Histological evaluation of pure NOTES – related complications in a survival animal study

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Abstract
Considered as an extension of both laparoscopic surgery and interventional endoscopy, natural orifice transluminal endoscopic surgery (NOTES) is emerging as a new alternative of minimal invasive surgery. Literature on the gastrointestinal complications of this novel technique is sparse. The goal of this study was the histopathologic evaluation of postoperative complications in a NOTES experimental animal group. Ten female pigs (Sus scrofa domesticus) underwent transgastric endoscopic oophorectomy and tubectomy followed by gastric closure using OTSC clips. Fourteen days after surgery, the animals were sacrificed. Based on the gross examination during necropsy focused excisional biopsies were performed. Gross and microscopic evidence of gastric ulcer distal to the puncture site and perigastric lymph node abscess were found on one animal. Histological evaluation plays a determinant role in the correct evaluation of postoperative complications of pure NOTES procedures.

Keywords: pure NOTES, histopathology, natural orifice transluminal endoscopic surgery, complications, minimal invasive surgery, survival.

Background
Although just a few years passed since Kalloo AN et al. [1] had published the first animal report on transgastric endoscopy, the acronym of natural orifice transluminal endoscopic surgery (NOTES) has become a familiar term in surgery. This concept represents/stands for a novel surgical technique involving the endoscopic access of the abdominal cavity in a scarless manor via one of the bodies’ natural orifices: mouth, anus, vagina, urethra. The specific anatomical structure is accessed by a flexible endoscope after puncturing one of the hollow viscera (esophagus, stomach, vagina, colon, rectum, urinary bladder, etc.). As expected, safety is one of the key issues that need to be solved before accepting the introduction of this technique into clinical practice. For the same reason the evaluation of the postoperative gastric function is one of NOSCAR recommendations regarding animal laboratory studies for NOTES [2, 3].

Acute gastric ulcer is considered a gastric wall defect extending beyond muscularis mucosa and usually healing with scar formation, being the consequence of a variety of factors who induce damage of the gastric mucosa: in debilitating illnesses, sepsis, trauma or in patients with severe central nervous system injury [4]. It is known for decades that surgery itself can involve an important biological stress, in particular when associated with hemorrhage or local infection, because of gastric ischemia and hypoxia [5, 6]. Minimal invasive surgical techniques (including NOTES) having advantages such as low immunologic impact, fast recovery, etc., are less likely to determine such post-operative events. On the other hand, transgastric surgery has a specific range of complications partially unknown or unevaluated, direct consequence of the uncommon access route used.

The need to establish a histological diagnosis has been stressed since the end of the nineteenth century when Ruge and Veit had introduced the surgical biopsy as an essential diagnostic tool [7]. During the 20th century, histopathology has earned its place as one of the most reliable diagnostic asset associated to the surgical intervention.

As for open and laparoscopic approaches, in NOTES, an important aspect regarding the evaluation of postoperative complications is to use the adequate criteria and the histopathological analysis of the specimen is one of them.

Although clinical reports on NOTES were published [8, 9], most of the studies to this date were performed on animals, mostly porcine and canine [10–12]. The high level of similarity between the anatomy of human and pig’s upper GI tract makes the porcine model a good option for our experimental study.

Materials and Methods

Subjects and preoperative care
The protocol was in accordance with Romanian laws for animal use and care and the directives of the European Community Council (No. 86/609/EEC) and was reviewed and approved by the Craiova Medical University Animal Care and Use Committee/ institutional review board.
For this study a total of 10 female domestic swine (*Sus scrofus domesticus*) that weighed between 20 and 40 kg were placed in quarantine five days before the planned procedure. The animals were removed from woodchip bedding 72 hours before surgery to minimize gastric bezoars and were fasted 24 hours before the operation but allowed to drink water *ad libitum*. The animals were transferred to a surgical lab facility on the day of the procedure. A single dose of parenteral antibiotics were given at the start of the intervention. All ten animals were assigned to pure NOTES procedure under general anesthesia with oro-tracheal intubation.

**Transgastric access and closure**

After gastric irrigation with normal saline an esogastroscopy was performed without the use of an overtube to evaluate the aspect of gastric lumen and to identify an adequate spot for gastrotomy creation. No endoscopic lesion of the gastric mucosa was identified at this stage. The procedure continued with the translumenal access through a 3 to 5 mm full-thickness incision followed by balloon dilation (Figure 1).

![Figure 1 – Gastric access site dilatation using endoscopic balloon.](image1)

The actual intervention consisted of the endoscopic evaluation of the peritoneal cavity and oophorectomy, done under endoscopically maintained capnoperitoneum, without any additional laparoscopic trocars (Figure 2).

![Figure 2 – Endoscopic view of peritoneal cavity.](image2)

At the end of the procedure the pneumoperitoneum was aspirated, the endoscope was withdrawn and the access site was closed with OTSC clips (OTSC; Ovesco Endoscopy GmbH, Tübingen, Germany) (Figure 3).

![Figure 3 – Translumenal access site closure using an OTSC clip (endoscopic view).](image3)

All the procedures were performed by a team comprising of surgeons, endoscopists, anesthetists and veterinary technician. No significant complication occurred during intraoperative time.

**Postoperative care**

All animal survived and were monitored daily for signs of distress (pain, eating habits, bowel movements, clinical signs of sepsis). One animal developed signs of infection on postoperative day 10. Intramuscular antibiotics were given for three days with significant clinical improvement. None of the animals received acid suppressors for the postoperative follow-up. All other animals appeared to thrive in the postoperative period. Swine were euthanized 14 days after procedure.

**Results**

The abdominal organs were examined to determine the presence or absence of abscesses, intraperitoneal adhesions or any other sign of inflammation, according to the predetermined biopsy protocol (Figure 4). The gross examination looked for the size, form, margins and localization of any pathologic process.

![Figure 4 – Stomach: anterior face and perigastric lymph nodes.](image4)

Special attention was paid to the gastric macroscopic aspect, considering the two critical aspects in natural orifice endoscopic surgery: the transluminal access and closure. For this purpose, the stomach was resected and evaluated for any sign of lesions like ulceration, inflammation, etc. No signs of perforation or leaks were
observed at the inspection of the peritoneal cavity. To further confirm that the stomach was later removed and a pressure test for leakage was performed in every case. All leak tests showed negative.

Abscesses on the greater curvature and multiple enlarged perigastric lymph nodes, sizes varying from 0.5 to 1.5 cm were observed in one animal, the same who developed signs of infection postoperatively. A 0.5 cm ulceration was revealed after opening the stomach, situated on the mid anterior wall, close to the greater curvature and distally from the transgastric access site (Figure 5).

The specimens were processed and analyzed at the Morphopathology Department of the Emergency County Hospital of Craiova. The histological specimen was fixated in neutral formaldehyde 10% followed by paraffin inclusions and a Microtome HM was used to perform 5 µm thick sections. All biologic material was stained using Hematoxylin and Eosin and examined at 4× and 10× magnifications by an investigator who was blinded to the type of intervention and time of harvest.

The histopathologic analysis of the first specimen — perigastric lymph node — revealed signs of sinusal histiocytosis, as evidence of local inflammation (Figure 6).

No such lesions were observed at the time of intervention so they were considered surgery-related. In this case, during gastric manipulation, the OTSC clip was dislodged and recovered at the opening of the stomach. Identification of the translumenal access site was impaired by the excellent healing of the gastrotomy and by the clip dislodgement.

Excisional biopsies were obtained based on the gross aspect of the specimen during the necropsy protocol: a 3 cm in diameter gastric fragment including the ulcer site previously described and a 1.5 cm perigastric lymph node. Some general rules for this procedure were followed. Special care was taken not to crush the tissue with forceps when performing the biopsies. Once the tissue fragments were obtained they were placed immediately into separate containers and sent for histopathological examination.

For the second specimen consisting of a gastric fragment at the level of the ulceration microscopy revealed an acute gastric ulcer. The pathologic process involved all the mucosa reaching all gastric layers to tunica muscularis interna (Figures 7–9). Evidence of necrotic detritus was also present at the level of the ulcer site (Figure 10).
**Discussion**

The first step into understanding postoperative complications is to document their presence and to investigate the circumstances in which they occurred. To do this adequate tools have to be used, such as gross and histological examination.

Regardless of the importance of histopathologic evaluation in experimental surgery, to this date, little efforts were reported to assess the microscopic changes in the stomach during the postoperative recovery period. Only a few NOTES animal experiment reports offer specific information on the gross aspect of organs and a detailed histological examination during postoperative recovery period [13–15].

It is well known that the usual location for stress ulcers is the gastric fundus and for 15% of patients with Curling ulcer, both gastric and duodenal lesions are present [16–20]. These ulcers are most commonly round in configuration and less than 2 cm in greatest diameter. [17, 19] The microscopic aspect shows sharp borders between the ulcerated region and the adjacent mucosa, with limited or no evidence of fibrosis. Numerous acute inflammatory cells and important vascular congestion are other key features in acute gastric ulcer [21].

Obviously, it is well known that any alteration in the thin balance between the acid aggression and the mucosal barrier is the cause of acute gastric ulcer. Still, the causes of this alteration in case of postoperative stress ulcer are difficult to understand and are partially unknown.

This is even more difficult to understand in case of endoscopic transluminal surgery, which is supposed to be less traumatic than open surgery.

Several factors, such as local infection, lack of postoperative gastric anti-acid protection, restrictive alimentary regime, use of NSAIDs or the surgical stress may be involved as potential causes [22, 23].

In our study, we believe that postoperative sepsis was the main cause for stress ulcer.

As we pointed out, and as previously confirmed by numerous studies, sepsis is a potent ulcerogenic promoter especially when associated to surgery [24]. The etiology of postoperative intra-abdominal infection may reside in the suboptimal preparation of gastric cavity before surgery in case of one animal and, in a lesser degree, by the high-level disinfection instead of sterilization of the endoscope. This hypothesis is supported by other authors. Several researches tend to confirm our hypothesis [12, 25, 26]. More, lack of gastric protection, both intraoperative against thermal lesions and postoperative anti-acid medication might have augmented the risk of ulcerogenesis. Some authors used anti-acid medication during the postoperative period in case of transgastric oophorectomy, with good results [14]. Also, adequate endoscopic platform capable of sealing the transgastric access site is demanded in order to have a safe, leak-proof intervention. This is even more obvious in case of an endoscopic mediastinoscopy, which requires perfectly sterile operation, in order to avoid serious complications – such as mediastinitis – with disastrous results.

**Limitations**

We identified several limitations in our study. First, the use of an animal model has some drawbacks and may not be ideal. Never the less, this is a general problem concerning experimental surgery and not specific to NOTES animal experiments. Still, given the availability, the cost, the relative anatomical similarities and safety, swine has proven its usefulness for the development of transgastric procedures [27]. We are also well aware of the limited number of cases and the need of a control group. Following this idea and keeping in mind the low impact no statistical evaluation was performed.

A natural tendency would have been to draw a comparison between laparoscopic and transgastric groups, yet we considered that there are only limited elements to compare when referring to postoperative follow-up and complication rates. One major problem is that transluminal endoscopic surgery has the potential for peritoneal contamination due to intraoperative leaks via the punctured access organ [28]. Peritoneal fluid prelevation during and after the operation would have indicated the intraperitoneal contamination and bring the quantitative and qualitative proof of the microorganisms involved. The lack of postoperative endoscopic evaluation a few days before the necropsy did not allowed us to have a dynamic evaluation of the pathologic process.
Conclusions

Even if it is very appealing to most of the patients, the “pure” natural orifice transluminal endoscopic surgery should not skip some ethical aspects regarding the safe introduction of this innovative technique in the clinical field. Little is known about the postoperative complications of this kind of surgical approach. For this purpose, a thorough macroscopic and histopathologic evaluation of postoperative complications in each research group is mandatory in order to optimize the technique and allow better pre- and postoperative planning.

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References


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