpart, making the presence of invasion the sole distinguishing characteristic between the two.[5] In this report, we present a case of serous cystadenocarcinoma with duodenal, vascular, and neural invasion. We also

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review the literature and discuss the current diagnostic techniques and principles of management.

Materials and Methods

A systematic review of the literature was performed utilizing PubMed and MEDLINE searches. Articles were identified using the search terms: pancreas and serous cystadenocarcinoma. Nineteen articles were included in the analysis. Data are presented as mean±standard error of the mean.

Results

A 70-year-old man presented with upper gastrointestinal (GI) bleeding and abdominal pain. There was a duodenal ulcer with no evidence of malignancy on esophagogastroduodenoscopy (EGD), and an abdominal computed tomography (CT) scan revealed a 5.7-cm cystic mass in the head of the pancreas which was diagnosed by core needle biopsy as a serous cystadenoma. The patient was treated for presumed duodenal ulcer disease leading to resolution of symptoms and scheduled for observation of his pancreatic mass.

Three months later, the patient returned with recurrent coffee-ground emesis and abdominal pain. CT scan showed enlargement of the pancreatic mass to 6.5×8 cm and central dystrophic calcifications with new pancreatic and biliary ductal dilatation (Fig. 1). Repeat EGD identified a bleeding duodenal ulceration, and biopsies were consistent with a "benign" serous cystadenoma.

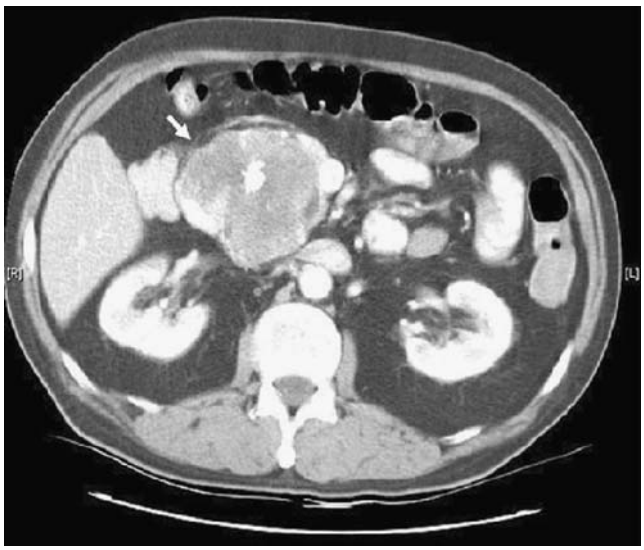

Fig. 1 Contrast-enhanced CT scan demonstrating a large mass measuring 6.5×8 cm in the head of the pancreas (*arrow*) with central dystrophic calcifications.

Upon surgical consultation, an elective pancreaticoduodenectomy was planned due to the increased size of the tumor, recurrent bleeding, and erosion into the duodenum. Laparotomy revealed a large mass in the head of the pancreas with no evidence of gross metastatic disease or invasion of the mesenteric vessels. The patient's postoperative course was complicated by delayed gastric emptying requiring temporary gastrostomy and feeding jejunostomy tubes.

On gross examination, the mass measured 9×8×6 cm, and there was marked, aggressive invasion of the duodenum beyond the level of the muscularis propria (Fig. 2). Histology demonstrated microcysts lined by clear cells without mucinous cytoplasm (Fig. 3). Microscopic vascular and perineural invasion were also seen, further distinguishing this lesion from a benign serous cystadenoma (Figs. 4 and 5). All resection margins and 17 lymph nodes were uninvolved.

Immunochemical stains for keratin AE1/3, 7, and 19 and CAM 5.2 were positive. Stained samples of the mass also showed weak immunoreactivity for carcinoembryonic antigen (CEA). Keratin 20 staining was negative. DNA content analysis by flow cytometry demonstrated no evidence of aneuploidy.

The final diagnosis was serous cystadenocarcinoma with duodenal, vascular, and neural invasion. At last follow-up 7 years post-operatively, the patient is doing well without clinical or radiographic evidence of recurrent disease.

Literature review yielded 25 reports of serous cystadenocarcinoma (Table 1).[5–23] The average age at presentation is 68±2 years, and 60% of patients affected are female (28% male; in 12% of cases, sex was not reported). Presenting complaints included abdominal pain (24%), upper GI bleeding (12%), weight loss (8%), palpable mass (8%), jaundice or abnormal serum liver enzymes (8%), and nonspecific abdominal complaints (8%).

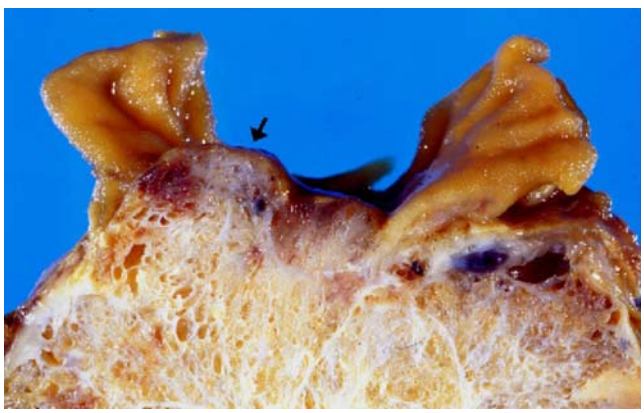

Fig. 2 Gross appearance of the tumor. Note invasion of the duodenum through the muscularis propria and submucosa with ulceration of the overlying mucosa (*arrow*).

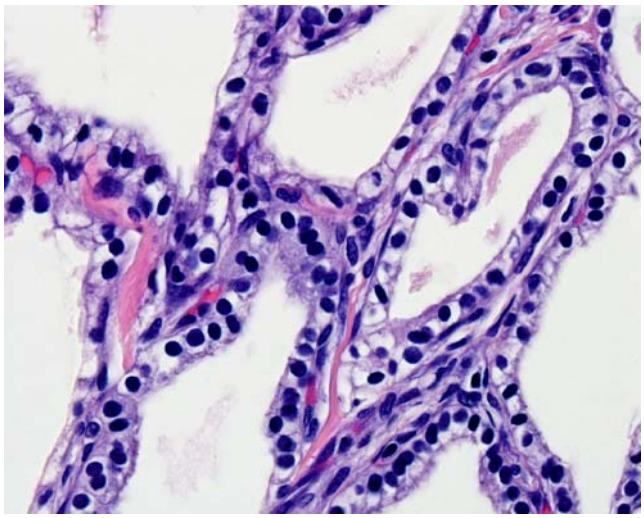

Fig. 3 Microscopic appearance of the tumor. The tumor is composed of multiple cysts lined by cuboidal cells with clear cytoplasm.

The mean diameter of serous cystadenocarcinoma was 10±1 cm (range, 2.5–19 cm). Lesions exhibit both of the hallmarks of malignancy: local invasiveness and distant metastasis with most tumors associated with local invasion of the spleen (8%), small intestine (4%), stomach (4%), adrenal gland (4%), or microscopic invasion of vascular and neural tissues. Synchronous or metachronous liver metastases were frequently noted (36%), along with metastasis to regional lymph nodes (12%), bone marrow (4%), and lung (4%). Mean survival was 36±11 months (range, <30 days to 120 months) among cases with follow-up (n=11), and ten (91%) of these patients were still alive when reports were published including seven (64%) patients with metastatic disease.

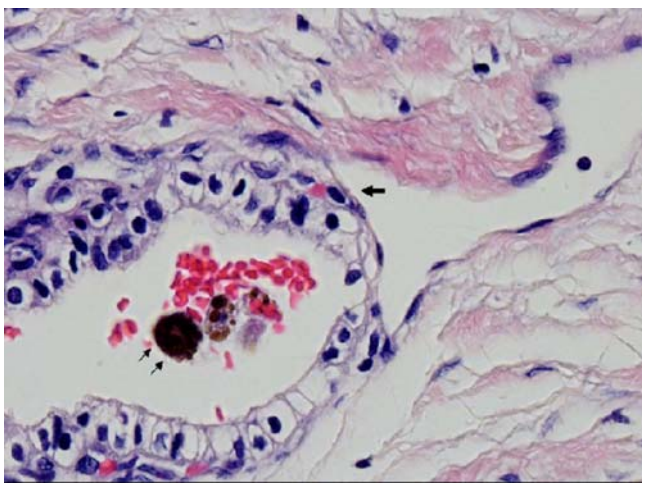

Fig. 4 Vascular invasion. Tumor erodes through the lumen of the vessel (*arrow*). Red blood cells and hemosiderin (*double arrow*) are present.

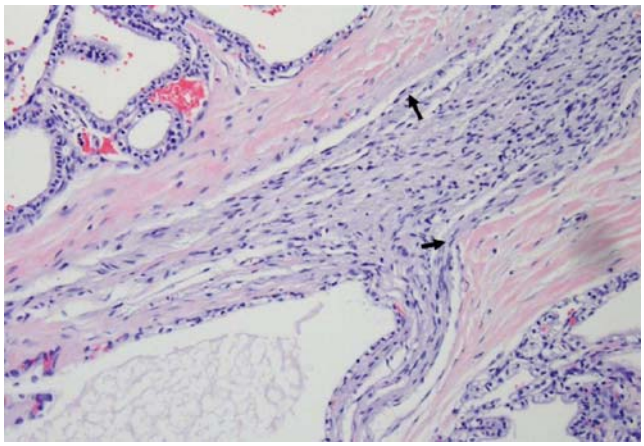

Fig. 5 Neural invasion. Tumor cells are noted within the nerve sheath (*arrows*).

Discussion

The preoperative differentiation between a benign serous cystadenoma and malignant serous cystadenocarcinoma remains difficult. Indeed, the correct diagnosis of serous cystadenocarcinoma was not made pre-operatively in any of the cases, including the current one.[5–23] The benign and malignant variants appear identical histologically, with the only distinguishing feature being gross or microscopic evidence of invasiveness. Thus, the utility of cytology or histology obtained from core needle biopsy is limited.[18]

The current practice for management of serous cystadenomas of the pancreas is to observe asymptomatic lesions thereby avoiding the potential morbidity and mortality associated with a major operation.[13,14,18,24,25] We agree with this conservative approach. Nevertheless, clinicians should be aware of the possibility for malignant transformation in serous cystic neoplasms and should maintain an index of suspicion when certain clues appear. These include the onset of new symptoms, worsening of symptoms, or rapid enlargement of the mass. In these cases, resection may be indicated, despite the lack of objective evidence for malignancy obtained from preoperative imaging, endoscopy, and biopsies.

In the current case, a serous cystadenocarcinoma was diagnosed without evidence of distant metastasis but with extensive tumor invasion into surrounding structures, both grossly and microscopically. To our knowledge, this is the first example of serous cystadenocarcinoma with extensive duodenal, vascular, and neural invasion but no distant metastases.

Conclusion

Our case report is illustrative of the management strategy for serous cystic lesions of the pancreas despite the

Table 1 Characteristics of Pancreatic Serous Cystadenocarcinoma Reported in the Literature

Author	Publication year	Patient age	Patient gender	Signs/symptoms	Tumor size (cm)	Metastases	Procedure	Outcome	Note
George et al.[5]	1989	70	M	Hemorrhage from gastric varices	11	Synchronous in stomach and liver	DP	Operative death due to hemorrhage	
Friedman[6]	1990	74	F	NA	19×16×10	Synchronous in liver, lungs, bone marrow, adrenal glands, LN	NA	NA	
Kamei et al.[7]	1991	72	F	Jaundice	10	No	Total pancreatectomy	NA	
Okada et al.[8]	1991	63	F	Abdominal pain	12	Metachronous in liver	DP	Alive 1 year later	
Yoshimi et al.[9]	1992	63	F	Abdominal pain	12	Metachronous in liver	DP	Alive 3 years later	
Ohta et al.[10]	1993	64	M	Urinary frequency	2.5×2.5×2	No	Enucleation	Alive 9 months later	
Widmaier et al.[11]	1996	71	M	Abnormal liver function	4	Synchronous in LN	Pylorus-preserving partial pancreatico-duodenectomy	Alive 1 year later	
Ishikawa et al.[12]	1998	63	F	Abdominal pain	12	Metachronous in liver	DP	NA	
Siech et al.[13]	1998	NA	NA	NA	NA	NA	NA	NA	2 cases reported
Eriguchi et al.[14]	1998	65	F	Palpable abdominal mass	16	Synchronous and meta-chronous in liver	DP, Microwave coagulo-necrotic therapy	Alive 10 years later	
Abe et al.[15]	1998	71	F	Palpable abdominal mass	12×8.5×5	Synchronous in LN	DP, splenectomy	Alive 2 years later	
Schmidt-Rohlfing et al.[16]	1998	52–74	2 M, 2 F	NA	NA	NA	NA	NA	4 cases reported
Kimura and Makuuchi[17]	1999	53, 66	F, M	NA	5, 3	No	NA	NA	2 cases reported
Horvath and Charbot[18]	1999	81	F	NA	6	NA	NA	NA	
Wu et al.[19]	1999	57	F	Hematemesis	NA	Synchronous and meta-chronous in liver	NA	NA	
Strobel et al.[20]	2001	56	F	Abdominal pain, weight loss	14×7×4	Metachronous in liver	Pylorus-preserving total pancreatico-duodenectomy	Alive 3 years later	
Shintaku et al.[22]	2005	85	F	Fatigue, intermittent diarrhea	12×9×7	Direct extension to spleen	Distal gastrectomy, DP	Alive 10 months later	
Friebe et al.[21]	2005	80	F	Abdominal pain, anorexia, weight loss	8×7×7	Direct extension to spleen	DP, splenectomy	Alive 1 year later	
Galanis et al.[23]	2007	NA	NA	NA	NA	Synchronous and meta-chronous lesion in liver	NA	NA	2 cases reported
Current	–	70	M	Hematemesis, abdominal pain	9×8×6	Direct extension to duodenum	PPW	Alive 7 years later	

DP distal pancreatectomy, *NA* not available, *LN* lymph node, *PPW* pylorus-preserving Whipple resection

presence of an initially unrecognized malignancy: the progression of symptoms and increase in size of the mass triggered curative resection. The excellent prognosis associated with serous cystadenocarcinoma justifies an aggressive approach to surgical resection, even in older patients. This is especially so since major pancreatic resections are now done with very low mortality and morbidity rates in major centers around the world.[26]

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J Gastrointest Surg (2009) 13:1869
DOI 10.1007/s11605-009-0954-z

MULTIMEDIA ARTICLE

Laparoscopic Versus Open Enucleation for Solitary Insulinoma in the Body and Tail of the Pancreas

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Received: 4 January 2009 / Accepted: 12 June 2009 / Published online: 18 July 2009

Abstract
Background Insulinomas, benign in the vast majority, are the prevailing pancreatic endocrine tumors amenable to surgical resection which is beneficial in most instances. This study aimed to compare the results of laparoscopic vs. open surgery enucleation of insulinomas.
Methods From October 1999 to June 2008, 12 case series of enucleation for benign insulinoma in the body and tail of the pancreas were identified through retrospective review of medical records. Main outcome measures were recurrent hypoglycemia, conversion to open procedure, complications, and length of hospital stay.
Results Seven patients were addressed with open and five with laparoscopic procedure. Mean age was 55 years (36–69). Lesions were identified preoperatively (via computed tomography and endoscopic ultrasonography) in 5/7 in the open and 4/5 in the laparoscopic group. Intraoperative ultrasound identified the rest of insulinomas. One conversion to the open approach was mandatory because the insulinoma was resting on the portal vein. The mean operative time and hospital stay was 92 min (66–126)/14 days (11–22) for the open and 121 min (89–187)/11 days (5–18) for the laparoscopic procedure (including conversion) ($p<0.5$ in both comparisons). Pancreatic fistula rate was respectively 28.57% (2/7) and 20% (1/5) ($p=0.65$). Mortality was nil. Mean follow-up was 54 months (3–109). Recurrent hypoglycemia was documented in one patient of the laparoscopic group ($p=0.46$) but blood glucose concentrations remained stable with diazoxide.
Conclusion Laparoscopic insulinoma enucleation seems to be a feasible and safe approach associated with reduction in hospital stay and comparable rates of pancreatic fistula in relation to open surgery.

Keywords Pancreatic insulinoma · Laparoscopic enucleation · Intraoperative ultrasound

Electronic supplementary material The online version of this article (doi:10.1007/s11605-009-0954-z) contains supplementary material, which is available to authorized users.

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J Gastrointest Surg (2009) 13:1870–1873
DOI 10.1007/s11605-009-0883-x

HOW I DO IT

Laparoscopic Duodenojejunostomy for Superior Mesenteric Artery Syndrome—How I Do It

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Received: 5 December 2008 / Accepted: 24 March 2009 / Published online: 9 April 2009

Abstract
Introduction Superior mesenteric artery (SMA) syndrome is a well-described condition involving mechanical compression of the third part of the duodenum by the SMA and the aorta, resulting in proximal obstruction.
Discussion Although there are a handful of case reports describing various techniques of laparoscopic duodenojejunostomy, a technique that involves creating the anastomosis in the infracolic compartment provides a more dependent stoma for the patient.
Conclusion This is a safe, effective, and relatively simple procedure for the experienced minimally invasive surgeon.

Keywords Superior mesenteric artery syndrome · Laparoscopy · Duodenojejunostomy · Wilkie syndrome

Introduction

To date, more than 400 cases of superior mesenteric artery (SMA) syndrome have been reported.[1,2] It is possible that this is an underestimation of the condition, as many such cases are probably not reported. SMA syndrome is characterized by symptoms of upper gastrointestinal obstruction such as nausea and vomiting, post-prandial epigastric pain, anorexia, and weight loss.[1,3,4] It is caused by compression of the third part of the duodenum, as it passes between the SMA and the aorta.

A narrowed angle between these two arteries may be seen in various situations: in patients who experience rapid weight loss (leading to a reduction in the amount of mesenteric fat surrounding the SMA), external cast compression, anatomic variants (a short/high ligament of Treitz or an unusually low origin of the SMA), and spinal cord injury and/or spinal surgery.[3–8] In particular, patients with traumatic spinal cord injuries are predisposed to this condition due to rapid weight loss, prolonged supine positioning, and the use of spinal orthoses.[9]

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Diagnosis and Initial Treatment

Traditionally, the diagnosis of SMA syndrome has been made with fluoroscopy and/or mesenteric angiography. However, computed tomography angiography with multiplanar three-dimensional reconstructions has recently been found to be more specific, more informative, and less invasive than mesenteric angiography.[10] Diagnostic criteria include (1) a reduction in the aortomesenteric angle from the normal 28–65° to <22°, (2) a decrease in the aortomesenteric distance from the normal 10–28 to <8 mm, and (3) gastric and proximal duodenal dilatation with obstruction of the third part of the duodenum (Fig. 1).[1,10,11] Upper endoscopy is indicated to confirm external compression where the SMA crosses the third part of the duodenum and to rule out an intrinsic abnormality.

The initial management of SMA syndrome is conservative. Fluid and electrolyte imbalances are corrected, the stomach is decompressed with a nasogastric tube, and nutritional support is instituted with either nasojejunal feeds or total parenteral nutrition. Gastric promotility agents such as metoclopramide may also be helpful.[1,3,11]

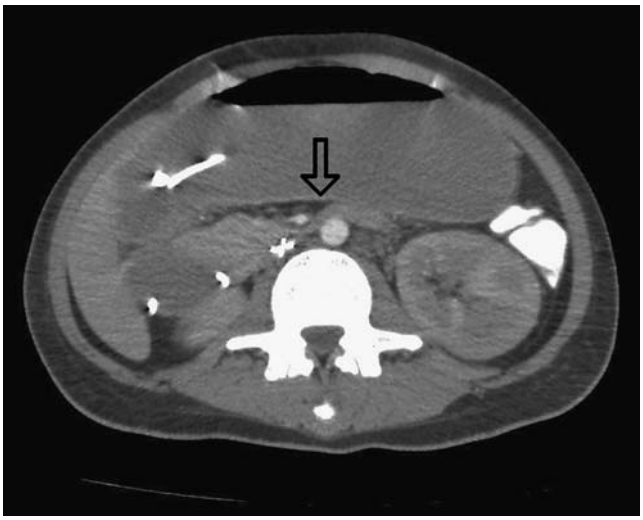

Figure 1 CT scan showing compression of third part of duodenum by the SMA against the aorta (*arrow*). The aortomesenteric angle in this patient was measured at 7° on a lateral reconstruction.

Laparoscopic Duodenojejunostomy

Preoperative Evaluation

If placement of a feeding tube is not possible, or if the condition persists, surgical relief of the obstruction may be required either via a bypass procedure (duodenojejunostomy or gastrojejunostomy) or by mobilization of the duodenum (division of the ligament of Trietz[3,12]). The most successful approach for treatment of SMA syndrome is a duodenojejunostomy.[3] Although there are only a handful of case reports on laparoscopic duodenojejunostomy, this is a relatively simple procedure for the experienced minimally invasive surgeon. Initial reports describing this technique began with a Kocher maneuver and side-to-side (sometimes retrocolic[11]) duodenojejunostomy at the mid-section of the second part of the duodenum.[11,13–15] Three more recent reports have excluded the need for a Kocher maneuver in order to create a more dependent stoma using the third part of the duodenum in the infracolic region.[2,4,16] This is the approach we prefer and will describe in detail below.

Operating Room Setup

Gastric decompression via a nasogastric tube should occur for at least 3 days before operation. Rapid sequence intubation is preferred in these patients due to the potential for aspiration. Preoperative antibiotics are given, and the patient is placed in the lithotomy position with the legs extended in stirrups. The right arm is tucked at the patient's side. The operating surgeon stands between the legs, and the assistant stands to the patient's left side. A laparoscopic monitor is placed at the head of the bed, to the patient's right side. Required instruments for the case include two 12-mm disposable ports, two 5-mm ports, a 10-mm 30-degree laparoscope, atraumatic graspers, hook cautery, a needle driver, scissors, and a 12-mm laparoscopic stapler with 45-mm white cartridges (Endo GIA Universal, Auto Suture, Norwalk, CT, USA).

Surgical Procedure

Initial entry can be performed with either an open (Hasson) technique in the infra-umbilical position or with a Veress needle in the left upper quadrant. A 12-mm port is inserted. Pneumoperitoneum is established to 12–14 mmHg, and a 30° camera is inserted near the umbilicus. The patient is positioned in 20° to 30° of reverse Trendelenburg. The right-handed 12-mm working port is positioned approximately 5 to 10 cm below the left costal margin, just above the level of the umbilicus. A left-handed 5-mm working port is placed lateral to the rectus sheath on the patient's right side, and a second 5-mm assistant's port is placed just below the left subcostal margin. The assistant uses this port to elevate the transverse mesocolon. Port placement is shown in Fig. 2.

Initial abdominal exploration usually shows a decompressed enlarged stomach. The transverse colon is located and elevated by grasping an inferior colonic epiploica. Dilated second and third parts of the duodenum will now be visible in the infracolic compartment, and the ligament of Treitz can be easily identified toward the patient's left side. Dissection of the visceral peritoneum and base of the transverse mesocolon over the distal second part of duodenum and third part proximal to the superior mesenteric vessels is performed with laparoscopic scissors. This exposes the duodenum just proximal to the site of obstruction. A tilt to the patient's left side may improve exposure during dissection. A loop of jejunum approximately 10 to 15 cm

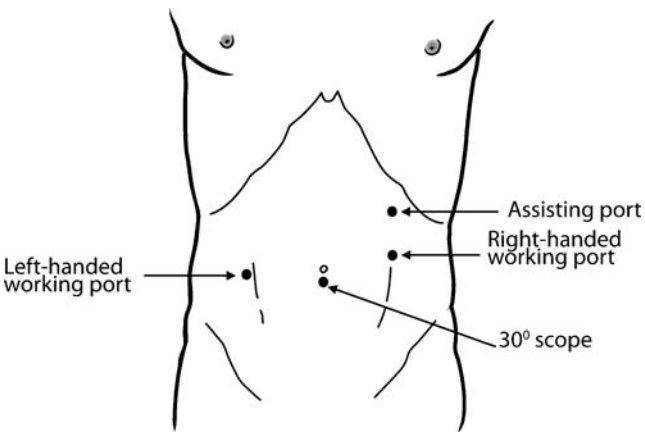

Figure 2 Trocar site placement for performing a laparoscopic duodenojejunostomy. The infra-umbilical port was inserted first using an open technique.

from the ligament of Treitz is brought over to this segment of duodenum, and both portions of bowel are held in apposition by the surgeon (Fig. 3).

Two intracorporeal 2-0 Vicryl stay sutures are placed. The most caudal portion of the second part of the duodenum is used to create a dependent stoma. Two small anti-mesenteric enterotomies are made with the hook cautery and gently dilated with an atraumatic grasper. The surgical assistant grasps the more proximal stay stitch and provides traction in a cephalad direction. The surgeon grasps the distal suture and carefully inserts a 45-mm laparoscopic stapler into both lumens through the right-handed working port (Fig. 4). We prefer to use a vascular white cartridge for the side-to-side anastomosis, as we believe this achieves better staple line hemostasis. A running intracorporeal 2-0 Vicryl suture is then used to close the remaining enterotomy. The assistant should continue grasping the proximal stay stitch to prevent the inadvertent inclusion of the posterior suture line to the running closure. The end of the 2-0 Vicryl suture is then tied to a separately placed suture. A single stitch is placed at the apex of the staple line to reduce tension.

Two small white gauzes are inserted into the abdomen and placed around the staple line. An atraumatic grasper must be used to compress the jejunum distal to the anastomosis. Methylene blue is injected slowly through the nasogastric tube to look for an anastomotic leak. A leak is repaired with a stitch or stitches. If the leak persists, conversion to an open approach may be appropriate.

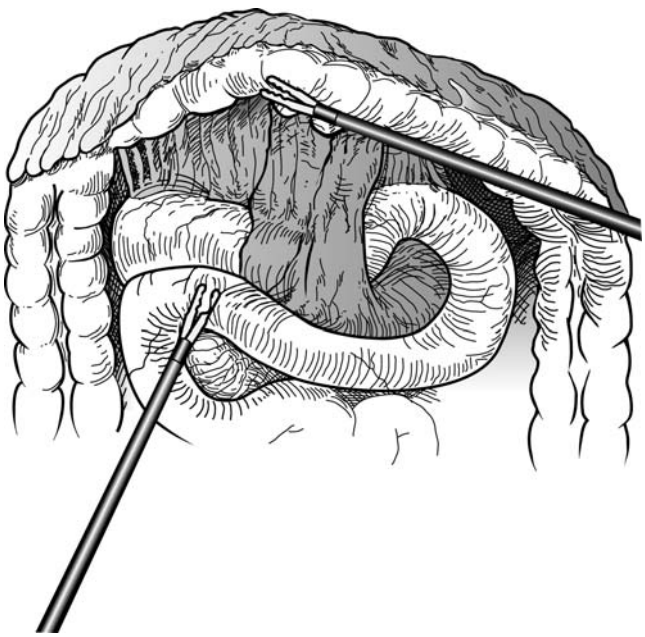

Figure 3 Laparoscopic view once the peritoneum over the proximal third part of the duodenum has been cleared away (adapted from Richardson and Surowiec,[4] p. 378, Copyright 2001, with permission from Elsevier).

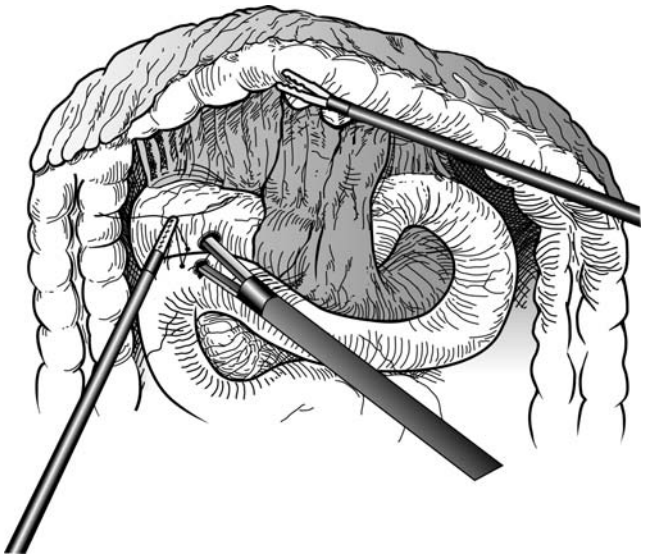

Figure 4 A laparoscopic stapler is inserted through both enterotomies to create a side-to-side duodenojejunal anastomosis while the proximal stay suture is grasped to provide counter-traction (adapted from Richardson and Surowiec,[4] p. 378, Copyright 2001, with permission from Elsevier).

Although possibly unnecessary, we place a 15-Blake drain through the assistant's 5-mm port in the right upper quadrant. This is positioned over the staple line. The transverse colon is replaced over the anastomosis. The abdomen is desufflated, the ports are removed, and all port sites are closed in standard fashion.

Postoperative Care

A nasogastric tube is left in situ initially. A postoperative contrast study with gastrografin should be performed on the first postoperative day. If the study shows free flow of contrast into the jejunum and no leak is visualized, the nasogastric tube is removed, and a clear liquid diet is commenced. The Blake drain should be removed once the patient is tolerating a fluid diet, and the output is less than 25 cc over 24 h. In consultation with a dietician, some patients will require additional nutritional support via a nasoenteric tube just distal to the pylorus. If necessary, a post-pyloric tube can be placed via endoscopic or radiological guidance once a postoperative contrast study confirms a patent anastomosis.

Conclusion

Laparoscopic duodenojejunostomy can be recommended as a safe and appropriate management option for SMA syndrome that fails conservative therapy. There have been no major complications reported for the technique de-

scribed above, and all cases (to our knowledge) have resulted in the successful resolution of symptoms.[2,4,16] This procedure offers patients the benefits of a minimally invasive approach and reduces the risk of incisional hernia formation from an open approach.

Acknowledgment We thank Quoc Nguyen from the Medical Art & Design, Royal Adelaide Hospital for creating Fig. 2 and for adapting Figs. 3 and 4 to illustrate our operative technique. We also thank Professor Glyn Jamieson for reviewing this manuscript.

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J Gastrointest Surg (2009) 13:1874–1887
DOI 10.1007/s11605-009-0935-2

REVIEW ARTICLE

Preemptive Surgery for Premalignant Foregut Lesions

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Received: 19 February 2009 / Accepted: 20 May 2009 / Published online: 10 June 2009

Abstract
Introduction Preemptive surgery is the prophylactic removal of an organ at high risk for malignant transformation or the resection of a precancerous or "early" malignant neoplasm in an individual with a hereditary predisposition to cancer. Recent advances in molecular diagnostic techniques have improved our understanding of the biologic behavior of these conditions. Predictive testing is an emerging field that attempts to assess the potential risk of cancer development in predisposed individuals. Despite substantial improvement in these forms of testing, all results are imperfect. This information often becomes an important tool that is used by healthcare providers to evaluate the risk–benefit ratio of various risk modifying strategies (i.e., intensive surveillance or preemptive surgery).
Methods A systematic literature review was performed using Medline and the bibliographies of all referenced publications to identify articles relating to preemptive surgery for premalignant foregut lesions.
Results and Discussion In this review, we outline the controversies surrounding predictive risk assessment, surveillance strategies, and preemptive surgery in the management of high-grade dysplasia (HGD) in Barrett's esophagus (BE), hereditary diffuse gastric cancer (HDGC), bile duct cysts, primary sclerosing cholangitis (PSC), and pancreatic cystic neoplasms. Resection of BE is supported by the progressive nature of the disease, the risk of occult carcinoma, and the lethality of esophageal cancer. Prophylactic total gastrectomy for HDGC appears reasonable in the absence of accurate screening tests but must be balanced by the impact of surgical complications and altered quality of life. Surgical resection of biliary cysts theoretically eliminates the exposed epithelium to decrease the lifetime risk of cholangiocarcinoma. Liver transplantation for PSC remains controversial given the scarcity of donor organs and inability to accurately identify high-risk individuals. Given the uncertain natural history of pancreatic cystic neoplasms, the merits of selective versus obligatory resection will continue to be debated.
Conclusions Preemptive operations require optimal judgment and surgical precision to maximize function and enhance survival. Ultimately, balancing the risk of surgical intervention with less invasive interventions or observation must be individualized on a case-by-case basis.

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Keywords Preemptive · Prophylactic · Precancerous · Foregut · Surgery

Introduction

Refinements in multimodality therapy have improved survival for patients with upper gastrointestinal malignancies. Unfortunately, the majority of patients are diagnosed with advanced cancers that often recur following treatment. Certain individuals have a hereditary predisposition to developing cancer. It has been postulated that chronic environmental exposure to a variety of carcinogens causes

epithelial cancers in genetically susceptible individuals. Precursor or "pre-cancerous" lesions have been identified for most malignancies. Investigators have sought to use molecular diagnostic techniques to define the natural history or biologic behavior of these lesions. High-risk individuals may opt for a risk-modifying strategy, including intensive surveillance or preemptive surgery, to either promote early detection or diminish/eliminate the future prospect of malignancy, respectively.

Increasing utilization of molecular diagnostic techniques has afforded many individuals an appraisal of their cancer risk. While this has opened new avenues to cancer risk assessment and diagnosis, it remains an imperfect science. The information gained from predictive testing is merely a risk estimate and not a guarantee of cancer developing in the future. Adding to this limitation are the uncertainties of surveillance strategies to identify cancer at its earliest stage in high-risk individuals. Preemptive surgery is being considered and, in some instances, recommended to address the high organ-specific cancer risk. Individuals undergoing preemptive surgery are likely to be younger and in better overall health than their older counterparts, and resection associated morbidity or mortality will have a profound impact on these individuals, relative to a longer anticipated life expectancy and loss of productive years. This will demand even greater precision and vigilance from the surgeon in order to minimize perioperative morbidity and mortality in these otherwise healthy individuals.

In this review, we will examine the impact and controversies surrounding molecular diagnostic techniques, surveillance strategies, and preemptive surgery in the risk assessment and management of premalignant upper gastrointestinal lesions. Primary emphasis will be on high-grade dysplasia in Barrett's esophagus, hereditary diffuse gastric cancer (HDGC), primary sclerosing cholangitis, bile duct cysts, and pancreatic cystic neoplasms (PCNs).

High-Grade Dysplasia in Barrett's Esophagus

Barrett's esophagus (BE) is the abnormal transformation of the esophageal squamous lining into columnar epithelium in response to chronic gastroesophageal reflux disease (GERD). The prevalence of BE in the general population is estimated to be less than 1%.[1] Even in patients with symptoms of GERD, BE has been confirmed in less than 15% of cases.[2] BE has also been linked to an increased risk for esophageal adenocarcinoma. Due to the aggressive behavior and lack of specific symptoms associated with esophageal cancer, most patients will have advanced disease at the time of diagnosis. Even with aggressive multimodal therapy, the reported 5-year survival rates for patients with esophageal adenocarcinoma are less than 30%.[3] Endoscopic surveillance has been recommended as a tool to enhance the early detection of BE-associated cancers. Unfortunately, reflux symptoms or the presence of BE cannot reliably identify all individuals at risk for esophageal adenocarcinoma. Up to 40% of patients with esophageal adenocarcinoma have no prior history or symptoms of reflux disease.[4] Even in symptomatic cancers, prior evidence of BE was documented in less than 5% of patients.[5]

Chronic exposure of the lower esophageal lining to hydrochloric acid and bile has been associated with progressive dysplasia and eventually the development of adenocarcinoma. Up to 28% of patients will progress from low-grade (LGD) to high-grade (HGD) dysplasia. It has been estimated that 16–59% of high-grade dysplastic lesions will become cancers.[6,7] The absolute risk for developing adenocarcinoma within BE is 0.5% per patient-year.[6,8–11] Interestingly, infection with cagA+ *Helicobacter pylori* has been reported to reduce the risk of adenocarcinoma forming within BE.[12,13] It is hypothesized that the effects of chronic acid reflux are reduced by *H. pylori* infection due to the development of chronic atrophic gastritis; eradication of *H. pylori* may potentially increase the risk of esophageal adenocarcinoma in susceptible individuals.

Diagnosing dysplastic lesions remains a challenge. Interobserver agreement among pathologists for the diagnosis of LGD and HGD is approximately 50% and 85%, respectively.[14] There is no reliable method to determine which patients with HGD will progress to carcinoma. In patients with HGD, 13–40% of the resected esophageal specimens contained occult adenocarcinomas.[15,16] It is likely that the preoperative detection of occult HGD-associated cancers varies with the experience of the endoscopist, techniques and technologies utilized, and the number of biopsies that are taken.

Preemptive esophageal resection is supported by the progressive nature of BE, the risk of occult carcinoma, and the lethality of advanced esophageal cancer. The entire region of metaplastic esophagus, including the regional lymph nodes, should be resected.[17] Transhiatal or transthoracic (TTE) esophagectomy are equivalent operations when performed by experienced surgeons.[18,19] The major differences between these operative approaches are a higher perioperative mortality rate and increased respiratory complications with TTE and a greater likelihood for recurrent laryngeal nerve injury, anastomotic leak, and stricture with a transhiatal approach.[18,19] Esophagectomy with preservation of the vagal nerves is being evaluated as an alternative approach with potentially less early and late morbidity than en bloc esophagectomy.[20] Minimally invasive esophagectomy (MIE), consisting of thoracoscopic or laparoscopic mobilization of the esophagus and stomach

with either an intrathoracic or cervical anastomosis, is an alternative to open esophageal resection. Benefits of MIE potentially include less operative blood loss, earlier return of gastrointestinal function, and shorter hospital stay.[21] The published data supporting MIE are primarily retrospective, single-institution case series; therefore, the reported results may not be generalizable. Further maturation of these data and controlled trials are needed before MIE can be considered the oncologic equivalent of open esophagectomy.[22,23] A multicenter Eastern Cooperative Oncology Group phase II study (ECOG 2202) evaluating MIE is ongoing and may answer questions regarding the efficacy of this approach.[24,25]

The literature on esophagectomy for HGD was analyzed to assess the role of preemptive surgery (Table 1).[26–62] Occult-invasive esophageal adenocarcinoma was detected in resection specimens from 219 of 695 (31.5%) patients with HGD. Early-stage disease (54% stage I) occurred predominantly, with 5-year survival ranging from 67% to 90%. Perioperative morbidity following esophagectomy was 37.8%. Despite the potential for complications, the 30-day mortality remained low (<5%) across the studies. These findings are consistent with previously reported data and suggest that improved outcomes may be anticipated with preemptive esophagectomy in properly selected patients and in the hands of experienced, high-volume surgeons.

Endoscopic surveillance has been proposed as an alternative to preemptive surgery. In a study by Schnell et al.,[7] only 12 out of 75 veterans (16%) with HGD and no biopsy detectable cancer developed esophageal adenocarcinoma in the first year of an intensive endoscopic surveillance program. Most of the cancers were detected at an early stage. The remaining 63 patients (84%) with HGD did not develop malignancy during the surveillance period (mean, 7.3 years; range, 0.5–12.3 years). Endoscopic surveillance was, therefore, recommended as a safe alternative to esophagectomy provided that 1 year of intensive endoscopic surveillance did not detect carcinoma.

Minimally invasive techniques such as thermal ablation, photodynamic therapy (PDT), and endoscopic mucosal resection (EMR) are investigational alternatives to preemptive esophagectomy. These techniques attempt to ablate or resect the metaplastic esophageal mucosa (Table 2). Proponents of these interventional techniques offer them as safe alternatives to esophagectomy. It has been hypothesized that the newly resurfaced squamous epithelium has a decreased malignant potential compared to BE. Critics of these modalities argue that these treatments may incompletely remove the area at risk, require specialized equipment and personnel, and may complicate future surveillance.

EMR can be used to excise the esophageal epithelium and any associated superficial lesions. The affected area is elevated by the submucosal injection of solutions (saline, dilute epinephrine, or polyethylene glycol) to facilitate endoscopic removal.[63] EMR is indicated for lesions that are less than 2 cm in diameter, involve less than one third of the wall circumference, and have not invaded the esophageal submucosa. The addition of chromoendoscopy or narrow-band imaging may facilitate the identification of abnormal mucosal regions.[64] The potential benefits of EMR include accurate pathologic staging, preservation of a functional esophagus, and reduced morbidity and mortality (compared to esophagectomy).[65] EMR also provides a therapeutic option for patients who are not candidates for surgical resection. Critics argue that complete removal of the BE requires multiple procedures by an experienced team of highly specialized providers. Additional concerns include the risk of metachronous lesions, the need for long-term surveillance, the failure of these procedures to address the underlying causes of the BE, and the absence of long-term follow-up studies.[66] Complications from EMR include bleeding, perforation, and stenosis (although the risk is less than for ablation or PDT); some of these problems may be managed endoscopically at the time of the EMR procedure.[67] The risk for esophageal stricture can be diminished by limiting the extent of resection at each endoscopy and by performing staged resections of the Barrett's epithelium several weeks apart.[64]

PDT produces tissue destruction through the generation of oxygen free radicals from photosensitizing agents exposed to light of specific wavelength. PDT combined with acid suppression therapy has been shown to eradicate HGD, but the risk of developing invasive carcinoma is not eliminated.[68,69] Laser ablation uses neodymium/yttrium–aluminum–garnet or neodymium/potassium titanyl phosphate to partially burn the abnormal esophageal epithelium. Both ablative techniques may result in the incomplete destruction of areas of BE.[70]

Preemptive esophagectomy remains the de facto standard of care for BE. The promising development of alternative therapies provides an appealing and less-invasive treatment option. Further refinements in these techniques and long-term outcome data are necessary.[71] Ultimately, we believe that both anatomic and molecular information will be used to individualize treatment for BE to prevent the development of esophageal cancer.

Hereditary Diffuse Gastric Cancer

One to 3% of gastric cancer is associated with a cancer syndrome.[72] HDGC is a rare, early-onset gastric cancer associated with an autosomal dominant germline mutation in the E-cadherin gene, CDH1. Approximately 50 families with HDGC have been reported in the literature.[73] Histologically, it appears as diffuse, submucosal foci of

Table 1 Esophagectomy for High-Grade Dysplasia in Barrett's Esophagus

Author	BE+HGD (number of cases)	Occult invasive adenocarcinoma (number of cases)	AJCC Stage						Morbidity (number of cases)	30-day post-operative mortality (number of cases)	Percent survival for occult adenocarcinoma
			0	I	IIA	IIB	III	IV			
Skinner et al.[40]	3	2	–	–	–	–	–	–	–	–	–
Schmidt et al.[39]	2	2	0	1	1	0	0	0	–	–	–
Lee et al.[31]	4	3	0	1	0	0	2	0	1	1	50 (1.9 years)
Hamilton et al.[30]	5	2	1	2	0	0	0	0	–	–	–
Reid et al.[37]	4	0	–	–	–	–	–	–	1	1	50 (0.9 years)
DeMeester et al.[28]	2	0	1	0	0	0	0	0	–	–	–
Altorki et al.[26]	8	4	0	3	1	0	0	0	2	0	100 (17 years)
McArdle et al.[33]	3	2	2	0	0	0	0	0	–	–	–
Pera et al.[35]	18	9	9	6	2	1	0	0	4	0	67 (5 years)
Rice et al.[38]	16	6	0	6	0	0	0	0	11	0	94 (1.4 years)
Levine et al.[32]	7	0	–	–	–	–	–	–	–	1	86 (2 years)
Streitz et al.[41]	9	2	–	–	–	–	–	–	–	–	–
Cameron et al.[27]	13	1	–	–	–	–	–	–	–	–	–
Peters et al.[36]	9	5	4	1	0	0	0	0	–	0	100 (1 years)
McDonald et al.[34]	1	0	1	0	0	0	0	0	–	–	–
Edwards et al.[29]	11	8	0	4	2	0	2	0	3	0	–
Ortiz et al.[52]	2	0	2	0	0	0	0	0	–	–	–
Ferguson et al.[46]	15	8	3	7	1	0	0	0	11	0	82 (5 years)
Cameron et al.[42]	19	2									
Cartambone et al.[43]	6	3	0	2	1	0	0	0		0	83
Falk et al.[45]	28	2	8	2	0	0	0	0	–	–	–
Patti et al.[53]	11	4	0	1	1	2	0	0	2	0	–
Nigro et al.[51]	14	6	–	–	–	–	–	–	–	–	–
Nguyen et al.[50]	12	2	3	2	0	0	0	0	5	0	100 (1 years)
Zaninotto et al.[62]	13	5	–	–	–	–	–	–	8	0	79 (5 years)
Headrick et al.[47]	54	14	5	7	3	1	3	0	31	1	86 (5 years)
Incarabone et al.[48]	5	3	–	–	–	–	–	–	–	0	–
Romagnoli et al.[57]	21	8	0	7	0	1	0	0	–	–	–
Tseng et al.[60]	60	18	0	13	4	0	1	0	15	1	88 (5 years)
Thomson et al.[59]	6	1	1	1	0	0	0	0	–	–	–
Reed et al.[55]	49	18	31	10	3	3	2	0	2	1	83 (5 years)
Sujendran et al.[58]	17	11	0	9	1	1	0	0	7	0	73 (2.6 years)
Moraca et al.[49]	23	6	–	–	–	–	–	–	20	0	85
Rice et al.[56]	111	50	0	46	1	3	0	0		0	86 (10 years)
Chang et al.[44]	9	1	–	–	–	–	–	–	–	0	–
Williams et al.[61]	35	6	4	5	0	1	0	0	13	0	97 (2.6 years)
Prasad et al.[54]	70	5	4	5	0	0	0	0	27	1	90 (5 years)
Totals	695	219 (31.5%)	75 (28.2%)	144 (54.1%)	24 (9%)	13 (4.9%)	8 (3%)	0	151/400 (37.8%)	6/558 (1.1%)	67–90% (5 years)

signet ring cell carcinoma. The average age of onset is less than 40 years. For those who carry a germline E-cadherin mutation, penetrance is incomplete with a 67% and 83% risk for developing HDGC by the age of 80 years in men and women, respectively.[74] Individuals as young as 14 years of age have been diagnosed with HDGC.

Cadherins are calcium-dependent transmembrane glycoproteins found at adherens junctions. Their role in cell–cell interactions helps to define cellular polarity, organize tissue structure, and maintain tissue integrity. One subtype, E-cadherin, functions as a tumor suppressor gene. E-Cadherin mutations lead to the loss of cellular growth control, derangements in tissue architecture, and increased cellular invasiveness into surrounding tissues.[75–77] More than 60 different E-cadherin gene mutations have been identified; however, not all of these contribute to the development of HDGC. Inactivating mutations predominate, and a founder mutation has been proposed.[78] CDH1 germline mutations affect a single allele. A "second hit" targeting the complementary allele is required to inactivate E-cadherin. Epigenetic hypermethylation, which occurs in 40–80% of sporadic and 50% of HDGCs, provides the second hit.[79,80]

The International Gastric Cancer Linkage Consortium (IGCLC) has issued guidelines for identifying individuals who may benefit from genetic counseling and testing for HDGC: (1) individuals with two or more first- or second-degree relatives with documented diffuse gastric cancer, one of whom was diagnosed before the age of 50 years or (2) individuals with three or more first- or second-degree relatives with diffuse gastric cancer regardless of the age of onset.[81,82] Additional testing criteria put forth by other groups include individuals with: (1) isolated cases of diffuse gastric cancer occurring before the age of 35 years; (2) two or more family members having gastric cancer with at least one case of diffuse gastric cancer diagnosed before 50 years of age; (3) lobular breast cancer in multiple first- or second-degree relatives with or without diffuse gastric cancer; and (4) a history of both diffuse gastric cancer and lobular breast cancer.[83,84] These criteria are not universally accepted.

Twenty-five percent of families that meet or exceed the IGCLC diagnostic criteria for HDGC will have a germline mutation in CDH1.[81] This specific mutation can be used to screen the remaining at-risk family members. It has been recommended that all positive genetic test results should be confirmed by a clinical laboratory prior to disclosure. Based upon our current understanding of HDGC, we believe that individuals that test negative for germline CDH1 mutations likely possess the same risk for developing diffuse gastric carcinoma as the general population. As a result, they may not require further testing or surveillance. This rule will need to be re-evaluated as additional mutations are discovered.

The optimal age to begin testing for germline CDH1 mutations in individuals at risk remains controversial. Although eight cases of HDGC have been reported in individuals 20 years of age or younger, the cited risk for HDGC in these patients may be less than 1%.[85] Nevertheless, some have recommended that screening tests begin at 18 years of age (when informed consent can legally be obtained from the affected individual).

Although all epithelial sites are theoretically at risk for malignant transformation in CDH1 germline mutation carriers, the most common first site of malignancy is the stomach. Exposure to dietary carcinogens, *H. pylori* infection, reflux of bile acids, and chronic gastritis may influence the higher propensity for gastric cancers compared to secondary sites.[86] Secondary malignancies of the breast, colon, and prostate have been reported in families with HDGC mutations.[87–89] The early demise of individuals with undetected and untreated HDGC has made it historically difficult to accurately assess the risk of other associated cancers. As long-term follow-up data emerge in patients treated by preemptive gastrectomy, the true incidence and relative importance of these cancers will become known. Lobular breast carcinoma appears to occur with increased frequency in CDH1 mutation carriers.[87–89] The lifetime risk for breast cancer in women bearing a germline CDH1 mutation ranges from 39% to 52% by the eighth decade of life.[74,78] Some believe that these women should begin screening mammography (or magnetic resonance imaging) at 25 years of age, similar to recommendations established for BRCA1 and BRCA2 associated hereditary breast cancer.[85,90]

Intensive endoscopic screening and surveillance (every 6 months) in CDH1 germline mutation carriers has been suggested for early detection of diffuse gastric cancer. The submucosal location of the disease and absence of associated mucosal abnormalities create diagnostic limitations for conventional gastroscopy.[81] Random biopsies at periodic intervals detect less than half of cancers, even when multiple (>45) cancer foci are present.[91] Since more than one third of lesions occur within the body-antral transition zone of the stomach, selective surveillance or targeting this region may improve the sensitivity of endoscopic screening.[92] Discriminatory techniques, such as chromoendoscopy and dual-band imaging, may potentially increase the diagnostic yield of endoscopic surveillance. It remains likely that all conventional endoscopic modalities will fail to identify lesions smaller than 4 mm in diameter.[93]

The accuracy of conventional light and chromoendoscopy for the detection of diffuse gastric carcinoma in 68 germline CDH1 mutation carriers has been summarized in Table 3. Chromoendoscopy was used in 48 of 68 (71%) patients undergoing gastroscopy. Gastric cancer was diagnosed in 22 (32%) these patients using endoscopy. Twenty-five of the remaining 46 individuals having negative endoscopic evaluation underwent surgical resec-

Table 2 Comparison of Preemptive Esophagectomy, Endoscopic Mucosal Resection, and Mucosal Ablative Therapies

Method	Occult adenocarcinoma	Morbidity (%)	Mortality	Recurrence (%)	Comment
Ablative therapy (PDT, laser, and RFA)[157]	Unable to assess	0–40	Rare	0–47	High incidence of esophageal stricture, available in specialized centers only, expensive, and requires continued endoscopic surveillance
Endoscopic mucosal resection[63,158]		0–30		0–40	Assesses depth of invasion and enables complete resection. May require more than one session. Surgical resection remains an option. Requires continued endoscopic surveillance
Preemptive esophagectomy	13–40%	38	<5%		Standard of care. Evaluates regional lymph nodes

tion. Eighty-eight percent (22/25) were diagnosed with foci (range 1–318) of diffuse gastric cancer following total gastrectomy. Data on the remaining 21 individuals was not reported. Endoscopy, including chromoendoscopy, failed to detect small (<4 mm) lesions and underestimated the true cancer burden within the stomach. Based on these findings, the utility of surveillance endoscopy for CDH1 mutation carriers remains unproven, even with the addition of chromoendoscopy.

Total gastrectomy is the only viable treatment capable of preventing the lethal consequences of invasive gastric cancer in CDH1 germline mutation carriers. Three-fourths of CDH1 germline mutation carriers undergoing preemptive gastrectomy are diagnosed with occult diffuse gastric carcinoma, which is consistent with the expected penetrance of this autosomal dominant disease.[73,78,83,90,92,94–99] Gastrectomy was actually therapeutic and not preventative in these individuals. The lesions were uniformly of early stage (T1N0Mx), which portends a favorable prognosis for these individuals who would otherwise succumb to a more advanced stage of disease if left untreated. What remains unclear, however, is if outcomes for early-stage diffuse gastric cancer are similar to stage I sporadic (non-HDGC) gastric cancer.

Preemptive gastrectomy in the appropriately selected CDH1 germline mutation carrier requires the complete resection of all gastric mucosa. Intraoperative endoscopy and frozen section analysis of the esophageal margin to confirm the adequacy of proximal resection is recommended, since it may be difficult to identify the proper location of the gastroesophageal junction.[97] The proper extent of lymphadenectomy (i.e., D1 vs. D2) for preemptive surgery is unknown. Reconstruction of the gastrointestinal tract following gastrectomy is routinely performed with Roux-en-Y esophagojejunostomy.

Preemptive gastrectomy for HDGC remains controversial. Supporting arguments for total gastrectomy include the findings that endoscopy has inadequate sensitivity to detect HDGC for it to be used as a diagnostic or surveillance strategy, most CDH1 mutation carriers have occult carcinoma, surgery offers the only curative therapy, and experienced centers report a low morbidity and mortality for surgery. On the contrary, incomplete penetrance among CDH1 mutation carriers means that approximately one-third of individuals would derive no benefit from total gastrectomy. Unidentified CDH1 mutations likely exist, making it impossible to identify all at-risk individuals. Long-term survival rates following gastrectomy for HDGC are unknown. The impact of surgical complications (10–20% morbidity and 3–6% mortality), nutritional/functional deficiencies (10–15% permanent weight loss), and altered quality of life may influence the decision to proceed with preemptive surgery in this young patient population.[97]

Bile Duct Cysts and Primary Sclerosing Cholangitis

Cholangiocarcinoma is a rare cancer that arises from premalignant biliary tract conditions that have the common predisposing triad of chronic inflammation, infection, and biliary obstruction.[100] Bile duct cysts (BDC) and primary sclerosing cholangitis (PSC) are the two most common etiologies in Western case series.[101] Early diagnosis is difficult because signs and symptoms are often nonspecific. Despite aggressive surgical resection, long-term survival is poor (2-year survival is estimated at 13%).[102]

BDCs likely arise from a congenital abnormality of the pancreaticobiliary ductal junction that results in the chronic reflux of pancreatic secretions into the biliary tree. It has been hypothesized that the inflammation leads to cystic dilatation of the bile duct. Five types of BDCs are described in the Todani–Lenriot classification system (Table 4).[103,104] The incidence of cholangiocarcinoma in BDCs ranges from 12% to 28% and increases with the age at presentation.[104,105] Cancer may develop anywhere within the biliary tract, including the cyst wall and gall bladder.[106] The chronic exposure of carcinogens within stagnant bile likely

Table 3 Endoscopic Detection of HDGC in Germline CDH1 Mutation Carriers

Author	Number of germline CDH1 mutation carriers evaluated by endoscopy	Employing chromo-endoscopy (%)	Number of patients with cancers diagnosed at endoscopy	Number of patients with negative endoscopy and cancer at gastrectomy
Chun et al.[94]	5	2/5 (40%)	0	5
Huntsman et al.[96]	2	0	2	0
Charlton et al.[92]	6	4/6 (66.7%)	5	1
Van Kouwen et al.[159]	1	0	1	0
Suriano et al.[83]	6	U	0	5
Shaw et al.[93]	33	32/33 (97%)	12*	NR
Gaya et al.[95]	2	0	1	0
Newman et al.[99]	1	0	0	0
Norton et al.[90]	10	10/10 (100%)	0	10
Kaurah et al.[78]	1	0	1	0
Chung et al.[73]	1	0	0	1
Total	68	48/68 (71%)	22/68 (32%)	22/25 (88%)

*Surgical findings were only reported for the 12 patients with endoscopically detected cancer that underwent total gastrectomy. *NR* not reported

transforms the biliary epithelium into cancerous lesions. Simple biliary cyst drainage does not appear to prevent the development of cholangiocarcinoma.[107] Preemptive resection of BDCs has been recommended as the most aggressive treatment in these patients. Surgical excision eliminates the epithelium at risk, abolishes pancreaticobiliary reflux, and restores bile flow. Japanese data show that the incidence of cholangiocarcinoma after complete cyst excision is 0.7%.[108]

PSC is an autoimmune disease characterized by periductal inflammation and multifocal intra- and extra-hepatic biliary strictures.[109] Most individuals will progess to biliary cirrhosis, portal hypertension, and ultimately liver failure. Nearly three fourths of individuals with PSC have underlying inflammatory bowel disease (IBD), whereas less than 10% of individuals with IBD develop PSC.[101,110] Despite this association, the severity and duration of IBD does not appear to influence the incidence of PSC.[111] Similarly, the duration of PSC does not affect the incidence of cholangiocarcinoma.[112] In addition, medical and surgical management of IBD do not decrease the long-term risk of PSC.

The pathogenesis of cholangiocarcinoma within BDCs and PSC is characterized by progressive alterations in the bile duct epithelium, from metaplasia to dysplasia, and then carcinoma. Several genetic mutations, such as K-ras (80–100%), p53 (40%), E-cadherin, vascular endothelial growth factor, and epidermal growth factor receptor have been identified.[113] Bile duct epithelial dysplasia likely represents a precancerous condition that may ultimately lead to the development of cholangiocarcinoma. This model has been previously demonstrated in breast, colorectal, and pancreatic adenocarcinomas. Dysplasia may indicate a "field defect" and prompt clinicians to perform additional diagnostic investigation and possibly surgical resection of the areas at highest risk. The histologic recognition of dysplasia remains problematic, with only moderate inter-observer agreement among pathologists.[114] Accurate and reproducible recognition of dysplasia is prudent before treatment decisions are finalized.

The lifetime risk of cholangiocarcinoma in patients with PSC has been estimated at 7–15%.[112] Higher rates (27–42%) have been reported in autopsy studies.[101,113] In PSC, bile duct dysplasia is frequently found in regions adjoining areas of carcinoma and may precede cholangiocarcinoma by 18 months.[115–117] Nearly 20% of patients with PSC-associated cholangiocarcinoma in one series had additional areas of dysplastic biliary epithelium.[114] Furthermore, biliary dysplasia is more common in patients who progress to cholangiocarcinoma than in end-stage PSC patients without cancer.

PSC-associated cholangiocarcinoma has a dismal outcome. Patients typically present with unresectable or distant metastatic disease. The median survival is often less than 5 months.[118] The presence of nonspecific symptoms, the inability of diagnostic studies to discriminate between PSC-

Table 4 Bile duct cysts[103,104]

Type	Description	Prevalence (%)
I	Extrahepatic, fusiform	79
II	Extrahepatic diverticulum	2.6
III	Intraduodenal	4
IV	Multiple extrahepatic or both extra- and intrahepatic	13
V	Multiple intrahepatic	<1

induced strictures and early cholangiocarcinomas, inaccuracies in the pathologic diagnosis of cancer, and liver failure secondary to chronic biliary obstruction are several factors that may explain the delayed diagnosis and aggressive biologic behavior of this disease. Treatment with ursodeoxycholic acid can transiently improve abnormal liver biochemistries and may reduce the incidence of cholangiocarcinoma.[119] Liver transplantation is the only therapeutic option for end-stage PSC. Five- and 10-year survival rates after liver transplantation for PSC have been reported to approach 80% and 60%, respectively.[120] Ten percent of patients transplanted for PSC in one series had occult cholangiocarcinoma identified in their resected livers. Patients with incidentally identified cholangiocarcinoma had survival that was comparable to "sporadic" cholangiocarcinoma, as long as the tumors were <1 cm in size and there was no lymph node involvement.[121] One- and 5-year survival rates are 59% and 36%, respectively, for hilar cholangiocarcinoma without lymph node involvement.[122] Few patients with lymphatic metastases survive beyond 5 years.

Early liver transplantation for PSC has been proposed as a strategy to prevent the development of cholangiocarcinoma and improve the survival of patients with PSC. Implementation of this preemptive operation is limited by the scarcity of donor organs. The unpredictable course of PSC, the complexity of prognostic modeling (Mayo risk score), and the lack of reliable biological markers (e.g., CA 19-9, CEA, p53, or K-ras) make it difficult to identify individuals at high risk for developing cholangiocarcinoma. Serum CA 19-9 (>129 U/ml) is an unreliable screening tool for detecting cholangiocarcinoma in the setting of PSC, with a positive predictive value of 56%.[123] Liver transplantation for PSC is associated with more frequent complications, including vascular and infectious complications, acute rejection, recurrent PSC, exacerbation of IBD, and the development of colorectal cancer.[121] The timing of liver transplantation relative to colectomy for IBD adds complexity to the decision-making process. Sophisticated protocols have been developed for transplantation in early-stage PSC-associated cholangiocarcinoma. Neoadjuvant chemoradiotherapy administered prior to hepatic transplantation for stages I and II hilar cholangiocarcinoma has been shown to improve survival and local recurrence rates compared to resection alone in a small single-institution series of highly selected patients.[124] Preemptive liver transplantation in PSC remains controversial, and further investigation is required before it can be broadly recommended.

Pancreatic Cancer

Adenocarcinoma of the exocrine pancreas is a uniformly fatal disease. Most patients have advanced disease at the time of presentation. Both medical and surgical treatments have a limited impact on the natural history of this disease. Risk factors associated with the development of pancreatic cancer include cigarette smoking, diet, chronic pancreatitis, morbid obesity, diabetes mellitus, and occupational exposures.[125] Precursor pancreatic conditions are also associated with an increased risk for pancreatic adenocarcinoma and can be classified into five groups (Table 5).[126,127]

Sporadic pancreatic cancer appears to arise in the setting of progressive dysplasia of the pancreatic duct epithelium. Pancreatic intraepithelial neoplasia (PanIN) is characterized by an abnormal mucinous epithelium and graded on a scale from 1 to 3. PanIN-1 represents a flattened or papillary mucinous epithelium, PanIN-2 has the additional feature of nuclear abnormalities, and PanIN-3 signifies carcinoma in situ.[126] Genetic mutations in K-ras, Her2-neu, p16, DPC4, p53, and BRCA2 accumulate in advanced PanIN.[128] The natural history of PanIN likely culminates in the development of an invasive pancreatic adenocarcinoma.

A minority of pancreatic cancers are associated with heritable conditions (Table 5).[129] An autosomal dominant pattern has been identified in less than 10% of "hereditary" pancreatic cancer.[130,131] Germline BRCA2 gene mutations have been detected in 17% of familial pancreatic cancers.[132] The risk of pancreatic cancer is 18-fold higher in kindreds with two affected family members and ≥57-fold more likely when three family members are affected.[133] Peutz–Jeghers syndrome is caused by an autosomal dominant inheritance of mutations in the serine/threonine kinase 11 (STK11) gene and is characterized by the presence of mucocutaneous pigmentations and multiple hamartomatous intestinal polyps. The risk of developing pancreatic cancer is 132-fold higher than in the general population, with a 36% lifetime risk.[134,135] Familial atypical multiple mole melanoma syndrome (FAMMM) is characterized by the presence of 50 or more dysplastic nevi or melanoma in two or more first- or second-degree relatives. Pancreatic cancer occurs in 25% of FAMMM families. Germline mutations in cyclin-dependent kinase 2A gene (CDKN2A) occur in FAMMM and increase the risk for developing pancreatic cancer 13- to 22-fold, with a cumulative risk of 17% by age 75.[136] Hereditary nonpolyposis colorectal cancer syndrome is caused by germline mutation in mismatch repair genes, and the risk for pancreatic cancer is estimated to be less than 5%.[129] Other conditions predisposing to pancreatic cancer include hereditary pancreatitis, neurofibromatosis (type 1), multiple endocrine neoplasia (type 1), and von Hippel–Lindau (VHL) syndrome.[137] Adenocarcinoma of the pancreas is rare in VHL syndrome. Pancreatic neuroendocrine tumors (PNET) occur in fewer than 10% of VHL cases and have similar malignant potential to the familial or sporadic forms of PNET.[138] Screening high-risk patients for clinically

occult pancreatic cancer remains experimental. Although several different strategies using CA 19–9, computed tomography (CT), and endoscopic ultrasound (EUS) have been tested at individual centers, no reliable method of earlier detection has been identified.

PCNs represent a continuum of disease ranging from benign inflammatory pseudocysts to cystic tumors that may harbor invasive cancer. PCNs are most often incidentally discovered in individuals undergoing imaging studies for other indications. The natural history of PCNs is unknown. Most of our existing information comes from large retrospective case series. Subtypes of PCNs include mucinous cystadenomas (MCNs) and intraductal papillary mucinous neoplasms (IPMNs). MCNs are mucin-containing multilocular lesions that occur predominantly in the body and tail of the pancreas. These tumors arise within a unique ovarian stroma that suggests a possible hormonal etiology. As such, they predominantly affect women in the fourth and fifth decades of life. The reported prevalence of invasive carcinoma in MCN ranges from 6% to 36%.[139] IPMNs are cystic dilatations of the pancreatic ductal system (i.e., main pancreatic duct, its side branches, or mixed). The main pancreatic duct type is more likely to be associated with high-grade cytology and malignant behavior than the branched-duct type. The prevalence of cancer in the main-duct and side-branch IPMNs ranges from 57% to 92% and 6% to 46%, respectively.[139]

The histologic classification of PCN has important clinical implications that may help estimate the malignant potential of these lesions. CT, magnetic resonance cholangiopancreatography (MRCP), and EUS can differentiate IPMN from serous and mucinous cystic neoplasms. The paucity of cellularity within the cyst fluid aspirate decreases the accuracy of endoscopic cytologic analysis. Sophisticated molecular fluid analysis may ultimately provide the answer to this question.[140] The cyst fluid concentration of carcinoembryonic antigen (CEA) can differentiate mucinous (high CEA; >192 ng/ml) from nonmucinous cysts. Although extremely high CEA values suggest malignant PCN, this relationship has not been conclusively established.[141,142] The aggregate clinicopathologic information derived from the combination of CT, MRCP, EUS, and cyst fluid analysis (i.e., tumor markers, cytology, etc.) helps the clinician narrow the differential diagnosis. Ultimately, complete surgical removal of the cystic neoplasm is the only definitive method for establishing a diagnosis.

As with other premalignant foregut lesions, the risk of surgical resection must be individualized to the patient and balanced with the likelihood that his/her PCN will transform into a malignant lesion or contains an occult carcinoma. Pancreatectomy is associated with a 30–50% risk of complications, including hemorrhage (2–4%), anastomotic leak (pancreatic fistula, 8–19%; bile leak, <5%), delayed gastric emptying (19–35%), development of diabetes mellitus, and wound infections (10%).[143] Although mortality rates at high volume centers may be as low as 1–3%, these outcomes may not be generalizable to the at-large surgical community.[144,145] Obligatory versus selective resection of PCN continues to be debated.[146–152] Clarification of the PCN sub-type is critical for surgical decision making. We believe that all main duct IPMN, large (>2–3 cm) branched duct-type IPMN or MCN with

Table 5 Pre-malignant pancreatic conditions

Group	Description	Estimated risk of pancreatic cancer
PanINs	PanIN-1 (A or B) PanIN-2	
	PanIN-3	
Cystic lesions	Intraductal papillary mucinous neoplasms	~50% (main duct); ~25% (side-branch) lifetime
	Mucinous cystic neoplasms	~30% lifetime
Inherited conditions	Familial pancreatic cancer	18-fold[160]
	Peutz-Jeghers Syndrome	132-fold (~36% lifetime)[161]
	Hereditary non-polyposis colorectal cancer syndrome (HNPCC)	<5% cumulative risk[129,160]
	Hereditary pancreatitis	50–100-fold[160,162]
	Familial atypical multiple mole melanoma syndrome (FAMMM)	13–22-fold[163]
	BRCA2 mutation	10-fold[160]
Chronic pancreatitis		2–9-fold[164,165]
Inherited endocrine neoplasms	Neurofibromatosis, type 1	
	Multiple endocrine neoplasia, type 1 von Hippel–Lindau syndrome	Rare

concerning features (i.e., cyst wall complexity, mural nodularity, pancreatic ductal dilation, or associated mass), and symptomatic PCN should be considered for resection.[146,147,153] The inability to exclude an associated malignancy may justify resection in appropriately selected patients. The operative approach is determined by the location of the lesion within the pancreas, presence of multifocal disease, and the estimated biological behavior of these tumors. Lesions in the pancreatic head are treated by pancreaticoduodenctomy, and tumors in the body and tail are best approached by a distal pancreatectomy (+/− splenectomy). Central or median segmental pancreatectomy is an uncommon operation that has been traditionally used for indolent lesions where conservation of the pancreatic parenchyma is essential.[154] Total pancreatectomy should be approached with caution in patients with multiple PCNs due to the complete pancreatic exocrine and endocrine insufficiency.[155,156]

Conclusion

Forestalling the onset of cancer by the early recognition and eradication of premalignant lesions represents the theoretical basis for preemptive surgery. Accurate identification of patients at highest risk is essential. Limitations in predictive testing and the uncertain biologic behavior of these lesions curtail surgical enthusiasm. The emergence of new screening and diagnostic innovations has given clinicians additional information that may assist with individual risk assessment. The diagnosis of a heritable or premalignant condition has a profound impact on the screening of both the patient and his/her relatives. This raises important ethical questions about the age at which to offer predictive testing and preemptive operations. We believe that these measures should be instituted when the age-specific risk for cancer exceeds the operative mortality of preemptive surgery.

Radical surgical resection remains the most aggressive and definitive risk modifying strategy for patients with premalignant foregut lesions. “Preemptive surgery” for these individuals is often therapeutic rather than preventative. The long-term consequences of these operations remain unknown. The impact of perioperative morbidity and mortality cannot be underestimated. Surgical resection of malignant neoplasms prior to regional or distant metastases has been historically associated with improved recurrence-free and cancer-specific survival rates. It is unclear if earlier surgical intervention will modify these outcomes. Preemptive surgery also may not eliminate the future risk of associated malignancies. It is imperative to recognize that this is an evolving field, with more uncertainty than certainty. It remains unlikely that well-designed clinical trials and level I evidence will be able to be generated in these rare syndromes. Balancing the risk of surgical intervention with less invasive interventions or observation must be individualized on a case-by-case basis.

Acknowledgments The authors would like to thank Patricia Schaddelee for proofreading this manuscript.

Grant support None

Prior presentations None

Permissions None

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J Gastrointest Surg (2009) 13:1888
DOI 10.1007/s11605-009-0805-y

GI IMAGE

A Tale of Colostomy Bag in Poor: GI Image

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Received: 13 December 2008 / Accepted: 3 January 2009 / Published online: 24 January 2009

Keywords Colostomy · Bag · Poor

A 57-year-old woman underwent abdominoperineal resection for rectal growth. She had end colostomy. Initially, she applied medical colostomy bag. During her hospital stay, she used to collect empty containers of intravenous fluids used on her. Nobody enquired for this. On follow-up, she was found to have used these empty containers as colostomy bags. On questioning, it was found out that the poor lady was not in the position to afford colostomy bags for her use.

It has been rightly said that colostomy can be the beginning of a new and healthier life. The quality of life for patients who have undergone total rectal resection for carcinoma is impaired by the artificial intestinal stoma .[1] In developed countries, the selection of colostomy appliance is a personal choice seeking the best and comfortable no matter how costly, but in poor countries, this is plausible. In underdeveloped countries, sometimes, a patient has no desire of even to dream of having modern colostomy appliance. What is affordable for them is demonstrated in this GI image (Figs. 1 and 2). A beautiful design of routinely used intravenous fluid is shown here (Fig. 1). The woman made hole with margins inverted in this plastic bottle for use as appliance for end colostomy (Fig. 2) because she cannot afford to buy colostomy appliance life long. Whether to admire the patient or curse poverty for this idea is undecided, but she has no other option.

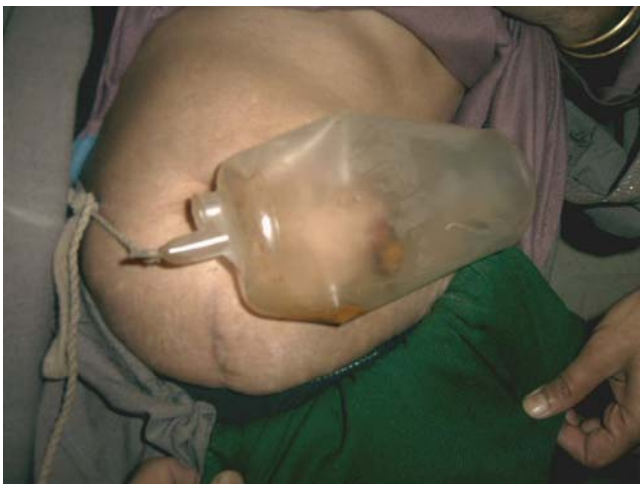

Figure 1 A makeshift colostomy bag made out of empty container of intravenous fluid.

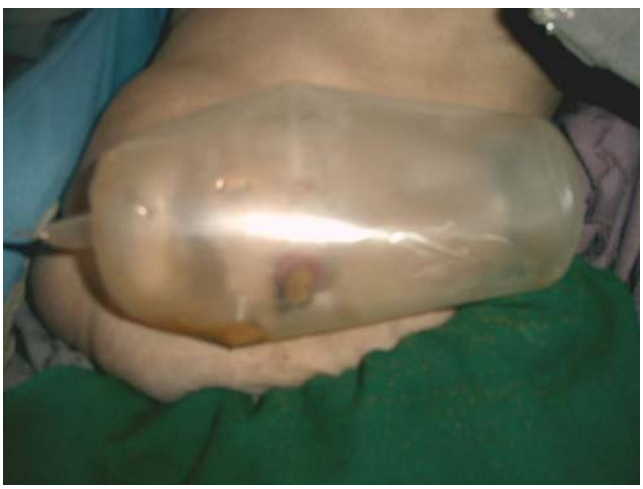

Figure 2 The woman made a hole with margins inverted in the plastic bottle that served as her colostomy bag.

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J Gastrointest Surg (2009) 13:1889–1891
DOI 10.1007/s11605-009-0976-6

EDITORIAL

Complete Mesocolic Excision—A Marker of Surgical Quality?

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Received: 2 May 2009 / Accepted: 21 July 2009 / Published online: 5 August 2009

Keywords Complete mesocolic excision · Colon cancer · Quality assurance

Quality assurance in surgery has never been more important. As public awareness and lay access to educational resources increase, the onus is on the surgical community to provide a consistently excellent standard of care. Nowhere is this more evident than the field of oncology. The establishment of the multidisciplinary care model ensures that patients are afforded timely and appropriate specialist referral,[1] and an international vogue towards a patient-led service is evident in recent years.[2] While involvement of chemo- and radiation-oncologists undoubtedly improves disease-free survival, there is an increasing body of evidence pointing to the primacy of surgical technique.[3] Natural evolution of practice produced enhanced results,[4] but a more active approach to establishment of guidelines and implementation of strict protocols has been adopted.[5] The concept of variation in outcome dependent upon the individual surgeon performing the operation is not new[6] but certainly adds weight to the argument for subspecialization in the light of the ongoing volume-outcome debate.[7]

Heald was first to describe total mesorectal excision,[8] and while the technique may not have been entirely original, there is no doubt but that it has revolutionized the worldwide management of rectal cancer.[9] It involves the formal resection of an intact tumor specimen with its full lymphatic drainage and blood supply within a predefined operative plane. However, until now, it has been difficult to attribute improvement in patient outcome specifically to technique alone, and the contribution of a concurrent global enhancement of rectal cancer care cannot be discounted. A recent study, however, succeeded in isolating adequate plane in rectal cancer surgery as an independent prognostic factor (irrespective of (neo)-adjuvant radiotherapy) and found it to be more important than resection margins, thus challenging traditional dogma.[10] Short-course pre-operative radiotherapy combined with adequate plane surgery almost abolished recurrence at 3 years, thus allowing the consideration of rectal cancer as a curable entity. The authors describe a progressive improvement in technique (and thus outcome) over the study period and suggest that the process of executing the trial alone may have contributed to this. With modification of technique and standardization of adjuvant therapies, rectal cancer now demonstrates an equivalent, if not better, disease-free survival to stage-equivalent colon cancer[11] (whose management, until now, has been poorly standardized).

The relatively new concept of complete mesocolic excision in the management of colon cancer represents far more than evolution in operative technique. It attempts to extrapolate the advances in rectal cancer management and translate the vast survival advantage to colon cancer. This reflects the vogue towards quality assurance[12] and international standardization of cancer care. While many guidelines govern the diagnosis of colon cancer,[13] far fewer attempt to legislate for specifics of operative technique. Many surrogate markers for excellence in cancer management have been adopted. Number of lymph nodes resected has been endorsed to benchmark operative quality[14] at certain disease stages[15] (with 12 considered adequate),[16]

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and specialist trained surgeons are more likely to deliver this standard.[17] The impact of hospital volume on outcome following surgery for colon cancer[18] is less certain than the convincing evidence pertaining to solid organ tumors,[19] but the importance of the individual surgeon's demonstration of technical credentials has been consistently highlighted.[20] Formal feedback from pathologists to surgeons regarding resection margins and planes of dissection is becoming the norm,[21] and quality of histopathological reports has been improved by introduction of a standardized pro-forma.[22] Clinical audit is well established as a professional requirement,[23] and anonymous reporting in many jurisdictions allows for open and transparent analysis of outcomes.[24] There is widespread awareness of the importance of opportunistic screening (colonoscopy[25] or CT colonography[26]) resulting in diagnosis at far earlier disease stage.[27]

Although the idea of complete mesocolic excision is still embryonic, early results are encouraging. Only evidence from retrospective trials is available to date, but the potential survival advantage resulting from careful intact specimen dissection is undeniable.[28] If nothing else, discrepancies in current practice have been highlighted, and the surgical community has been made aware that the time is ripe for formal standardization of operative practice. A recent study attributed improved cancer-free survival, reduced loco-regional recurrence, increased lymph node harvest, and decreased morbidity to the formal introduction of a clearly described operative technique for colonic resection.[29] This involved separation of mesocolic and parietal planes and true central ligation of supplying arteries and draining vessels at their roots. While this could be considered nothing more than good oncological surgical practice, its widespread introduction as standard of care would undoubtedly translate to improved cancer-specific survival. Pathologists are in an ideal position to police the maintenance of high quality dissection, and their move toward subspecialization will certainly aid the optimization of the quality assurance process for colon cancer.[30] Open communication and appraisal of technique in the forum of a regular multidisciplinary meeting provides an invaluable feedback opportunity for surgeons striving to optimize patient care.

Complete mesocolic excision is the latest addition to a plethora of surrogate markers for high quality care in operative management of colon cancer. Specifics of the technique are most likely less important than the generalized concept. It may, in fact, be its accompaniments (in the form of surgeon cognizance of anatomical planes, careful pathological evaluation, multidisciplinary communication) that afford survival benefit. However, it allows specific instructions to be issued to international surgeons involved in the operative management of colon cancer and, undoubtedly, has a valuable role to play in the overdue implementation of a quality assurance strategy in the management of colon cancer.

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J Gastrointest Surg (2009) 13:1892
DOI 10.1007/s11605-008-0532-9

LETTER TO THE EDITOR

Does a 48-Hour Rule Predict Outcomes in Patients with Acute Sigmoid Diverticulitis?

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Received: 7 March 2008 / Accepted: 14 April 2008 / Published online: 1 July 2008

We read with interest the paper by Evans[1] in which the author demonstrated that a noticeable drop in leucocyte count and temperature over the first 48-h of medical management predicted early discharge on oral antibiotics. We recently undertook an audit of all patients with acute sigmoid diverticulitis, over a 4-month period, which supported these findings.

Our audit reviewed all admissions to the surgical admissions unit of our district general Trust to improve surgical practice and thereby patient care, focussing on management and length of stay. Of all 33 patients admitted, none developed any complications or required surgical interventions. Further, neither intolerance of oral intake, nor opioid need, precluded discharge — despite this, however, mean length of stay was 3 days.

It has been shown that 70–85% of episodes of sigmoid diverticulitis resolve with medical management,[1] and, further, Broderick-Villa et al.[2] have shown that the risk of recurrent diverticulitis after initial non-operative management was significantly lower than previous reports. Therefore, this study has important implications for current surgical practice.

There are currently no published guidelines in the UK, and other studies have shown, as has our audit, that there is no consensus between practitioners regarding management.[3] Combining the results of Evans' study, current ASCRS guidelines[4] and our audit, we have written a local guideline to encourage best practice and a reduction in unnecessary in-patient stay. If admission is necessary, then decreasing inflammatory markers within 48-h is an indication for discharge, as complications appearing after this have now been shown to be unlikely.

A 48-h rule should have a significant impact on length of stay of patients with acute sigmoid diverticulitis, with consequent benefits for the patient and substantial financial savings for our Trust.

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