Assessing transgastric Natural Orifice Translumenal Endoscopic Surgery prior to clinical implementation

Anders Meller Donatsky

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Tutors: Jacob Rosenberg, Søren Meisner, Peter Vilmann & Lars Nannestad Jørgensen

Official opponents: Peter Bytzer, Michael Bau Mortensen & Per-Ola Park

Correspondence: Center for Perioperative Optimization, Department of Surgery, Herlev Hospital, Herlev Ringvej 75, 2730 Herlev. Denmark.
E-mail: andersdonatsky@gmail.com

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ARTICLES INCLUDED IN THE THESIS

Study I
Donatsky AM, Andersen L, Nielsen OL, Holzknecht BJ, Vilmann P, Meisner S, Jørgensen LN, Rosenberg J. Pure natural orifice transluminal endoscopic surgery (NOTES) with ultrasonography-guided transgastric access and over-the-scope-clip closure: a porcine feasibility and survival study. Surg Endosc 2012; 26: 1952-62. [1]

Study II
Donatsky AM, Vilmann P, Meisner S, Jørgensen LN, Rosenberg J. Transgastric pure-NOTES peritoneoscopy and endoscopic ultrasonography for staging of gastrointestinal cancers: a survival and feasibility study. Surg Endosc 2012; 26: 1629-36. [2]

Study III
Donatsky AM, Holzknecht BJ, Arpi M, Vilmann P, Meisner S, Jørgensen LN, Rosenberg J. Oral chlorhexidine and microbial contamination during endoscopy - possible implications for transgastric surgery. A randomised clinical trial. Surg Endosc 2013; 27: 1914-22. [3]

Study IV
Donatsky AM, Andersen L, Nielsen OL, Meisner S, Jørgensen LN, Rosenberg J, Vilmann P. Gastrotomy healing after endoscopic ultrasonography guided pure transgastric peritoneoscopy: A randomized blinded study in a pig model. [4]

INTRODUCTION

The posttraumatic catabolic state of the human body as a response to systemic injury was described in the early 1930'ies [5]. Studies showing that surgery elicited a similar systemic metabolic response soon followed [6, 7]. These metabolic and inflammatory changes represent the surgical stress response that results in increased demands on organ functions and is associated with increased morbidity and mortality, especially in the elderly and in patients with severe co-morbidity.

With the concept of minimal invasive surgery, the goal is to minimize the incisions and thereby reduce the surgical stress response thus resulting in improved postoperative recovery. Since the introduction of laparoscopy in the late 80'ies, this technique has been shown beneficial compared with open surgery for a wide variety of surgical procedures [8]. In an effort to minimise the surgical trauma even further, Kalloo et al. described transgastric (TG) peritoneoscopy in a pig model in 2004 [9]. With this publication the term Natural Orifice Transluminal Endoscopic Surgery (NOTES) was introduced. The concept of NOTES is to achieve access the peritoneal cavity through one of the body's natural orifices. Although not termed NOTES at the time, transvaginal (TV) nephrectomy had been described 2 years earlier by Gettman et al. [10]. The NOTES technique holds several promising benefits but also barriers that hinder clinical implementation [11]. The theoretical advantages of NOTES compared to laparoscopy are reduced postoperative pain due to none or fewer abdominal incisions, decreased incidence of wound infections and incisional hernias, less intraperitoneal adhesions, decreased inflammatory response, faster convalescence and finally

improved cosmesis. NOTES as a minimal invasive procedure has been evaluated against laparoscopy in animal models. Although contradictions were found, the majority of studies support an inflammatory and cardiopulmonary response similar to or less profound than that of laparoscopy [12-23]. Studies have also found that TG peritoneoscopy can be performed at a lower intraabdominal pressures resulting in a less profound cardiopulmonary response compared with laparoscopy [24, 25]. The preliminary experimental results support the concept of NOTES as a minimal invasive approach to the abdomen, thus establishing the foundation for continued research into the implementation of NOTES in clinical practice.

The challenges for safe implementation of NOTES are numerous. Peritoneal access must be achieved without risk of iatrogenic lesions to adjacent organs, and closure needs to be reliable to prevent leakage. Decontamination regimens need to be effective to prevent infectious complications. Some challenges have been solved with the concept of hybrid NOTES, where access through the natural orifice is supplemented with one or more trocars through the abdominal wall. With this approach, access can be achieved under visual guidance. The additional trocars also allow for transabdominal instrumentation to ensure sufficient tissue traction and triangulation for dissection. Lastly, closure can be achieved, controlled and inspected through laparoscopy. Although hybrid-NOTES reduces the number of abdominal trocars in comparison with conventional laparoscopy, the concept of scarless surgery is lost. In a pure-NOTES setting, a given procedure is solely achieved through one of the natural orifices of the body thus completely avoiding the use of abdominal incisions. If pure-NOTES are to be implemented in clinical practice, it is a necessity that the before mentioned challenges are solved satisfactorily.

Objective of PhD thesis

The objective of this PhD thesis was to evaluate the safety aspects of TG pure-NOTES prior to clinical trials. The feasibility and safety of endoscopic ultrasonography (EUS) guided access and Over-The-Scope-Clip (OTSC) closure was evaluated in two experimental studies. The risk of infectious complications and the effectiveness of decontamination were also examined. The effect of oral chlorhexidine and bacterial contamination during gastroscopy was examined in a randomised clinical trial. Finally TG peritoneoscopy and ultrasonography were evaluated for the staging of upper gastrointestinal cancer.

ETHICAL CONSIDERATIONS

For studies I, II and IV approvals were obtained from the Danish Experimental Animal Inspectorate, The Danish Ministry of Justice (Journal-nr. 2010/561-1854 for studies I and II; Journal-nr. 2012-15-2934-00035 for study IV). All animal studies were performed at The Laboratory Animal Facility, The Faculty of Health Sciences, University of Copenhagen. Study III was approved by the Regional Committee on Biomedical Research Ethics (H-2-2010-068), the Danish Data Protection Agency (HEH.afd.D.750.89-6) and registered at www.clinicaltrials.gov (NCT01154530) before enrolment of patients. All participants were enrolled after written informed consent had been acquired.

PRESENTATION OF INCLUDED STUDIES

Study I: Pure natural orifice transluminal endoscopic surgery (NOTES) with ultrasonograhpy-guided transgastric access and over-the-scope-clip closure: a porcine feasibility and survival study.

Aim

The aim of this study was to evaluate the feasibility and safety of EUS guided TG access and OTSC closure for a diagnostic pure-NOTES procedure.

METHOD

Survival experiments were performed in 10 pigs. Antibiotic prophylaxis with intravenous (IV) cefuroxime and metronidazole were administered. Intraluminal EUS with Doppler was performed to locate a safe point of entry through the gastric wall. EUS guided TG puncture was performed. Correct needle placement in the peritoneal cavity was controlled by instillation of sterile saline. If placed correct a guidewire was advanced through the needle, and the TG fistula tract was dilated with 18 mm balloon. The videoendoscope was then advanced to the peritoneal cavity. In all 10 pigs a peritoneoscopy combined with intraperitoneal EUS was performed. These results are presented in study II. To achieve closure of the gastrotomy an over-the-scope-clip was applied.

Survival was assessed at postoperative day (POD) 14, at which time the pigs were euthanized, and a necropsy was performed.

Primary outcome parameters were uncomplicated follow-up and survival until POD 14, intraoperative complications, pathological lesions related to access and closure, macroscopic full wall closure and microscopic full thickness healing of the gastrotomy. Secondary outcome parameters were procedural time for access and closure, signs of infection, adhesion formation, culture samples from the peritoneal cavity and histology of the excised gastrorrhaphies.

Quantitative data were expressed as median and range or number and percent.

RESULTS

Results are summarised in table 1 and 2. All pigs survived until POD 14. EUS guided TG access was achieved without any intraoperative complications in all pigs in a median time of 25 minutes (range 12-62 minutes). No iatrogenic lesions related to EUS guided access were found at ne

cropsy. Median time for closure was 11 minutes (range 3-28 minutes) with macroscopic full wall closure in 9/10 of the excised gastrorrhaphies. One case had a mucosal fissure in relation to a broken OTSC. Histology showed ulcerations and severe inflammation with micro abscesses in all the excised gastrorrhaphies. Based on the definition, full thickness healing was not achieved in any case. Small lesions of localised to multifocal granulation and fibrous tissue on the peritoneal surface were the only pathology found in the abdominal cavity in 6/10 pigs. Fibrinous lesions were present in minute and moderate amounts in two cases respectively. The later had a solitary abscess adjacent to the access site. The last two cases had extensive fibrinous lesions and multiple abscesses in the peritoneal cavity. Chronic abscesses was thus present in 3/10 pigs. The omentum adhered to the access site in 5/10 pigs. Further adhesion formations were only found in three cases of abscesses formation. Bacterial growth was only present in samples taken from the abscesses.

Table 1 Summary of outcome parameters ($n = 10$)

Outcome parameters	Results
Survival (POD 14)	10/10
Uncomplicated follow-up (POD 14)	9/10
Intraoperative complications	0/10
Gross lesions related to access (necropsy)	0/10
Macroscopic full-thickness closure (necropsy)	9/10
Full-thickness healing (histology)	0/10

POD postoperative day

CONCLUSION

EUS guided TG access proved feasible and safe. Over-the-scope-clip provided immediate closure but the histopathology raises concerns regarding healing and risk of perforation. Further measures are needed to prevent contamination and intraabdominal infection.

Limitations

A primary limitation with this study is that it is an experimental descriptive study based on a pig model. While such a design cannot directly be correlated to the human clinical setting, it offers the opportunity to test the feasibility of new surgical techniques and to assess technical and safety aspects prior to clinical trials. Thus, the experimental descriptive design serves as proof of concept.

The results regarding infectious complications are difficult to extrapolate to the human setting. The species causing infectious complications seen in this study are part of the normal flora of the upper respiratory tract and stomach of pigs [26, 27]. Infections with these types of bacteria are zoonotic in humans and thus not part of the human flora [28-30]. In the present study the only precaution against infectious complications was the administration of preoperative antibiotics. With this precaution, signs related to contamination were seen in all animals. More elaborate decontamination regimens are thus required.

A limitation when interpreting the result is the study size compromised of only 10 pigs in total. The size of the present study was limited by financial reasons.

The technique used for gaining access in this study lacks reproducibility as evident from procedural time and the use of several different instruments for creation of the TG fistula. The main reason for this is the lack of NOTES specific endoscopes and instruments making handling and execution of a specific procedure difficult. This study found no iatrogenic lesions to adjacent organs or bleeding when EUL/Doppler were used, but the study size is not large enough to fully assess the actual risk of intraoperative

Table 2 Detailed overview of pathology, histology, and microbiology[a]

Animal no	Access technique	Gastrorrhaphy									Full-thickness healing	Peritoneal cavity				
		OTSC	Mucosal ulceration (macroscopic exmination)	Serosa (macrocopic examination)			Gastric wall (microscopic evaluation)					Adhesions	Abscesses (*n*)	Peritoneal grannulation	Fibrin deposits	Microbiology
				Focal chronic fibrous peritonitis	Greater omentum adhesion	Absces	Microscopic ulceration	Severe inflammation	Micro abscesses	Foreign body giant cells						
1	–	In situ	0	+	+	0	+	+	+	0	0	0	0	Multifocal	0	0
2	–	In situ	0	+	0	0	+	+	+	+	0	0	0	Localized	0	0
3	S	In situ, superficial	0	0	+	+	+	+	+	+	0	+	1	Multifocal	++	AP
4	–	In situ	0	+	0	0	+	+	+	0	0	0	0	Multifocal	0	0
5	S	In situ	0	+	0	0	+	+	+	0	0	0	0	Localized	0	0
6	B	In situ	0	+	+	0	+	+	+	0	0	0	0	Multifocal	0	0
7	S, N	In situ	+	+	+	0	+	+	+	0	0	0	0	Localized	+	0
8	–	In situ, broken	+	+	+	0	+	+	+	0	0	+	>20	Disseminated	+++	AP, PM
9	–	In situ	0	+	0	0	+	+	+	0	0	+	>35	Disseminated	+++	AP
10	–	In situ	0	+	0	0	+	+	+	0	0	0	0	Localized	0	0

OTSC over-the-scope clip, *AP Arcanobacterium pyogenes, PM Pasteurella multocida*

For access technique, (–) indicates transgastric needle puncture and balloon dilation, S indicates sphincterotome, B indicates bougie, and N indicates needleknife. For pathology, histology, and microbiology 0 indicates absence, and + indicates presence of the specific parameter. For fibrin desposits, + indicates minute, ++ indicates moderate, and +++ indicates extensive

complications related to access. When looking at the time used for gaining access ranging from 12 – 62 minutes, it is evident that access to the abdomen is not always easy. All the procedures in the present study were performed by two experienced endoscopists indicating that the pure TG NOTES technique is a very demanding procedure. At present it is a procedure for specialist endoscopists only and for future widespread clinical application a comprehensive training program on animal models or NOTES simulators would be mandatory.

Although OTSC provided immediate closure, the histology raised concerns of late postoperative spontaneous perforation. Based on the simple descriptive design of this study, it cannot be concluded whether OTSC closure is a reliable method for gastrotomy closure. Longer postoperative follow-up could help assess the risk of late perforation and randomisation to different lengths of postoperative follow-up could be used to assess the healing process. To fully assess the actual risk of leakage larger studies would be essential to avoid type 2 statistical errors.

Study II: Transgastric pure-NOTES peritoneoscopy and endoscopic ultrasonography for staging of gastrointestinal cancers: a survival and feasibility study.

Aim

The aim of this study was to evaluate the feasibility of intraluminal EUS combined with TG pure-NOTES peritoneoscopy and intraperitoneal EUS for GI cancer staging in a porcine survival model.

METHOD

The results presented here are based on the same animals as presented in study I. Before TG access an intraluminal EUS was performed. After TG access a peritoneoscopy and an intraperitoneal EUS were performed.

Whether or not adequate visualisation of predetermined anatomical structures could be obtained was recorded for intraluminal EUS, peritoneoscopy and intraperitoneal EUS respectively. The anatomical structures had been selected based on their clinical relevance for evaluating the operability of GI cancers. Intraluminal EUS, peritoneoscopy and intraperitoneal EUS consisted of 15, 13 and 9 structures of interest, with one point scored for each structure adequately identified to a maximum score of 15, 13 and 9 points.

Primary outcome parameter was visualisation scores. Secondary parameter was procedural time.

Quantitative data was expressed as median and range or number and percent. Mann-Whitney's test was used to test for declining procedural time with increasing experience of the surgeons. A p-value less than 0.05 was considered statistically significant.

RESULTS

Results are summarised in table 3 and 4. The TG-NOTES diagnostic procedure was completed with success all 10 pigs. Median total procedural time was 94 minutes (range 74-130 minutes). Median time used for intraluminal EUS, peritoneoscopy and intraperitoneal EUS was 11 min (range 7–14 min), 10 min (range 6–23 min) and 13 min (range 8–20 min), respectively. A significant decline in procedural time was only found for intraperitoneal EUS with a median reduction of 8 minutes (p = 0.03).

The median score for intraluminal EUS, peritoneoscopy and intraperitoneal EUS was 15 of 15 possible points (100 %, range 14-15), 12 of 13 possible points (92 %, range 8-13), and 6 of 9 possible points (67 %, range 1-8). For intraluminal EUS the common bile duct, hepatic artery and the superior mesenteric artery remained to be visualised in three separate cases. For peritoneoscopy there was difficulty with adequate visualisation of the left liver lope, left hemi-diaphragm, and gallbladder. For intraperitoneal EUS the inferior mesenteric artery, left liver lope, inferior caval vein and the aorta proved difficult to visualise.

Table 3 Visualisation scores according to the predefined record form

Pig	Intraluminal EUS (max = 15)	Intraperitoneal EUS (max = 9)	Peritoneoscopy (max = 13)
1	15	7	13
2	14	6	12
3	14	1	11
4	15	5	8
5	15	6	12
6	14	6	11
7	15	7	12
8	15	7	10
9	15	5	10
10	15	8	12
Median	15	6	12
Range	14–15	1–8	8–13

EUS endoscopic ultrasonography

CONCLUSION

Intraluminal EUS combined with TG pure-NOTES peritoneoscopy and intraperitoneal EUS proved feasible. Although diagnostic modalities lacking individually, the combined technique provided adequate visualisation of anatomic structures relevant for minimal invasive staging of GI cancers.

Anatomical structure		Intraluminal EUS	Peritoneoscopy	Intraperitoneal EUS
Upper abdomen	Right liver lobe	10/10	9/10	8/10
	Left liver lobe	10/10	6/10	3/10
	Gallbladder	10/10	8/10	9/10
	Common bile duct	9/10	–	–
	Spleen	10/10	10/10	–
	Right upper quadrant	–	10/10	–
	Left upper quadrant	–	10/10	–
	Right diaphragm	–	9/10	–
	Left diaphragm	–	8/10	–
	Stomach	–	10/10	–
Lower abdomen	Right lower quadrant	–	10/10	–
	Left lower quadrant	–	10/10	–
	Descending colon	–	10/10	–
	Urinary bladder	–	10/10	–
	Pancreas	10/10	–	–
	Right kidney	–	–	7/10
Retroperitoneum	Left kidney	10/10	–	–
	Hepatic veins	10/10	–	–
	Hepatic artery	9/10	–	–
Vessels	Portal vein	10/10	–	–
	Celiac artery	10/10	–	–
	Upper abdominal aorta	10/10	–	–
	Lower abdominal aorta	–	–	7/10
	Superior mesenteric artery	9/10	–	–
	Inferior mesenteric artery	–	–	1/10
	Caval vein	10/10	–	6/10
	Splenic artery and vein	10/10	–	–
	Right iliac artery and vein	–	–	8/10
	Left iliac artery and vein	–	–	9/10

Table 4 Achieved visualisation of individual anatomical structures (n = 10)

Limitations

The combination of intraluminal EUS and TG peritoneoscopy and intraperitoneal EUS in a single procedure could allow for faster cancer diagnostics, TNM classification and assessment of resectability for upper GI cancers. A primary limitation is the study being a descriptive experimental study evaluating the feasibility in a pig model. Although somewhat similar in anatomy, the results cannot be directly correlated to humans.

A more accurate evaluation would have been to compare TG versus laparoscopy either in a randomised cross over design or allocation to either technique. The present study simply evaluated whether or not TG NOTES was capable of identifying key structures relevant for cancer staging. In this design it is difficult to clearly define when a specific visceral surface is adequately visualised, such as the different surface areas of the liver. The lower surface of the liver was thus not inspected due to difficulties in lifting the liver using only an endoscopy. The study could have been optimised by implanting foreign objects or thermal lesions to imitate peritoneal carcinomatosis in hard to reach places.

A limitation for clinical use of the combined technique is that air filled intestinal loops from the preliminary intraluminal EUS can subsequently complicate the safety of TG access and limit adequate visualisation during the intraabdominal exploration.

The learning curve for pure TG procedures is certainly very long, especially if it is performed by an individual that is not already an experienced endoscopist. In the present study procedural times for the first five cases were compared with the last five cases. A sample size of 10 pigs would be too small to adequately assess a long learning curve. This becomes evident if procedural time used for access and closure reported in study I is compared to that of study IV. Access and closure were the only parameters that were standardised between these two studies, thus allowing for comparison, but no reduction in procedural time were found. It is known that simple graphical representation of the learning curve is inadequate to assess surgical performance [31]. Thus the present study is not designed to evaluate this aspect.

Study III: Oral chlorhexidine and microbial contamination during endoscopy - possible implications for transgastric surgery. A randomised clinical trial.

Aim

The primary aims of this study were to evaluate the effect of oral chlorhexidine on the level of microbial contamination during gastroscopy and secondary the effect of PPI on bacterial load of endoscope and stomach culture samples as well as a possible species specific effect of chlorhexidine.

METHOD

The effect of oral chlorhexidine was evaluated in a prospective single blinded randomised clinical trial. Patients referred for gastroscopy were approached and assessed for eligibility. After informed consent, participants were block randomised to one of two groups. The control group did not receive any kind of oral decontamination. The intervention group received mouth rinse with 2 cl of 0.2 % chlorhexidine for 1 minute. The gastroscopy was then performed in accordance with the referred indication. Two sample cultures were taken from each participant. The first sample was a stomach aspirate, and the second sample was taken from the endoscope immediately after the procedure. Quantification and identification of microorganisms were performed blinded to group allocations.

CFU counts for the endoscope samples acted as a surrogate measure for the potential contamination level of the peritoneal cavity, had the procedure been a TG NOTES procedure.

Primary outcome parameter was CFU counts in culture samples from the endoscope. Secondary outcome parameters were CFU counts in stomach aspirates, the influence of PPI on CFU counts and species specific effect of chlorhexidine on microorganisms with abscess forming capabilities.

Calculation of sample size and power of the study could not be performed prior to enrolment on the grounds of insufficient data in the literature. Enrolment continued until 50 participants had been allocated to each group for final analysis. Quantitative data are expressed as median and range or number and percent. Mann-Whitney U test for independent samples and Chi Square test were used to compare the two groups. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 160 patients were approached, and 109 accepted to participate in the study. Due to losses and in accordance with the protocol, fifty participants were randomly assigned to each group for final analysis (Figure 1).

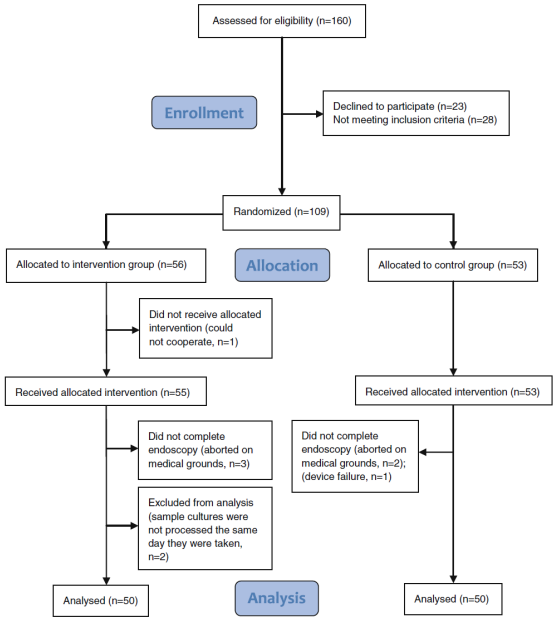

Fig. 1 Flow diagram of the inclusion and randomization process

The two groups had comparable baseline demographics with the exception of age and number of instrumentations (Table 5).

	Control ($N = 50$)	Intervention ($N = 50$)	p
Gender (male)	33 (66 %)	24 (48 %)	0.222
Age (years)	51.5 (19–74)	63 (20–90)	0.008*
BMI (kg/m^2)	23.9 (18–42)	23.5 (16–35)	0.238
Number of instrumentations	2 (0–7)	1 (0–8)	0.044*
Procedure time (min)	7 (2–30)	6 (2–16)	0.182
PPI treatment	28 (56 %)	23 (46 %)	0.424
PPI daily dosage (mg)	40 (20–80)	40 (20–80)	0.079
Propofol sedation	40 (80 %)	33 (66 %)	0.176
Benzodiazepine sedation	5 (10 %)	10 (20 %)	0.262
Oropharyngeal anaesthesia	8 (16 %)	16 (32 %)	0.1

Table 5 Baseline demographics. Data is presented as median (range) or number (percent). BMI body mass index, PPI proton pump inhibitor
* statistically significant p < 0.05

Chlorhexidine resulted in a significant reduction of the median CFU count in the endoscope sample (4,240 CFU/ml versus 36,270 CFU/ml, p=0.001) (Table 6).

	Control (N=50)	Intervention(N=50)	p
Gastric aspirate (CFU/ml)	55 (0-740,000)	95 (0-3,820,000)	0.651
Endoscope sample (CFU/ml)	36,270 (0-1,950,000)	4,250 (0-660,000)	*0.001

Table 6 Quantification results in regard to allocation. Data are presented as median (range). CFU colony-forming units
* statistically significant p < 0.05

PPI treatment was associated with significantly higher median CFU counts in both the endoscope samples (p=0.049) and the stomach aspirates (p=0.004) (Table 7).

Table 7 Effect of PPI treatment on quantification results

	Sample type	PPI	No PPI	p
Control ($N = 50$)	Gastric aspirate (CFU/ml)	575 (0–740,000)	5 (0–300,000)	0.026*
	Endoscope sample (CFU/ml)	60,740 (0–1,950,000)	20,460 (0–630,100)	0.145
Intervention ($N = 50$)	Gastric aspirate (CFU/ml)	370 (0–3,820,000)	30 (0–550,200)	0.079
	Endoscope sample (CFU/ml)	8,500 (50–315,000)	4,000 (0–660,000)	0.271
Groups combined ($N = 100$)	Gastric aspirate (CFU/ml)	370 (0–3,820,000)	10 (0–550,200)	0.004*
	Endoscope sample (CFU/ml)	37,040 (0–1,950,000)	8,800 (0–660,000)	0.049*

Data are presented as median (range)
PPI proton pump inhibitor; *CFU* colony forming units
* Statistically significant, $p < 0.05$

There were no species specific effects of chlorhexidine (Table 8). Microorganisms with abscess forming capabilities were equally present in the two groups.

Table 8 Species-specific effect of chlorhexidine mouth rinse

	Gastric aspirate		p	Endoscope sample		p
	Control ($N = 50$)	Intervention ($N = 50$)		Control ($N = 50$)	Intervention ($N = 50$)	
Micro-organisms involved in abdominal abscess formation						
Staphylococcus aureus	1 (2 %)	2 (4 %)	1	0 (0 %)	4 (8 %)	0.12
Beta-hemolytic streptococci[a]	0 (0 %)	0 (0 %)	N/A	1 (2 %)	2 (2 %)	1
Non-hemolytic streptococci	21 (42 %)	26 (52 %)	0.42	46 (92 %)	48 (96 %)	0.68
Escherichia coli	4 (8 %)	1 (2 %)	0.36	3 (6 %)	4 (4 %)	1
Klebsiella pneumoniae	1 (2 %)	1 (2 %)	1	1 (2 %)	1 (2 %)	1
Serratia marcescens	1 (2 %)	2 (4 %)	1	1 (2 %)	0 (0 %)	1
Other *Enterobacteriaceae*[b]	1 (2 %)	1 (2 %)	1	3 (6 %)	3 (6 %)	1
Anaerobic bacteria[c]	1 (2 %)	1 (2 %)	1	7 (14 %)	5 (10 %)	0.76
Candida albicans	7 (14 %)	7 (14 %)	1	1 (2 %)	1 (2 %)	1
Other *Candida* species[d]	2 (4 %)	3 (6 %)	1	0 (0 %)	0 (0 %)	N/A
Less pathogenic bacteria						
Branhamella catarrhalis	6 (12 %)	7 (14 %)	1	30	20	0.07
Neisseria species	0 (0 %)	1 (2 %)	1	5 (10 %)	4 (8 %)	1
Haemophilus species[e]	2 (4 %)	1 (2 %)	1	7 (14 %)	4 (8 %)	0.52
Kingella kingae	0 (0 %)	0 (0 %)	N/A	0 (0 %)	1 (2 %)	1
Coagulase-negative staphylococci	7 (14 %)	9 (18 %)	0.79	13 (26 %)	15 (30 %)	0.82
Stomatococcus species	0 (0 %)	0 (0 %)	N/A	0 (0 %)	3 (6 %)	0.24
Coryneform gram-positive rods	5 (10 %)	6 (12 %)	1	14 (28 %)	6 (12 %)	0.08
Lactobacillus species	3 (6 %)	3 (6 %)	1	0 (0 %)	2 (4 %)	0.49
Bacillus species	0 (0 %)	0 (0 %)	N/A	1 (2 %)	0 (0 %)	1

The number of samples in which the specific micro-organism was found
N/A not applicable
[a] Lancefield group A, C and G in one sample from one patient each
[b] *Citrobacter braakii*, *Citrobacter koseri*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Enterobacter aerogenes*, and *Enterobacter cloacae* were found in one sample from one patient each. *Proteus vulgaris* was found in one sample from two patients, in one of which also grew *Escherichia coli*
[c] Anaerobic gram-negative rods were found in 14 samples from 13 patients. Gram-negative cocci in one sample from 2 patients, gram positive cocci in both samples from 1 patient, gram-positive rods in one sample from 1 patient. Gram-negative rods also were present in all of these samples
[d] Three samples with *Candida dubliniensis*, one *C. tropicalis*, and one non-*albicans* not further identified from one patient each
[e] *Haemophilus parainfluenzae* in one sample from five patients, *H. influenzae* and *H. parahaemolyticus* each in four samples from three different patients, *H. haemolyticus* in one sample from one patient

CONCLUSION

The use of oral chlorhexidine is an effective, simple and economic way to reduce contamination prior to TG NOTES. As bacteria are still present, chlorhexidine should be combined with other measures into a standardized decontamination regimen. PPI treatment should be paused prior to TG procedures.

Limitations

Despite being an RCT, the study lacked a power calculation prior to enrolment. This limitation could have been eliminated if a pilot study quantifying bacterial contamination of the endoscope had been performed before the study commenced. A retrospective power calculation could be performed but the role of retrospective power analyses is controversial [32]. Retrospective power analyses should mainly be reserved for studies with non-significant results, thus acting as the foundation for future studies. Alternatively, an interim analysis could have been used during the study period.

There are other limitations associated with the methodology of this study. The gastric aspirate sample could have been contaminated with bacteria from the mouth and/or oesophagus and as such not be representative of the actual bacterial load of the stomach. This risk of cross contamination could have been minimised with the use of sterile overtubes although increasing the total budget of the study. As the endoscope can be contaminated with bacteria both during advancement and retrieval of the endoscope, the results presented in this study is most likely an overestimation of the actual load potentially introduced to the abdominal cavity during an actual TG procedure. Finally the procedures were not performed in a sterile surgical setting also contributing to possible cross contamination from the environment. For logistical reasons it was not possible to perform sterile procedures as patients were enrolled, and the procedures performed in a busy out-patient setting.

The groups were not comparable with regard to age and total number of instrumentations. A significant higher age was observed in the intervention group. It could be speculated that higher age represents poorer oral hygiene and increased bacterial load. This would have weakened the effect of chlorhexidine rather than enhancing the effect and as such this would strengthen the results presented here. A significantly higher number of instrumentations were observed in the control group, potentially accounting for a higher bacterial load in this group due to repeated cross contamination, thus weakening the results. An explanation for the observed differences in baseline demographics is most likely due to random imbalance and would probably have been balanced out if a larger sample had been used. To ensure homogeneity between the two groups, more extensive eligibility criteria could have been used, but at the expense of prolonging the study period.

Study IV: Gastrotomy healing after endoscopic ultrasonography guided pure transgastric peritoneoscopy: A randomised blinded study in a pig model.

Aim

The primary aim of this study was to evaluate the healing process of OTSC closed gastrotomies after pure-NOTES TG peritoneoscopy, and secondly to evaluate the effect of a combined decontamination regimen.

METHOD

Survival experiments were performed in 7 pigs. A multimodal decontamination approach was used consisting of IV metronidazole and cefuroxime, oral chlorhexidine, sterile overtube and gastric lavage with 1L sterile saline suspension with metronidazole and cefuroxime. Access to the peritoneal cavity and closure of the gastrotomy was achieved as described in study I. With the videogastroscope in the peritoneal cavity a peritoneoscopy was performed.

Each animal was randomised to either 14 or 28 days follow-up. The surgeon, assistants and staff were blinded to the respective allocations. Survival was assessed at POD 14 and 28 respectively where euthanasia and necropsy were performed. Histological evaluations were performed blinded to the respective allocations.

Primary outcome parameters were macroscopic full wall closure, microscopic full thickness healing and histological signs of inflammation in the excised gastrorrhaphies. Secondary outcome parameters were procedural times (total, access and closure), intraoperative complications, pathological lesions related to the procedure, signs of infection and adhesion formation in the peritoneal cavity.

Quantitative data were expressed as median and range or number and percent.

RESULTS

Results are summarised in tables 9, 10 and 11. Three pigs were allocated to 14 and four pigs to 28 POD follow-up. One pig allocated to 28 POD was euthanized prematurely due to deteriorating health. The remaining pigs had uneventful recovery and survived the respective follow-up periods.

Animal no.	Follow-up allocation	Access			Closure				Pathologist´s assessment
	(euthanized)	Puncture	Method	Time	OTSC	Endoclips	Time	In situ	
	(POD)	(n)		(minutes)	(n)	(n)	(minutes)		(POD)
1	28	1	s,b	41	1	0	6	0	28
2	14	1	s,b	22	1	0	6	+	14
3	28	1	s,n,b	53	1	0	17	0	28
4	28 (6)	4	s,n,b	66	1	0	7	+	14
5	14	1	n,b	43	1	0	15	+	14
6	28	3	b	49	1	2	14	0	28
7	14	2	s,n,b	43	1	0	5	0	28

Table 9 Allocation and characteristics of access and closure for each animal as well as the blinded assessment of follow-up allocation by the pathologist. Animal number represents the consecutive order in which the animals had surgery. Postoperative day (POD), sphincterotome (s), needle knife (n), balloon dilation (b), Over The Scope Clip (OTSC). For clips in situ at necropsy (+) indicates presence of and (0) indicates absence of this parameter.

Total procedure time was median 95 minutes (range 68 – 105 minutes). Median time for EUS guided access was 43 minutes (range 22 – 66 minutes). Closure of the gastrotomy lasted median 7 minutes (range 5 -12 minutes). Correct needle tip placement was only achieved with the first try in 4 pigs. The remaining 6 required repeated punctures. Immediate advancement of the balloon over the guidewire was only possible in a single case. The use of sphincterotomes and/or needleknifes were required for the rest. A single OTSC was applied in all pigs. In one pig the additional use of 2 endoclips were necessary before mucosal closure was deemed sufficient.

One intraoperative complication occurred. A mucosal tear in the oesophagus occurred during the passage of the OTSC. It did not require intervention, and necropsy revealed that the mucosal tear had fully healed.

Allocation	Animal no.	Peritoneal cavity			
		Peritoneal granulation	Absces (n)	Fibrin deposits	Adhesions
14 POD	2	0	0	0	0
	5	0	1	0	0
	7	0	0	0	0
28 POD	1	0	0	0	0
	3	0	0	0	0
	4	+++ (dfp)	0	0	0
	6	0	0	0	0

Table 10 Macroscopic pathology in the peritoneal cavity found during necropsy for each animal. Diffuse fibrinopurulent peritonitis (dfp). For peritoneal granulation, fibrin deposits and adhesions other than omentum, (0) indicates none, (+) indicates slight, (++) indicates moderate and (+++) indicates severe.

Allocation	Animal no.	Mucosal ulceration		Serosa (macroscopic)		Gastric wall (microscopic)						Full thickness healing
						Inflammation						
		Macroscopic	Microscopic	Focal peritonitis	Omentum adhesion	Overall	Acute	Chronic	Abscesses	FBGC	Ischemia / Necrosis	Per protocol / clinical relevance
14 POD	2	0	+++	0	+	+++	+++	+	+	+	0 / 0	0 / 0
	5	0	+++	0	+	+++	+++	+	+	+	0 / 0	0 / 0
	7	0	+	0	+	+	0	+	0	+	0 / 0	0 / +
28 POD	1	0	+	0	0	+	0	+	0	+	0 / 0	0 / +
	3	0	+	0	+	+	0	+	0	+	0 / 0	0 / +
	4	0	+++	0	0	+++	+++	+	+	+	0 / 0	0 / 0
	6	0	0	0	+	+	0	+	0	0	0 / 0	+ / +

Table 11 Results for full thickness healing based on the macroscopic and microscopic pathology of each individual gastrorrhaphy. Foreign body giant cells (FBGC). For focal peritonitis, omentum adhesion, microscopic abscesses, FBGC, ischemia/necrosis and full thickness healing, (+) indicates presence of and (0) indicates absence of the specific parameter. The term per protocol adheres to the definition of closure given in the method section. For the parameters ulceration and inflammation (overall/acute/chronic), (0) indicates none, (+) indicates slight, (++) indicates moderate and (+++) indicates severe.

Pathological lesions were found in 2/7 pigs. Necropsy of the pig euthanized due to deteriorating health revealed fibrinopurulent peritonitis. The gastrorrhaphy was intact, and there were no signs of bowel perforations. With this pig access was difficult and required repeated punctures and the use of several instruments. As no pathology indication iatrogenic lesions were found it could be speculated that the repeated punctures have resulted in increased contamination of the peritoneal cavity giving rise to the infection. With the other case, a single encapsulated abscess was found in the omentum. The omentum adhered to the serosa side in 5/7. No other adhesions were present in any of the pigs. Macroscopically the gastrorrhaphies were all without mucosal ulcerations. Microscopy revealed extensive ulceration, severe inflammation and micro abscesses in 3 gastrorrhaphies. Two were allocated to 14 POD and the last to 28 POD but euthanized prematurely. In three gastrorrhaphies (two allocated to 28 POD, one to 14 POD) only minute ulcerations and slight to moderate chronic inflammation were present. In the last gastrorrhaphy allocated to 28 POD, no mucosal ulcerations were present and only slight chronic inflammation. The OTSC was no longer in situ in these last four cases. Adhering to the definition of histology proven full thickness healing, this was only achieved in a single case. With respect to clinical relevance, sufficient healing was deemed achieved in all gastrorrhaphies with no or minute ulceration and slight chronic inflammation. Thus, clinical relevant healing was achieved in 4/7 pigs in total, and in all pigs allocated to and surviving until 28 POD.

CONCLUSION

OTSC provided easy and immediate closure. Clinical relevant full thickness healing had been achieved on POD 28. Despite no iatrogenic complications, EUS guided access lacked reproducibility, and the technique requires further refinement. Infectious complications still occurred despite the implementation of a multimodal decontamination regimen.

Limitations

Extrapolating to the human setting is difficult as the results are based on an experimental design in a pig model. Although the pigs were allocated in a randomised fashion, the study remains descriptive as the size and lack of power calculation limit the use of statistical analysis. The sample size was restricted due to financial reasons, but we anticipated that the study would still give valuable information as a hypothesis-generating pilot study.

Histology proven full thickness healing was only achieved in a single case based on the protocol definition. It could be argued that this definition was too strict, and that a clinical full thickness healing could be deemed achieved in another 3 gastrorrhaphies with only minute microscopic erosion and slight chronic inflammation. With this definition, full thickness healing was achieved in one pig allocated to 14 POD, and in all pigs allocated to and surviving until 28 POD. Thus, sufficient healing of OTSC closed gastrotomies seems achieved somewhere between 14 and 28 days postoperatively.

The secondary aim was to evaluate the effect of multimodal decontamination on the risk of contamination and infection in a TG pure-NOTES setting, thus originating from the access route. A potential bias is the use of Veress needle for pneumoperitoneum, as this carries a risk of contamination from the transabdominal puncture. The consequent practise of abdominal disinfection at the puncture site and the use of a sterile needle should reduce this risk of bias.

In the initial animal model (study I and II) the pigs were given per oral proton pump inhibitor treatment with

omeprazol 100 mg daily until POD 7. This was not administered in the present study. The reason for this is the difficulty with which to administer and ensure correct dosage of per oral medication to a pig. Medication could have been given intravenously but this setup also has several drawbacks. IV access can be achieved through a vein in the ear or a central venous catheter. Both methods carry a high risk of accidental displacement, and replacement requires sedation. Due to environmental factors and the nature of the pig, IV access also carries a risk of infection.

DISCUSSION

The objective of this thesis was to investigate the safety and feasibility of pure TG NOTES. In the experimental studies, the use of EUS/Doppler guided access was found to be safe but difficult thus lacking reproducibility and requiring further refinement. For closure, the OTSC delivered safe and reliable closure and sufficient postoperative healing. Oral chlorhexidine was effective to reduce the bacterial load, but abscess forming bacteria were still present. Despite the use of an extensive decontamination regimen, TG surgery still carried a high risk of infectious complications in the pig model. As standalone diagnostic modalities, the visualisation capabilities of both TG peritoneoscopy and intraabdominal EUS was lacking due to difficulties with intraabdominal manoeuvrability. When the two modalities were combined together with intraluminal EUS, the technique provided sufficient abdominal exploration in an experimental pig model.

In the following sections, the literature on safety of TG pure-NOTES in regards to key issues concerning access, closure and contamination will be discussed. Moreover, the limitations of TG peritoneoscopy in cancer staging will be reported.

Transgastric access

How to achieve safe and reproducible access to the peritoneal cavity is one of the primary concerns of TG NOTES [11, 33]. Survival experiments in animal models have been used to assess the feasibility and safety of different access techniques. Access is typically acquired through the anterior gastric wall. This location is presumed safest based on the evidence acquired from percutaneus endoscopic gastrostomy (PEG) tube placement introduced in the early 80'ies [34]. Meta-analyses regarding the use of PEG have shown low procedure related morbidity and mortality [35]. The vast majority of these complications are not relevant when performing TG NOTES as they are associated with the prolonged use of the PEG tube for nutrition.

The described techniques for access are basically variances of the same principle. TG puncture can be achieved with a combination of needle puncture, electrocautery with needle knife, and the use of sphincterotomes. When a TG incision or fistula has been created, it is dilated with either endoscopic dilators or balloon to accommodate the passage of the endoscope to the peritoneal cavity. Guidewires can be used to ease the passage and stabilise the use of the different endoscopic instruments.

It has been shown that direct endoscopic gastrotomy creation with needle knife carries a high risk of organ injury [36], and that an endoscopic chosen access point compares poorly to the ideal safe point of access determined through laparoscopy [37]. These findings emphasise the need for technique modifications to safeguard against iatrogenic organ lesions, bleeding, and electrocautery lesions to adjacent organs and bowel perforation. A hybrid procedure with simultaneous laparoscopic visualisation and instrumentation can facilitate safe TG access [36], but this method does not adhere to the NOTES principle of scarless surgery. Another technique is based on the same principle as PEG tube placement. Modifications of this technique have also been associated with low complication rates [38-40]. Creation of preliminary pneumoperitoneum has also been shown to facilitate TG puncture and access in an experimental study [41], as well as in humans scheduled for laparoscopic Roux-en-Y gastric bypass [42]. Because the scope of this thesis was TG pure-NOTES, these access techniques will not be discussed further.

One pure-NOTES access technique is based on endoscopic submucosal dissection (ESD) primarily developed for resection of early stage gastric cancer [43]. Here access is acquired by first injecting saline into the submucosa. The mucosa is cut and the submucosal layer is dissected to create a submucosal tunnel (SMT). An incision to the peritoneal cavity is made at the end of the tunnel and dilated with balloon. The SMT technique has been evaluated in experimental series with minimal risk of complications [44-48]. The primary strengths of this technique are that the tunnel provides protection against peritoneal soiling and eases closures. A primary concern is the risk of mucosal tearing along the length of the tunnel due to endoscope manoeuvring or specimen extraction [49]. Another variation is submucosal endoscopy with mucosal flap safety valve (SEMF) technique [50]. Here a submucosal working space is created by high pressure carbon dioxide injection, chemically softening of connective tissue, and balloon dissection [51].

Another way to prevent iatrogenic lesions is to determine a safe point of entry through ultrasonography. EUS guided TG puncture has been shown to minimise complication rates compared to blind puncture [52]. This risk could be minimised even further with the creation of hydroperitoneum to displace adjacent organs [53]. In this study, a total volume of 3 L was instilled through the Veress needle. Although this minimised complications, such a large volume could complicate the subsequent peritoneoscopy due to pockets of fluid hindering visualisation.

The experimental studies presented in this thesis evaluated the feasibility and safety of EUS/Doppler guided

transmural needle puncture. After puncture saline was infused, creating a fluid cushion that displaced adjacent organs and allowing advancement of a guidewire with subsequent balloon dilation of the fistula tract [1, 4]. In regard to reproducibility, the procedural time range indicates high difficulty and that the technique needs further refinement before implementation in clinical practice. This is especially evident from 3 cases where numerous TG punctures were required before access could be achieved. In one pig these repeated punctures probably lead to contamination and generalised peritonitis. In general it was difficult to advance the balloon over the guidewire, often requiring the use of both needle knife and sphincterotome to predilate the fistula tract. The reason was the fact that the balloon instrument frequently was caught on the muscular layer of the gastric wall exposed in the fistula tract despite the use of a guidewire. In a single case an endoscopic bougie dilator was used with great success. Predilation with plastic bougies has been evaluated in one study which found that their use facilitated creation of the gastrotomy [38].

Several techniques for TG access have been described in the literature and show promise. However, sufficient evidence of safety, efficiency and reproducibility is not available due to study designs and sample sizes. Larger randomised experimental studies are needed to fully describe the safety profile of the various techniques. At present, the access techniques could be applied in humans with the simultaneous use of laparoscopy to provide safety i.e. as hybrid procedures.

Gastrotomy closure

It has been reported that a gastrotomy does not necessarily require closure when the muscular layer is simply dilated and not cut to allow passage of the endoscope [54, 55]. Nevertheless, a 100 % reliable closure has to be developed before widespread implementation of TG surgery in humans. Leak rates as low as 1 % have been deemed unacceptable due to the risk of peritonitis and associated morbidity [11, 56]. The difficulty with which to acquire easy and reliable closure is evident in the number of different methods evaluated to date, such as commercially available endoclips, T-tags/bars/anchors, loops, staplers, fibrin glue, bioabsorpable plugs, laser, endoscopic omentoplasty [57-67], utensils primarily designed for other indications [68, 69], newly developed endoscopic utensils like the Over-The-Scope-Clip [70, 71], and prototype instruments such as the Eagle Claw and Padlock-G clip [72, 73]. Another problem is how to assess for closure reliability. In this respect, there is huge heterogeneity in study designs using both ex vivo and in vivo designs with or without survival. The assessment methods applied to test for reliable closure and healing are numerous such as air and water burst pressure, contamination samples, macroscopic inspection, and microscopic examination with varying histological definitions of closure/healing sufficiency. This lack of consensus makes simple comparison of closure techniques impossible [56]. The majority of human reports on TG NOTES to date rely on the hybrid technique thus having the choice of closing/inspecting the gastrotomy through laparoscopy [74].

When Kalloo et al. first described the feasibility of TG peritoneoscopy in 2004, closure was achieved by applying mucosal endoclips [9]. Here no leakage was observed in 5 pigs. It has been speculated that simply closing the mucosa carries a high risk of leakage and that full thickness closure involving al the layers of the stomach wall is preferable as performed in laparoscopic and open surgery.

Several techniques for closure after SMT access have been evaluated. In one study, the gastrotomies were not closed resulting in a mucosal defect with necrotic tissue and abscess formation in the wall as well as localised peritonitis. The conclusion was that the mucosa required closure with either clips or anchors if adequate healing was to be achieved [44]. It has been shown that mucosal endoclips provides no leakage or signs of intraabdominal pathology after SMT access [47, 75]. Other techniques have been evaluated as well. One study evaluated the use of fibrin glue VS endoclips and found no leakage in either group [65]. The only difference was in procedural time indicating the ease and simplicity of closure using fibrin glue. Another study evaluated closure of both the mucosal incision with endoclips and the seromuscular incision with implantation of acellular porcine dermal matrix [76]. Procedural time for closure was long due to the difficulty of loading and delivering the matrix into the submucosal space. In this study, several matrix related complications also occurred. The last technique that has been described closes the mucosal flap by deploying full thickness tissue anchors [50]. This technique carried a risk of iatrogenic organ penetration due to blind TG deployment of anchors. Overall, it seems that closure after SMT access can be achieved with relative ease by applying mucosal endoclips with or without fibrin glue.

The use of tissue anchors, T-bars, or T-tags have also been evaluated for closure after direct TG access. The use of anchors have been shown to give reliable closure with high burst pressures and no leakage in a non-survival design [63]. Survival experiments are supporting high bursting pressures without leakage together with macroscopically full thickness healing [57, 77, 78]. An adapted version uses loop-anchors thus closing the gastrotomy in a purse string fashion with similar high bursting pressures [79]. Histological evaluations of closures are contradictive. One study found sufficient healing without signs of infection or abscesses [60]. Another study describe inferior layer-to-layer transmural healing for T-tags compared to using endoclips [80]. While yet another report inferior closure with endoclips compared to that of loops and clips [61]. Ulcerations, transmural necrosis, foreign body material, and microabscesses have also been described [81]. As mentioned earlier, there is also the risk of iatrogenic complications due to

transmural needle puncture required for delivering the anchors [77, 81, 82].

With the use of the OTSC system, closure can be achieved with relative ease. The majority of studies evaluating OTSC closure reported mean procedural times in the range of 6-12 minutes [83-89], although mean procedural time has been reported as high as 27 minutes [90]. One study found that longer procedural time was linked to the increased difficulty of closure when the OTSC had to be deployed with the endoscope in a J-position [91]. In the majority of cases, a single OTSC is required for closure. The application of a single OTSC has been shown to give sufficient closure of gastric wall defects reaching 18 – 20 mm [92, 93]. Simultaneously, it is recommended that defects reaching 18mm and above requires two OTSC for sufficient closure [92, 93]. Although contradictive results have been reported, the bursting pressure of OTSC closed gastrotomies have in the majority of randomised studies been found comparable to and higher than that achieved with the gold standard of hand sutured closure [78, 86, 89, 94]. The results presented in the two experimental studies included in this thesis also support the use of the OTSC system with regards to easy closure without immediate leakage and with sufficient healing reached after 28 days of postoperative follow-up [1, 4]. The results from recent reviews evaluating the OTSC for closure of iatrogenic gastrointestinal perforations and fistulas lends further supports to the clinical use of the OTSC system [95, 96].

The existing evidence on TG closure is limited by small sample sizes that cannot predict leakage rates of around 1 %. The small samples could thus account for the overall contradictive results presented in studies evaluating closure techniques conducted to date. The heterogeneity in study designs makes comparison of closure techniques difficult, and a definite conclusion as to what technique delivers the most promising results cannot be made. Too make future comparison easier, a design for testing closure techniques compromised of several testing modalities have been proposed [56]. With regards to test for closure integrity per-operatively in future clinical trials on TG pure-NOTES, one group has described a reproducible endoscopic pressure monitoring system for the measurement of intragastric pressure to demonstrate the presence of gastric leakage. They found the system reliable and comparable to that of contrast based radiographic leak testing [97]

Transgastric peritoneoscopy for cancer staging

Less invasive procedures have been shown to minimise suppression of the immune response [98]. Thus minimal invasive surgery provides better preservation of the immune function [99]. Although the clinical relevance of observed differences in immune function between open and minimally invasive surgery is not fully determined, it has been speculated that a better preserved postoperative immune function might have a positive influence on tumour recurrence and survival rates [100]. To support this notion laparoscopic assisted resection seems to provide better cancer related survival than open surgery for the treatment of non metastatic colon cancer [101]. As a minimal invasive approach, one study analysed 474 patients undergoing cancer surgery for the possible application of the NOTES technique [102]. A potential for the application of NOTES was present in 11 % with staging of gastrointestinal tumours being the main indication (45 %). In theory NOTES as a minimal invasive approach could have potential in the diagnosis, staging, and treatment of gastrointestinal cancer. In the above described study caution for clinical implementation was advised due to the potential technical difficulties, arising from abdominal adhesions (30 %) and intraoperative orientation (20 %) [102].

Several studies have evaluated the efficacy of TG peritoneoscopy in animal models. One study evaluated TG peritoneoscopy and intraperitoneal EUS of the liver [103]. The main difficulty observed was how to achieve adequate visualisation in the upper abdomen, specifically the inferior and the right lateral part of the liver. The same group compared TG peritoneoscopy to laparoscopy using a non-inferiority design in both an animal model and human cadavers [104, 105]. To simulate peritoneal carcinomatosis, small beads were used. Both studies found TG peritoneoscopy to be inferior to laparoscopy. For the TG approach, the missed beads were primarily located in the region of the liver. Another study has also evaluated TG peritoneoscopy against laparoscopy in an animal model [106]. The ability to detect electrocautery markings simulating intraperitoneal metastases was examined. The sensitivity for detecting lesions was 78.5 % for laparoscopy versus 38.9 % for the TG approach ($p<0.001$). Similarly, biopsy capability was better with laparoscopy ($p<0.01$). Concluding that in the current form, TG NOTES is unsuitable for sufficient exploration of the abdominal cavity.

The manoeuvring capabilities of existing endoscopes coupled with retroflexion are the primary reasons for visualisation of the upper abdomen being difficult and time consuming. To solve this problem one study used an image registration system with real time tracking of the endoscope in relation to a three dimensional reconstruction of the anatomy. This setup provided enhanced navigation with improved efficacy and ease of intraabdominal exploration [107].

The efficacy of TG peritoneoscopy has been evaluated in a human setting. In a series of 20 patients scheduled for laparoscopic Roux-en-Y gastric bypass, adequacy of TG exploration of the 4 quadrants was evaluated prior to the procedure [108]. The study found no limitations with visualisation of the abdomen. A limitation with the study is that it did not provide a clear description of what parameters the decision of adequate visualisation was based.

Although TG peritoneoscopy is inferior to laparoscopy in detecting all lesions, it can be argued that in the diagnosis of peritoneal carcinomatosis this has no clinical

relevance. It is the presence of peritoneal carcinomatosis regardless of the actual number of lesions that influences the decision of operability. In that regard the TG approach could be comparable to laparoscopy. To support this statement, one study has evaluated laparoscopic staging of pancreatic head masses versus the TG approach in 20 human subjects [109]. Two separate surgeons, blinded to each other's findings, performed the procedures. A total of six discrepancies were found. Five discrepancies were in favour of laparoscopy. Four of these were small lesions located in the right upper quadrant and right liver lope. Three of these were benign. The last lesion was malignant but was no longer present for repeated biopsy during the TG approach. The fifth lesion missed during the TG approach was located on the anterior abdominal (also benign). The last discrepancy was in favour of the TG approach. Several small plagues in the left upper quadrant were missed during laparoscopy, these also tested benign. The final blinded decision to proceed with palliative or curative surgery was the same in 95 % of cases. The only disagreement was in the case of malignant lesion no longer present for biopsy during the TG approach. The initial series consisting of 10 patients reported difficulties in visualising the upper abdomen sufficiently, specifically the gallbladder and the right lobe of the liver, consistent with the difficulties reported in animal series [110]. To overcome this obstacle, the same group has evaluated a steerable flexible trocar (overtube) in 10 patients scheduled for Roux-en-Y gastric bypass [111]. The overtube is advanced into the abdominal cavity and can be articulated and locked into position to provide a stable platform guiding the endoscope, allowing for greater mobility of the endoscope in the upper abdomen. The system shows promise but has not yet been sufficiently evaluated.

It has been shown that location of the gastrotomy can influence the ability to adequately manoeuvre and explore the abdominal cavity [112]. Furthermore, one study showed that the method used for gaining access can influence the ability to localise points of interest and manoeuvre the endoscope to a desired location [113]. In this study direct incision of the gastric wall provided significantly higher localisation and touch scores than submucosal tunnelling. Access through submucosal tunnelling has on the other hand been shown to allow for in-line endoscope positioning in regards to predetermined abdominal locations of interest [75]. In the experimental study presented in this thesis (study II), a tendency towards achieving higher visualisation scores due to better manoeuvrability for both peritoneoscopy and intraperitoneal EUS was found when access was achieved through the antrum of the stomach (unpublished data). The study was not designed to evaluate this aspect, and thus no conclusions can be made. The optimal TG access site remains to be determined and should be evaluated in future studies. The visualisation results presented in this thesis support the use of TG access for cancer staging when combining peritoneoscopy with both intra and extra luminal EUS [2]. Small series are emerging, supporting the use of TG peritoneoscopy with biopsy in humans [114, 115]. The procedure has even been performed under conscious sedation in an endoscopic unit [116], thus paving the way for TG NOTES cancer diagnostics in an outpatient setting. At present, a major limitation is the risk of inadequate inspection of structures, especially in the upper abdomen. This limitation is primarily based on technical difficulties related to the endoscopes being used, thus emphasizing further research and support from the industry to drive new technical innovations.

Transgastric contamination and risk of infection

Aseptic technique and sterility of the abdominal skin as to prevent infectious complications is easily achieved in open and laparoscopic surgery. Achieving an aseptic approach is much more difficult for TG NOTES. Here infection could arise from contamination with bacteria from the mouth, oesophagus, and stomach. Thus infection prevention was primarily identified as one of the limiting barriers for clinical implementation of the TG technique [11, 33].

The reported incidence of infectious complications after TG procedures are contradictive, ranging from 0 to 100 % [117, 118]. Several modalities to prevent contamination have been used in animal studies, most often in combination. These modalities are IV antibiotic prophylaxis, disinfection of the mouth, the use of sterile overtubes, high-level disinfection of endoscopes, instruments and equipment, proton pump inhibitor treatment, and gastric lavage with saline and/or antiseptic solutions.

Primarily it was speculated that PPI had to be used preoperatively to prevent peroperative leakage of acidic content resulting in chemical peritonitis. However, in an experimental rat model, it was found that the use of PPI resulted in a higher rate of peritoneal contamination and abscess formation [119]. One human study evaluated contamination of the peritoneal cavity after TG peritoneoscopy in patients scheduled for LRYGB [120]. The use of PPI was associated with increased bacterial load of the stomach and increased contamination of the abdomen, although this did not lead to increased risk of infectious complications. The results presented in Study III of this thesis support that PPI treatment increases the bacterial burden of the stomach and contamination of the endoscope. Despite this the design does not allow for an assessment of whether this increase is linked to a higher risk of infectious complications [3]. The present evidence seems to support the discontinuance of PPI prior to TG surgery. To facilitate postoperative healing it could be speculated that PPI treatment should be initiated postoperatively.

Only a limited number of studies have evaluated the effect of gastric lavage as a standalone modality. Results regarding the effect of lavage are contradictive [121-123]. This could in part be explained by the different solutions

being studied, although another study found that preprocedural lavage had no effect on intra abdominal bacterial burden or subsequent infections regardless of the solution being used (saline and antibiotics) [124]. In this study, lavage with betadine or chlorhexidine was not evaluated. A more recent ex vivo study found that both topical betadine and chlorhexidine were significantly more effective in reducing the bacterial burden of the stomach than no lavage and lavage with saline or antibiotics [125]. Intraperitoneal lavage as a standalone modality has been evaluated in a single study which found no difference when compared to placebo [126].

Combined regimens consisting of several decontamination modalities have also been evaluated with contradictive results in animal models. One study found that a combined regimen was effective in reducing peritoneal bacterial contamination, but despite a significant reduction infectious complications were still present [127]. Another study with a strict regimen compromised of both IV AB, high-level disinfection of equipment, triple lavage, and the use of a sterile overtube found peritoneal contamination levels comparable to that of laparoscopy [128]. Yet another study compared contamination from TG access with that of laparoscopy and open surgery [129]. Here evidence of contamination was present at the end of the TG procedure but without clinical relevant infections. One study evaluating the effect of sterile equipment found that non-sterile conditions invariably lead to infections [130]. An infection rate of 100 % in a non-sterile group was significantly reduced to 0 % in the sterile group. The bacterial flora found in the peritoneal cavity in this study consisted primarily of oral flora. It has been proposed that this could account for the fact that several modalities such as gastric lavage have no clinical effect [118].

When comparing the results from study I and IV of this thesis, the improved decontamination regimen used in study IV reduced the amount of intraabdominal pathology found during necropsy [1, 4]. This could be taken as a measure that the level of contamination had been decreased with the use of a combined regimen. Although the rate decreased, infectious complications were still present. In study III it was shown that oral chlorhexidine was very effective in reducing the bacterial load of the endoscope during gastroscopy [3]. This makes oral chlorhexidine an effective and cheap agent with which to reduce the contamination level when performing TG surgery.

To sum up, the evidence from experimental animal models is contradictive. Results seem to support a combined approach to effectively reduce contamination. The sample sizes are too small to fully assess the actual incidence of intraabdominal infectious complications, and the question regarding where to sterilise and what solutions to use remain unanswered.

Founded in the contradictive evidence, it has been proposed that the pig model is unsuitable for evaluating contamination and infection [124]. To support this statement are results from a retrospective review of 100 patients enrolled in different pre-NOTES protocols [131]. Here cross-contamination was observed in as many as 21 % but without any infectious complications. The study concluded that bacterial contamination secondary to TG access is clinically insignificant due to either the species or bacterial load. A primary limitation is that half of these cases are from a study evaluating contamination in relation to LSRYGB and thus not related to an actual TG NOTES procedure [132]. Although limited in the total number of patients, the results from human series on TG procedures published to date support that infectious complications are rare [133, 134]. Even though contamination in the human setting does not seem to amount to clinical infection, a decontamination regimen could help reduce subclinical peritoneal reaction and thus minimise adhesion formation.

Based on evidence available, a grade C recommendation was proposed in 2011 stating that no preoperative preparation is necessary before TG access to the abdomen in humans [117]. Until this aspect is fully assessed, it seems only ethical to use combined regimens when performing TG NOTES. Based on the results presented, such a regimen could comprise oral chlorhexidine, gastric lavage with either betadine or chlorhexidine, the use of a sterile overtube, prophylactic AB with IV metronidazole and cefazoline, and the conduction of the procedure in a sterile setting using sterile instruments and endoscopes.

CONCLUSION

In the experimental studies, EUS guided TG access was found technical feasible without any iatrogenic organ lesions or gastric haemorrhaging requiring intervention. The technique lacked reproducibility, and in one case the high difficulty resulted in repeated punctures and subsequent peritonitis. OTSC closure was found to be easy, quick, and reliable with sufficient healing achieved within a time span of 14 to 28 days postoperatively.

In an RCT, oral chlorhexidine was found to significantly reduce the bacterial load on the endoscope when performing gastroscopy, thus potentially making it an effective and cheap way of minimising TG procedure related contamination. In this study, simultaneous PPI treatment was found to significantly increase not only the bacterial load of the stomach but also contamination of the endoscope. It should thus be recommended that PPI be discontinued prior to TG procedures.

In the first animal study, only IV AB prophylaxis was administered to prevent postoperative infectious complications resulting in areas of localised peritonitis in all cases together with an intraabdominal abscess rate of 30 %. In the second animal study, a combined effort was made to prevent contamination and infection implementing the knowledge gained from the RCT. Disregarding the case with peritonitis after repeated punctures, no animals had peritoneal lesions representative of postoperative peritoni-

tis thus being a clinical marker for reduced contamination. Despite this, an intraabdominal abscess was still present in a single case. When reviewing the preliminary results from human series it is doubtful whether the animal model is suitable to assess infectious complications following TG NOTES. The evidence is limited by small samples, and caution should be taken making combined regimens a necessity in human studies until fully evaluated.

The combination of intraluminal EUS with TG peritoneoscopy and extraluminal EUS was found feasible and provided sufficient evaluation of the abdomen. The technique has potential for minimal invasive staging of upper gastrointestinal cancers. The upper abdomen is difficult to visualise, and technical advantages in especially NOTES specific endoscopes are required before the procedure can be implemented in routine practice.

In conclusion, we did not feel that the existing evidence and our own experience rectified the progression of TG pure-NOTES from animal models to human series. Although smaller series support the feasibility in humans with no risk of infectious complications, there exist a need for further research to fully describe the safety profile and further refinement of the technique. Although NOTES is categorised as a minimal invasive approach, the endoscopes and instruments available at present leads to a significant increase in procedural time with minimal patient benefits when compared to laparoscopy.

FUTURE PERSPECTIVES

With the use of conventional endoscopes in the peritoneal cavity, manoeuvrability is limited, and it is difficult to maintain spatial orientation and stabilisation, triangulation, and tissue traction for dissection. If TG NOTES are to be implemented in routine clinical practice, it is essential that the industry can provide multitasking platforms with NOTES specific endoscopes and instruments. This is essential before possible benefits of TG NOTES can be evaluated against the gold standard of laparoscopy in randomised clinical trials. With NOTES specific endoscopes, the relevance of access location with respect to adequate visualisation would probably be insignificant.

Regarding the risk of contamination and infection, it would seem that this aspect is not easily evaluated in experimental studies based on animal models. It can be discussed whether or not it is ethically justifiable to proceed to human series without first having the safety aspect fully clarified. A dilemma exists if the results from animal studies are not applicable to a human setting. Limited data from human series and pre-NOTES protocols seem to show little risk of infectious complications. Cautious evaluation in well-designed and controlled trials need to be conducted in humans to assess this aspect, including clearly defined parameters for terminating the study ahead of time assessed by an impartial safety committee. Based on the results available standardization of TG surgery should be dictated through consensus amongst NOTES organisations.

SUMMARY

The objective was to investigate whether transgastric Natural Orifice Transluminal Endoscopic Surgery (NOTES) could be implemented safely in clinical practice. The experimental studies proved ultrasonography guided access through the stomach to be feasible and safe without iatrogenic complications. Although the technique was safe, further development is needed to increase reproducibility and reduce the procedural time used for gaining access. Closing the gastrotomy after the procedure can be performed easily by application of an endoscopic clip (Over-The-Scope-Clip). Microscopic evaluation of excised gastrorrhaphies revealed that sufficient healing had been achieved after long-term follow-up. A fundamental problem with TG peritoneoscopy is the lack of NOTES specific endoscopes. With the combination of intraluminal EUS and peritoneoscopy with extraluminal EUS, it was possible to achieve sufficient visualisation of anatomical structures of interest in the diagnostics and staging of upper gastrointestinal cancers. Another problem with TG NOTES is the risk of intra-abdominal infections. Using a multimodal decontamination regimen reduced the rate of intra-abdominal pathology, but the risk of intra-abdominal abscess formation as a result of contamination from the access route was still present. To reduce this contamination, mouthwash with chlorhexidine was effective in a human randomised study. The same study also found significant higher bacterial load in the stomach of patients using proton pump inhibitor, emphasising the need to pause PPI prior to future TG interventions. Whether the risk of infectious complications after TG NOTES is comparable between animals and humans is debatable. Despite this, the subject of infectious complications and the safety profile of the TG technique require further research. Based on the evidence available in the literature and current experience, clinical implementation to the benefit of patients does not seem justifiable at present time.

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