Assessing transgastric Natural Orifice Transluminal Endoscopic Surgery prior to clinical implementation

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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

ARTICLES INCLUDED IN THE THESIS

Study I


Study II


Study III


Study IV

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 ultrasoundography (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 ultrasonography-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 videendoscope 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 gastrostomy 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 gastrostomy. 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 gastrostomies.

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 ulcers 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)

<table>
<thead>
<tr>
<th>Outcome parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (POD 14)</td>
<td>10/10</td>
</tr>
<tr>
<td>Uncomplicated follow-up (POD 14)</td>
<td>9/10</td>
</tr>
<tr>
<td>Intraoperative complications</td>
<td>0/10</td>
</tr>
<tr>
<td>Gross lesions related to access (cropsy)</td>
<td>0/10</td>
</tr>
<tr>
<td>Macroscopic full-thickness closure (cropsy)</td>
<td>9/10</td>
</tr>
<tr>
<td>Full-thickness healing (histology)</td>
<td>0/10</td>
</tr>
<tr>
<td>POD postoperative day</td>
<td></td>
</tr>
</tbody>
</table>

### 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

<table>
<thead>
<tr>
<th>Animal no</th>
<th>Access technique</th>
<th>Gastrorrhaphy</th>
<th>Peritoneal cavity</th>
<th>Full-thickness healing</th>
<th>Adhesions</th>
<th>Abscesses (n)</th>
<th>Peritoneal granulation</th>
<th>Fibrinous deposits</th>
<th>Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Multicellular</td>
</tr>
<tr>
<td>2</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Multicellular</td>
</tr>
<tr>
<td>3</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Multicellular</td>
</tr>
<tr>
<td>4</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Multicellular</td>
</tr>
<tr>
<td>5</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Localized</td>
</tr>
<tr>
<td>6</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Multicellular</td>
</tr>
<tr>
<td>7</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Localized</td>
</tr>
<tr>
<td>8</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Disseminated</td>
</tr>
<tr>
<td>9</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Disseminated</td>
</tr>
<tr>
<td>10</td>
<td>In situ</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Localized</td>
</tr>
</tbody>
</table>

**Table Notes:**

- OTSC: over-the-scope clip
- AP: Azotobacterium prutenae
- PM: Paenibacillus multivaculifer

For access technique, (+) indicates transgastric needle puncture and balloons dilation, S indicates sphincterotomy, B indicates bougie, and N indicates needlefistula. For pathology, histology, and microbiology, 0 indicates absence, and + indicates presence of the specific parameter. For fibrinous deposits, ++ 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).

Table 3 Visualisation scores according to the predefined record form

<table>
<thead>
<tr>
<th>Pig</th>
<th>Intraluminal EUS</th>
<th>Intraperitoneal EUS</th>
<th>Peritoneoscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(max = 15)</td>
<td>(max = 9)</td>
<td>(max = 13)</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Median</td>
<td>14–15</td>
<td>1–8</td>
<td>8–13</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Anatomical structure</th>
<th>Intraluminal EUS</th>
<th>Peritoneoscopy</th>
<th>Intrapерitoneal EUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper abdomen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right liver lobe</td>
<td>10/10</td>
<td>9/10</td>
<td>8/10</td>
</tr>
<tr>
<td>Left liver lobe</td>
<td>10/10</td>
<td>6/10</td>
<td>3/10</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>10/10</td>
<td>8/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Common bile duct</td>
<td>9/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spleen</td>
<td>10/10</td>
<td>10/10</td>
<td>-</td>
</tr>
<tr>
<td>Right upper quadrant</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Left upper quadrant</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Right diaphragm</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Left diaphragm</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stomach</td>
<td>-</td>
<td>-</td>
<td>10/10</td>
</tr>
<tr>
<td>Lower abdomen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right lower quadrant</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Left lower quadrant</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Descending colon</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pancreas</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Right kidney</td>
<td>-</td>
<td>-</td>
<td>7/10</td>
</tr>
<tr>
<td>Retropertoneum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left kidney</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hepatic veins</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>9/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal vein</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Celiac artery</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Upper abdominal aorta</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lower abdominal aorta</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Superior mesenteric</td>
<td>9/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inferior mesenteric</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cecal vein</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Splenic artery and vein</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Right iliac artery and vein</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Left iliac artery and vein</td>
<td>10/10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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.


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).

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

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).

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).

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

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: Gastroscopy 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 videogastro-

scope 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 gastrotomies. 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.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Follow-up allocation</th>
<th>Access</th>
<th>Closure</th>
<th>Pathologist’s assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>2</td>
<td>stay</td>
<td>4</td>
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<tr>
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</tr>
<tr>
<td>6</td>
<td>28</td>
<td>1</td>
<td>stay</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>2</td>
<td>stay</td>
<td>4</td>
</tr>
</tbody>
</table>

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 ScopeClip (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 needleknives 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.
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.

<table>
<thead>
<tr>
<th>Allocation</th>
<th>Animal no.</th>
<th>Peritoneal cavity</th>
<th>Abcesses (n)</th>
<th>Fibrin deposits</th>
<th>Adhesions</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+++ (dfp)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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 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 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.
omепразол 100 мг дневно до POD 7. Това не бе администра-рирано в настоящия студий. Причината за това е трудно-стта, с което да се администрира и да се установи коректна доза на перорално лекарство до свързване. Лекарството би могло да бъде дадено интраареночно, но този вариант има няколко недостатъка. IV достъп може да бъде достигнат през тунел в ухото или през централен венозен категер. Оба метода имат висока риска от случайно нейното влияние, и заместването изисква предпазна обработка. Доатомични фактори и природа на свинчето, IV достъп също влошава риска от инфекция.

**DISCUSSION**

Целта на настоящата трудност беше да изследваме безопасността и възможността за репродуктивен TG NOTES. В експерименталните изследвания, употребата на EUS/Doppler управлението беше открито да бъде безопасно, но трудно, поради недостиг възможност на манипулация в преден гръбначен проход. За закриване, OTSC предостави безопасност, но трудност, изисквайки некоректна репродуктивност и изисквайки заместване.

Приносът на тези изследвания за определение на рисках и безопасността на TG NOTES по отношение ключеви изисквания за достъп, закриване и инфекция, също съвпадат с точките, че възможността за репродуктивен TG NOTES, тези достъпни техники не бяха накратко обсъжданите.

**Transgastric access**

Как да осигурим безопасен и репродуктивен достъп до пърете-нога на коприна е една от основните задачи на TG NOTES [11, 33]. Създаването на преден гастростомичен (PGE) тунел е внедрен в клиничната практика от началото на тунелите [34]. Метаанализите по отношение на хемостомията (PEG) показват нисък рисък за усложнения и смъртност [35]. Възможно е да се разгледат някои от някои от тези усложнения, но не са свързани с пърквата на PEG тунел за хранене.

Възможностите на достъпа са базални варианти на същата принцип. Тъй като се осъществява съвместно със стъпка и крепеж, радиокауерът се напълва с чесък воал и се подрежда със стъпка и крепеж.
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, bioabsorbable 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 all 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 contradictory results have been reported, the bursting pressure of OTSC closed gastrostomies 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 support 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 contradictory 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 lobe. 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 plaques 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 gastrostomy 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 endoscopy 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 endoscop 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 contradictory, 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 antisepic 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 contradictory [121-123]. This could in part be explained by the different solutions
being studied, although another study found that prepro-
cedural 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 re-
ducing 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 decon-
tamination modalities have also been evaluated with
contradictive results in animal models. One study found
that a combined regimen was effective in reducing perito-
eal bacterial contamination, but despite a significant re-
duction 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 con-
tamination 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 sig-
nificantly 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 de-
creased 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 con-
tamination level when performing TG surgery.

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

Founded in the contradictory evidence, it has been
proposed that the pig model is unsuitable for evaluating
contamination and infection [124]. To support this state-
ment are results from a retrospective review of 100 pa-
tients enrolled in different pre-NOTES protocols [131].
Here cross-contamination was observed in as many as 21 %
but without any infectious complications. The study con-
cluded that bacterial contamination secondary to TG ac-
cess 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 recommenda-
dation 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 per-
forming 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 cefa-
zoline, 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 le-
sions 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 signifi-
cantly reduce the bacterial load on the endoscope when
performing gastroscopy, thus potentially making it an ef-
fective 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 endo-
scope. It should thus be recommended that PPI be discon-
tinued prior to TG procedures.

In the first animal study, only IV AB prophylaxis was
administered to prevent postoperative infectious complica-
tions 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 intra-abdominal 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 gastrotomies 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.

LITTERATURE


