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SSAT POSTER PRESENTATION

Surgeon Perceptions of Natural Orifice Translumenal Endoscopic Surgery (NOTES)

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Abstract
Introduction If proven feasible and safe, Natural Orifice Translumenal Endoscopic Surgery (NOTES) would still need acceptance by surgeons if it were to become a mainstream approach.
Methods Three hundred fifty-seven surgeons responded to a preliminary survey describing NOTES and were asked to rate the importance of various surgical considerations and (assuming availability and safety) if they would choose to undergo and/or perform cholecystectomies by NOTES or laparoscopy and why.
Results The risk of having a complication was considered most important. NOTES was theorized to be riskier and to require greater skill than laparoscopy but to potentially cause less pain and convalescence. Nearly three-fourths (72%) of surgeons expressed interest in NOTES training which correlated with younger age, SAGES membership, minimally invasive surgery specialization, and flexible endoscopic volume. Forty-four percent would like to introduce NOTES cholecystectomy into their practices. Among those not preferring NOTES, 88% would adopt NOTES if data showed improved outcomes over laparoscopy. Finally, only 24% would choose to undergo cholecystectomy themselves by NOTES, believing it to be too new and riskier than laparoscopy.
Discussion The risk of having a complication is the greatest concern among surgeons, and safety will affect NOTES acceptance.
Conclusion The results of this survey seem to justify more focused future investigations.

Keywords NOTES · Flexible endoscopy · New technology · Surgery · Attitude of health personnel

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Introduction

Natural Orifice Translumenal Endoscopic Surgery (NOTES) is a surgical approach that combines elements of flexible endoscopy and laparoscopic surgery. It is currently being studied in research labs and in limited clinical studies. Since the first report of NOTES procedures in experimental animals in 2004,[1] NOTES has generated excitement among surgeons and gastroenterologists. The ability to offer even less invasive surgical techniques than conventional laparoscopy has inherent merit. NOTES procedures could theoretically be accompanied by less pain, shorter recovery time, and absent or reduced abdominal wall incisions when compared to laparoscopic operations. These considerations have resulted in a new direction of research and led to the creation of the Natural Orifice Surgery Consortium for Assessment and Research.

This consortium, comprised of leaders of the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) and the American Society for Gastrointestinal Endoscopy, has identified key elements to limit patient harms as well as potential barriers to NOTES requiring further investigation, and has allocated industry-sponsored grant money to researchers studying these areas of interest.[2]

To date, the majority of research pertaining to NOTES has been confined to studies in large animal or human cadaver models. This research has consisted of studies of various NOTES procedures, alternative translumenal access sites (gastric, colonic, urethral, and vaginal), investigations of the physiology of NOTES, and the application and testing of new technologies including those for the closure of access sites. Most of this research has been clustered at a relatively small number of academic medical centers and performed by limited numbers of skilled researchers and physicians. However, the number of researchers investigating NOTES is growing, and NOTES and NOTES-assisted minimally invasive operations have been reported in a small number of patients under experimental, IRB-approved protocols.[3–7]

For NOTES to advance beyond animal studies and anecdotal human case reports and become a mainstream surgical procedure, it will need to be accepted and embraced by both patients and physicians. The combined perceptions of these groups will affect the demand for NOTES as an alternative to current minimally invasive surgical techniques. The understanding of patient and physician perceptions of NOTES is, thus, important to help guide the trajectory of physician training, research efforts, and the allocation of research and development funding.

Recently we found that if given the choice in a hypothetical scenario, a majority of patients would prefer to have cholecystectomy via NOTES rather than by laparoscopy.[8] Patients indicated that when making a decision to undergo surgery, their most important consideration was the risk of suffering a complication, followed in decreasing importance by time to full recovery, amount of postoperative recovery time, and length of hospital stay (LOS). As the hypothetical surgeon's experience decreased and the risk of complications increased, there was a corresponding diminution in patient preference for NOTES. Furthermore, most patients preferring the NOTES approach to cholecystectomy would still choose this technique if it had a slightly greater risk of complications (2% vs. 1%) but not if associated with a markedly higher risk of complications (10% vs. 1%). In order to better understand surgeons' perceptions of NOTES, we conducted an opinion survey of surgeons from three major surgical societies.

Material and Methods

A 75-item survey was offered electronically to members of the Society for Surgery of the Alimentary Tract (SSAT), SAGES, and the American College of Surgeons (ACS) after obtaining permission from each organization, as well as institutional review board approval from Northwestern University and Legacy Health System. Survey subjects were solicited via direct email messaging (SSAT) and email newsletters (SAGES Mini-Scope, ACS NewsScope) which briefly described the study and provided an internet hyperlink to a secure online survey (SurveyMonkey.com). In this manner, the study hyperlink was distributed to approximately 45,000 physicians. Eighty-five percent of these emails were sent via the ACS NewsScope, 11% were sent through the SAGES Mini-Scope, and 4% were distributed via direct email to SSAT members. Whereas the hyperlink to the survey could only be included within the electronic newsletters of the ACS and SAGES, the email sent by the SSAT was a focused, direct request for participation in the study.

The posted survey included a brief introduction describing the basic concepts of NOTES and (assuming safety and availability) how NOTES might be applied to cholecystectomy in the setting of symptomatic cholelithiasis (Appendix 1). Demographic information was then collected, and surgeons were asked to rate the importance of procedure-specific considerations including cost, complication risk, length of hospitalization, anesthesia type (general anesthesia vs. conscious sedation), cosmesis, and postoperative pain and recovery time. Surgeon perceptions of NOTES, laparoscopy, and traditional open procedures were subsequently measured with respect to these surgical considerations on an analog scale of 0–5. Questions aimed at assessing surgeons' interest in NOTES, or lack thereof, and the reasons for these sentiments were also posed (Appendix 2).

Data were collected anonymously and coded numerically. Responses were downloaded in Microsoft Excel before analysis for significance using SPSS 14.0 (SPSS, Chicago, IL, USA). Only responses from completed surveys were recorded. Significance was determined using chi-square and Wilcoxon signed ranks tests as well as forward stepwise logistic regressions.

Results

Three hundred fifty-seven surgeons completed the questionnaire. Overlap was present among society memberships with 85.4% of respondents belonging to the ACS, 66.4% to the SSAT, and 56.9% to SAGES (39.5% were members of all 3 societies, Table 1). The overall response

Table 1 Surgeon Demographics

Surgeons surveyed	357
Age (mean)	46
Age<60 years old	85.7%
Society membership	
ACS	85.4%
SSAT	66.4%
SAGES	56.9%
SSAT and ACS	57.4%
SAGES and ACS	52.1%
SAGES and SSAT	42.0%
ACS, SSAT, and SAGES	39.5%
Specialty	
Minimally invasive	22.7%
Gastrointestinal	21.8%
General surgery	20.7%
Colorectal	9.5%
Surgical oncology	7.3%
Hepatobiliary	7.3%
Other	10.7%
Heard of NOTES	87.7%
Perform flexible endoscopy in <10% cases	66.1%

rate from the study was less than 1% (0.79%). Among the 1,977 email messages sent via the SSAT, 181 members (9.2%) followed the link to the survey. It cannot be determined how many of these SSAT members completed the survey or what proportion of the total 237 respondents from the SSAT were directly attributable to this email message.

The median surgeon age was 46 years, 66.1% reported using flexible endoscopy in less than 10% of their cases, and 65.2% listed their specialty as either gastrointestinal (21.8%), minimally invasive surgery (MIS; 22.7%), or general surgery (20.7%, Table 1). In deciding upon a surgical approach, the risk of a complication was the most important consideration to surgeons and complication risk, recovery time, amount of postoperative pain, and length of stay were each felt to be to be more important than cosmesis, cost, or anesthesia type ($p<0.005$; Fig. 1). When NOTES was compared independently to laparoscopy and laparotomy, it was felt to require significantly greater technical skill and be associated with less pain and shorter recovery, while having higher costs and increased risk of complications than the other approaches ($p<0.05$; Fig. 2).

Seventy-two percent of these surgeons expressed an interest in becoming trained in NOTES, and 47% of subjects felt that it would eventually become a mainstream surgical approach. When interest in becoming trained in

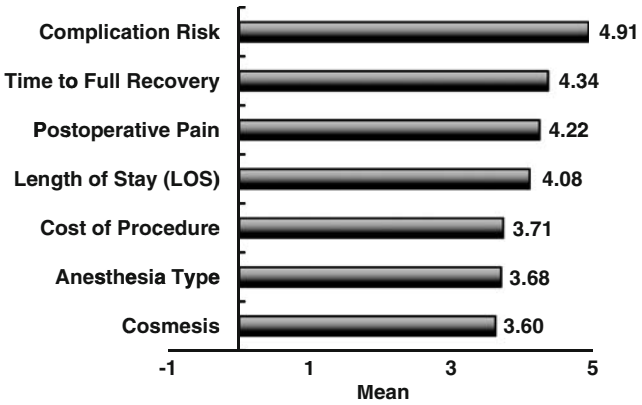

Figure 1 Surgeon considerations *1* unimportant, *2* somewhat unimportant, *3* neither important nor unimportant, *4* somewhat important, *5* important. Complication risk, time to full recovery, postoperative pain, and LOS were each significantly more important than anesthesia type, procedure cost, or cosmesis (Wilcoxon Signed Ranks $p<0.005$).

NOTES was analyzed by society, 81.3% ($p<0.001$) of SAGES members indicated that they were interested. Although a majority of SSAT members (70.5%) and ACS members (71.5%) were interested in NOTES training, this was not statistically significant. In addition, 71.9% of SSAT and 69.3% of ACS members responding this way were also members of SAGES (Table 2). Data analysis using a forward stepwise logistic regression of physician characteristics that were found to be significant during chi-square analysis was performed to avoid confounding errors due to overlapping characteristics. This showed that age less than 60, minimally invasive surgery (MIS) specialization and SAGES membership correlated

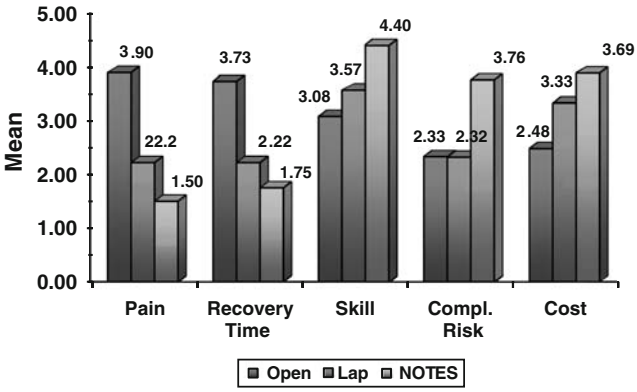

Figure 2 Procedure perceptions *0* none, *1* very low, *2* low, *3* moderate, *4* high, *5* highest. NOTES was perceived to be associated with less pain and shorter recovery, while requiring greater skill and having increased costs and risk of complications when compared independently to open and laparoscopic surgery (Wilcoxon Signed Ranks $p<0.001$).

Table 2 Interest in Becoming Trained in NOTES by Society Membership and MIS Specialization

Society/MIS specialty	% NOTES interest	% SAGES members (among those with NOTES interest)	% MIS specialization (among those with NOTES interest)
SAGES	81.3% ($p<0.001$) ($N=165$)	100% ($N=165$)	35.3% ($N=65$)
SSAT	70.5% ($N=167$)	71.9% ($N=120$)	28.7% ($N=48$)
ACS	71.5% ($N=218$)	69.3% ($N=151$)	28.9% ($N=63$)
MIS specialization	90.1% ($p<0.001$; $N=73$)	89.0% ($N=65$)	100% ($N=73$)

significantly with increased interest in NOTES training while the performance of flexible endoscopy in less than 10% of their practices was predictive of decreased interest (Table 3).

In addition, when surgeons were asked the question: "Assuming NOTES was feasible, available in your hospital, and that you were trained to operate in this fashion, would you choose to perform NOTES rather than laparoscopy as the preferred surgical approach for cholecystectomy?," 44% of those surveyed answered affirmatively. If the complication rate for NOTES was slightly higher (2% vs. 1%) compared to laparoscopy, 61% of these surgeons would still prefer NOTES while only 3% would still prefer NOTES if the complication rate was significantly higher (10% vs. 1%). Surgeons choosing NOTES over laparoscopic cholecystectomy would also be less likely to do so if they had to travel farther to perform the procedure, with 76% willing to travel to another hospital in the same city to perform the procedure and only 41% and 13% willing to still perform the procedure if they had to travel 25 and 100 miles, respectively. Among the 56% of surgeons who would not prefer to perform cholecystectomy by NOTES, 88% indicated that they would change to a NOTES approach if data demonstrated improved outcomes vs. laparoscopy (Table 4). However, when surgeons were asked whether they would choose to personally undergo NOTES cholecystectomy if it were currently available, only 26% of surgeons opted for NOTES over laparoscopy, with most of these individuals citing that it was too new and more risky (Fig. 3, Table 5).

Table 3 Surgeon Characteristics Correlating with Interest in Becoming Trained in NOTES

Variable	Odds ratio	*P* value[a]	95% C.I.	
			Lower	Upper
Age<60	6.56	<0.01	3.30	13.05
MIS specialty	2.57	<0.03	1.11	5.92
SAGES membership	2.11	<0.01	1.23	5.92
Less than 10% endoscopy	0.44	<0.01	0.24	0.81

[a] Analysis using forward stepwise logistic regression demonstrated significantly increased interest in NOTES with age less than 60 and MIS specialization and decreased NOTES interest with low endoscopy practice volume

Discussion

This is the first study examining the perceptions of surgeons at large regarding Natural Orifice Translumenal Endoscopic Surgery, providing valuable insight into surgeon interest in NOTES and allowing identification of potential barriers to its adoption. When the data collected in this study are compared to some of the findings from our earlier survey of patient opinions, a number of similarities and differences are apparent. Most notably, the considerations of greatest importance to surgeons when considering a surgical approach are the same as those for patients when deciding which surgical procedure to undergo. Both groups felt that the risk of suffering a complication due to surgery was the most important consideration, followed by time to full recovery, postoperative pain, and LOS, in that order. In addition, these four considerations were each judged to be significantly more important than procedure cost, anesthesia type, or cosm-

Table 4 Surgeon Interest in NOTES

Interested in becoming trained in NOTES	72%
Believe NOTES will eventually become a mainstream procedure	47%
Would prefer to perform cholecystectomy by NOTES if it was feasible and safe	44%
Among surgeons not preferring to perform NOTES cholecystectomy, would change to NOTES if data showed improved outcomes	88%

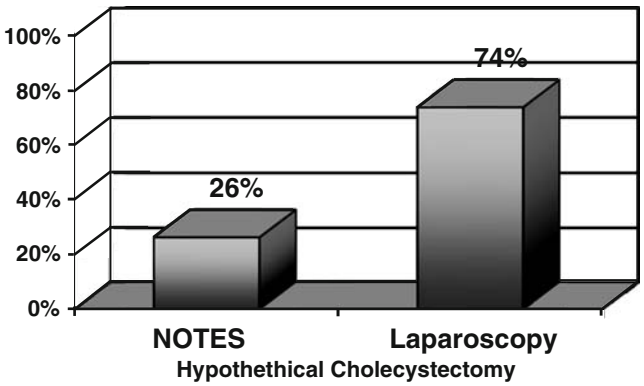

Figure 3 Percentage of surgeons who would choose to personally undergo NOTES cholecystectomy.

esis. As was found for patients, cosmesis was the least important concern for surgeons.

Surgeon perceptions were also similar to our earlier findings for patients in that they believed NOTES would be associated with less pain and shorter recovery time, but would require greater skill than either laparoscopy or open surgical procedures. In contrast to patients, who equate a NOTES approach with less risk and cost, surgeons believe NOTES carries greater risks and costs than laparoscopic or open procedures. This may explain why only 26% of surgeons would be willing to personally undergo NOTES vs. laparoscopic cholecystectomy and may be further supported by the fact that among surgeons opting not to undergo NOTES 79% felt it was too risky and another 70% felt it was too new. This finding is similar to findings by Windsor et al. that the "absence of long-term results" and potential complications were major factors in the slow introduction and adoption of laparoscopic inguinal hernia.[9] A frequently voiced concern among surgeons completing the survey was the risk of leakage from the enterotomy necessary for access to the abdominal cavity. Given how important the risk of a surgical complication is to both surgeons and patients, it is likely that the differences in the perceived risk of NOTES accounts for some of the difference between patient and surgeon preference to personally undergo a NOTES procedure.

Table 5 Reasons Given by Surgeons for Choosing Not to Personally Undergo NOTES Cholecystectomy

NOTES more risky	79.4%
NOTES too new	70.0%
See no advantage to NOTES	47.9%
Other	18.3% (Enterotomy risk, 66%)
Do not like concept of NOTES	6.2%

While the majority of surgeons would not elect to personally undergo a NOTES cholecystectomy, it is interesting that 72% of those surgeons surveyed would be interested in becoming trained in NOTES, and roughly half of the surgeons surveyed believed that NOTES will eventually become a mainstream surgical procedure. Not surprisingly, younger surgeons, minimally invasive surgeons, and SAGES members displayed greater interest in NOTES. Interestingly, Escarce et al. likewise demonstrated that surgeons 30–40 years of age adopted laparoscopic cholecystectomy earlier than older surgeons.[10] It is possible that surgeons, particularly those with a vested interest in minimally invasive surgery and endoscopy, would want to become trained in NOTES in the event that it becomes a commonly accepted minimally invasive surgical approach with better outcomes than laparoscopy. This is echoed by the fact that although only 44% of surgeons would chose to perform a NOTES cholecystectomy even if it was judged to be feasible and safe, 88% of these surgeons would switch to the NOTES approach if it demonstrated improved outcomes versus laparoscopy. A study examining adoption of laparoscopic cholecystectomy likewise showed "[m]ore than three fourths of adopters identified the desire to keep up with the state-of-the-art and improved patient outcomes as very or extremely important reasons for adoption".[11] Furthermore, informal discussions suggest that some surgeons feel they did not adopt laparoscopy early enough in its development and so not want to "miss the boat" if NOTES becomes mainstream.

There are several limitations and potential biases with our study that need to be discussed. The low response rate of the survey is readily apparent. Using direct email rather than utilizing electronic newsletters may have improved the response rate from SAGES and ACS members, as 9.1% of SSAT members who were directly emailed followed the link to the survey. Only completed surveys were included in the study and the length of this broad, opinion survey may have further contributed to the low response rate. Our surgeon demographic may also not have been representative of the entire general surgery cohort, with a selection bias toward academic surgeons and/or those interested in minimally invasive surgery. This is represented in the fact that 57% and 66% of surgeons were members of SAGES and SSAT, respectively. Furthermore, over 60% of the SSAT and ACS members who responded to the study were also members of SAGES. Sampling the entire cohort of surgeons may or may not demonstrate greater skepticism toward NOTES. Interestingly, although one would expect academic surgeons to be early adopters of new surgical technology, Escarce et al. showed that

surgeons with a full-time faculty appointment were slower to adopt laparoscopic cholecystectomy than private practice surgeons.[10]

The language used in the survey may also bias the results of our study. The survey posited that "appropriate instrumentation" for NOTES is available and that "you or another physician…is fully trained and credentialed to perform a NOTES cholecystectomy". Such instrumentation, training, and credentialing issues have not been thoroughly addressed thus far in the early stages of NOTES, so our results may not apply to the present status of the practice. At the very least, our results give us a glimpse into the decision making process of surgeons on the adoption of new surgical technologies.

Conclusion

Although only one fourth of surgeons would currently choose to undergo a NOTES cholecystectomy themselves and 53% thought it would not become a mainstream approach, a large majority would be interested in becoming trained in NOTES if it were clinically available and easily accessible. Interest in NOTES is affected by a surgeon's age, SAGES membership, and specialization in minimally invasive surgery, as well as flexible endoscopic volume, with younger age, and increased volume of minimally invasive and flexible endoscopic procedures being predictive of increased interest. The results of this study also demonstrate that the risk of a procedure-related complication is the most important concern for surgeons contemplating NOTES. This sentiment echoes our earlier patient survey which suggested that the majority of patients would prefer to undergo NOTES cholecystectomy as long as their surgeon was well trained, and the risks of the procedure were not significantly greater than for laparoscopic cholecystectomy. These findings suggest that the acceptance by surgeons of NOTES will be contingent upon evidence of its safety and the findings of this preliminary study may serve as a framework for more focused studies in the future.

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Appendix 1

Physician Survey of Natural Orifice Translumenal Endoscopic Surgery (NOTES)

Your assistance is requested for a survey on NOTES (Natural Orifice Translumenal Endoscopic Surgery) that is a new approach proposed for common surgical procedures. NOTES is a hybrid procedure that combines elements of laparoscopic surgery and flexible endoscopy. In NOTES procedures, a flexible endoscope (inserted orally or rectally) is used to make an incision in the stomach or colon and then passed through this incision into an insufflated abdomen. The intent is to perform surgery inside of the abdomen that typically requires a traditional, open, or laparoscopic approach. Your responses will be helpful in evaluating surgeons' opinions of this new concept.

For the purposes of this survey, we are asking you to imagine a patient with gallstones that have been causing pain for several months. You counsel this otherwise healthy patient to have a cholecystectomy and inform the person that the procedure can be done by two possible methods. The first is the standard laparoscopic approach which is performed in the operating room under general anesthesia and takes about an hour to perform. There is an approximately 1% incidence of major complications associated with laparoscopic cholecystectomy. Now, assume that the appropriate instrumentation is available at your hospital and that you or another physician at your institution is fully trained and credentialed to perform a NOTES cholecystectomy. The NOTES approach currently involves general anesthesia. Once anesthetized, a special operating endoscope would be inserted into the patient's stomach. The stomach would be insufflated with carbon dioxide gas, and an incision created to allow the scope to be advanced into the abdominal cavity which also would be insufflated with carbon dioxide. The scope would have appropriate instruments for grasping, applying clips and/or ties and dissecting the gallbladder off the liver as in the laparoscopic procedure. The gallbladder would then be placed inside a specimen bag and pulled back into the stomach. The gastrotomy would be closed from the inside with full thickness sutures or the equivalent. The scope and gallbladder would subsequently be removed and the patient awakened. In this scenario, there would be no skin incisions or dressings and the patient would be discharged home the same day.

Please answer the following questions considering this information. This multiple choice survey takes approximately 5–10 min to complete and there are no right or wrong answers—we are simply interested in your honest opinions on this new technology. In order to finish the survey, a response is required for each question and respondents that return to an incomplete survey will be taken to the point where they left off. Study participation is voluntary and anonymous and concludes with completion of the survey. Thank you for your time.

Appendix 2

Background: (check the appropriate box or fill in the blank)

Your age: _____

Your sex: □ Male □ Female

Employment: □ Employed □ Retired or Unemployed

Do you perform any of the following procedures?
- Flexible endoscopy □ Yes □ No
- Open surgery (other than minor surgical procedures) □ Yes □ No
- Laparoscopic surgery □ Yes □ No

Have you completed residency training in a general surgery program? □ Yes □ No

Are you currently a general surgery resident? □ Yes □ No

Are you currently in a surgical fellowship? □ Yes □ No

How many years ago did you start your practice (upon completion of residency/fellowship)?
□ ≤ 5 □ 6-10 □ 11-20 □ 21-30 □ > 30

If you are a surgical specialist or subspecialist (or are training to become one), what is your specialty?
□ Colorectal □ Hepatobiliary □ Vascular □ Cardiothoracic
□ Minimally Invasive Surgery □ Surgical Oncology □ Trauma □ Pediatric Surgery
□ Gastrointestinal □ Transplant □ Critical Care □ Plastics
□ Other _____

Were you familiar with NOTES before this study? □ Yes □ No

Were you familiar with laparoscopic surgery before this study? □ Yes □ No

Are minimally invasive approaches currently available for the surgeries you commonly perform(ed)? □ Yes □ No

What percent of your procedures do (or did) you perform via a minimally invasive approach?
□ 0% □ < 10% □ 10-25% □ 26-50% □ 51-75% □ > 75%

What percent of your procedures do (or did) you perform laparoscopically?
□ 0% □ < 10% □ 10-25% □ 26-50% □ 51-75% □ > 75%

Are you comfortable performing basic flexible endoscopy? □ Yes □ No

Are you comfortable performing advanced flexible endoscopy?
(e.g. stents, ablations, mucosectomies, etc) □ Yes □ No

Do you regularly perform flexible endoscopy? □ Yes □ No

What percent of the procedures you perform involve flexible endoscopy?
□ 0% □ < 10% □ 10-25% □ 26-50% □ 51-75% □ > 75%

Would you be interested in becoming trained to perform NOTES procedures? □ Yes □ No

Do you foresee NOTES becoming a mainstream approach for abdominal operations? □ Yes □ No

How long do you think it will be until NOTES becomes a mainstream approach for abdominal operations?
□ 1 year □ 3 years □ 6 years □ 10 years □ Never

When considering the choice of a surgical approach, rate the following characteristics: (circle the corresponding number)

	Important	Somewhat Important	Neither Important nor Unimportant	Somewhat Unimportant	Unimportant
- Cost of the procedure	5	4	3	2	1
- Risk of having a complication	5	4	3	2	1
- Length of hospital stay	5	4	3	2	1
- Type of anesthesia (sedation vs. general)	5	4	3	2	1
- Cosmetic result	5	4	3	2	1
- Amount of postoperative pain	5	4	3	2	1
- Time it takes to return to full activity	5	4	3	2	1

Based upon the description in the introduction or on your own knowledge and understanding of NOTES, please indicate your estimation of the following characteristics of the procedure: (circle the corresponding number)

	None	Very Low	Low	Moderate	High	Highest
- Amount of postoperative pain	0	1	2	3	4	5
- Cost of the procedure	0	1	2	3	4	5
- Risk of having a complication	0	1	2	3	4	5
- Degree of skill needed by the surgeon	0	1	2	3	4	5
	(Zero)	(Very Short)	(Short)	(Moderate)	(Long)	(Longest)
- Time it takes to return to full activity levels	0	1	2	3	4	5

Based upon the information provided to you in the introduction or on your own knowledge and understanding of laparoscopic surgery, please indicate your perception of the following characteristics: (circle the corresponding number)

	None	Very Low	Low	Moderate	High	Highest
- Amount of postoperative pain	0	1	2	3	4	5
- Cost of the procedure	0	1	2	3	4	5
- Risk of having a complication	0	1	2	3	4	5
- Degree of skill needed by the surgeon	0	1	2	3	4	5
	(Zero)	(Very Short)	(Short)	(Moderate)	(Long)	(Longest)
- Time it takes to return to full activity levels	0	1	2	3	4	5

Based upon your own knowledge and understanding of traditional "open" surgery, please indicate your perception of the following characteristics: (circle the corresponding number)

	None	Very Low	Low	Moderate	High	Highest
- Amount of postoperative pain	0	1	2	3	4	5
- Cost of the procedure	0	1	2	3	4	5
- Risk of having a complication	0	1	2	3	4	5
- Degree of skill needed by the surgeon	0	1	2	3	4	5
	(Zero)	(Very Short)	(Short)	(Moderate)	(Long)	(Longest)
- Time it takes to return to full activity levels	0	1	2	3	4	5

NOTES vs. Laparoscopic Surgery:

1.) Who do you think the laparoscopic approach might be best for? (select ONLY one)

□ Infants □ Children □ Adults □ Elderly adults □ Anyone □ No one

2.) Who do you think the NOTES approach might be best for? (select ONLY one)

□ Infants □ Children □ Adults □ Elderly adults □ Anyone □ No one

3.) Assuming NOTES was feasible, available in your hospital, and that you were trained to operate in this fashion, would you choose to perform NOTES rather than laparoscopy as the preferred surgical approach for cholecystectomy?

□ Yes □ No

3a.) If you answered No to question #3, would you refer to another provider who was skilled and experienced in NOTES? □ Yes □ No

3b.) If you answered No to question #3, would you change your mind if:

- Other surgeons in your city were marketing NOTES approaches? □ Yes □ No

- Published data showed improved outcomes? □ Yes □ No

- Your volumes of laparoscopic and open procedures started to significantly decrease? □ Yes □ No

3c.) If you answered Yes to question #3, would you still use it as your approach of choice for cholecystectomy if: (check the appropriate box for EACH yes/no question)

I.) The complication rate was

a.) slightly higher (2% vs. 1%)? □ Yes □ No

b.) significantly higher (10% vs. 1%)? □ Yes □ No

II.) The patient had to pay

a.) slightly more (<$100)? □ Yes □ No

b.) significantly more ($100 - $1,000)? □ Yes □ No

c.) out of pocket ($12,000)? □ Yes □ No

IV.) If you had to go to another hospital

a.) in your town? □ Yes □ No

b.) 25 miles away? □ Yes □ No

c.) 100 miles away? □ Yes □ No

d.) 500 miles away? □ Yes □ No

4.) If you or a family member were the one who needed gallbladder surgery and your surgeon was fully qualified to do either approach (NOTES or laparoscopy), which one would you prefer? (select ONLY one)

□ Laparoscopic

□ NOTES

4a.) If you chose the laparoscopic approach, why did you choose laparoscopy? [Answer *only if* you chose *laparoscopic* for question #4. Check the appropriate box(es)]

- NOTES is too new □ Yes □ No
- I see no advantage to NOTES over laparoscopic surgery □ Yes □ No
- NOTES sounds more risky than laparoscopic surgery □ Yes □ No
- NOTES sounds more painful than laparoscopic surgery □ Yes □ No
- Recovery time sounds longer for NOTES □ Yes □ No
- I don't like the thought of something being removed from my mouth or anus. □ Yes □ No
- Other: (explain) _____

4B.) If you chose NOTES, why did you choose this approach? [Answer *only if* you chose *NOTES* for question #4. Check the appropriate box(es)]

- NOTES avoids incisions in the abdominal wall and leaves no scars on the skin □ Yes □ No
- NOTES sounds less risky than laparoscopic surgery □ Yes □ No
- NOTES sounds less painful than laparoscopic surgery □ Yes □ No
- Recovery time sounds shorter for NOTES □ Yes □ No
- Other: (explain) _____

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ORIGINAL ARTICLE

ERCC1 and XRCC1 Gene Polymorphisms Predict Response to Neoadjuvant Radiochemotherapy in Esophageal Cancer

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Abstract
Introduction Neoadjuvant treatment strategies have been developed to improve survival of patients with locally advanced esophageal cancer. Since only patients with major histopathological response benefit from this therapy, predictive markers are needed. We examined a panel of selected gene polymorphisms to predict response to neoadjuvant radiochemotherapy (cisplatin, 5-fluorouracil, 36 Gy) in esophageal cancer patients.
Materials and method Genomic DNA was extracted from paraffin-embedded tissues of 52 patients. Allelic genotyping was performed by real-time polymerase chain reaction using allele-specific TaqMan probes and correlated with therapy response.
Results Single-nucleotide polymorphism *ERCC1 C118T* was predictive for therapy response ($p<0.003$). Within the TT genotype group of 25 patients, 20 (80%) did not respond to chemoradiation. Of 20 patients with heterogeneous C/T genotype, 14 (70%) were major responders. The CC genotype (seven patients) was not of predictive importance. ERCC1 polymorphism was significantly ($p<0.02$) associated with formation of lymph node metastases. Predominant GG genotype of XRCC1 *A194G* was not predictive; however, the rarely occurring AA genotype was response-associated and the A/G variant was associated with nonresponse. Fifteen additionally analyzed polymorphisms did not show any correlation.
Conclusion Our data support the role of ERCC1 as a predictive marker for therapy response. Single-nucleotide polymorphisms of ERCC1 and XRCC1 could be applied to further individualize treatment strategies.

Keywords Single-nucleotide polymorphism · Nucleotide excision repair · Multimodality treatment · Chemo-radio-sensitivity · Response prediction

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Introduction

Esophageal squamous cell and adenocarcinoma are common malignancies worldwide.[1] Patients with locally advanced esophageal cancer have a dismal prognosis despite complete surgical resection.[2] This fact prompted many investigators to apply neoadjuvant treatment strategies in an effort to improve survival.[3,4] Several meta-analyses of randomized trials have shown encouraging results; however, they revealed that only patients with major histopathologic response clearly benefited from treatment.[5–8] Further evaluation following neoadjuvant chemoradiation has shown that major histomorphologic regression, i.e., <10% vital residual tumor cells or complete pathologic response, is one of the most significant prognostic factors.[9] In addition, neoadjuvant therapies are expensive and may lead to therapy-associated complications.[7] Accordingly, the development of validated predictive and prognostic markers

may not only be helpful in identifying patients who are at high risk, but they will also be critical in selecting more efficient treatment strategies with the means of tailoring a targeted and effective therapy to the molecular profile of the patient while minimizing life-threatening toxicities.

The cancer genomics research on single-nucleotide polymorphism (SNP) variation as well as recent release of the completed initial phase of a haplotype map of the human genome provides an opportunity for the discovery and analysis of cancer-promoting genes and the detection and validation of molecular markers of prognosis and response prediction. Glinsky[10] and Mei et al.[11] revealed that cancer therapy outcome predictor genes manifest a common feature of SNP patterns reflected in population-specific profiles of SNP genotype and allele frequencies. Hu et al.[12] performed genome-wide detection of chromosomal changes using a single-nucleotide polymorphism array. The science of pharmacogenomics is emerging as a useful molecular tool to investigate the disparity in drug efficacy.

Common polymorphisms in DNA repair genes may alter individual's capacity to repair damaged DNA; deficits in repair capacity may lead to genetic instability affecting carcinogenesis and therapy response.[13,14] Depending on their location, SNPs may cause disease or contribute to the risk for a disease and may help us discover new ways to diagnose, treat, and even prevent disease.[15]

We analyzed allelic variations of candidate genes which have been specified as predictive for response to multimodality treatment in former studies of different carcinomas for their impact to predict response to our therapy regimen of esophageal cancer.

Materials and Methods

Study Population, Demographic Data, and Neoadjuvant Therapy

Patients with locally advanced resectable esophageal cancer (cT2–4, Nx, M_0) were selected from a recently reported prospective observation trial investigating neoadjuvant radiochemotherapy for esophageal cancer followed by surgery.[9] None of the patients had had prior radiotherapy and /or chemotherapy. Briefly, cisplatin (20 mg/m^2 per day) was administered as a short-term infusion on days 1–5 and 5-fluorouracil (5-FU; 1,000 mg/m^2 per day) as a continuous infusion over 24 h on days 1–5. Radiation was delivered in daily fractions of 1.8 Gy to a total dose of 36 Gy using a multiple-field technique. Standardized transthoracic en bloc esophagectomy with two-field lymphadenectomy was performed 4–5 weeks after completion of chemoradiation. Postresectional tissue samples from 52 patients (median age 59 years, range 38 to 73) were available for this study. Clinical data are summarized in Table 1. Informed consent was obtained from each patient and the scientific protocol was approved by the local ethics committee.

Histopathologic Response Classification

The degree of histomorphologic regression was classified into four categories: grade I: >50% vital residual tumor cells (VRTC), grade II: 10–50% VRTC, grade III: nearly complete response with <10% VRTC, and grade IV: complete response.[16,17] This analysis was performed by two independent staff pathologists who were blinded for all

Table 1 Clinical and Histopathological Parameters and ERCC1 C118T (rs11615) Genotype Distribution for 52 Esophageal Cancer Patients

Parameter	*N*=52 (%)	TT [%]	CC [%]	C/T [%]	*P* value
Gender					
Male	43 (82.7)	47	17	37	ns
Female	9 (17.3)	56	0	44	
Histology					
Squamous cell					
Carcinoma	31 (59.6)	36	19	45	ns
Adenocarcinoma	21 (40.4)	67	5	28	
ypN-category[a]					
N0	23 (44.2)	29	8	63	0.004
N1	28 (53.8)	64	18	18	
Regression grade[b]					
Major response	22 (42.3)	23	13	64	0.003
Minor response	30 (57.7)	67	13	20	
ypM-category					
ypM0	47 (90)	45	15	40	ns
ypM1	5 (10)	80	0	20	

Distribution of genotype in percentage

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[a] Histopathological lymph node category after neoadjuvant therapy according to UICC

[b] Minor response: ≥10% vital residual tumor cells, major response <10% VRTC

other clinical data (S.E.B. and H.P.D.). Due to prognostic implications, regression grades III and IV were classified as major histomorphologic response compared to grades I and II constituting minor histopathologic response. Histopathologic tumor regression is the most significant independent prognostic indicator.[9] Tissue samples were chosen based on histopathologic response classification. Thirty were classified as minor and 22 as major histopathologic responders.

Selection of Potentially Predictive Gene Polymorphisms

To select candidate SNPs for response prediction, a systematic review based on MEDLINE database was performed. Selection criteria were either association with clinical or histopathologic tumor response or prognosis to multimodality treatment. Seventeen candidate SNPs were identified and included in our study (Table 2).[18–28]

Allelic Discrimination by TaqMan SNP Genotyping Assays

Paraffin-embedded tissues from resection boundaries containing exclusively normal cells were collected and genomic DNA was extracted using the QIAamp kit (Qiagen, Hilden, Germany).

Genomic DNA was directly used as template for detection of single-nucleotide polymorphisms by real-time PCR by TaqMan 7900HT (Applied Biosystems, Darmstadt, Germany). SNP analysis involves the discrimination between single-nucleotide changes by two allele-specific probes labeled with different fluorophores. Homozygous genotype (x) was detected by VIC 5'allele and homozygous genotype (y) by Fam 5' allele, whereas heterozygous genotype (x/y) was visualized by detection of both fluorescent signals.

Amplification mixtures contained 10-ng genomic DNA from paraffin-embedded tissues, 200 μM dNTPs and 900 nM primer. Primer und probes were purchased from Applied Biosystems, Darmstadt, Germany. Assay identification numbers are listed in Table 3 (online only). PCR conditions were as follows: initial denaturation for 10 min at 95°C, followed by 40 cycles of 15 s 92°C and 60 s 60°C. By quantification of the distinct allele/marker fluorescence signal contributions, the allelic content of each sample was determined by multicomponent algorithm yielding three allelic clusters representing the genotypic constituents: allele x homozygous, allele y homozygous, as well as heterozygous genotype (Fig. 1).

Statistical Analysis

Univariate analysis SNP data were analyzed with nonparametric statistical methods. Chi-squared test and, if neces-

Table 2 SNPs with Putative Predictive or Prognostic Impact for Different Cancer

Gene	Rs	Cancer	Response	Prognosis
Akt1	rs4375597			Glinsky[10]
c-erbB-2 (HER-2/neu)	rs1801200	Breast		Cox et al.[18]
ERCC1	rs3212986	Colon rectum		Moreno et al.[19]
	rs11615	Colon rectum		Zhou et al.[20]
FGFR4	rs351855	Esophagus	Wu et al.[21]	Gordon[22]
GSTP1	rs1695	Esophagus		Wu et al.[21]
	rs1138272			
MDR1	rs1045642	Esophagus	Wu et al.[21]	
MGMT	rs12917	Colon rectum		Moreno[19]
MTHFR	rs1801131	Esophagus		Wu et al.[21]
		Rectum		Terrazzino et al.[23]
	rs1801133	Lung	Takehara et al.[27]	
TERT	rs6882077			Glinsky[10]
TS	rs699517	Rectum	Terrazzino et al.[23]	
		Esophagus		Dong et al.[24]
	rs2790	Colon rectum		Morganti et al.[25]
		Colon rectum		Marcuello et al.[26]
		Lung		Takehara et al.[27]
XRCC1	rs25487	Esophagus	Wu et al.[21]	Wu et al.[21]
		Colon rectum		Moreno et al.[19]
		Cervix	Chung et al.[28]	
	rs1799782	Cervix		
XRCC3	rs861539	Colon rectum		Moreno[19]

Akt1 v-akt murine thymoma viral oncogene homolog 1, *c-erbB-2* erythroblastic leukemia viral oncogene homolog 2, synonyme: HER-2/neu, *ERCC1* excision repair cross-complementing 1, *FGFR4* fibroblast growth factor receptor 4, *TS* thymidylate synthetase, *EGFR*, epidermal growth factor receptor, *GSTP1* glutathione S-transferase p1, *MDR1* multidrug resistance 1, *MGMT* methylguanine-DNA methyltransferase, *MTHFR* methylenetetrahydrofolate reductase, *TERT* telomerase reverse transcriptase, *XRCC1*, 3, X-ray repair complementing defective repair

Table 3 Description of Analyzed SNP Genotyping Assays

Gene/SNP assay number	SNP ID/AA change	Cytoband	SNP bases	Mutation	Function
AKT1 C_26352820	rs4375597 None	14q32.33b	C/T	Intron	Serine–threonine protein kinase, survival factor
C-ERBB-2 C_7452451	rs1801200 I655V	17q12c	A/G	Missense	Growth factor receptor, tyrosine kinase
ERCC1 C_2532948	rs3212986 C8092A	19q13.32a	A/C	Missense	Excision repair complementing factor 1, nucleotide excision repair
ERCC1	rs11615	19q13.32a	C/T	Silent	
C_2532959	N118N				
FGFR4 C_3166614	rs351855 R388G	5q35.2d	A/G	Intron	Fibroblast growth factor receptor 4
GSTP1 C_3237198	rs1695 I105V	11q13.2a	G/A	Missense	Glutathionine *S*-transferase p1, detoxification of platinum agents
GSTP1	rs1138272	11q13.2a	C/T	Missense	
C_1049615	A114V				
MDR1 C_7586657	rs1045642 None	7q21.12a	A/G	Intergenic	Multidrug resistance, encoding P-glycoprotein
MGMT C_3157955	rs12917 None	10q26.3b	C/T	Intergenic	DNA repair
MTHFR C_850486	rs1801131 None	1p36.22a	G/T	Open reading frame	Methylentetrahydrofolate reductase increases amount of folate, enhancing action of 5FU
MTHFR	rs1801133	1p36.22a	G/A	Intergenic	
C_1202883	None				
TERT C_31881542	rs6882077 None	5p15.33d	A/G	Intergenic	Telomerase (ribonucleoprotein polymerase)
TS C_7486263	rs2790 None	18p11.32c	A/G	UTR3	DNA synthesis, 5-FU metabolism
TS	rs699517	18p11.32c	C/T	UTR3	
C_7486269	None				
XRCC1 C_622564	rs25487 Q399R	19q13.31a	C/T	Missense	X-ray repair complementing defective repair, base excision repair
XRCC1	rs1799782	19q13.31a	A/G	Missense	
C_11463404	R194W				
XRCC3 C_8901525	rs861539 None	14q32.33a	A/G	Missense	X-ray repair complementing, double-strand-break repair

A adenin, *C* cytosine, *G* guanine, *T* thymidine, *SNP* single-nucleotide polymorphism, *ID* identification, *AA* amino acid, *UTR* untranslated region

sary, Fisher's exact test were used to examine the difference between gene polymorphisms of samples of patients with major or minor response. *P* values less than 0.05 were classified as significant.

Multivariate analysis In addition binomial logistic regression methods were used to determine the strength of influence of the selected SNPs upon response prediction.

Survival analysis The median follow-up time of all patients was calculated using the time between study entry and the date of the procedure and the time between study entry and the date of censoring for censored patients.[29] The median follow-up time of the patients was 6.1 years (range 3.1–10.2 years). All living patients had follow-up of more than 2 years.

Kaplan–Meier plots were used to describe survival distribution.[30] The log-rank test was applied to evaluate for survival differences.[31] In addition, 95% confidence intervals (95% CI) for the different survival curves were calculated. Postoperative mortality was not included in the calculation of prognosis. The 30-day postoperative mortality was 3.0%. The multivariate analysis of survival used Cox regression analysis to identify independent prognostic variables. The level of significance was set to $p<0.05$.

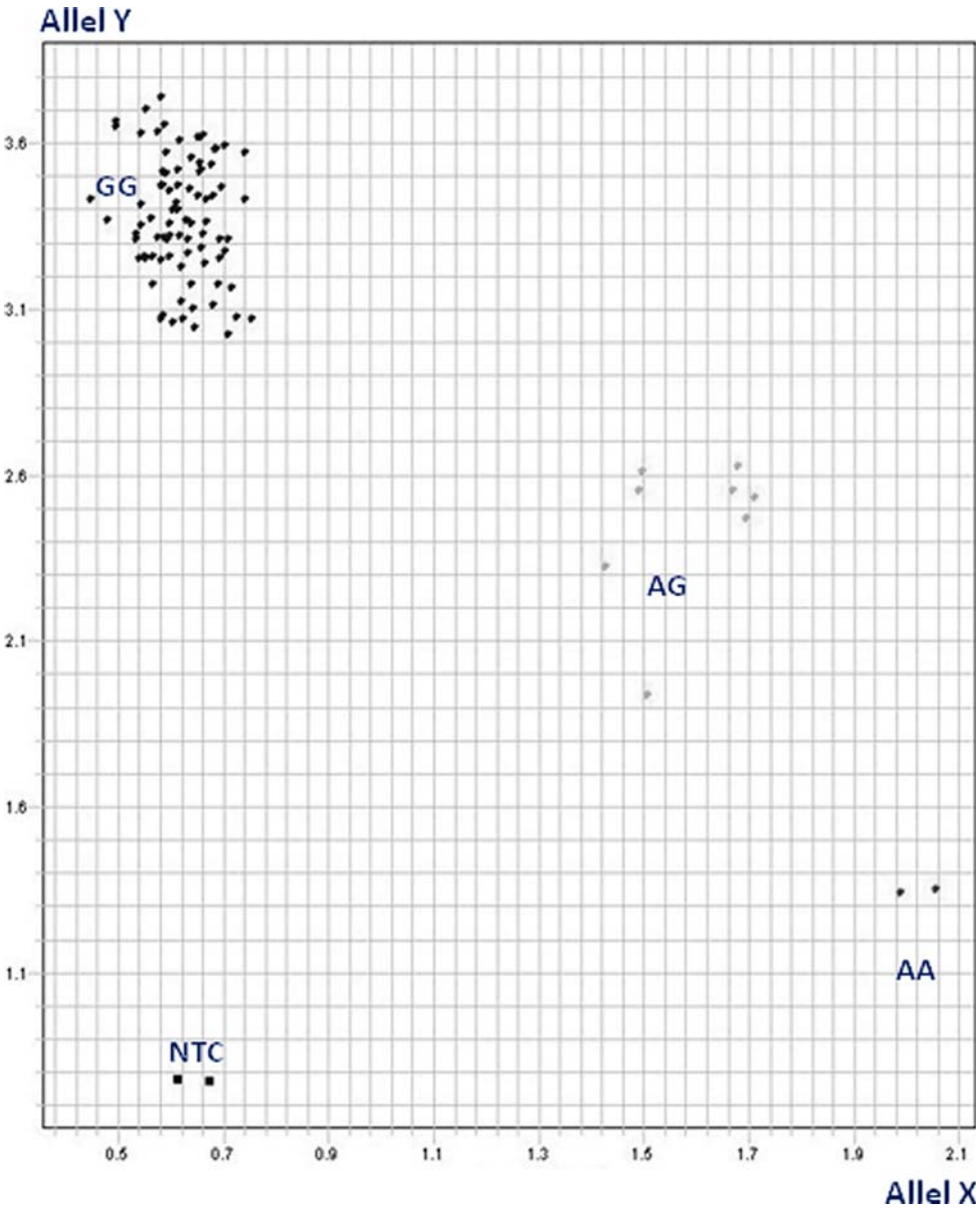

Figure 1 Allelic discrimination plot XRCC1 SNP rs1799782. Discrimination between the three XRCC1 genotypes AA, A/G, and GG based on TaqMan quantification; *NTC*: nontemplate control.

Results

Single-Nucleotide Polymorphisms and Therapy Response

In the present study, 52 patients with esophageal cancer were genotyped for 17 single-nucleotide polymorphisms of 12 different genes by real-time quantitative TaqMan SNP genotyping assays, listed in Table 3. The pattern of allelic variations was examined for association with response to neoadjuvant radiochemotherapy. Fifteen SNPs did not show any association with therapy response (Table 4).

XRCC1 A194G (rs1799782) and Therapy Response

Forty-five patients (87%) revealed homozygous GG genotype for XRCC1 with similar distribution of responding and nonresponding patients (47% and 53%). Only one patient, a responder, was identified with homozygous AA genotype. Six patients revealed the heterozygous genotype and they all did not respond to neoadjuvant radiochemotherapy. This correlation of XRCC1 SNP rs1799782 with major or minor histopathological response was significant ($p<0.05$). Data are shown in Table 5. SNP analysis could be applied to prevent six patients (11%) from noneffective treatment.

ERCC1 C118T (rs11615) and Therapy Response

ERCC1 SNP rs11615 correlated with response to neoadjuvant therapy as well ($p<0.003$). Twenty-five patients with TT genotype comprised 20 (80%) minor responder and five major responders. Allelic variation CC was not of prognostic importance: three (43%) responders, four (57%)

Table 4 Association of Allelic Genotyping with Therapy Response

Polymorphism	*N*=52 (100%)	Regression grade		*P* value
		Major response [%]	Minor response [%]	
AKT1/rs4375597				nr
CC	0			
TT	52 (100%)	42	58	
CT	0			
C-ERBB-2/rs1801200				ns
AA	32 (62%)	47	53	
GG	4 (8%)	50	50	
AG	16 (30%)	31	69	
ERCC1/rs3212986				ns
AA	2 (4%)	50	50	
CC	35 (67%)	34	66	
AC	15 (29%)	60	40	
ERCC1/rs11615				0.003
TT	25 (48.0%)	20	80	
CC	7 (13.5%)	43	57	
CT	20 (38.5%)	70	30	
FGFR4/rs351855				ns
AA	8 (15%)	37	63	
GG	26 (50%)	42	58	
AG	18 (35%)	44	56	
GSTP1/rs1138272				ns
CC	45 (87%)	44	56	
TT	1 (2%)	0	100	
CT	6 (11%)	33	67	
GSTP1/rs1695				ns
GG	4 (8%)	25	75	
AA	20 (38%)	40	60	
GA	28 (54%)	46	54	
MDR1/rs1045642				ns
AA	13 (25%)	54	46	
GG	12 (23%)	25	75	
AG	27 (52%)	44	56	
MGMT/rs12917				ns
CC	43 (83%)	42	58	
TT	2 (4%)	0	100	
CT	7 (13%)	57	43	
MTHFR/rs1801131				ns
GG	21 (40%)	43	57	
TT	10 (20%)	30	70	
GT	21 (40%)	48	52	
MTHFR/rs1801133				ns
GG	8 (15%)	38	62	
AA	24 (46%)	42	58	
GA	20 (39%)	45	55	
TERT/rs6882077				nr
AA	0			
GG	52 (62%)	42	58	

Table 4 (continued)

Polymorphism	*N*=52 (100%)	Regression grade		*P* value
		Major response [%]	Minor response [%]	
AG	0			
TS/rs2790				ns
AA	36 (69%)	47	53	
GG	2 (4%)	50	50	
AG	14 (27%)	29	71	
TS/rs699517				ns
CC	29 (56%)	55	45	
TT	6 (11%)	33	67	
CT	17 (33%)	23	77	
XRCC1/rs25487				ns
CC	28 (54%)	36	64	
TT	4 (7%)	75	25	
CT	20 (39%)	45	45	
XRCC1/rs1799782				$p<0.05$
AA	1 (2%)	100	0	
GG	45 (87%)	47	53	
AG	6 (11%)	0	100	
XRCC3/rs861539				ns
AA	6 (11%)	33	67	
GG	15 (29%)	47	53	
AG	31 (60%)	42	58	

Minor response: ≥10% vital residual tumor cells, major response <10% VRTC

ns not significant, *nr* not relevant, *A* adenin, *C* cytosine, *G* guanine, *T* thymidine, *SNP* single-nucleotide polymorphism

Table 5 Clinical and Histopathological Parameters and XRCC1 *A194GA* (rs1799782) Genotype Distribution for 52 Esophageal Cancer Patients

Parameter	*N*=52 (%)	AA [%] *N*=1	GG [%] *N*=45	A/G [%] *N*=6	*P* value
Gender					ns
Male	43 (82.7)	2	84	14	
Female	9 (17.3)	0	100	0	
Histology					
Squamous cell					ns
Carcinoma	31 (59.6)	0	87	13	
Adenocarcinoma	21 (40.4)	5	86	0	
ypN-category[a]					ns
N0	23 (44.2)	0	88	12	
N1	28 (53.8)	4	86	10	
Regression grade[b]					<0.05
Major response	22 (42.3)	4	96	0	
Minor Response	30 (57.7)	0	80	20	
ypM-category					ns
ypM0	47 (90)	2	87	11	
ypM1	5 (10)	0	80	20	

Distribution of genotype in percentage

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[a] Histopathological lymph node category after neoadjuvant therapy according to UICC

[b] Minor response: ≥10% vital residual tumor cells, major response <10% VRTC

nonresponders, whereas heterogenous C/T genotype indicated therapy response: 14 patients (70%) with major response and six patients (30%) with minor response, Table 4. Multivariate analysis applying the most relevant genes revealed ERCC1 SNP (CC + TT vs C/T) as an independent variable for therapy response (p=0.007).

Gene Polymorphisms and Clinicopathological Data

Seventeen SNPs have been analyzed in 52 patients with advanced esophageal cancer for association with clinicopathological parameters. Sixteen gene polymorphisms did not show any clinicopathological correlation (data not shown). TT allele of ERCC1 rs11615 was more often present in adenocarcinoma (67% TT) than in squamous cell carcinoma (36% TT). This difference between the two histological types was not significant. Association of ERCC1 SNP rs11615 with formation of lymph node metastases was significant (p<0.02), Table 1. The amount of lymph node metastases was differently distributed among the three genotype groups. The median lower quartile–upper quartile (LQ–UQ) TT=2 (0–3), CC=1 (0–4), and C/T=0 (0–0.8) are visualized in Fig. 2.

Spearmen's coefficient of rank correlation (rho)=−0.38 (95% Cl: −0.59 to −0.12) revealed a correlation between ERCC1 genotype and lymph node metastases (p=0.007).

Gene Polymorphisms and Survival

Patients' survival was directly dependent on response to neoadjuvant radiochemotherapy (Fig. 3a). The response-predictive group with heterozygous C/T polymorphism of ERCC1 had a better 5-year survival (40%) than the groups with the homozygous TT or CC genotypes (16%; Fig. 3b).

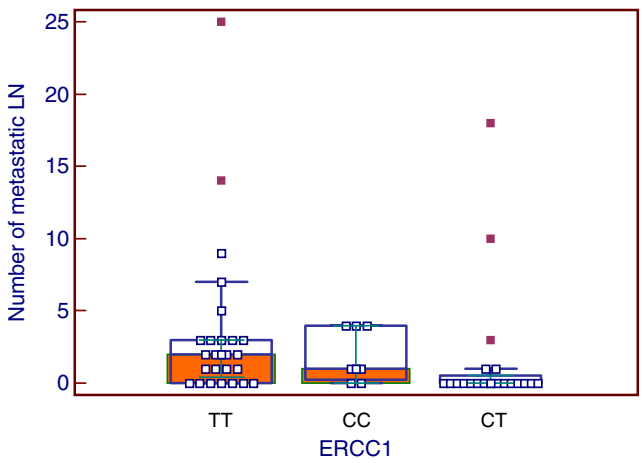

Figure 2 Correlation of ERCC1 single-nucleotide polymorphism with the number of lymph node metastases for each patient (p=0.007). *Box*-and-*whisker* plot (medians, *error bars*: 95% confidence interval for median), ERCC1 genotype: 1, TT; 2, CC; 3, C/T, *LN*: lymph nodes.

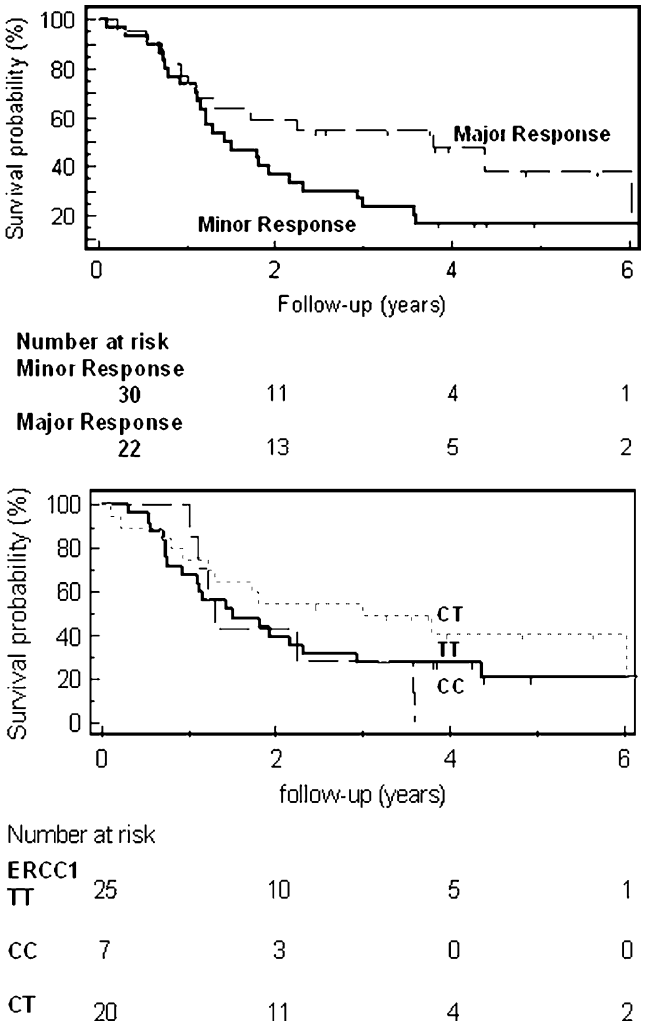

Figure 3 ERCC1 *C118T* single-nucleotide-polymorphism-dependent survival analyses. Kaplan–Meier analyses of (**a**) responding and nonresponding patients after neoadjuvant RTx/CTx, **b** response-predicting C/T genotype group and nonresponse-predicting CC genotype group. Minor response: ≥10% vital residual tumor cells, major response <10% VRTC.

However, this difference in Kaplan–Meier curves was not significant since the response indicating group still contained six (30%) nonresponders in addition to the 14 (70%) major responder, and the group indicating minor response included five (20%) major responders in addition to the 20 (80%) minor responders. Therefore, none of the SNPs analyzed was appropriate for prediction of prognosis.

Discussion

Established diagnostic methods are insufficient[32] to allow tailored multimodality treatment. The present study suggests that single-nucleotide polymorphisms can be used to identify responders and nonresponders to a common 5-

fluorouracil, cisplatin, and radiation-based neoadjuvant therapy.

With detection of ERCC1 polymorphism, we were able to discriminate between response- and nonresponse-predicting genotypes, i.e., 20 patients out of 52 would have been prevented from noneffective neoadjuvant treatment. However, five patients with TT genotype would have missed their chance of a potentially responsive therapy. For this reason, additional markers are needed to further individualize the treatment. XRCC1 proved to be an additional candidate marker for response prediction. The rarely occurring AA and AG genotypes of XRCC1 polymorphism rs1799782 show strong specificity for prediction of response. The only detected AA-genotyped patient was a major responder; all of the six detected AG-bearing patients were minor responders. Since these genotypes occurred with low frequency (2% AA; 11% AG), the response-predictive impact still has to be confirmed by a large number of patients. Probably a combination of predictive SNPs is necessary to improve sensitivity and specificity for response prediction.

ERCC1 is part of the nucleotide excision repair (NER) complex involved in repair of platinum-induced interstrand and intrastrand cross-links.[33–35] Whereas ERCC1 acts on larger lesions covering 20–25 nucleotides, XRCC1 belongs to the base excision repair (BER) system removing small lesions around the damaged base.[14,34] Since efficient DNA repair capacity seems to be a critical mechanism of resistance to platinum drugs,[33] we conclude that combined NER and BER pathways are important for therapy outcome.

There are previous studies describing the predictive impact of XRCC1 *Arg399Gln* polymorphism for therapy response in esophageal, lung, and cervical cancer treated with platinum-based neoadjuvant therapy.[21,36,28] Concerning the polymorphism *A194G* additionally analyzed in this study, there are so far only reports that associate this SNP with decreased risk of cancer reviewed by Goode et al.[14,37,38] The present study is the first one showing an association of XRCC1 *A194G* with response prediction.

ERCC1 *C118* genotype is associated with treatment response in NSCLC,[20,39] ovarian cancer,[40,41] and colorectal cancer.[42–44] Our results are in accordance with these reports.

Results from recent studies show that ERCC1 mRNA expression is predictive for response to neoadjuvant radiochemotherapy in esophageal and gastric cancer.[45–51] The present data indicate that the specific ERCC1 polymorphism rs11615 is an additional parameter to predict therapy response. Although the analyzed ERCC1 polymorphism represents a silent mutation, it seems to play a central role in resistance mechanisms. The single-nucleotide change C to T at codon 118 converts a codon of common usage (AAC) to a less used codon (AAT), both coding for asparagine. This change results in a decreased ERCC1 gene expression, which impairs repair activity.[42] The missense mutation of XRCC1 results in a change of protein structure. Increased expression of ERCC1 and XRCC1 might be related with better DNA repair and a worse response on therapy.[52]

Although we analyzed additional 15 polymorphisms of candidate genes recently discussed in literature, only ERCC1 and XRCC1 SNPs proved to be of prognostic impact. We cannot exclude random association between polymorphisms and therapy response. However, gene expression[45] and protein expression analysis[51] of ERCC1 proved to have predictive impact for therapy response.

This is the first study reporting on significant differences of ERCC1 and XRCC1 polymorphisms (rs11615 and rs1799782) between the responding and nonresponding patients to the commonly applied 5-FU, cisplatin, and radiation-based neoadjuvant therapy in esophageal cancer. This association has to be verified by a larger group of patients and is only predictive for the applied therapy regimen. Our study cohort was based on the therapy modus and included squamous cell and adenocarcinomas. The main goal of our study was to evaluate therapy response. Both histological entities of esophageal cancer are sensitive to neoadjuvant radiochemotherapy. This has been demonstrated by Schneider et al.[9]

Conclusion

In conclusion, the present results support the role of ERCC1and XRCC1 polymorphisms as predictors of response. By analysis of ERCC1 polymorphism rs11615, 20 nonresponding patients out of 52 patients, i.e., 67% of 30 nonresponders, could be prevented from noneffective and potentially harmful therapy. A smaller group of five patients with TT genotype would miss their chance of a potentially responsive treatment. These polymorphisms might be applied for further individualization of neoadjuvant radiochemotherapy in locally advanced esophageal cancer.

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ORIGINAL ARTICLE

A New Technique for Measurement of Pharyngeal pH: Normal Values and Discriminating pH Threshold

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Abstract
Introduction Identifying gastroesophageal reflux disease as the cause of respiratory and laryngeal complaints is difficult and depends largely on the measurements of increased acid exposure in the upper esophagus or ideally the pharynx. The current method of measuring pharyngeal pH environment is inaccurate and problematic due to artifacts. A newly designed pharyngeal pH probe to avoid these artifacts has been introduced. The aim of this study was to use this probe to measure the pharyngeal pH environment in normal subjects and establish pH thresholds to identify abnormality.
Methods Asymptomatic volunteers were studied to define the normal pharyngeal pH environment. All subjects underwent esophagram, esophageal manometry, upper and lower esophageal pH monitoring with a dual-channel pH catheter and pharyngeal pH monitoring with the new probe. Analyses were performed at 0.5 pH intervals between pH 4 and 6.5 to identify the best discriminating pH threshold and calculate a composite pH score to identify an abnormal pH environment.
Results The study population consisted of 55 normal subjects. The pattern of pharyngeal pH environment was significantly different in the upright and supine periods and required different thresholds. The calculated discriminatory pH threshold was 5.5 for upright and 5.0 for supine periods. The 95th percentile values for the composite score were 9.4 for upright and 6.8 for supine.
Conclusion A new pharyngeal pH probe which detects aerosolized and liquid acid overcomes the artifacts that occur in measuring pharyngeal pH with existing catheters. Discriminating pH thresholds were selected and normal values defined to identify patients with an abnormal pharyngeal pH environment.

Keywords Gastroesophageal reflux disease (GERD) · Laryngopharyngeal reflux (LPR) · 24-h pH monitoring · Pharynx · Esophagus

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Introduction

Respiratory and laryngeal symptoms such as hoarseness, throat clearing, chronic cough, asthma, and laryngospasm can occur in patients with typical symptoms of gastroesophageal reflux disease (GERD).[1] They also can occur in the absence of typical GERD symptoms.[2] In this setting, there are no specific clinical or pathological findings to identify reflux as the cause of the laryngopharyngeal symptoms and existing diagnostic tests lack sufficient sensitivity and specificity to confirm the diagnosis.[3]

The current practice to identify gastroesophageal reflux as a cause of laryngopharyngeal symptoms is to detect increased esophageal acid exposure by a pH probe with dual sensors, one placed 5 cm above the upper border of the lower esophageal sphincter (LES) determined by manometry and a second placed in the proximal esophagus near the

lower border of upper esophageal sphincter (UES). If abnormal acid exposure is measured at both levels, it is inferred that the laryngopharyngeal symptoms are due to reflux.[4] If abnormal esophageal acid exposure is measured only in the upper probe, the relationship of reflux to laryngopharyngeal symptoms is less certain. Clinical experience with this approach has been mixed with only a minority of patients responding well to treatment.[5,6] This has led some investigators to place the proximal pH sensor in the pharynx in an effort to improve the diagnostic accuracy of reflux induced respiratory and laryngeal symptoms.[7]

Measuring pharyngeal pH has unique problems that make interpretation of the pH record difficult. There is a high frequency of artifacts in the pH recordings due to drying of the pH sensor, the accumulation of mucous or food on the sensor or the interruption of electrical continuity due to the loss of contact of the reference electrode with the mucosa. Complex criteria have been described to differentiate between these artifacts and true changes in pH caused by reflux.[8] These criteria have restricted computer reading of the pH record and required laborious hand analysis.

A new pH sensor has been designed specifically to monitor the pharynx. This sensor detects aerosolized or liquid acid, resists drying, and does not require contact with fluid or tissue for electrical continuity. The probe has a teardrop shape with the sensor oriented downward to avoid becoming covered with food or mucus (Fig. 1). The aim of this study was to measure pharyngeal pH with this newly designed sensor in a large series of normal subjects and to propose discriminating pH threshold to identify patients with abnormal pharyngeal pH environment.

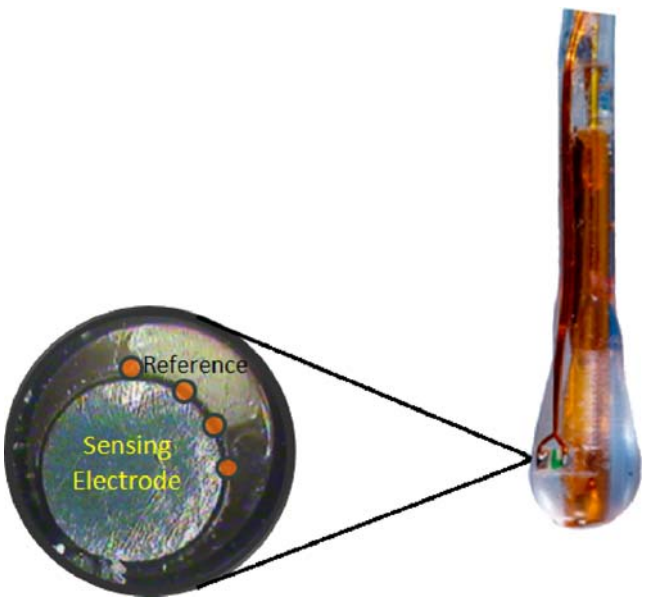

Figure 1 A magnified photograph of the Restech® pharyngeal pH probe showing the downward-oriented teardrop shape of the probe and the special proximity of the reference and sensing electrodes.

Materials and Methods

The goal was to recruit a minimum of 50 normal volunteers between the ages of 18 and 75 years. All volunteer subjects were questioned regarding the presence of GERD symptoms including heartburn, regurgitation, dysphagia, the presence of a known motility disorder or esophageal stricture, current or previous heavy alcohol or tobacco use, nasal obstruction or recent nasal surgery, anticoagulation therapy, and potential pregnancy. Subjects who answered yes to any of these questions were excluded. Out of 250 subjects screened, 78 asymptomatic volunteers were identified for participation in this study. These subjects underwent video esophagram, esophageal manometry, and esophageal pH monitoring with a catheter containing dual-pH sensor, one placed in the distal and the other in the proximal esophagus to exclude occult esophageal reflux disease. Pharyngeal pH monitoring was performed using the new pH probe. For the subjects' convenience, pharyngeal pH monitoring was performed in the same day as esophageal pH monitoring.

Technique of Esophageal pH Monitoring

Esophageal Manometry

Esophageal manometry was performed in the supine position using an eight-channel water-perfused catheter with lateral openings placed 5 cm apart and oriented radially 45° from each other. At the start of the study, all recording channels were placed in the stomach. The catheter was withdrawn in 1-cm increments every 20 s. The position of the catheter was recorded in centimeters from the ala of the nostril. The motility record was assessed using a commercially available software program (Polygram® Net, Medtronic Inc., Minneapolis, MN, USA).

Ambulatory pH Monitoring Using a Catheter with Dual-pH Sensor

A dual-pH probe was positioned in the esophagus with the distal sensor 5 cm above the upper border of the LES determined by manometry and the proximal sensor within 5 cm of the lower border of the UES. The subjects were instructed to remain in the upright or sitting position until retiring to bed in the evening, not to eat or drink between meals, refrain from chewing gum or smoking, and to go about their normal duties at home or at work. Patients were instructed to eat the meals in one sitting, accompanied only

by water, milk, coffee, or tea. Carbonated beverages, alcohol, and fruit drinks with an acid pH were not permitted. Subjects were instructed to lie flat at night, if possible, with a single pillow. Medications effecting gastrointestinal function were not allowed during the monitored period. Subjects were asked to keep a diary of events that included the beginning and the end of meals, and the times of retiring in the evening and rising in the morning. Subjects returned the following day and the data were downloaded from the recording units to a personal computer and analyzed using commercially available software (Polygram® Net, Medtronic Inc., Minneapolis, MN, USA). Distal and proximal esophageal acid exposure was expressed using six components of the 24-h record and the calculated pH score for a pH threshold of <4 (Table 1).

Technique of Pharyngeal pH Monitoring

Restech® Pharyngeal pH Sensor Technology

The Restech® pH probe (Respiratory Technology Corp., San Diego, CA, USA) contains a newly developed pH sensor based on proven antimony technology. The antimony sensor changes voltage potential relative to the pH of its surrounding environment. The sensor design includes both antimony and reference electrodes bound tightly together into a miniaturized package less than 1 mm in diameter. The sensor is mounted at the tip of the probe rather than placed on the side of the shaft, as in traditional pH probe designs. The combination of miniaturization and geometric positioning of the reference electrodes allows for the sensor to operate in the environment of the pharynx without drying out. Condensation from exhaled breath continually saturates the sensor with moisture. Miniaturization of the electrode also allows the measurement of hydrogen ion concentration in both liquid and aerosolized droplets. The tip of the probe contains a light-emitting diode (LED) that aids the clinician in catheter placement. The pH is measured at a frequency of two times per second and transmitted wirelessly to a data recorder.

Table 1 Assessment of 24-h Esophageal Acid Exposure

Percent total time pH<4
Percent upright time pH<4
Percent supine time pH<4
Number of reflux episodes
Number of reflux episodes ≥5 min
Longest reflux episode (minutes)
Composite score[a]

[a] The 24-h composite pH score is the sum of the scores for each of the six components calculated by the formula: [(patient value − mean)/mean)SD] + 1

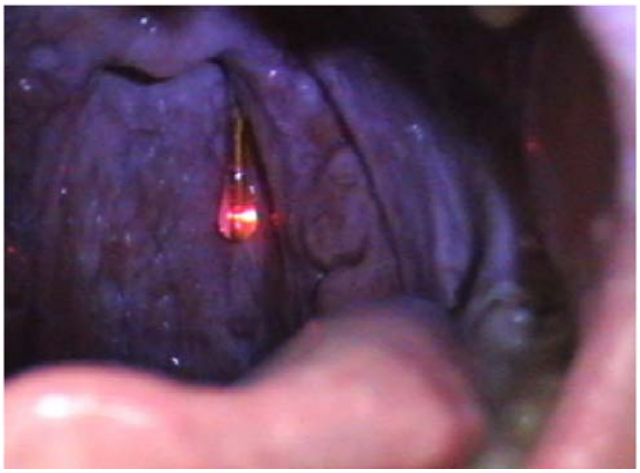

Figure 2 A photograph showing the Restech® pharyngeal pH probe properly positioned in a subject with a 5-mm flashing LED light that can be used as a guide to place the probe 5–10 mm below the uvula.

Restech® Pharyngeal pH Probe Preparation and Placement

The Restech® pH sensor was calibrated in solutions of pH 7 and pH 4 prior to use. The nasal passage was topically anesthetized using Q-tips soaked with 2% lidocaine. The sensor was inserted until the flashing LED was seen in the back of the subject's throat and then positioned so that the flashing light was 5–10 mm below the uvula. The length of the LED light is 5 mm and serves as a useful guide for placement (Fig. 2). The catheter was secured to the patient's face, as close to the nares as possible using a Tegaderm™ and then passed over the ear and secured to the neck with a second Tegaderm™. The transmitter at the end of the catheter was either taped to the skin or attached to the subjects' clothing using a clip-on case. A data recorder was attached to the patients' belt. Patients were asked not to shower during the recording period and to keep a diary indicating the time of the meal periods and the time spent in the supine and upright positions. The meal periods were excluded in the analyses of pharyngeal pH recordings. The esophageal and pharyngeal pH data were collected by two different recording devices. The timers of both data recorders were synchronized prior to the start of the monitoring period to assure simultaneous monitoring of esophageal and pharyngeal pH. The Restech® data recorder was downloaded to a proprietary software program and correlated with the patient's diary. Data from the esophageal pH probe with dual sensor were also exported to the same software. This program allowed simultaneous comparison of the pH records to determine the temporal relationships between the pH changes in the distal esophagus, proximal esophagus, and pharynx.

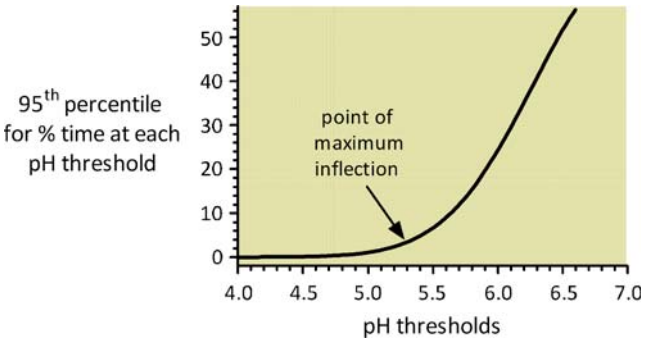

Figure 3 An example of the mathematical graphic model used to determine the discriminating pH threshold. The 95th percentile values for percent time the pH is below various pH thresholds is plotted to construct a curve. The point of maximum inflection is calculated using the equation for the plotted curve. The equation for this illustrated curve is: $y = 4.1852x^3 - 50.952x^2 + 202.45x - 261.19$.

Determination of pH Thresholds and Normal Pharyngeal Acid Exposure

Pharyngeal pH recordings performed with the Restech® probe prior to the study showed that changes in the pharyngeal pH environment can be caused by the reflux of gastric juice, i.e., true reflux events, alteration in salivary flow during sleep and awake periods, and small fluctuations due to noise in the recording system. The best discriminating threshold should detect the majority of true reflux events while minimizing the influence of saliva and the noise of the system.

The best discriminatory pharyngeal pH threshold was determined using a mathematical graphic methodology in which the 95th percentile value for the percent time the pH was below 4, 4.5, 5, 5.5, 6, and 6.5 was calculated and plotted to construct a curve. The slope in the curve reflects the noise in the system (Fig. 3). The horizontal portion of the curve represents the thresholds that are less affected by the noise of the system but fail to recognize many true reflux events. The vertical portion of the curve represents thresholds that are more affected by the noise of the system but detect higher number of true reflux episodes. The equation that defined the curve was used to calculate the point of its maximal inflection. This is the point at which the ability to detect true reflux events is maximized while the noise of the system is minimized as illustrated in Fig. 3.

Determination of the Pharyngeal Composite pH Score

A composite pH score was calculated for the pH threshold identified by applying the same method used to calculate the composite pH score for esophageal pH monitoring. This required calculating for each subject the scores for each component by the formula:

$$\frac{\text{subject value} - \text{mean of 55 normal subjects}}{\text{SD}} + 1$$

The sum of the three component scores (percent time below threshold, number of reflux episodes, and duration of the longest episode) equals the pharyngeal composite pH score.

Results

Among the 78 volunteers, 12 had abnormal distal esophageal acid exposure (DeMeester Score>14.7); five had a hiatal hernia larger than 2 cm on video esophagogram, and six had technical difficulties with either their esophageal (n=3) or pharyngeal (n=3) pH recorders rendering their tracings unusable. These 23 subjects were excluded. In the remaining 55 normal subjects, pharyngeal pH monitoring was performed with the new pharyngeal pH sensor without encountering artifacts or technical problems.

The study population consisted of these 55 normal subjects. There were 28 males and 27 females with a median age of 28 years (range 19–72). A representative pharyngeal pH tracing is shown in Fig. 4. The pattern of the pharyngeal pH environment was visibly different between the upright and supine periods. Further, the mean pharyngeal pH was significantly higher in the upright than supine period (Fig. 5). Consequently, separate analyses were done for the upright and supine periods.

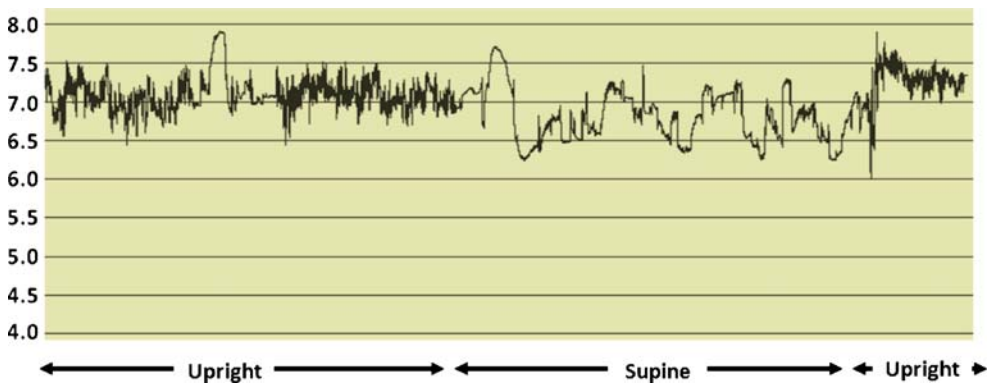

Figure 4 A representative 24-h pharyngeal pH tracing from a normal subject. The upright and supine periods can be identified easily by the pattern of the pH recording.

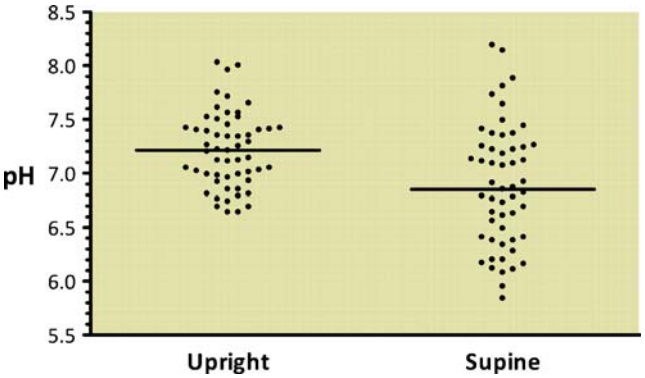

Figure 5 The mean values for the pharyngeal pH environment in each subject are plotted for the upright and supine periods. The mean pharyngeal pH was higher in the upright period (7.2 vs. 6.8, $p<0.0001$, Wilcoxon matched-pairs test).

The mean, median, interquartile range, and 95th percentile values for the components of pharyngeal pH monitoring at different pH thresholds are shown in Tables 2 and 3. The 95th percentile values for percent time the pH was below the various pH thresholds for the upright and supine periods are plotted in Fig. 6. The point of maximal inflection of the curves was at the pH of 4.8 for the supine period and 5.6 for the upright period. These were selected as discriminating pH thresholds and were rounded off to 5.0 for the supine period and 5.5 for the upright period.

Since pharyngeal pH exposure for the upright and supine periods were calculated separately, only three of the six components (percent time, number of episodes, and duration of longest episode) were used to calculate the composite score. The 95th percentile values for pharyngeal pH exposure at the discriminating pH thresholds in the

Table 2 Normal Values for 24-h Pharyngeal pH Monitoring in the Upright Position at Different pH Thresholds ($n=55$)

	Mean	Median	IQR		95th percentile
			25th percentile	75th percentile	
Ph<4.0					
% Time	0.0	0.0	0.0	0.0	0.0
No. of episodes	0.0	0.0	0.0	0.0	0.0
Longest episode	0.0	0.0	0.0	0.0	0.0
No. of episodes ≥5 min	0.0	0.0	0.0	0.0	0.0
pH<4.5					
% Time	0.0	0.0	0.0	0.0	0.0
No. of episodes	0.0	0.0	0.0	0.0	0.0
Longest episode	0.0	0.0	0.0	0.0	0.0
No. of episodes ≥5 min	0.0	0.00	0.0	0.0	0.0
pH<5.0					
% Time	0.004	0.0	0.0	0.0	0.021
No. of episodes	0.073	0.0	0.0	0.0	1.00
Longest episode	0.021	0.0	0.0	0.0	0.118
No. of episodes ≥5 min	0.0	0.0	0.0	0.0	0.0
pH<5.5					
% Time	0.015	0.0	0.0	0.001	0.133
No. of episodes	0.255	0.0	0.0	0.0	1.20
Longest episode	0.068	0.0	0.0	0.0	0.71
No. of episodes≥5 minutes	0.0	0.0	0.0	0.0	0.0
pH<6.0					
% Time	0.846	0.170	0.0	0.65	6.29
No. of episodes	5.33	1.0	0.0	5.0	40.2
Longest episode	1.98	0.010	0.0	1.29	12.83
No. of episodes ≥5 min	0.218	0.0	0.0	0.0	2.0
pH<6.5					
% Time	6.55	1.32	0.074	8.42	32.9
No. of episodes	34.18	10.0	2.0	43.0	154.4
Longest episode	27.12	2.85	0.18	14.2	144.1
No. of episodes ≥5 min	1.66	0.0	0.0	2.0	10.0

Table 3 Normal Values for 24-h Pharyngeal pH Monitoring in the Supine Position at Different pH Thresholds (n=55)

	Mean	Median	IQR		95th percentile
			25th percentile	75th percentile	
pH<4.0					
% Time	0.68	0.0	0.0	0.0	1.26
No. of episodes	0.16	0.0	0.0	0.0	1.00
Longest episode	1.16	0.0	0.0	0.0	5.93
No. of episodes ≥5 min	0.09	0.0	0.0	0.0	0.2
pH<4.5					
% Time	0.92	0.0	0.0	0.0	1.54
No. of episodes	0.22	0.0	0.0	0.0	1.20
Longest episode	1.88	0.0	0.0	0.0	7.11
No. of episodes ≥5 min	0.11	0.0	0.0	0.0	0.2
pH<5.0					
% Time	1.33	0.0	0.0	0.0	5.15
No. of episodes	0.55	0.0	0.0	0.0	4.0
Longest episode	2.91	0.0	0.0	0.0	18.97
No. of episodes ≥5 min	0.18	0.0	0.0	0.0	0.2
pH<5.5					
% Time	3.98	0.0	0.0	5.07	23.9
No. of episodes	3.38	0.0	0.0	3.0	16.2
Longest episode	9.79	2.71	0.0	6.11	52.7
No. of episodes ≥5 min	0.76	0.0	0.0	1.0	4.4
pH<6.0					
% Time	13.94	3.51	0.0	22.8	55.1
No. of episodes	10.95	4.00	0.0	17.0	45.0
Longest episode	27.8	5.8	0.0	33.8	152.3
No. of episodes ≥5 min	2.51	1.0	0.0	4.0	10.2
pH<6.5					
% Time	31.1	23.0	1.9	60.7	77.9
No. of episodes	24.95	16.0	2.00	34.0	114.0
Longest episode	74.5	34.9	2.27	98.6	334.2
No. of episodes ≥5 min	3.84	4.0	0.0	7.0	10.0

upright and supine positions and their RYAN composite score values are shown in Table 4.

Discussion

Acid-related laryngeal ulcerations and granulomas were first described in 1968.[4] Since that time, acid reflux has been implicated as the cause of several laryngeal and pharyngeal symptoms including hoarseness, globus sensation, chronic cough, otalgia, and laryngospasm.[9] Acid reflux has also been implicated as the cause of laryngeal stenosis and carcinoma.[2] Of interest, only a minority of these patients have typical reflux symptoms such as heartburn and regurgitation.[1] Further, even when abnormal distal esophageal acid exposure is confirmed by 24-h pH monitoring, the effectiveness of antireflux surgery in eliminating laryngopharyngeal reflux (LPR) symptoms is not predictable.[10] These results have led to the monitoring of the esophagus using a catheter with dual-pH sensors, one located in the distal and the other in the proximal esophagus, to better identify acid reflux as the etiology of LPR symptoms.[7]

Clinical experience has shown that even when monitored with catheters containing dual-pH sensors the ability to predict relief of LPR symptoms by acid suppression therapy or antireflux surgery is inconsistent. Studies by Wo and colleagues[11] and Cool and colleagues[12] claim that there is no convincing evidence that proximal esophageal pH monitoring predicts response to acid-suppressive therapy in patients with LPR symptoms. Further, Wo and colleagues have reported that only 25% of patients with increased proximal esophageal acid exposure were relieved of their LPR symptoms following antireflux surgery.[5] In

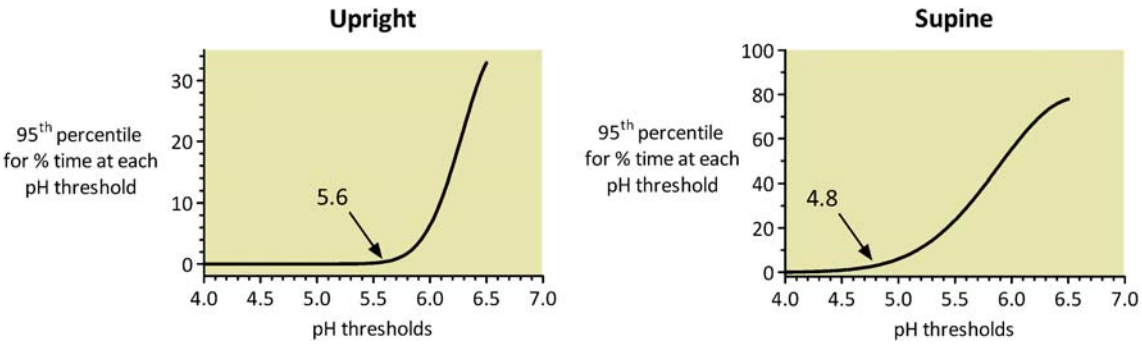

Figure 6 The mathematical graphic model plotting the 95th percentile values for percent time below different pH threshold values is shown for the upright and supine periods. The equations that define these curves were used to calculate the point of maximum inflection (*arrows*) to identify the best discriminating thresholds.

contrast, Patti and colleagues reported that cough resolved after antireflux surgery in 83% of patients in whom a correlation between cough and reflux was found during proximal esophageal pH monitoring.[6]

These results have prompted investigators to monitor the pharynx.[7] There are several problems with this approach. Technical artifacts are common due to drying of the sensor or disruption of the electrical circuit between the reference and sensing electrodes. Food and mucous can also accumulate on the sensor interfering with the ability to detect acid in the pharynx.[13,14] The Restech® pharyngeal pH probe and sensor utilized in this study has been designed to avoid these limitations, and we did not observe artifacts or technical problems with its use.

A pH of 4 is used as a threshold in distal esophageal pH monitoring[15] based on studies showing that heartburn is associated with esophageal exposure to a pH less than 4.[16] No such typical symptom exists to define a pH threshold in pharyngeal pH monitoring. In addition, there is a pH gradient in the esophagus when reflux occurs due to neutralization of the refluxed gastric juice by swallowed saliva.[17] Consequently, a pH threshold higher than 4 is likely needed to identify abnormal pharyngeal exposure to gastric juice.

In this study, we characterized the pH environment of the pharynx in normal subjects and have shown that the mean pharyngeal pH is lower during the supine period than during the upright period (6.8 vs.7.2, $p<0.0001$). This is because salivary flow is reduced during the night resulting in a lower pharyngeal pH. Consequently, we propose that the upright and supine periods should be analyzed separately using different pH thresholds. When using the chosen discriminating pH threshold, we found that a drop in pharyngeal pH was more frequent and prolonged in the supine compared to the upright position. This is likely also due to the decreased production of saliva during the sleep.

We used a mathematical graphic model to identify the best discriminating pH thresholds to detect the changes in the pharyngeal pH environment during the upright and supine period. This methodology allowed us to select pH thresholds for the two periods in which detection of true reflux episodes was maximized while the noise of the system was minimized. The percent time pH was below these selected thresholds, the number of episodes in which the pH dropped below these thresholds, and the duration of the longest episode were measured and integrated into a pharyngeal pH (RYAN) score for the upright and supine periods. The calculated threshold for the upright period was pH 5.5 and for the supine period 5.0. The normal RYAN composite score for these periods was 9.4 and 6.8, respectively.

Selecting discriminating pH thresholds and defining normal values are necessary first steps toward establishing the utility of this newly designed probe. The mathematical graphic methodology used in this study is a reasonable approach for selecting a discriminating pH threshold for the pharyngeal environment. The selected discriminating thresholds and normal values reported in this study need to be validated by collecting a registry of patients with LPR symptoms who have an abnormal pretreatment pharyngeal pH environment and show relief of symptoms and normalization of pharyngeal pH environment with acid suppression therapy or antireflux surgery.

Finally, an abnormal pharyngeal pH environment can be caused by decreased salivary production, change in bacterial flora of the pharynx, and reflux of gastric juice into the pharynx.[18,19] Only the latter is likely to be associated with LPR symptoms. The pharyngeal pH records in symptom-

Table 4 The 95th Percentile Values (Normal) for the Components and Composite Score of Pharyngeal pH Exposure at the Discriminating pH Thresholds

	Upright pH<5.5	Supine pH<5.0
% Time	0.13 min (8 s)	5.15 min (309 s)
No. of episodes	1	4
Longest episode (min)	0.71	18.97
RYAN[a] Score	9.41	6.79

[a] Composite pH score for pharyngeal acid exposure

atic patients need to be interpreted keeping these other etiologies in mind.

Conclusion

A new pharyngeal pH probe which detects aerosolized and liquid acid overcomes the artifacts that occur in measuring pharyngeal pH with existing catheters. New discriminating pH thresholds were selected to identify patients with abnormal pharyngeal pH environment. The discriminating thresholds and normal values reported in this study need to be validated by patients with LPR symptoms who respond to acid suppression therapy or antireflux surgery.

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ORIGINAL ARTICLE

Inhibition of Nucleostemin Upregulates CDX2 Expression in HT29 Cells in Response to Bile Acid Exposure: Implications in the Pathogenesis of Barrett's Esophagus

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Abstract
Background Barrett's esophagus (BE), a squamous-to-columnar metaplasia, may originate from growth-promoting mutations in metaplastic stem cells. Nucleostemin is a protein highly expressed in undifferentiated embryonic stem cells. The objectives of this study were to explore the potential role of nucleostemin in the pathogenesis of BE
Methods The expression profiles of 30,968 genes were compared between BE and normal esophageal tissues ($n=6$ in each group) by using oligo microarray. Three siRNA plasmid expression vectors against nucleostemin, *pRNAi-1*, *pRNAi-2*, and *pRNAi-3*, were constructed and transfected into HT29 cells. In addition, HT29 cells were exposed to 100–1,000 μM chenodeoxycholic acid (CDC), a bile acid, for 2, 12, and 24 h, and then messenger RNA and protein expressions of nucleostemin and CDX2 were determined by reverse-transcriptase polymerase chain reaction and Western blotting.
Results Four hundred and twenty-six differentially expressed genes were detected in BE; 142 were upregulated and 284 downregulated. Nucleostemin was downregulated while CDX2 was upregulated. *In vitro*, all the recombinant plasmids inhibited the nucleostemin expression in transfected HT29 cells, with *pRNAi-1* being the most effective. CDX2 expression was significantly increased in *pRNAi-1*-transfected HT29 cells, compared with that in the empty plasmid (*pRNAT-U6.1*/*Neo*) transfected or untransfected HT29 cells. In addition, CDX2 expression was increased whereas nucleostemin expression was decreased in a dose- and time-dependent manner in HT29 cells treated with CDC.
Conclusion These findings suggest that the inhibition of nucleostemin expression in "esophageal stem cells" in response to bile acid exposure may be involved in the pathogenesis of BE through upregulating CDX2 expression.

Keywords Barrett's esophagus · Nucleostemin · CDX2 · HT29 cell · Oligomicroarray

Introduction

The incidence of esophageal adenocarcinoma has increased at a rate that is among the highest of all cancers.[1,2] The major risk factor for esophageal adenocarcinoma is the presence of Barrett's esophagus (BE), a premalignant neoplastic lesion that is characterized by intestinal metaplasia replacing the normal squamous esophageal epithelia.[3] The presence of BE increases the overall risk of adenocarcinoma by 40-fold.[4]

Nucleostemin, a newly found p53-binding protein, exists mainly in the nucleoli of stem cells and some various cancer cells but is not expressed in committed and terminally differentiated cells.[5] Nucleostemin helps regulate proliferation of both cancer cells and stem cells and is considered as a useful marker of undifferentiated human adult bone marrow stem cells.[6] It has been demonstrated that nucleostemin is expressed, to a certain extent, in normal esophageal squamous mucosa and increasingly expressed in esophageal squamous carcinoma.[7] However, its role in the pathogenesis of BE and subsequently esophageal adenocarcinoma is yet to be elucidated.

In the present study, we first performed a genome-wide assessment of gene in endoscopic biopsy specimens taken

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from BE patients and those with normal esophageal mucosa, using an oligo microarray method. We observed that CDX2 was expressed, but nucleostemin was not detected in BE tissues. We hypothesized that nucleostemin downregulates CDX2 expression, and the loss of nucleostemin expression in the esophageal "stem cells" may result in activation of CDX2 expression, leading to the intestinal differentiation and subsequent formation of intestinal metaplasia. It has been demonstrated that HT29 cells can be used to serve as an *in vitro* model for the study of the effects of different components of gastroduodenal refluxate on cellular and molecular events in the development of Barrett's esophagus.[8] To test our hypothesis, we further determined the effects of silencing nucleostemin expression on the expression of CDX2 in HT29 cells with the RNAi technique to see whether siRNAs that target nucleostemin transduction would enhance CDX2 expression in vitro. In addition, we observed the expression of nucleostemin and CDX2 in HT29 cells after chenodeoxycholic acid (CDC) exposure.

Materials and Methods

Tissue Specimens

Endoscopic tissue biopsies taken from the BE areas of six patients (n=6) and from six subjects with normal esophageal mucosa (n=6) were provided by the Gastroenterology Research Institute, Southwest Hospital, Third Military Medical University, Chongqing, China. Routine histopathologic examinations were performed to confirm the diagnosis by experienced gastrointestinal pathologists. BE was defined as any columnar-lined mucosa above the gastroesophageal junction, which was further confirmed by Alcian blue staining. Intestinal metaplasia was defined by the presence of barrel-shaped goblet cells in normal gastroesophageal junction.[9]

The study protocol was approved by the Ethic Committee of the Third Military Medical University, and written informed consent was provided by all study subjects.

RNA Preparation

Total sample RNA was extracted by a single-step method. Briefly, the tissues were ground and homogenized using the Trizol reagent (Invitrogen Life Technologies, CA, USA) for extraction of total RNA, according to the instructions of the manufacturer. The integrity of total RNA was checked by 1.2% formaldehyde agarose gel electrophoresis showing the 28S and 18S bands. Total RNA with OD260/OD280>1.8 was used for microarray experiments

Detection of Gene Expression Profiles in Tissue Specimens by Oligomicroarray

Total RNA from BE and matched normal tissue were labeled with cyanine 3-dUTP and cyanine 5-dUTP by direct labeling method (Perkin Elmer Life Sciences, USA: Micromax Direct labeling kit). Labeled probes were denatured at 95°C for 5 min and hybridized with a human oligo microarray (University Health Network, Microarray Center, Toronto, Canada) in a hybridization chamber (Corning Life Sciences, USA) at 65°C water bath for 18 h. Before hybridization, slides were prehybridized in 5× saline–sodium citrate buffer (SSC), 0.1% sodium dodecyl sulfate (SDS), and 1% bovine serum albumin solution at 65°C for 45 min to prevent nonspecific hybridization. After hybridization, the slides were washed in 2× SSC with 0.1% SDS, 0.1× SSC with 0.05% SDS, and 0.1× SSC sequentially for 20 min each and then spin-dried. The microarray image was scanned by Gene Pix 4200A scanner (Axon Instruments Inc., Foster City, CA, USA) and analyzed by Gene Pix Pro 6.0.1.27 software (Axon Instrument). Differentially expressed genes, which were defined as genes with twofold or greater difference in the expression between BE and normal esophageal tissues in four out of the six chips, were further analyzed for functional gene clusters using GeneSpring software GXV. The normalized ratio of Cy5 intensity to Cy3 intensity greater than 2.0 or less than 0.5 was considered as upregulated or downregulated gene expression, respectively.

Cell Line and Culture

Human colon adenocarcinoma cell line, HT29, was obtained from the American Type Culture Collection (Manassas, VA USA). HT29 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Gibco-BRL, Grand Island, NY, USA), 50 U/mL of penicillin, and 50 μg/mL of streptomycin. The cells were detached from the flasks before subculturing by the removal of the medium and the addition of 1 mL of 0.25% trypsin and incubation at room temperature for 3 to 5 min.

Construction of the siRNA Plasmid Expression Vectors and Transfection of Plasmids

Three siRNAs targeted against nucleostemin were designed by a program available online (www.genscript.com), namely, nucleostemin I (GTGGACAGGTGCCTCATTA), nucleostemin II (ACAGAGGCTTGAAGAACTA), and nucleostemin III (GAAGCTGTACTGCCAAGAA). siRNA-expressing plasmids were constructed by cloning the siRNA sequences into *pRNAT-U6.1*/*Neo* via *Bam*HI and *Hind*III digestion. The plasmids were extracted following the manufacturer's instruction and then sequenced to confirm the correct insertion. The new plasmids were

named *pRNAi-1*, *pRNAi-2*, and *pRNAi-3*, respectively, and the concentration and purity of the plasmids were detected by ultraviolet spectrophotometry. The plasmids were stored at −20°C for subsequent experiments.

HT29 cells were seeded on six-well culture plates and grown to 80~90% confluence before the transfection. The recombinant *pRNAi-1*, *pRNAi-2*, and *pRNAi-3* were used for the transfection in the corresponding experimental groups. Lipofectamine™ 2000 alone was used for the transfection in the blank control group whereas the empty plasmid *pRNAT-U6.1/Neo* was used in the negative control group. The culture medium was replaced with the fresh medium containing calf serum (150 mL/L) at 6 h posttransfection. Forty-eight hours later, the transfected cells were selected by G418 (600 μg/mL; Huamei Biotechnology Company, Beijing, China) until positive clones were discovered after 14 days. The cells were cultured and finally selected by G418 (300 μg/mL) for a further 10 days. Single clones were selected to build a stable transfected cell line.

Treatment of HT29 Cells with CDC

After 70% confluence, HT29 cells were placed in serum-free Roswell Park Memorial Institute 1640 for 24 h and then exposed to 100, 500, and 1,000 μM CDC (Sigma Chemical Co., St. Louis, MO, USA) in serum-free medium for 2, 12, and 24 h, respectively. Cells were harvested at the end of each time point with 0.25% trypsin solution.

Detection of Protein Expression of Nucleostemin and CDX2 in HT29 Cells by Western Blot Analysis

Cells were washed three times with ice-cold sterile phosphate buffer solution (PBS), then lysed in radio-immunoprecipitation assay (Beyotime Co., China) with 10 mM phenylmethylsulfonyl fluoride (Beyotime Co.) for 30 min on ice. The lysate was centrifuged at 16,000×*g* for 15 min at 4°C. Then, the supernatant was transferred to clean microfuge tubes. Protein concentration was measured by the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA), as recommended by the manufacturer.

Proteins (25 μg) were separated by 12% SDS polyacrylamide gel electrophoresis and then transferred to nitrocellulose membrane (0.45 mm). Each membrane was then blocked for 1 h at room temperature with 5% dehydrated skim milk; the membranes were incubated overnight at 4°C with a goat polyclonal antinucleostemin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, 1:200) and a mouse monoclonal anti-CDX2 antibody (Santa Cruz, 1:100), and for the detection of nucleostemin (62 kDa) and CDX2 (33 kDa). β-actin (42 kDa) was also detected with a mouse monoclonal antibody (Sigma, St. Louis, MO, USA) as a loading control. Membranes were washed in 3% dry nonfat milk in PBS containing 0.05% Triton X-100 and incubated with antigoat or antimouse peroxidase-conjugated secondary antibody (Amersham Pharmacia Biotech, Berkshire, UK, 1:10,000) for 30 min. Immunoblots were revealed by using an enhanced chemiluminescence system (Amersham Pharmacia Biotech). Densitometric analyses were performed using Quantity one software (version 4.2.2, Bio-Rad USA).

Detection of mRNA Expression of Nucleostemin and CDX2 in HT29 Cells by Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from each sample using the Total RNA Extract Kit (Omega) following the manufacturer's instructions. The concentration of RNA was measured by spectrophotometry. Total RNA was reverse-transcribed to complementary CDNA (cDNA) with reverse-transcriptase reagents (Toyobo Co., Japan) according to the manufacturer's protocol. Two-microgram cDNA was amplified in a total volume of 25 μL under the conditions recommended by the manufacturer. The cycling conditions were 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 64°C (for primers of nucleostemin) for 30 s or 60°C (for primers of CDX2) or 58°C (for primers of β-actin), and 72°C for 60 s, and a final extension of 72°C for 10 min. Polymerase chain reaction (PCR) products were separated on a 1.5% agarose gel and viewed by ethidium bromide staining. Amplification of human β-actin served as an internal standard. The gene primers are shown in Table 1.

Statistical Analysis

All data were expressed as mean±standard deviation (SD) and analyzed by analysis of variance. All data were analyzed with SPSS 10.0 software. A P value of <0.05 was considered as statistical significant.

Table 1 Sequence and Size of Primers Used for RT-PCR Amplification of *Nucleostemin*, *CDX2* Gene, and β-*actin* Genes

Gene	Primer	Sequence	Product size (bp)
β-actin	Sense	GTTGCGTTACACCCTTTCTTGACA	446
	Antisense	GCACGAAGGCTCATCATTCAAAA	
Nucleostemin	Sense	GAAACAGAGGCTTGAAGAACTAA	223
	Antisense	GGAGGCTTCGATCACCTTTTTA	
CDX2	Sense	ACCAGGACGAAAGACAAATATCGA	85
	Antisense	TGTAGCGACTGTAGTGAAACTCCTTCT	

Results

Gene Expression Pattern in BE and Normal Tissues

From the original number of 30,968 gene probes, a total of 426 genes were identified to be differentially expressed genes in all six chips; 142 were upregulated and 284 were downregulated in BE compared with the normal esophageal mucosa (Fig. 1). Among these differentially expressed genes, nucleostemin downregulation was 0.34±0.09-fold, while CDX2 upregulation was 3.58±0.97-fold in BE, compared with the normal esophageal mucosa.

Identification of Constructed Recombinant Plasmids and Confirmation of Transfection of the Vectors

The recombinant plasmids were identified to have correct sequences by DNA sequencing analysis, and the resulting sequencing confirmed that the DNA chains had been ligated to the vectors. Efficiency of transfection was evaluated by fluorescence microscopy after transfection of a vector containing the gene encoding a green fluorescent protein at 2 weeks, and nearly 100% of cultured HT29 cells transfected with *pRNAi-1* were positive for the green fluorescent protein (Fig. 2).

Protein and mRNA Expression of Nucleostemin and CDX2 in Transfected HT29 Cells

Nucleostemin protein expression were downregulated significantly in HT29 cells after transfection with *pRNAi-1*, *pRNAi-2*, and *pRNAi-3* (all $P<0.05$; Fig. 3a, b). Since the *pRNAi-1* was the most effective vector, it was selected for further experiment on the effect of RNAi on the expression of CDX2. It was shown that CDX2 protein expression in HT29 cells transfected with *pRNAi-1* was significantly increased, compared with that in HT29 cells transfected with *pRNAT* or untransfected HT29 cells (Fig. 3c, d).

Figure 1 Image of gene expression profiles in Barrett's esophagus tissue.

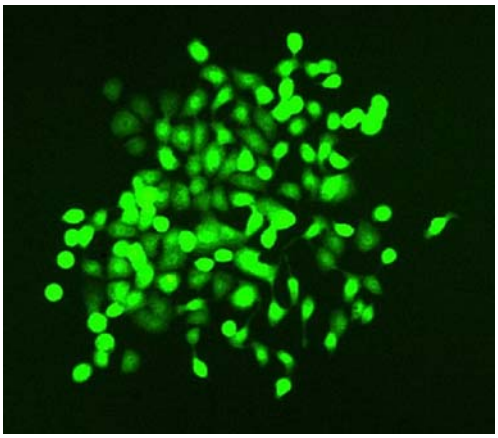


Figure 2 Expression of green fluorescent protein in HT29 cells after transfection with *pRNAi-1* (×200, under a fluorescence microscope).

The expression of *nucleostemin* mRNA was significantly inhibited in HT29 cells transfected with siRNA-expressing vectors, *pRNAi-1*, *pRNAi-2*, and *pRNAi-3*, compared with that in HT29 cells transfected with *pRNAT* or untransfected HT29 cells (all $P<0.05$). It was noticed that *pRNAi-1* was the most effective (Fig. 4a, b). In addition, CDX2 expression in HT29 cells transfected with *pRNAi-1* was noticeably stronger than that in HT29 cells transfected with *pRNAT* or untransfected HT29 cells (Fig. 4c, d).

Protein and mRNA Expression of Nucleostemin and CDX2 in HT29 Cells Exposed to CDC

A low level of CDX2 protein expression was detected in HT29 cells without CDC exposure. CDX2 protein expression was highly upregulated by CDC treatment in a dose- and time-dependent fashion. Although CDC exerted no significant effect of on CDX2 protein expression in HT29 cells at 100 μM for up to 24 h and at 500 μM for up to 12 h, CDX2 protein expression was significantly increased after treatment with 500 μM CDC at 24 h or 1,000 μM CDC at 2 h, with the maximal effect being achieved with 1,000 μM CDC at 24 h. Furthermore, nucleostemin protein expression was decreased in a dose- and time-dependent fashion in HT29 cells treated with CDC (Fig. 5, A1, B1, and C1).

After exposure to CDC, nucleostemin mRNA expression was significantly downregulated but CDX2 mRNA expression was significantly upregulated at all time points (i.e., 2, 12, and 24 h) in a dose- and time-dependent fashion, especially at the concentration of 1,000 μM CDC (Fig. 5, A2, B2, and C2).

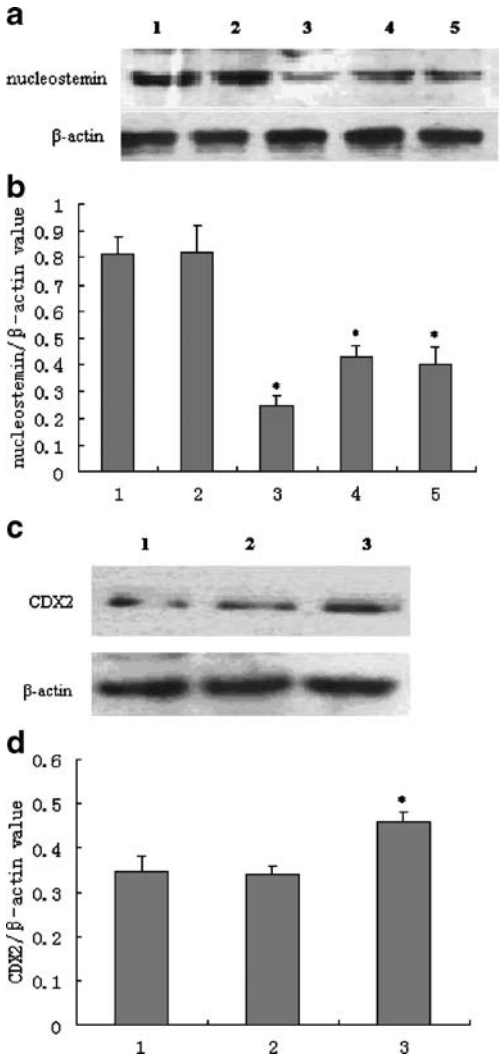


Figure 3 Western blot assay of nucleostemin protein and CDX2 protein expression in HT29 cells. **a** Nucleostemin protein expression shown in the Western blot assay. **b** Nucleostemin protein expression shown in a densitometric analysis. *1*, untransfected HT29 cells; *2*, HT29 cells transfected with *pRNAT*; *3*, HT29 cells transfected with *pRNAi-1*; *4*, HT29 cells transfected with *pRNAi-2*, and *5*, HT29 cells transfected with *pRNAi-3*. **c** CDX2 protein expression in HT29 cells, as shown in Western blot assay. **d** CDX2 protein expression shown in a densitometric analysis. *1*, untransfected HT29 cells; *2*, HT29 cells transfected with *pRNAT*; *3*, HT29 cells transfected with *pRNAi-1*. The densitometric analysis of nucleostemin protein and CDX2 protein over β-actin protein data are expressed as mean±SD of three experiments. *, $P<0.05$, compared with untransfected HT29 cells and HT29 cells transfected with *pRNAT*.

Discussion

Although there is great interest in the pathogenesis of BE, little is known regarding the mechanism of cellular metaplasia or precise cell origin of this lesion. In the present study, we found, for the first time, that nucleostemin is persistently expressed in HT29 cells but is not in biopsy specimen of human BE, and inhibition of nucleostemin expression results in the upregulation of expression of CDX2, a caudal-related homeobox gene and intestinal transcription factor essential for intestinal development or intestinal metaplasia of the esophagus.[10,11] Our results suggest that there is an association between nucleostemin and CDX2 in the development of BE. While the exact mechanisms of the interaction in esophageal cells remain to be elucidated. It is conceivable that inhibition

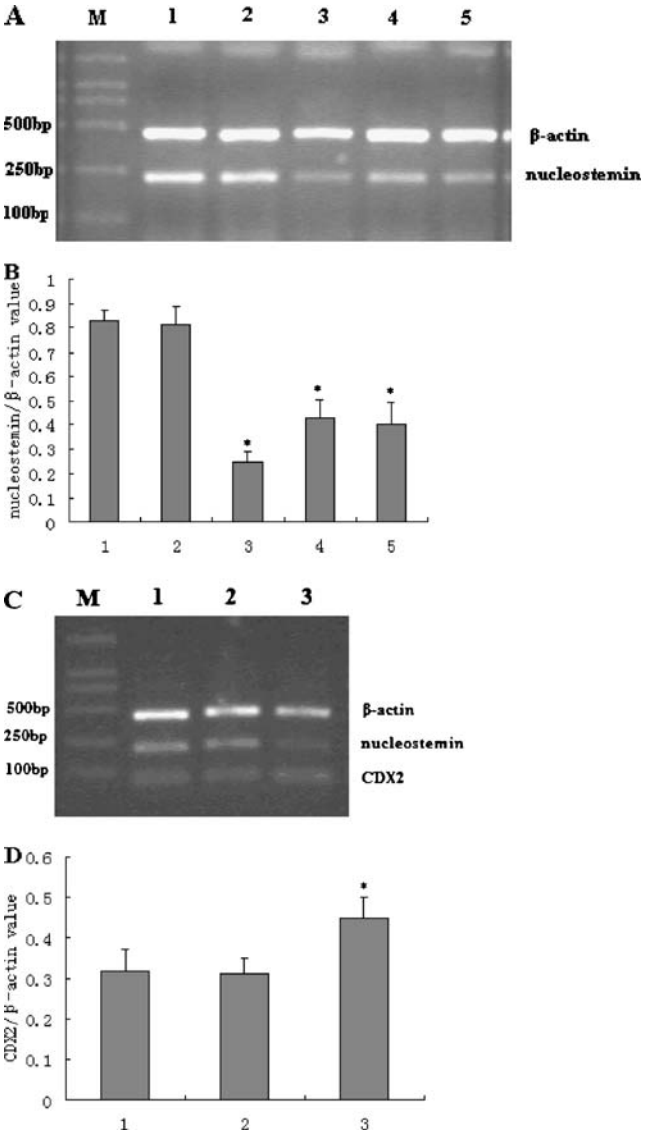


Figure 4 Expression of *nucleostemin* mRNA and *CDX2 mRNA* expression in HT29 cells as detected by RT-PCR (β-actin was used as a control). **a** *M*, DL2000 marker; *1*, untransfected HT29 cells; *2*, HT29 cells transfected with *pRNAT*; *3*, HT29 cells transfected with *pRNAi-1*; *4*, HT29 cells transfected with *pRNAi-2* (for *nucleostemin* mRNA only); and *5*, HT29 cells transfected with *pRNAi-3* (for *nucleostemin* mRNA only). **b** The densitometric analysis of *nucleostemin* mRNA over β-actin mRNA data is expressed as mean±SD of three experiments. **c** *1*, untransfected HT29 cells; *2*, HT29 cells transfected with *pRNAT*; *3*, HT29 cells transfected with *pRNAi-1*. **d** The densitometric analysis of *CDX2* mRNA over β-actin mRNA data is expressed as mean±SD of three experiments. *, $P<0.05$, compared with untransfected HT29 cells and HT29 cells transfected with *pRNAT*.

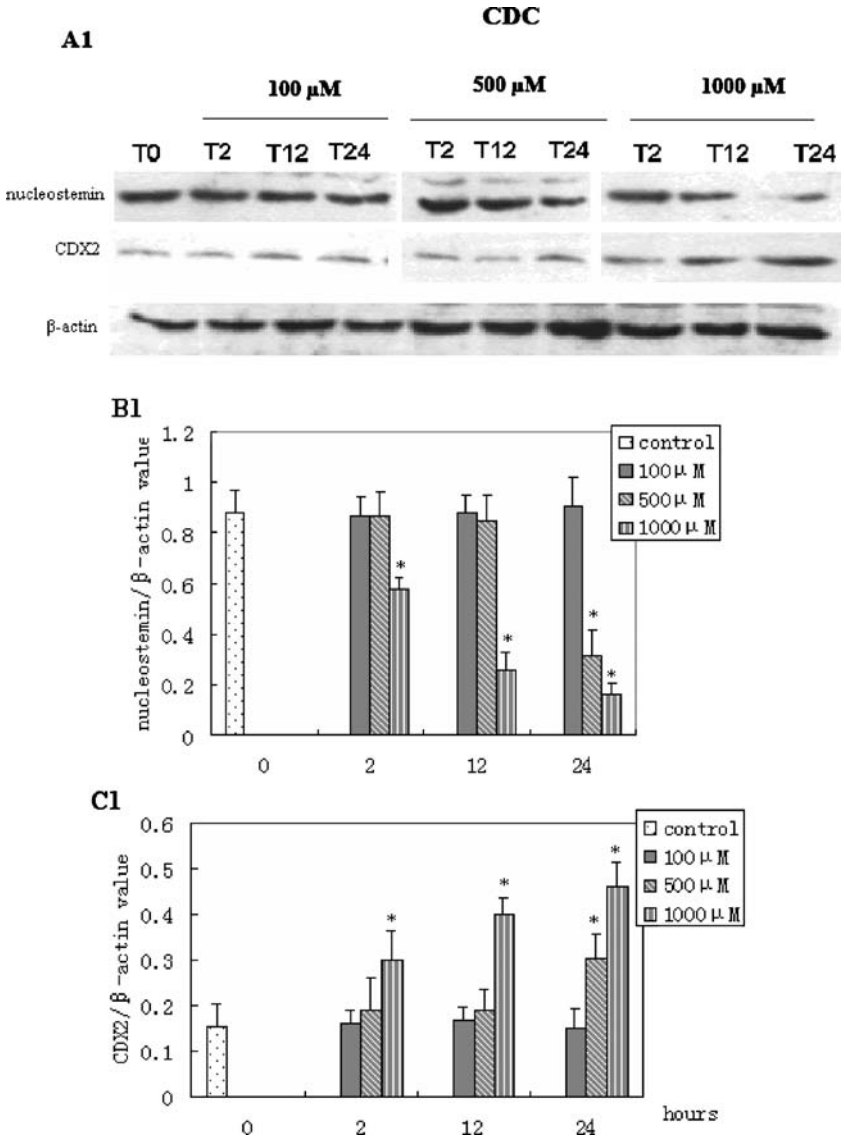


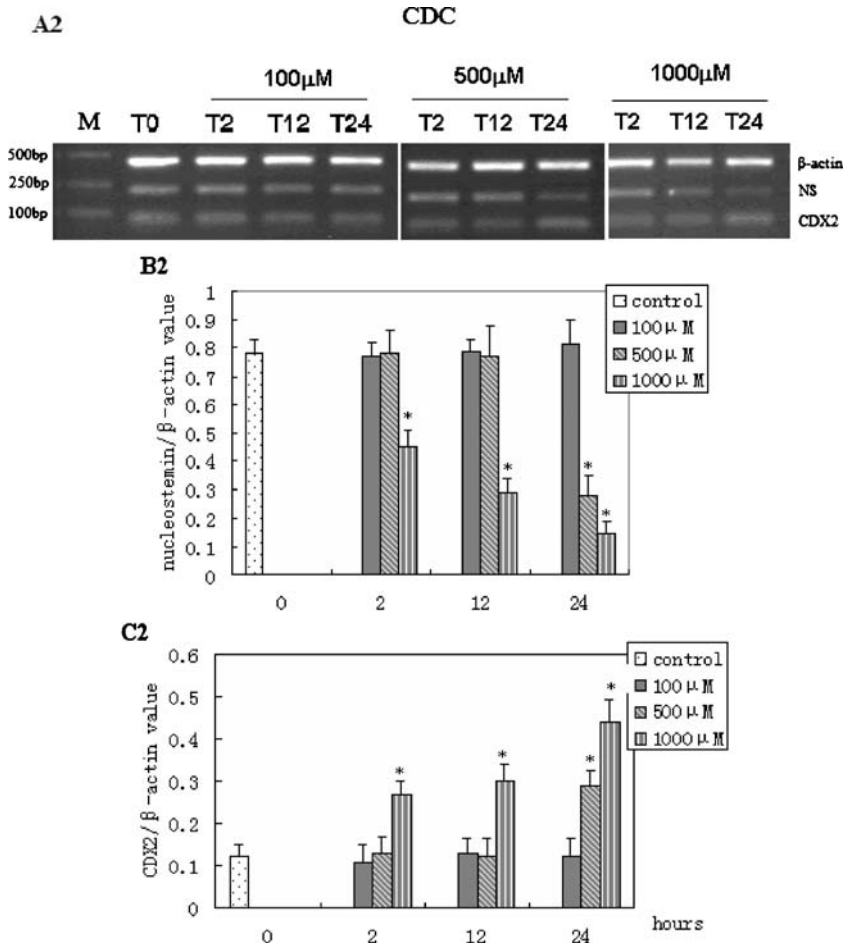
Figure 5 Effects of chenodeoxycholic acid (*CDC*) on the production of nucleostemin and CDX2 in HT29 cells as shown in Western blot assay and RT-PCR. (*A1*) After incubation with various concentrations of CDC (100, 500, or 1,000 mM) for 2, 12, and 24 h, protein (25 μg) was extracted and subjected to Western blot analysis as described in Fig. 3. (*B1*) and (*C1*) Results are expressed as the mean (SD) of three experiments. (*A2*) Effect of chenodeoxycholic acid (CDC) on the mRNA expression of *nucleostemin* and *CDX2* in HT29 cells, as detected by RT-PCR (β-actin was used as a control). *M*, DL2000; *T* indicates time points (i.e., 2, 12, and 24 h). After incubation with various concentrations of CDC (100, 500, or 1,000 μM) for 2, 12, and 24 h. (B2) and (C2) Results are expressed as the mean (SD) of three experiments. *, $P<0.05$, compared with untreated HT29 cells.

of nucleostemin expression in esophageal stem cells promotes the cells to differentiate toward an intestinal epithelial lineage by upregulating CDX2.

It has been well established that chronic gastroesophageal reflux disease (GERD) is the most important etiological factor for BE and adenocarcinoma.[12] It is widely accepted that chronic GERD leads to inflammation and ulceration of the esophageal squamous mucosa, which if persistent and recurrent, leads to columnar metaplasia and eventually to "intestinal" metaplasia. Growing evidence suggests that bile reflux is important in the etiology of BE. In animal studies, bile acids, especially in acid environments, accumulate in esophageal mucosal cells and cause cell membrane and tight junction dissolution.[13] This process allows acid and activated pepsins access to the submucosal region, precipitating more severe injury. Bile acids also increase the gastric fluid pH to 3–5, a range which promotes phenotypic differentiation of cardiac-type mucosa toward specialized intestinal-type glandular epithelium.[14] Several human studies have identified esophageal bile reflux as a risk factor for BE.[15] In addition, studies specifically investigating BE risk and duodenogastric reflux have reported a correlation between bile acid levels in refluxate and the presence of BE.[16]

The molecular and genetic events underlying the pathogenesis of BE, particularly the cell of origin, are poorly understood.[17] Stem cells are present throughout embryonic development as well as in several organs of the adult. They constitute a pool of undifferentiated cells with the remarkable ability to perpetuate through self-renewal while also retaining the potential to terminally differentiate into various mature cell types.[18] Recently, there is *in vitro* and experimental evidence to support the possibility that pluripotent stem cells may be derived from either undifferentiated mesenchymal cells in the lamina propria or the

Fig. 5 continued.



bone marrow.[19,20] Accumulating clinical and experimental studies suggest that the esophageal mucosal gland ducts harbor stem cells capable of differentiating into the columnar epithelium.[21–24] Detailed analysis of mitotic figures in the esophageal epithelium combined with immunohistochemical staining for proliferating cells has also demonstrated that cells in the flat interpapillary basal layer are candidates for esophageal epithelial stem cells.[25] There is also accumulating evidence that the squamous-to-columnar metaplastic sequence occurs through an intermediate, or transitional, phase characterized by the presence of an epithelium that shows combined squamous and columnar features, termed "multilayered epithelium.[21–26]

CDX2 is a nuclear transcription factor that has an important role in the early differentiation and maintenance of the intestinal epithelial phenotype.[27] CDX2 is specifically expressed in the small and large intestines and has been shown to activate other intestinal differentiation genes.[28,29] In normal intestinal epithelium, CDX2 is expressed in most cell lineages.[10] Squamous epithelial cells of normal human esophagus do not express CDX2, while submucosal glands weakly express CDX2 protein in the cytoplasm. In human BE, CDX2 is expressed in both goblet and nongoblet cells.[30–32] In esophageal adenocarcinoma, a high level of CDX2 expression was usually associated with well or moderate differentiation.[33,34] CDX2-mediated expression of cell adhesion proteins such as e-cadherin, LI-cadherin, and claudin-2 appears to play a role both in maintaining intestinal cell morphology and polarity.[35] Recently, CDX2 has been shown to be a useful marker of intestinal metaplasia in the diagnosis of Barrett esophagus.[36]

Because of the difficulty in establishing an appropriate culture model of esophageal stem cells, the effects of bile acids on esophageal stem cells have not been fully tested. We postulate that the nucleostemin may actually arise from stem cells and that these cells are the ones responding to bile acid exposure. To approve this hypothesis, we used the HT29 human colon adenocarcinoma cell line as an *in vitro* model for esophageal stem cells because they have the capacity to differentiate in vitro in response to changes in their extracellular environment and because in their differentiated state the polarized HT29 cells with an apical microvillus border show ultrastructural resemblance to the differentiated cell phenotype of BE.[37,38] Using these cells, we investigated the effect of CDC on the expression of nucleostemin and CDX2 in HT29 cells in vitro and found that exposure to bile

acids inhibits nucleostemin but activates CDX2 expression. In addition, our results also support that HT29 cells may serve as an in vitro model for studying the mechanism underlying the effect of bile acids or other gastroduodenal refluxate components on cellular and molecular biology of BE.

Identification of stem-cell-specific proteins and elucidation of their novel regulatory pathways may help in the development of protocols for the control of the self-renewal and differentiation of the stem cells.[39] Nucleostemin is a newly discovered nucleolar protein present in both embryonic and adult stem cells and also in several human cancer cell lines.[5] This protein is abundantly expressed while the cells are proliferating in an early multipotential state, but it almost disappears at the start of differentiation. Thus, it has been considered that it may be involved in the regulation of proliferation of these cells and can be used as a marker of undifferentiated human adult bone marrow stem cells.[6] Nucleostemin may play an essential role in the specification and/or maintenance of intestinal progenitor cells. Characterization of the zebra fish phenotype will likely provide additional insight into the functional role of nucleostemin in the intestine.[40] The fact that nucleostemin expressed in HT29 cells, but not in the differentiated cells of adult BE, suggests that HT29 cells share a common characteristics with esophageal stem cells and any factors that results in the loss of nucleostemin expression would lead to the intestinal differentiation and the subsequent development of BE. This is in agreement with observation in a study of rodent stem cells that nucleostemin expression was downregulated in mature and terminally differentiated cells, compared with their precursor neural stem cells.[5]

The key steps in the molecular pathogenesis of BE are still largely unknown. It has been shown that the intestinal transcription factor, CDX2, may play a key role in the early columnar differentiation of what are presumably the esophageal stem cells known to be present in the basal layer of esophageal epithelium.[41] In the present study, we observed that CDX2 was overexpressed in the BE biopsy tissue but weakly expressed in HT29 cells, which is consistent with previous observations.[42] Moreover, exposure to a bile acid, CDC, induced CDX2 expression in HT29 cells. These findings suggest that the activation of CDX2 in response to bile acids is associated with the pathogenesis of BE.

CDX2 expression has been reported to be regulated by phosphatase and tensin homolog deleted from chromosome 10, tumor necrosis factor α, and butyrate in colon cancer cells, such as Caco-2 and HT-29.[43,44] It has been reported that chronic acid exposure upregulated the expression of CDX2 in primary squamous epithelial cells of mouse esophagus and in cultured rat esophageal keratinocytes and human esophageal epithelial cells, and the nuclear factor kappa B (NF-κB) pathway plays a critical role in this process.[41,45,46] It has been known that bile acids upregulate both CDX2 and MUC2, a goblet cell-specific factor, in normal esophageal and cancer cell lines and activate the NF-κB and p38 MAPK pathways, which further activate CDX2 expression to regulate downstream genes.[44,47–49] In the present study, the inhibition of nucleostemin activated the expression of CDX2 in HT29 cells. All these data demonstrate that multiple regulatory factors including nucleostemin may have contributed to CDX2 activation in human esophageal epithelial cells in response to gastroesophageal reflux. The potential mechanisms of interaction would be that nucleostemin activates CDX2 promoter via NF-κB and stimulates production of CDX2 in HT29 cells, and thus we could use the mutation analysis of CDX2 promoter to identify the NF-κB binding sites that are responsible for the nucleostemin-induced activation of CDX2 in future studies.

The extracellular environment is known to play an important role in cell proliferation and differentiation. Bile acids upregulate both intestinal differentiation factor CDX2 and goblet cell-specific gene MUC2 in normal esophageal and cancer cell lines.[49] Bile-acid-stimulated expression of the farnesoid X receptor enhances the immune response in BE.[50] Results from mutation analysis of CDX2 promoter suggested that two NF-κB binding sites were responsible for the bile-acid-induced activation of the CDX2 promoter.[41] In the present study, we found that CDC exposure upregulated CDX2 gene expression and downregulated nucleostemin gene expression in a dose- and time-dependent manner in HT29 cells. These findings support the role of bile acids in the pathogenesis of BE.

Conclusion

There is an increased nucleostemin expression but decreased CDX2 expression in BE tissues. *In vitro*, inhibition of nucleostemin results in an increased expression of CDX2. In addition, CDC dose-dependently increases CDX2 production and decreases nucleostemin production in HT-29 cells. These findings suggest that the inhibition of nucleostemin expression in "esophageal stem cells" may be involved in the pathogenesis of BE through upregulating CDX2 expression. Further studies are needed to investigate whether the inhibition of nucleostemin results in the activation of the CDX2 promoter via a transcription factor binding site (e.g., NF-κB).

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ORIGINAL ARTICLE

Obesity and Gastroesophageal Reflux: Quantifying the Association Between Body Mass Index, Esophageal Acid Exposure, and Lower Esophageal Sphincter Status in a Large Series of Patients with Reflux Symptoms

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Abstract
Introduction Obesity and gastroesophageal reflux disease (GERD) are increasingly important health problems. Previous studies of the relationship between obesity and GERD focus on indirect manifestations of GERD. Little is known about the association between obesity and objectively measured esophageal acid exposure. The aim of this study is to quantify the relationship between body mass index (BMI) and 24-h esophageal pH measurements and the status of the lower esophageal sphincter (LES) in patients with reflux symptoms.
Methods Data of 1,659 patients (50% male, mean age 51±14) referred for assessment of GERD symptoms between 1998 and 2008 were analyzed. These subjects underwent 24-h pH monitoring off medication and esophageal manometry. The relationship of BMI to 24-h esophageal pH measurements and LES status was studied using linear regression and multiple regression analysis. The difference of each acid exposure component was also assessed among four BMI subgroups (underweight, normal weight, overweight, and obese) using analysis of variance and covariance.
Results Increasing BMI was positively correlated with increasing esophageal acid exposure (adjusted R^2=0.13 for the composite pH score). The prevalence of a defective LES was higher in patients with higher BMI (p<0.0001). Compared to patients with normal weight, obese patients are more than twice as likely to have a mechanically defective LES [OR=2.12 (1.63–2.75)].
Conclusion An increase in body mass index is associated with an increase in esophageal acid exposure, whether BMI was examined as a continuous or as a categorical variable; 13% of the variation in esophageal acid exposure may be attributable to variation in BMI.

Keywords Obesity · Gastroesophageal reflux disease (GERD) · BMI · Comorbidity · Ambulatory pH monitoring · Lower esophageal sphincter (LES)

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Introduction

Gastroesophageal reflux disease (GERD) is a major health problem. Epidemiologic studies have shown that the prevalence of GERD in Western countries is approaching 20%.[1] This increased prevalence appears to be accelerating. A meta-analysis conducted in 2007 of reports published over the past 20 years suggested that the prevalence has increased by 4%/year in the Western world.[2] In North America, the incidence increased 5% annually between 1992 and 2005.[2]

Obesity has also increased in prevalence during the same period of time.[3] In 1980, the National Health and Nutrition Examination Survey II (NHANES II) reported that the

prevalence of obesity among US adults between the ages of 20 and 75 was 15%. By 2003–2004, the NHANES III study reported that the prevalence of obesity had more than doubled in the 25 years between the studies.[4] It is predicted that by the year 2020, 77.6% of men will be overweight and 40.2% obese; the corresponding predictions for women are 71.1% overweight and 43.3% obese.[5]

The parallel rise in GERD and obesity suggests a link between the two. A recent meta-analysis of 20 studies reported a positive association between increasing body mass index (BMI) and the presence of GERD within the USA.[6] Further, in many chronic diseases such as cardiovascular diseases, cancer, arthritis, and diabetes, obesity appears to be a substantial etiologic factor. Therefore, it is reasonable to enquire if obesity may contribute to the increased prevalence of GERD. However, the literature on this subject is conflicting.[7–11] This conflict may be due to differences in the definition of GERD: surveys that define GERD based on symptom questionnaires may be over-inclusive,[8,10] whereas those based on complications of GERD such as esophagitis, Barrett's esophagus, or esophageal adenocarcinoma are too restrictive.[12–15]

To establish a more convincing relationship between obesity and GERD, the diagnosis of GERD must be made with greater precision. The most objective method of defining GERD is 24-h esophageal pH monitoring. Additional insight into the physiological mechanism underlying the relationship between obesity and GERD requires studies such as esophageal manometry. The invasive nature of these tests precludes their application to large populations of patients. For this reason, there is no large study that has correlated BMI with esophageal acid exposure and lower esophageal sphincter (LES) function. The aim of this study is to quantify the relationship between BMI and esophageal acid exposure and LES status in a large number of symptomatic patients.

Methods

Data were collected on 2,723 subjects with foregut symptoms referred to the Esophageal Diagnostic Laboratory at USC University Hospital between October 1998 and August 2008 who underwent esophageal pH monitoring. The subjects were weighed by laboratory personnel on arrival at the esophageal laboratory. In most cases, height was also measured, but in a small minority of patients, self-reported height was used. BMI was calculated as weight in kg/(height in m)^2. The World Health Organization categories of BMI were used to group the patients into four standard categories: underweight <18.5, normal weight 18.5–24.9, overweight 25–29.9, and obese ≥30. All subjects had esophageal manometry of the LES and esophageal body and 24-h esophageal pH monitoring. Subjects were excluded if there was a technical problem with the test, if the studies were conducted while on acid suppression medication, or if they had a history of previous foregut surgery. Subjects found to have a named motility disorder of the esophageal body (achalasia, diffuse esophageal spasm, and nutcracker esophagus) were also excluded. As a result, 638 subjects were excluded. Of the remaining 2,085 subjects, 1,659 underwent a detailed assessment of the LES using slow motorized pull-through manometry. These 1,659 subjects constituted the study population of this investigation.

Esophageal Manometry

All drugs interfering with foregut function were discontinued for at least 48 h before the study. After an overnight fast, a 12 French 8-channel water-perfused motility catheter (Arndorfer Medical Specialties, Greendale, WI, USA) was passed through the anesthetized nostril into the esophagus and into the stomach. The manometry study was conducted and analyzed as previously described.[16]

Detailed assessment of the LES was performed using slow motorized pull-through manometry. The conduct and analysis of this method has been reported by us previously.[17] This technique has been shown to have superior accuracy and reproducibility compared to the standard manometry.[17] Three characteristics of the LES were assessed: pressure, total length, and abdominal length. When all three components of the LES were normal, the LES was considered mechanically normal and when one or more components were abnormal, the LES was considered mechanically defective.

For the purpose of graphical representation, the LES was stratified on an ordinal scale of 0–3, according to the number of LES components (resting pressure and total and abdominal length) within the normal range: 0, all components defective; 1, only one component normal; 2, two components normal; 3, all three components normal.

Detection of Hiatal Herniation

The presence of hiatal hernia was defined manometrically by the presence of a double hump pattern. This pattern is created by separation of the manometrically observed high-pressure zone in the distal esophagus into two distinct locations with a near-baseline pressure between.[18]

Ambulatory 24-h Esophageal pH Monitoring

Acid-suppression medications were discontinued 3 days (H_2-blocking agents) or 14 days (proton pump inhibitors) before the study. The pH catheter with an antimony sensor

was calibrated in a standard buffer solution at pH 1 and 7 before and after monitoring. The catheter was passed transnasally in order to position the pH sensor 5 cm above the manometrically determined upper border of the LES. Subjects were given dietary instruction to be followed throughout the 24-h monitoring period. They were also asked not to eat or drink between meals; to avoid carbonated beverages, alcohol, or fruit juices; to remain upright (sitting, standing, or walking) throughout the day; and to lie flat at night for sleep. They were instructed to keep a diary for the 24-h period indicating the time of meals, when they went to bed, when they got up, and when symptoms occurred. Esophageal acid exposure was expressed by the standard parameters, namely, the percent time pH was <4 for the total monitored period, and the time spent in the upright and supine positions, the number of reflux episodes, the number of reflux episodes longer than 5 min, and the duration of the longest reflux episode. From these six values, a composite pH score (DeMeester score) was calculated using a commercial software program (PolyGram®). The software also measured the percent time pH was <4 during the 2 h immediately following a meal (post prandial period).

The study was approved by the Institutional Review Board (Reference number, HS-07-00573).

Statistical Analysis

The esophageal 24-h pH components and the composite pH score were compared across BMI groups using analysis of variance (ANOVA) to detect an overall difference. The Bonferroni post hoc test was used to detect differences between pairs of groups. The relationship of each 24-h esophageal pH component with BMI was studied using linear regression and multiple regression analysis to derive the unadjusted and adjusted slope and the 95% confidence intervals. Age, sex, hiatal hernia, and LES status that were identified as significant risk factors in the univariate analysis were used as regressors in all models. The difference of each acid exposure parameter was also assessed among four BMI groups using analysis of variance and covariance.

We further assessed the significance of the difference of the adjusted slope between men and women using the multiple regression model.

In order to explore whether there is a threshold in BMI above or below which there is no relationship between pH and BMI, we repeated the analysis of covariance for each cut point of BMI from 25 through 35 and derived the difference of the adjusted means, the 95% confidence interval (CI), and R^2.

To assess the relationship between BMI and LES status, we examined the risk of a mechanically defective LES in the four BMI groups adjusting for age, sex, and hiatal hernia using the logistic regression model from which we derived the adjusted odds ratios and the 95% CI.

We used the SAS statistical analysis system (The SAS System Release 8.02, SAS Institute, Cary, NC, USA) for all analyses. The MATLAB program (MATLAB, The MathWorks; Natick, MA, USA) was used to create a three-dimensional model showing interaction between BMI, LES status, and esophageal acid exposure. This was performed by plotting an "empiric spline" surface. A modified ridge estimator was used to generate this surface on a two-dimensional grid.

Results

Of the 1,659 subjects, 835 were women (50%) and 824 men (50%). The mean±SD for BMI was 27.7±5.4, and the mean age was 51.4±14.2. The demographic and physiologic characteristics of the study population including the distribution by BMI categories are shown in Table 1. The

Table 1 Demographic and Physiologic Characteristics of the Study Population (n=1659)

Mean age (SD)	51.4 (14.2)
Mean BMI (SD)	27.7 (5.4)
Sex	
Male	824 (50.3%)
Female	835 (49.7%)
BMI categories	
Underweight (%)	16 (1.0%)
Normal (%)	530 (32.0%)
Overweight (%)	640 (38.6%)
Obese (%)	473 (28.5%)
LES status	
Defective (%)	776 (46.8%)
Normal (%)	883 (53.2%)
Hiatal hernia	
Present	715 (43.1%)
Absent	944 (56.9%)
Esophageal pH monitoring components[a]	
% Total time	8.1 (13.0)
% Upright time	9.4 (28.9)
% Supine time	6.7 (14.1)
Number of episodes	99 (116)
Number of episodes >5 min	3.8 (5.6)
Longest episode (min)	17.1 (25.9)
% Post prandial time	11.7 (14.9)
Composite pH score	30.4 (39.1)

[a] Mean (SD)

Table 2 Esophageal 24-h pH Components and Composite pH Score Compared Across BMI Groups

	Underweight (n=16)	Normal (n=530)	Overweight (n=640)	Obese (n=473)	p value[a]
% Total time	1.28±1.73	4.76±8.95	9.16±15.06	10.81±13.09	<0.0001
% Upright time	1.77±2.45	5.10±7.50	11.59±44.37	11.51±13.31	<0.0001
% Supine time	0.21±0.44	3.39±7.90	7.36±15.05	9.55±17.33	<0.0001
Number of episodes	28.6±29.0	60.0±72.4	108.8±122.1	131.8±134.2	<0.0001
Number of episodes >5 min	0.44±1.26	2.21±3.39	4.12±5.96	5.09±6.61	<0.0001
Longest episode	3.81±5.31	11.58±15.60	19.00±26.59	21.30±32.49	<0.0001
% Post prandial time	2.73±4.24	7.45±10.62	12.63±15.78	15.41±16.70	<0.0001
Composite pH score	5.57±6.25	17.73±23.18	33.44±40.60	41.42±46.92	<0.0001

Underweight <18.5, *Normal* 18.5–24.9, *Overweight* 25–29.9, *Obese* ≥30

[a] One-way analysis of variance

mean±SD for the components of the pH record by BMI groups are shown in Table 2. A significant stepwise increase in all parameters of esophageal acid exposure was observed with increasing BMI category. The differences between individual BMI groups using a global measure of esophageal acid exposure (composite pH score) is shown graphically in Fig. 1.

Other Factors

In addition to BMI, we investigated the role of age, sex, hiatal herniation, and presence of a defective LES on esophageal acid exposure. Table 3 provides a comparison of the mean composite pH score between subgroups of these factors. Older age, male sex, hiatal herniation, and presence of a mechanically defective LES are all significant contributing factors for higher composite pH score. The above factors were then included as regressors in the multiple regression analysis where we studied the linear relationship between esophageal acid exposure with BMI.

Multiple Regression Analysis

We studied the relationship of each measure of esophageal acid exposure with BMI using age, sex, hiatal hernia, and LES status as regressors. Table 4 provides the adjusted slope (increase in pH parameter per unit increase of BMI), 95% confidence interval, and percent of variation explained by BMI (R^2). For each component, the adjusted increase in that component per unit increase of BMI was significant (p<0.0001). On the basis of the adjusted R^2 value, 13% of the variability in the composite pH score may be attributable to variation in BMI.

Each unit increase in BMI was associated with an increase in the composite pH score of 1.46 (95% CI, 1.13–1.79) unit. This increase in composite pH score was higher in men [1.79, CI (1.18–2.39)] than in women [1.31, CI (0.92–1.69)]; however, the difference was not statistically significant (p=0.19).

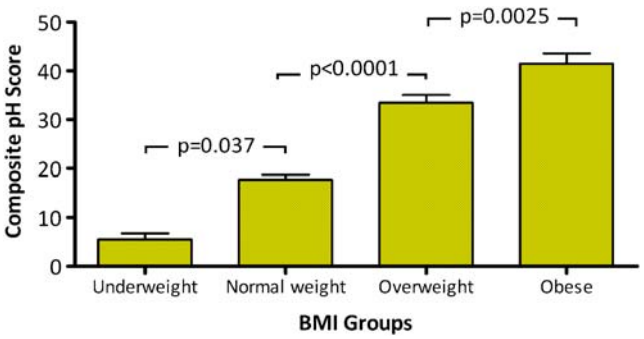

Figure 1 Composite pH score (mean, SE) across BMI groups. A significant difference between all BMI groups was observed (p< 0.0001, ANOVA). Post hoc tests used to calculate the statistical significance of differences between each two adjacent individual BMI groups.

Table 3 Comparison of the Composite pH Score by Risk Factor

Risk Factor	Subgroup	N	Mean ± SD	p value[a]
Age	≥55	704	32.3±38.5	0.004
	<55	955	29.1±39.5	
Sex	Male	824	35.5±40.3	<0.0001
	Female	835	25.4±37.3	
Hiatal hernia	Present	715	33.7±38.4	<0.0001
	Absent	944	28.0±39.5	
LES	Defective	776	20.7±25.4	<0.0001
	Normal	883	20.7±48.0	

[a] Wilcoxon two-sample test

Table 4 Multiple Regression Analysis for Esophageal 24-h pH Components and Composite pH Score on BMI Adjusted for Age, Sex, Hiatal Hernia, and LES Status

	Adjusted slope	95% CI of slope	Adjusted R^2	Adjusted p value
% Total time	0.35	0.24, 0.46	0.0869	<0.0001
% Upright time	0.37	0.11, 0.63	0.0254	0.0046
% Supine time	0.38	0.26, 0.50	0.0795	<0.0001
Number of episodes	4.41	3.44, 5.38	0.1275	<0.0001
Number of episodes >5 min	0.18	0.13, 0.23	0.0949	<0.0001
Longest episode	0.62	0.39, 0.85	0.0486	<0.0001
% Post prandial time	0.48	0.36, 0.61	0.0881	<0.0001
Composite pH score	1.46	1.13, 1.79	0.1264	<0.0001

Exploration for BMI Thresholds

We further explored to determine if a threshold existed in BMI above or below which the positive relationship between pH and BMI ceased to exist. Table 5 shows the difference in the adjusted mean and R^2 for each cut point of BMI from 25 through 35 for the percent total time pH<4. It can be observed that the relationship is constant and that there is no distinct threshold above which the effect of BMI is maximal.

LES Status and BMI

The prevalence of a mechanically defective LES increased in higher BMI groups (p<0.0001, Fig. 2). Table 6 presents the adjusted odds ratio and 95% CI for the risk of LES by different risk groups including BMI group, age, sex, and hiatal hernia status. Hiatal hernia and obesity and overweight were shown to have a significant effect on risk of a defective LES, but age and sex had no significant effect. The relative contribution of BMI and LES pressure to esophageal acid exposure was demonstrated by plotting these parameters in a three-dimensional graph. Figure 3 shows this interaction.

Discussion

Two common diseases of contemporary Western society are GERD and obesity. Despite the many parallels between their epidemiology and presumed etiology, the relationship between the two disease processes remains incompletely understood. The major finding of this study is that the degree of esophageal acid exposure is strongly associated with increasing weight. This was true whether BMI was examined either as a continuous variable or as a categorical variable. The relationship is even stronger after adjusting for the known effect of age, sex, hiatal herniation, and LES status. Other workers, including ourselves, have reported comparable findings in much smaller series.[19–21] The major strength of the current study lies in the very large number of subjects studied, with a wide range of BMI and esophageal acid exposure.

For every unit increase in BMI, the percent total time pH<4 increased by 0.35% (95% CI, 0.24–0.46). This effect

Table 5 Exploration of BMI Cut Point for Detecting Difference in Percent Total Time pH<4

BMI cut-point	Difference of adjusted means	95% CI for difference	Adjusted R^2	Adjusted p value
<25 vs. ≥25	−3.86	−5.17, −2.54	0.0838	<0.0001
<26 vs. ≥26	−3.33	−4.56, −2.10	0.0811	<0.0001
<27 vs. ≥27	−3.42	−4.64, −2.20	0.0823	<0.0001
<28 vs. ≥28	−3.40	−4.63, −2.17	0.0817	<0.0001
<29 vs. ≥29	−3.74	−5.00, −2.47	0.0841	<0.0001
<30 vs. ≥30	−3.18	−4.52, −1.84	0.0776	<0.0001
<31 vs. ≥31	−3.06	−4.50, −1.62	0.0752	<0.0001
<32 vs. ≥32	−2.99	−3.55, −1.43	0.0734	0.0002
<33 vs. ≥33	−3.09	−4.82, −1.37	0.0724	0.0004
<34 vs. ≥34	−3.48	−5.37, −1.59	0.0728	0.0003
<35 vs. ≥35	−4.33	−6.43, −2.23	0.0746	<0.0001
<36 vs. ≥36	−3.63	−6.04, −1.21	0.0703	0.0032

Analysis of covariance on percent total time pH<4 comparing BMI < and ≥ cut point adjusted for age, sex, hiatal hernia, and valve status

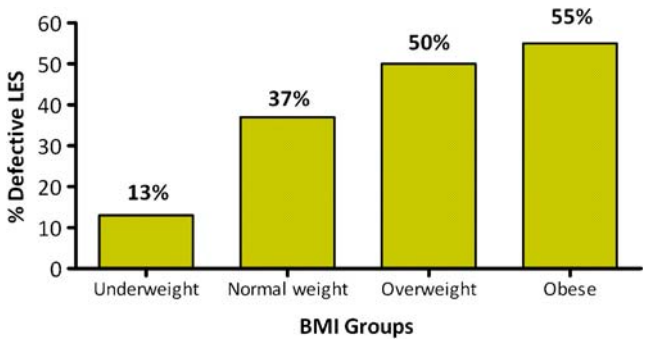

Figure 2 Prevalence of a defective LES across BMI groups ($p<$ 0.0001, chi-square test).

of BMI on esophageal acid exposure appears to be continuous, since no particular BMI cutoff point was associated with a larger difference in the percent total time pH<4 (Table 5). The R^2 value calculated by multiple regression analysis in our series indicates that 13% of the change in esophageal acid exposure may be explained by variation in the BMI. This degree of association is much greater than the values typically reported for other recognized relationships between BMI and obesity-related comorbidities. Studies correlating BMI and blood pressure have reported values ranging from 5% to 9%, and the magnitude of correlation between BMI and blood sugar and high-density lipoprotein-cholesterol and triglycerides is even less.[22,23]

In concert with the greater degree of reflux observed in heavier subjects, we also observed a greater frequency of a mechanically defective LES. We found that age and sex have minimal effect on the status of the LES. Compared to patients with normal weight, obese patients were more than twice as likely to have a defective LES [OR=2.12(1.63, 2.75)]. In those with a hiatal hernia, the likelihood of a defective LES was also twice as great as those without hiatal herniation [OR=2.36 (1.93–2.89)]. These two observations suggest that the effect of obesity on the LES status is almost as great as the effect of hiatal herniation.

Reflux of gastric juice usually results from either a defective LES or transient loss of LES pressure. We focused on the correlation between presence of a defective LES and increasing BMI. Other workers have reported that obesity is associated with increased frequency of transient lower esophageal sphincter relaxation.[24] It is therefore clear that obesity has the potential to affect both these two mechanisms of reflux.

The effect of increasing BMI on the different components of pH record may shed light on the mechanism of obesity-induced reflux. For each unit increase in BMI, the increase in percent post prandial time was 0.48 compared to 0.35 for the percent total time. This may reflect the eating habit of obese subjects.

We also found that the association between BMI and esophageal acid exposure was stronger during the supine period compared to the upright position. One potential explanation is that the influence of increased intra-abdominal pressure found in obesity may be maximal in the supine position.

The relative contribution of BMI and LES status to esophageal acid exposure can be conceptualized in a three-dimensional model showing the interaction between BMI and LES and esophageal acid exposure. As BMI increases and the status of the LES deteriorates, esophageal acid exposure peaks, suggesting an additive effect (Fig. 4).

We acknowledge several limitations of this study. The absence of endoscopic data in the study subjects precludes any comment on the relation between BMI and esophageal mucosal damage. The identification of hiatal herniation in the study population was based on manometric criteria since consistent radiologic information was not available for all subjects. However, manometric identification of hiatal herniation has been reported to be highly specific when compared to endoscopic evaluation.[25] The sensitivity of our manometric identification appears higher than that of the other reports, most likely because of the greater accuracy of identifying the double hump in the artifact-free tracings produced by the slow motorized pull-through technique.[18,25]

Another potential limitation is selection bias related to the referral pattern of the subjects. Although our esoph-

Table 6 Logistic Regression Analysis for LES Status on BMI Group, Age, Sex and Hiatal Hernia Adjusted for Parameters in the Model

Parameter	Adjusted OR	95% CI	Adjusted p value
BMI group			
Obese vs. normal	2.115	1.632, 2.747	<0.0001
Overweight vs. normal	1.687	1.320, 2.161	<0.0001
Underweight vs. normal	0.238	0.037, 0.880	0.0620
Age: per year increase	1.004	0.996, 1.011	0.3211
Sex: male vs. female	1.021	0.831, 1.254	0.8418
Hiatal hernia: present vs. absent	2.359	1.926, 2.894	<0.0001

Number of cases in model, 1,659 (776 defective valve; 883 normal valve). R^2, 0.0942

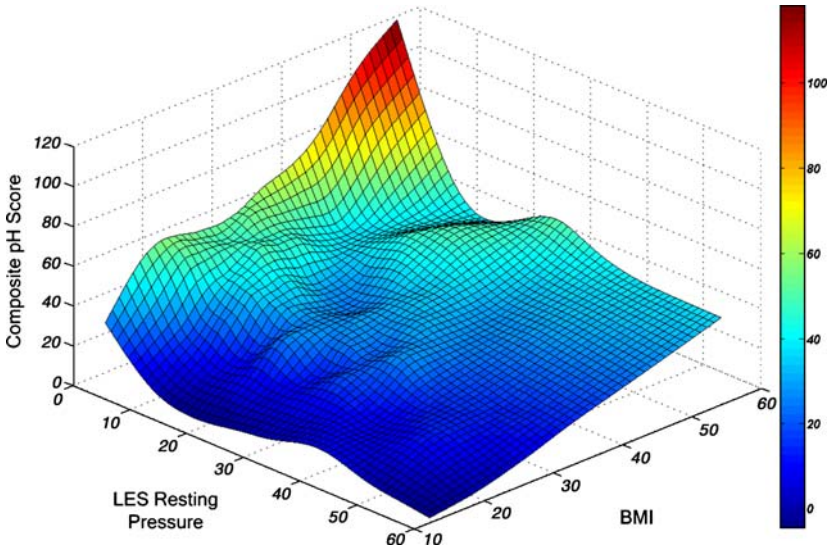

Figure 3 Interaction between LES resting pressure, BMI, and esophageal acid exposure in all subjects (n=1659).

ageal laboratory is a recognized referral center for patients with complex esophageal diseases, we deliberately excluded those complex patients such as those with prior gastric or esophageal surgery, or with named motility disorders, whose results would be irrelevant to the understanding of the general relationship. It is also important to emphasize that subjects were referred to the diagnostic esophageal laboratory for the physiologic studies and not specifically for a surgical opinion. We recognize that the subjects in this study were all symptomatic patients, and the findings cannot be extrapolated to the asymptomatic population.

This relationship between BMI and esophageal acid exposure suggests that the same environmental influences are responsible for the epidemic of both diseases in contemporary Western society. There is evidence that the volume and fat content of the diet are associated with increased esophageal acid exposure.[26] In addition, high caloric diets have been shown to increase esophageal acid exposure.[27,28] It is therefore likely that the same dietary habits can promote both diseases. The healthcare implications of our study are potentially far reaching. For example, the reduction of weight by surgical or pharmacological intervention has reduced obesity-related comorbidities such as diabetes and cardiovascular disease: the possibility that weight reduction may also reduce or prevent the development of the complications of GERD, including reflux-induced lung disease, Barrett's esophagus, and esophageal adenocarcinoma, is ripe for further study.

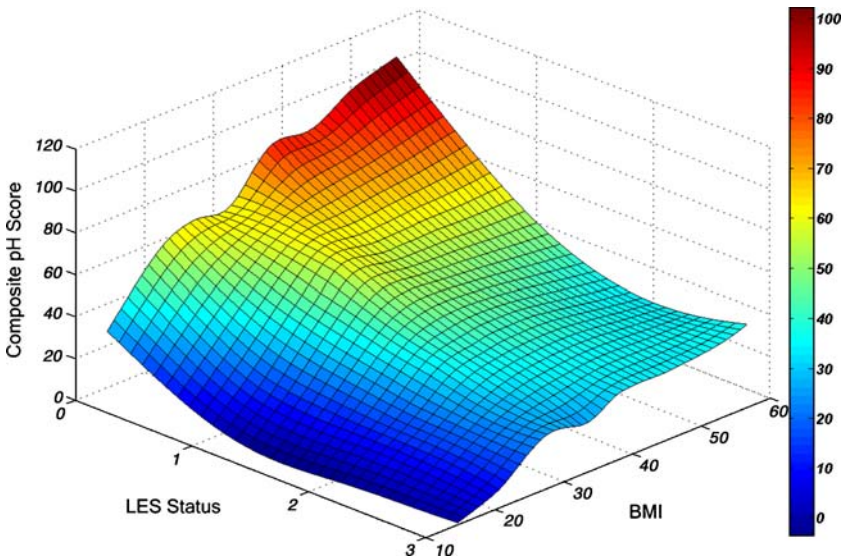

Figure 4 Interaction between LES status, BMI, and esophageal acid exposure in all subjects (n=1659). LES status stratified on an ordinal scale of 0–3, according to the number of LES components (resting pressure and total and abdominal length) within the normal range: *0* all components defective, *1* only one component normal, *2* two components normal, *3* all three components normal.

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ORIGINAL ARTICLE

Distal Intestinal Obstruction Syndrome (DIOS) in Patients with Cystic Fibrosis After Lung Transplantation

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Abstract
Background Cystic fibrosis (CF) is the commonest inherited life-threatening disease in Caucasians. With increased longevity, more patients with CF are developing gastrointestinal complications including the distal intestinal obstruction syndrome (DIOS), in which ileocecal obstruction is caused by viscid mucofeculent material. The optimal management of DIOS is uncertain.
Methods The medical records of all patients with CF who underwent lung transplantation at this institution during a 15-year period were reviewed. The definition of DIOS required the presence of both clinical and radiological features of ileocecal obstruction.
Results One hundred twenty-one patients with CF underwent lung transplantation during the study period. During a minimum 2-year follow-up, there were 17 episodes of DIOS in 13 (10.7%) patients. The development of DIOS was significantly associated with a past history of meconium ileus (odds ratio 20.7, 95% C.I. 5.09–83.9) or previous laparotomy (odds ratio 4.93, 95% C.I. 1.47–16.6). All six patients who developed DIOS during the transplantation admission had meconium ileus during infancy, and five had undergone pretransplant laparotomy for CF complications. First-line treatment for all patients was a combination of medication (laxatives, stool softeners, and bowel preparation formulas). This was successful in 14 of the 17 DIOS but needed to be given for up to 14 days. The other three patients required laparotomy with enterotomy and fecal disimpaction. This provided definitive resolution of DIOS except in one patient who presented late and died despite ileal decompression and ileostomy.
Conclusions DIOS occurred in approximately 10% of CF patients after lung transplantation. Patients with a history of meconium ileus or previous laparotomy are at high risk of developing DIOS. Patients with DIOS require early aggressive management with timely laparotomy with enterotomy and possible stoma formation when non-operative therapy is unsuccessful.

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Keywords Cystic fibrosis · Lung transplantation · Distal intestinal obstruction syndrome · Intestinal obstruction · Meconium ileus.

Introduction

Cystic fibrosis (CF) is the most common inherited life-threatening disease in Caucasians, with an incidence of approximately one per 3,500 live births.[1] Lung function deterioration remains the primary determinant of survival. Since 1983, when lung transplantation was first performed on a patient with CF, transplantation has been further refined and is now a standard therapy for patients with end-stage disease. Almost one third of lung transplants are

performed for CF.[2] Improvements in diagnosis and therapy in recent decades have led to a marked increase in longevity, and median survival for CF patients is currently estimated at 36.9 years.[1]

Distal intestinal obstruction syndrome (DIOS), previously called "meconium ileus equivalent," is a syndrome that can occur in patients with CF at any time after infancy but is commonest in adolescence or adulthood. There is no standard definition for DIOS, but the syndrome is characterized by partial or complete bowel obstruction due to the abnormal accumulation of viscid mucofeculent material in the terminal ileum and cecum.[3] An important factor in the etiology of this viscid material is pancreatic insufficiency. Other etiologic factors include dehydration, constipating medications, and immobility.[4]

The reported clinical features of DIOS range from those of minor partial intestinal obstruction (constipation, any abdominal distension) through to those of severe obstruction and its complications. Probably because of this wide range in diagnostic criteria and definition, the reported incidence of DIOS in adults with CF ranges from 4.5% to 41.3%.[5]

The combination of an increase in the number of CF patients undergoing lung transplantation and the increased longevity of these patients seems to have resulted in more patients developing post-transplantation DIOS, but the number of DIOS patients is nevertheless small. Gastrointestinal surgeons, even those who work in a center where lung transplantation is performed, are unlikely to have a large experience with DIOS and may be completely unfamiliar with the syndrome. This may be a factor contributing to the mortality associated with this condition in some series.[6] After noting an increase in the number of patients with DIOS at our institution, with some poor outcomes, we undertook this study in order to improve our management of this condition. In particular, we sought to clarify the indications for operative therapy.

Methods

The prospectively collected computer database of all patients who have undergone lung transplantation at this institution was reviewed in accordance with our institutional ethics committee guidelines. Patients with CF were identified and their demographic and clinical data retrieved from the database and the medical records. All patients with CF who underwent lung transplantation over the 15-year period from 1st August 1989 to 1st August 2004 were included. DIOS was defined as occurring when patients had both clinical and radiological (Fig. 1) evidence of ileocecal obstruction. All episodes of DIOS in the study population between 1st August 1989 and 1st August 2006 were studied, thus providing a minimum 2-year follow-up for all patients, and were incorporated in the final data sets. Details of pretransplant workup, preoperative preparation, operative details, and postoperative management were recorded. There were no exclusion criteria.

Fisher's exact test was used to compare proportions between two groups. Continuous data were compared using the Mann–Whitney U test. All P values are two-sided. SPSS version 10.0.5 software (SPSS, Chicago IL, USA) and GraphPad Instat3 software (GraphPad Software, San Diego CA, USA) were used. All values are shown as median with (range) or as number of patients with (percentage). Statistical analysis was conducted based on the patients who had an episode of DIOS rather than the individual episodes.

Results

A total of 121 transplants were performed on 121 patients with cystic fibrosis during the study period. Details of the transplant operation and overall outcomes are provided

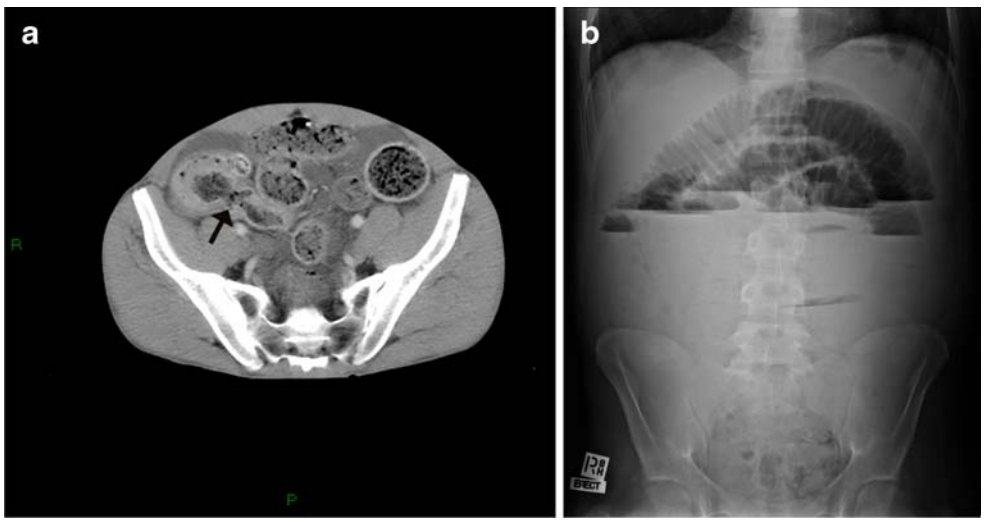

Figure 1 **a** Abdominopelvic CT scan with oral and intravenous contrast showing mucoviscous material within the terminal ileum and cecum (*arrow*), cecal wall thickening, and ascites. **b** Plain abdominal radiograph showing high-grade small-bowel obstruction. In more severe cases, the mucofeculent material may fill the entire alimentary tract, resulting in a "gasless abdomen" on plain X-ray.[37]

elsewhere.[7,8] Sixty-two (51%) of the patients were male, the mean age at transplant was 27 years (range 13–56 years), and the mean duration of follow-up was 67 months (range 1–179 months). One hundred twelve patients were taking pancreatic supplementation pretransplantation. Forty-eight (40%) patients died during the study period. All patients received four tablets of coloxyl with senna in divided doses and sorbitol 20 ml daily during the postoperative period. There was no preoperative bowel preparation protocol.

During the minimum 2-year follow-up period, 13 (10.7%) patients had 17 episodes of DIOS. No patient had more than two DIOS episodes. As shown in Table 1, the development of DIOS was unrelated to age at transplantation, sex, or use of pancreatic enzyme supplements. Season, including spring and summer versus autumn and winter, was also not a significant factor.

Six of the 17 DIOS episodes occurred in six patients during the transplantation admission at a median 7 days (range 3–14 days) post-transplant. All patients had meconium ileus in infancy, and five had undergone a pretransplant abdominal operation. The other 11 episodes occurred during readmissions (for DIOS) between 5 months and 10 years after transplantation. Most of the pretransplant laparotomies were performed for meconium ileus (five patients) or were probably performed for meconium ileus (three patients: one right hemicolectomy; one intestinal perforation, one small bowel obstruction; these patients were not classified as having had meconium ileus because of uncertainty of diagnosis), but two patients underwent laparotomy for biliary complications of CF.

As shown in Table 1, a history of meconium ileus (by definition in infancy) and of pretransplant abdominal operation were both significantly associated with the development of DIOS after transplantation. DIOS occurred in ten of 15 patients with a history of meconium ileus compared to three of 93 without this history and in six of 22 patients who had undergone pretransplant abdominal operation compared to seven of 99 patients without this history.

First-line treatment for all patients was a combination of laxatives, stool softeners, and bowel preparation formulas. The median and mean number of days to resolution of the DIOS with "conservative" management was 3 days and 3.67 days, respectively (range 1–14 days). Osmotic solutions given were Gastrografin (nine patients), sorbitol (seven patients), Glycoprep (two patients), and sodium picosulphate (Picolax, Picoprep) or lactulose (both two patients). Six patients received coloxyl with senna tablets, and five were given the emulsifier Polysorbate 80 (Tween 80, ICI Americas, Wilmington, DE, USA). Enemas were fleet (six patients), coloxyl (three patients), travade (three patients), or microlax enemas (two patients). The stool softener Agarol was given to three patients. This medical therapy provided successful treatment, defined as bowel opening and resolution of abdominal pain, in 14 of the 17 DIOS episodes (ten of the 13 patients) after 1 to 14 days. Bowel opening was accompanied by marked relief of abdominal pain and distension in all cases.

Four laparotomies were required in three patients after failure of medical therapy. Two of these operations were performed because of suspected intestinal ischemia, but viable bowel was found at operation in both patients. Successful resolution of DIOS was achieved by fecal disimpaction via ileostomy in one patient, but a lack of awareness of DIOS by the treating surgical team resulted in inadequate disimpaction being performed in the other patient. This patient required a second laparotomy after abdominal compartment syndrome, with consequent respiratory failure that developed. Hard putty-like stool extending proximally from the cecum and terminal ileum to the duodenal–jejunal flexure in this patient was found at laparotomy. Despite adequate fecal disimpaction being performed with an end ileostomy during the re-laparotomy, the patient died of respiratory disease 25 days postoperatively. The other patient underwent laparotomy for possible appendicitis complicating DIOS. Operative findings were of DIOS only, and fecal disimpaction performed through a cecotomy was curative.

Table 1 Risk Factors for Developing DIOS

	DIOS episode	No DIOS episode	*P* value	Odds ratio (95% confidence interval)	Relative risk (95% confidence interval)
Number of patients	13	108	–	–	–
Male	9 (69%)	53 (49%)	0.247	–	–
Age at transplant (years)[a]	26 (13–56)	25.5 (16–44)	0.243	–	–
Duration of follow-up (years)	6 (3–13)	7 (2–16)	0.814		
Pancreatic supplementation	12 (92%)	100 (93%)	0.964	–	–
History of neonatal meconium ileus	10 (71%)	15 (14%)	<0.0001	20.7 (5.09–83.9)	12.8 (3.81–43.1)
History of pretransplantation bowel operation	6 (46%)	16 (15%)	0.014	4.93 (1.47–16.6)	3.86 (1.44–10.4)

[a] Continuous data shown as median and (range)

Discussion

Distal intestinal obstruction syndrome, previously termed meconium ileus equivalent, is a well-recognized complication in patients with CF.[4,9–12] This study is one of the largest series of patients with cystic fibrosis who have undergone lung transplantation. The relatively large patient numbers and the strict criteria used for DIOS diagnosis, with both clinical and radiologic evidence of ileal obstruction required, suggests that the 10% incidence of DIOS in our study is likely to be an accurate estimate of the risk of developing DIOS after lung transplantation. The actual incidence of DIOS may be higher than this, however, as some patients with early DIOS are successfully treated or even self-treat without hospitalization. Our DIOS incidence of one in ten patients is comparable with most studies' estimates of 10–20%,[13–16] although the reported range is wide (2–41.3%).[5,17–19]

Similar to the reports by Minkes et al. and Gilljam et al., this study confirms that pretransplantation abdominal operation is a significant risk factor for developing DIOS.[4,14] Whether a past history of meconium ileus is a risk factor for DIOS in nontransplant patients has been disputed, with conflicting findings in previous reports,[4,5,13,20–23] but we found that previous meconium ileus was the strongest predictor of developing DIOS; patients with this history had a 12.8 times increased risk of DIOS compared to patients with no history of meconium ileus (Table 1). These findings suggest that meconium ileus and previous abdominal operations may be markers for more severe alimentary tract disease, especially worse mucoviscidosis and hypomotility, and hence a higher risk of DIOS.

The findings also indicate that patients at high risk of DIOS post-transplant can be identified preoperatively. In consequence, consideration should be given to initiating preventative therapy for DIOS pretransplantation for these high-risk patients. Boyle et al. from the John Hopkins Adult CF Program report that the routine use of GoLytely (Braintree Laboratories; Braintree, MA, USA) pretransplantation at their institution has virtually eliminated DIOS.[24] At our institution, patients routinely received simple laxatives (sorbitol, coloxyl with senna) after transplantation, but our results indicate that this regimen is inadequate for the patients most likely to develop DIOS. In most cases, we have inadequate time between admission and transplantation for DIOS prophylaxis immediately prior to transplant. An alternative strategy is to initiate some preventative therapy when patients join the transplant waiting list.

Successful treatment of DIOS requires early diagnosis with exclusion of alternative pathologies. In our experience, this requires a computed tomography (CT) scan with oral contrast showing mucoviscous material filling the distal small bowel (Figs. 1a and 2), especially since adhesional small-bowel obstruction, for which DIOS standard treatment may be hazardous, is a differential diagnosis in the patients with a history of previous laparotomy. Plain abdominal X-ray is less helpful for diagnosis but may have a role in monitoring the degree of obstruction and intestinal diameter (Fig. 1b). As with other patients with intestinal hypomotility, operation should be avoided if at all possible because of the risk of later mechanical obstruction due to adhesions, as well as the morbidity and potential mortality associated with surgery in this group of patients.[25] Initial medical management includes rehydration and early reintroduction of pancreatic supplementation.[14] *Nil per os* and nasogastric aspiration are indicated if there is evidence of high-grade obstruction. Other conservative measures such as minimizing narcotic use and early mobilization, if possible, may also be beneficial.

Numerous studies advocate the use of osmotic solutions in the treatment of DIOS.[9,11,18,26] Glycoprep® (Macrogol 3350, multiple manufacturers), GoLytely® and NuLytely (both Braintree Laboratories, Braintree, MA, USA), and Klean-prep® (Norgine Ltd, Harefield, Middlesex, UK) are osmotic agents containing polyethylene glycol. They have water and electrolyte concentrations that are iso-osmotic with normal gastrointestinal contents, thus avoiding large fluid shifts on administration. Recommended doses are typically 20–40 ml kg^{-1} h^{-1} up to a maximum of 1 L/h. Gastrografin® is a hypertonic contrast medium that has been widely described as both an oral and an enema treatment for DIOS. Its use was first described by Noblett in 1969 after observing relief of obstruction following a diagnostic Gastrografin enema.[27]

Complications with Gastrografin enemas for treating DIOS, including necrotizing enterocolitis, shock, perforation, and death, have all been reported.[28] In a review by Rescorla et al., the success rate for treating DIOS with Gastrografin enemas was approximately 55% with a perforation rate of 11%.[12] As perforations were occurring despite low infusion pressures, it was hypothesized that the osmotic properties of undiluted Gastrografin were responsible, and trials have subsequently shown that diluted Gastrografin is safer and equally effective.[19,29] Enema protocols include 100 ml diluted four times with water and administered up to twice daily. Oral Gastrografin can be considered in those not vomiting. An example of a standard regimen is 100 ml Gastrografin diluted with 400 ml water or juice on day 1, and half doses on subsequent days should this be required. Shidrawi et al. have published a small series of emergency colonoscopic enemas where 500 ml of half-strength Gastrografin mix was introduced at the limit of the examination. There were no complications relating to the procedure, and resolution was achieved in 14 out of 16 DIOS episodes.[17]

N-Acetylcystine (Parvolex®) administered orally, via a nasogastric tube, or as an enema has been used in both the

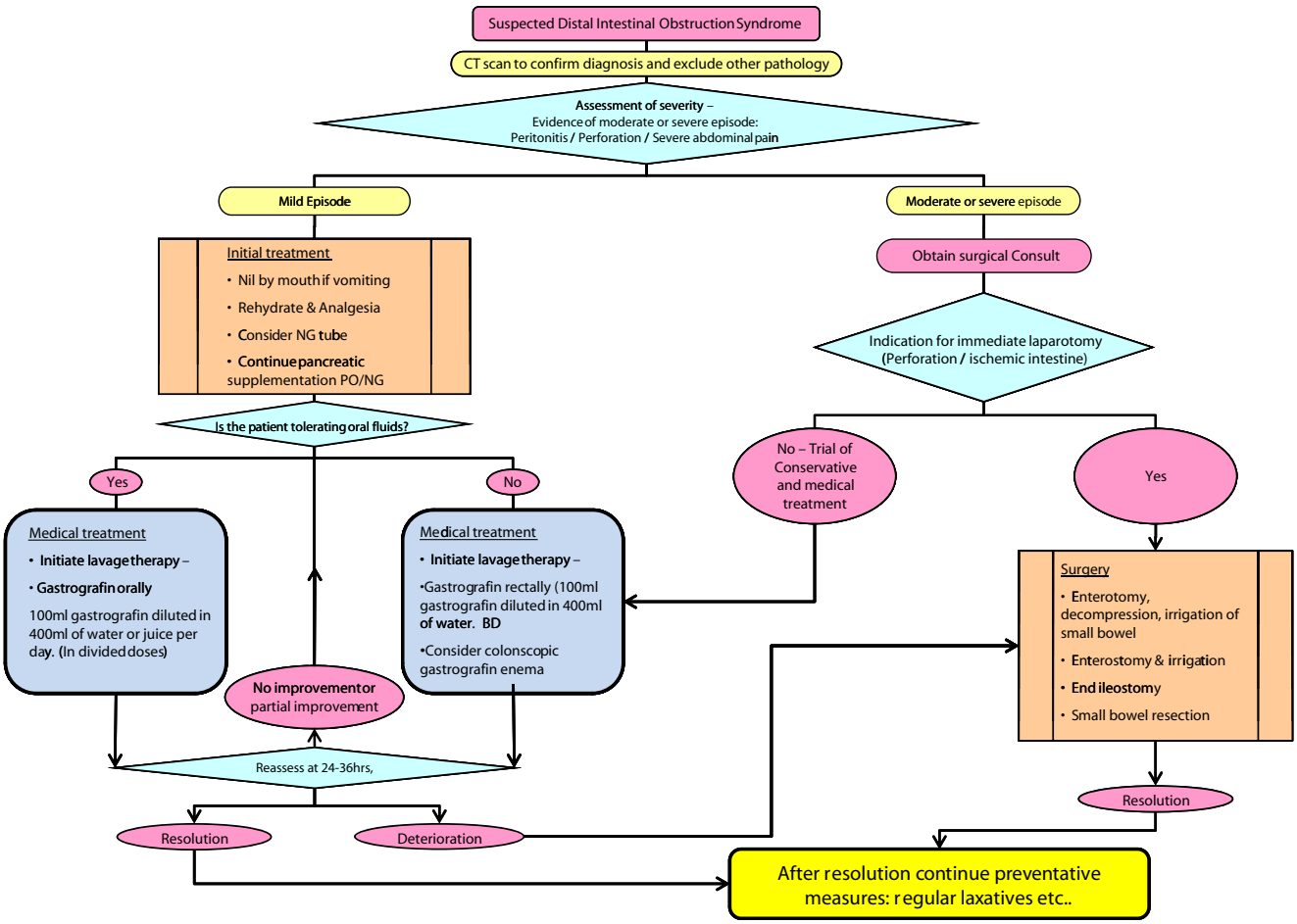

Figure 2 Flow chart for diagnosis and management of DIOS.

prevention and treatment of DIOS with varying degrees of success.[6,12,15] The *N*-acetylcystine is thought to act as a mucolytic that disrupts the protein matrix of the inspissated plug in the distal ileum. Typical doses are 10 ml of solution three times a day (10–20 g in 100 ml concentrate) orally or 100 ml of a 50% solution given as an enema.[29] Complications associated with this therapy range from hypernatremia to acute hypomagnesaemia.[30,31]

Surgery is generally considered when medical treatment has failed or when there are indications for immediate laparotomy, such as intestinal ischemia or perforation. Prior to the first report of surgery for meconium ileus in 1948 by Hiatt et al. (enterotomy and saline irrigation), this condition was almost always fatal.[32] There have subsequently been a diverse range of surgical procedures described for meconium ileus, all of which emphasize the need for decompression of the inspissated material. Decompression is either achieved by enterostomy with or without irrigation or resection of the affected bowel with primary anastomosis.[33,34] A simpler method of performing enterostomy and irrigation was described by Fitzgerald et al., who used an appendicectomy stump as the enterostomy for irrigation of Gastrografin directly into the terminal ileum and reported that the resulting wall defect was easier to close.[35] This surgical technique was used with good effect in one patient in this study. Some advocate giving warm isotonic sodium chloride solution mixed with mineral oil via the nasogastric tube intra-operatively.[36]

Conclusions

Approximately one in ten patients with cystic fibrosis will develop the distal intestinal obstruction syndrome following lung transplantation. Previous meconium ileus or pretransplantation abdominal operations are highly significant risk factors for developing this syndrome, and consideration should be given to implementing DIOS prevention pretransplantation in these patients. This will not always be possible because of the short time interval between admission and transplantation in some patients, but routine preventative strategies that are instituted at transplant listing could be appropriate. A high index of suspicion, early diagnosis, and aggressive non-operative treatment are

essential for successful treatment. Laparotomy, which generally involves enterotomy and evacuation of the lumenal contents, is indicated when medical therapy has failed.

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ORIGINAL ARTICLE

Abdominal Computed Tomography for Diagnosing Postoperative Lower Gastrointestinal Tract Leaks

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Abstract
Background Computed tomography (CT) is the most readily available imaging tool for diagnosis of postoperative lower gastrointestinal tract (LGIT) leak. The accuracy and sensitivity of CT for diagnosing a leak from a hollow viscous or anastomotic bowel leakage are still not well established. This retrospective study was conducted in order to define the role of CT in this setting.
Study Design The medical records of patients who underwent early relaparotomy (within 30 days) due to LGIT leak following a previous surgery in our department between 1998 and 2006 were reviewed. The ones whose abdominal CTs were done within 72 h prior to the repeated surgery with the aim of ruling out an intraabdominal infection or leak were studied, and the results were compared to the postsurgical findings.
Results Seventy patients were reoperated shortly following abdominal surgery due to postoperative LGIT leak. Forty-one of them had undergone 45 CT studies within 72 h before reoperation. Another 29 patients underwent a second procedure based on clinical presentation. Reoperation was done after an interval of 7.3±4.4 days in patients who underwent CT studies and after 4.5±2.3 days in patients without CTs (p=0.003). Preoperative CTs identified only 47% of the leaks.
Conclusions CT studies on patients shortly after abdominal surgery are not definitive. A negative CT study does not rule out LGIT leak. Clinically based decision making and exploratory relaparotomy still do play a role in those patients with suspicion for LGIT leak.

Keywords Computed tomography · Lower gastrointestinal tract · Leak

Introduction

Undiagnosed intestinal anastomotic leak or perforated bowel carries a hazardous outcome. It has been described to occur from 2% to 50% of patients undergoing colorectal surgery. It may result in the need for further interventions, including the incursion of percutaneous drains, proximal diverting ostomy, and even complicated major surgeries. Such leaks are responsible for increased perioperative morbidity and mortality and may account for one third of the deaths following colorectal surgery.[1] Due to the severity of the complications that are associated with leaks from the gastrointestinal (GI) system, it is essential to identify them promptly and manage them appropriately. Some leaks present abruptly, precluding the need for imaging studies to establish the diagnosis. Often, however, the presenting signs may be subtle and confusing, suggesting other less alarming etiologies, whereupon different imaging modalities are used to identify the presence of an anastomotic leak.

None of the available imaging tools was proven to have a superior sensitivity and specificity over the others.[2,3] Although the accuracy and sensitivity of computed tomography (CT) for diagnosing a leak from a hollow viscous or

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anastomotic bowel leakage are not well established, CT is nevertheless widely used for this purpose. Moreover, in the postoperative setting, CT may be less reliable because most the expected postoperative CT features such as extraluminal air or focal bowel wall thickening may overlap with the CT findings that result from a clinically important GI leak.[4] Moreover, in patients who had sustained blunt abdominal trauma, CT with and without oral contrast was not reliable in diagnosing intestinal injuries,[5] while CT could predict the need for explorative laparotomy in penetrating abdominal trauma.[6] In a recently published report on 85 patients with surgically proven GI tract perforation, it was possible to predict the site of the perforation based on the CT findings in 86% of the patients.[7] In that study, however, all the patients presented with acute abdominal pain, and none had undergone recent abdominal surgery.

We conducted this retrospective study to define the accuracy and efficiency of CT imaging to diagnose early postoperative LGIT leaks in patients who were reoperated within 30 days for clinical and/or radiologic evidence of a leak and in whom a leak was confirmed by relaparotomy. Furthermore, we will try to learn about the CT's ability to identify those with insidious clinical presentation but require surgical reintevention.

Materials and Methods

Patients who were reoperated within 30 days of an index abdominal surgery in our general surgery department from 1998 to 2006 were identified in hospital computerized records. The medical records of 70 patients who underwent early relaparotomy for LGIT leak were reviewed. Patients where the leak site was documented in the LGIT and their primary operation was not related to the LGIT were included in the study. The patients were reoperated due to suspected LGIT leak, missed injury, or anastomotic dehiscence following a previous surgery, a suspicion that was confirmed during the second operation. Leak was defined as enteral content in the peritoneal cavity combined with documented anastomotic dehisence or missed enteral injury, which needs surgical reintevention. Patients who were found to have abdominal abscess without documented leak or false positive CT findings were excluded from the study (one patient). Forty-one of the study patients underwent an abdominal CT within 72 h prior to the repeat surgery with the aim of ruling out intraabdominal infection or leak. An additional 29 patients, where presentation was severe and includes generalized peritonitis or systemic deterioration were reoperated based on their clinical presentation only. However, the operating surgeon decision making plays a major role in this group.

The CT studies were performed on a four-MDCT scanner. Oral contrast was administered to all patients. During the study period, there was no established CT protocol for diagnosis of intestinal leakage, and rectal contrast material was given sporadically, according to the surgeon and radiologist decision. Specifically for our patients, rectal contrast was administered for colon operations when the oral contrast did not progress to reach the operation site or routinely for left-sided colonic and rectal operations, when leak was not documented with oral contrast. Rectal contrast material was required in six patients. Intravenous contrast material was administered in all patients, excluding those with impaired renal function and allergy history. Overall, 32 studies were performed with intravenous contrast.

Patients where CT did not reveal LGIT leak were initially treated conservatively with NPO and wide spectrum antibiotics. When systemic deterioration or generalized peritonitis developed, patients were reoperated on, and leak was documented in all cases.

The preoperative radiological findings, according to staff radiologist report, were evaluated and compared with the physical intraoperative findings. CT data were classified into four groups: leak (defined as extraluminal contrast material), high probability for leak (if there was a large amount of free intraperitoneal air or fluid), low probability for leak (if the CT revealed a low amount of air and/or fluid), and normal (in the absence of any findings). Thereafter, the accuracy of CT findings was evaluated for small bowel, large bowel, and left-sided large bowel leaks, separately.

Statistical Analysis

Quantitative data were expressed as means ± standard deviation. Prevalences were analyzed using the chi-square test. The *t* test was used to compare the intervals between operations. Significance was set at $p<0.05$.

Results

During the study period, 70 patients (0.006% of overall abdominal operations for the time period) were reoperated shortly following the first surgery due to LGIT leak. Forty-one of them (24 men and 17 women, mean age 63.8± 17 years) with insidious clinical presentation underwent 45 CT studies within 72 h before they were reoperated. Thirty-two CTs were performed 24 h before reoperation. Additional six and seven CTs were performed within 48 and 72 h before reoperation, respectively. Another 29 patients (19 men and ten women, mean age 56±21 years) underwent the second procedure based solely on their clinical presentation.

The initial surgery was in the large bowel for most of the patients, followed by the small bowel. The sites and

primary operations for both groups are summarized in Table 1. There were no significant differences between the two groups (x^2=4.478, df=5, p=0.431). The need for a diversion procedure, which could be a factor indicative of the severity of peritonitis, was similar for both groups. The same surgical procedures were performed in both groups (Table 2; x^2=1.967, df=3, p=0.579).

The mean interval until reoperation was 7.3±4.4 days in patients who underwent CT studies compared to 4.5± 2.4 days in patients who were reoperated without CTs (t= 3.12, df=66, p=0.003). The intervals between operations for one patient who underwent two reoperations (one with a preoperative CT and one without) were not available and did not included for this calculation.

For the 41 patients who underwent CT prior to reoperation, the leak site was the small bowel in 19 and the large bowel in 22 patients. It was from the anastomosis or suture line in 29 (71%) patients and from a missed enterotomy in 12 (29%).

The preoperative CT findings were negative or low probability for leak in 24 out of 45 (53%) of all the studies (Table 3). Interestingly, the negative CT findings contributed to delaying the intervention for over 24 h in ten out of 41 patients. The leakage sites in these patients distributed equally between large and small bowel (Table 4).

A LGIT leak had been detected by the CT in two of those six patients where contrast material was administered per rectum. However, both patients were classified as highly descriptive for leak prior to rectal administration of contrast material. Rectal contrast did not add to CT accuracy for diagnosis of leak in another four patients classified as low probability for leak or no leak. In one patient with ileorectal anastomosis where contrast material reaches the rectum efficiently and classified as low probability for leak, the decision was made not to perform CT enema. The correlation between left-sided leak, i.e., left colon and rectum, and CT findings with and without CT enema is shown in Table 5. No contrast material enemas were performed separately.

Due to the heterogeneity of patients in both groups, in terms of site and indication for primary operations, emergency or elective procedures, and cancer or benign diseases, a comparison of outcome would have been unreliable.

Table 1 Sites and Types of Primary Operations

Site and primary operation[a]	CT group (n)	Non-CT group (n)
Stomach	7	2
Gastrectomy (total/partial)	4	0
Morbid obesity procedure	3	2
Small bowel	8	10
Small bowel resection	4	5
Jejunostomy	1	2
Stricturoplasty	1	1
Closure of ileostomy	2	1
Lysis of adhesions	0	1
Colon and rectum	19	13
Right hemicolectomy	7	3
Left hemicolectomy/ sigmoidectomy	3	6
Total/subtotal colectomy	6	0
Rectal resection	3	4
Appendectomy	0	1
Ventral hernia repair	3	1
Other	4	2

CT computed tomography

[a] p=not significant

Table 2 Surgical Procedures at Reoperation

Surgical procedures at reoperation[a]	CT group n (%)	Non-CT group n (%)
Diversion ± repair ± drainage	27 (66)	18 (62)
Primary repair ± drainage	10 (25)	5 (17)
Drainage	1 (2)	1 (4)
Resection and anastomosis	3 (7)	5 (17)

CT computed tomography

[a] p=not significant

Discussion

Postoperative GI leaks are life-threatening complications which carry a high mortality rate.[8,9] Early diagnosis and treatment may improve prognosis, but insidious presentation and, unfortunately, nonspecific signs and symptoms similar to other postoperative complications make the diagnosis much more difficult to establish. Accordingly, various imaging investigations are frequently requested, but there is considerable variability in their ability to differentiate between contained leaks that can be managed expectantly from noncontained lower GI tract leaks that require urgent repair,[2,3] and CT is still the most widely

Table 3 Preoperative CT Findings Confirmed by Operative Findings

Preoperative CT finding	CT studies, n (%)
Leak	9 (20)
High probability for leak	12 (27)
Low probability for leak	17 (38)
No leak	7 (15)

CT computed tomography

Table 4 Preoperative CT Findings Confirmed by Operative Findings, Classified Per Small and Large Bowel

Preoperative CT finding	CT studies for patients with small bowel leak, *n* (%)	CT studies for patients with large bowel leak, *n* (%)
Leak	5 (24)	4 (17)
High probability for leak	3 (14)	9 (37)
Low probability for leak	9 (43)	8 (33)
No leak	4 (19)	3 (13)
	21	24

CT computed tomography

available and effective tool. The radiological definition of significant leak from LGIT is not well-defined and varies widely among different studies,[2] making it difficult to compare them. Most postoperative CT features apparently overlap widely between patients with and without clinically important anastomotic leak.[4,10] Consequently, CT may be a less reliable test in this specific group of patients. For this reason, we conducted the current study on intraoperatively confirmed LGIT leaks in order to explore the efficacy of CT in this setting.

Our results support previously reported data[1,10] that emphasize the low sensitivity of CT for diagnosing LGIT leak in the early postoperative period. CT studies detected a leak from the GI tract in only 20% of our patients. Furthermore, even if the group of patients whose CT findings were highly suggestive for a leak were combined with the leak group, the sensitivity rate of 47% in this specific group of patients is still low. Our results are in agreement with a previous study by Nicksa et al.[1] where only 48% of CT studies correctly diagnosed LGIT leak. In contrast, other studies[11–13] found the CT to be good for diagnosing LGIT anastomotic leaks, but they included small numbers of patients, and the leak was not surgically proven in some of them.

Since only six of our patients had contrast material administered transrectally, we could not draw any conclusions about its diagnostic value. However, in those six patients with left-sided leak, CT enema did not change the final conclusion or the treatment plan. This was confirmed also in a recently published study by Nicksa et al.[1] where rectal contrast material was administered routinely, but no significant changes were reported.

The current study is unique in that all the LGIT leaks were confirmed intraoperatively. As such, it is the only study thus far that accurately assessed the sensitivity of CT in diagnosing postoperative clinically significant leaks, a condition that calls for urgent operative intervention. Furthermore, in order to reduce the variability correlated with radiologist experience, only CT findings reported by a staff radiologist were considered for the study.

Negative CT findings contributed to less aggressive treatment approach and possibly postponed necessary surgical intervention in 24% of our patients in whom the clinical presentation was insidious. Noteworthy, all patients were symptomatic at the time of first negative CT. A repeat CT or reoperation was indicated due to the continuation or worsening of those symptoms, though we assume that they were related to the same pathology, i.e., leak. However, in order to decrease the probability of false-negative test, only CTs that took place within 3 days before reoperation were considered. Notably, 38 out of 45 CT studies were performed within 48 h before reoperation.

Moreover, an issue to be considered was the significantly longer interval before surgical intervention among our patients who underwent CT compared to those who did not. It is reasonable to consider that the CT findings contribute to faulty diagnosis and delayed intervention. This may play a role in higher morbidity and mortality rates[14,15] as well as the choice of surgery. Establishment of the disadvantages of negative CT findings in patients with postoperative leaks and the possible effects on patient outcome await further studies.

Based on the above findings, we caution our colleagues in the application of the results of CT studies in patients suspected of having LGIT leaks shortly after surgery. A negative CT study may be misleading and may contribute to delayed appropriate intervention. However, CT may diagnose other pathologies and may prevent unnecessary operation. Accordingly, we suggest that, for the time being, the decision to take the patient with a high level of suspicion of having an anastomotic leak into the operating room should be based on clinical findings, with careful interpretation of the imaging findings.

Table 5 Preoperative CT Findings Confirmed by Operative Findings, Classified Per Left-Sided Large Bowel Leak with and Without Rectal Contrast

Preoperative CT finding[a]	Left-sided leak	
	Without contrast material administered per rectum (n=12)	Contrast material administered per rectum[b] (n=12)
Leak	2	4
High probability for leak	5	3
Low probability for leak	4	4
No leak	1	1

CT computed tomography

[a] p=not significant

[b] Indicated in six patients

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2008 SSAT POSTER PRESENTATION MANUSCRIPT

Colorectal Surgical Specimen Lymph Node Harvest: Improvement of Lymph Node Yield with a Pathology Assistant

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Abstract
Introduction Adequate lymph node harvest from colorectal cancer specimens has become a standard of care, influencing both staging and survival. To improve lymph node harvests at our hospital, a pathology assistant was trained to meticulously harvest lymph nodes from colorectal cancer specimens. An analysis of trends in lymph node harvests over time is presented.
Methods The number of harvested lymph nodes from 391 consecutive colorectal cancer pathology reports was retrospectively reviewed from a single community hospital over 8 years (1999–2006). This spanned 4 years prior to the training of the pathology assistant and 4 years after.
Results From 1999–2002, the mean number of harvested lymph nodes varied from 12.2 to 14.4. The percentage of specimens achieving 12 lymph nodes was 50–67%. From 2003–2006, the mean number of harvested lymph nodes increased to 18.4–20.7, while the percentage of specimens achieving 12 lymph nodes was 83–87%. Both of these improvements achieved statistical significance with p values of <0.00001.
Conclusions Over time, lymph node harvests at our hospital dramatically improved. The training of a pathology assistant to harvest the lymph nodes from colorectal cancer specimens dramatically affected lymph node harvests and can be a crucial component of pathologic analysis of these specimens.

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Keywords Colorectal neoplasms · Lymph node excision · Pathology · Surgical

Introduction

Lymph node harvests in surgically removed colorectal cancer specimens have become increasingly important. Many authors have demonstrated that the number of harvested and pathologically examined lymph nodes affects staging[1–4] and potentially survival.[3–8] Some authors have also noted that the total number of negative lymph nodes and/or the ratio of positive to negative nodes is an independent prognostic factor in colorectal cancer survival.[9,10] Although there is some controversy about the survival benefit,[11] achieving an adequate lymph node assessment (usually defined as at least 12) has become a standard of care for colorectal cancer.[12,13] As the importance of adequate lymph node harvest and

pathologic assessment has become clearer, institutions involved in cancer care have sought to improve lymph node harvests. However, very little has been published regarding specific factors, procedures, or techniques which improve lymph node harvests. The question as to whether the key to adequate lymph node assessment is dependent on the surgical removal of a large enough mesentery or a meticulous enough pathologic dissection has been debated both in the literature and other forums.[14–16] Undoubtedly, as this issue has gained increasing awareness, both surgeons and pathologists have made extra efforts to insure adequate staging of their colorectal cancer patients.

We were interested in the trend in lymph node harvests at our institution over time during the last 8 years as lymph node harvest importance has become better understood. We hypothesized that our lymph node harvests have improved over time with the increasing national attention on this issue. We also hypothesized that the hiring and training of a pathology assistant (Mr. Bowles) to harvest lymph nodes from colorectal cancer specimens had resulted in a positive impact on the lymph node yield at our institution.

McKay-Dee Hospital Center, owned by Intermountain Healthcare, is a 317 bed community hospital which provides comprehensive medical and surgical care. Located in Ogden, UT (2006 population 78,000),[17] it serves patients primarily in Weber, Morgan and northern Davis Counties (2006 populations 213,000, 8,100 and 276,000 respectively)[17] in northern Utah. However, it serves as a tertiary referral center and draws patients from a vast geographic area including all of northern Utah, north of Salt Lake City, as well as southwestern Wyoming and southeastern Idaho. It has an American College of Surgeons Committee on Cancer accredited cancer care program.

Methods

Three hundred ninety-one consecutive surgically removed colorectal adenocarcinoma pathology reports were retrospectively reviewed from Jan. 1999 to Dec. 2006—a total of 8 years. We excluded local transanal rectal cancer excisions. The primary data we looked at were the number of lymph nodes examined and reported for each cancer. The study was approved by the Intermountain Healthcare Institutional Review Board. Comparison of mean numbers of lymph nodes reported from year to year was done using the Students T test statistic. Comparison of percentages of specimens achieving at least 12 lymph nodes from year to year was done using the Chi-square statistic. Statistical significance was assumed at the $p<0.05$ level.

In terms of procedures for pathologic processing of these specimens, colorectal resection specimens for malignancy are transported to the McKay-Dee Pathology Department by operating room personnel. Some are sent prior to fixation for intraoperative consultation to grossly assess margin adequacy or to confirm that an early lesion or polyp site is included in the specimen. Intraoperative consultation is performed by the responsible pathologist. The pathology assistant (PA) then assumes responsibility for these specimens as well as the other resection specimens that are not sent for intraoperative consultation and are received in formalin.

The PA is supervised by three pathologists, each of whom is responsible for essentially identical numbers and types of specimens. Certified as a histology technician, he had approximately 20 years of experience in surgical pathology gross examination including a children's hospital and a community hospital prior to starting his employment at McKay-Dee Hospital Center.

The pathology assistant (PA) spent the first 2 years of his employment working under the supervision of the pathologists, while the pathologists maintained complete responsibility for colorectal carcinoma specimens. Training in gross examination and lymph node retrieval, again under the direct supervision of the pathologists, occurred in the third year. In subsequent years, the PA assumed primary responsibility for retrieval of lymph nodes, following the approach detailed below.

The PA documents and dictates the size of the specimen and the size and the location of the tumor. Distance to margins, (proximal, distal, and circumferential radial margin) is documented prior to the shrinkage that occurs due to formalin fixation. Any unusual or irregular gross findings are reviewed with the pathologist. If serosal changes, raising the possibility of peritoneal invasion by tumor are noted, these areas are marked with ink. The PA then removes the mesocolic adipose tissue from the entire specimen, with the exception of the tissue at the level of the tumor. Approximately 1 cm of tissue is left in contiguity with the tumor, and it is examined at the time of submission of the bowel segment and tumor sections by the pathologist. In addition, removal of adipose tissue of low anterior and rectosigmoid specimens stops at the level of the peritoneal reflection.

The removed adipose tissue is placed in at least twice as much *Dissect Aid™ as there is tissue and left in this solution for a minimum of 4 h, but more often overnight (Dissect Aid is a special fixative for easier, quicker lymph node recovery. It turns lymph nodes white in the surrounding tissue mass making them simple to find. Since Dissect Aid fixes and dehydrates simultaneously, it will also firm up fatty tissues making them easier to handle. Paraffin infiltration is quick and complete. Routine H & E and special stains, including immunoperoxidase, all work well with tissues fixed in Dissect Aid.).[18] The removed adipose tissue with lymph nodes is then sectioned at approximately 3 mm intervals to retrieve the lymph nodes. The lymph nodes are

white against a yellow-tan translucent background of altered adipose tissue (see Fig. 1). Unless there is grossly apparent tumor involving multiple lymph nodes, all lymph nodes are submitted with documentation of numbers per tissue cassettes and how it was handled (e.g., "A3: one lymph node, bisected; A4: four lymph nodes; A5: one lymph node, serially sectioned") in order to maintain an accurate total node count. Lymph nodes are not separated into anatomical locations (e.g., proximal, tumor, distal), unless the surgeon has indicated a special interest by providing orientation of nodes (e.g., "stitch marks *Decal Chemical Corporation, Tallman, NY, USA highest lymph node"). The pathologist is responsible for submitting the sections of the bowel segment and tumor and also maintaining an accurate total node count. Dissect Aid was used by pathologists prior to the PA's assuming responsibility for node retrieval. These procedures are all consistent with published national standards.[19]

Results

The most important results are displayed in Table 1. For each year 1999–2006, the total number of colorectal cancer specimens is given, followed by the average number of lymph nodes and the percentage of specimens that had greater than 11 lymph nodes in each succeeding column. The differences are remarkable. Average lymph node harvest for the years 1999–2002 were all between 12.2 and 14.4. The percentage of specimens achieving 12 lymph nodes during these years varied from 50% to 67%. From 2003–2006, the average number of lymph nodes examined per specimen increased to 18.4 to 20.7. The percentage of specimens reaching 12 lymph nodes during those years was 83–87%. Comparing 1999–2002 with 2003–2006, the difference in the average lymph node harvest reached a p value of <0.00001 (T test). Comparing the percentage of specimens with at least 12 lymph nodes between 1999–2002 and 2003–2006, the difference reaches a p value of <0.00001 (Chi-square). The division in the table noted by "Training of a PA" denotes that time period where Mr. Bowles took over responsibility for dissecting our colorectal cancer specimens in 2003.

Table 1 Lymph Node Harvests and Percentage of Specimens Achieving 12 Lymph Nodes for each Year

Year	No. of specimens	Mean no. of LN[a]	Percent specimens> 11 LN[b]
1999	18	13.3	67
2000	48	12.2	50
2001	53	14.3	55
2002	49	14.4	67
Training of PA			
2003	40	20.7	83
2004	50	20.6	84
2005	75	18.4	87
2006	58	20.0	86

The division marked by "Training of PA" indicates the time frame where the PA took over responsibility for all specimen lymph node processing, i.e., at the beginning of 2003

LN lymph nodes

[a] $p<0.00001$ years 1999–2002 compared to years 2003–2006

[b] $p<0.00001$ years 1999–2002 compared to years 2003–2006

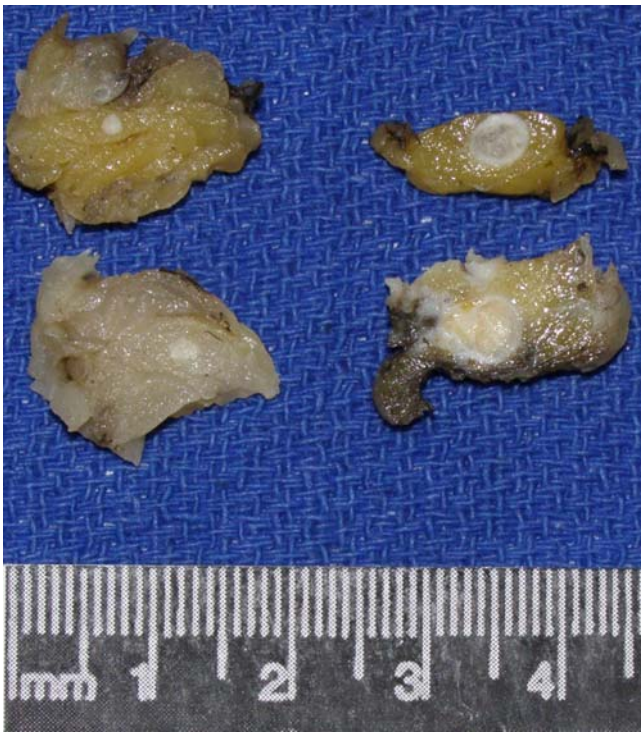

Figure 1 Colonic mesenteric lymph nodes fixed with Dissect Aid. The lymph nodes are the lighter areas within the specimens.

It is thought that it might be more difficult to harvest 12 lymph nodes in rectal specimens.[3,14,15] This could be due to a smaller mesentery and due to the effect of neoadjuvant radiation therapy which has become much more common in the treatment of rectal cancers. Although this review does not include data on which specimens had neoadjuvant therapy, some comparisons can be made. For the first 4 years, 1999–2002, there were a total of 36 non-stage IV rectal cancers (21% of all specimens). The average number of lymph nodes assessed in these specimens each year was 12.3, 13.0, 10.6, and 12.4 respectively—virtually identical to the averages for all specimens, for those years. Additionally, 42% of the rectal specimens achieved 12 nodes, only a little lower than the colon specimens. For the years 2003–2006, the average number of lymph nodes for

the 38 rectal specimens (17% of total) was 29.0, 17.8, 24.0, and 18.3, respectively (One rectal specimen was excluded from this analysis because it was a re-resection at the site of an anastomotic recurrence.). For these years, the percentage of rectal specimens with at least 12 lymph nodes was 82%. Thus, for the second 4-year period, lymph node harvests of rectal specimens were nearly as high as the colon specimens, as they were in the preceding 4 years, and the percentage achieving 12 nodes was statistically identical.

Subset analysis of the 2006 specimens was done, the results being illustrative. For the colon specimens in 2006, the average lymph node harvest is 22.4 with a range of 8–37, while the rectal specimens averaged 18.3 with a range of 5–34. A two-tailed Student's *T* test of these two means gives a *p* value of 0.07, which does not achieve statistical significance but may be meaningful. Based on that, we cannot say with certainty that rectal lymph node harvests are the same as the colon lymph node harvests and may be slightly lower on average. However, we believe that achieving the 12 node standard in rectal specimens is usually achievable. In 2006, the percentage of colon specimens achieving at least 12 nodes was 91%, and the percentage of rectal specimens achieving at least 12 nodes was 83% (excluding the one specimen which was a re-resection). Chi-square *p* value on achieving 12 nodes between the colon and rectal specimens in 2006 is 0.67.

We looked at harvests from stage IV specimens and from laparoscopic specimens as well. The numbers of these cases were small, but there was no apparent significant difference in lymph node harvests in these specimens compared to the other specimens during the same time frames.

Discussion

Obtaining adequate lymph node harvests from surgical colorectal adenocarcinoma specimens is clearly multifactorial. Surgeons need to resect enough mesentery for adequate lymph node assessment, and pathologists need to carefully dissect the resected mesentery to obtain as many lymph nodes as possible for analysis. This is a time and labor-intensive process. In addition to fastidious dissection, other techniques can reveal more lymph nodes for harvest, including the use of Dissect Aid as noted in the "Methods" section. The Dissect Aid is particularly helpful in retrieving small lymph nodes that can be missed even by an experienced dissector. Although we think that Dissect Aid or similar solutions maximizes node retrieval, it is important to note that it was in use prior to delegating the responsibility for node retrieval to the PA, and thus is not likely to be related to the improvement in node retrieval. Although we made no cost analysis of using Dissect Aid and our pathology assistant to harvest lymph nodes, we believe the cost is offset by the freeing of our pathologists' time from this tedious duty to do other things, and we clearly believe that the cost is more than justified by the more complete lymph node retrieval and staging data.

It is interesting to compare our data with nationally published data. An abstract, presented at the American Society of Clinical Oncology in 2007, looked at lymph node harvest data from NCCN institutions in 2005–2006 as well as SEER data from 2002. Although these two databases are vastly different in terms of time frame and hospital setting, the data are remarkably consistent with our own. They noted that 45% of stage I–III colorectal cancer specimens in the SEER database in 2002 achieved 12 lymph nodes, whereas the NCCN data from 2005 to 2006 showed 89% compliance with the 12 lymph node guideline.[20]

A large analysis of over 2,400 colorectal specimens over a 45-year period demonstrated that specimens with a larger number of lymph nodes analyzed had a much higher probability of finding positive nodes.[21] In our data, the average number of lymph nodes in specimens with negative nodes was 16.8 as opposed to 18.1 lymph nodes in specimens with positive nodes. Although suggestive that node positive specimens had a higher number of lymph nodes analyzed, it did not reach statistical significance ($p=0.165$; *T* test.) The same author emphasizes the importance of looking at all recoverable nodes including those that may be only 1–2 mm in size, a practice which we enthusiastically support.

We believe the substantial improvement in lymph node harvest in colorectal cancer specimens over the last 8 years at our institution is largely attributable to the training of a pathology assistant to fastidiously dissect colorectal mesentery and carefully search for nodes. The PA has greater time to devote to this task than pathologists and works in an environment with fewer distractions. Since the pathology assistant performs this task more frequently than any single pathologist, it is likely that ongoing proficiency exceeds that of any single pathologist. Another advantage is that a more uniform sampling for specimen examination occurs, since one person does most of the specimens instead of three pathologists with varying interests and amounts of time to devote to this task. We also believe that the importance placed on adequate lymph node harvests has had some influence on surgical technique with larger mesenteric resections, but the effect of this is admittedly more difficult to quantify.

Our data demonstrate that close attention to pathologic standards which are data-driven can clearly improve the quality of pathological analysis and consequently improve patient care. We were pleased to see the rapid and impressive improvement in our results following efforts to meet the nationwide standard of pathologic care for colorectal cancer specimens. Intermountain Healthcare, which owns

and operates 20 hospitals in Utah and Idaho, has made the 12-lymph node standard a system-wide Quality Assurance goal for 2008. We anticipate that many health care organizations and professional societies will do the same.[22]

Conclusion

A retrospective review of 391 consecutive colorectal adenocarcinoma pathology reports at a single community hospital was undertaken to follow the trend in lymph node harvests over an 8-year period. This time frame coincided with increased national recognition of the importance of adequate lymph node harvests for colorectal cancer staging. During this time, a pathology assistant was hired and trained to meticulously dissect colorectal mesentery and prepare as many lymph nodes as possible for pathologic analysis.

A highly statistically significant improvement in lymph node harvests was seen after this pathology assistant began processing all colorectal cancer specimens. This improvement has been sustained over a 4-year time frame. Fastidious dissection of colorectal mesentery clearly improves lymph node yields in colorectal cancer specimens. As medical institutions and national organizations focus on lymph node harvests as a Quality Assurance standard, factors that clearly improve lymph node harvests are becoming increasingly important. An appropriately trained and motivated pathology assistant is among the best of measures to ensure adequate lymph node assessment and accurate colorectal cancer staging.

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ORIGINAL ARTICLE

Volumetric and Functional Recovery of the Remnant Liver After Major Liver Resection with Prior Portal Vein Embolization

Recovery After PVE and Liver Resection

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Abstract
Introduction Portal vein embolization is an accepted method to increase the future remnant liver preoperatively. The aim of this study was to assess the effect of preoperative portal vein embolization on liver volume and function 3 months after major liver resection.
Materials and methods This is a retrospective case-control study. Data were collected of patients who underwent portal vein embolization prior to (extended) right hemihepatectomy and of control patients who underwent the same type of resection without prior portal vein embolization. Liver volumes were measured by computed tomography volumetry before portal vein embolization, before liver resection, and 3 months after liver resection. Liver function was assessed by hepatobiliary scintigraphy before and 3 months after liver resection.
Results Ten patients were included in the embolization group and 13 in the control group. Groups were comparable for gender, age, and number of patients with a compromised liver. The mean future remnant liver volume was 33.0±8.0% prior to portal vein embolization in the embolization group and 45.6±9.1% in the control group ($p<0.01$). Prior to surgery, there were no significant differences in future remnant liver volume and function between the groups. Three months postoperatively, the mean remnant liver volume was 81.9±8.9% of the initial total liver volume in the embolization group and 79.4±11.0% in the control group ($p>0.05$). Remnant liver function increased up to 88.1±17.4% and 83.3±14% respectively of the original total liver function ($p>0.05$).
Conclusion Preoperative portal vein embolization does not negatively influence postoperative liver regeneration assessed 3 months after major liver resection.

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Keywords CT volume · Liver · Surgery · Interventional radiography · Liver regeneration

Abbreviations

FRL	Future remnant liver
FRLF	Future remnant liver function
FRLV	Future remnant liver volume
HBS	Hepatobiliary scintigraphy
PVE	Portal vein embolization
RLF	Remnant liver function
RLV	Remnant liver volume
TLF	Total liver function
TLV	Total liver volume
TV	Tumor volume

Introduction

Portal vein embolization (PVE) has been widely accepted as an effective means to increase the future remnant liver volume (FRLV) in patients requiring extensive liver resection. The safety and efficacy of PVE have been confirmed by several studies and a recent meta-analysis.[1–4] PVE induces atrophy of the ipsilateral liver segments with concomitant compensatory hypertrophy of the future remnant liver (FRL). Preoperative PVE is recommended when the FRLV is less than 30–40% of the total liver volume (TLV) as determined by computed tomography (CT) volumetry, depending on the presence of underlying liver disease (e.g., steatosis, cholestasis).[5,6]

Liver regeneration is generally assessed by CT volumetry. Liver volume, however, does not necessarily represent liver function during liver regeneration.[7,8] Liver function can accurately be assessed by technetium-99m mebrofenin hepatobiliary scintigraphy (^{99m}Tc-mebrofenin HBS).[7,9]

The underlying mechanism of liver regeneration after partial liver resection or PVE is not fully understood. One suggested trigger for regeneration of the nonembolized liver lobes after PVE or resection is the instant increase in portal blood flow to the FRL.[10–12] When right PVE is performed, the portal blood flow is preoperatively diverted to the left liver lobes. As a consequence, minimal changes in portal blood flow are induced at the time of partial liver resection and therefore, this trigger for posthepatectomy liver regeneration is lacking. Our hypothesis is therefore that preoperative PVE might hamper postoperative liver regeneration. The aim of this study was to evaluate the effect of preoperative PVE on postoperative liver volume and function 3 months after major liver resection.

Materials and Methods

Patients

Eighteen patients underwent PVE of the right portal system prior to (extended) right hemihepatectomy at our institution between January 2005 and November 2007. Only those patients in whom a complete set of CT scans was obtained were included in the study, i.e., a four-phase CT scan prior to PVE, 3–4 weeks after PVE (before liver resection), and 3 months after liver resection (n=10). In all the patients, HBS was performed before PVE and in nine patients 3 months after liver resection.

Patients who had undergone (extended) right hemihepatectomy without prior PVE in the same period and of whom a CT scan had been obtained prior to and 3 months after liver resection were included in the control group (n=13). Twelve of the 13 patients underwent HBS prior to PVE, which was repeated 3 months after liver resection in 11 patients. Patient characteristics, including gender, age, and number of patients with a compromised liver were compared for both groups.

Indications for surgery in the control group were colorectal metastasis (n=5), hilar cholangiocarcinoma (n=4), hepatocellular carcinoma (n=1), and other metastases (n=3). In the PVE group, the indications were colorectal metastasis (n=5), hilar cholangiocarcinoma (n=1), hepatocellular carcinoma (n=3), and neuroendocrine tumor (n=1). Postoperative complications were subdivided into "minor" (grades I and II) or "major" (grades III, IV, V) according to the revised 2004 Clavien classification.[13]

CT Volumetry

Liver volumes were measured using CT. The total liver, the FRL, and tumor mass were manually delineated on each 5-mm slide of the portal phase images. The TLV, tumor volume (TV), and FRLV were calculated using dedicated software (Mx-View 3.52, Philips Medical Systems, The Netherlands; Fig. 1). The percentage FRLV before PVE was calculated by:[14]

$$\%\mathrm{FRLV}_{\mathrm{pre-PVE}} = \left(\frac{\mathrm{FRLV}_{\mathrm{pre-PVE}}}{(\mathrm{TLV}-\mathrm{TV})_{\mathrm{pre-PVE}}}\right) \times 100\%$$

To obtain the percentage, FRLV after PVE was computed by:

$$\%\mathrm{FRLV}_{\mathrm{pre-op}} = \left(\frac{\mathrm{FRLV}_{\mathrm{pre-op}}}{(\mathrm{TLV}-\mathrm{TV})_{\mathrm{pre-PVE}}}\right) \times 100\%$$

The remnant liver volume (RLV) 3 months after liver resection was calculated as a percentage of the initial total functional liver volume (TLV-TV):

$$\%\mathrm{RLV}_{3\ \mathrm{months}} = \left(\frac{\mathrm{RLV}}{(\mathrm{TLV}-\mathrm{TV})_{\mathrm{pre-PVE}}}\right) \times 100\%$$

Hepatobiliary Scintigraphy

HBS was performed using ^{99m}Tc-mebrofenin as previously described.[7] Briefly, after injection of 85 MBq of ^{99m}Tc-mebrofenin (Bridatec; GE-Amersham Health), dynamic images were acquired with a γ-camera (Diacam, Siemens, Milwaukee, WI, USA) for 60 min. During the first 10 min, 60 frames of 10 s were acquired (liver uptake phase) followed by 50 frames of 1 min (liver excretion phase). Total hepatic ^{99m}Tc-mebrofenin uptake rate was calculated as described by Ekman et al.[15] On preoperative scan,

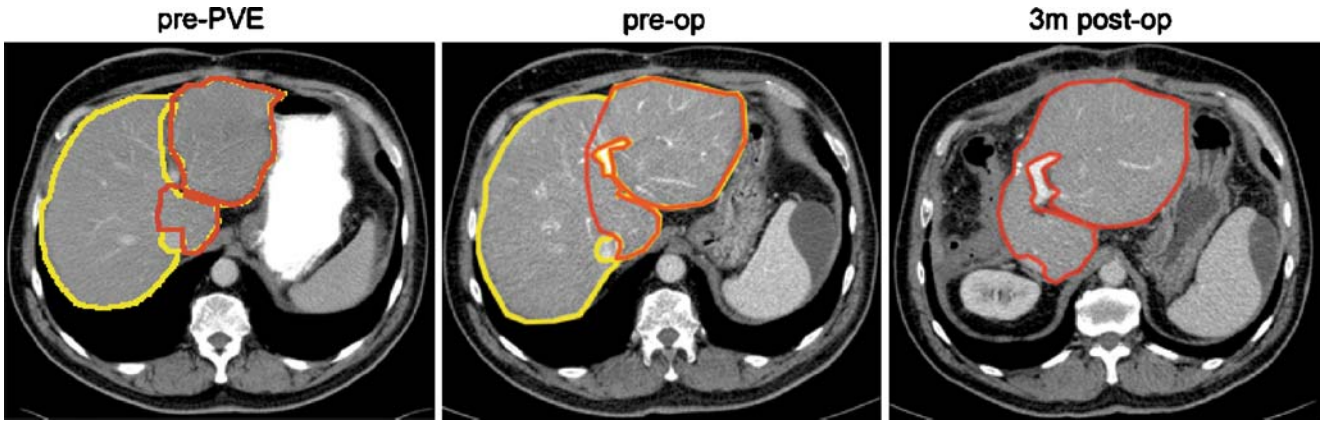

Figure 1 CT cross section of the liver showing total liver (*yellow delineation*) and the future remnant liver (*red delineation*). CT volumetry showed that the future remnant liver was markedly increased 3 weeks after portal vein embolization (*pre-op*, 507 ml) compared to before portal vein embolization (*pre-PVE*, 392 ml). Three months after partial liver resection, the remnant liver volume almost reached its original total liver volume. For interpretation of the references to color in this figure legend, the reader is referred to the online version of this article.

regions of interest (ROIs) were drawn around the total liver, the heart (serving as blood pool), and the total field of view. From these ROIs, three time–activity curves were generated. The total hepatic ^{99m}Tc-mebrofenin uptake rate, representing total liver function (TLF), was calculated as percent per minute (of the injected dose) based on these three parameters. Calculations of the hepatic ^{99m}Tc-mebrofenin uptake rate were performed using measured values obtained between 150 and 350 s postinjection to ensure that hepatic uptake calculations were performed during a phase of homogenous distribution of the agent in the blood pool, before occurrence of the rapid phase of hepatic excretion. To compensate for differences in individual metabolic requirements, the TLF was divided by the body surface area and expressed as percent per minute per square meter.

Portal Vein Embolization

PVE was performed in patients in whom the estimated FRLV, based on CT volumetry, was <30% in case of normal liver parenchyma and <40% in patients with compromised liver parenchyma due to steatosis, cholestasis, or fibrosis. PVE was performed using the ipsilateral percutaneous transhepatic approach. After retrograde catheterization via a peripheral portal branch (segment 6 or 7), the right portal trunk and intrahepatic tributaries were occluded using a combination of polyvinyl alcohol particles (300–500 μm, Cook, Bloomington, IN, USA) and platinum coils of various sizes (Tornado embolization microcoil, Cook).

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD). An independent sample *t* test was performed to assess the difference in future remnant liver volume and function between the two groups prior to surgery. A mixed analysis of variance was conducted to assess whether there were PVE and time differences in CT volumetry and HBS outcomes between the two groups after liver surgery. The correlation between variables was tested using the Pearson correlation coefficient *r*. All tests were two-tailed and differences were evaluated at the 5% level of significance.

Results

Patient characteristics are shown in Table 1. There were no significant differences between the two groups with respect to gender, age, and number of patients with a compromised liver.

The FRLV was based on the actual removed part of the liver. Prior to resection, the %FRLV was calculated taking into account the maximum volume of liver that would need to be resected to achieve complete removal of all lesions. In some patients, the extent of the resection was less than expected based on intraoperative findings, resulting in a higher $\%FRLV_{pre\text{-}PVE}$.

The $\%FRLV_{pre\text{-}PVE}$ was 33.0±8.0% in the PVE group compared to a $\%FRLV_{pre\text{-}op}$ 45.6±9.1% in the control group (p=0.002). Three to 4 weeks (mean 23 days) after PVE, the $\%FRLV_{pre\text{-}op}$ increased to 41.6±9.5%, resulting in no significant difference between the two groups prior to liver resection (p=0.33). Liver scintigraphy showed a mean ^{99m}Tc-mebrofenin uptake rate in the total liver of 7.90±1.5%/min/m^2 in the control group and 7.11±1.6%/min/m^2 in the PVE group before any intervention (p=0.24).

The increase in percentage remnant liver volume from preoperatively to 3 months after major liver surgery was not

Table 1 Patient Characteristics of Patients Undergoing Liver Resection with (PVE Group) or Without (Control Group) Prior Portal Vein Embolization

	PVE group (n=10)	Control group (n=13)	p value
Female/male	6/4	8/5	n.s.[b]
Mean age in years (range)	56.1 (49–74)	55 (39–71)	n.s.[c]
Compromised/noncompromised	6/4	7/6	n.s.[b]
Standard/extended hemihepatectomy	5/5	10/3	n.s.[b]
Postoperative complications (minor/major[a])	5 (3/2)	7 (4/3)	n.s.[b]
Mean ± SD %FRL volume before PVE	33.0±8.0	45.6±9.1	<0.01[c]
Mean ± SD %FRL volume preoperative	41.7±9.5	45.6±9.1	n.s.[c]
Mean ± SD %FRL 3 months after liver resection	81.9±8.9	79.4±11.0	n.s.[c]
Mean ± SD FRL function before PVE	7.1±1.6	7.9±1.5	n.s.[c]
Mean ± SD FRL function 3 months after liver resection	6.2±1.8	6.5±2.1	n.s.[c]

Both groups were comparable for gender, age, number of patients with a compromised liver, postoperative complications, and preoperative future remnant liver volume. Future remnant liver volume before PVE was significantly smaller in the PVE group than in the control group, which was equalized 3–4 weeks after PVE. Three months after major liver resection, the remnant liver gained up to 80% of its initial total functional liver volume in both groups

[a] According to the revised 2004 Clavien classification (7): minor = grades I and II; major = grades III and above

[b] Pearson's chi-square test

[c] Independent sample T-test

different between the two groups (p=0.81). Three months after surgery, the mean RLV in the PVE group was 81.9±8.9% of the initial total liver volume compared to 79.4±11.0% in the control group (p=0.57; Table 1; Fig. 2). In addition, the postoperative increase in liver function did not differ between both groups (p=0.471). Three months postoperatively, the RLF regained 88.1±17.4% of the original total liver function in the PVE group compared to 83.3±14% in the control group (p=0.50; Fig. 3). No correlation was found between liver volume and function (r=0.13, p=0.59).

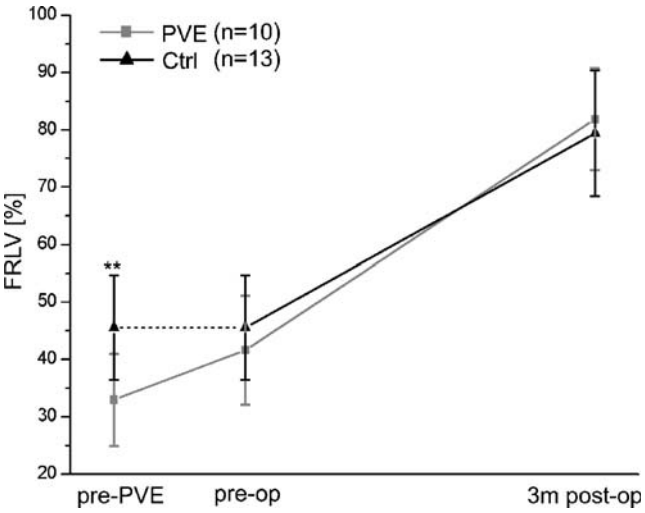

Figure 2 CT volumetry data. Mean percentage of (future) remnant liver volume (*FRLV*) in relation with initial total functional liver volume. Prior to PVE (*pre-PVE*), this percentage was significantly lower in the group requiring PVE (**p<0.01). Three to 4 weeks after PVE (*pre-op*), the FRLV increased with 8.7% in the PVE group, leading to comparable values in the two groups. Three months after partial liver resection (3 m post-op), remnant liver volumes reached approximately 80% of initial total functional liver volume in both groups.

Discussion

The main goal of this study was to evaluate the influence of PVE on volumetric and functional liver regeneration after

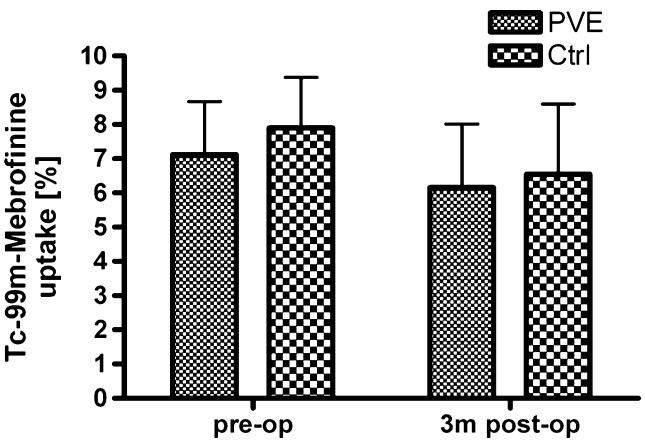

Figure 3 Uptake of ^{99m}Tc-mebrofenin by the total liver prior to any intervention and 3 months after partial liver resection. There were no significant differences in uptake between the PVE and the control groups at both time points. The remnant liver function reached 88.1% and 83,3%, respectively, of the original total liver function in both groups (p=0.50).

major liver resection. CT volumetry was performed prior to PVE and surgery. The increase of the %FRLV after PVE (% $FRLV_{pre\text{-}op-pre\text{-}PVE}$) was 8.7% in 23 days. In a recent meta-analysis, a mean increase of 11.9% was reported 29 days after PVE.[16] However, results between the various studies are difficult to compare due to substantial differences in the time interval between PVE and subsequent CT volumetry and the different techniques of embolization. For example, Farges et al. observed an increase in FRL of 16% 4–8 weeks after PVE[17] whereas Elias et al. reported an increase of 13% 1 month after PVE.[18] Ribero et al.[19] and Madoff et al.[20] showed an increase of 8.8% and 7.7%, 2–8 and 2–4 weeks after PVE, respectively, using a calculation based on body surface area.

Three months after partial liver resection, the remnant liver volume regenerated to approximately 80% of its original total volume in both groups. Liver function increased to 83% in the control group and to 88% in the PVE group. There was no correlation between volumetric and functional recovery, confirming the postulation that liver volume does not necessarily reflect liver function during liver regeneration.[7]

To our knowledge, there are no studies that compared postoperative liver volume increase and functional increase after partial liver resection in patients with and without prior PVE. Although there could have been a difference in initial regenerative response following liver resection, our results show comparable restoration rates of liver volume 3 months after (extended) hemihepatectomy in both groups.

Most data on the process of hepatocyte regeneration have been obtained from animal or in vitro studies. The time course of liver regeneration after PVE and after partial liver resection appears to be similar as has been shown in a rat model.[21] Although various mediators and pathways involved in liver regeneration have been described, the initial trigger of the entire process remains elusive.[22–25] The instant change in portal blood flow after partial liver resection is believed to be a trigger for liver regeneration. Experimental studies have shown decreased posthepatectomy liver regeneration in rats receiving a portacaval shunt.[26,27] When performing PVE prior to surgery, the change in portal blood flow is negligible in case of a standard right hemihepatectomy and less profound in case of an extended right hemihepatectomy because the portal blood had already been diverted to the left portal vein at the time of PVE. Our study shows that the liver regenerates up to 80% of its original total liver volume 3 months after major liver resection, in spite of prior PVE.

One might speculate that instead of the change in portal blood flow, the change in arterial blood flow after hepatic resection induces liver regeneration. A study in rats showed that ligation of the hepatic artery alone did not affect liver regeneration.[28] However, it is questionable whether the rat model is an appropriate surrogate model for studying the effects of altered hepatic arterial blood flow on liver regeneration or function. It is possible that the hypertrophy response of the remnant liver is slower after prior PVE in the first weeks after liver resection, but this ultimately did not result in dissimilar liver volumes after 3 months.

Conclusion

PVE does not hamper the regenerative capacity of the FRL after partial liver resection. The remnant liver regenerates up to approximately 80% of its initial total liver volume and over 83% of its original total liver function 3 months after major liver resection with or without prior PVE.

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ORIGINAL ARTICLE

Gemcitabine-Based Adjuvant Chemotherapy Improves Survival After Aggressive Surgery for Hilar Cholangiocarcinoma

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Abstract
Background The prognosis of hilar cholangiocarcinoma is dismal although aggressive surgery including major hepatectomy has been performed. The aim of this study was to clarify useful prognostic factors and the usefulness of gemcitabine-based adjuvant chemotherapy for patients with hilar cholangiocarcinoma who had undergone aggressive surgical resection.
Methods Medical records of 42 patients with hilar cholangiocarcinoma who underwent surgical resection were reviewed retrospectively. Univariate and multivariate models were used to analyze the effect of various clinicopathological factors on long-term survival.
Results Overall 1-, 3-, and 5-year survival rates of the 42 patients with hilar cholangiocarcinoma were 81%, 42%, and 30%, respectively (median survival time, 21.5 months). Univariate analysis revealed that adjuvant gemcitabine-based chemotherapy, tumor differentiation, lymph node metastasis, and surgical margin status were associated significantly with long-term survival ($P<0.05$). Furthermore, use of a Cox proportional hazards regression model indicated that only adjuvant gemcitabine-based chemotherapy was a significant independent predictor of a favorable prognosis ($P=0.035$). The toxicity of adjuvant gemcitabine-based chemotherapy was mild. Five-year actuarial survival rates of patients who did or did not receive adjuvant gemcitabine-based chemotherapy were 57% and 23%, respectively ($P=0.026$).
Conclusions Postoperative adjuvant gemcitabine-based chemotherapy may be a promising strategy to improve survival after surgical resection for hilar cholangiocarcinoma. A prospective randomized study should be done to confirm the results of this study.

Keywords Hilar cholangiocarcinoma · Prognostic factor · Postoperative adjuvant chemotherapy · Gemcitabine · S-1

Introduction

Cholangiocarcinomas are relatively rare clinical entities that comprise less than 2% of all new cancer cases per year in the USA,[1] and they have been usually divided into three categories based on tumor location: intrahepatic, hilar, and distal.[2,3] According to the previous literatures, about 60% to 80% of cholangiocarcinomas are found in the perihilar bile duct.[1,4–7] However, the prognosis of hilar cholangiocarcinoma is dismal because this tumor often invades the portal vein and the hepatic artery and metastasizes to lymph nodes and liver. Because complete surgical resection provides the only curative treatment option in hilar cholangiocarcinoma, several surgeons have advocated aggressive surgical resection including major hepatectomy, extended lymphadenectomy, and vascular resection to improve the survival of this disease.[8–11] However, despite the use of aggressive surgery, the 5-year survival rate of hilar cholangiocarcinoma has remained 20% to 40% although the mortality and morbidity rate have gradually decreased.[2,8–24] Therefore, adjuvant therapeutic modalities including chemotherapy or radiotherapy are needed for long-term survival. Although there is no established

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adjuvant therapy for cholangiocarcinoma at present, new anticancer drugs, including gemcitabine,[25] oxaliplatin,[26] capecitabine,[27] and S-1,[28] have recently been reported as useful for patients with unresectable biliary carcinomas.

In our institute, adjuvant gemcitabine-based chemotherapy was started after aggressive surgery for hilar cholangiocarcinoma in 2002.[29] The aim of this retrospective study was to clarify useful prognostic factors and the usefulness of the gemcitabine-based adjuvant chemotherapy for patients with hilar cholangiocarcinoma who had undergone aggressive surgical resection. Cases treated at a single institution were assessed with univariate and multivariate survival analysis.

Patients and Methods

Patient Population and Preoperative Workup

Medical records for 42 patients with hilar cholangiocarcinoma treated at the Department of Surgery, Hiroshima University Hospital, between January 1990 and December 2007 were reviewed retrospectively. All patients underwent tumor resection with the aim of achieving cure and had a confirmed pathological diagnosis. Patients with intrahepatic cholangiocarcinoma, who did not undergo resection of extrahepatic bile duct, were excluded from this analysis.

Preoperative workup included ultrasonography, computed tomography, endoscopic retrograde cholangiography, percutaneous transhepatic cholangiography, and intraductal ultrasonography to evaluate the local or distant extension of the tumors. The tumors were classified by their anatomic location, which was reported by Bithmuth and Corlette.[30] If jaundice was identified preoperatively, endoscopic retrograde biliary drainage (ERBD) or percutaneous transhepatic biliary drainage (PTBD) were performed to reduce the cholestatic liver damage. In addition, preoperative percutaneous transhepatic portal embolization (PTPE) for the liver segment to be resected was utilized to induce compensatory hypertrophy of the future remnant liver if the estimated resection liver volume, which was calculated by computed tomography, exceeded 60% of the whole liver.

Surgical Procedures

All surgical resections included right trisegmentectomy, right hemihepatectomy, left hemihepatectomy, left trisegmentectomy, and hilar bile duct resection with or without caudate lobectomy. If the tumor invaded the pancreatic head, pancreatoduodenectomy was also performed. All 42 patients underwent dissection of the regional lymph nodes, which included the nodes along the common hepatic artery, nodes in the hepatoduodenal ligament, and posterior pancreaticoduodenal nodes. Intraoperative pathological assessment of the proximal or distal bile duct transection lines was performed with frozen tissue sections. If the bile duct transection line was positive for cancerous cells, further resection of the bile duct was performed to the maximum extent possible. After completion of tumor resection, biliary continuity was restored by a Roux-en-Y biliary-enteric anastomosis.

Pathological Investigations

After tumor resection, hematoxylin and eosin staining was performed. All specimens were examined pathologically, and each tumor was classified as well-differentiated, moderately differentiated, or poorly differentiated adenocarcinoma according to the predominant pathological grading of differentiation. Perineural invasion, hepatic invasion, and lymph node metastasis were all examined pathologically. Surgical margins were considered positive if infiltrating adenocarcinoma was present at the proximal hepatic transection line, distal bile duct transection line, or dissected periductal soft tissue margins. The final stage of hilar cholangiocarcinoma was examined pathologically according to the TNM classification system of malignant tumors published by the International Union Against Cancer (UICC), 6th edition.[31]

Postoperative Adjuvant Chemotherapy

Gemcitabine-based postoperative adjuvant chemotherapy was administered beginning in 2002. Eligibility criteria for gemcitabine-based postoperative adjuvant chemotherapy included an Eastern Cooperative Oncology Group performance status of 0 to 1, an adequate bone marrow reserve (white blood cell count>3,000 per cubic millimeter, platelet count>100,000 per cubic millimeter, hemoglobin level> 8 g/dl), and adequate renal (serum creatinine concentration <1.5 mg/dl) and liver function (total serum bilirubin concentration<3 mg/dl). The patients who were offered postoperative adjuvant chemotherapy had two options after surgical resection. The patients with UICC stage IA disease received intravenous gemcitabine at a dose of 700 mg/m^2 biweekly, while the patients with UICC stage IB, IIA, or IIB disease received intravenous gemcitabine at a dose of 700 mg/m^2 on day 1 and orally administered S-1 at a dose of 50 mg/m^2 for seven consecutive days, followed by a 1-week pause of chemotherapy. All patients received ten cycles of adjuvant chemotherapy every 2 weeks. Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria scale (version 2.0). An additional course was withheld if toxicity of grade 3 was observed or if the patient's condition did not improve sufficiently to fit eligibility criteria. Neither external beam radiation nor

intraoperative irradiation was given to any of the patients during the study period.

Survival

Patients were followed regularly in outpatient clinics at 3-month intervals by undergoing a blood test, ultrasonography, and computed tomography for up to 5 years after surgery. Information on outcomes more than 5 years after surgery was collected by telephone or personal interview. For patients who died, survival time after surgery and the cause of death were recorded. For surviving patients, postoperative survival time and status of recurrence were recorded. Survival analyses on ten clinical factors (gender, age, Bithmuth–Corlette classification, presence of preoperative jaundice, use of percutaneous transhepatic cholangiodrainage, use of PTPE, operative procedure, type of hepatectomy, postoperative complication, and use of adjuvant chemotherapy) and seven pathological factors (tumor differentiation, perineural invasion, hepatic invasion, lymph node metastasis, surgical margin status, UICC pT factor, and UICC stage) were performed with univariate and multivariate methods.

Statistical Analysis

The χ^2 test was used for comparison among two groups. Survival curves were constructed using the Kaplan–Meier method, and differences in survival curves were compared by univariate log-rank (Mantel–Cox) test. Factors found to be significant on univariate analysis were subjected to multivariate analysis using a Cox proportional hazards model. $P<0.05$ was considered statistically significant. Statistical analysis was performed with the Macintosh version of StatView (version 5.0; SAS Institute, Cary, NC, USA).

Results

The 42 eligible patients included 26 men and 16 women (median age, 68 years; range, 37–81 years), and 26 patients (62%) were more than 65 years old. According to the Bithmuth–Corlette classification, one, four, nine, nine, and 19 patients had type I, type II, type IIIa, type IIIb, and type IV tumors, respectively. Preoperative jaundice was identified in 25 patients (60%). For reduction of serum bilirubin levels or preoperative workup, PTBD was performed for 27 patients while seven patients underwent ERBD. Percutaneous transhepatic portal embolization was performed for seven patients including five patients who underwent left trisegmentectomy and two patients who underwent right hemihepatectomy. Right hemihepatectomy, left hemihepatectomy, right trisegmentectomy, and left trisegmentectomy were performed for 16, 13, two, and five patients, respectively. All hepatic resections included caudate lobectomy. However, of six patients who underwent hilar bile duct resection, caudate lobectomy was performed for two patients. Pancreatoduodenectomy was performed only for one patient who underwent right hemihepatectomy. Thirty-day operative deaths occurred in three patients (7%) among the 42 patients. The cause of death was postoperative hepatic failure (two patients) and rupture of aneurysm of the common hepatic artery (one patient). Both patients with hepatic failure were not diagnosed with liver cirrhosis at the time of the liver resection. One patient who underwent right hemihepatectomy developed intra-abdominal bleeding 1 day after surgery and reoperation was performed for hemostasis. However, he died of hepatic failure due to subsequent intra-abdominal abscess. Another patient underwent right hemihepatectomy. The resected liver of this patient showed severe cholestasis with microabscess formation pathologically. He died of hepatic failure 28 days after surgery. In addition, the morbidity rate was high (22/42, 52%). The leading postoperative complication was biliary fistula in 12 patients (29%). However, all 12 patients were treated conservatively by leaving the drains in place, and the fistulae resolved. Other complications were intra-abdominal abscess in four patients, postoperative bleeding in three patients, hepatic failure in two patients, and stenosis of biliary-enteric anastomosis in one patient. Two patients with postoperative bleeding required further surgery (Table 1).

Pathologically, tumors were identified as well-differentiated adenocarcinoma in 20 patients (48%), moderately differentiated adenocarcinoma in 19 patients (45%), and poorly differentiated adenocarcinoma in three patients (7%). Perineural invasion and hepatic invasion were identified in 35 patients (83%) and 28 patients (67%), respectively. There were 19 tumors (45%) with lymph node metastasis and 23 (55%) without lymph node metastasis. Thirty-one patients (74%) had negative surgical margins. According to the TNM system, nine patients (21%), four patients (10%), ten patients (24%), and 19 patients (45%) were diagnosed with stages IA, IB, IIA, and IIB disease, respectively (Table 1).

Overall survival rates for the 42 patients were 81% at 1 year, 42% at 3 years, and 30% at 5 years (median survival, 21.5 months; range, 1 to 161 months; Fig. 1). Tumor recurrence occurred in 21 patients. The sites and nature of recurrence in these patients included liver metastases ($n=5$), peritoneal dissemination ($n=8$), and local disease ($n=8$). Eighteen patients died of recurrent disease, and one died of rupture of thoracic aortic aneurysm 2 months after surgery. Each one patient with local, hepatic, and peritoneal recurrence was still alive at the time of this

Table 1 Clinicopathological Characteristics of 42 Patients with Hilar Cholangiocarcinoma

Factors	No. of patients
Clinical factors	
Gender	
Male	26
Female	16
Age (mean±SD, years)	66.3±8.9 (range, 37–81)
Bithmuth–Corlette classification	
I	1
II	4
IIIa	9
IIIb	9
IV	19
Preoperative jaundice	
Yes	25
No	17
Percutaneous transhepatic biliary drainage	
Yes	27
No	15
Preoperative portal embolization	
Yes	7
No	35
Operative procedure	
Right hemihepatectomy	16
Left hemihepatectomy	13
Right trisegmentectomy	2
Left trisegmentectomy	5
Hilar bile duct resection	6
Postoperative complication	
Yes	22
No	20
Operative death	
Yes	3
No	39
Initial recurrence site	
Liver	5
Peritoneum	8
Local	8
Pathological factors	
Tumor differentiation	
Well differentiated	20
Moderately differentiated	19
Poorly differentiated	3
Perineural invasion	
Yes	35
No	7
Hepatic invasion	
Yes	28
No	14
Lymph node metastasis	
Yes	19
No	23
Surgical margin	
Positive	11
Negative	31
UICC pT factor	
pT1	10
pT2	6
pT3	26
UICC stage	
IA	9
IB	4
IIA	10
IIB	19

writing. Six patients have survived for more than 5 years. However, of the six 5-year survivors, two died of recurrent disease after the 5-year mark, and one remained alive with recurrent disease for 81 months after surgery.

In order to clarify the usefulness of gemcitabine-based adjuvant chemotherapy, 38 patients, excluding three patients with 30-day operative death and one patient who died of rupture of thoracic aortic aneurysm 2 months after surgery, were analyzed. Clinicopathological details of the 38 patients are summarized in Table 2, according to the presence or absence of adjuvant gemcitabine-based chemotherapy. Eighteen patients (43%) received postoperative adjuvant chemotherapy.

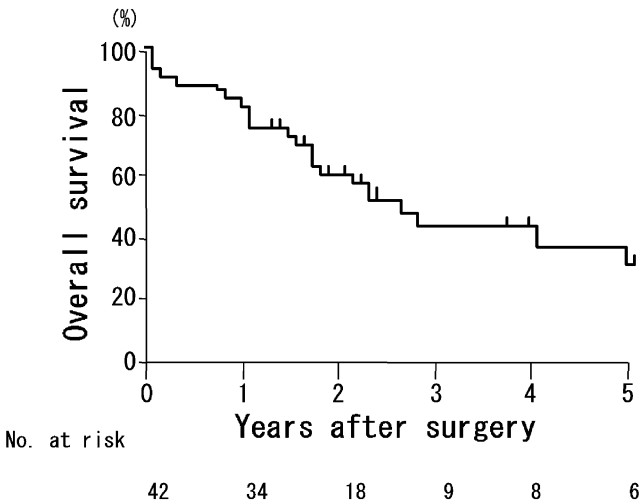

Figure 1 Overall survival in 42 patients who underwent resection for hilar cholangiocarcinoma.

Table 2 Comparison of Clinicopathological Factors of 38 Patients with Hilar Cholangiocarcinoma Who Did or Did Not Receive Adjuvant Chemotherapy

	Adjuvant chemotherapy		*P* value
	Present (n=18)	Absent (n=20)	
Clinical factors			
Gender			
Male	10	12	0.782
Female	8	8	
Age (years)			
<65	7	8	0.944
≥65	11	12	
Bithmuth–Corlette classification			
Type I, II	1	4	0.188
Type III, IV	17	16	
Preoperative jaundice			
Yes	9	13	0.350
No	9	7	
Percutaneous transhepatic biliary drainage			
Yes	7	17	0.003
No	11	3	
Preoperative portal embolization			
Yes	4	2	0.302
No	14	18	
Operative procedure			
Hepatectomy	15	18	0.544
Hilar bile duct resection	3	2	
Type of hepatectomy			
Right-sided hepatectomy	6	9	0.688
Left-sided hepatectomy	8	9	
Postoperative complication			
Yes	10	9	0.516
No	8	11	
Pathological factors			
Tumor differentiation			
Well	12	8	0.100
Moderate–poor	6	12	
Perineural invasion			
Yes	15	17	0.888
No	3	3	
Hepatic invasion			
Yes	9	16	0.052
No	9	4	
Lymph node metastasis			
Yes	8	9	0.973
No	10	11	
Surgical margin			
Positive	5	5	0.846
Negative	13	15	
UICC pT factor			
pT 1, 2	8	7	0.552
pT 3	10	13	
UICC stage			
IA, IB	6	6	0.825
IIA, IIB	12	14	

P value is the result of a χ^2 test. Three patients with operative death and one patient who died for rupture of thoracic aortic aneurysm 2 months after surgery were excluded

Gemcitabine plus S-1 was administered to 13 patients with UICC IIA and IIB disease while five patients with UICC stage IA disease received gemcitabine alone. Adjuvant chemotherapy was started between days 18 and 78 (median day 29) following surgery. All patients were given the full number of ten cycles of the intended chemotherapy. Toxicity during chemotherapy was mild although nausea was commonly observed. Thrombocytopenia and leukopenia of grade 3 were observed in each one of the 18 patients. However, hospitalization was not required for toxicity, and there were no treatment-related deaths in any of the patients. PTBD was performed more frequently for patients who did not receive adjuvant chemotherapy, compared with patients who received adjuvant chemotherapy. However, other 15 clinicopathological factors did not differ between two groups.

Seventeen clinicopathological factors were investigated to determine their prognostic significance. The results of the log-rank test are shown in Table 3. Thirteen factors including gender, age, Bithmuth–Corlette classification, preoperative jaundice, use of PTPE, performance of PTBD, operative procedure, type of hepatectomy, postoperative complication, perineural invasion, hepatic invasion, UICC pT factor, and UICC stage did not influence postoperative survival by univariate survival analysis. In contrast, univariate analysis revealed that postoperative adjuvant chemotherapy (P=0.026), tumor differentiation (P=0.001), lymph node metastasis (P=0.023), and surgical margin status (P=0.007) were associated significantly with survival. These factors were entered into multivariate analysis with a Cox proportional hazards model, and only use of postoperative adjuvant chemotherapy (P=0.035) remained independently associated with survival (Table 4). Five-year survival rates of patients who did or did not receive postoperative adjuvant chemotherapy were 57% and 23%, respectively (Fig. 2).

Table 3 Univariate Survival Analysis of Prognostic Factors for 38 Patients with Hilar Cholangiocarcinoma

Factors	No. of patients	5-year survival rate (%)	*P* value
Clinical factors			
Gender			
Male	22	46	0.126
Female	16	0	
Age (years)			
<65	15	44	0.863
≥65	23	16	
Bithmuth–Corlette classification			
Type I, II	5	0	0.107
Type III, IV	33	36	
Preoperative jaundice			
Yes	22	25	0.108
No	16	49	
Percutaneous transhepatic biliary drainage			
Yes	24	29	0.163
No	14	39	
Preoperative portal embolization			
Yes	6	0	0.620
No	32	37	
Operative procedure			
Hepatectomy	33	34	0.600
Hilar bile duct resection	5	0	
Type of hepatectomy			
Right-sided hepatectomy	17	29	0.435
Left-sided hepatectomy	15	38	
Postoperative complication			
Yes	19	21	0.899
No	19	40	
Adjuvant chemotherapy			
Yes	18	57	0.026
No	20	23	
Pathological factors			
Tumor differentiation			
Well	19	50	0.001
Moderate–Poor	19	18	
Perineural invasion			
Yes	32	25	0.162
No	6	75	
Hepatic invasion			
Yes	25	24	0.054
No	13	55	
Lymph node metastasis			
Yes	17	25	0.023
No	21	38	
Surgical margin			
Positive	10	0	0.007
Negative	28	41	
UICC pT factor			
pT 1,2	15	52	0.177
pT 3	23	24	
UICC stage			
IA, IB	12	47	0.264
IIA, IIB	26	29	

P value is the result of a log-rank (Mantel–Cox) test. Three patients with operative death and one patient who died for rupture of thoracic aortic aneurysm 2 months after surgery were excluded

Discussion

High mortality rates (0–15%) and morbidity rates (14–66%) have been reported in surgical treatment for hilar cholangiocarcinoma because major hepatectomies required for complete resection of the tumors (Table 5).[2,8–24] The leading cause of death was postoperative hepatic failure in the previous reports,[8,23] and preoperative biliary drainage and portal vein embolization were utilized to prevent this unfortunate complication by several surgeons.[24,32] In this series, preoperative biliary drainage was routinely performed for patients with jaundice, and portal vein embolization was utilized recently depending on the future remnant liver volume. As a result, mortality rate and morbidity rate of this study were 7% and 52%, respectively, which are consistent with those of the previous reports.

Many investigators have attempted to find useful prognostic factors for hilar cholangiocarcinoma after surgical resection, using multivariate survival analysis (Table 5).[2,8–24] According to these reports, potential factors include nodal involvement,[8,10,13,15,22–24] pathological grading of differentiation,[8,9,10,12,14–16,21] pathologically curative resection,[2,8,12–17,19,21–23] preoperative serum bilirubin level,[12,18] gender,[8,18] and operative procedure.[9,16,17] In the current study, univariate analysis showed that postoperative adjuvant chemotherapy, tumor differentiation, nodal involvement, and surgical margin status were associated significantly with survival,

Table 4 Multivariate Survival Analysis of Prognostic Factors for Patients with Hilar Cholangiocarcinoma

Factors	Hazard ratio	95%CI	*P* value
Adjuvant chemotherapy			
Yes	1.0	1.11–14.7	0.035
No	4.04		

P value is the result of a Cox proportional hazards model

CI confidence interval

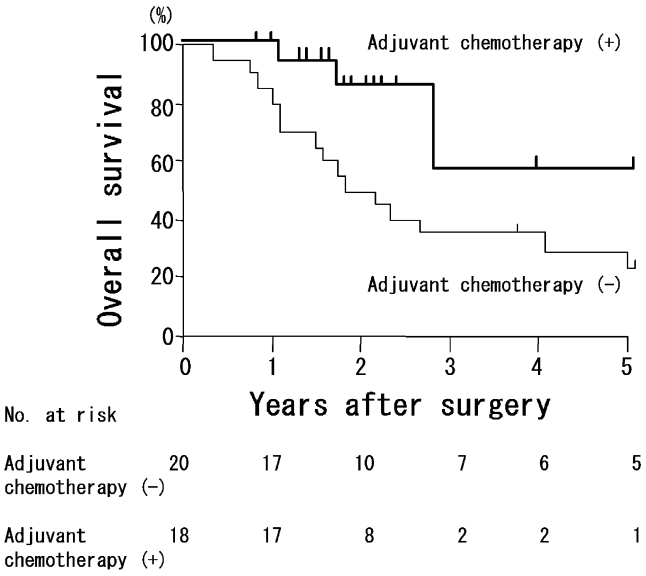

Figure 2 Comparison of postoperative survival in patients who did or did not receive postoperative adjuvant chemotherapy following surgical resection of hilar cholangiocarcinoma (P=0.026).

which is almost similar to the previous reports. However, only adjuvant chemotherapy was an independent prognostic factor of long-term survival by multivariate analysis. To our knowledge, there have been no reports that adjuvant chemotherapy is found to be an independent factor after resection of hilar cholangiocarcinoma. The reason is that we select new anticancer drugs including gemcitabine or S-1 as an adjuvant chemotherapy regimen, and these new drugs may contribute to long-term survival of patients who received adjuvant chemotherapy, we think.

There have been few reports regarding postoperative adjuvant therapy for hilar cholangiocarcinoma including chemotherapy or radiotherapy, and, to date, randomized controlled studies on adjuvant therapy for hilar cholangiocarcinoma have never been seen in the literature. With regard to adjuvant radiotherapy, several retrospective analyses have suggested that radiotherapy augments survival in patients with hilar cholangiocarcinoma.[6,33–35] Todoroki et al.[33] retrospectively analyzed 63 patients who underwent resection of a hilar cholangiocarcinoma and reported that actuarial 5-year survival was significantly better in the resection plus radiotherapy group (39%) compared with the resection-alone group (14%). In addition, Gerhards et al.[34] reported that, with a review of 91 patients with hilar cholangiocarcinoma, overall median survival time was significantly longer in patients treated with adjuvant radiotherapy than in those who underwent resection without additional radiotherapy (24 versus 8 months). However, in many of these retrospective reports, the survival advantage was mainly found in patients who had microscopically positive resection margins. In contrast,

Table 5 Recent Reports on Resectional Treatment of Hilar Cholangiocarcinoma

Author	Year	No. of patients	Mortality (%)	Curative resectability (%)	Median survival (months)	5-year survival rate (%)	Prognostic factors by multivariate analysis
Our series	2008	42	7	74	22	30	AC
Miyazaki et al.[23]	2007	161	7	63	–	–	R, N, PVR, HAR
Hasegawa et al.[22]	2007	49	2	73	45	40	R, N
Witzigmann et al.[21]	2006	60	8	70	23	22	R, G
Dinant et al.[20]	2006	99	15	31	–	27	None
Hemming et al.[19]	2005	53	9	80	22	35	R
Rea et al.[18]	2004	46	9	80	28	26	Hep, Bil, BT, Gender
Ramesh et al.[17]	2004	46	7	70	28	22	R, AT, OP
Kondo et al.[9]	2004	40	0	95	27	–	OP, G, Stage
Ebata et al.[10]	2003	160	9	83	–	–	G, N, PV
Jarnagin et al.[16]	2001	80	10	78	35	27	G, R, OP
Todoroki et al.[15]	2000	101	9	14	21	28	R, N, Bith, G
Neuhaus et al.[14]	1999	80	8	55	–	22	R, PN, LY, G
Kosuge et al.[8]	1999	65	9	52	28	33	R, G, GB, N, Sex
Klempnauer et al.[13]	1997	151	10	77	21	26	R, N, pT
Nakeeb et al.[2]	1996	109	4	26	19	11	R, Alb, Sep
Su et al.[12]	1996	49	10	49	14	15	Bil, G, R

AC adjuvant chemotherapy, *R* pathologically curative resection, *N* nodal involvement, *PVR* portal vein resection, *HAR* hepatic artery resection, *G* pathological grading of differentiation, *Hep* presence of hepatitis, *Bil* preoperative serum bilirubin level, *BT* blood transfusion, *AT* adjuvant therapy, *OP* operative procedure, *PV* portal vein invasion, *Bith* Bithmuth–Corlette classification, *PN*, perineural invasion, *LY* lymphangiosis carcinomatosa, *GB* transmural extension to gallbladder, *pT* UICC pT factor, *Sep* postoperative sepsis, *Alb* preoperative serum albumin level

the Johns Hopkins group reported that postoperative radiotherapy had no survival benefits with a review of 50 patients who underwent resection of hilar cholangiocarcinoma.[36] A similar result of no survival effect of postoperative radiotherapy for resected hilar cholangiocarcinoma was reported by Sagawa et al.[37] Thus, radiation therapy seems to have no definite benefits after resection of hilar cholangiocarcinoma.

Reports concerning postoperative adjuvant chemotherapy or chemoradiation for hilar cholangiocarcinoma are scarce, and these reports often combine intrahepatic cholangiocarcinoma, distal cholangiocarcinoma, and gallbladder carcinoma. Kim et al.[38] reported a survival advantage of adjuvant concurrent chemotherapy and maintenance 5-fluorouracil (5-Fu) chemotherapy for patients with resected extrahepatic cholangiocarcinoma (including hilar and distal) by univariate survival analysis, with a retrospective analysis of 84 resected cases. In addition, Takada et al.[39] reported that there was no apparent difference in a 5-year survival rate between patients with and without adjuvant chemotherapy (using mitomycin C plus 5-Fu), with a randomized controlled study on 118 patients with cholangiocarcinoma who mainly consisted of patients with distal cholangiocarcinoma. Based on these reports, there are also no apparent advantages in adjuvant chemotherapy or chemoradiotherapy for patients with resected hilar cholangiocarcinoma. Recently, new anticancer drugs including gemcitabine,[25] oxaliplatin,[26] capecitabine,[27] and S-1[28] have been reported to be effective for patients with biliary carcinoma who are not amenable to surgical resection. However, to date, there have been no reports concerning survival effects of these new drugs after surgical resection of hilar cholangiocarcinoma. In the present series, we used mainly a gemcitabine plus S-1 regimen as postoperative adjuvant chemotherapy after surgical resection of hilar cholangiocarcinoma. Gemcitabine has been reported to inhibit the growth of cholangiocellular carcinoma cell lines.[40] In a recent phase II study, the response rates of patients with unresectable bile duct carcinoma to gemcitabine or S-1 have been reported to be 22% to 36%[25,41] and 21%,[28] respectively. Moreover, gemcitabine plus S-1 therapy has been associated with an excellent survival benefit in patients with unresectable[42] or resected[43] pancreatic carcinoma. In the current study, a significant survival benefit was observed in patients with adjuvant chemotherapy compared with patients without adjuvant chemotherapy by univariate analysis, and multivariate analysis showed that adjuvant gemcitabine-based chemotherapy was an only independent favorable prognostic factor.

Five-year actuarial survival rates of resectional treatment for hilar cholangiocarcinoma have been reported to range from 11% to 40%, including operative deaths and R0, R1, and R2 resections (Table 5).[2,8–24] Seyama et al.[24] reported zero mortality and 5-year actuarial survival rate of 40% and claimed the importance of preoperative biliary drainage and portal vein embolization. Moreover, Witzigmann et al.[21] reported that neoadjuvant photodynamic therapy before surgery resulted in 5-year actuarial survival rate of 42%. In this series, the 5-year actuarial survival rate for all patients who underwent resection was 30%, which is a similar result to the previous reports. However, the 5-year actuarial survival rate in the small subgroup of adjuvant gemcitabine-based chemotherapy in the present study was 57% including R0 and R1 resection, which was an excellent result compared with the previous reports. We believe that gemcitabine-based chemotherapy is a promising adjuvant strategy to improve long-term survival after resection of hilar cholangiocarcinoma, although the result in this study is based on a small number of patients and follow-up periods are relatively short. Further studies on a larger number of patients are needed to determine the usefulness of this new adjuvant chemotherapy for hilar cholangiocarcinoma.

The frequency of nodal involvement has been reported to range from 16% to 56% in patients with hilar cholangiocarcinoma who underwent surgical resection,[2,8–24] and was 45% in this series. The literature provides conflicting results concerning the relationship between nodal involvement and survival. However, many author showed an apparent effect of nodal involvement on survival, as described above.[8,10,13,15,22,23] In the present study, patients with nodal involvement showed significantly worse survival by univariate analysis, although statistical significance was not obtained by multivariate analysis. Moreover, five of six 5-year survivors did not exhibit nodal involvement. One 5-year survivor with nodal involvement had nodal metastasis along the common hepatic artery and received adjuvant gemcitabine plus S-1 therapy after surgery. Adjuvant chemotherapy may contribute to longer survival of patients with nodal involvement.

Surgical margin status proved to be an independent prognostic factor in many reports.[2,8,12–17,19,21–23] According to the previous literature, curative (R0) resection was performed in 14–95% of patients undergoing surgical resection (Table 5).[2,8–24] In the current study, the rate of patients resected with negative margin was 74%, and there were no 5-year survivors in patients with positive surgical margin. However, three patients with positive surgical margin (R1 resection), which received adjuvant gemcitabine plus S-1 chemotherapy, have remained alive without recurrence for 20 to 29 months at the time of this writing. Adjuvant chemotherapy with gemcitabine has been reported to improve survival of not only patients with R0 resection but also patients with R1 resection in pancreatic carcinoma.[44] Adjuvant gemcitabine-based chemotherapy

for hilar cholangiocarcinoma may also improve survival of the patients with positive surgical margin.

The major site of initial recurrence after resection of hilar cholangiocarcinoma has been reported to be locoregional recurrence, even after curative resection is performed.[45] However, Hasegawa et al.[22] reported that 60% of the patients with R0 operations developed distant metastases including hepatic and peritoneal recurrence after resection of hilar cholangiocarcinoma. In addition, Kondo et al.[9] reported that, of the nine patients who died of disease after R0 resection, the causes of death included peritoneal seeding in five patients and hepatic metastasis in two patients. In this series, of 14 patients who developed recurrence after R0 resection, nine patients (64%) developed distant metastases (peritoneum in six and liver in three). These results indicate that curative surgical resection is not sufficient, and additional systemic treatment strategies, not locoregional therapy, are mandatory for long-term survival of hilar cholangiocarcinoma. Based on these results, we believe that adjuvant chemotherapy is a preferable strategy compared with radiotherapy to improve survival after surgical resection of hilar cholangiocarcinoma.

In this study, six patients have survived for more than 5 years after surgical resection. However, three of the six 5-year survivors developed recurrence and two died of disease after the 5-year mark. Jarnagin et al.[16] reported that, of the nine actual 5-year survivors after surgical resection of hilar cholangiocarcinoma, six died of disease recurrence and progression. Five-year survival seems to be no guarantee of cure.

In conclusion, postoperative adjuvant gemcitabine-based chemotherapy may improve survival after surgical resection for hilar cholangiocarcinoma. Further studies on larger numbers of patients, including randomized controlled trials, are required to confirm the usefulness of adjuvant gemcitabine-based chemotherapy.

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ORIGINAL ARTICLE

Comparative Analysis of Outcomes in Living and Deceased Donor Liver Transplants for Primary Sclerosing Cholangitis

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Abstract
Introduction Primary sclerosing cholangitits (PSC) is a progressive fibrosing cholangiopathy eventually leading to end-stage liver disease (ESLD). While literature for deceased donor liver transplantation (DDLT) for PSC abounds, only a few reports describe live donor liver transplant (LDLT) in the setting of PSC. We present a single-center experience on survival outcomes and disease recurrence for LDLT and DDLT for ESLD secondary to PSC.
Aim The aim of this study was to analyze survival outcomes and disease recurrence for LDLT and DDLT for ESLD secondary to PSC.
Patients and Methods A retrospective review of 58 primary liver transplants for PSC-associated ESLD, performed between May 1995 and January 2007, was done. Patients were divided into two groups based on donor status. Group 1 (n=14) patients received grafts from living donors, while group 2 (n=44) patients received grafts from deceased donors. An analysis of survival outcomes and disease recurrence was performed. Recurrence was confirmed based on radiological and histological criteria.
Results Recurrence of PSC was observed in four patients in LDLT group and seven in DDLT group. Retransplantation was required in one patient in LDLT group and nine patients in DDLT group. One patient (7%) among LDLT and six patients (14%) among DDLT died. The difference in patient and graft survival was not statistically significant between the two groups (patient survival, p=0.60; graft survival, p=0.24).
Conclusion This study demonstrates equivalent survival outcomes between LDLT and DDLT for PSC; however, the rate of recurrence may be higher in patients undergoing LDLT.

Keywords Liver transplant · Primary sclerosing cholangitis · Living donor · Deceased donor · Outcomes · Recurrence

Introduction

Primary sclerosing cholangitits (PSC) is a progressive fibrosing cholangiopathy characterized by inflammatory

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and fibrotic bile duct lesions forming multiple strictures and ectatic dilatations of the intra- and extrahepatic biliary system,[1–3] eventually leading to recurrent episodes of cholangitis and secondary fibrosis and cirrhosis. Mounting evidence now exists, which supports liver transplantation as the optimal treatment for decompensated liver disease with a 5-year graft survival in the range of 65.2% to 79%.[4–7] Recent studies based on deceased donor liver transplantation (DDLT) suggest that PSC can recur.[6–11] While literature for deceased donor liver transplantation for PSC abounds,[6–11] only a few reports describe live donor liver transplant (LDLT) in the setting of PSC-associated end-stage liver disease (ESLD).[12] Unlike with the DDLT population, the postoperative course in the LDLT group may be affected by the possible shared genetic background between the recipient and the donor, impacting long-term outcomes. It is unclear whether the outcome of LDLT is equivalent to or different from that of DDLT for PSC. We present a single-center experience on survival outcomes and disease recurrence for LDLT and DDLT for ESLD secondary to PSC.

Patients and Methods

A retrospective review was conducted of all primary liver transplants performed at our center from May 1995 to January 2007. Fifty-eight liver transplants were carried out for PSC-associated ESLD. Diagnosis of PSC was based on clinical signs and symptoms of jaundice, pruritus, and cholangitis, as well as the endoscopic retrograde cholangiopancreatography or transhepatic cholangiography findings of multiple strictures and dilatations of the intrahepatic and extrahepatic biliary ducts. The characteristic findings of PSC were further confirmed in the explant liver specimen with histologic sections showing overall bile duct loss, concentric and obliterative periductal fibrosis, and atrophy of ductal epithelium. In addition, other potential causes of progressive cholestatic liver disease including primary biliary cirrhosis (PBC), sarcoidosis, choledochal cysts, and chronic obstruction secondary to biliary stone disease were microscopically and grossly excluded upon pathologic examination. With a meticulous analysis of the radiographic and clinical data, the distinction between recurrent PSC and ischemic cholangiopathy was deliberated with all the available clinical, radiological, and biochemical evidence. All strictures were related to PSC recurrence and not to variant anatomy. Evidence of recurrence was further suggested by allograft biopsy showing a variety of bile duct alterations including epithelial damage, reduction in bile duct numbers, and in some circumstances background changes of an inflammatory infiltrate, portal edema, and cholangiolar proliferation.

Patients were divided into two groups based on donor status. Group 1 (n=14) comprised nine men and five women with a mean age of 44±12 years, who received grafts from living donors. Group 2 (n=44) consisted of 34 men and ten men with a mean age of 43±11 years, who received grafts from deceased donors. All living related donors underwent a pretransplant liver biopsy which was found to be microscopically normal and in particular negative for latent PSC. The mean Model for End-Stage Liver Disease (MELD) score was 12±5 in group 1 and 16±9 in group 2. The mean overall follow-up was 41.5±

Table 1 Patient Characteristics in LDLT vs. DDLT

	LDLT (n=14)	DDLT (n=44)	p value
Age	44±12 (median43)	43±11 (median42)	0.62
LOS	12±3 (median13)	25±27 (median11)	0.79
Males	9	34	
Females	5	10	
Race			
Caucasian	13	38	
African American	0	6	
Hispanic	1	0	
Blood group			
A	8	11	
B	0	3	
AB	0	2	
O	6	27	
Missing	0	1	
MELD score	12±5 (median10)	16±9 (median14)	0.25
Follow-up days	57.2±35.9	41.5±24.8	0.13

MELD Model for End-stage Liver Disease, *LOS* length of stay, *LDLT* live donor liver transplant, *DDLT* deceased donor liver transplant

24.8 months in group 1 and 57.2±35.9 months in group 2 (Table 1). In group 1, the mean duration to transplant after diagnosis was 57.8±42.2 months, and none of the patients had a colectomy pretransplant. The demographics of patients in group 1 are summarized in Table 2.

All hepatic resections in living donors were performed by a single surgeon with cavitron ultrasound surgical aspirator (Valley Lab, Boulder, CO, USA), unipolar electrocautery, liga clips, prolene sutures, and silk ties.

Statistical Analysis

Means of continuous variables were compared by *t* tests and correlations by Pearson's test. Categorical variables were compared by chi-square testing. Odds ratios were calculated using logistic regression. Statistical analysis was performed with SPSS Windows-based version 15.0 (SPSS, Chicago, IL, USA).

Results

Recurrence

Recurrence of PSC was observed in four patients in LDLT group and seven patients in DDLT group. Among recipients of living donor grafts, four patients experienced PSC recurrence as determined by radiological and histological criteria (Table 3). One patient had received the graft from spouse, and the remaining five patients had biologically related donors. The mean time to recurrence was 219 days in this patient who required retransplant (Table 2). This patient's cholangiogram showed diffuse beading and irregularity of the ducts (Fig. 1). Eventually, the graft was lost, with histologic confirmation of the diagnosis of PSC recurrence in the explant; the patient retransplanted and is now doing well.

Among deceased donor allograft recipients, seven patients developed recurrence (Table 3). The suspicion of recurrence was based initially on elevated liver function tests (LFTs) with a cholestatic picture and confirmed with cholangiography that demonstrated multiple intrahepatic biliary strictures. Evidence of recurrence was further confirmed by allograft biopsy. Three patients required retransplantation, two for recurrent disease, pathologically confirmed on explant examination, and one for a non-PSC type of biliary stricture. Of these three, one patient died 8 months after retransplant due to sepsis and multisystem organ failure. Of the other four, three were managed with percutaneous biliary drainage, and one did not require radiological intervention over a mean follow-up period of 77.3±19.0 months. One of the three patients requiring percutaneous drainage had only stenosis of hepatic duct at confluence on percutaneous transhepatic cholangiogram (PTC); however, the biopsy was suggestive of recurrent PSC. The remaining two patients had multiple intrahepatic bile duct strictures.

Out of the remaining 47 patients with no recurrence, 29 patients required a PTC for elevated liver function tests. Of these, 18 had a normal cholangiogram and 11 patients had biliary anastamotic strictures on cholangiogram.

Retransplant

Graft loss was defined as graft failure requiring retransplantation or as a result of death. Retransplantation was required in one patient in LDLT group and nine patients in DDLT group (Table 3). The living donor recipient who required retransplantation had graft failure related to recurrent PSC ($n=1$). Among deceased donors, retransplantation was required in nine patients for the following indications: hepatic artery thrombosis (HAT; $n=3$), recurrent PSC ($n=2$), primary nonfunction ($n=2$), hepatitis C viral infection ($n=1$), and non-PSC-related biliary stricture ($n=1$). Three patients with HAT required a retransplant 0.3, 0.8, and 1.5 months after primary transplant, respectively. Three of the nine patients who required retransplant died 60.9, 2.0, and 24.6 months after primary transplant due to sepsis and multisystem organ failure.

Survival

One patient (7%) among live donor recipients and six patients (14%) among deceased donor recipients died (Table 3). Amongst the former, the patient who died had developed refractory ascites after transplant and required the placement of a Denver shunt. The shunt later became infected, leading to removal of the stent followed by serial paracentesis and drain placement, resulting eventually in the death of the patient from liver failure, 36.7 months after primary transplant. Amongst the deceased donor recipient group, the most common cause of death was sepsis with multisystem organ failure ($n=4$). Out of these four patients, one developed PSC recurrence, for which the patient was retransplanted 16.4 months later but died of sepsis and multisystem organ failure 24.6 months after primary transplant.

One patient, who was found to have a co-existing cholangiocarcinoma at explant biopsy, developed abdominal wall metastases and died of metastatic cholangiocarcinoma 13.1 months after transplant. In another patient who passed away at home, the cause of death could not be ascertained.

Actuarial overall patient and graft survival at 1, 2, 3, and 5 years was 96%, 94%, 90%, 88%, and 89%, 87%, 83%, 81%, respectively (Fig. 2a). Actuarial patient survival at 1, 2, 3, and 5 years was 100%, 100%, 87%, and 87% for

Table 2 Demographics and Outcomes in LDLT

Case	Age	Sex	donor	Graft type	ABO	MELD score	LOS	Follow-up (months)	Pretransplant treatment	Warm Ischemia Time	Explant biopsy	Colectomy	Duration to Txp (months)	Recurrence (days)	Retransplant	Survival (days) graft	Survival (days) patient	Current status
1	40	M	Sister	Right lobe	A	6	11	7.1	No	0:53	Active cirrhosis	No	30.0	No	No	217	217	Alive
2	37	M	Brother	Right lobe	O	23	11	35.2	Yes	0:44	Active cirrhosis, adenocarcinoma (well differentiated) of gall bladder	No	60.0	Yes (400)	No	1,071	1,071	Alive
3	40	F	Mother	Right lobe	A	17	14	35.6	Yes	1:01	Active cirrhosis	No	168.0	No	No	1,084	1,084	Alive
4	57	F	Spouse	Right lobe	A	Pre-MELD	7	36.7	No	0:58	Active cirrhosis	No	84.0	Yes (219)	Yes	1,117	1,117	Alive
5	53	M	Spouse	Right lobe	A	16	16	55.9	No	0.36	Active cirrhosis	No	48.0	No	No	1,701	1,701	Died
6	62	M	Nephew	Right lobe	A	Pre-MELD	8	70.8	Yes	0:36	Active cirrhosis, dysplasia	No	36.0	No	No	2,156	2,156	Alive
7	57	F	Son	Right lobe	O	Pre-MELD	9	77.9	No	0:51	Active cirrhosis	No	60.0	No	No	2,373	2,373	Alive
8	59	M	Sister	Right lobe	O	7	15	12.6	No	0:41	Active cirrhosis	No	40.0	No	No	385	385	Alive
9	40	F	Son	Right lobe	A	Pre-MELD	16	65.7	No	0:33	Active cirrhosis severe dysplasia	No	36.0	Yes (540)	No	2,000	2,000	Alive
10	38	F	Brother	Right lobe	O	6	10	36.8	Yes	0:30	Fibrosis, chronic active hepatitis	No	72.0	Yes (1900)	No	1,120	1,120	Alive
11	19	M	Aunt	Right lobe	O	10	14	37.2	Yes	0:35	Active cirrhosis	No	36.0	No	No	1,134	1,134	Alive
12	28	M	Brother	Right lobe	A	10	14	0.9	Yes	0.45	Active cirrhosis	No	38.0	No	No	28	28	Alive
13	47	M	Spouse	Right lobe	O	12	17	32.6	Yes	0:54	Active cirrhosis	No	12.0	No	No	994	994	Alive
14	50	M	Son-in law	Right lobe	A	Pre-MELD	12	75.6	Yes	1:07	Active cirrhosis	No	24.0	No	No	2,268	2,268	Alive

MELD Model for End-Stage Liver Disease, *LOS* length of stay, *Txp* transplant, *M* male, *F* female

Table 3 Recurrence, Retransplant, and Death

	LDLT (n=14)	DDLT (n=44)	P value
Recurrence	4 (28%)	7 (16%)	0.29
Retransplant	1 (7%)	9 (20%)	0.25
Death	1 (7%)	6 (14%)	0.5
Retransplant			
Cause			
HAT	0 (0%)	3 (7%)	
Primary nonfunction	0 (%)	2 (5%)	
Recurrent PSC	1 (7%)	2 (5%)	
Biliary stricture	0 (0%)	1 (2%)	
Hepatitis C	0 (0%)	1 (2%)	
Total	1 (7%)	9 (20%)	
Death			
Sepsis	0 (0%)	4 (9%)	
Metastatic cholangiocarcinoma	0 (0%)	1 (2%)	
Unknown	0 (0%)	1 (2%)	
Hepatic failure	1 (7%)	0 (0%)	
Total	1 (7%)	6 (14%)	

LDLT live donor liver transplant, *DDLT* deceased donor liver transplant, *HAT* hepatic artery thrombosis, *PSC* primary sclerosing cholangitis

LDLT and 95%, 93%, 87%, and 87% for DDLT, respectively (Fig. 2b). Actuarial graft survival at 1, 2, 3, and 5 years was 100%, 100%, 87%, and 87% for LDLT and 86%, 84%, 78%, and 78% for DDLT, respectively (Fig. 2c). Difference in patient and graft survival was not statistically significant between the two groups (patient survival, p=0.60; graft survival, p=0.24).

Discussion

Liver transplant is the definitive treatment of complications from primary sclerosing cholangitis, namely recurrent cholangitis and liver failure. It has been shown that PSC is fraught with not only the risk of recurrence (as with PBC) but at an increased rate and at an earlier point than with other autoimmune processes. Its progressively fibrosing nature remains unamenable to any other form of therapy. DDLT for PSC is widely reported, while the literature on LDLT for PSC remains sparse.[13–17] The incidence of PSC recurrence in DDLT is approximately 20% (6–37%), diagnosed around 4 years after transplantation.[5,7–11,18–22] To date, few other studies have reported the outcome of LDLT for PSC from biologically related donors.[23–32] Yamigawa et al. reviewed 66 patients with PSC who underwent LDLT in Japan. The 5-year survival rate was 72%, and the rate of recurrence diagnosed on histological and cholangiographic findings was 25%.[12] Another report evaluated recurrence with a longer follow-up and a recurrence rate of 50%, when restricted to cases of biologically related live donors.[30] This series, though it presents with the longest follow-up period after LDLT for PSC described in the literature to date, being limited to nine patients, led the authors to conclude that the results obtained from their study have a large confidence interval, are prone to type 2 error, and would require confirmation by a larger series.

In our series, the overall rate of recurrence was 28%, and in biologically related live donors, it was 37%. However, none of the patients required retransplant. The patient who received graft from spouse was diagnosed with recurrent PSC and presented with typical radiologic images of non-anastomotic biliary strictures of the intrahepatic biliary tree with beading and irregularity (Fig. 1), which occurred 219 days post-LDLT which is consistent with recurrent PSC.

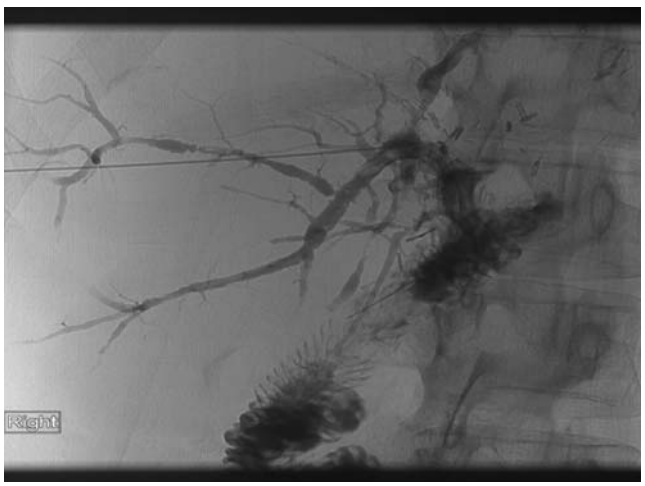

Figure 1 Cholangiogram in a LDLT recipient with PSC recurrence showing diffuse beading and irregularity of ducts.

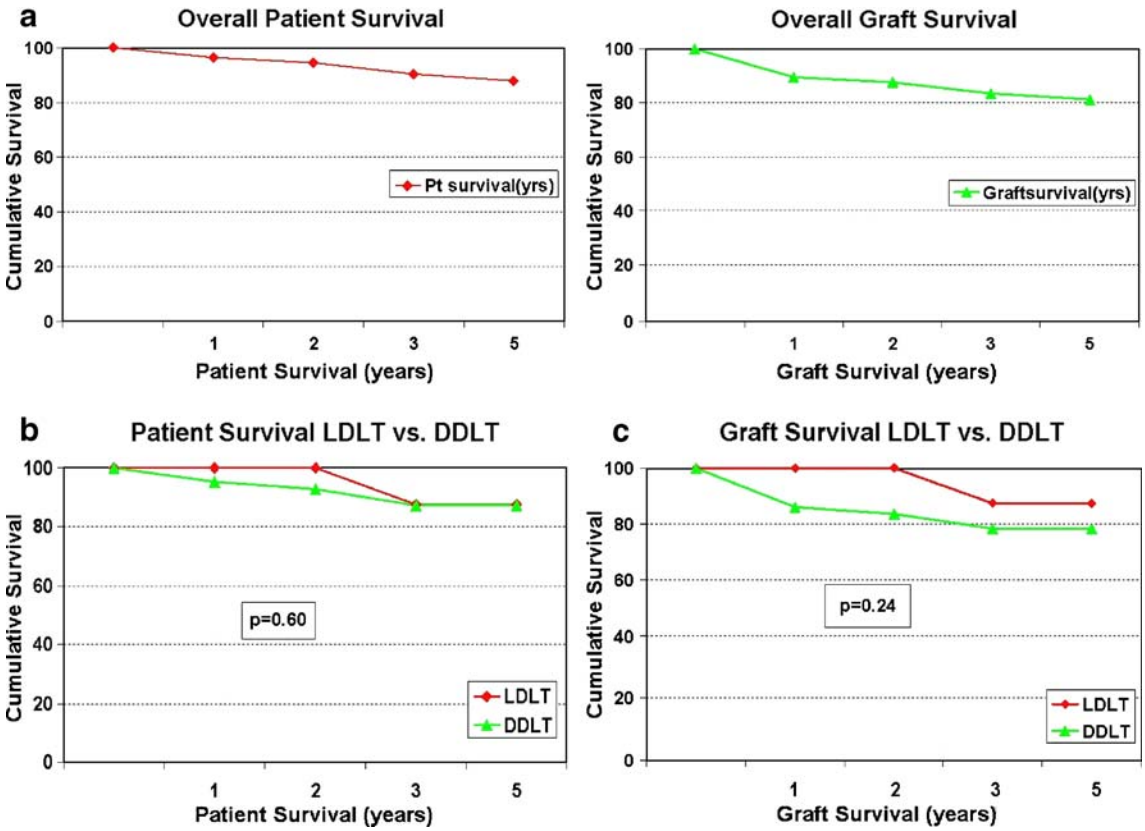

Figure 2 **a** Actuarial overall patient survival and actuarial overall graft survival. **b** Actuarial patient survival in LDLT vs. DDLT. **c** Actuarial graft survival in LDLT vs. DDLT.

While the precise etiology and pathogenesis of PSC remain unknown, the involvement of both immunologic as well as genetic factors has a strong but difficult to estimate influence.[33] An association between susceptibility to the development of PSC and human leukocyte antigen (HLA) gene complex was investigated by Tamura et al.[30] reporting the HLA-B8DR3 haplotype to be more common among PSC patients than among control patients, but this difference was not statistically significant with regard to recurrent PSC. In their series of nine cases of recurrent PSC among 49 PSC patients after DDLT, HLA-B8DR3 disparity did not seem to affect the outcome. Whether it is the associated HLA genes per se or some other closely linked genes that are responsible for the recurrence is yet to be determined; however, LDLT for PSC might offer a unique opportunity to examine the genetic aspects involved in disease recurrence. Current literature remains, at best, speculative with regards to a faster rate of recurrence with LDLT. This is being blamed on the hereditary commonality of donor and recipient as the association HLA B8 and PSC is recognized as is that of HLA DR2 and HLA DR3 haplotypes with PSC. Futugawa et al. have recently reported lower graft survival rates in PSC patients undergoing LDLT. Our study, being retrospective in nature, cannot account for a number of confounding factors which may influence the outcomes reported.

In conclusion, our study, though limited by its small sample size, demonstrates equivalent survival outcomes between patients who underwent DDLT or LDLT for PSC; however, the rate of recurrence may be higher in patients undergoing LDLT. In a majority of patients, this did not lead to graft loss or affect patient survival in our long follow-up period. The superior graft quality, as well as the favorable elective timing of LDLT, conferred marginally better patient and graft survival over DDLT in our analysis. Based on our results and those of others, we suggest doing a pooled analysis of data from different centers to develop a better understanding of the genetic aspects involved in disease recurrence.

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Conflicts of Interest None.

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SSAT POSTER PRESENTATION

Assessment of "Gene–Environment" Interaction in Cases of Familial and Sporadic Pancreatic Cancer

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Abstract
Introduction Pancreatic cancer (PC) is the fourth leading cause of cancer death in the United States. This study characterizes one of the largest national registries of familial PC (FPC) and sporadic PC (SPC), focusing on demographics, clinical factors, self-reported environmental and occupational lifetime exposures, and survival status.
Background Reported risk factors for PC include advancing age, a family history of PC, high-risk inherited syndromes, cigarette, cigar, and pipe smoking, exposure to occupational and environmental carcinogens, African-American race, high fat/high cholesterol diet, obesity, chronic pancreatitis, and diabetes mellitus.
Patients and Methods This retrospective cross-sectional, case-only analysis includes cases of FPC (n=569) and SPC (n=689) from the Johns Hopkins National Familial Pancreas Tumor Registry (NFPTR) enrolled between 1994 and 2005.
Results FPC smokers with environmental tobacco smoke (ETS) exposure were diagnosed at a significantly younger mean age (63.7 years) as compared to FPC non-smokers without ETS exposure (66.6 years; p=0.05). Non-smoker ETS-exposed cases were diagnosed with PC at a significantly younger mean age (64.0 years) compared to non-smoker non-ETS-exposed cases (66.5 years) (p<0.0004). The mean age at diagnosis for Ashkenazi Jewish SPC subjects was significantly younger (by 2.1 years) than Ashkenazi Jewish FPC cases (p=0.05). In addition, Ashkenazi Jewish FPC subjects who smoked were diagnosed 5.9 years earlier than Ashkenazi Jewish FPC non-smokers (p=0.05). The median length of survival for unresected FPC cases was significantly shorter (168 days) as compared to unresected SPC cases (200 days) (p=0.04). Survival was improved in resected cases, 713 days for FPC cases and 727 days for SPC cases, but was not significantly different between the groups (p=0.4). Mild to moderate multiplicative interaction was found between a family history of PC and exposure to asbestos, environmental radon, and environmental tobacco smoke (ETS), as evidenced by odds ratios >1.0.
Conclusions These are the first data to show that occupational and environmental exposures may act synergistically with inherited or acquired genetic polymorphisms, resulting in earlier occurrence of PC. Exposure to cigarette smoking and ETS exposure in non-smokers when younger than 21 years of age are associated with a younger mean age of diagnosis in FPC and SPC cases and Ashkenazi Jewish smokers, when compared to non-exposed cases. Risk prediction models which take into account environmental exposures as well as family history may more accurately predict the risk of PC. High-risk individuals will likely benefit from early identification of pre-malignant lesions and molecular profiling, as methods of early detection, prevention, and personalized therapy.

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Keywords Pancreas cancer · Gene–environment interaction · Familial pancreas cancer · Occupational exposures · Environmental exposures · Age at diagnosis

Introduction

In the United States, pancreas cancer (PC) is the fourth leading cause of cancer death in men and women with 37,680 new cases and approximately 34,290 deaths expected in 2008.[1] Inherited genetic risk factors account for a proportion of the cases of pancreatic cancer. Six high-risk familial syndromes [hereditary pancreatitis, hereditary non-polyposis colorectal cancer (HNPCC), hereditary breast and ovarian cancer, familial atypical multiple mole melanoma (FAMMM), Peutz–Jeghers syndrome, and ataxia–telangectasia] account for approximately 20% of the familial aggregation in PC.[2] Familial pancreatic cancer (defined as at least a pair of first-degree relatives in a kindred without one of the six syndromes) accounts for another 5% to 10% of all cases.[3] However, the majority of all PC cases are not in either of these groups, implicating unidentified gene mutations, interaction with genetic polymorphisms, and gene– environment interactions. Identifying these variables will be critical for early detection and personalized treatment strategies.

Findings from case–control, cohort, and registry studies indicate a relationship between environmental exposures and cases of PC, including personal cigarette smoking, environmental tobacco smoke (ETS) exposure, and chemical exposures (such as coal gas, coal tar pitch derivatives, and machine cutting fluids).[4] Cigarette smoking has been causally linked to the development of PC.[5,6] A recent meta-analysis of 82 case–control, cohort, and nested case–control epidemiological studies on cigarette, pipe, or cigar smoking found that the population attributable risk for smoking and PC was 20%.[7] Other risk factors associated with the development of PC include advancing age (only 13% of affected individuals are <60 years of age at diagnosis, and 50% are >75 years at diagnosis), African-American race, Ashkenazi Jewish heritage, diabetes mellitus, chronic pancreatitis, high fat/cholesterol dietary intake, obesity, and sedentary lifestyle.[8] Possible gene–environment interactions have been suggested, but largely remain to be defined. Cigarette smoking alone is a contributing factor in approximately 25–30% of the cases of PC and is the most consistently reported risk factor.[9–11]

Environmental tobacco smoke (ETS), also known as passive smoking or second-hand smoke, is comprised of a combination of sidestream cigarette smoke (85%) and mainstream smoke (15%).[12,13] ETS from cigarettes and cigars contains 43 known carcinogens (such as carbon monoxide, nicotine, cyanide, ammonia, benzene, nitrosamines, vinyl chloride, arsenic, and hydrocarbons) and is classified as a Group A carcinogen.[14] ETS is known to cause lung cancer in humans; however, the data for breast, bladder, gastrointestinal, and childhood cancers are inconclusive.[15] Villeneuve et al. explored the relationship between ETS and PC, reporting an odds ratio of 1.2. (95% CI 0.60–2.44), suggesting a weak association between PC and ETS exposure in non-smokers reporting ETS exposure in childhood and as an adult.[16] The evidence linking occupational exposures to PC is inconsistent. A number of previous epidemiological investigations have suggested excess risk of pancreatic cancer in certain occupations.[17–21]

The purpose of this retrospective, case-only descriptive study was to examine the relationships between self-reported occupational and environmental exposures, cigarette smoking (referred to as "smoking"), ETS exposure, and usual occupation and cases of FPC and SPC in a large registry of PC patients.

Patients and Data Collection

The study population and data for this cross-sectional, case-only study were derived from the Johns Hopkins National Familial Pancreatic Tumor Registry (NFPTR). All procedures and informed consent forms related to the NFPTR have been previously approved by The Johns Hopkins Institutional Review Board. This specific study was also approved by the Johns Hopkins Bloomberg School of Public Health, Office for Research Subjects, Committee on Human Research. The sample consisted of a subset of 1,258 cases of FPC and SPC who were previously enrolled and had given informed consent to participate in the NFPTR as of April 15, 2005.

Study exclusion criteria included: unconfirmed diagnosis of PC (adenocarcinoma) by either pathology or cytology report or death certificate data, failure to sign informed consent prior to initiation of the study, failure to complete the NFPTR questionnaire, primary residence outside the US, and age at diagnosis ≤18 years. Enforcement of these criteria resulted in the exclusion of 102 cases. The final sample consisted of 569 individuals in the FPC group and 689 individuals in the SPC group. The study sample included 673 men (53.5%) and 585 women (46.5%). The median age of the cohort was 65.0 years (range 26–94 years).

All participants in the study answered a questionnaire including demographic information (age, sex, race, education), past history of cancer, pancreatitis, gallstones, diabetes mellitus, or cholecystectomy, smoking history, ETS exposure or second-hand smoke, usual or longest occupation, exposure to 20 occupational/environmental carcinogens, a history of living near industrial areas, X-ray exposure prior to diagnosis, and surgical resection status. The NFPTR questionnaire was originally adapted from a questionnaire extensively tested for

reliability and validity in patients with hereditary colorectal cancer.[22] Though updated several times to add questions on newly identified PC risk factors, the current questionnaire has been in use since 1999.

Definitions

PC was defined as infiltrating ductal adenocarcinoma of the pancreas in this study. The following definitions of key terms are used: FPC is defined as two or more first-degree relatives (mother, father, sister, brother) with PC in a kindred. SPC is defined as a kindred in which one or more family members have been diagnosed with pancreatic cancer, but not two first-degree relatives. Smoking indicates a personal (past or current) history of smoking cigarettes. ETS is defined as regularly spending more than 1 h per day in a room where someone else smoked. Multiplicative interaction is considered to be present when the relative difference in the risk of an outcome, between cases exposed and those not exposed to a putative factor, differs as a function of a third variable. Usual occupational is considered the occupation worked at longest by the patient.

Statistical Analyses

A sample size calculation (Quanto®, Version 1.0)[23] was performed using significance levels of α=0.05 and β=0.20, an assumed effect size of 15%, and a PC population risk of 9/100,000. The sample size calculation determined that there was adequate power ($1-\beta$=0.80) to detect differences between the FPC and SPC groups with 1,258 subjects. Data from the NFPTR patient questionnaires were entered into an Excel® program spreadsheet and transferred using STAT-Transfer®, to Stata® Special Edition 7.0 and Stata®, Version 8.0, for statistical analyses. The data were characterized using exploratory data analysis methods,

Table 1 Demographic Characteristics of the National Familial Pancreatic Tumor Registry Study Sample

Characteristic	FPC group	SPC group	Entire cohort	*p* value
Type of PC, *N* (%)	569 (45.2)	689 (54.8)	1,258	
Age at diagnosis, years, mean (SD)	64.5 (11.1)	63.8 (11.7)	64.1 (11.4)	0.31[a]
Age at diagnosis, years, median			65	
Range, years	31–94	26–90	31–90	
Sex, *N* (%)				
Male	301 (52.9)	372 (54.0)	673 (53.5)	0.70[b]
Female	268 (47.1)	317 (46.0)	585 (46.5)	
Race, *N* (%)				
Caucasian	529 (93.0)	647 (93.9)	1,176 (93.4)	0.77[b]
African-American	11 (1.9)	14 (2.0)	25 (2.0)	
Hispanic	7 (1.2)	7 (1.0)	14 (1.1)	
Asian	4 (0.7)	7 (1.0)	11 (0.9)	
Native American	7 (1.2)	10 (1.5)	17 (1.4)	
Other	5 (0.9)	2 (0.3)	7 (0.6)	
Missing or unknown	6 (1.1)	2 (0.3)	8 (0.6)	
Ashkenazi Jewish heritage, *N* (%)	75/471 (15.9)	94/515 (18.3)	169/986 (17.1)	0.33[b]
Educational level achieved, *N* (%)				
<11th grade	68 (12.0)	74 (10.7)	142 (11.3)	0.46[b]
High school graduates	153 (26.9)	161 (23.4)	314 (25.0)	
Some college	104 (18.3)	135 (19.6)	239 (19.0)	
College graduates	117 (20.6)	159 (23.1)	276 (21.9)	
Post-graduate	116 (20.4)	136 (19.7)	252 (20.0)	
Missing	11 (1.9)	24 (3.5)	35 (2.8)	
Questionnaire completion, *N* (%)				
Index case	126 (22.1)	338 (49.1)	464 (36.9)	<0.001[b]*
Family member/proxy	443 (77.9)	351 (50.9)	794 (63.1)	
Route of entry into Registry, *N* (%)				
JHMI recruitment	79 (13.9)	299 (42.0)	378 (30.1)	<0.0004[b]*
Internet recruitment	380 (66.8)	270 (39.2)	650 (51.7)	<0.0004[b]*
Outside referral to Registry	101 (17.8)	107 (15.5)	208 (16.5)	0.30[b]
Missing	9 (1.6)	13 (1.9)	22 (1.8)	

Tests of significance: *$p \leq 0.05$

JHMI Johns Hopkins Medical Institutions

[a] Two-sample *t* test

[b] χ^2 test

Table 2 Clinical Characteristics of the Sample

Patient characteristics	FPC cases	SPC cases	*p* value
Age at diagnosis, years	64.5	63.8	0.31[b]
Age at death, years, mean	65.6	65.9	0.60[b]
Prior pancreatitis (%)	11.9	20.9	<0.001[a]
Prior cholecystectomy (%)	32.7	46.0	<0.001[a]
Diabetes mellitus (%)	25.2	27.8	0.031[a]
Other cancers prior to PC diagnosis (%)	21.8	19.2	0.23[a]

[a] χ^2 analysis
[b] Two-sample *t* test, $p \leq 0.05$

frequency distributions, and chi-square testing, and univariate and multivariate analyses. Two-sample *t* tests and analysis of variance were used to examine sample means between the FPC and SPC groups. A *p* value of ≤0.05 was accepted as significant. Survival time was assessed using Kaplan–Meier survival analyses. Differences between survival in the groups were determined using the Log-rank and Wilcoxon tests for significance. In addition, linear and logistic regression models were developed and tested.

Results

Demographic Features

The demographic characteristics of the sample (n=1,258) are presented in Table 1 and include 569 FPC cases and 689 SPC cases, spanning the period between January 1, 1994 and April 15, 2005. The mean age at the time of diagnosis for the entire cohort was 64.1 years, ranging from 26 to 94 years (mean age at diagnosis in the FPC group was 64.5 years, SPC group was 63.8 years). The median age at diagnosis was 65 years. Males and females were nearly equally distributed in the cohorts. The sample was predominantly Caucasian (93.4%), with essentially equal racial distribution between the FPC and SPC groups. More SPC cases (18.3%) reported an Ashkenazi Jewish background than did FPC cases (15.9%). High school graduates (25%) compromised the largest educational group in the sample, followed by college graduates (21.9%). The FPC and SPC groups differed significantly by who completed the NFPTR questionnaire (i.e., the index case or a family member proxy), with more questionnaires in the FPC group being completed by a family member proxy (77.9%) than in the SPC group (50.9%). The majority of the FPC group entered the NFPTR via the internet website (66.8%). For the SPC group, the most common route of entry (42.0%) was via Johns Hopkins Medical Institutions recruitment.

Clinical Characteristics

Initial analysis of the FPC and SPC cohorts revealed important differences in clinical characteristics; while mean age at diagnosis and death were comparable, survival was longer in both the unresected (200 days) and resected (727 days) SPC group, compared to the FPC group (168 days vs. 713 days, respectively). However, only in the unresected groups was this difference significant (p= 0.04). The Kaplan–Meier probability of surviving 730 days (2 years) for all resected SPC and FPC cases was 47.4%, as compared to 8.6% for all unresected cases. The FPC and SPC groups differed with regard to certain clinical characteristics (Table 2), such that SPC patients were significantly more likely to have had prior pancreatitis, prior cholecystectomy, and a history of diabetes mellitus. There were a total of 256 other cancers (affecting 20.4% of the entire study sample) reported prior to the diagnosis of PC, which were equally distributed between the FPC (21.8%) and SPC (19.2%) groups. The five most commonly reported other types of cancer, in order of occurrence, were skin (basal cell and melanoma), breast, colon, prostate, and lung cancer.

Exposure History

Smoking history was comparable (57% to 60%) in the FPC and SPC groups (Table 3); however, mean cigarette consumption was significantly higher for the SPC group, as was lifetime ETS exposure. In both groups, smoking resulted in a younger mean age at diagnosis, as compared to the non-smoking group (Table 4). However, only in the FPC patients did this difference achieve significance (p=

Table 3 Smoking and ETS Characteristics of the FPC and SPC Groups

Exposure variable	FPC cases	SPC cases	*p* value
Smoking	57%	60%	0.20
Cigarettes per day, mean number	11	13	0.01
Lifetime ETS exposure	79%	84%	0.02

ETS environmental tobacco smoke

Table 4 Mean Age at Diagnosis Variations between FPC and SPC Groups

Variable	FPC Cases: Mean Age at Diagnosis (years)	*p*-value	SPC Cases: Mean Age at Diagnosis (years)	*p*-value
Never Smoked Cigarettes (reference)	65.6 (reference)	----	63.9 (reference)	0.15**
Regular smokers: > 5 cigarettes / day	63.4	0.02*	62.6	0.26*
No Reported ETS Exposure (reference)	65.3 (reference)	----	65.8 (reference)	0.75**
ETS exposure, < 21 years of age	59.6	0.001*	56.7	<0.0004*
ETS exposure, 21- 40 years of age	61.2	0.01*	59.5	<0.0004*
ETS exposure, 41- 60 years of age	65.7	0.73*	66.9	0.38*

* p – value compared to respective reference value

** p – value compares FPC to SPC cases

ETS = environmental tobacco smoke

0.02). For all smokers in the study, the mean pack-year smoking history was 35.1 pack-years. No classic dose–response relationship was observed, as those with the most pack-years of smoking were diagnosed at the oldest mean age. Importantly, FPC patients who smoked were diagnosed significantly earlier (2.2 years) than the non-exposed group (Table 4). Similarly, when ETS exposure in non-smokers was assessed using 20-year exposure strata, FPC and SPC cases exposed when younger than 21 years of age were diagnosed at significantly younger ages than the non-exposed groups (5.7 years earlier for the FPC cases and 9.1 years earlier in the SPC group). A similar significant effect on age of diagnosis was seen when the primary ETS exposure occurred between the ages of 21 and 40 years. This difference persisted after controlling for the effect of who had answered the NFPTR questionnaire, whether the index case or a family member proxy.

Of the more than 20 occupational and environmental exposures assessed by the NFPTR questionnaire, exposure to asbestos, pesticides and herbicides, residential radon, coal products, welding products, and radiation were the most commonly reported (Table 5). The proportion of occupational or environmental exposure items left unanswered or answered as unknown varied from 5% to 30% per item, and was not significantly different between the groups. To be consistent in the analysis of these items, the decision was made to exclude missing or unknown responses from the analysis. There were significantly higher frequencies of asbestos and residential radon exposure in the FPC group, as compared to the SPC group.

Crude interaction was assessed under a multiplicative model and did not detect interaction between a family history of PC and smoking (Table 6). However, mild to moderate multiplicative interaction was present when ETS exposure, occupational asbestos and residential radon were assessed, with odds ratios greater than 1.0, and *p* values all less than 0.02.

Table 5 Occupational and Environmental Exposures in the FPC and SPC Groups

Exposure	FPC cases (%)	SPC cases (%)	*p* value
Occupational asbestos	28.0	17.5	<0.004
Pesticides and herbicides	13.4	13.5	0.9
Residential radon	11.2	5.0	<0.001
Coal products	10.2	10.9	0.7
Welding	10.2	8.9	0.4
Radiation	8.6	8.5	0.9

Table 6 Multiplicative Interaction between Family History of PC and Smoking, ETS, Asbestos, and Radon Exposure Compared to the SPC Group

Exposure variable	Odds ratio	Confidence intervals (95%)	*p* value	Multiplicative interaction
Ever smoker	0.86	0.68–1.09	0.20	None
ETS exposure, between 41 and 60 years of age	1.31	1.04–1.65	0.02	Mild
Wood dust	1.49	0.86–2.61	0.13	None
Occupational asbestos	1.83	1.31–2.56	<0.002	Mild
Residential radon	2.39	1.39–4.30	<0.0008	Moderate

ETS environmental tobacco smoke

Due to the high prevalence of inherited *BRCA2* gene mutations in Ashkenazi Jewish populations, this group was analyzed as a subset with regard to smoking. Non-smoking Ashkenazi Jews with FPC were diagnosed at a significantly older age (70.3 years) than those who smoked (64.4 years), with an age difference of 5.9 years ($p=0.05$). An age-at-diagnosis difference was also found in Ashkenazi SPC cases non-smokers compared to Ashkenazi SPC cases smokes (65.2 years for non-smokers vs. 63.8 years for smokers), but the difference did not achieve significance.

Usual occupation was determined by the respondent's answer to the question, "What was your usual occupation, or the job held longest?" From the responses, occupations were categorized using a modified version of the U.S. Department of Labor Standard Occupational Classifications (SOC) System into seven categories.[24] The SOC classification is based on work performed, jobs that require similar skills, and jobs that have similar educational requirements and experience. However, occupational exposures are not specifically outlined in the SOC classification. The mean length of employment at the usual job was 26.5 years, which did not differ significantly between the FPC and SPC groups. Data were analyzed by age at diagnosis for workers reporting early exposure to ETS (less than 21 years of age and between 21 and 40 years of age) compared to those without early ETS exposure, adjusting for smoking status (Table 7). ETS-exposed workers in the following categories; law and education, office work, skilled labor, healthcare professionals (medicine, nursing, pharmacology, radiology, mortician science, social work, veterinary medicine, laboratories and dentistry), and computer occupations were diagnosed at significantly younger ages than their non-ETS-exposed counterparts. Healthcare professionals and computer workers reporting early ETS exposure were the most susceptible to early PC diagnosis.

Discussion

Understanding the influence and interplay between the genetic and environmental factors involved in the development of PC will help guide a modern era of early detection and personalized treatment strategies for this devastating disease. Prior epidemiological studies have focused on identifying causative environmental risk factors, in addition to determining genetic predisposition for PC, but the in-depth examination of occupational and environmental exposures as predictors of PC is far from complete. Limitations imposed by incomplete and/or lack of environmental exposure assessment, the prolonged disease latency (20 years) and the typically short time between diagnosis, treatment, and death have all slowed progress in this regard. This study analyzed the clinical characteristics, the impact of cigarette and ETS exposure on age at PC diagnosis, self-reported environmental and occupational exposures, and longest job held from a large national registry of FPC and SPC patients.

Our data indicate that there is an age-at-diagnosis effect from exposure to ETS early in one's life, up to age 40 years.

Table 7 Mean Age at Diagnosis by Occupation and Early ETS Exposure, Adjusted for Smoking

Occupational category	Mean age at diagnosis if ETS at <21years of age	Mean age at diagnosis if no ETS at <21years of age	*p* value
Lawyers and education	61.6	66.4	<0.001
Office workers	61.7	66.5	<0.001
Skilled labor	61.4	66.5	<0.001
Healthcare professionals	60.4	66.7	<0.0001
Computer workers	56.5	66.5	<0.001

Occupations were categorized using a modified version of the U.S. Department of Labor Standard Occupational Classifications (SOC) System
ETS environmental tobacco smoke

This finding is not surprising as ETS contains the same carcinogens as cigarettes, but aggregation with other air pollutants may well intensify the physiochemistry of ETS such that the effects may be more carcinogenic in non-smokers than the mainstream smoke that regular smokers inhale.[25–27] Iodice and colleagues reported a similar finding, but noted that competing causes of tobacco-related morbidity may account for this finding and expressed concern that it may be an artifact.[7] Another explanation is that there is a synergistic effect between tobacco exposure and inherited genetic alterations or single-nucleotide polymorphisms (SNPs) that leads to the development of PC. McWilliams et al. identified the SNP (XPF/ERCC4 at D312N or D711D) as being associated with an increased risk of PC in heavy smokers in a case–control study of PC.[28] In lung cancer, attempts to establish such an association between ETS and the risk of lung cancer have resulted in a small, though perhaps disputable increased risk.[29,30]

From this study, we have elucidated a number of environmental influences that interact with the distinct genetics of the FPC and SPC groups. First, it is notable that a significantly earlier age of diagnosis of PC was found in Ashkenazi Jewish smokers, compared to non-smokers in this population. There is a high prevalence of mutations of BRCA2 and related genes in the Ashkenazi Jewish population. These data suggest that, in Ashkenazi Jews in whom BRCA2 is not mutated and not decreased (i.e., is proficient), BRCA2 may offer protection against the deleterious effects of smoking. In addition, the finding in this study that, among FPC cases, there was significantly more reporting of exposure to occupational asbestos and residential radon (Table 5) may indicate their biological importance in the tumorigenesis process in the familial form of PC. This relationship is further supported by the evidence of multiplicative interaction (Table 6) between a family history of PC and exposure to either occupational asbestos or residential radon.

It is noted that this descriptive case-only study suffers from the inherent difficulties of using a retrospective database and self-reported data. The problems associated with obtaining longitudinal, quantitative assessments of ETS, asbestos, and residential radon exposure are also substantial. Limitations imposed by incomplete and/or lack of environmental exposure assessment restrict generalizability of the findings; however, an important function of epidemiological studies is to inform directions for future mechanistic investigations.

Our data reveal significantly higher rates of prior pancreatitis and cholecystectomy in the SPC patients, as compared to FPC patients (Table 2). This observation may be confounded by diagnosis delays in SPC patients, or may represent increased medical surveillance in diagnosis of FPC. It is interesting that there is no difference in the rate of other cancers between the SPC and FPC groups (Table 2), especially since PC is typically diagnosed later in life, and some of the reported exposures that are linked to PC (smoking, ETS, asbestos, and radon) are also linked to other neoplasms, most notably lung cancer.

These data offer preliminary evidence of an acquired or inherited genetic alteration of a dominant genome maintenance gene, a DNA repair gene, and/or genetic polymorphisms as partners in FPC. As interest in translational science expands into molecular risk assessment, individuals with early life ETS and smokers will likely benefit from early identification of pre-malignant lesions (such as IPMNs and PanINs) and molecular profiling as methods of cancer prevention and personalized cancer treatment. Risk prediction models, such as PancPRO, which take into account environmental exposures as well as family history may more accurately predict the risk of pancreatic cancer.[30] Our results imply that unaffected individuals from families with a history of PC who smoke, and who had early life ETS exposure, or are exposed to certain occupational and environmental carcinogens may benefit from screening and early identification of pre-malignant lesions.

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SSAT POSTER PRESENTATION MANUSCRIPT

Pancreatic Acinar Cell Carcinoma: A Multi-institutional Study

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Abstract
Introduction The presentation and outcome of patients with acinar cell carcinoma (ACC) of the pancreas compared to the more common ductal cell adenocarcinoma (DCA) may be distinct. This study combines the experience with ACC from multiple academic institutions to better understand its natural history and outcomes.
Methods This study is a multi-institutional retrospective review of patients with ACC.
Results Between 1988 and 2008, 17 patients were identified with pathologically confirmed ACC. Median age at presentation was 59 years. Common presenting symptoms were abdominal pain (60%), back pain (50%), and weight loss (45%). Fifteen patients underwent 16 operations: pancreaticoduodenectomy (nine), distal pancreatectomy (four), and exploratory laparotomy (three). Mean tumor size was 5.3 cm. American Joint Commission on Cancer tumor stages were stage I (two), stage II (eight), stage III (four), and stage IV (three). Overall, 1- and 5-year survival rates were 88% and 50%, respectively. In resected cases (13), 1- and 5-year survival rates were 92% and 53%, respectively. Median survival in resected cases was 61 months. This is in contrast to 1,608 patients with ductal cell adenocarcinoma who underwent resection identified from recent literature reports where the average median survival was only 24 months. There was no discernable difference in the outcomes of patients with ACC between United States and Germany patients.
Conclusion Acinar cell carcinoma of the pancreas is rare and appears to have a presentation and outcome distinct from the more common pancreatic DCA. Based upon these data, the outcome of patients with ACC is superior to that of DCA.

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Introduction

Acinar cell carcinoma accounts for only 1% of all primary pancreatic neoplasms, even though the pancreas is composed predominantly of acinar cells by volume (82%).[1] The first case of acinar cell carcinoma (ACC) in the literature was described by Berner in 1908. Berner[2] described the histological characteristics of ACC as well as the clinical presentation of subcutaneous fat necrosis now known to be secondary to lipase hypersecretion by the tumor. Although most pancreatic cancers are not metabolically active, ACC may secrete pancreatic enzymes systemically and in some cases cause a syndrome that is characterized by fever, polyarthritis, subcutaneous fat nodular necrosis, and eosinophilia.[3] This syndrome is now recognized as lipase hypersecretion syndrome.[4–9]

The topography of acinar cell carcinoma favors a head of the pancreas distribution, but ACC may occur in any portion of the pancreas.[10,11] Pathologically, ACC are usually well circumscribed. Microscopically, ACC is a markedly cellular tumor with "minimal stroma" (Fig. 1a–e). Due to its rarity, little is known regarding the outcome and predictors of survival in patients with ACC when compared to pancreatic ductal cell adenocarcinoma (DCA). Reports in the literature on ACC are mixed with some articles showing a poorer prognosis with ACC[12,13] and others showing a better prognosis when compared to DCA.[10,11,14] Even though the recent literature has suggested a better prognosis, there remains skepticism that this may be due to the inclusion of mixed acinar–

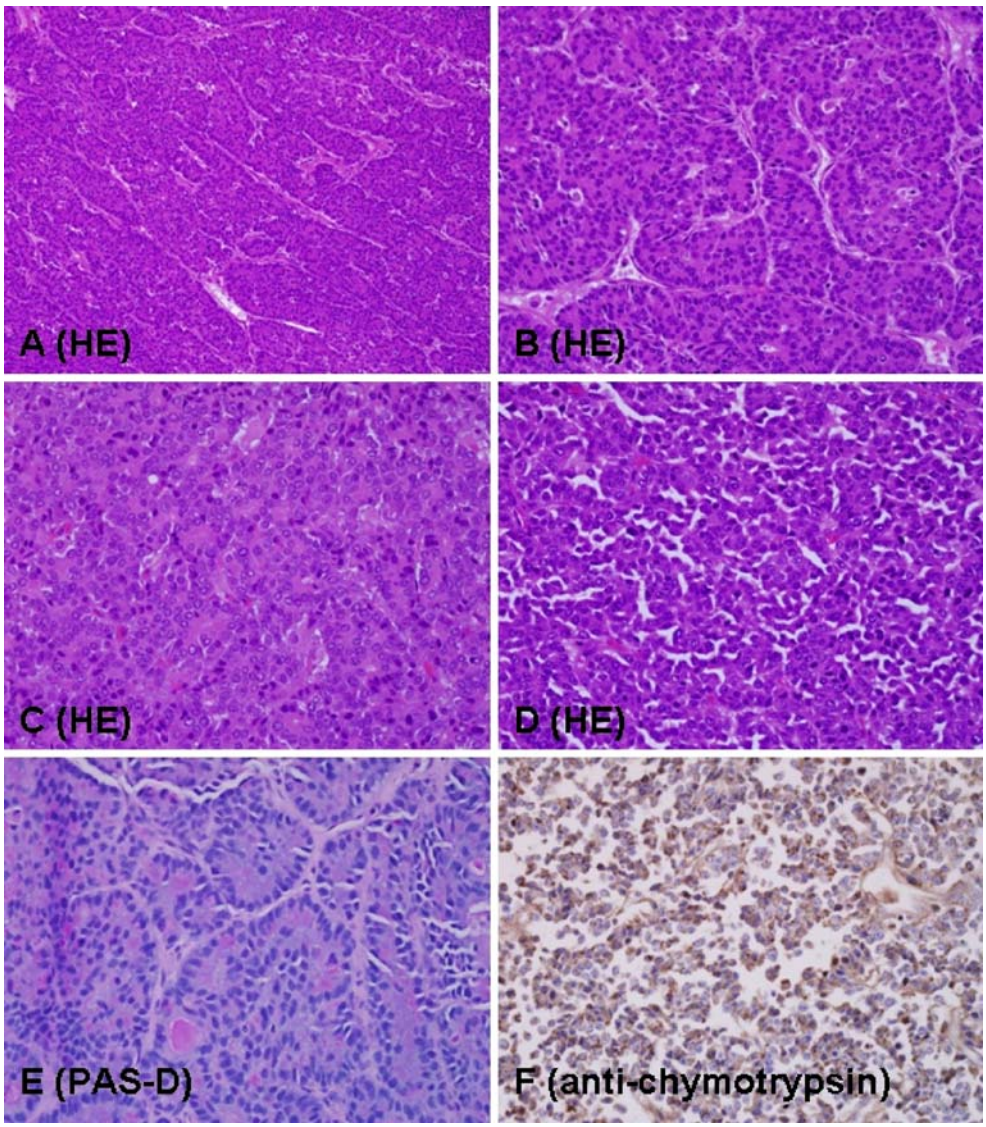

Figure 1 Microscopic architectural patterns of acinar cell carcinoma. ACC is a markedly cellular tumor with minimal stroma. The cytoplasm is eosinophilic and granular. The nuclei are relatively uniform and show presence of a large, central nucleoli. Furthermore, there is a variable mitotic rate ranging from 0.5 to 2 high power fields.[10,11] **a** Trabecular pattern (H&E). **b**, **c** Acinar pattern (H&E). **d** Solid pattern (H&E). **e** PAS-positive stain showing zymogen granules.[11] **f** Immunohistochemical stain for chymotrypsin.[8,19]

endocrine differentiation tumors. Endocrine tumors by their very nature have a better prognosis which may skew the data presented.

Furthermore, there are no robust data to support differential surgical management or other therapeutic options.[15] Even the largest single institutional series are small and treat only a few patients with ACC.[10,11] Thus, the objective of our study was to combine the experience with ACC from multiple academic institutions in the USA and Germany (where ACC was originally described) to better understand the natural history and outcomes of patients with this rare form of pancreatic cancer.

Materials and Methods

Assurances These studies have been conducted in strict compliance with the Indiana University School of Medicine Institutional Review Board (IRB) as well as each respective institution's (Dresden, Mannheim, and Vanderbilt) IRB.

Patient Data Each institution (Indiana University, University Hospital Dresden, University Hospital Mannheim, and Vanderbilt University) searched their prospectively collected surgical and pathologic databases for all cases of acinar cell carcinoma of the pancreas from 1988 to 2008. Clinical information was also obtained from patient medical records and office charts at each institution. United States patients were cross-referenced to each institution's corresponding cancer registry to determine outcomes and to the national social security database to confirm survival status. The follow-up for this study ended September 2008. Tumor size was calculated as the maximum cross-sectional diameter determined by pathology if the tumor was surgically removed. In cases where surgical pathology was not performed, tumor size was calculated as the maximum cross-sectional diameter on computed tomography. Every case of ACC presented in this multi-institutional study was re-reviewed and confirmed on secondary review to be ACC by a pancreatic pathologist at each respective institution.

Statistical Analysis Survival time was calculated from the date of diagnosis to the date of death or the last date known to be alive. The Kaplan–Meier method was used to calculate mean and median survival. Log-rank tests were performed to test for differences in survival between patients who did and did not receive radiation, patients who did and did not receive chemotherapy, and patients who did and did not have resection. For all tests, $p<0.05$ was considered significant.

Table 1 Patient Characteristics

Patient	Institution	Age (years)	Stage	Size (cm)	Operation	Survival (months)	Recurrence	Chemo/ Rad	Alive/ Dead
1	IU	59	IIA	4	Distal pancreatectomy	132	None	−/−	Alive
2	IU	72	IB	10	Distal pancreatectomy	13	None	−/−	Dead
3	IU	55	IIA	2.5	Pancreaticoduodenectomy	13	None	−/−	Alive
4	IU	68	IIA	7.8	Distal pancreatectomy	13	Distant	−/−	Dead
5	IU	69	IIA	5	Pancreaticoduodenectomy	23	None	−/−	Alive
6	IU	45	III[a]	7	Gastrojejunostomy	16	n/a	+/+	Alive
7	IU	53	IV	3.7	None	5 days	n/a	−/−	Dead
8	IU	46	IA	1.2	Pancreaticoduodenectomy	4	None	−/−	Alive
9	Mannheim	71	IIA	7.4	Pancreaticoduodenectomy	13	Local	−/−	Dead
10	Mannheim	59	III	6	Pancreaticoduodenectomy	63	Liver	−/−	Dead
11	Mannheim	66	IIA	5	Pancreaticoduodenectomy	19	Liver	−/−	Dead
12	Dresden	64	III	10	Distal Pancreatectomy	18	Liver	+/−	Alive
13	Dresden	71	IV[b]	3.2	Exploratory Laparotomy	16	None	−/−	Dead
14	Dresden	59	III	5.3	Pancreaticoduodenectomy	11	Liver	+/−	Dead
15	Dresden	65	IIB	4.1	Pancreaticoduodenectomy[c]	16	None	+/−	Alive
16	Vanderbilt	54	IIB	3	Pancreaticoduodenectomy	89	None	+/+	Alive
17	Vanderbilt	52	IV	4.6	None	67	n/a	+/−	Dead

[a] Patient was found to have extensive superior mesenteric artery and vein involvement

[b] Patient found to have liver metastasis on exploratory laparotomy

[c] Patient was the patient found to be unresectable on initial operation, received neo-adjuvant chemotherapy, and on re-exploration was found resectable

Results

Patients Seventeen patients with pathologically confirmed ACC of the pancreas were collectively identified between 1988 and 2008. These 17 patients were treated at four different institutions (two USA and two Germany; Table 1). The mean and median age of presentation was 60 and 59 years, respectively. Common presenting symptoms were abdominal pain (60%), back pain (50%), weight loss (45%), and nausea/vomiting (29%). Tumor location was predominantly in the head (13), but also occurred in the body/tail (four). No patient with a head cancer presented with jaundice. Laboratory studies showed a median CA 19-9 of 17 (range 5.6–27), with no patients out of the normal serum range. Four patients were found to have elevated serum lipase levels up to 4,151 U/L). These patients, however, lacked classic clinical manifestations of hyper-lipase secretion syndrome.

Pathologic Staging and Treatment Of the 17 patients, two were found to have metastatic disease on preoperative evaluation and never underwent surgical resection. Both of these patients were managed non-operatively. Of the remaining 15 patients, all underwent exploratory laparotomy (Fig. 2). Twelve patients were found to be surgically resectable at initial operation. One patient with regionally advanced tumor on initial exploration underwent subsequent chemotherapy and was then resected with negative margins at re-exploration. Surgical resections performed included nine pancreaticoduodenectomies and four distal pancreatectomies. The remaining two patients who were taken to the operating room were unresectable due to regionally advanced disease (one) or liver metastases (one). The patient with regionally advanced disease underwent palliative gastrojejunostomy and adjuvant chemotherapy.

Margins of surgical resection were negative (R0) in 12 patients and positive (R1) in one patient. The one patient with an R1 resection had a pancreaticoduodenectomy. The mean tumor size was 5.3 cm. American Joint Commission on Cancer tumor stages were: stage I (n=2), stage II (n=8), stage III (n=4), stage IV (n=3). Fifteen cases had surgical pathology (13 resections, one intraoperative incisional biopsy, and one transcutaneous core biopsy). The latter was a liver biopsy confirming a metastatic ACC. The remaining two patients were diagnosed on cytopathology

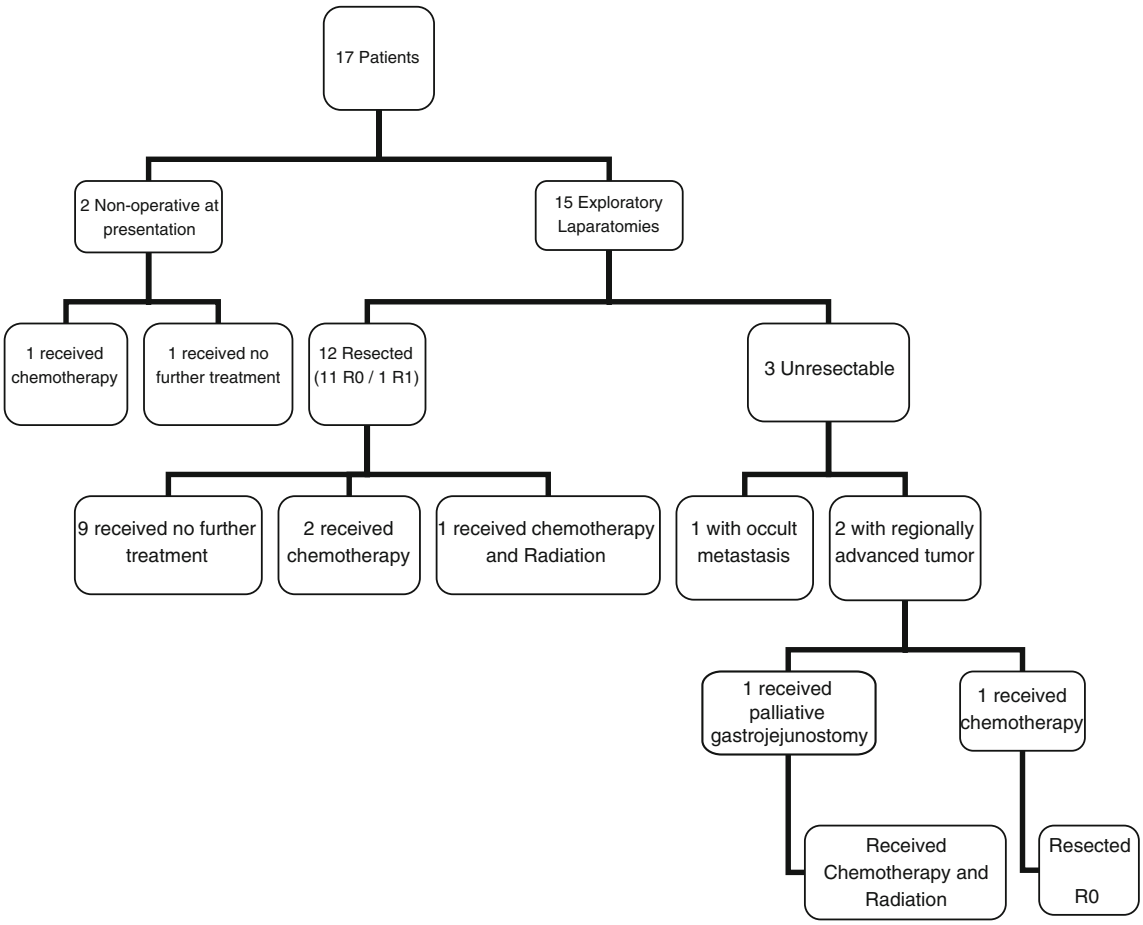

Figure 2 Schematic of patient treatment.

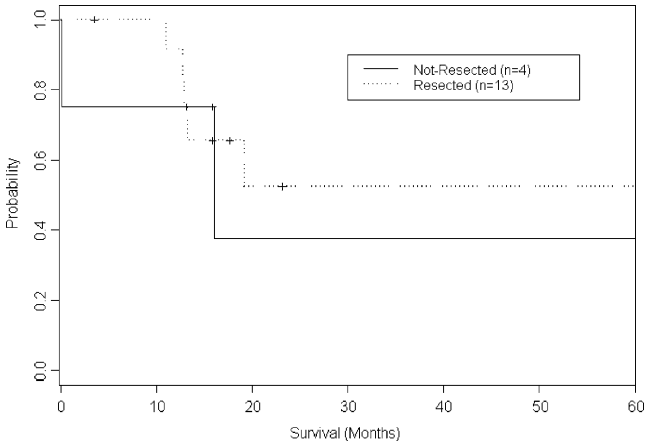

Figure 3 Survival differences among resected and not-resected patients. Probability of survival from date of diagnosis by resection is shown. Not resected (n=4), resected (n=13).

from endoscopic retrograde cholangiopancreatography brushings. The pathologic diagnosis was based in part on the morphology (H&E stain) in all cases. Special staining (PAS-D) and additional immunohistochemical stains for anti-chymotrypsin were performed in eight cases. In one case, ultrastructural studies with electron microscopy were required to confirm the presence of zymogen granules. None of the patients on re-review by pathology had endocrine differentiation or a mixed acinar–endocrine differentiation.

Survival and Recurrence Outcomes The overall 1- and 5-year survival rates of all patients in our study were 88% and 50%, respectively. Kaplan–Meier estimates of median and mean survival times were 19 and 39 months, respectively. Of the subgroup of patients who underwent surgical resection (13), 1- and 5-year survival rates were 92% and 53%, respectively, with a median survival of 61 months (Fig. 3). The longest survivor in the series to date (remains alive) is a patient with stage II disease who has lived 11 years after primary resection. Resection, chemotherapy, and radiation had no statistical significant effect on survival, but numbers in subgroups were small (Table 2). Among resected patients, six patients to date have been found to have tumor recurrence. Five patients were found to have metastasis to the liver (four) and supraclavicular nodes (one) in follow-up. The one patient with an R1 resection after a pancreaticoduodenectomy experienced a local recurrence. None of the patients who experienced recurrence received further surgical treatment. Presentation and survival outcome of patients did not significantly differ in the USA compared to Germany (Table 3).

Discussion

Acinar cell carcinoma is an uncommon solid epithelial exocrine tumor. In this multi-institutional study, we examined a 20-year primarily surgical experience with ACC. By combining multiple institutional experiences, we sought to better define the clinical presentation, pathology, treatment, survival outcomes, and patterns of recurrence of patients with ACC. These parameters distinguish ACC from other invasive pancreatic neoplasms, particularly the most common, pancreatic DCA.

The clinical presentation of ACC is unique compared to other invasive cancers of the pancreas. This is most pronounced in patients with ACC in the head of the pancreas. Despite a majority of ACC in our study being located in the head of the pancreas, the classic presentation of painless obstructive jaundice in these patients did not occur. In contrast, patients with ACC presented mainly with pain and weight loss. Furthermore, the serum tumor marker CA19-9, commonly elevated in invasive DCA, was not elevated in any patients with ACC.

The pathological characteristics of ACC are also quite unique compared to other invasive cancers of the pancreas. Most remarkable pathologically is the large size of ACC on presentation. Despite the large size of ACC, margins of resection were consistently negative in all but

Table 2 Survival of Patients with ACC as a Function of Treatment

	Alive	Dead	Median survival (months)	95% C.I. (months)	p value
Chemo + RT					0.1619
Yes	2	0	Not yet achieved	–	
No	6	9	19.2	(12.9, 66.7)	
Chemotherapy					0.2447
Yes	4	2	66.7	(66.7, –)	
No	4	7	19.2	(12.9, –)	
Resection					0.5058
Yes	7	6	62.7	(13.3, –)	
No	1	3	16.1	(0.2, 66.7)	
Overall survival	8	9	23.1	(15.9, 132.0)	

The two patients who received radiation are still alive, so the median survival cannot be estimated with the current data

There are not enough deaths to estimate the 95% confidence interval in all cases

Table 3 United States vs Germany Patients with ACC

	USA	Germany
Patients (*n*)	10	7
Mean (median) age (years)	57.3 (55)	65.0 (65)
Mean size (cm)	4.88±2.67	5.86±2.26
Mean survival (months)	47	31
Median survival (months)	67	19

one patient who underwent resection in this series. Furthermore, stage of ACC relative to the size of the tumor also appeared low, with nearly 60% of patients having stage I or II cancers in this series. Theoretically, this may be due in part to a selection bias since recognition of ACC over other more common invasive pancreatic malignancies at more advanced stages may not allow for optimal capture of ACC patients with advanced stage disease.

The survival of ACC in patients who underwent resection as well as in patients who were not surgical candidates appeared to be favorable when compared to other invasive cancers (i.e., DCA) of the pancreas. There were not enough non-operative patients in this series to make meaningful conclusions about the positive influence of surgical resection on ACC survival outcomes. Taken in context with the existing literature, the treatment of ACC does not appear unlike other invasive cancers of the pancreas insofar as surgery appears to be the first option in fit candidates.

Based upon the more favorable survival data relative to DCA, it is surprising that the patterns of recurrence of ACC were similar to pancreatic adenocarcinoma, i.e., the majority of recurrences were distant, not local. This suggests that ACC, like other invasive pancreatic cancers, is often a systemic disease despite preoperative staging suggesting local confinement. Patients who received adjuvant therapy did not have worse survival outcomes compared to patients who did not receive adjuvant therapy. One patient in our series underwent neoadjuvant therapy after staging laparotomy, which suggested unresectability, and went on to have an R0 resection. Other studies[15] corroborate similar outcomes in a few patients with ACC who underwent a neoadjuvant approach. Although there were not enough patients in this series to make meaningful conclusions about the influence of adjuvant or neoadjuvant therapy on survival outcomes in patients with ACC, the data are certainly encouraging that some patients appeared to derive benefit from this approach.

Due to the rarity of ACC, it is difficult to fully power outcome and natural history studies of patients with this disease. A thorough review of the literature reveals a collection of fairly small institutional series,[10–12,15] with the exception of one large registry study from Japan[4] (Table 4). Some of the older literature reports suggest that patients with ACC have a poorer prognosis when compared to more recent reports. Cubilla and Fitzgerald[13] and Webb[12] report an overall mean survival of 5–7 months in patients with ACC. Seth et al.[15] in a more recent study reports a median survival post-resection of 33 months. The disparity in survival in earlier series compared to more recent series may be explained in part by differences in preoperative tumor stage. Webb reports 75% stage IV disease in his patient population in contrast to Seth (14%) and our series (18%). In addition, most of the survival figures in the earlier literature quoted overall survival for the entire series of patients which included a relative minority of patients who underwent surgical resection. The more recent literature includes more surgically treated patients and better highlights the distinction between overall survival and survival post-resection. Similar to the study by Seth et al., the current study represents a primarily surgical series of patients

Table 4 Recent Case Series of ACC

Author	*N*	MET	Median age (years)	Mean tumor size (cm)	Overall MS (months)	Resected MS (months)[a]
Matos[b] (2008)	17	0	59	5.3	39	61 (13)
Schmidt (2008)	865	NA	66	5.9 (median)	24	25
Wisnoski (2008)	672	NA	56 (mean)	NA	47 (median)	123 (median)
Seth (2008)	14	2	57	3.9	NA	33 (14)
Holen (2002)	39	2	60	NA	19	36 (18)
Klimstra (1992)	28	9	62	10.8	18.1	18 (18)
Cubilla (1979)	6	NA	54	5 (median)	6.5	NA (0)
Webb (1977)	11	4	54 (mean)	6.4	5	NA

N number of patients, *MET* number of mixed endocrine tumors, *NA* not available, *MS* mean survival

[a] Number of resected cases

[b] Current study

with ACC and reports a relatively long mean survival (39 months).

In comparing ACC to the much more common DCA, we compared survival results to other recent literature reports of DCA. We identified 1,608 patients in the recent literature who underwent resection for DCA. The average median survival in resected patients with DCA was 24 months compared to 61 months in resected patients with ACC. The 5-year survival with DCA was 23% compared to 53% for patients with ACC. Overall, we found patients with ACC to have a better survival when compared to DCA.[16–23]

The objectives of this study were to try to improve our understanding of the presentation, pathology, treatment, survival outcomes, and patterns of recurrence of patients with ACC. Although this objective was met in part, there still are insufficient patient numbers to make meaningful conclusions about the influence of treatments. The strengths of the current study include a multiple institution experience with the ability to gather more patients than we would have with a single institution study. Registry studies have a significant advantage of greater numbers of patients, e.g., Kitagami (Japanese Cancer Registry)[4] and, more recently, Wisnoski et al.[24] and Schmidt et al.[25] who looked at the SEER and NCDB databases, respectively. Registry studies, however, are often not able to assess the specific details of patient presentation, operative parameters, and treatment. Furthermore, it is more difficult to control for pathologic review or coordinate a re-review of pathological specimens when a registry is used. The importance of an accurate diagnosis cannot be overstated. Major differences in outcomes may arise if endocrine and mixed-endocrine tumors are not excluded in analysis of ACC.[26] Our study excluded patients with tumors expressing endocrine and mixed-endocrine features.

The diagnostic characterization of ACC has undergone an evolution in the last two to three decades. Previously, a combination of morphology and electron microscopy was used to make the diagnosis of ACC. With the advent of immunohistochemistry and antibodies to trypsin, chymotrypsin and lipase in the last two decades, the diagnosis of ACC can now be made with these immunohistochemical stains. The most common neoplasm in the differential diagnosis of patients with ACC is the well-differentiated pancreatic endocrine neoplasm. Immunohistochemical stains for neuroendocrine markers, synaptophysin and chromogranin, are helpful in differentiating between the two neoplasms. Mixed acinar–endocrine neoplasms may occur, and it is extremely difficult to distinguish pure acinar cell neoplasms from mixed acinar–endocrine neoplasms based on morphology alone. Immunohistochemical studies are essential in the characterization of these tumors.[27] Neoplasms exhibiting >25% of both cell types should be designated mixed acinar–endocrine neoplasms.[26,28]

Worthy of mention is that our series included one patient who was found to have the rare intraductal acinar cell carcinoma (IACC) variant on final pathology. This patient presented with recurrent pancreatitis. On original evaluation, the patient was thought to have a main duct involved intraductal papillary mucinous neoplasm (IPMN) but after resection was found on pathology to have an IACC. Few case reports exist in the literature regarding IACC.[28–32] The patient with IACC in our series had the smallest tumor size in our series. This might be explained by an early symptomatic presentation due to its intraductal location.

It is unclear why ACC is such a rare tumor in comparison with DCA, particularly since acinar cells are so much more abundant than ductal cells in the pancreas. Some scientists speculate that ACC is rare because acinar cells undergo metaplasia into ductal cells when they experience genetic instability.[24,25,33–35] Acinar cell metaplasia may occur through upregulation of matrix metalloproteinase 7 (MMP-7)[33–35] or inhibition of the Mist1 protein known to be involved in differentiation, development, and maintenance of the different stages of pancreatic cell development.[36]

In conclusion, ACC is a rare pancreatic tumor with favorable prognosis compared to the more common DCA. Preoperative differentiation of ACC from DCA is difficult. Nonetheless, both require aggressive surgical resection. Importantly, some locally advanced ACC have responded to a neoadjuvant approach allowing resection of a downstaged tumor, so a combined modality approach should be considered in such patients.

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ORIGINAL ARTICLE

Operative Re-intervention Following Pancreatic Head Resection: Indications and Outcome

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Abstract
Background This study analyzed indication and outcome regarding operative re-intervention following pancreatoduodenectomy (PD) and pancreatogastrostomy (PG) with special emphasis on complications related to redo surgery.
Patients and Methods Two hundred eighty-five patients who underwent PD with PG between 1989 and 2008 were identified from a pancreatic resection database and indications for repeat surgery were registered. Patients with and without reoperation were analyzed with regard to gender, age, underlying disease, length of hospital stay, mortality rate, and postoperative complications.
Results Thirty-one patients (11%) underwent operative reintervention. Early intra-abdominal extraluminal postoperative bleeding was the main cause for redo surgery followed by abdominal abscesses. Thirteen percent of patients with and 1.9% without secondary surgery died during the postoperative course. Forty-five percent of reoperated patients had to undergo at least one more operation resulting in doubling of the length of hospital stay. There was no correlation between patients' gender, age, and underlying disease and the need for operative reintervention. However, redo surgery was associated with higher incidence of delayed gastric emptying, pancreatic fistula and bleeding, and non-surgery related complication. Intra-abdominal bleeding and abscesses, insufficiencies of bilio-digestive and gut anastomosis, wound infections, and pancreatitis were observed significantly more often in patients with secondary surgery.
Conclusions Complications after pancreatic resection that require operative re-intervention are associated with a notably increased mortality, ranging between 13% and 60%. Apart from the surgeon's experience in selecting patients and his/her personal technical skills in performing a pancreaticoduodenectomy, timely anticipation and determined management of postoperative complications is essential for improving the outcome of this operation.

Keywords Operative re-intervention · Redo surgery · Repeat laparotomy · Additional operation · Secondary surgery · Kausch–Whipple procedure · Partial pancreaticoduodenectomy · Pancreatic resection

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Introduction

Pancreaticoduodenectomy remains one of the most formidable operations for the abdominal surgeon.[1–3] It is not only a technical challenge, but also demanding for patients. The persistent high incidence of morbidity following pancreatic head resection refers to the complexity of surgery with multiple anastomoses of different types, a consuming underlying malignancy and patients who are usually in an advanced stage of life.[4] Under these circumstances, the need for secondary surgery due to post-operative complications exposes the patient to a considerably increased mortality risk as operating in these situations easily becomes a hazardous venture. Therefore, the aim of the present study was to evaluate indications and outcome as

well as prevention potentialities of operative re-interventions after pancreaticoduodenectomy.

Material and Methods

Between 1989 and 2008, 285 patients with PD and PG reconstruction were identified from a pancreatic resection database. Patients were evaluated for resectability preoperatively by thin-section CT angio scans and general operability according to a protocol of our Department of Anesthesiology. Jaundiced patients had endobiliary stent placement by endoscopic retrograde cholangiopancreaticography (ERCP). Magnetic resonance cholangiopancreatography (MRCP), upper GI endoscopic ultrasound, PET-CT, and diagnostic laparoscopy were only used in exceptional cases.

PD was performed via bilateral subcostal incision as a classic Kausch–Whipple procedure, with distal gastrectomy and reconstruction of the alimentary tract according to either Billroth II or Roux-en-Y. Following single-layer hepaticojejunostomy, the pancreatic remnant was anastomosed with the posterior gastric wall using a seromuscular purse-string suture (0–2/0 PDS) and four to six interrupted sutures (4/0 Monocryl) between gastric mucosa and pancreatic capsule.[5] No pancreatic duct stents were used. All patients received perioperative antibiotic and postoperative weight adapted thrombosis prophylaxis. A pancreatic secretion inhibitor (octreotide 300 μg/d s.c.) was administered on individual decision of the surgeon. Four soft drainages were placed next to anastomoses and patients were monitored on ICU for at least one night. The nasogastric tube was removed as soon as the daily output was less than about 500 ml and oral intake was permitted from postoperative day 2.

Patients with and without secondary operation were analyzed regarding gender, age, length of hospital stay, hospital mortality, morbidity in detail,[6] and need for third and consecutive operations. Data until the end of 1999 were collected retrospectively and prospectively thereafter. Surgical complications were classified as major if reoperation was required and as minor if conservative or interventional procedures were sufficient. Until 2005, pancreatic fistula was defined as persistent secretion of >50 ml/d amylase-rich fluid (more than three times the normal amylase plasma levels) for more than 6 days from the drains placed in the vicinity of the pancreatogastrostomy or anastomotic disruption confirmed by X-ray or operation. Since then, ISGPF definition has been adopted.[7] Catmaker 1.1 (Centre for Evidence-Based Medicine, Oxford, UK) and Microsoft Excel were used for data collection and analysis. Chi^2, Student's t and Fisher's exact tests were used as appropriate. A p value <0.05 was considered statistically significant.

Results

Patients

Of the 285 resections, 128 (45%) were for pancreatic adenocarcinoma, 67 (24%) for ampullary cancer, 21 (7%) for distal bile duct cancer, 35 (12%) for chronic pancreatitis, and 34 (12%) for miscellaneous reasons. There were 58% male and 42% female patients with an average age of 64 (±10) years. There were no statistical differences between patients with and without reoperation regarding underlying disease (pancreatic adenocarcinoma, distal bile duct and papillary cancer, and chronic pancreatitis), gender, and age. Table 1 compares a first series between 1989 and 1999 with a second series between 2000 and 2008. There was a highly significant decrease in postoperative in-hospital deaths (7.3% vs. 0.6%) between the two series with no differences regarding postoperative complications (43% vs. 57%) and reoperations (10.2% vs. 11.9%). The overall morbidity and mortality rates for all patients were 52% and 3%, respectively (Table 2).

Reoperations

Thirty-one (11%) patients required at least one operative reintervention during their postoperative stay. Compared to the "near-zero-mortality" of the whole cohort, mortality in these patients increased to 13% (Table 2). Accordingly, mortality in patients without need for reoperation felt below 2% (Table 3). Mortality occurred in both groups on average at day 29 after the index operation with slight differences in the standard deviation. Septic shock with consecutive multi-organ failure and pulmonary complications were the most common causes of death. Forty-five percent of patients with redo surgery required at least one more surgical intervention during the further course. Accordingly, the length of hospital stay more than doubled in these patients (Fig. 1).

Figure 2 shows the most common indications for secondary surgery. Early extraluminal intra-abdominal bleeding (29%) followed by infectious fluid collections

Table 1 Morbidity, Mortality and Incidence of Secondary Surgery in Patients with Pancreaticoduodenectomy and Pancreatogastrostomy between 1989 and 1999 and between 2000 and 2008

Period	Mortality (%)	Morbidity (%)	Reoperation (%)	Remarks
1989–1999 (n=109)	7.3	43	10.2	Retrospective analysis
2000–2008 (n=176)	0.6	57	11.9	Prospective analysis

Table 2 Overall Morbidity and Mortality and Incidence of Redo Surgery with Related Mortality in Percent after Pancreatic Resection

Center (year)	Morbidity	Overall mortality	Reoperation	Reoperation mortality	Remarks
Mainz[1] (1999)	25	6	8.6	37	Data on morbidity indicates surgery-related complications only
Bern[19] (2000)	38	2.1	3.9	23	Including DPPHR
Liverpool[4] (2002)	54	5	9	25	Cancer cases only
Heidelberg[9] (2003)	36	2	4	16	All pancreatic resections including left-sided, DPPHR etc.
Mannheim[20] (2003)	30	3.1	7,2	36	
Ann Arbor[21] (2004)	28	3.7	3.7	60	
Toulouse[22] (2005)	46	11	20	43	Multicenter study comparing PJ vs. PG; data on reoperation mortality indicates inclusion of interventional procedures
Bonn (2008)	52	3	11	13	
Mean (n=2,067)	**38 (784)**	**3.5 (72)**	**7.2 (149)**	**28 (42)**	**Relative risks from pooled data of all studies**

The list lays no claim on completeness. The series are not immediately comparable and include different operative techniques

DPPHR duodenum-preserving pancreatic head resection, *PJ* pancreaticojejunostomy, *PG* pancreatogastrostomy

(23%) were the main cause for additional operations in these patients. In case of early hemorrhage, indications were depending on bleeding severity either Hb-decrease in the routine hemogram and/or a general patient's deterioration which led to further diagnostics. Technical mishaps such as slipped ligatures or incomplete transfixation sutures, disintegration of thermocoagulated vessels, and diffuse bleeding due to coagulopathy were observed. Retroperitoneal cut surface and splenic vessels were mainly affected. The operative strategy, which was indicated on average on postoperative day 5, ranged from local control of bleeding (individual approach according to bleeding site) up to abdominal packing with subsequent laparotomies. In two cases, hemorrhage of splenic artery and at gastroenterostomy resulted in considerable insufficiency of the pancreatic anastomosis which was redone after successful control of bleeding. In case of intra-abdominal abscesses, relaparotomy with lavage and (re-) placement of drainages was performed after failure of CT-guided drainage placement.

In contrast to hemorrhage, septic complications are typically not advanced at the early postoperative days. If it was not the patient's condition (including drainage quality) that concerned us we initiated further diagnostics in case of persistently high or sharply increasing inflammatory parameters. In these patients reoperations were performed on postoperative day 12 on average (range, 4–24 days).

Further indications included complications at the laparotomy site (19%) and enterogenous complications (13%), e.g. ischemia, ileus (Fig. 2). In three cases, a wound hematoma or wound infection was surgically sanitized and three dehiscences were closed with interrupted all layer sutures. Due to gut ischemia in two patients, segmental intestinal resection was indicated. A perforation of the anterior gastric wall due to either an ulceration or nasogastric tube or both was treated by excision and oversewing. A stenosis of the Braun entero-enterostomy resulted in an ileus and was managed by redoing the anastomosis.

Table 3 Comparison of Postoperative Course in Patients with and without Secondary Surgery after Pancreaticoduodenectomy

Re-Operation	Re-Reoperation	Length of stay	Complications			
			Grade V (mortality; %)	Grade IV and III (ICU, invasive; %)	Grade I and II (bed-side; %)	Overall (%)
With (n=31)	45%	41 days	13	100	100	100
Without (n=254)	0	20 days	1.9	15	31	46

Statistical analysis revealed significant differences ($p<0.001$) for all comparisons. Grading refers to "Classification of surgical complications adopted for pancreatic surgery" by DeOliveira et al.([6]) Grade IV and III includes need for invasive therapy and organ dysfunction with ICU stay and grade II and I includes bed-side therapy with no need for further intervention

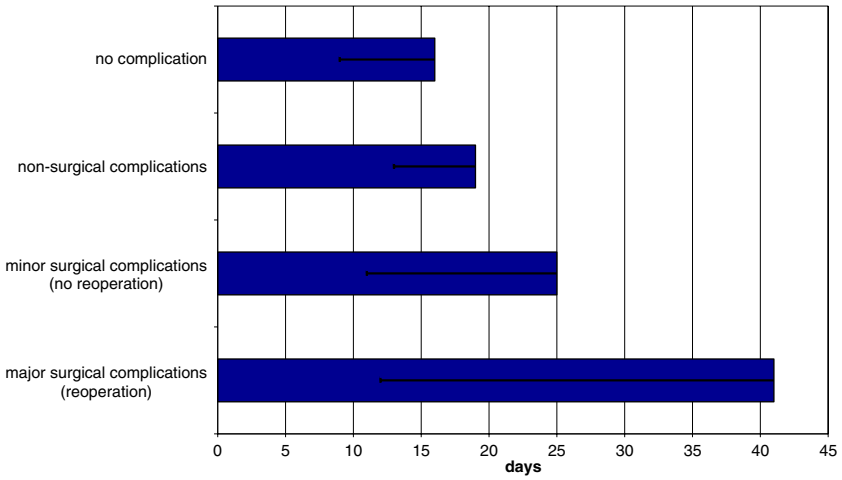

Figure 1 Length of hospital stay according to complication severity. There was a statistically highly significant difference ($p<0.001$) regarding length of hospital stay between patients without additional operation (*first three bars*, mean 20 days) and patients requiring secondary surgery.

Bile-leakage (10%), pancreatitis (3%), and bleeding at the pancreatic anastomosis (3%) were less commonly observed indications for operative reintervention (Fig. 2). One early fistula of the bilio-digestive anastomosis was immediately re-explored and oversewn. A volume-rich leakage due to upper abdominal ischemia following a post-thrombotic stenosis of the celiac trunk forced us to break up the anastomosis with blind closure of the hepatic duct and transhepatic drainage followed by successful reconstruction 5 months later. An aberrant bile duct of the right liver lobe was accidentally ignored and closed during surgical re-exploration. Necrectomy with drainage replacement, pancreatic duct blocking, and closure of the gastrotomy was performed in one case due to necrotizing stump pancreatitis with progressive multi-organ failure. In one instance of complete pancreatic stump necrosis with concomitant hemorrhage, completion pancreatectomy was performed. As published elsewhere, hemorrhage of the pancreatogastrostomy can be controlled easily by endoscopy and injection of hemostatics.[8] Nevertheless, this procedure failed in one patient and the bleeding anastomosis was oversewn via gastrotomy of the anterior gastric wall.

Postoperative Course

The incidence of ten postoperative complications was compared between patients with and without additional surgery (Tables 3 and 4). Delayed gastric emptying and pancreatic fistula occurred more often in patients with secondary surgery. However, the difference did not reach statistical significance. Non-surgical complications and pancreatogastrostomy hemorrhage occurred significantly more often in patients with secondary surgery ($p<0.01$). In terms of figures, significant differences were observed for wound infections, intra-abdominal hemorrhage, abdominal abscesses, pancreatitis, and bile- and entero-leaks, which occurred more often in patients after operative reintervention.

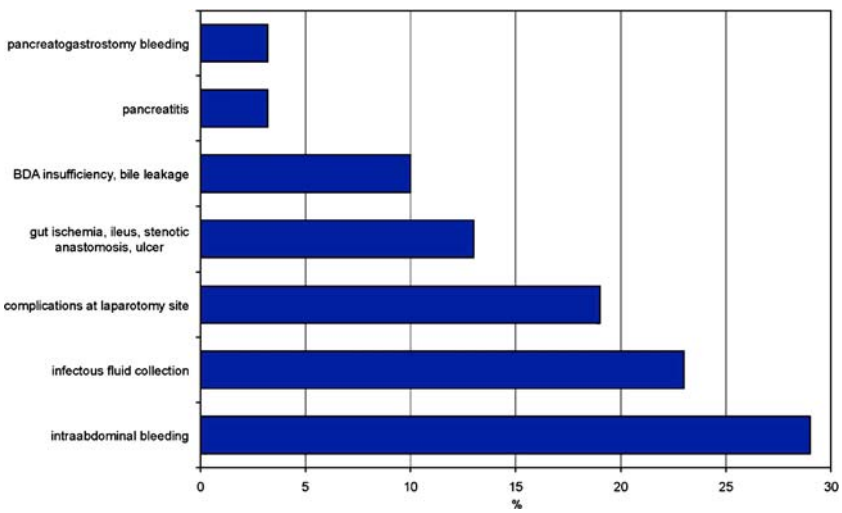

Figure 2 Own indications for repeat laparotomy after pancreaticoduodenectomy.

Table 4 Complications in Patients with and without Secondary Surgery after Pancreaticoduodenectomy in Percent

	Not significant		$p<0.1$		Significant					
Reoperation	DGE	Pancreatic fistula	Non-surgical mortality	PG-bleeding	Wound infection	Abdominal bleeding	Abdominal abscess	Pancreatitis	Bile-leak	Entero-leak
With (n=31)	29	13	32	9.7	32	29	26	9.7	9.7	6.5
Without (n=254)	17	7.1	18	3.2	4.7	1.6	2.8	1.2	0.4	0

DGE delayed gastric emptying, *PG* pancreatogastrostomy

Discussion

Pancreatic resection remains an intervention of particular significance, often technically challenging and with logistic demands for preoperative diagnostics and perioperative management.[9] With persisting mortality rates of about 2–5%, the focus lies on attempts to lower morbidity rates, especially since postoperative complications considerably contribute to the remaining overall mortality. Moreover, secondary surgery after pancreatic resection together with the underlying intra-abdominal complication can also be held responsible for most of the extra-abdominal complications. Therefore, timely anticipation and detailed knowledge about indications for reoperations in these patients together with a determined complication management is essential for further lowering mortality rates.

In our series, postoperative hemorrhage was the most frequently observed complication that required secondary surgery after pancreaticoduodenectomy. In general, the management of hemorrhage depends on the time of onset: early versus late hemorrhage, and on the bleeding localization: intraluminal versus intra-abdominal. According to the International Study Group of Pancreatic Surgery (ISGPS), early postpancreatectomy hemorrhage occurs within the first 24 h after the index operation and late hemorrhage thereafter.[10] Reactionary early hemorrhage is most likely caused by technical failure of appropriate hemostasis, e.g. slipped ligatures, incomplete transfixation sutures, or insufficient coagulated smaller vessels, or less often by an underlying perioperative coagulopathy. The need for prompt emergency reoperation in these situations is beyond doubt. The per definition timeframe of 24 h, however, seems—according to our experience—somewhat tight, as we had to perform secondary surgery due to hemorrhage on average on postoperative day 5. This finding is in accordance with other reports attributing early hemorrhage to the first 3 to 7 days following the index operation.[11–13] We cannot offer a clear cut off day between early and late hemorrhage, as, among others, bleeding intensity, patient's condition, need for ICU-therapy, and the probability of a causative septic complication do have an effect on the decision finding towards immediate operation or time-consuming radiological intervention. Nevertheless, the earlier (and more intense) the bleeding appears the more likely we would decide to operate.

Delayed hemorrhage, on the other hand, is often secondary to an anastomotic leak with subsequent erosion of the retroperitoneal vasculature (e.g. gastro-duodenal artery) or formation of pseudoaneurysms.[14] Here, (super-) selective angiography with interventional coil embolization or endovascular stenting is able to achieve efficacious hemostasis with a fairly high success rate.[13] In contrast, emergency surgery for secondary hemorrhage remains the solution for hemodynamically unstable patients or after failure of an angiographic approach.[11] Nevertheless, one should bear in mind that even if an interventional approach is successful, surgery is still likely to be required to deal with the underlying cause of hemorrhage. This is more feasible in stable patients.[15] Endoscopy would be the procedure of choice for intraluminal bleeding sites and is especially suitable for pancreatogastrostomy hemorrhage, as this anastomosis is easily accessible with this procedure. In case of bleeding at the pancreaticojejunostomy, however, an operative approach is usually mandatory as this anastomosis lies beyond endoscopic accessibility and the jejunal loop is filled with blood clots.[12]

Following early extraluminal bleeding, intra-abdominal infectious fluid collections were the second main cause for additional operations in our cohort. This was an unexpected observation as the procedure of choice for this complication is radiologically guided percutaneous drainage placement.[16] However, in two instances, organized hematomas were causative and too viscous for suction irrigation. Moreover, most of these operative interventions were carried out between 1989 and 1999. To date, the more sophisticated potentialities of interventional radiology have brought this indication for secondary surgery close to zero. Accordingly, the availability of more sophisticated interventional procedures has contributed to significantly reduce the need for secondary surgery in recent patients.

The general consensus is for conservative management of pancreatic fistula in the absence of peritonitis, sepsis,

hemorrhage, or organ failure.[5] As shown in our study, most leaks of the pancreatic-enteric anastomosis run a benign course if properly drained and maintained by reduction of oral intake and adequate nutritional support, i.v. antibiotics if necessary, and close monitoring.[2] Despite conflicting publications and an ongoing discussion[17] we would administer octreotide for about 7 days in this situation.

Operative reintervention is usually mandatory in otherwise uncontrollable general sepsis with progressive organ failure or in case of complete anastomotic breakdown. The degree of destruction and inflammation in the retroperitoneum as well as the condition of the pancreatic stump will determine the surgical strategy, while the severity of clinical instability will also set limitations regarding extent and duration of the operative procedure. Oversewing or redoing of the anastomosis has little value as these methods are rarely successful. We were able to do so in only two cases of early anastomotic breakdown due to hemorrhage and without any concomitant pancreatitis. Completion pancreatectomy, on the other hand, will probably salvage the situation, but the procedure is technically demanding and hazardous. We performed one completion pancreatectomy due to severe pancreatitis with concomitant hemorrhage and anastomotic breakdown, but the patient died after 11 follow-up operations with abdominal lavage and 59 postoperative days. Accordingly, other centers and our institution have abandoned this procedure from their complication management armory.[9,18] Local debridement and extensive peripancreatic drainage with and without occlusion of the pancreatic duct represents probably the best feasibility in these situations. A temporary takedown of anastomoses might be helpful to achieve better clarity of the situs, as they completely obliterate the view of the operative field. Especially the subsequent reconstruction is challenging but accurate identification of anatomy and complete evacuation of septic deposits often mandates this approach.

Fistulation or leakage of the bilio-digestive anastomosis was less frequently observed than pancreatico-enteric insufficiencies and it is rarely seen in fatal postoperative courses. Here, management depends on time of onset and output rate. Early leakages within the first one or two postoperative days result usually from a technical problem and oversewing or redoing the anastomosis is purposeful. In most instances, smaller anastomotic leakages later on with good drainage and in the absence of biliary peritonitis can be managed conservatively. Since an endoscopic approach to the anastomosis is in most instances impossible, ERCP with nasobiliary drain placement is not an option for these patients. Also, in singular cases aberrant and surgically missed bile ducts can be made responsible for postoperative fistula. Gastro-enterostomies and entero-enterostomies after pancreaticoduodenectomy are very rarely prone to insufficiencies and thus do not play a major role in the postoperative course in our and other series.

Subsequent operations were required in nearly 50% of patients with redo operations. The majority of these were necessary in patients with septic complications and the need for open abdominal lavage with secondary abdominal wall closure. According to acute pancreatitis, closed packing, or closed continuous lavage might be an alternative approach that could reduce the need for subsequent surgical intervention, especially in patients with peritonitis.

In summary, complications after pancreatic resection that require operative re-intervention are associated with a notably increased mortality rate ranging between 13% and 60% and prolongate the hospital stay according to complication severity. Main indications for secondary surgery were early extraluminal intra-abdominal hemorrhage and abscesses. Almost half of the reoperated patients required at least one more operation. There was no correlation between patients' gender, age, and underlying disease and the need for operative reintervention following pancreatoduodenectomy.

While primary pancreas resection requires significant expertise, operative reintervention due to postoperative complications are even more demanding. Especially in the case of a complicated postoperative period after the index operation, access to the complication site can be a fortuitous trial. The operating field with its changed anatomy is hypervascularized and postoperative adhesions protract the surgical approach. In case of pancreaticojejunostomy, the jejunal loop is relatively fixed owing to the end-to-side hepaticojejunostomy and the gastrojejunostomy, which makes access to the anastomosis even more difficult. In addition, patients in these situations are often in a critical condition. The continuous and close postoperative observation of the patient is of paramount importance to timely diagnose severe complications and early diagnosis and experienced management of these complications can improve outcome and save lives. The continuous development of specialist units (high throughput centers) with increased experience in postoperative complication management and the necessary resources for interdisciplinary treatment might further improve operative mortality rates following pancreaticoduodenectomy.

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ORIGINAL ARTICLE

Molecular Analysis of *PIK3CA*, *BRAF*, and *RAS* Oncogenes in Periampullary and Ampullary Adenomas and Carcinomas

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Abstract
Background Mutations of *KRAS* are known to occur in periampullary and ampullary adenomas and carcinomas. However, nothing is known about *NRAS*, *HRAS*, *BRAF*, and *PIK3CA* mutations in these tumors. While oncogenic *BRAF* contributes to the tumorigenesis of both pancreatic ductal adenocarcinoma and intraductal papillary mucinous neoplasms/carcinomas (IPMN/IPMC), *PIK3CA* mutations were only detected in IPMN/IPMC. This study aimed to elucidate possible roles of *BRAF* and *PIK3CA* in the development of ampullary and periampullary adenomas and carcinomas.
Methods Mutations of *BRAF*, *NRAS*, *HRAS*, *KRAS*, and *PIK3CA* were evaluated in seven adenomas, seven adenomas with carcinoma in situ, and 21 adenocarcinomas of the periampullary duodenal region and the ampulla of Vater. Exons 1 of *KRAS*; 2 and 3 of *NRAS* and *HRAS*; 5, 11, and 15 of *BRAF*; and 9 and 20 of *PIK3CA* were examined by direct genomic sequencing.
Results In total, we identified ten (28.6%) *KRAS* mutations in exon 1 (nine in codon 12 and one in codon 13), two missense mutations of *BRAF* (6%), one within exon 11 (G469A), and one V600E hot spot mutation in exon 15 of *BRAF*. *BRAF* mutations were present in two of five periampullary tumors. All mutations appear to be somatic since the same alterations were not detected in the corresponding normal tissues.
Conclusion Our data provide evidence that oncogenic properties of *KRAS* and *BRAF* but not *NRAS*, *HRAS,* and *PIK3CA* contribute to the tumorigenesis of periampullary and ampullary tumors; *BRAF* mutations occur more frequently in periampullary than ampullary neoplasms.

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Keywords Ampullary cancer · Periampullary · *KRAS* · *BRAF* · *PIK3CA*

Introduction

Ampullary cancers account for 5% of all gastrointestinal tract malignancies.[1] Tumors of the ampulla of Vater include tumors arising in the ampulla (intra-ampullary type), tumors arising in the periampullary region of the duodenum (periampullary type), or tumors involving both the intra-ampullary and periampullary region of the duodenum (mixed periampullary and intra-ampullary type).

Ampullary adenocarcinomas often are identified in association[2] with adenoma precursor lesions. Ampullary carcinomas represent approximately 10% of cancers resected via the Whipple procedure (pancreaticoduodenec-

tomy).[3] The peak age incidence is in the 8th decade, with men more commonly affected than women.[2] The 5-year survival rates of patients with resected ampullary carcinoma are reported to be 33–50%,[4] and thus significantly better than the observed 10–20% 5-year survival rate of patients with resected conventional pancreatic ductal adenocarcinoma.[5] These data suggest that differences in tumor biology may also be an explanation for the relatively good survival of patients with this disease. The biological characteristics of periampullary and ampullary adenocarcinomas have not been extensively studied. Genetic alterations of the *KRAS* oncogenes,[6] the *p53*,[7] *p16*,[8] and *MADH4*(*SMAD4*/*DPC4*)[9] tumor suppressor genes, all commonly altered in pancreatic cancer,[10] have also been described in periampullary and ampullary cancer, although at lower frequencies.[11]

Oncogenic point mutations in the three human *Ras* genes (*NRAS*, *HRAS*, *KRAS*) have been detected in a wide variety of human cancers. Since the discovery of the role of *RAS* oncogenes in tumorigenesis, an increasing focus has been set to define its oncogenic signal transduction pathway and the mitogen-activated protein kinase (MAPK) pathway has emerged as an important link between membrane-bound Ras proteins and the nucleus,[12,13] involving the kinase cascade Raf–MEK–ERK (*MEK*, MAPK/ERK kinase; *ERK*, extracellular signal-related kinase).[14] Signaling through the MAPK cascade is transduced by guanosine triphosphate loading of Ras leading to the activation of Raf kinase. *BRAF* mutations have been described in about 15% of all human cancers and are known to have a mutational hot spot at codon 600, which is reported to account for 91% of *BRAF* mutations in human cancers.[15] We and others have previously shown that oncogenic *BRAF* contributes to the tumorigenesis of pancreatic ductal adenocarcinoma and IPMN/IPMC of the pancreas.[16–18]

Phosphatidylinositol-3 kinases (PI3Ks) constitute a large and complex family of lipid kinases.[19–21] They play an important role in several cellular functions, such as proliferation, differentiation, chemotaxis, survival, trafficking, and glucose homeostasis,[19] activating diverse cellular target proteins such as the survival signaling kinase AKT/PKB.[19,20,22] A tumorigenic role has been proposed for the *PIK3CA* gene that encodes the catalytic p110α subunit of phosphatidylinositol 3-kinase belonging to the class IA of PI3Ks.[19,21] Previously, Samuels et al.[23] reported mutations in *PIK3CA* in several tumor types. In the study by Samuels et al.[23] three *PIK3CA* mutational hot spots were described and found to affect the helical (exon 9) and catalytic (exon 20) protein domains. Similar to colon tumors, *PIK3CA* mutations also clustered in the three hot spot regions (exons 9 and 20) in gastric carcinomas.[23,24] Other independent studies in hepatocellular carcinomas, breast carcinomas, lung cancers, ovarian carcinomas, brain tumors, head and neck squamous cell carcinomas, and intraductal papillary mucinous neoplasms of the pancreas have since supported and emphasized the oncogenic potential of *PIK3CA* in the development of cancer.[24–29] We and others have previously shown that *PIK3CA* mutations occur in ~10% of IPMN/IPMC but not in the pancreatic ductal adenocarcinoma.[23,29,30] In the present study, we analyzed the mutational status of *BRAF*, *NRAS*, *HRAS*, *KRAS*, and *PIK3CA* to elucidate a possible role of these genes in the tumorigenesis of periampullary and ampullary adenomas and carcinomas.

Materials and Methods

Patients and Tissue Samples

Surgical paraffin-embedded adenoma and carcinoma samples from 31 patients (female n=17, male n=14, median age 63.6 years, range 40–85 years) were obtained from the archival tissue collection of the Columbia University Medical Center. Acquisition of the tissue specimens was approved by the Institutional Review Board of Columbia University Medical Center and performed in accordance with Health Insurance Portability and Accountability Act regulations. In detail, we analyzed 35 tumor areas including seven adenomas, seven adenomas with carcinoma in situ (CIS), and 21 carcinomas of the periampullary and ampullary region. The anatomic distribution of the tumors included 24 ampullary tumors, sox tumors involving both ampulla and periampullary duodenal region, and five periampullary duodenal tumors. Of the five periampullary tumors analyzed, two tumors involved the papilla of Vater whereas three tumors were located within 2 cm of the papilla and were classified as periampullary tumors of duodenal origin (see Table 1 for a more detailed register.)

DNA Samples for Mutation Analysis

All tissue samples were handled in an environment free of polymerase chain reaction (PCR) products. Paraffin-embedded tumor samples were microdissected by hand. Surrounding nontumorous tissue or tissue derived from a tumor-free specimen of the same patient served as the corresponding normal control. Genomic DNA was extracted using QIAmp DNA Mini Kit (Qiagen, Valencia, CA, USA). The procedures were performed according to the manufacturers' instructions for paraffin-embedded tissues.

Exons 1 of *KRAS*, 2 and 3 of *NRAS* and *HRAS*, exons 5, 11, and 15 of *BRAF*, and exons 9 and 20 of *PIK3CA* were analyzed by PCR amplification of genomic DNA and direct sequencing of the PCR products. Genomic DNA (40 ng per sample) was amplified with primers that had been designed to specifically amplify the codons 12 and 13 of *KRAS* or

Table 1 Summary of the Sample Data and Mutation Status of the Lesions Investigated

Sample no.	Age	Sex	Lesion analyzed	Anatomic location Ampullary	Anatomic location Periampullary	pTNM	Stage	KRAS mutation	BRAF mutation
1	76	M	CA + CIS	X		pT3N0	II		
2	74	M	CA + CIS	X	X	pT2N0	II		
3	72	F	CA + CIS		X	pT3N1	III		
4	58	M	Adenoma	X	X	[a]	0	G13D	
5	49	M	CA + CIS	X	X	pT3N1	III	G12D	
6 [a]	63	F	CA, small cell	X		pT3N1	III		
6 [a]			Adenoma + CIS	X					
7	72	F	CA + CIS	X		pT2N0	II		
8	65	F	Adenoma	X		[a]	0		
9	66	F	Adenoma	X		[a]	0		
10	71	F	CA + CIS	X		pT3N1	III		
11	56	F	Adenoma + CIS	X		[a]	0		
12	65	F	CA + CIS	X		pT3N1	III		
13	40	M	Adenoma	X		pT2N0	II		
14	73	M	CA + CIS	X		pT3N1	III		
15	71	M	CA + CIS	X		pT2N0	II		
16	70	F	CA + CIS	X	X	pT3N0	II	G12D	
17	78	M	CA + CIS	X		pT1N0	I		
18	52	M	Adenoma + CIS		X -D	pT1N0	I	G12V	G469A
19	68	M	CA + CIS	X		pT1N0	I		
20	68	M	CA + CIS	X		pT3N0	II	G12D	
21 [a]	85	F	CA + CIS	X		pT2N0	II	G12R	
21 [a]			Adenoma + CIS	X				G12R	
22	44	F	Adenoma	X		[a]	0		
23	64	F	CA + CIS	X		pT3N1	III		
24	67	F	CA + CIS	X		pT3N0	II	G12V	
25 [a]	51	M	Adenoma + CIS		X-D	pTisN0	0		
25 [a]			Adenoma		X	[a]	0		
26	44	F	Adenoma	X	X	[a]	0	G12D	
27	77	F	CA + CIS	X		pT2N0	II		
28 [a]	40	M	CA + CIS	X		pT3N0	II		
28[a]			CA[b]	X					
29	71	F	Adenoma + CIS	X	X	pTisN0	0		
30	78	F	Adenoma + CIS		X-D	pT4N1	III		V600E
31	43	M	CA	X		pT3N1	III	G12V	

CA invasive carcinoma, *CIS* carcinoma in situ, Anatomic location of tumor: *ampullary* periampullary or both, *D* duodenal mucosa adjacent to ampulla not involving papilla of Vater, *TNM* stage of tumor in Whipple resection specimen (Tis: carcinoma in situ)

[a] Different areas of same tumor were analyzed.

[b] High-grade/giant cell

each exon and its exon/intron boundaries in the *NRAS*, *HRAS*, *BRAF*, and *PIK3CA* loci, respectively. The primers were adopted from those published in the literature to omit analyzing the *BRAF* and *KRAS* pseudogenes.[31–33] Before sequencing, all PCR products were purified, using QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). Sequencing was performed with ABI's 3100 capillary automated sequencers at the DNA core facility of Columbia University Medical Center. All samples found to have genetic alteration in the target genes were subsequently sequenced in the reverse direction to confirm the mutation. The mutations were then further verified by sequencing of a second PCR product derived independently from the original template.

Results

In the present study, 35 periampullary and ampullary adenomas and carcinomas were analyzed for mutations in the *BRAF*, *NRAS*, *HRAS*, *KRAS*, and *PIK3CA* genes. We performed sequencing analyses of codons 12 and 13 of exon 1 of *KRAS*, the entire exons 2 and 3 of *HRAS* and *NRAS*, exons 5, 11, and 15 of *BRAF*, and exons 9 and 20 of *PIK3CA* in all these specimens. These regions included the most common *HRAS*, *NRAS*, *KRAS*, *BRAF*, and *PIK3CA* mutations previously observed in human cancers.[23,31–33]

Two *BRAF* mutations (6% of all samples, 66% of periampullary tumors originating from the duodenum) were identified in our set of samples: one exon 15 hot spot mutation at nucleotide 1799 (G**T**G→G**A**G), leading to an amino acid change from valine to glutamic acid (V600E) and one exon 11 mutation at nucleotide 1406 (G**G**A→G**C**A), leading to an amino acid change from glycine to alanine (G469A), which has also been described previously (Fig. 1 and Table 1). Interestingly, both mutations were found in periampullary adenomas with CIS originating from the duodenal mucosa. We did not identify any mutation in exon 5.

KRAS mutations were found in ten (28.6%) of the 35 samples (Fig. 2 and Table 1). Nine tumors carried a mutation of codon 12 and one a mutation of codon 13. In detail, we identified four *KRAS* mutations within the 14 adenomas (28.6%) and six mutations in the 21 ampullary and periampullary carcinomas (28.6%). The distribution of *KRAS* mutations showed a single mutation in all observed cases. The coexistence of KRAS and BRAF mutations was observed in a periampullary adenoma sample (see Table 1). All mutations proved to be somatic since none of them was detected within the matching normal tissues (Fig. 1). No mutation was detected in the *HRAS*, *NRAS*, and *PIK3CA* loci.

Discussion

Carcinomas of the ampulla of Vater and the periampullary region are distinguished from conventional pancreatic ductal adenocarcinoma clinically and pathologically, but the relationship of these tumor types at the genetic level is still being investigated. *KRAS* gene mutations have been demonstrated in periampullary and ampullary adenomas, even in areas of low-grade dysplasia. In addition, there is a strong correlation (93%) between the *KRAS* gene mutation found in ampullary adenomas and their associated infiltrating carcinomas,[34,35] indicating that *KRAS* gene mutations in ampullary cancer, when present, occur early in tumorigenesis. Mutant Kras constitutively activates the Raf–MEK–ERK–MAP kinase pathway, which mediates cellular response to various growth signals.[36,37] Unlike pancreatic ductal adenocarcinoma, where *KRAS* is mutated at a frequency close to 100%.[38,39] approximately 60% of ampullary carcinomas do not harbor an active *KRAS* mutation. This suggests that a relatively large percentage of periampullary and ampullary adenomas and carcinomas might use alternative ways to activate the RAS–RAF–MEK–ERK–MAP kinase pathway.

BRAF, a serine/threonine kinase located immediately downstream in RAS signaling, has been examined and found to be mutated in a variety of human malignant

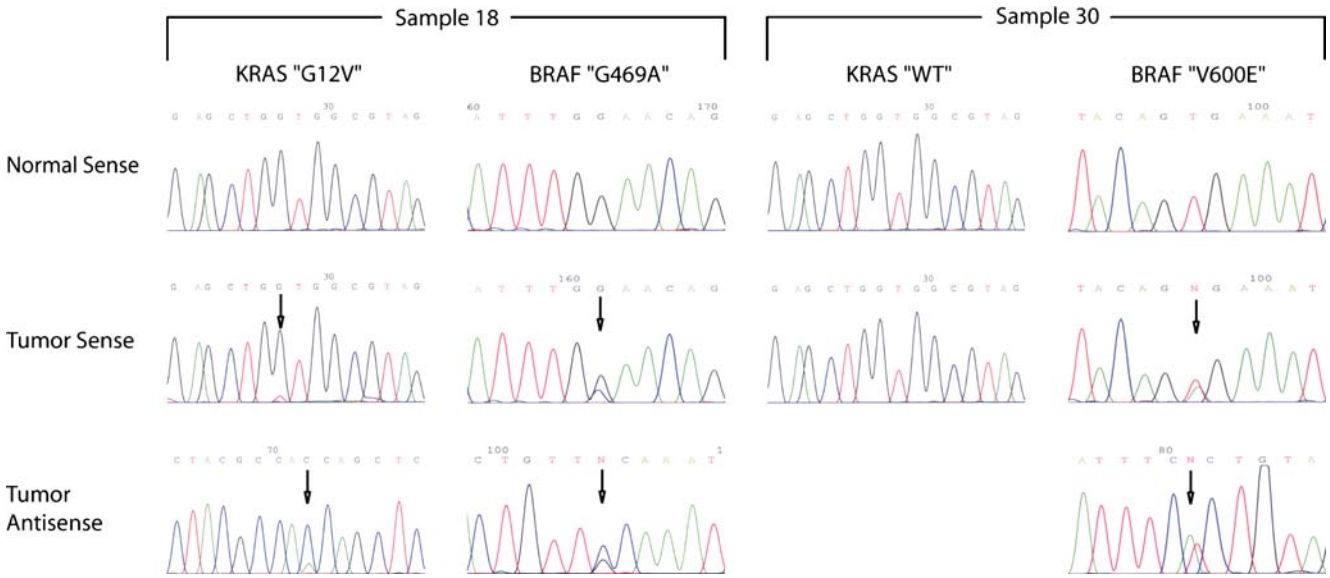

Figure 1 Somatic *BRAF* mutations found in two duodenal periampullary lesions with their respective KRAS status (*WT*, wild type). One of the BRAF mutations (V600E) was a hot spot mutation. All mutations were confirmed to be somatic.

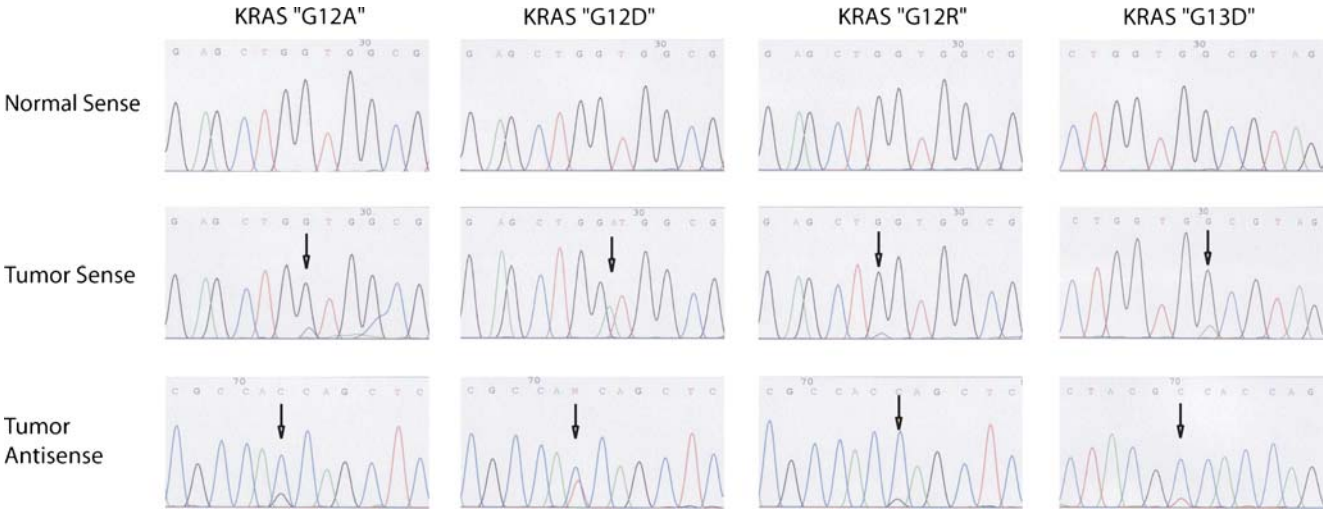

Figure 2 Four KRAS mutant codons were identified in ten specimens. Nine tumors carried a mutation of codon 12 and one a mutation of codon 13. Representative of each mutant codon is shown here. All codon changes were somatic.

neoplasms. Here, we report two somatic *BRAF* mutations out of 35 (6%) periampullary and ampullary neoplasms examined. Our data are in concordance with the literature, which described a trend for *BRAF* mutations in cancer types harboring *KRAS* mutations.[31] *BRAF* is known to have a mutational hot spot at nucleotide 1799, which is reported to account for 91% of *BRAF* mutations in human cancers[15,31,33,40] (V600E). All mutations observed in our set of neoplasms were within exon 11 and 15. These mutations are not only the predominant type in melanoma but also in colon cancer and sarcoma.[31] Transfection assays revealed that these mutations were active in vitro and stimulate the activity of the ERK pathway in vivo.[31] Davies et al. showed that Ras function was not required for the growth of cancer cell lines with the V600E mutation. Mutations at exon 11, codon 469, of *BRAF* have been found in several tumors and appear to be the second most frequent mutation of *BRAF* in human cancers.[15] The ^{G469A}*BRAF* mutant in particular has been shown to have similar activity to ^{V600E}*BRAF* and is also generated through a single-nucleotide substitution but accounts for less than 1% of mutations.[31] In our study, *KRAS* and the ^{G469A}*BRAF* mutation occur simultaneously in one periampullary adenoma of duodenal origin. It has been observed previously that *BRAF* mutations, other than *BRAF* V600E, coexisted with *RAS* mutations.[31] The *BRAF* V600E mutation seems to uncouple cells from their proliferation requirement of *RAS*, and mutation of *RAS* was not detected in any of the tumors carrying this particular mutation.[31] However, *in vitro* data indicated that ^{V600E}*BRAF* mutants can be further activated by mutant *RAS*, whereas other *BRAF* mutants remain dependent on *RAS* function.[31] A previous study on pancreatic ductal adenocarcinoma revealed that the V600E mutation occurred in two of nine xenografted tumors retaining wild-type copies of the *KRAS*, *NRAS*, and *HRAS* genes, but none in 72 xenografted carcinomas with *KRAS* mutations within exons 11 and 15.[16] In contrast, another study on pancreatic adenocarcinoma found both *KRAS* and *BRAF* V600E mutations coexisting in two cases.[17] Previously, we were able to show that, in IPMN, *KRAS* mutations coexist with *BRAF* mutations, other than the V600 mutation.[18] Cells with activating mutations in both *KRAS* and *BRAF* had a substantially higher B-Raf kinase activity and ERK 1/2 phosphorylation activities than those with *BRAF* mutation alone.[31] So tumors with both *BRAF* and *KRAS* mutations might have an accelerated course in terms of development or progression. *BRAF* mutations have been detected in the early stages of colon cancer and melanoma development.[41,42] These observations are in concordance with our results, where both *BRAF* mutations occurred in periampullary adenomas with CIS, indicating that *BRAF* mutation, when present, is an early event in tumorigenesis.

In summary, we found two *BRAF* and ten *KRAS* and no mutations of *HRAS*, *NRAS*, and *PIK3CA* in 35 periampullary and ampullary adenoma and carcinoma samples. The rarity of *PIK3CA* mutation in periampullary and ampullary adenoma and carcinoma is potentially significant, given that *PIK3CA* mutations was previously reported in 10% of IPMN but not in pancreatic ductal adenocarcinoma.[23,29,30] There appears to be a divergent role for *PIK3CA* in the tumorigenesis of IPMN/IPMC and adenocarcinoma. Another intriguing aspect of our results is that both *BRAF* mutations were found in two of a total of three (66%) periampullary adenomas of duodenal origin and none in ampullary cancer. All mutations found proved to be somatic. This is the first mutational study of *BRAF*, in periampullary and ampullary neoplasms. Our data indicate that *BRAF* mutations do not play a major role in the

tumorigenesis of ampullary carcinomas but are of importance in periampullary lesions, specifically those of duodenal origin. Targeted mutation of *BRAF* is an early event, which suggests that alteration of RAS–RAF–MEK–ERK–MAP kinase pathway by *BRAF* mutation together with *RAS* mutation plays an important role in periampullary tumorigenesis.

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CASE REPORT

Neuroma of the Bile Duct: A Late Complication After Cholecystectomy

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Abstract
Introduction Extrahepatic biliary obstruction with a mass in the common bile duct and elevated CA 19-9 level is often due to cholangiocarcinoma.
Case Report We present a case of a 71 year-old woman who presented with an extrahepatic biliary obstruction and a mass in the common bile duct 45 years after cholecystectomy. Pathologic analysis revealed a bile duct neuroma. We present the preoperative imaging, operative management, pathologic diagnosis, and literature review of this rare condition.

Keywords Biliary obstruction · Bile duct neuroma · Cholangiocarcinoma · CA 19-9

Case Presentation

A 71-year-old woman presented with a several-month history of intermittent right upper quadrant abdominal pain and jaundice. She had lost 50 lb over the past several months due to loss of appetite. Review of systems was otherwise negative. Her past medical history was significant for congestive heart failure and chronic renal insufficiency. Her past surgical history was remarkable for a history of cholecystectomy and common bile duct exploration complicated by an intra-abdominal abscess in 1963. On physical exam, she was afebrile, and her abdominal exam was benign. White blood cell count was normal. Other pertinent lab values included total bilirubin 1.7 mg/dl, direct bilirubin 0.3 mg/dl, alkaline phosphatase 237 U/l, CA 19-9 113.1 U/ml (normal range <34.9 U/ml). A computed tomography scan of the abdomen revealed no masses in the pancreas or periampullary region. There was no periportal lymphademopathy. There was a possible 1–2 cm mass noted in the common bile duct. Magnetic resonance cholangiopancreatography (MRCP; Fig. 1) and endoscopic retrograde cholangiopancreatography (ERCP; Fig. 2) revealed a 1–2 cm mass in the common bile duct. Brushings for cytology were indeterminate but suspicious for malignancy. A biliary stent was placed to relieve the biliary obstruction. She was taken to the operating room with a presumptive diagnosis of cholangiocarcinoma for exploratory laparotomy, periportal lymphadenectomy, extrahepatic bile duct resection, and Roux-en-Y hepaticojejunostomy reconstruction. There were extensive adhesions encountered in the right upper quadrant and the porta hepatis, likely from her prior postoperative abscess. There was a firm 2-cm mass present in the mid-common bile duct. Due to the adhesions and inflammatory changes, the dissection was difficult. A periportal lymphadenectomy was performed. At the time of surgery, it was not possible

Meetings presented at: None

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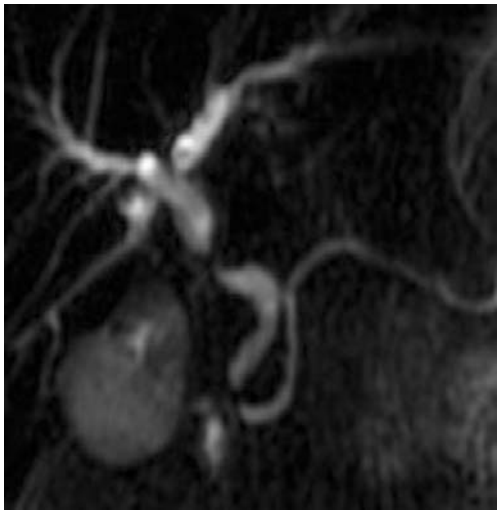

Figure 1 MRCP shows focal smooth mass occupying lesion found in the midcommon bile duct. There is bilateral intrahepatic biliary dilation.

to differentiate the process as a benign finding vs. a malignancy. All margins at surgery were free of malignancy as were the celiac and periportal lymph nodes. Pathologic analysis of the surgical specimen revealed a bile duct neuroma (Figs. 3 and 4). She was discharged home on postoperative day # 6 after an unremarkable hospital course.

Discussion

Neuroma of the biliary tree was first described in 1928 by Husseinoff.[1] Since that time, there have been a total of 84 cases of biliary obstruction due to neuromas reported in the worldwide literature.[2] The majority of these reports come from non-English literature. Neuromas most commonly occur in the cystic duct stump after a cholecystectomy and have been described after both laparoscopic and open cholecystectomy. Common bile duct exploration has also been associated with neuroma formation.

Neuroma formation is thought to be precipitated by posttraumatic nerve cell growth after surgery.[2–6] It is a nonneoplastic disorganized proliferation of axons, Schwann cells, and perineurial cells in a fibrocollagenous stroma and

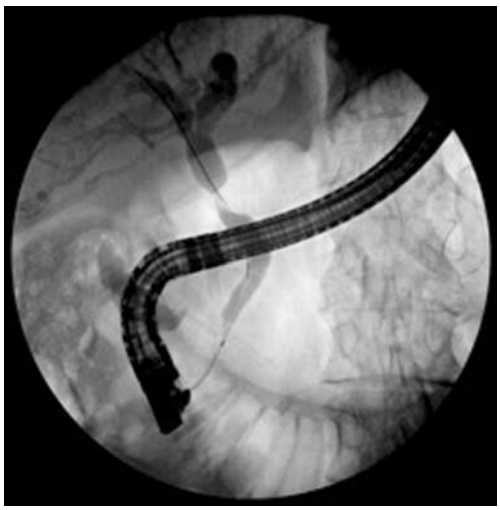

Figure 2 ERCP shows contrast within the common bile duct, demonstrating a circumferential smooth filling defect in the mid-common bile duct. Bilateral intrahepatic and common hepatic ducts are dilated.

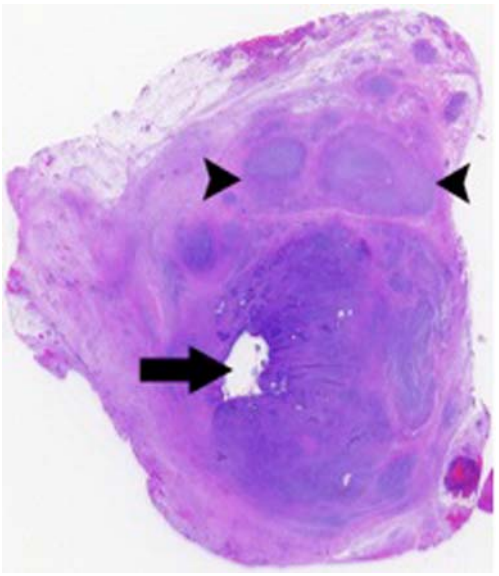

Figure 3 H&E stain of a cross section of the resected bile duct showing eccentric thickening of the wall and severe narrowing of the bile duct lumen (*arrow*). Parent nerve fascicles are noted at the periphery of the lesion (*arrowheads*).

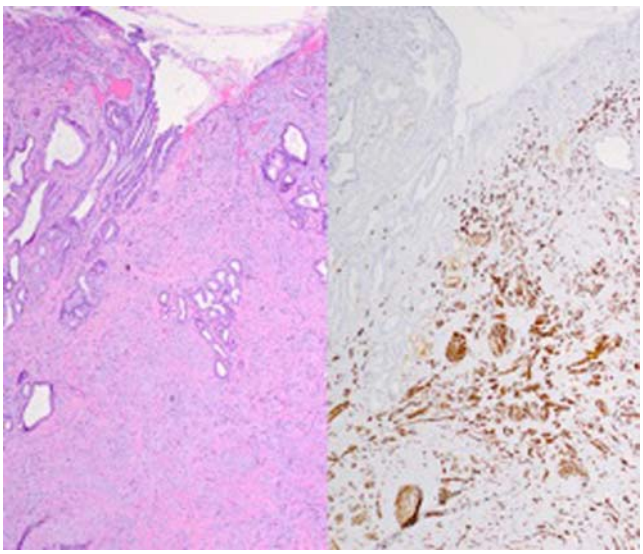

Figure 4 H&E stain (*left*) and S-100 protein immunohistochemical stain (*right*) showing haphazard proliferation of nerve fibers, highlighted by S-100 immunohistochemical stain, in between and compressing the peribiliary glands.

affects nerves that are encased in Schwann cells.[2] Nerve hypertrophy in response to injury is similar to that occasionally seen with extremity amputations. The sympathetic and parasympathetic fibers arising from the greater and lesser splanchnic nerves are involved.[4] Although there has been no definitive mechanism describing the dysregulated growth pattern, there has been the suggestion of increased levels of fibroblast growth factor and its receptor in traumatic neuromas.[3]

There are reports in the literature of biliary tree neuroma presenting from several months to 40 years after cholecystectomy.[2–6] Our patient underwent cholecystectomy with common bile duct exploration in 1963. Her 45-year interval between surgery and symptomatic presentation with bile duct neuroma is the longest interval ever reported between the initial procedure and the development of symptoms.

Bile duct neuroma has been demonstrated in up to 10% of post cholecystectomy patients at autopsy in one study.[3] The vast majority of these patients remain asymptomatic for life. A very small proportion of patients eventually develop symptoms. Symptomatic patients tend to present with intermittent symptomatic right upper quadrant pain and jaundice. The vast majority of these patients are diagnosed in retrospect when the surgical pathology specimen is examined.[2–6] Indeed, in most cases, the leading differential diagnosis is cholangiocarcinoma due to the similarity of presentation. The associated biliary obstruction is not treated well in the long term with biliary stenting. Due to the need for biliary decompression and, in most cases, to obtain the correct diagnosis, surgery is indicated.[2–6] The most widely advocated approach in the literature is extrahepatic bile duct resection with negative margins, periportal lymphadenectomy, and Roux en-Y hepaticojejunostomy.[2–6] Given that the diagnosis is almost never made preoperatively, and cholangiocarcinoma remains high on the differential, a simple excision and hepaticojejunostomy may not be adequate. Unless the diagnosis is definitively known preoperatively, an aggressive resection with formal periportal lymphadenectomy is advocated.

Our patient had an elevated CA 19-9 level of 113.1 U/ml (normal range is<34.9 U/ml) prior to surgery. Although CA 19-9 levels are often elevated with biliary malignancy such as cholangiocarcinoma, elevated levels are not specific for this diagnosis.[5,7] CA 19-9 levels have also been reported to be elevated in other settings such as liver disease, ascending cholangitis, and pancreatitis. Mann et al. studied all patients at their institution with CA 19-9 levels above 34. Patients with benign conditions generally had modest elevations of CA 19-9 (mean 102 U/ml, interquartile range (IQR) 50–264). There was also a correlation with bilirubin levels. With the relief of the obstruction, both the bilirubin and CA 19-9 levels returned to normal. In contrast, patients with malignancy generally had much higher CA 19-9 levels (mean 910 U/ml, IQR 263–6,170). Ultimately, the authors concluded that they were unable to discriminate between benign and malignant disease based on the magnitude of CA 19-9 elevation alone.[7]

Others have described the value of preoperative testing to delineate between benign and malignant biliary strictures. Bain et al. studied factors associated with malignant vs. benign strictures of the biliary tree. Total bilirubin >75 μmol/l (4.3 mg/dl), longer stricture length (30 vs. 9.2 mm), and the presence of intrahepatic biliary dilation were all suggestive but not diagnostic for malignant biliary stricture.[8] A stricture in midbile duct is uncommon for cholangiocarcinoma, which most often presents distally in the head of the pancreas or proximally at the confluence (Klatskin). This may provide a clue to the diagnosis. A stricture in this location makes one think of gallbladder cancer when the gallbladder is present, but in its absence, perhaps benign neuroma should be higher on the differential. Most authors currently support imaging of the extrahepatic biliary tree with ERCP or MRCP preoperatively; however, definitive diagnosis has not been described based on imaging alone.[6]

In light of the difficulties with prospective diagnosis and the common presentation with biliary obstruction, these patients may be best served with an aggressive approach including formal biliary resection if they are medically fit for surgery. An aggressive surgical approach may aid in the definitive diagnosis as well as providing definitive biliary decompression.

Financial interests None

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CASE REPORT

Single Incision Laparoscopic Splenectomy: The First Two Cases

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Abstract
Aims Single incision laparoscopic procedures are presumed to be as a step towards pure natural orifice transluminal endoscopic surgery. However, loss of requirement of any perforation of visceral organ and endoscopic equipment make this technique more popular and easily performable. Herein we report two splenectomy cases where single incision surgery (SILS) technique was performed.
Cases Two females of 28 years old and 22 years old with the diagnoses of ITP underwent single incision laparoscopic splenectomy. Preoperatively with the receival of steroid therapy, thrombocyte counts were 92,000/m^3. A 2-cm umbilical incision was used for the placement of three (5 mm) trocars. One 5-mm videoscope (30°) and roticulated laparoscopic dissector/grasper were the main tools during surgical procedure. Spleen was removed with a plastic removal bag through the umbilical trocar incision. The whole procedure ended in 110 and 150 min in both cases without any problem.
Results Two patients were discharged on third and second postoperative days with the thrombocyte counts of 174,000/m^3 and 400,000/m^3, respectively.
Conclusion Although there were some procedures performed with single incision technique like cholecystectomy, prostatectomy, and partial nephrectomy, as far as we are concerned this is the first report about laparoscopic splenectomy performed with single incision surgery technique.

Keywords Single incision surgery · Splenectomy · Laparoscopy

Laparoscopic surgery is a well-established alternative to open surgery across disciplines. Although the magnitude of impact varies by procedure, in general, the benefits of laparoscopy on postoperative pain, cosmesis, hospital stay, and convalescence are widely recognized. Current efforts are aimed at further reducing the morbidity associated with minimally invasive surgery. To this end, two recent innovations are being developed, either pure or hybrid: natural orifice transluminal endoscopic surgery (NOTES), whereby intraperitoneal access is gained through the mouth, anus, vagina, or urethra and the viscus-of-entry is perforated to reach the surgical target; and embryonic natural orifice transumbilical endoscopic surgery (E-NOTES), wherein the surgical scar is virtually concealed within the umbilicus, an embryonic natural orifice.[1,2] Transumbilical surgery either can be performed with one port having three working channels or three separate trocars introduced through the same umbilical incision. The latter technique is entitled laparoendoscopic single site incision (LESS) or single incision laparoscopic surgery (SILS).[3]

Our institution began performing LESS since January 2009, and subsequently we developed a technique for laparoendoscopic single site splenectomy. To our knowledge, we herein report the first SILS splenectomy cases.

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Cases

All patients were vaccinated against pneumococci (Pneumovax 23, Boehringer) 2 weeks prior to the operation, and

received 1 g sulbactam/ampicillin intravenously as a preoperative prophylaxis. All patients were informed about the details of the surgical procedure and informed consents were taken.

The first case was a 28-year-old female patient with the diagnosis of ITP. Both cases underwent single incision splenectomy. Preoperatively with the receival of steroid therapy, thrombocyte counts were 92,000/m^3. The second case was a 22-year-old female, again with the diagnosis of ITP. Her preoperative thrombocyte counts were 100,000/mm^3. Preoperative abdominal computerized tomography of both cases was normal and did not reveal any accessory spleen.

Surgical Technique

Patients were placed in a semilateral position on the right side with left arm fixed over the head and a cushion placed under the right side. The surgeon and the assistant stood on the right side of the patient with the monitor placed on the opposite side of the patient. Under general anesthesia, a 2-cm complete umbilical skin incision of 2 cm was made. Pneumoperitoneum was performed through the umbilicus with a Veress needle in closed technique. After the completion of 12 mmHg CO_2 pneumoperitoenum, the "three ports" with the size of 5 mm were placed into the abdominal cavity through this 2-cm umbilical incision (Fig. 1). The patient was then put in a reverse Trendelenburg position with the right side rotated down. We have routinely used a rigid 30°, 5-mm laparoscope and a standard rigid 5-mm laparoscopic instrument for all procedures. Once the laparoscope, grasper, and dissector were placed, the overall procedures were similar to the procedures performed in a three-port laparoscopic splenectomy. The most difficult part of this technique was working instruments that were crossing each other and roticulated.

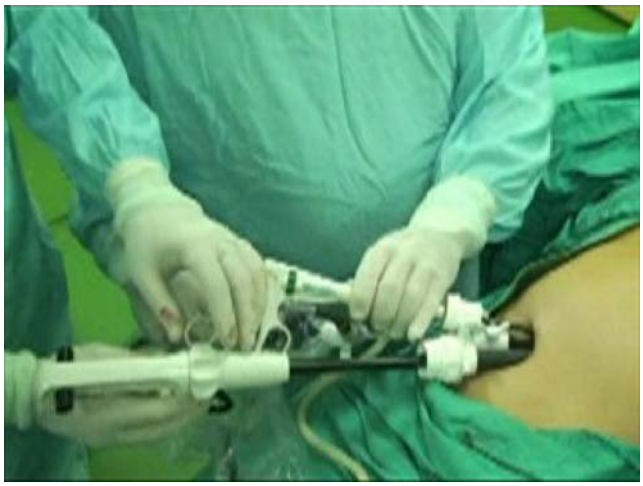

Figure 2 One of the 5-mm trocars replaced with a 15-mm trocar for the final hilum ligation with endoscopic vascular stapler.

The 5-mm telescope was introduced under both instruments and over both instruments changing according to the surgical step of the procedure. Nothing different from the three-trocar laparoscopic splenectomy technique was performed. The first step was the liberation of the inferior pole of the spleen with the dissection of the splenocolic ligament. As the second step, gastrosplenic ligament was opened and lesser sac was explored to expose the splenic hilum. During all these steps, at least one of the roticulated grasper and dissector equipment was used. Including these tools, a 5-mm Ligasure standard laparoscopic straight hook was also used. Following the completion of splenic hilum dissection, one of the 5-mm trocars was replaced with a 15-mm trocar to be able to introduce the endoscopic stapler with white cartridge (Fig. 2). At this time, the whole spleen was dissected and liberated other than hilum a small piece of phrenosplenic ligament. Finally, the hilum was ligated and cut with this stapler (Fig. 3) and the spleen was

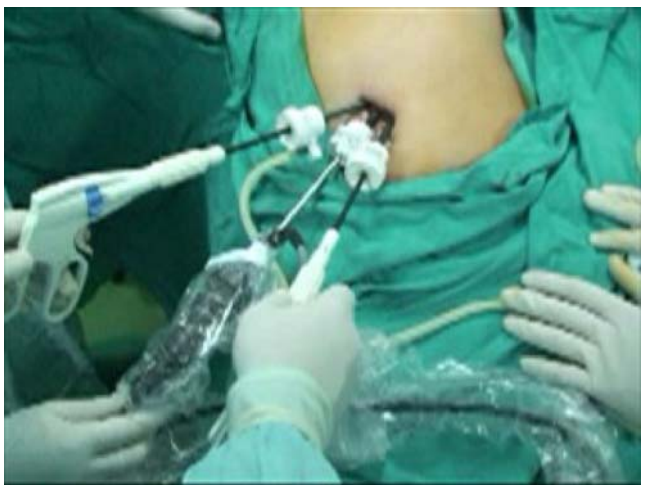

Figure 1 Transumbilical three 5-mm trocars.

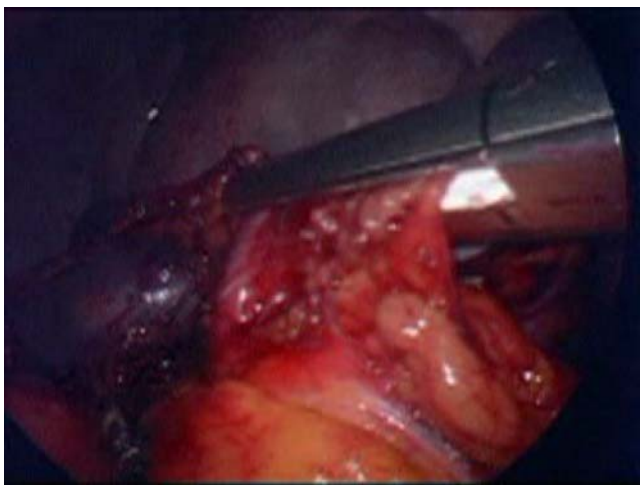

Figure 3 Hilum ligation with an endoscopic vascular stapler.

removed with a retrieval bag that was introduced through the same 15-mm trocar. The spleen was morcellated in this bag before removal. An aspirative silicone drain was placed in splenectomy lodge through the other 5 mm trocar and finally the 15 mm trocar site closed with a polypropylene (no. 0) suture.

Postoperative Period

Patients received oral food at postoperative eighth hour and mobilized. Drains of both cases were removed on the first postoperative day. The first and the second cases were discharged on the third and second postoperative days, respectively. Postoperative pain was assessed by visual analog scale.[4] Postoperative pain scores of the cases on the first postoperative day were 2/10. Although pain seemed to be minimized compared with the regular laparoscopic approach, the number of patients was too small to make any conclusions. Postoperative follow-up did not reveal any umbilical wound complication (Fig. 4).

Discussion

The introduction of laparoscopy in the early 1990s ushered in a new era in the surgical treatment of human diseases. Evolution of minimally invasive techniques has furthered an impulsion in the surgical community to reduce the invasiveness of laparoscopic surgery. To achieve this goal, surgeons have anticipated limiting the number of abdominal incisions (as in SILS) or eliminating them completely (as in natural orifice transluminal endoscopic surgery [NOTES]).[5]

To date, however, experience with SILS is still in its infancy, with fewer than 80 published cases reported for all indications and no splenectomy cases. As clinical experience with SILS increases, it is imperative that we critically evaluate two important questions: First, does SILS compromise on current standards of surgical care? Second, are the true benefits of SILS restricted to only improved cosmesis, or are there benefits with respect to convalescence and postoperative recovery?

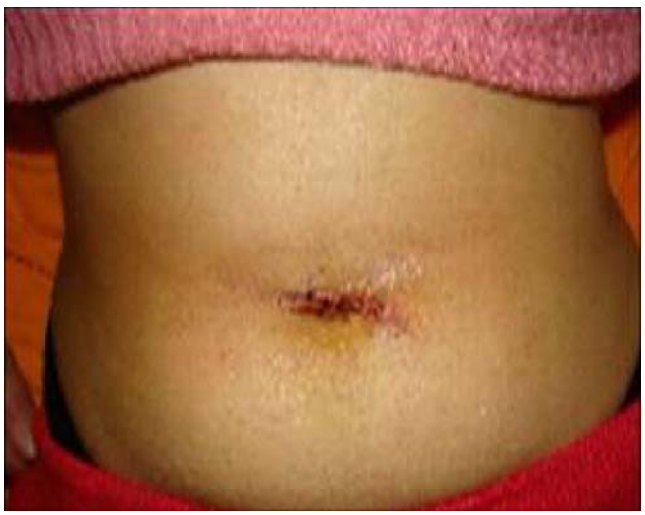

Figure 4 Postoperative umbilical wound site.

Raman's findings in single incision nephrectomy cases underscore that, in the hands of an experienced laparoscopic surgeon, SILS nephrectomy is equally efficacious to conventional laparoscopic nephrectomy without compromising on surgical or postoperative outcomes.[6] Interestingly, despite this series representing their initial SILS experience, they noted no differences in any operative variables compared to conventional laparoscopy. Anecdotally, they observed no increased difficulty in their cases compared to radical nephrectomy, although they presume that there may be future cases in which dense retroperitoneal inflammatory reaction may prove a challenging obstacle.

In SILS, since all instruments were closely packed together, clashing of instruments and the laparoscope was common. It has a unique learning curve, principally in navigating the instruments within a limited range of motion and needs significant coordination between the surgeon and the camera holder. The surgeon also has to be adapted to counterintuitive movements due to frequent crossing of the instrument shafts at the point of entry into the abdominal cavity.

Other than nephrectomy, prostatectomy was also successfully performed by Kaouk et al. They performed single-port laparoscopic radical prostatectomy in four patients diagnosed with prostate cancer.[7] Patients with early-stage prostate cancer (T1c), no previous pelvic surgery, and a body mass index <35 kg/m^2 were selected for single-port laparoscopic radical prostatectomy. A multichannel port was inserted transperitoneally through a 1.8-cm umbilical incision. No additional extraumbilical instruments or ports were inserted. Urethrovesical anastomosis was performed using free-hand interrupted suturing and extracorporeal knot tying. One of their patients developed a rectourethral fistula that was noted 2 months after surgery and was managed with a mucosal advancement flap.

The sleeve gastrectomy is routinely performed using five and up to seven laparoscopic trocars with enlargement of one of the trocar sites for extraction of the gastric specimen. Kevin et al. described the first case of laparoscopic sleeve gastrectomy performed through a single laparoscopic incision.[8] Hodgett et al. recommend single incision cholecystectomy for patients with uncomplicated gallbladder pathology and biliary anatomy not distorted by inflammation.[9] After comparison of 29 cases of standard multiport laparoscopic cholecystectomy with SILS, they

concluded that it is a safe alternative to standard laparoscopic cholecystectomy and can be done with comparable operative times. Randomized controlled trial to document not only safety and feasibility but also patient satisfaction, postoperative pain, and cosmesis should be performed to be able to comment on.

Minimal invasive splenectomy history started with Delaitre in 1991 and widened its range including massive splenomegaly cases.[10] The ultimate point in minimal invasiveness was three-trocar laparoscopic splenectomy. Application of SILS in solid organ surgery like nephrectomy led us to our laparoscopic splenectomy experience in SILS splenectomy. Herein we performed single incision splenectomy in two cases of ITP successfully without sacrificing the standard principles of splenectomy. To our knowledge, these are the first SILS splenectomy cases reported in literature.

Single-port laparoscopy has had a positive effect on standard laparoscopy. Undoubtedly, single-port or single incision laparoscopy, even with flexible instrumentation, is technically more challenging than straight laparoscopy; however, we are still in the initial learning curve. This new technique of single-port surgery has brought to light various extra aspects of standard laparoscopy and seems to have facilitated these cases as well.

In experienced hands of minimally invasive surgery, SILS splenectomy is equally efficacious to conventional laparoscopic splenectomy without compromising surgical standards of care. Although SILS splenectomy may offer a subjective cosmetic advantage, validated patient-outcome data are required to more objectively address this final comment. Prospective comparison between SILS and conventional laparoscopic procedures is mandatory to more clearly define the exact impact of single incision surgery.

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Long Mesentericoportal Vein Resection and End-to-End Anastomosis Without Graft in Pancreaticoduodenectomy

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Abstract
Introduction The feasibility and safety of pancreaticoduodenectomy (PD) combined with long segmental mesentericoportal vein (MPV; >5 cm) resection and end-to-end anastomosis without graft has rarely been demonstrated.
Materials and methods Eight patients with pancreatic head adenocarcinoma underwent PD combined with long MPV resection between August 2006 and May 2008 in Peking University School of Oncology.
Results By liver mobilization and Cattell–Braasch maneuver, direct and tension-free end-to-end anastomosis was easily performed even when the resected segment of the MPV was longer than 5 cm. All the eight patients experienced uneventful recovery without severe complications.
Conclusions PD with long MPV resection and direct end-to-end anastomoses is safe and effective.

Keywords Pancreatic carcinoma · Pancreaticoduodenectomy · Vascular resection

Introduction

Pancreaticoduodenectomy (PD) remains the only potential curative treatment for carcinoma of the pancreatic head. The presence of metastatic disease or invasion of local structures ensures that most patients are not operative candidates at presentation.[1] Historically, involvement of regional vasculature by pancreatic carcinoma has been considered a contraindication to curative resection. However, because of technical advances in vascular surgery, the involvement of the superior mesenteric vein (SMV) or portal vein (PV) is no longer an absolute contraindication for radical surgery.[2] It has been reported that the presence of segmental mesentericoportal vein(MPV) resection combined with PD could significantly increase the R0 resection rate without sacrificing the morbidity or mortality.[3,4] It was also popularly accepted that a vascular graft or conduit would be necessary when the resected segment of the MPV extends longer than 3 to 4 cm.[2,5] Vessel transplantation will risk the operation by increasing rates of infection, blood loss, thrombosis, and extension of operative time.

Between August 2006 and May 2008, we performed PD with long segmental (>5 cm) SMV–PV resection in eight patients with locally advanced pancreatic head cancer. Patient characteristics, preoperative examination, surgical data, and pathologic data are shown in Tables 1 and 2. By Cattell–Braasch maneuver, liver mobilization, and early retropancreatic dissection, the tension at the anastomosis was significantly reduced and R0 resection was successfully achieved. As a result, the use of vascular graft was avoided. All the eight patients experienced uneventful recovery without severe complications.

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Table 1 Demographic and Preoperative Data

Patient no.	Gender	Age (years)	Bilirubin (μmol/L)		Liver function (U/L)		Tumor size (cm)
			TB	CB	ALT	SLT	
1	F	78	13.6	2.9	17	15	6
2	F	73	12.1	1.4	21	23	3
3	F	67	351.0	252.8	64	112	4
4	F	65	165.3	116.5	56	62	7
5	F	63	226.8	176.2	49	57	6
6	M	61	11.4	4.0	12	15	5
7	M	60	273.9	191.3	24	19	7
8	F	46	271.1	178.6	162	81	5

TB total serum bilirubin, *CB* conjugated serum bilirubin, *ALT* alanine transaminase, *AST* aspartate transaminase

Materials and Methods

Surgical Procedure

A bilateral subcostal incision was used as the standard approach. A careful abdominal exploration was performed to exclude the presence of distant metastases and peritoneal dissemination.

The hepatic pedicle was dissected with mobilization of the gallbladder. Vessels including the common hepatic artery, celiac axis (CA), PV, and those in the hepatic pedicle were skeletonized for lymphadenectomy. The duodenum and the head of pancreas were Kocherized and reflected medially so that the paraaortic lymph node involvement could be excluded and the superior mesenteric artery (SMA) could be fully exposed to exclude the tumor invasion. Kocherization of the head of the pancreas and duodenum needs to be extended past the aorta with opening of the ligament of Treitz to allow enough ventral and lateral retraction to visualize the SMV and SMA posteriorly. Lymph nodes and soft tissue located on the right and posterior aspects of the SMA were removed.

After the division of the stomach, the pancreas was transected at the level of the pancreatic isthmus, left anteriorly to the mesentericoportal venous axis, followed by retroperitoneal dissection with sharp division of soft tissues anterior to the aorta and at the right aspect of the SMA in order to obtain disease-free margins and fully mobilize the uncinate process from the SMA. In this step, the uncus is exposed up to the right aspect of the SMA and easily dissected off the artery. The origin of the inferior pancreaticoduodenal artery, which usually originates from the first jejuna artery, is identified. The extrahepatic bile duct was divided above the entry of the cystic duct just before the removal of the surgical specimen to prevent the possible dragging of PV–SMV. The surgical margins of the bile duct, pancreatic neck, and uncinate process were routinely examined by frozen section. In case of positivity in the former two margins, an additional resection was performed until a negative margin was obtained. If the uncinate margin on the SMA or retroperitoneal soft tissue margin is positive, metal clips were labeled for later radiation therapy.

After the previous procedures, when the involvement of the MPV was confirmed, Cattell–Braasch maneuver was performed by mobilizing the right colon and incising the visceral peritoneum to the ligament of Treitz.[6] This maneuver facilitated cephalad displacement of the SMV

Table 2 Surgical and Pathologic Data

Patient no.	Surgery	Resected vein (cm)	Nakao's classification[17]	SMV–PV pathology	Margin pathology		
					Bile duct	Pancreatic neck	Retroperitoneal
1	PD+RHC	7	C	+	−	−	−
2	PD+RHC	5	B	−	−	−	−
3	PD	6	C	+	−	−	+
4	PD	6	C	+	−	−	−
5	TP	6	B	+	−		−
6	PD	5	D	+	−	−	−
7	PD	5	C	+	−	−	−
8	PD	7	B	−	−	−	−

RHC right hemicolectomy, *TP* total pancreatectomy

through the mobilization of the retroperitoneal attachments of the mesentery. The falciform ligament, right coronary ligament, and right triangular ligament of the liver were dissected, so that the liver could be lowered by packing several Mikulicz pads superiorly and posteriorly. These two procedures are important to facilitate approximation of the two to-be-reconstructed SMV–PV ends and made the end-to-end anastomosis in a tension-free fashion.

At this time, the splenic vein was ligated and divided and an en bloc resection of the involved vein together with PD was performed. We usually do not perform tangential resection of the SMV if tumor involvement is suspected.

End-to-end anastomosis was performed using 5-0 Prolene continuous sutures to reconnect the SMV–PV. A 1–1.5 cm of growth factor was preserved for later vessel expansion. Right before the end of the anastomosis, the Satinsky clamp on the mesenteric side was removed to cleanse the possible clots. Since the uncinate process had been mobilized from behind previously, the specimen could be removed immediately after the division of the SMV–PV, which dramatically reduced the blood exclusion time. In this series, the blood exclusion time was confined to within 15 min in all cases. On completion, the anastomosis was checked to make sure the vessels were filling well and no narrowing or tension at the site. We then performed the rest of the gastrointestinal reconstructions of the Whipple procedure.

In most patients, the size of the two ends matches well for reconstruction with no need for specific management. In one patient, the successful anastomosis was achieved by making the SMV end into an oblique shape to deal with the size discrepancy. Concomitant right hemicolectomies were conducted in two patients due to tumor involvement of the transverse mesocolon.

Postoperative Management

According to the general principles of surgical and supportive care, the standard postoperative treatment includes hemodynamic monitoring with a central venous catheter, urinary catheter, fluid balance, and adequate replacement of electrolytes. The nasogastric tube was removed when flatus has been expelled. Parenteral antibiotics and octreotide acetate were administered to all patients prophylactically. Patients were given total parenteral nutrition (PN) for the first 4–5 days after surgery. Then, enteral nutrition (EN) was added until finally replaced PN. EN was administered through a feeding jejunostomy tube.

Results

R0 resection was performed successfully in seven cases. The blood exclusion time was confined to within 15 min in every case. No anticoagulant drug was used perioperatively or postoperatively. One patient underwent total pancreatectomy for positive pancreatic margin on frozen section. The other patient had microscopically positive retroperitoneal margin, but no further surgical measure could be taken. Metal clips were labeled in place for postoperative radiation. Stress ulcer happened in one patient 7 days postoperatively and was successfully managed using conservative measures. No other severe morbidity (vessel resection-related complications, obvious pancreatic or bile leakage) was observed. No postoperative mortality, which was defined as deaths within 30 days postoperatively, occurred in this series. No narrowing in blood vessel or thrombosis was detected by ultrasound either 2 weeks or in the regular follow-up every 3 months after the surgery. No symptomatic left-sided portal hypertension was observed in this group in the postoperative period and follow-up.

Discussion

Tumor involvement of the SMV or PV in the absence of extension to the SMA or CA should be considered as a function of tumor location or tumor size, rather than an indicator of biologic aggression.[3,4] In patients with ductal adenocarcinoma of the pancreatic head, PD with SMV–PV resection seems justified in order to achieve a R0 resection when a close adhesion between the tumor and the venous wall is the only obstacle for resection. It was reported that the rate of R0 resections after PD with SMV–PV resection could be as high as 82%.[7]

With the development and popularization of vascular surgery, PD with SMV–PV resection is getting widely accepted around the world. In some experienced centers, the rate of VR during PD for pancreatic adenocarcinoma is around 40%.[8,9]

The modified technique of PD with early retropancreatic dissection and vascular skeletonization before digestive or pancreatoenteric continuity be interrupted has been described before.[10,11] We believe that the modification is especially appropriate for PD with PV–SMV resection because (1) it facilitates the exposure of SMA before pancreatoenteric continuity is interrupted, which can reduce the rate of nonradical PD when SMA is involved; (2) it avoids palliative resection owing to the involvement of the retroperitoneal margin which happens frequently in cases of invasion of the SMV;[12] (3) it shortens the vascular clamping time, so the possibility of bowel congestion and ischemia/reperfusion liver injury greatly reduced; and (4) it results in the tumor being attached only to the involved veins, so clamping and division may be easier and safer.

After the resection of the involved segment of PV–SMV, venous continuity was usually restored by a direct end-to-

end anastomosis. It is believed that vascular graft (artificial or autogenous) should be used when the resected segment is longer than 4 cm, which makes a tension-free anastomosis impossible.[2,5] By liver mobilization and Cattell–Braasch maneuver, the tension at the anastomotic site was reduced significantly. As a result, venous reconstruction was satisfactory with a tension-free end-to-end anastomosis in every single case, even though the resected segments were longer than 5 cm. We believe that this technique can make a successful end-to-end anastomoses after the resection of PV–SMV as long as 10 cm, which will be a very rare event and implies that almost all the direct end-to-end reconstructions is possible without grafts.

The other prominent advantage of our procedure is that it could be accomplished within 15 min, which would be much longer when graft of autogenous or prosthetic vein is used because at least two anastomoses are required. It avoids the arterial occlusion or a mesenteric–systemic bypass when longer anastomosis time is needed.[13] Also, the bowel congestion and ischemia/reperfusion liver injury are greatly reduced.

The application of Cattell–Braasch maneuver was described in detail before in reconstruction of SMV–PV.[14] But we believe that the combination of liver mobilization would make the approximation of the two ends and reconstruction easier without much additional work.

It was reported that about 30% of patients who underwent the PV–SMV resection did not have pathologically confirmed tumor invasion.[15,16] It happened to two patients in our series. It would be unjust if these patients are excluded from curative resection just because of the suspected vascular invasion, which actually is inflammatory adhesion.

Although end-to-end anastomoses could be successfully accomplished in most patients using the procedure we described, we have to point out that it is not the case for all conditions. Besides the absolute length of the SMV–PV, there are some other anatomic factors, such as the location of the first jejunal branches, body habitus of the patient, thickness of the mesenteric root, and inferior mesenteric vein variant into the SMV, are also very important in the decision for end-to-end versus insertion of a vascular conduit.

Conclusions

In conclusion, we performed modified PD with long segmental (>5 cm) SMV–PV resection in eight patients with pancreatic head carcinoma. No postoperative mortality and sever morbidity was observed. The blood exclusion time was confined to be within 15 min in all cases. No anticoagulant drug was used. Although our data is still limited due to the number of the cases, our results suggested that with the procedure we used, PD with long segmental (>5 cm) SMV–PV resection is safe and feasible.

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REVIEW ARTICLE

A Review of Risk Scoring Systems Utilised in Patients Undergoing Gastrointestinal Surgery

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Abstract
Introduction Adequate stratification and scoring of risk is essential to optimise clinical practice; the ability to predict operative mortality and morbidity is important. This review aims to outline the essential elements of available risk scoring systems in patients undergoing gastrointestinal surgery and their differences in order to enable effective utilisation.
Methods The English literature was searched over the last 50 years to provide an overview of systems pertaining to the adult surgical patient.
Discussion Scoring systems can provide objectivity and mortality prediction enabling communication and understanding of severity of illness. Incorporating subjective factors within scoring systems can allow clinicians to apply their experience and understanding of the situation to an individual but are not reproducible. Limitations relating to obtaining variables, calculating predicted mortality and applicability were present in most systems. Over time scoring systems have become outdated which may reflect continuing improvement in care. APACHE II shows the importance of reproducibility and comparability particularly when assessing critically ill patients. Both NSQIP in the USA and P-POSSUM in the UK seem to have many benefits which derive from their comprehensive dataset. The "Surgical Apgar" score offers relatively objective criteria which contrasts against the subjective nature of the ASA score.
Conclusion P-POSSUM and NSQIP are comprehensive but are difficult to calculate. In the search for a simple and easy to calculate score, the "Surgical Apgar" score may be a potential answer. However, more studies need to be performed before it becomes as widely taken up as APACHE II, NSQIP and P-POSSUM.

Keywords Critical illness · Critical care · Surgery · Risk assessment · Peri-operative care · Prognosis · High dependency unit · Scoring systems

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Abbreviations

APACHE	Acute Physiology and Chronic Health Evaluation
ASA	American Society of Anaesthesiologists
AVPU	Alert/Voice/Pain/Unresponsive (conscious level)
BUPA	British United Provident Association
CRIA	Cardiac Risk Index Assessment
ICU	Intensive Care Unit
E-PASS	Estimation of Physiologic Ability and Stress
GCS	Glasgow Coma Scale
HDU	High Dependency Unit
ICNARC	Intensive Care National Audit and Research Centre
Max-Fax	Maxillary Facial

MPM	Mortality Prediction Model
NSQIP	National Surgical Quality Improvement Programme
Op	Operation
POSSUM	Physiological and Operative Severity Score for EnUmeration of Mortality and Morbidity
P-POSSUM	Portsmouth POSSUM
SMS	Surgical Mortality Score
SRS	Surgical Risk Score
SAPS	Simplified Acute Physiology Score
SaO_2	Arterial Oxygen Saturations

Introduction

The assessment of the potential risks of peri-operative mortality and morbidity is increasingly important in the provision of health care. There is a growing realisation that providers need to ensure appropriate resource allocation and enable informed decision making by the recipient.[1] Adequate stratification and scoring of risk should, therefore, be considered essential to aid clinical practice. Assessment may occur at various points throughout the patient's journey through the health care system and can be grouped into three stages relating to the operation. Pre-operative assessment when planning an intervention can help quantify the potential risks of a procedure for the client and peri-operative (physiological) assessment may determine the most suitable setting for further care by stratifying risks, while post-operative scores may alter management.

There are a variety of risk scoring systems in use derived from different populations of patients for a variety of purposes and each has their limitations. Scoring systems that were appropriate for patients undergoing gastrointestinal surgery were examined. As surgical patients account for up to 70% of the workload[2] of general intensive care units (ICUs), risk scoring systems that related to ICU and critically ill patients were also reviewed. Clinically, these assessments can provide a framework for stratifying risk and identify patients that may require in the change of management which may include admission to a higher level of care. This review aims to outline the essential elements of these systems and their differences in order to enable effective utilisation.

Methods

A search was performed of the English literature over the last 50 years to provide an overview of validated risk scoring systems that exist pertaining to the general surgical patient. Principal databases searched were the National Library for Health (including the Cochrane database) and Ovid (Medline; 1950–2008). Search terms used were risk assessment, scoring systems, surgery, mortality and morbidity as well as derived terms. Secondary references were obtained from primary articles. Papers that introduced, validated and developed the scoring systems discussed in this article were also assessed. Articles pertaining to paediatric, thoracic, vascular, plastic, cardiothoracic, burns or trauma patients were excluded to increase homogeneity. Risk scoring systems relating to specific surgical conditions or obesity were beyond the scope of this review.

Overview of Risk Scoring Systems and Models

A number of scoring systems exist which have been applied to patients who are acutely ill. In patients undergoing surgery, these risk scoring systems can be broadly categorised into three groups (see Table 1), which relate to the timing of the assessment in relation to the surgical procedure. Outcome is generally measured in terms of mortality as it is a definitive endpoint and easy to measure. A few scores predict both morbidity and mortality, while some indicate morbidity alone yet almost none seem to measure quality of life or return to pre-existing function. In order to understand the limitations of the scores described, the methodology of the scoring system has been listed alongside the dataset from which it was originally derived, and these are summarised in Table 1.

Pre-operative Scores

American Society of Anaesthesiologists Score

Widely used as a surrogate for operative risk, the American Society of Anaesthesiologists (ASA) score was originally devised to grade the patients "in relation to physical status only"[3] (see Table 2). It is incorporated in a number of other scoring systems as discussed later. The ASA score is subjective and based on clinical evaluation "only", although objective test results will indirectly affect the clinician's assessment.[4,5] Although not intended for use as a risk scoring system, the ASA score has been used for this purpose in part due to the simplicity of the tool, its universal use and allowance for individual patient parameters.

Limiting factors in its applicability are the aforementioned of subjectivity, its lack of specificity inherent in its design and wide inter-observer variability.[4–8] The ASA score has been used to categorise pre-operative risk and is a good indicator of post-operative mortality.[9] It does not, however, provide a quantitative assessment of

morbidity and mortality risk and is better at risk stratification.

Surgical Risk Scale

Sutton et al[10] devised the Surgical Risk Scale (SRS) as a comparative surgical audit tool (see Table 2). When prospectively validated, it appeared to be effective at predicting mortality. The ASA score is combined with the Confidential Enquiry into Peri-operative Deaths category and British United Provident Association operative grade resulting in a score from 3 to 15, each of which relates to a likely mortality score. The use of the ASA makes it a partly subjective scoring system as described above. The SRS has been shown to have a similar accuracy[11,12] to Portsmouth Physiological and Operative Severity Score for EnUmeration of Mortality and Morbidity (P-POSSUM) especially in higher risk patients[11] yet was easier to calculate.[12]

Cardiac Risk Index Assessment

Goldman et al. described the original Cardiac Risk Index Assessment (CRIA) in 1977.[13] A number of other cardiac risk assessment tools have been developed: a modification by Detsky et al. in 1986[14] is the mainstay of the American College of Physician's guidelines, while a further derivation for scheduled surgery by Lee et al. in 1999 was named the Revised CRIA.[15] The advent of more specific cardiac investigations such as trans-thoracic echocardiography has not resulted in better pre-operative risk assessment[16] but can add significant information in high-risk patients.[17] These CRIA tools are limited in that they do not correlate particularly well with peri-operative mortality and appear to be poorer than the ASA score.[18]

Peri-operative Physiological Scores

Acute Physiological and Chronic Health Evaluation

The relatively complex scoring system, the Acute Physiological and Chronic Health Evaluation (APACHE) II, has been derived from large American ICU patient databases.[19,20] While it does not specifically assess surgical patients,[2] Goffi et al.[21] found that APACHE II could be used pre-operatively "with caution", in both elective and emergency surgical patients outside of the ICU or High Dependency Unit (HDU) setting.

The second version of APACHE reduced the number of variables to 12 from the original 34 required. A further derivation, APACHE III does not seem to be more accurate than APACHE II in the ICU population in the UK[20] and in some studies has been shown to be poorer when used to look at surgical patients[22,23] and patients with gastrointestinal disease.[23] The lack of transparency as well as the original licensing cost has deterred a number of ICUs from using APACHE III and IV.[24,25] The latter has become more complex due to an expansion of disease groups but is now available to the public domain. Overall, while widely used and well-understood, calculating APACHE II is complex and time consuming; furthermore, the raw data is not always easily obtainable, particularly outside that of the ICU setting.

Intensive Care National Audit and Research Centre Model

In the UK, the recently published the Intensive Care National Audit and Research Centre (ICNARC) model by Harrison et al, in 2007,[26] allows for the type of surgery being performed. Data were drawn prospectively from a UK population (excluding Scotland). Factors used to derive a model were drawn from APACHE III and other models. The ICNARC model does not seem to be limited to any patient sub-groups; therefore, there are no stated exclusions (even encompassing paediatric patients). In particular, it is well suited to the UK population and it compares favourably with other models such as MPM_0-II, SAPS II and APACHE II.

Simplified Acute Physiology Score

The Simplified Acute Physiology Score (SAPS) is assigned after 24 h of ICU admission and is another derivation of APACHE. The second version, SAPS II, which uses the original 13 physiological variables, also factors in the type of admission (elective or emergency; medical or surgical) and chronic health points (acquired immune deficiency syndrome, metastatic cancer and haematological malignancy).[27] It has been trialled extensively with some reporting improved predictive ability.[28] However, many others have found it less effective in different countries and subgroups[29] with poor goodness of fit. The original authors acknowledge that it is dated[27] and requires modification and although attempts to modify it have had limited success.[27,30] An updated version SAPS[31] has had very poor uptake with little in the way of validation after being available for over 3 years. With its inherent weaknesses, APACHE II is preferred to SAPS II in most units.

Early Warning Systems

Early warnings systems provide a way to calculate quickly an instant assessment of physiological status to ensure that patients who are acutely ill on the wards are detected.[1] In HDU patients that were surgical, basic parameters such as

Table 1 Risk Scoring System

Risk scoring system	Primary author and year	Patient sample/ validation	Patient population type	Number of hospitals involved	Number of variables	Variable categories	Strengths of score	Weakness of score
Pre-operative scores								
ASA	M Saklad 1941	Not derived or validated	N/A	N/A	No finite number	Subjective assessment	Common. Simply. Easily applied. Allows subjective interpretation	Not designed as an op risk score. Not specific. Subjective. Inter-observer variability. Not predictive
SRS	R Sutton 2002	4 308/2 780	Surgical-various	One Hospital	3	Scheduling/ op complexity/ ASA	Easy to calculate. Effective mortality predictor. Broad application.	Does not allow for effect of operation and surgeon. Broad categories.
Goldman Cardiac Risk Index	L Goldman 1977	1 001/Not validated	Adults >40 for non-cardiac op	One Hospital	9	Physiological/ age/ emergency / chronic health	Assesses major cause of peri-operative complications.	No evidence better than any other score at predicting mortality. Predicts only cardiac complications
Peri-operative scores								
APACHE II	WA Knaus 1985	805/5 815	Acute ICU admissions. US population	13 hospitals	15	Physiological/ age/ chronic health/ surgery	Widely used. Comparable.	Complex. Hard to calculate. Poor applicability to UK and surgical patients.
ICNARC model	DA Harrison 2007	216 626/ 30 000	Acute ICU admissions. (UK)	163 hospitals	31	Physiological/ Age/ Admission/CPR	UK based. Good comparison with other scores. No exclusions.	Large amounts of data. Ongoing model with further studies required. Large number of diagnostic codes
SAPS II	JR Le Gall 1993	8 500/ 4500	Acute ICU admissions. USA and UK	137 units	15	Physiological/ admission type/ chronic health	Data burden less than APACHE II. Initial good fit	Limited when wider studies performed. Can rank severity but needs modification
Post-operative scores								
MPM_0 -III	TL Higgins 2007	74 518/5 0307	Acute ICU admissions. US population	98 Hospitals	16	Physiological/ age/ surgery/ cancer	Low data burden. Good predictive properties. Recent update. Avoids incorporating ICU care	Some exclusions. Not as good discriminator, APACHE IV or SAPS III

P-POSSUM	DR Prytherch 1998	2 500/7 500	General Surgical	1 Hospital	18	Physiological/ operative	Better fit, POSSUM especially low risk. Linear so predicts individual outcome	Day-case patients not studied. Specialities require modification
E-PASS	Y Haga 2001	902	Elective Gastro-intestinal	6 Hospitals	9	Physiological/operative/ ASA/age	Prospective.	Complex to derive. Elective patients only
NSQIP	SF Khuri 1997	117 000/ 8 593	Non-cardiac surgical	44 veteran affairs hospitals	66	demographic, clinical, lab, intra and post-op	Massive prospective database. Coefficients. Simpler bedside variant	Complex. Subjective data including ASA and age. Applicability to other populations uncertain
Surgical APGAR	A Gawande 2007	303/767	General and vascular	1 Hospital	3	Blood loss, mean arterial pressure, heart rate	Simple, applicable, relates to outcome	Variation in estimated blood loss. Limited outcome groups therefore only broad assessment
SMS	VG Hadjianastasiou 2004	6 595/4 494	Surgical-various	1 Hospital	6	Specialty/age/gender/ operative factors	Easily obtainable variables. Stratified relation of SMS to mortality	Requires list of reference op times. Comparative audit tool

heart rate and respiratory rate can detect differences between those patient groups which required ICU within 8 h compared to those who did not.[32] Other parameters such as blood pressure, temperature and AVPU level of consciousness could, according to Subbe et al.,[33] identify medical patients at risk of deterioration. Overall early warning systems relate poorly to ICU or HDU[34] and do not provide an idea about prognosis and are not designed around peri-operative general surgical patients.

Post-Operative Scores

Mortality Prediction Model

The Mortality Prediction Model (MPM)[35] is normally scored at admission to ICU/HDU with data from within the first hour (MPM_0) although older versions could be scored after 24 or 48 h (MPM_{24} and MPM_{48}, respectively). The burden of data collection is low and relates to the following: emergency admission, resuscitation, cancer, chronic renal failure, heart rate, systolic blood pressure, infection, previous ICU admission within 6 months, surgery, age and GCS. Values are assumed to be normal when measurements have not been taken. The data allow for greater completeness and subsequently a higher degree of consistency.[36] It does not use the worst criteria during the first 24 h unlike APACHE and, therefore, can provide a more defined way of comparing admissions to different ICUs.[36] MPM_0-III,[37] which can be downloaded from the internet, shows better characteristics regarding expected ICU outcomes as compared to APACHE II. Higgins et al.[37] found that by adjusting prediction at the extremes of the model, MPM_0-III was a better predictor than MPM_0-II. Limitations of the MPM are that some sub-groups are excluded (e.g. cardiac surgery, myocardial infarction and ICU readmissions) and while only recently updated, APACHE IV and SAPS III still obtain better discrimination. This may be a result of the simplicity of the model with only 16 independent variables being required.

Physiological and Operative Severity Score for EnUmeration of Mortality and Morbidity

The POSSUM predicts the probability of surgical mortality for a range of surgical sub-populations and allows comparison of performance.[38] The 12 physiological factors (see Table 3) can be determined pre-operatively, and the system is designed for a default value of one for missing data.

Electively[39] or peri-operatively, its use has not been validated with regard to outcome or need for ICU or HDU admission either. The timing of the acquisition of data is

Table 2 ASA Grade, CEPOD Category and Operative Course Comprising Surgical Risk Score

Scoring system	Score	Description
ASA		American Society of Anaesthesiologists Grading
I	1	Healthy patient
II	2	Mild systemic disease, no functional limitation
III	3	Moderate systemic disease, definite functional limitation
IV	4	Severe systemic disease that is a constant threat to life
V	5	Moribund patient, unlikely to survive 24 h with/without operation
CEPOD		Confidential Enquiry into Peri-operative Deaths
Elective	1	Routine booked non-urgent case, e.g. varicose veins or hernia
Scheduled	2	Booked admission, e.g. cancer of the colon
Urgent	3	Cases requiring treatment within 24±48 h of admission, e.g. obstruction
Emergency	4	Cases requiring immediate treatment, e.g. faecal peritonitis, perforation
BUPA		British United Provident Association
Minor	1	Removal of sebaceous cyst, skin lesions, upper GI endoscopy
Intermediate	2	Unilateral varicose veins, unilateral hernia repair, colonoscopy
Major	3	Appendicectomy, open cholecystectomy
Major plus	4	Gastrectomy, any colectomy, laparoscopic cholecystectomy
Complex major	5	Anterior resection, oesophagectomy

important as discussed later.[40] Furthermore, POSSUM has variable usage across different specialities, which has led to specialty-specific derivations of POSSUM, especially in oesophageal[41,42] and colorectal surgery.[43,44] These have ideally increased predictive power at the expense of decreasing cross-specialty comparison.

In POSSUM, the lowest predictable expected mortality is 1%. This value equates to the rate for all patients undergoing general surgery so POSSUM will effectively exaggerate mortality rates in minor operations. POSSUM is not readily applied to individual patients, as it is based on an exponential equation and the calculated prediction is based on groups. These problems as well as that of "goodness of fit"[45] have led to a more broad-based derivation, known as the P-POSSUM score.[46] The benefits of P-POSSUM include a lower baseline prediction of 0.2% while linear individual mortality and also morbidity can be predicted. Specialty-specific modifications seem to have improved the prognostic features of P-POSSUM, and it has become widely used and accepted as a risk scoring system.

Estimation of Physiologic Ability and Stress

A Japanese comparative audit tool called Estimation of Physiologic Ability and Stress (E-PASS) has been developed.[47] This uses coefficients to combine pre-operative factors (heart-disease, pulmonary disease, diabetes, performance status) with operative aspects (ratio of blood loss to body weight, operative time, type of operation/incision). E-PASS also incorporates age and the ASA score. It has been evaluated in those undergoing elective gastrointestinal surgery[47] and used to predict complications.[47,48] Oka et al.[49] found that E-PASS was a good predictor of morbidity and severity of illness. It seems similar to POSSUM and P-POSSUM in its applicability[48] but is a complex system to score.

National Surgical Quality Improvement Programme

Derived from the Veteran Administration,[50] the National Surgical Quality Improvement Programme (NSQIP) has been applied in other US hospitals to provide risk adjusted 30-day outcome data principally relating to mortality. Data collected comprise of 66 variables, but Aust et al.[51] provide a simpler bedside derivation. This equation relies upon six main factors: Albumin, Age, ASA, emergency procedure, disseminated cancer and if operation is difficult. In the original derivation, other significantly weighted factors included resuscitation status, functional status, urea and weight loss. Applying it to other providers or other countries is limited by a lack of studies, the amount of data required and the complexity of the coefficients to tailor the data to outcome.

Surgical Apgar Score

The simplicity of the Apgar score in obstetric practice led to its worldwide uptake as an assessment tool. Gawande et al.[52] set out to derive a similar surgical model which they published in 2007. Using a retrospective dataset they used

Table 3 POSSUM Parameters

Physiological parameters	Operative parameters
Age	Mode of surgery
Cardiac status	Operation type/grade1
Respiratory status	Multiple procedures
Glasgow coma score	Peritoneal soiling
Pulse rate	Malignancy
Blood pressure	Intra-operative blood loss
Haemoglobin	
White cell count	
Serum sodium	
Serum potassium	
Urea	
Electrocardiogram	

multivariable logistic regression to derive intra-operative and pre-operative factors associated with surgical mortality and morbidity. The group then chose to use one of their models that relied solely on intra-operative factors as these were independent predictors of outcome. The three factors used were: estimated blood loss, lowest mean arterial pressure and lowest heart rate (or arrhythmias). This ten-point model was prospectively validated. Its strength, the authors state, is that it can be easily derived post-operatively. Similar to early warning systems, it uses important physiological criteria which can be assessed objectively. Criticisms of this scoring system are that operative blood loss can be subjective although the authors argue the wide categories allow for reasonably accurate estimation. The overall score can be used to discriminate which patients are likely to have a post-operative mortality or morbidity (Table 4).

Surgical Mortality Score

The Surgical Mortality Score (SMS) was designed to provide an audit tool to compare outcomes rather than a tool to assess severity of illness or suitability for admission to HDU or ICU.[53] It is effectively an odds ratio. At the lowest predicted value, the mortality rate was 0.08%, which accords well with other scoring systems. Hadjianastassiou et al.[53] simplified their data to provide a stratified classification of mortality which approximates to in-hospital mortality and is easier to use than the logistic equations of other scoring systems.

The SMS is not altered by variability in clinical intervention or timings regarding when to measure physiological variables that other systems suffer from. While it allows for the sub-speciality of surgery performed, like the derivations of P-POSSUM, it needs to be referenced regarding operative time. The reference operating times (in 652 categories) are available on-line. This does not enable the SMS score to be calculated easily and presumes that it can be generalised to other institutions.[6]

Discussion

The variety of scoring systems demonstrates how numerous the variables are that can be analysed to derive mortality and morbidity rates. The difficulty lies in choosing the most informative variables without having to collect and input large amounts of data. There is debate on which system to use, how complex systems need to be and when to score them. Furthermore, calculating the risk once the variables have been obtained can also be difficult (see limitations outlined in Table 1).

In order to predict risk, the individual surgical unit needs to be able to compare itself against the database and hospitals that the risk scoring system was derived from. One inherent problem is that the population data used to derive models are normally not contemporary. The change in the population with time, continuing advances in medical care, critical care outreach teams and improvement in outcomes for ICU mean that scoring systems will become dated: the applicability of scoring systems diminishes over time.[27,36,54]

The acute physiology and chronic health scores that combine to form the APACHE scoring systems are standard in the ICU and HDU setting but due to the burden of data collection seem to be limited when in the ward or Emergency Department. The ICNARC and SAPS models have been derived in part from APACHE, but both of these require fewer variables. The use of APACHE II shows the importance of reproducibility and comparability particular-

Table 4 Surgical Apgar score

For lowest heart rate, occurrence of arrhythmias score 0 (pathologic bradyarrhythmia, including sinus arrest, atrioventricular block or dissociation, junctional or ventricular escape rhythms and asystole)

Factor	Score (points)				
	0	1	2	3	4
Estimated blood loss (mL)	>1,000	601–1,000	101–600	<100	–
Lowest mean arterial pressure (mmHg)	<40	40–54	55–69	>69	–
Lowest heart rate (beats/min)	>85	76–85	66–75	56–65	<56

ly when assessing critically ill patients and APACHE II continues to be used as it is familiar although flawed. To facilitate change to another scoring system, several years of using the new and the old systems being trialled alongside each other is required.[36] This is necessary to ensure no loss of data, comparability and assess local applicability.

The most routinely utilised risk assessment used is the ASA score. Testimony to the power of the ASA score is that it is incorporated in a number of other scoring systems. The strength of the ASA score is that allows the clinician to weigh his/her experience and understanding of the situation and to allocate risk appropriately.[4] Several studies have shown the importance of subjective assessment; the surgeons' "gut feeling" was a good indicator of post-operative course, even when compared against POSSUM.[4,5] Surgeons were more accurate in predicting morbidity in elective surgery but underestimated the risk of complications in the more complex emergency setting.[5]

The weakness of the ASA score apart from its subjectivity, is its wide inter-observer variability.[4–8] As such, it is hard to use to compare units. The ASA grade has been shown to be a good predictor of post-operative mortality[9] and better in this regard than the Goldman CRIA.[18] The ASA score does not correlate with the requirement for ICU admission[2] or assessing changes in post-operative patients;[32] however, it correlates with early post-operative emergencies, which often lead to ICU admissions.[55]

Approximately 50% of surgical deaths are in patients scoring ASA III or IV.[56] Hall and Hall [57] found that if the ASA score was III or more and the age was over 60 years of age, this identified over 80% of the patients who died or had significant morbidity (prolonged stay in hospital, developed intra-peritoneal sepsis or were admitted to the ICU). Age may be a proxy for physiological reserve[6,21] and a surrogate marker for undeclared co-morbidity. This may explain its use in a number of scoring systems including APACHE, MPM, SMS and NSQIP. The SRS relies heavily upon the ASA score while allowing for urgency and type of operation. It appears easy to calculate as compared to P-POSSUM[12] with similar accuracy[11,12] especially in higher risk patients.[11] There seem to be few studies by other groups regarding the SRS despite its potential and applicability.

Both NSQIP in the USA and P-POSSUM in the UK seem to have many benefits which derive from their comprehensive dataset. Driven by inter-hospital comparison amongst other factors, they seem to be the current gold standards by which other tests are measured against. P-POSSUM with its linear analysis and ability to predict mortality for individual patients seems to be appropriate method of assessment for those undergoing surgery. The E-PASS system seems to offer similar applicability to P-POSSUM, but there are only a few published validation trials. In comparison the NSQIP has a wide usage in the USA with a large database and validated trials.

The comprehensive nature of data required for some risk scoring systems leads to an increased complexity of score calculation. In response to the complexity of some scores, a number of scoring systems relating to ICU can be run on individual computer systems either via the internet or as standalone programmes. Simple bedside scoring systems as outlined by Aust et al.[51] in relation to the NSQIP are more transportable and encourage use. In the paper by Gawande et al., the authors relate the search for a simple and easy to calculate score. Their "Surgical Apgar" score may be a potential answer; it seems to offer relatively objective criteria which contrasts against the subjective nature of the ASA score yet not require large amounts of data variables to give a meaningful assessment of risk.

Conclusion

So where does the last 35 years or so of risk scoring systems leave us? What it can do is:

- provide us with baselines upon which the probable mortality and morbidity can be determined
- framework for benchmarking, which may help to engender improvement
- allow a way of quantifying, recording and communicating risk
- provide an ICU or HDU with a basis for tailoring their intervention or for declining admission,[1] as knowing when to admit patients to ICU or HDU relies upon realising when there is no benefit.
- provide the clinician with a basis for declining surgery or for instituting a palliative and humane policy towards those who are likely to die despite our best interventions.[20]

There are a variety of risk scoring systems, each with their own limitations, as outlined above, and strengths. The subjective element of the ASA score seems to emphasise that there is role for clinical judgement in assessing patients. P-POSSUM and NSQIP are comprehensive but are difficult to calculate. The Surgical Apgar score has been created to provide an objective score that is easy to measure and calculate. While it has been validated, more studies need to be performed before the Surgical Apgar becomes as widely taken up as APACHE II, NSQIP and P-POSSUM.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

AC devised and wrote the preliminary draft. AC and SM performed the literature search and revised the article. DM supervised the project and wrote the introduction.

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REVIEW ARTICLE

Surgical Reintervention After Failed Antireflux Surgery: A Systematic Review of the Literature

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Abstract
Background Outcome and morbidity of redo antireflux surgery are suggested to be less satisfactory than those of primary surgery. Studies reporting on redo surgery, however, are usually much smaller than those of primary surgery. The aim of this study was to summarize the currently available literature on redo antireflux surgery.
Material and Methods A structured literature search was performed in the electronic databases of MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials.
Results A total of 81 studies met the inclusion criteria. The study design was prospective in 29, retrospective in 15, and not reported in 37 studies. In these studies, 4,584 reoperations in 4,509 patients are reported. Recurrent reflux and dysphagia were the most frequent indications; intraoperative complications occurred in 21.4% and postoperative complications in 15.6%, with an overall mortality rate of 0.9%. The conversion rate in laparoscopic surgery was 8.7%. Mean(±SEM) duration of surgery was 177.4±10.3 min and mean hospital stay was 5.5±0.5 days. Symptomatic outcome was successful in 81.1% and was equal in the laparoscopic and conventional approach. Objective outcome was obtained in 24 studies (29.6%) and success was reported in 78.3%, with a slightly higher success rate in case of laparoscopy than with open surgery (85.8% vs. 78.0%).
Conclusion This systematic review on redo antireflux surgery has confirmed that morbidity and mortality after redo surgery is higher than after primary surgery and symptomatic and objective outcome are less satisfactory. Data on objective results were scarce and consistency with regard to reporting outcome is necessary.

Keywords Gastro esophageal reflux disease · Antireflux surgery · Nissen fundoplication · Dysphagia · Reoperation

Introduction

Antireflux surgery for refractory gastroesophageal reflux disease (GERD) has satisfactory outcome in 85–90% of patients.[1–6] In the remaining 10–15%, reflux symptoms persist, recur, or complications occur. Dysphagia is a frequent complication of fundoplication.[7] The indications for reoperation are far from straightforward, varying from severe recurrent symptoms with a more than adequate anatomical result to recurrent abnormal anatomy without any symptoms at all. Studies on reoperations also show similar wide variations with a full range of abnormal anatomy, symptoms and objective failure documented by esophageal manometry, and pH monitoring.

In our recently published study on redo antireflux surgery, morbidity and mortality were higher than after primary antireflux surgery, with a symptomatic and objective success rate of 70% which is obviously inferior to the outcome of primary surgery.[4,8] Several other studies have been published describing causes of failure of conventional and laparoscopic antireflux surgery. Most studies have

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included only a small group of patients, so an adequate impression on the outcome of reoperation is hard to extract from such studies.

This study aims to summarize the currently available literature on surgical reintervention after primary antireflux surgery focusing on morbidity, mortality, and outcome in order to get a more complete overview of the results of redo antireflux surgery and to give guidelines about how patients should be informed on their chances of success.

Material and Methods

Search Strategy

A literature search was performed in three electronic databases, MEDLINE using the Pubmed search engine, EMBASE, and the Cochrane Central Register of Controlled Trials. The databases were searched for all years, up to November 2008. Search terms were entered to identify the relevant studies. Separate search terms were entered for the intervention, i.e., surgical reintervention, and the disease, i.e., GERD. For the disease, dysphagia was also used because this is a frequent indication for reoperation. For both the intervention and the disease, headwords in the thesaurus of the three databases [Medical Subject Heading (MeSH) Thesaurus in Pubmed and the Cochrane library and the Emtree Thesaurus in EMBASE] and free text words in title and abstract were used as search terms. The headwords from the thesaurus and the different synonyms for free text words were coupled by the Boolean operator "OR". The combination of search terms for the intervention and disease were subsequently coupled by the Boolean operator "AND". The free text words and headwords identified in the thesauruses are listed in Table 1.

Selection of Studies

The studies identified by the search strategy were independently selected by two reviewers (E.F. and W.D.) based on title, abstract, and full text. The literature was searched for randomized controlled trials, cohort studies, and case–control studies on the feasibility and/or outcome of surgical reinterventions. Studies in children, on other indications for primary surgery than GERD, conservative treatment of symptoms following primary antireflux surgery, surgical reintervention within 30 days after primary surgery, and patients cohorts with less than ten patients were not included. Only articles in English were included. Addition-

Table 1 Search Terms used in this Review

Intervention	Disease
Free text words in title and abstract of MEDLINE, EMBASE, and the Cochrane Library	
Refundoplication(s)	Gastro esophageal reflux
Redo	Gastro esophageal reflux disease(s)
Redo surgery	Gastro esophageal reflux disorder(s)
Redo surgical procedure	Gastro oesophageal reflux
Redo Nissen (fundoplication)	Gastro oesophageal reflux disease(s)
Redo antireflux procedure	Gastro oesophageal reflux disorder(s)
Redo antireflux surgery	Gastroesophageal reflux
Reoperative antireflux surgery	Gastroesophageal reflux disease(s)
Revisional surgery	Gastroesophageal reflux disorder(s)
Reoperation(s)	GERD
Reintervention(s)	GORD
Surgical revision(s)	Reflux disease(s)
Second look surgery	Esophagitis
	Oesophagitis
	Dysphagia
Headwords in the Medical Subject Head (MeSH) Thesaurus of Pubmed and the Cochrane library	
Reoperation	Deglutition disorders
Second-look surgery	Esophagitis
Headwords in the Emtree Thesaurus of EMBASE	
Reoperation	Stomach function disorder
Second look surgery	Dysphagia
	Esophagitis

ally, references of all selected publications were reviewed for other relevant studies. In case of a difference in opinion between the two reviewers about in- or exclusion of a study, the opinion of a third reviewer was decisive.

Analysis of Data from Selected Studies

Data of the selected studies were independently acquired by two reviewers (E.F. and W.D.). Study design, time period, number of patients, sex ratio, and mean age were retrieved from the studies. Based on the study design, each study was qualified by a level of evidence according to the Oxford Centre for Evidence Based Medicine Levels of Evidence.[9] Type and approach of primary antireflux interventions and reoperations, mean period between both interventions, causes of failure of primary surgery and perioperative information, i.e. intra- and postoperative complications, mortality, number and causes of conversions in case of laparoscopic reoperations, mean intraoperative blood loss, duration of reoperations, and hospital stay were also extracted from the included studies. Completeness of follow-up, number of patients available, mean duration of follow-up, method of obtaining outcome at follow-up, and the definition and percentage of patients with successful symptomatic and objective outcome were extracted from all studies.

Data Analysis

Data were analysed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Values were expressed as mean±SEM. Statistical analysis was not performed owing to the lack of statistically appropriate data from the included studies.

Results

General Results

One thousand six hundred twenty-five articles were eligible for further selection after removing duplicate hits, and finally, 73 articles met the inclusion criteria (Fig. 1). The references of these articles yielded eight more articles for inclusion. These articles had not been identified with the initial search strategy because of absence of abstracts in the databases or atypical description for the intervention or disease. Eventually, 81 articles were eligible for inclusion in this study. According to the Oxford Centre for Evidence Based Medicine Levels of Evidence, 27 studies had a level of evidence IIb (33.3%)[8, 10–35], two level of evidence IIIb (2.5%)[36, 37], and 15 level of evidence IV (18.5%)[38–52]. The remaining 37 studies (45.7%) were cohort studies, but a level of evidence could not be adjudged owing to unknown study design[53–89]. Baseline characteristics extracted from the individual studies are shown in Table 2.

Primary Antireflux Procedures

Total fundoplication performed by laparoscopy, laparotomy, or thoracotomy was the most frequently reported primary antireflux procedure followed by partial fundoplication (Table 3). The type of primary antireflux procedure was not reported in almost one third, and 241 patients (5.3%) underwent more than one previous operation before inclusion in the original studies.

Causes of Failure of Primary Antireflux Surgery

Causes of failure of the previous antireflux procedure were reported on 3,175 reoperations in total. Intrathoracic wrap migration, total or partial disruption of the wrap, and telescoping were the most common anatomical abnormalities encountered (Table 4). Esophageal motility disorder or erroneous diagnosis, i.e., another primary disease than GERD, were the causes of failure of the previous operation in 62 patients (2.0%). In 194 reoperations (6.1%), no cause of failure could be identified.

From six studies, it was shown that wrap disruption and telescoping were more frequent after conventional primary surgery, whereas disruption of hiatal repair and a tight wrap were more frequent after laparoscopic primary repair (Table 5).[18,49,61,67,84,85] Intrathoracic wrap migration was reported by Serafina et al.[85] to be more frequent after conventional primary procedures (13/17, 76.5% vs. 5/11, 45.5%), whereas Heniford et al.[67] showed that this was more frequent after laparoscopic primary repair (16/22, 72.7% vs. 13/33, 39.4%). In the study by Salminen et al.,[84] intrathoracic wrap migration was equal after conventional and laparoscopic primary surgery.

In five other studies,[8,11,12,31,72] it was shown that intrathoracic wrap migration and wrap disruption were more frequent in the case of recurrent reflux, whereas in the case of dysphagia, no cause of failure could be demonstrated more frequently (Table 5).

Indications for Reoperations

Recurrent reflux and dysphagia were the most frequent indications for reoperations (Table 3). In 1,435 reoperations (31.3%), the indication for reoperation was not reported. Preoperative symptoms were assessed by questionnaire in 26 studies (32.1%).[10,14,17,18,23–25,28,30,33,36,45,53,54,56,61–66,71,74,76,87,88] In most studies (93.8%), preoperative work-up consisted of esophagogastroduodenoscopy, barium

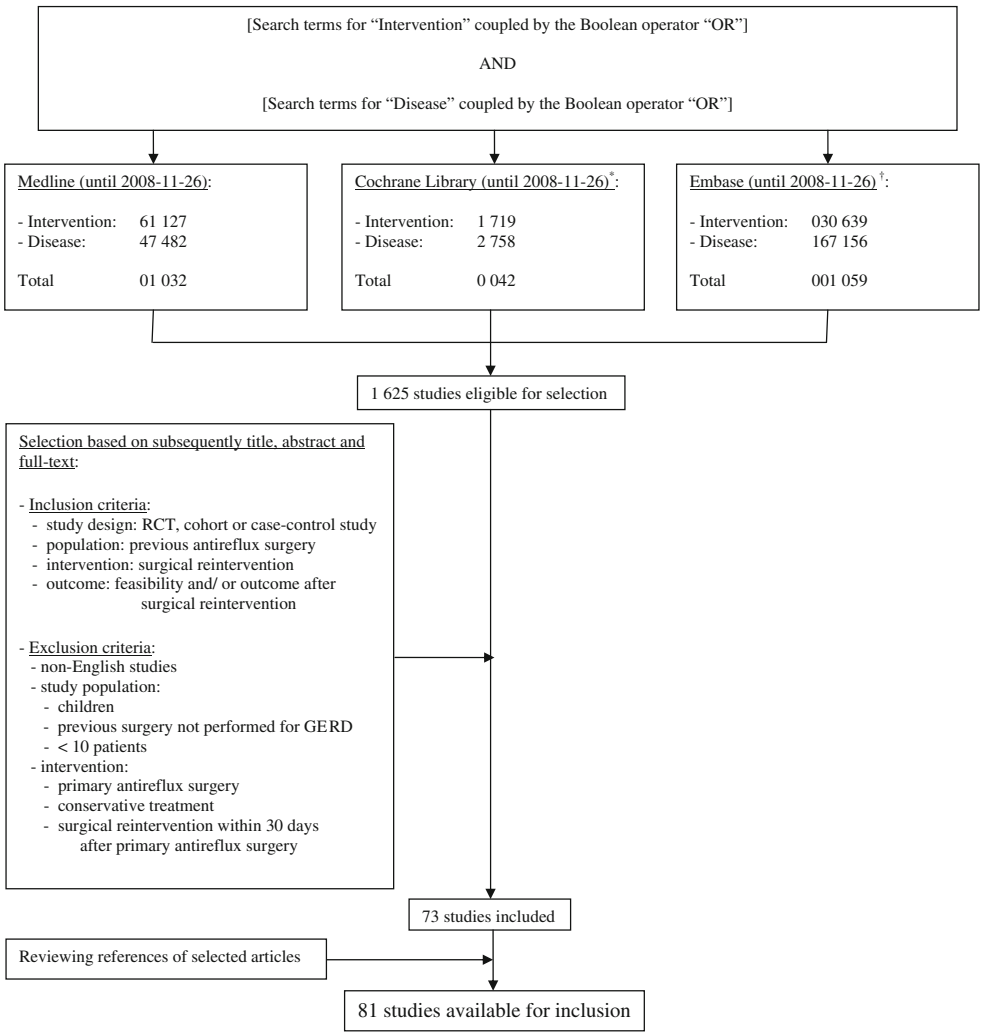

Figure 1 Results of Search Strategy and Selection of Studies.

* The Cochrane Central Register of Controlled Trials

† Embase only

Abbreviations: RCT, randomised controlled trial; GERD, gastroesophageal reflux disease

Table 2 Baseline Characteristics Extracted from the Included Studies

Number of patients (*n*)	4,509
Male	1,524 (33.8%)
Female	1,762 (39.1%)
Sex not reported	1,223 (27.1%)
Age (years)	51.3±0.8
Number of reoperations (*n*)	4,584
Study period (months)	10.8±0.7
Duration between primary surgery and reoperation (months)	38.3±4.1
Study design of the individual studies	
Prospective cohort study	27 (33.3%)
Retrospective cohort study	14 (17.3%)
Prospective case–control study	2 (2.5%)
Retrospective case–control study	1 (1.2%)
Not reported	37 (45.7%)

Values are given as mean±SEM unless otherwise stated

Table 3 Type and Indication of Primary Antireflux Procedures and Reoperations

	Primary procedures (n=4,750)	Reoperations (n=4,584)
Indication of operations		
Recurrent reflux	–	1,912 (41.7%)
Dysphagia	–	760 (16.6%)
Recurrent reflux and dysphagia	–	184 (4.0%)
Anatomical abnormality	–	114 (2.5%)
Gasbloat syndrome	–	31 (0.7%)
Miscellaneous	–	148 (3.2%)
Not reported	–	1,435 (31.3%)
Type of operations		
Total fundoplication	2,162 (45.5%)	2,397 (52.3%)
Partial fundoplication	471 (9.9%)	999 (21.8%)
Resection surgery	–	327 (7.1%)
Miscellaneous procedures	657 (13.8%)	737 (16.1%)
Not reported	1,460 (30.7%)	124 (2.7%)

swallow, and/or esophageal pH monitoring.[10–28,30–41,43–46,48–76,78,79,81–89]

Type and Route of Reoperations

Total or partial fundoplication was the most frequently performed reoperation (Table 3), whereas the type of reoperation was not reported in 124 patients (2.7%). The laparoscopic approach was used in 1,666 reoperations (36.3%); 1,589 reoperations (34.7%) were performed by the conventional (open) abdominal route and 1,041 (22.7%) by thoracotomy. The approach of reoperation was not reported in the remaining 288 reoperations (6.3%). More than one reintervention was performed in 75 patients (1.7%).

The esophagus was totally or partially resected during 125 reoperations (2.7%). The reasons to perform esophageal resection were severe esophagitis with or without Barrett metaplasia,[15,25,59] peptic stricture of the esophagus,[10,33,51,57,72,81] severely disturbed esophageal motility,[26,44,57,81] or short esophagus.[70,82] In 202 reoperations (4.4%), gastric resection was performed. Indications for this were alkaline reflux,[10] dense adhesions on attempted refundoplication,[33,59,86] or severe gastric paresis.[25,81]

Table 4 Causes of Failure of Previous Antireflux Procedure

	n=3,175
Anatomical abnormalities	
Intrathoracic wrap migration	885 (27.9%)
Wrap disruption	722 (22.7%)
Telescoping	448 (14.1%)
Para-esophageal hiatal herniation	195 (6.1%)
Hiatal disruption	167 (5.3%)
Tight wrap	168 (5.3%)
Stricture	60 (1.9%)
Wrong primary diagnosis	
Achalasia	37 (1.2%)
Esophageal spasms	7 (0.2%)
Sclerodermia	4 (0.1%)
Esophageal carcinoma	1 (0.03%)
Disturbed esophageal motility	13 (0.4%)
No cause for failure identified	194 (6.1%)
Miscellaneous	347 (10.9%)
Not reported	120 (3.8%)

Percentages exceed 100% since more than one cause of failure was found during several reoperations

Intra- and Postoperative Results

The different intra- and postoperative parameters were only reported in a subset of the original studies. Intraoperative complications were reported in 454 of 2,123 reoperations (21.4%) and were more frequent during laparoscopic than during open abdominal reoperations (150/770, 19.5% vs. 5/92, 5.4%). Laceration or perforation of the esophagus and/or stomach was the most common (Table 6). Postoperative complications were present after 546 of 3,491 reoperations (15.6%). Infectious, pulmonary, and cardiac complications were the most common postoperative complications (Table 6). Open abdominal reoperations were accompanied with more complications than laparoscopic reoperations (55/317, 17.4% vs. 98/642, 15.3%). Thirty-seven of 4,329 patients (0.9%) died intra- or postoperatively (Table 6). No mortality occurred in studies only reporting on laparoscopic reoperations, while the mortality rate was 1.3% in studies in which all reoperations were performed by a conventional abdominal approach.

Mean duration of reoperation was 177.4±10.3 min, mean intraoperative blood loss 205.5±35.6 ml, and mean

Table 5 Anatomical Abnormalities Depending on the Approach of Primary Surgery and the Indication of Reoperation

Anatomical abnormalities depending on the approach of primary surgery		
	Conventional (abdominal) approach (n=120)	Laparoscopic approach (n=132)
Wrap disruption	48 (40.0%)	24 (18.2%)
Telescoping	32 (26.6%)	10 (7.6%)
Hiatal disruption	23 (19.2%)	42 (31.8%)
Tight wrap	2 (1.7%)	24 (18.2%)
Miscellaneous	36 (30.0%)	42 (31.8%)
Anatomical abnormalities depending on the indication of reoperation		
	Recurrent reflux (n=234)	Dysphagia (n=118)
Intrathoracic wrap migration	104 (44.4%)	18 (15.3%)
Wrap disruption	109 (46.6%)	12 (10.2%)
No cause of failure	34 (14.5%)	51 (43.2%)
Miscellaneous	64 (27.4%)	54 (45.8%)

Percentages exceed 100% since more than one cause of failure was found during several reoperations

hospital stay 5.5±0.5 days. Comparing results of laparoscopic reoperations with laparotomy regarding the preceding parameters was not possible due to the small number of well-documented studies in the laparotomy group.

Reoperation was performed laparoscopically in 36.3% of all cases with a conversion rate of 8.7%. Causes of conversion were dense adhesions (n=57, 39.3%), severe intraoperative bleeding (n=11, 7.6%), poor visualization (n=3, 2.1%), and other (n=15, 10.3%). In the remaining 59 cases (40.7%), the reason for conversion was not reported.

Table 6 Intra- and Postoperative Results of Reoperations

Intraoperative complications	N=2,123[a]
Injury of esophagus and stomach	278 (13.1%)
Pneumothorax	73 (3.4%)
Hemorrhage	41 (1.9%)
Splenectomy	7 (0.3%)
Other	49 (2.3%)
Not reported	6 (0.3%)
Postoperative complications	N=3491[a]
Pulmonary complication	125 (3.6%)
Wound infection	64 (1.8%)
Leakage from alimentary tract	52 (1.5%)
Urinary tract infection	12 (0.3%)
Other infectious complications	48 (1.4%)
Cardiac complications	31 (0.9%)
Hemorrhage	22 (0.6%)
Other	136 (3.9%)
Not reported	56 (1.6%)
Causes of mortality	N=4,329[a]
Infectious	11 (0.3%)
Pulmonary	7 (0.2%)
Cardiac	4 (0.1%)
Miscellaneous	10 (0.2%)
Not reported	5 (0.1%)

[a] Total number of reoperations in which the intra- and postoperative complications and mortality rate were reported

Symptomatic Outcome after Reoperations

Symptomatic outcome after reoperation was determined in 79 studies (97.5%)[8,10–18,20–28,30–89] and reported as successful in 81% of patients, although with different definitions of success (Table 7). Data were obtained by questionnaires in 29 studies (36.7%),[8,10,11,16–18,20,22–24,27,28,30,34–37,42,45,46,48,49,54,55,61,69,71,80,84] by interview in 21 (26.6%),[13,25,31,38,41,47,52,53,57,60,62,65–68,73,74,78,82,83,85] and this was not reported in the remaining 29 studies (36.7%).[12,14,15,21,26,32,33,39,40,43,44,50,51,56,58,59,63,64,70,72,75–77,79,81,86–89] The mean success rate in studies only reporting on laparoscopic reoperations (17 studies)[11–13,23–25,28,31,35,39,41,48,50,53,61,70,85] was 84.2±2.5% and 84.6±3.4% in studies in which all reoperations were performed by a conventional abdominal approach (ten studies).[10,22,33,44,58,68,69,75,76,86] In patients in whom the reoperation was performed for symptoms only, 82.0±10.7% had successful symptomatic outcome,[47,79] and the success rate was 81.0±12.1% in patients with recurrent reflux documented by pH monitoring.[10,12,56,89] Comparing the outcome of total and partial refundoplication, Awad et al.[53] reported symptomatic success in 68% and 60% of patients, respectively. In two other studies,[11,45], however, no relationship between the type of fundoplication and the symptomatic outcome was found.

Objective Outcome after Reoperations

Objective outcome was reported in 696 patients (15.4%) in 24 studies (29.6%), without a definition of success[17,18,20] or

Table 7 Symptomatic and Objective Outcome after Reoperation

Definition of successful symptomatic outcome in the individual studies	Symptomatic outcome $n=79$	Objective outcome
Degree of symptoms at follow-up	25 (31.6%)	–
Patient satisfaction	22 (27.8%)	–
Satisfaction defined	6 (27.3%)	–
Satisfaction not defined	16 (72.7%)	–
Visick grading system	7 (8.9%)	–
Visick grading system combined with patient satisfaction	1 (1.3%)	–
Scores calculated from specific quality of life questionnaires	5 (6.3%)	–
Miscellaneous	5 (6.3%)	–
Not reported	14 (17.7%)	–
Patients available at follow-up	3 338 (74.0%)	581 (12.9%)
Duration of follow-up (months)	34.2±2.7	21.8±4.7
Patients with successful outcome	2 706 (81.1%)	455 (78.3%)

Values are given as mean±SEM unless otherwise stated

the number of successful cases,[14,17,18,20,28,49,87] however, in seven studies. In the remaining 17 studies, successful objective outcome was defined as normal acid exposure during pH monitoring in 11,[8,15,19,23,25,36,38,51,57,58,88] absence of esophagitis in four,[10,54,59,76] combination of these both in one,[75] and the absence of reflux during radiologic imaging in another one.[65] In these 17 studies, 78% had a successful objective outcome (Table 7). The mean success rate of laparoscopic reoperation (four studies[19,23,25,88]) seemed higher than in the case of a conventional abdominal approach (four other studies[10,58,75,76]), 85.8±5.6% and 78.0±10.1%, respectively.

Discussion

The often reported observations that morbidity and mortality are higher after redo antireflux surgery and symptomatic outcome is inferior to primary antireflux surgery have been confirmed in this systematic review on all studies currently available. Very few had a prospective study design, and in almost half of all, the type of analysis was not even reported. Moreover, most studies only present symptomatic outcome, and data on anatomy and function of the esophagogastric junction are scarce.

Morbidity was most frequently caused by direct injury of the esophagus and stomach during reoperation in the current review, and this was confirmed in our own data on redo surgery,[8] mainly as a result of increased complexity due to adhesions after the primary operation. Most primary interventions in the studies reviewed were performed by the conventional approach. Nowadays, with laparoscopy as the golden standard, less adhesions may be encountered if redo surgery is required. This might improve the outlook for these patients with a lower chance of iatrogenic organ damage, but this has to be proven in future studies. Although postoperative morbidity and mortality appeared to be lower after laparoscopic reoperations compared to the open abdominal approach, intraoperative complications occurred more frequently during laparoscopic surgery. These data, however, are not based on comparison between both approaches within individual studies, and therefore, this should, in our opinion, be interpreted with caution.

The cause of failure was recognized in 93.8% and mainly consisted of anatomical abnormalities or an erroneous indication for primary surgery. Disruption of hiatal repair and a too tight wrap were more frequently observed after the laparoscopic than after the open approach. This again underlines the difficulty of doing an adequate hiatal repair and creating a "floppy" wrap by laparoscopy. Achalasia was the most frequently reported incorrect diagnosis as the cause of failure, and this supports the inclusion of esophageal manometry and 24-h pH monitoring in the preoperative workup. It has also been suggested that a too tight fundoplication can cause an achalasia-like clinical picture.[90] Esophageal manometry shows, in those circumstances, a non-relaxing lower esophageal sphincter, but not an aperistaltic esophagus.[91]

Preoperative workup before reoperation is, apparently, not standardized but tailored to the cause of failure and the indication for reoperation. In the case of dysphagia, this consists of barium swallow to evaluate the esophageal and gastric anatomy and esophageal manometry to detect whether or not a motility disorder may be an (additional) cause of failure. In patients with reflux symptoms, extensive reevaluation is essential. Symptoms have been shown, however, to be bad predictors of pathological reflux after primary antireflux surgery[92] and unrelated to anatom-

ical wrap position.[93] Therefore, objective preoperative workup is equal to patients evaluated for primary antireflux surgery and consists of esophagogastroduodenoscopy, esophageal manometry, and 24-h pH monitoring, completed with barium swallow to evaluate the anatomy in addition to endoscopy.

Symptomatic outcome was described in most studies in this review with a success rate ranging from 56% to 100%. The definitions for success showed considerable variation and focus either on a more general or overall system or on specific symptoms with or without mentioning data on quality of life and the effect of surgery on quality of life aspects, compromising comparison between the individual studies. Patient satisfaction was a frequently used method for scoring symptomatic outcome. Patient's satisfaction is important and clinically highly relevant, but it does not directly refer to the specific symptoms of the disease, and consequently, this type of scoring does not provide insight in which aspects of the disease have improved and whether or not reflux symptoms have been exchanged by, for example, dysphagia. The Visick grading system, indicating that the disease was cured or improved with Visick grades I and II or unchanged or worsened in grades III and IV considered a symptomatic failure,[94] correlated well with postoperative daily reflux related symptoms and daily complaints of dysphagia in our patient group on redo antireflux surgery.[8]

Objective outcome was only reported in less than one third of the included studies in this review, with a mean success rate of 78%, which is slightly worse than after primary surgery. In our unit, all patients are encouraged to undergo stationary esophageal manometry and ambulatory 24-hr esophageal pH monitoring before and after primary as well as redo antireflux surgery primarily for quality control, but also to be able to correlate the functional results with symptoms and to understand possible future symptoms. Although previous studies have shown that for a good symptomatic outcome after primary surgery optimal anatomical and functional results are not a prerequisite,[92,93] more studies reporting the anatomical and functional status of the esophagus and stomach after redo surgery are required to outline a more complete overall picture of the outcome of redo antireflux surgery.

Conclusion

Redo antireflux surgery has a higher morbidity and mortality rate than primary antireflux surgery and symptomatic outcome is less satisfactory. Consistency with regard to reporting on symptomatic and objective outcome is necessary. Data on objective results after redo antireflux surgery are scarce and a plea can be made to subject all primary cases to full-scale evaluation, before and after antireflux surgery. Data to support this suggestion with evidence, like adequate cost-effectiveness studies, are lacking. The relative disappointing results of redo antireflux surgery with regard to morbidity, mortality, and symptomatic outcome support the opinion that redo surgery is tertiary referral center surgery and these centers should continue their efforts to collect prospective subjective and objective data.

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GI IMAGE

Primary Hepatic Osteosarcoma

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Extraskeletal osteosarcoma (EOS) is a rare entity. Most human cases have been described in the soft tissues of the limb.[1] Primary hepatic osteosarcoma is extremely uncommon with only seven cases reported in the world's literature. Of these cases, no patient survived more than 8 weeks from the time of diagnosis. We describe a young patient with a symptomatic primary hepatic osteosarcoma that was successfully treated by surgical resection and adjuvant chemotherapy. She is alive and tumor free 3 years after surgery.

Case Report

A healthy, athletic 19-year-old African American woman presented for a surgical evaluation of a newly diagnosed hepatic mass. The patient reported approximately 18 months of occasional and intermittent episodes of epigastric discomfort. Recently, she had developed symptoms of early satiety and anorexia. On physical exam, she had vague diffuse upper abdominal tenderness and a firm, palpable mass in the left upper quadrant extending to the midline. A calcified left upper quadrant mass was seen on abdominal X-ray taken during an emergency room visit. Laboratory evaluation was remarkable for moderate elevations of alkaline phosphatase and alpha fetoprotein.

To further evaluate the mass, a CT scan of the chest, abdomen, and pelvis was performed (Fig. 1), which showed a large heterogeneous mass occupying the left lobe of the liver with a 5-cm area of calcification in the anterior aspect. MRI demonstrated a single heterogeneously enhancing 15 × 10 × 13.8 cm lobulated mass inseparable from the left lobe of the liver (Fig. 2). The left portal vein was not visualized and there was no biliary dilatation. T1- and T2-weighted sequences showed a 5-cm area of signal hypointensity with multiple hypointense non-enhancing linear areas throughout the mass consistent with areas of calcification. A small amount of intraperitoneal free fluid was present. Staging chest and brain CT demonstrated no evidence for metastatic disease. Whole body Tc-99m MDP bone scan demonstrated no evidence of skeletal metastatic disease. The radiologic differential diagnosis includes sarcoma, including metastatic osteosarcoma, and fibrolamellar hepatocellular carcinoma.

At operation, a large, scarred lesion replacing the left hepatic lobe was immediately identified. The lesion extended across the falciform ligament into segment 4 of the liver. It was also adherent to, but did not invade, the stomach. The abdominal cavity was examined and no metastatic deposits were noted. The right hepatic lobe was examined with both palpation and intraoperative ultrasound, and was free of tumor. A left hepatic lobectomy was performed with en-bloc cholecystectomy using a combination of electrocautery and hydrojet dissection. The middle hepatic vein was not involved in the mass and was left in-situ. Her post-operative recovery was uneventful. She was discharged on the fifth post-operative day.

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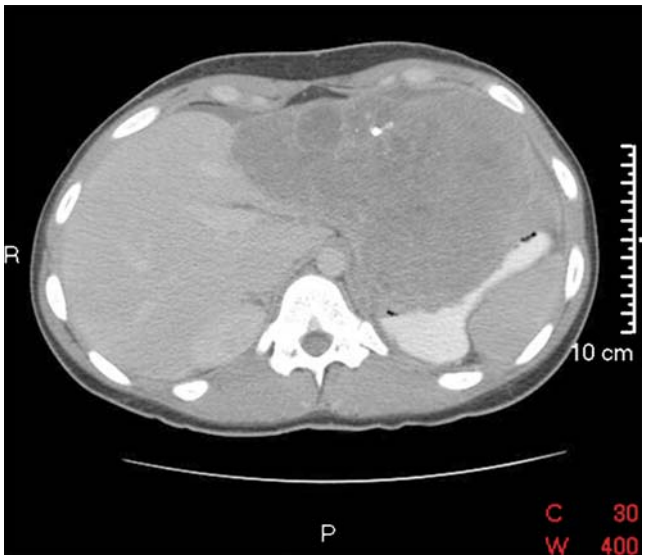

Figure 1 Post-contrast axial CT demonstrates a heterogeneous mass replacing the left lobe of the liver, hypoenhancing to liver parenchyma, with areas of calcification. It displaces the stomach posteriorly and inferiorly. The left portal vein is not visualized.

The patient received adjuvant chemotherapy with ifosfamide and mesna at 14 g/m^2 for two courses followed by cisplatin at 100 mg/m^2 with doxorubicin at 75 mg/m^2. Chemotherapy courses were administered at 3-week intervals. She received a total of four courses of ifosfamide and two courses of cisplatin and doxorubicin with a cumulative dose of doxorubicin of 150 mg/m^2. The patient has been followed clinically as well as by serial CT imaging of her abdomen, pelvis, and chest. She is alive without recurrence 36 months after surgery.

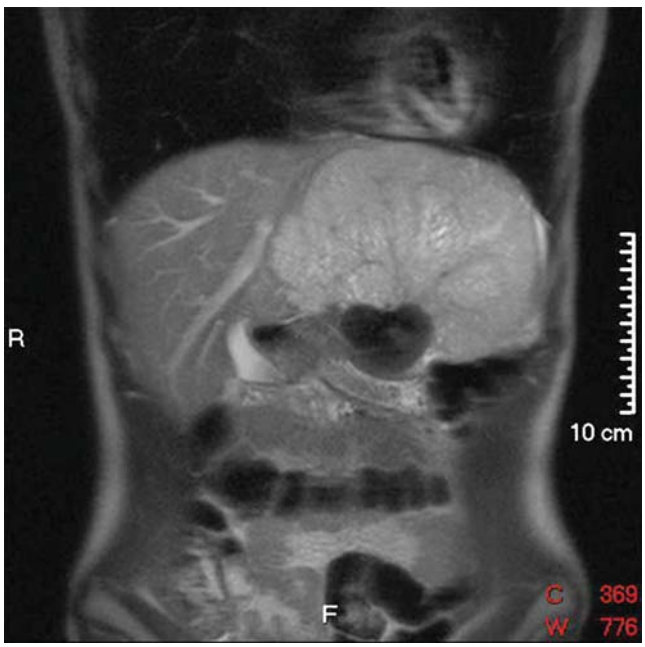

Figure 2 Coronal T2-weighted MR demonstrating the lobulated mass, predominantly hyperintense to liver. The calcified component is hypointense.

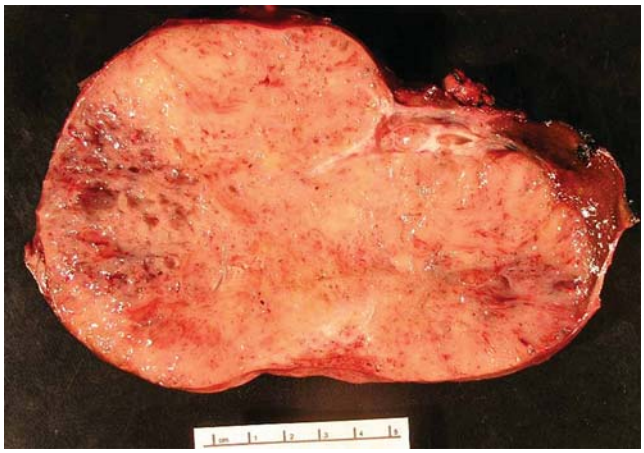

Figure 3 Cut surface of the resection specimen shows a tan-gray, rubbery, firm well-demarcated tumor with multifocal areas of hemorrhage and a thrombus at the right upper portion of the specimen. The *scale* shows centimeters.

Pathologic examination of the left lobe resection revealed a 15.0×13.8×12.0 cm, 1,390 g well-demarcated non-encapsulated tan-gray rubbery intraparenchymal mass with diffuse punctate hemorrhagic areas involving 95% of the specimen (Fig. 3). A focal 5.5×4.5×4.5 cm gray-white calcified area was present and a thrombus was in a large portal vein. The surrounding liver parenchyma was tan-brown and non-cirrhotic appearing. The specimen was processed with formalin fixation and paraffin embedding.

Histological examination showed an undifferentiated spindle cell neoplasm with foci of osteoid formation. The calcified area was composed of multiple osteoid islands circumferentially surrounded by undifferentiated malignant spindle cells. These pleomorphic spindle cells were characterized by indistinct cytoplasmic borders, vesicular

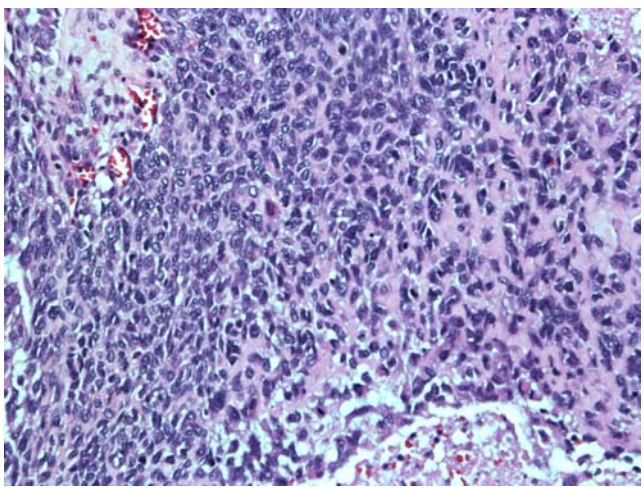

Figure 4 Malignant spindle cells with high mitotic activity, lacey osteoid formation, and necrosis (H&E ×20).

nuclei with inconspicuous nucleoli, and irregular nuclear membrane (Fig. 4). The spindle cells reacted with antibody directed against vimentin and there was no immunostaining for smooth muscle actin (SMA), S-100, desmin, epithelial membrane antigen (EMA), CD 57, or AE1/3. A high mitotic rate with greater than 80% staining for proliferative marker Ki-67 was seen. Tumor was present in the portal vein and in many intra-hepatic portal vein branches.

Discussion

Skeletal osteosarcoma is the most common malignant bone tumor in children and adolescents. Modern multimodality therapies including surgery and multiagent chemotherapy have produced 60–70% 3-year survival rates.[2] Extraskeletal osteosarcoma is a rare entity and case descriptions can be found throughout the literature mostly in the limbs and limb girdles.[3] Even fewer are reports of parenchymal osteosarcoma, but such cases have been documented in thyroid, kidney, gallbladder, breast, mesentery, liver, and colon.[4–10]

In adults, primary sarcomas of the liver are very uncommon. Attention has been focused particularly on angiosarcoma in connection with thorotrast and polyvinyl chloride.[11] Cases of primary hepatic fibrosarcoma and leiomyosarcoma have been reported as have undifferentiated sarcomas, though these tumors are seen almost exclusively in the pediatric age group.[12] Primary osteosarcoma of the liver is an exceedingly rare neoplasm of the liver which requires that the presence of other neoplastic components be excluded.[13] It is important to note that, after extensive radiologic imaging and physical examination, no evidence of a primary skeletal osteosarcoma was found in the patient described in this report. Sumiyoshi and Niho reported the case of a 52-year-old man who was hospitalized for hepatic failure.[14] He died 2 months after onset of symptoms. Autopsy revealed a cirrhotic liver with a large mass with histologic features of osteosarcoma. A literature search revealed a total of seven cases of primary osteosarcoma of the liver in humans and two case reports in animals.[8,13–20] We are unaware of a pre-operative diagnosis being made on any patient with this disease. This may be related to the rarity of the lesion or, alternatively, that if a biopsy were to return osteoid in a large liver tumor, various more common tumors would be suspected including hepatoblastoma, hepatic teratoma, malignant mesenchymoma, carcinosarcoma, hepatic angiosarcoma, or other hepatic sarcoma. Moreover, when mixed epithelial components are present, the pattern of differentiation is essential to characterize the tumor. This would be impossible with only a needle biopsy.

The age of patients with primary hepatic osteosarcoma in prior reports ranged from 52 to 73 years. Each previously described case resulted in either quick progression to death after diagnosis or the diagnosis was only established at autopsy. This case is notable for the young age at presentation (19 years old) and the prolonged disease-free survival she has experienced following surgery and adjuvant chemotherapy with multiagent chemotherapy.

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